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TOWARDS A SWEETER FUTURE

Sugarcane:
research towards efficient and sustainable production

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Sugarcane:

Research Towards Efficient

and

Sustainable Production

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MAINTAINING THE INTERNATIONAL COMPETITIVENESS OF THE AUSTRALIAN SUGAR INDUSTRY*

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ABSTRACT

The success of Australia's sugar industry has been built on its ability to compete in an imperfectly competitive world market. This success has been achieved through the continued development and implementation of new technology and improved practices throughout the industry. For example, Queensland's bulk sugar terminals lead the world in their performance. The Australian sugar industry must maintain its competitive edge for it to remain viable in the long term. Research is an important element in achieving this objective. To be effective the output from research must contribute to lowering the cost of production and must be implemented by the industry.

INTRODUCTION

Within five years, Thailand, Australia's major competitor in the Far East expects to increase its sugar production by around 45% to 7.7 million tonnes. The Cuban sugar industry is expected to further recover from its recent difficulties and Brazil has the potential to increase its sugar exports substantially. This scenario highlights the need for the Australian raw sugar industry to continue to strive for improved efficiency in order to retain its competitive edge in the world sugar market.

Australia's sugar industry has been built on its ability to compete in an imperfectly competitive sugar market (Borrell & Duncan 1991; Tyers & Anderson 1993). The on-going commitment to the development and the implementation of new technologies on the farm, in the factory, in transport, storage and handling, and in marketing, has resulted in Australia being one of the most efficient raw sugar producers in the world. However, past achievements are not enough. Australia's competitors are continuing to improve efficiency throughout their industries, often with the benefit of government subsidies.

In contrast to overseas industries, the Australian sugar industry receives little government assistance and is reliant on the world sugar price. Therefore, it is imperative that the technical development in the Australian raw sugar industry, at least, keeps pace with developments in competitor countries.

This paper focuses on the need for the Australian raw sugar industry to maintain its international competitiveness by continually moving through the boundaries of present technology and information. Implementing the results of innovative research and development will be instrumental in ensuring the Australian sugar industry retains a competitive advantage.

INTERNATIONAL COMPETITORS

A comparative study of eight leading sugar industries undertaken by Landell Mills Commodity Studies (1993) indicates that many of Australia's competitors are achieving significant productivity gains in both the field and the factory.

Cane production in Brazil has increased substantially, although the rate of increase has somewhat slowed in recent years (Fig. 1). Brazilian cane yields increased from 51.2 t/ha in 1985-86 to 62.8 t/ha in 1995-96, an increase of 23%. Prior to the collapse of the Former Soviet Union, Cuban cane yields were more than 50 t/ha on average. However, since 1990, Cuban production has suffered a severe decline, cane yields in the 1990s averaged just 41.8 t/ha. In Thailand, cane yields have increased from 38.8 t/ha on average in 1985-86 to more than 55.1 t/ha in 1995-96, an increase of 42% in the decade. Growth in the Brazilian and Thai sugar industries combined with the decline in Cuban production over the past 10 years, has meant a change in the source of competition for Australian producers.

Whilst Australia consistently produces more cane per hectare than its competitors, 80 t/ha in 1985-86 rising to 92 t/ha in 1995-96, competitors
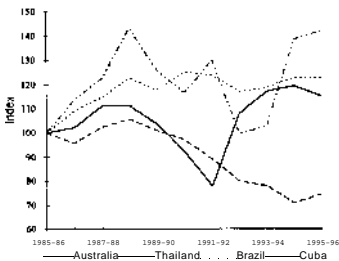
Fig. 1 *Trends in cane yields for selected countries [data source US Dept. of Agriculture (1995)].*

continue to close the gap. The pressure to identify innovative ways to continually achieve productivity gains, and thereby lower costs of production, is mounting.

In the milling sector, Australia leads the world in the recovery of sucrose from the cane crushed, with 90% of sucrose recovered. Australia has achieved this high standard by the improvement of existing technologies and implementation of new technologies. The transfer of milling technologies developed in Australia to other industries has contributed to a significant improvement in their performance. For example, sucrose recovery rates in Thai mills have increased from 68% on average in the 5 years 1971-75, to currently more than 80% (Landell Mills Commodity Studies 1991; 1993).

The main point to be taken from this analysis is that, although Australia is attaining high levels of performance in the field and factory, similar gains are being made elsewhere, often using techniques and technology developed in Australia. Both Thailand and Brazil are positioning themselves to be competitive, low cost producers.

TECHNICAL ADVANCEMENT AND EFFICIENCY

Australia is at the forefront of technological innovation in the world sugar industry in all areas of the industry's activities, *viz.* growing, milling, transport and handling logistics, and marketing.

At the farm level, benefits have arisen from larger scale farming, the introduction of improved sugar cane varieties (with genetic engineering further varietal gains are likely), mechanical cane harvesting, green cane harvesting, trash blanket ratoon management, and irrigation practices. The wide spread use of these improved techniques has reduced costs of production in the field. For example, the introduction of green

cane harvesting and trash blanket ratoon management over the past decade has resulted in lower cultivation and weed control costs. In some areas the associated improvement in soil moisture retention has reduced irrigation costs.

Similar gains have been made in sugar mills. Increased economies of size have seen the number of sugar mills in Australia fall from 33 in 1980 to 29 today. They are expected to crush almost 40 million tonnes of sugar cane this season. In Queensland the average mill throughput (tonnes of cane crushed) in the 1996 season will be in the order of 1.5 million tonnes of cane, up from 872,000 tonnes in 1985-86.

In the milling sector, the introduction of computer control and process automation has contributed to increased factory efficiency and reduced operating costs. Continuous processing technology has allowed mills to increase the efficiency of vacuum pans and centrifugals, reducing the number of units required. In addition, continuous crushing has lead to higher capital utilisation. In other words, existing mill capacity has been used more efficiently as a consequence of the changes. At the same time, Australian mills have been among the most successful in reducing sugar loss in the milling process (Landell Mills Commodity Studies 1993). Overall, as in the farm sector, these gains have lowered the unit costs of sugar production in Australia.

On the marketing front, the advanced technology and integrated logistics of the bulk sugar terminals have enabled Australia to co-ordinate export shipments overcoming a geographical disadvantage. Co-ordinated management of the bulk sugar terminals enables the Queensland Sugar Corporation to guarantee the on-time delivery of consistent high quality sugar suited to the specific needs of customers.

CASE STUDY: BULK SUGAR TERMINALS

Australian raw sugar remains competitive in the market place despite the increases in efficiency being achieved by our main competitors. One reason for this is the low cost of the industry's bulk sugar storage, handling and transport infrastructure. The distances and the congestion associated with moving sugar into and around Bangkok in Thailand and in the port of Santos in Brazil considerably hinder their raw sugar cost structure. Nevertheless, both the Thai and Brazilian sugar industries are investing in their port infrastructure in an effort to overcome these congestion problems.

In this competitive environment, the Queensland Sugar Corporation continually reviews all aspects of the bulk sugar terminal operations to ensure they are operating at world's best practice (Bureau of Industry Economics 1995). For example, terminals originally designed to receive sugar at 250 t/hr have been gradually upgraded so that, today, receiving rates are around 1,000 t/hr. Ship loading rates also have been progressively improved. Average loading rates are now around 2,000 tonnes per hour more than twice the original design rate. These changes have enabled greater intensity of use of the bulk storage facilities, and have significantly reduced labour and operating costs. These lower costs, and the faster turnaround time for ships at all terminals, have established Queensland as the world leader in the technical and economic efficiency of bulk storage and handling of raw sugar. These developments have also helped the Queensland sugar industry maintain a competitive advantage in the world market despite the high levels of government assistance and rapid growth in production efficiency being achieved by overseas competitors.

As is the case in other areas of the industry's activities, competitors are implementing some of the bulk handling technologies developed in Australia. To keep in front, the Australian industry needs to keep enhancing productivity either by increasing economies of scale or through the development and implementation of new technologies.

COST - PRICE SQUEEZE

Another facet of the challenge facing the Australian sugar industry is remaining competitive in the face of a continuing cost-price squeeze.
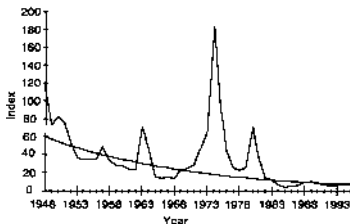
Fig. 2 *Terms of Trade - Australian sugar industry showing yearly variation and long term trendline [index is a ratio of prices received and paid by the raw sugar industry].*

Unlike many competitors, the Australian industry receives little government assistance. With a relatively small domestic market some 80-85% of Australian raw sugar production is exported. The replacement of the import embargo with a tariff in 1989, and the subsequent reductions in the level of the tariff, have exposed the Australian industry to the effects of world price fluctuations. All revenue from sales of Australian raw sugar is dependent on world sugar price movements.

Historically, the world sugar price has been cyclical with a broad pattern of high prices for one or two years, followed by a long period of low or relatively low prices. This pattern occurs because sugar production tends to expand rapidly in response to high prices but is much less responsive when prices fall.

The price fluctuations show only one side of the equation. Of more importance to the continued prosperity of raw sugar producers in Australia are the terms of trade they face (Fig. 2). The terms of trade are defined as the ratio of prices received for raw sugar produced to the prices paid for the inputs used to produce the commodity.

For agriculture in general, the deterioration in the terms of trade is driven, in large part, by world-wide increases in the technical efficiency of agricultural production as well as by the significant assistance that is offered to producers in some countries. The same forces cause the deterioration in the terms of trade for sugar.

The terms of trade facing the Australian sugar industry have fallen by 2-4% per annum on average. Whilst the estimated rate of decline depends on the period analysed, the long term downward trend is clear. The productivity gains identified above (field, factory, transport, storage and handling, and in marketing), have enabled the Australian industry to remain viable despite the relentless nature of the cost price-squeeze.

An irony of this is that, as new technologies developed in Australia are implemented in sugar industries around the world, they inevitably lead to a further deterioration in the terms of trade. This provides the imperative for Australia to stay at the forefront of sugar industry research and development across the range of industry activities. The Australian industry can keep ahead of the cost-price pressures by being a world leader in the development and application of research and technology. If we do not maintain our research activity, there is ample scope for competitors to close the productivity gap, exacerbating the cost-price pressures on Australian producers.

SUGAR INDUSTRY R&D

The sugar industry in Australia has a long history of funding industry research development and extension. The first levy 1 penny per ton, paid equally by cane growers and mill owners, was raised in the *Sugar Experiment Stations Act 1900* and first collected in 1901. This equates

to a levy of 37.1 cents per tonne in 1996 dollars. By comparison the actual levy collected in 1996 by the BSES and SRDC for industry R&D is 31 cents per tonne paid equally by cane growers and mill owners. Although the value of the levy has fallen on a dollar per tonne of cane basis, the total levy collected has increased with industry production, and in 1995 industry funding of BSES and SRDC was S9.6 million.

Areas of R&D which are expected to continue to be of importance to sustaining the competitiveness of the Australian sugar industry include cane production, harvesting, milling, storage handling and marketing. Raw sugar quality is an issue of particular importance and is likely to remain so.

Gains in the technical efficiency of the industry are important because, ultimately, it is technical progress which determines the boundaries of efficient production within the industry. Nevertheless, the technical innovations will only be implemented if the economic benefits of introducing the new technology or techniques exceed the costs of its introduction.

The greatest gains from new technology will arise when research results are disseminated quickly in a readily useable form. Therefore it is important that appropriate technology transfer mechanisms be established early in the research process or be developed as a part of the project.

CONCLUSION

The development of Australia's sugar industry has been built up on its ability to compete in a world market characterised by government intervention and a deteriorating terms of trade for sugar. The relative efficiency of the Australian industry has been achieved through the continued development and implementation of new technology and production techniques on the farm, in the factory, and in marketing the end product. The focus has been on improving the economic efficiency of the industry.

For the future, the challenge is to continue to make these gains. There is no alternative for the Australian sugar industry if it is to remain viable and profitable. The task for R&D providers is to produce results which can be disseminated quickly in a readily useable form. The real benefits from R&D are only realised when the new knowledge has been applied successfully.

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THE FUTURE OF THE MAURITIUS SUGAR INDUSTRY

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ABSTRACT

Sugarcane cultivation is the main agricultural activity in Mauritius and contributes c. 30% of net earnings. Sugar is sold primarily on preferential European Union markets at remunerative prices. Total sugar production has been declining primarily because of abandonment of cane cultivation which has been partly offset by higher sugar yield/ha. The industry has embarked on a programme of increasing productivity per unit area, reducing costs of production, and diversifying both within sugar and in crops grown in association with it, in order to remain sustainable and continue to play a dominant role in the Mauritius mixed economy. The role of R. D & E in attaining the targets set is discussed and the priorities identified are strategic research, extension and support to development.

INTRODUCTION

Sugarcane was introduced into Mauritius by the Dutch in 1639. Mahe de Labourdonnais. Governor General during the French occupation, gave a great impetus to the production of sugar on the island. At the beginning of the XIXth Century, there were 60 to 80 factories producing over 3000 t of sugar. Since that time, the sugar industry has been undergoing a constant process of expansion, modernization and centralization of factories. Today there are only 17 sugar mills that produce about 0.63 Mt sugar annually.

This paper reviews the current situation of the Mauritius sugar industry and identifies the factors that have contributed to its present status of development. The probable evolution of these factors will be forecasted and new factors that will have an important bearing on the industry will also be identified. From this analysis, three scenarios will be developed, and long-term goals will be set for the most likely one. The contribution of research, development and extension in meeting the above targets will be discussed.

THE INDUSTRY: 1970 TO 1990

Sugar dominated the Mauritian economy in the 1970s with a GDP of 25%. employment factor of 45% and represented 90% of exports. With the development of the manufacturing and tourism sectors, sugar now is still the main agricultural crop but represents only 10% GDP with employment factor of 15%, and 30% of exports. It was the highest net income earner up to 1992 but has, in 1993, been superseded by the manufacturing sector. Sugarcane production is well organized in Mauritius with strong components of co-ordination, research, training, development, marketing and export, finance, insurance and public relations.

Employment in the agricultural sector has been declining as a result of competition from other sectors of the economy. Small planters, particularly, have experienced a severe labour shortage. This has greatly contributed to increased costs of production and led to the decline of profitability.

The present area under agriculture is approximately 86600 ha of which approximately 81000 ha (95%) are under cane. Farms range from a fraction of a hectare to several thousand hectares. There are 17 miller-planters, 207 large planters, 31209 small planters and 1187 tenant planters. About 55% of the area belongs to miller-planters who produce about 61% of total production. A yield gap therefore exists between the two groups (Julien et al 1994). Total sugar production is currently 0.63 Mt, with an average sugar yield of 8 t/ha.

Sugar production increased from 1968 to 1973 and has stabilised and even shown a slight decline in the last decade. Sugar yield per unit area has shown a clear increasing trend during the same period which is solely attributable to an increase in cane yield as extraction rate has declined mainly due to an increase in extraneous matter and varieties with lower CCS.

Cane is planted in a variety of environments comprising four main soil types and annual rainfall from 800 mm to > 3 m. Cane planted in summer is harvested at 16-18 months and winter plantings are harvested at 13-14 months. Average number of ratoons is 7. Varieties specifically adapted to different soil types, climates and dates of harvest have been developed in Mauritius. Chemical weed control is generally practised, although some small planters still perform manual weeding. About half of the sub-humid area is irrigated on a total of 35000 ha. Surface, improved surface using siphons, overhead and drip irrigation are usually practised. Fertilizer recommendations are based on soil analysis and foliar diagnosis. Cane fields are not usually deficient in phosphorus and micro-nutrients, except for silicon in the superhumid zone, where pH is also generally low to very low. Complete fertilizers 17-8-25 or 17-2-27 (N-P-K) are applied at rates ranging from 700 to 950 kg/ha in the furrows at planting or surface banded along the cane rows in ratoons after harvest.

The ripening period starts just after flowering (normally May and June) and cane is harvested from June to December. Sucrose content generally increases up to end of October and shows a decline towards the end of November. Cane loading is highly mechanized among the large and miller-planters. With the shortage of labour, mechanical harvesting has been re-introduced and is expanding rapidly among this group of planters. Transport from field to mill as well as from mill to port is solely by lorries.

Mauritius has negotiated and obtained access to the remunerative European Union in the framework of the Sugar Protocol, and to the US market with the US Sugar Programme at prices which are currently more than twice that of world sugars. Most of the Mauritius sugar is sold on the preferential markets, namely EU (507000 t), USA (15000 t), EU Refiners' Deficit (85000 t) and world specials (10000 t). About 37000 tonnes are sold locally, and the balance is sold on the world market.

NEW DEVELOPMENTS

Markets

In 1995, Mauritius negotiated an additional quota of about 85000 tonnes for the EU Refiners' Deficit at a remunerative price for the next six years. However, market demand and sale price beyond 2001 will depend on maintenance of the Sugar Protocol and implementation of GATT.

Production

The increase in sugar yield per unit area has not been translated into an increase in total sugar production on account of a marked reduction in area cultivated (c. 400 ha/yr) in recent years, particularly from planters' land (Julien et al 1994). Area under cane cultivation will further reduce through growth of urbanization, tourism and industrialization, competition with other crops, and abandonment of sugarcane land. Yield per unit area will continue to increase by more than 1 % per year through

varietal improvement, development of irrigation, and improved agronomic and management practices through the implementation of recommendations of the Julien Report (Julien et al 1995).

Socio-economic factors
Costs of production: An ageing labour force and a reduction in agricultural labour will tend to maintain high costs of production which could, however, be offset by mechanization. The relative parity of the rupee with other major currencies will partly determine the trend of cost of production for other inputs. Centralization and increase in efficiency at factory level should reduce costs of production.

Socio-economic structure: It is forecasted that the initial steps in worker participation (viz. 20% of the shares of sugar factories allocated to small growers and work force of the industry) which have just been taken by Government will be consolidated and expanded, and will have a positive bearing on level of productivity.

Diversification
Diversification within sugar will progress further with a marked increase of electrical energy produced from bagasse, special sugars, ethanol and the recent development of organic sugar. Diversification of cropping in association with sugar, after initial success, is now primarily limited to the production of potatoes, because various economic and technological factors have limited production of other crops such as maize and beans. However, progress is expected in the future because the introduction of GATT will open new and more opportunities to diversify into other crops particularly for self-sufficiency.

LONG-TERM GOALS

Three scenarios have been considered:

(i) *Mauritius—A Mixed Economy:* Development based on sugarcane, tourism, services and industries.

(ii) *Sugarcane and Mauritius:* Negative economic factors for the industrial sector would lead to a decline of the manufacturing sector and Mauritius would again have to rely primarily on agriculture dominated by sugarcane with complementary sectors, services and tourism.

(iii) *Mauritius—The Green Island Paradise:* In view of negative economic factors for world sugar exports and preferential sales agreements, the growing of sugarcane would be viable for only local and highly remunerative export markets, and other options for the use of some of the sugarcane lands would have to be found.

The first scenario is considered the most likely one (Julien et al 1994), and Table 1 summarizes the likely status of the Mauritian sugar industry by 2020 based on this scenario.

Table 1 *A comparison of current production parameters with those targeted for year 2020*

	Current	Year 2020
Markets (tonnes)	650000	700000
Average price per tonne (A$)	830	800
Area under cultivation (ha)	81000	72000
Area irrigated (ha)	17000	30000
Area treated with ripeners (ha)	5000	15000
Yield of sugar (tonnes/ha)	8	10
Total production (tonnes)	648000	720000
Research (A$ million)	8	8

A$ = Australian dollars

ROLE OF RESEARCH, DEVELOPMENT AND EXTENSION

In view of the high level of technology developed through several decades of applied research in sugarcane husbandry, higher priority will be given in the future to strategic research, socio-economic studies, extension and development (MSIRI 1994). as outlined below:

Increasing total sugar production at the national level
Increasing sugar yield per unit area: Research will aim at: (i) increasing the efficiency of breeding and selection of cane varieties through analytical studies and the development of classical as well as molecular genetics; and (ii) improving sugarcane husbandry with particular emphasis on mechanization, ripening and irrigation.

Rehabilitation of abandoned cane lands: Surveys based on rapid appraisal techniques will be undertaken to identify the major reasons for abandoning cultivation of cane and discuss remedial actions with planters. The Land Index database and GIS techniques will be used for the planning and field layout of Land Area Management Units (LAMUs) during the process of rehabilitation (Jhoty 1995).

Yield gap between planters and millers: Global analysis and investigations at level of each factory area will be conducted to establish the evolution, of variation among planter groups, and factors responsible for, the yield gap of about 20% between miller planters and others.

Improving labour efficiency: As mechanization cannot be introduced under all conditions, research will aim at improving the performance of labour. This would also contribute to a reduction of costs of production through increased efficiency during field operations as well as at factory level, where losses due to delay in supply of cane would be minimized.

Land/crop research studies: Compilation and exploitation of relevant databases through statistical and spatial analyses are vital for identifying the land, management and socio-economic constraints affecting productivity (Julien et al 1987). A geographical information system for cane lands (GISCANE) has been developed as a supporting tool for carrying out these land/crop related research studies on productivity (Jhoty 1995).

Reduction of costs of production
Important issues identified are the optimal use of farm inputs, identification of the best technology in the field of crop husbandry, analysis of units of production, and improving management techniques for different planter groups. Priority will be given to cost of labour, fertilizers and transport.

Diversification within sugar
The major objective of diversifying within sugar is to make maximum economic use of the diversity of yield components of sugarcane (cane tops and trash and by-products of sugar processing). This includes maximum use of by-products and the production of derivatives of high value. Generation of electricity from bagasse will become more important in years to come, and agricultural and engineering research will be directed towards higher production of bagasse through breeding high fibre varieties and saving energy at factory level.

Experimental work for increasing yield of fields earmarked for organic sugar production (128 ha planted in 1995) so as to ensure its economic viability will be initiated.

Crop diversification
Growing other food crops in association with sugarcane has been adopted to maximize production of these crops for self-sufficiency (Wiehe et al 1987). A number of factors will influence die choice of the crop, e.g. length of maturation cycle, climatic adaptation, competition for water and nutrients, etc. Potato, maize, groundnuts, beans and tomatoes have been shown to be suitable crops for growing in interrows or on rotational land. Govinden (1995) has shown that our priorities at present should be potatoes, tomatoes, and maize to be used as a vegetable. To extend the planting season of potatoes and thus reduce the time of storage, there is a need for varieties that are tolerant to heat and bacterial wilt, which is particularly important in summer. Research inputs for maize will be reduced since, as a result of high cost of production, locally produced grain maize cannot compete with imported maize; however, the development of varieties for the island of Rodrigues will continue as well as selection of green cobs and

sweet corn varieties. The main research projects for improving tomatoes will include selection of cultivars for higher yield, improvement in fruit quality, disease and pest management, and post-harvest technology for keeping quality.

Monitoring environmental issues

R, D & E will aim at evaluating the impact of cane cultivation, milling and processing on the environment (Ricaud et al 1993). The major fields of study which will be pursued are:

(i)   The persistence and leaching of pesticides.
(ii)   The degradation of agro-chemicals in soils and their movement into surface and ground waters,
(iii)   The effects of mechanization on soil conservation,
(iv)   Erodibility and erosion of soils.
(v)   Monitoring and treatment of waste water, oil and gases from sugar factories.

Transfer of technology

Research findings only have value if they lead directly or indirectly to an advancement in technology which is adopted by producers. MSIRI is directly responsible for extension to medium (10-40 ha), large, and miller, planters. Extension for the small planters (about 35000 who own about 65000 plots < 10 ha. and average 0.8 ha) is conducted by the Farmers Service Corporation. In order to ensure that new developments are adopted by this last group, MSIRI work in close collaboration with Farmers Service Centres and Directorate of Agricultural Research and Extension officers, as well as with Sugar Estates Planters' Advisers. The use of improved extension methods and modern communication techniques such as visits, open days, videos, talks, etc will strengthen the linkage between MSIRI researchers and all categories of producers.

Development

The mandate of a research organization should also include support to the development of new technologies. This, in association with efficient extension, will contribute to the successful implementation of advanced technologies. A few examples, already undertaken by MSIRI are: (1) The use of GIS technology for locating the best position of a centre irrigation pivot and also as an aid to field layout and farm planning (Jhoty et al 1994); and (2) The production of maize and potato seeds. and cane nurseries to provide a wider range of disease-free planting material to growers.

New projects currently being started include: (1) The preparation of an irrigation suitability map: (2) An economic analysis of industrial field data to determine optimum cane cycles (number of ratoons) and the ratio of summer to winter planting; and (3) Economic evaluation of various new development projects such as mechanization of field operations.

CONCLUSION

Our vision should be *The Sugarcane Industry of Year 2000* as opposed to *The Sugar Industry of 1990s*. The role of R, D & E in attaining this goal is crucial.

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SUGAR PRODUCTION IN INDIA BY 2000 AD. 1. CONSTRAINTS AND STRATEGIES FOR INCREASING PRODUCTION AND PRODUCTION EFFICIENCY

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ABSTRACT

India is currently the world's largest producer of sugarcane, however, the sugar economy remains in a state of flux. Per capita consumption of sugar is c. 14.7 kg again world average of 25 kg. India's sugar need is c.18.4 Mt for domestic consumption by 1000 million people and about 2.0 Mt for export. Therefore, the Indian Sugar industry has a formidable task to produce 320 Mt of sugarcane by 2000 AD. To achieve this target, the area, cane productivity and sugar recovery have to be raised to 4.0 Mha, 80 t/ha and 10.5 % respectively, against the current levels of 3.78 Mha, 67 t/ha and 10.0%. The present production system is plagued with many problems such as regional imbalances in productivity, high cost of cultivation, fluctuation in cane area, lack of suitable varieties for saline, alkaline and water-logged conditions, lack of early maturing high sugar varieties, low sugar recoveries, low productivity of ratoons, and lack of suitable pricing and marketing policies.

INTRODUCTION

India produces 13 % of the world sugar output as well as 8 to 10 Mt of jaggery and *khandsari* (Open Pan). Despite being the largest producer of sugarcane in the world (estimated 255 Mt during 1994-95), its sugar production efficiency is not at the top level. The crop is grown on 3.78 Mha with average productivity of about 67 t/ha and average recovery of 10%. Sugarcane is the sole raw material for the second largest processing industry, where about 35 million skilled and unskilled workers are employed. In the organised sector, it is the largest single employer in the rural area. At present, there are 435 sugar mills in operation in comparison to just 139 mills in 1953. The sugar industry pays about A$ 2800 million annually to cane growers.

According to the estimates of the National Commission on Agriculture (Anon. 1976), the population of India may rise to about 1000 M by 2001 AD and is expected to swell to 1360 M by 2020 AD at present compound growth rate of 1.6% per annum. The estimated per capita consumption of sweeteners by 2001 AD will be around 32 kg (18.2 kg white sugar and 13.8 kg jaggery and *khandsari*).The fulfilment of this target is only possible if the cane area, productivity and sucrose recovery are raised to 4.0 M ha, 80 t/ha and 10.5 %, respectively. (Table 1) . The present scheme of horizontal expansion is not feasible because India has limited cropping area with unabated urban expansion. Vertical expansion seems to be the only possibility and this can be achieved by obviating genetic, physiological, biochemical, agronomic, and biotic and abiotic stresses of the crop. This paper briefly highlights various constraints limiting sugar production.

Table 1 *Projection of sugar production and consumption in India*

Year	Ansa (Mha)	Expected population (M)	Expected production (Mt)	Expected recovery (%)	Sugar output (Mt)	Percapita Consumption (kg/year) Sugar	Jaggery	Total
1990								
–91	3.68	840	241	9.85	12.05	14.7	10.7	25.0
2001	4.00	990	320	10.50	8.50	18.2	13.8	32.0
2010	4.25	1160	383	10.75	26.80	23.4	12.6	36.0

CONSTRAINTS IN SUGAR PRODUCTION: INDIAN SCENARIO

Environmental constraints

In me past, improved varieties have played a major role in increasing cane productivity. These varieties are generally selected for optimal conditions under spring planting with emphasis on high yield and moderate sugar content. Sugarcane is a 12-18 months crop and faces vagaries of nature in the form of temperature, frequent drought and water logging . The wide fluctuation in the yield and sugar recovery in tropics and sub-tropics is primarily due to weather conditions. The sub-tropical cane growing area which is about 65% of total area under cane contributes only 35 % of total sugarcane production.

Biotic and abiotic constraints

The biotic constraints which limit cane productivity are diseases, pests, rodents and weeds. The abiotic constraints are drought, flood, salt stress (salinity and alkalinity), frost, low temperature, mineral deficiency and wind injury. These stresses singly or in combination affect quality and cane yield (Table 2).

Table 2 *Major biotic and abiotic stresses affecting cane productivity*

Constraints	Percent loss in cane productivity	Affected areas in India
ABIOTIC STRESS		
Drought	15-20	All over but more in Central and Northern states
Wate-logging	5-20	U.P.,Bihar and Assam (c.30% in sub-tropics)
Saline/alkaline soils	10-20	All over (c. 7 M ha)
Frost and low temp	2-7	Northern India and Assam
Mineral deficiency	25-45	All over
BIOTIC STRESS		
Weeds	10-15	All over
Diseases	2-5	
Pests	2-5	

In recent times, diseases and pests are posing great threats to sugarcane production. Nearly 130 diseases and 160 insect pests have been recorded in sugarcane. The collective losses vary from 15 to 20% in terms of quality and quantum. In this regard, diseases like red rot have a profound effect in epidemic form and pose a serious threat to cane growers and the sugar industry.

Non-availability of Quality seed

Being a vegetatively propagated crop, cane setts carry many serious diseases such as red rot, smut. GSD, RSD, leaf scald.etc. Another related

constraint to this is low seed multiplication ratio (1:10). This constraint hinders rapid multiplication and spread of new varieties. Planting techniques such as STP (Spaced Transplanting method) and micropropagation are now being promoted in some areas to overcome this problem.

Late-planting

There are three major planting seasons for sugarcane.viz., spring, autumn and late-planting (April/May). In the western region of the sub-tropical zone, there is a practice to plant sugarcane after harvesting of wheat is over, i.e. April/May. This pushes the planting of sugarcane into the summer months. Due to late-planting, the early growth phase is subjected to high temperature (40-45 °C) and water stress, which impedes tillering pattern and consequently the number of millable stalks.

Yield plateau

Development of sugarcane varieties in the past has played an important role in increasing cane productivity. However, the major thrust today is to improve both yield and sucrose content in genotypes, which are negatively correlated. Therefore, for further improvement, one of the major challenges to sugarcane breeders is to modify breeding approaches to break the yield and sugar plateau. Unconventional methods, particularly biotechnological approaches, which are being tried in Australia could be promising in this direction. Furthermore, a directed approach to identify varieties which have the capacity and potential to thrive under sub-optimal conditions will have to be adopted.

Low sprouting in sub-tropics

In India, usually 2 to 3 bud setts are used as planting material. In tropical cane growing areas about 70-80% sprouting takes place whereas in sub-tropics it never exceeds 30-40%. This results in a poor stand of crop which subsequently affects cane productivity. Extensive work has been done to understand the activation and sprouting of sugarcane buds under sub-tropics (Solomon and Kumar 1987; Solomon et al. 1988; Solomon and Srivastava 1990). The studies conducted by Solomon et al (1993) have shown that pre-harvest foliar application of ethephon (at 500 mg/L) induced better sprouting of cane buds from treated setts under sub-tropical conditions.

Non-synchronised tillering

Tillering is the largest growing phase in sugarcane because millable cane forming tillers continue to emerge till July. Among the total tiller population, only 25-35% of tillers form millable canes or economic product. Tillers emerged during early phase (March- April) form about 70% of millable cane. However, tiller emergence continues followed by mortality which is a pure economic waste of plant energy. Solomon *et al* (1987,1993) made basic biochemical studies during tiller emergence, particularly in respect to nitrogen assimilating and carbohydrate mobilizing enzymes. However, a technology of *"Synchronized Tillering "* to obtain uniform crop stand is yet to be developed.

Low irrigation water availability

The water requirement of sugarcane is very high (2000-3000 mm) and accounts for nearly 30% of production costs.The impredictability of the South-West monsoon during recent years has led to increased dependency on irrigation resources for cane cultivation. It is estimated that nearly 43% of cane production comes from the 29% of the area which is fully irrigated, the remaining 71% of the area is rainfed or partially irrigated.

Lack of suitable varieties

The sugarcane research system has evolved and released a number of sugarcane varieties for commercial cultivation which have made significant improvement to sugar productivity. However, the present day varieties are unable to meet the complete demand of sugarcane gowers and sugar industry. This is largely due to the extension of cane cultivation into marginal and sub-marginal soils, unirrigated areas, and extension of cane crushing duration to about 180 days. This requires a wide spectrum of varieties suitable for early milling (October/November) till late crushing period (June and July). The field stability of many newly developed varieties, is therefore, a major issue in the cane production plan.

India has a huge collection of germplasm maintained by the Sugarcane Breeding Institute, Coimbatore. The total number of *Saccharum* sp., wild species and related genera is 4803. But the basic species germplasm utilized in India for evolving sugarcane varieties until 1980 was just 32. There is an urgent need for extensive exploration of this gene repository for developing suitable genotypes for future use.

Sustainability of crop

Sugarcane is grown on various soil types, most of them are deficient in N and a few of them are deficient in P and K. On average, a sugarcane crop (100 t/ha) removes 208 kg N, 53 kg P and 280 kg K , in addition to other major and micronutrients from the soil. The extensive cultivation has made most of the sugarcane soils deficient in N,P and K and many macro-and micro-nutrients. At present, nutrient replenishment cost is prohibitive due to reduction in subsidy and high cost of cane production.

Uneconomic ratoon crop

The national average cane production is greatly influenced by ratoon yield which contributes over 30% of the total sugarcane production. In the sub-tropical cane growing belt, 45-50% cane area is under ratoon crop, where yields are very poor. This is mainly because farmers consider it as a *"Gift Crop"* and in most of the cases recommended cultivation practices are not followed, resulting in poor yield and quality of successive ratoons.

Cane diversion in the decentralized sector

Unlike other cane growing countries,the entire cane produced in India is not processed for crystal sugar manufacture. During last two decades, only 40% of the sugarcane produced in the country was utilized for the manufacture of centrifugal sugar. Diversion into the Jaggery and *Khandsari* sector was as high as 60% (Table 3). This situation is quite contrasting to other major sugar producing countries of the world like Australia, South Africa and Cuba where most of the cane produced is used for sugar manufacture.

Table 3 *Sugarcane production and its utilization for Jaggery and Khandsari in India (Baboo and Solomon 1995)*

Year	Total cane production (Mt)	% used for Jaggery[1] and Khandsari[2]	Production of Jaggery and *Khandsari* (Mt)
1960-61	111.0	59.0	6.7
1970-71	126.3	57.8	7.4
1980-81	154.2	54.8	8.6
1990-91	241.0	39.0	8.4

[1] Jaggery: Prepared from clarified cane juice by open pan process
[2] Khandsari: Crystalline or coarse powder obtained from sugarcane juice by open pan process after clarification and concentration

In recent years, the use of cane for sugar production has increased at 5.5% per annum, primarily due to increase in cane price. Consequently, the cane supply ratio for vacuum pan sugar has increased to nearly 48 % during the last five years as against 33-35% during 50's and 60's. This peculiar change of the Indian Sugar Industry has a tremendous effect on the net production of centrifugal sugar. The decentralized sector which processes about 50% of cane does not operate under any definite government control. Contrary to this, the sugar industry is under the stringent control of Central as well as State governments which dictate prices of raw material, sugar, molasses and alcohol.

Sucrose losses in field, cane centres and sugar mills

The national approach to sugarcane cultivation is to maximize sugar production in time, space and inputs. Emphasis on cane tonnage with indifference to sugar content induced by the prevailing cane payment system is acting as a serious restraint to scientific exploitation of available genetic potential. The farmers' practice of harvesting cane 2 to 4 days prior to its transportation to a cane centre or sugar mill, lack of fast transport, and poor link roads, leads to deterioration in cane quality. There is no scientific harvesting and crushing schedule in many

cane growing areas, especially when the crushing season is extended into summer months when daily maximum temperatures range upto 42 - 46°C . This causes immense damage to the standing crop and harvested cane which is reflected in poor recovery (Solomon et al. 1990). It has been estimated that the Indian Sugar industry loses about AS 800 million every year due to sub-optimal sucrose recovery (Solomon 1994).

The usual time lag between harvesting and milling in sub-tropical areas ranges from 3 to 10 days. This results in 15-30 kg sucrose loss per tonne of cane milled (Solomon and Madan 1995). In addition to this, absence of a proper cane laundering system allows a lot of extraneous matter to enter the processing system along with cane. This is responsible for loss of sugar in molasses and imparts undesirable color to the final product. India has a large number of sugar mills with varying capacities (500- 8000 tonne cane/day). However, some of the plants use outmoded machinery and technology, and therefore processing losses are enormous compared to the other countries such as Australia, Mauritius, Brazil and Colombia.

Constraints in sugarcane mechanization
Sugarcane requires very high input of labour for various cultural operations. There has been little adoption in harbors for operations such as sett cutting, planting, harvesting, loading, etc. Mechanization of these operations will drastically cut down the labour requirement and cost of operations. This will also help in timely operations, which are otherwise difficult under present crop husbandry practices.

Sugarcane marketing constraints
Marketing of sugarcane is a complicated process and, unlike other agricultural produce, nearly 50% of total production is supplied to the organized sector, i.e. the sugar industry. The remaining produce is utilized in the decentralized sector out of which about 10-12% is used for seed, feed and chewing purposes. In the years of excess production, cane growers have to suffer heavy losses as they are bound to supply their produce to the local crusher since sugar mills stop their crushing operation after a certain period. However, in some areas, sugar mills are compelled to crush surplus cane during summer months when sugar recovery is extremely low. In sub-tropics, marketing of cane is arranged through co-operative cane societies and there is no direct contact between the growers and sugar mill. This results in enormous difficulties at all levels from alloting permits to cane payment.

Financial and managerial constraints
In recent past, cost of cultivation of sugarcane has increased many fold due to higher cost of fertilizers, pesticides and other inputs. According to an estimate, it has risen from AS 100/ha in 1950 to AS 750/ha in 1994-95. Deviation from the recommended package of practices results in loss of quality and yield of sugarcane. For example, spring planting of sugarcane should be completed by 15th March but in many areas, especially in sub-tropics, the planting of cane continues until May, i.e. after harvest of wheat. This results in loss of cane tonnage and poor recovery in plant crop.

Fixation of cane prices
In sugarcane cultivation the major policy decision, i.e., fixing of Statutory Minimum Price (SMP) is decided by Government of India

on the basis of the recommendations of Commission of Agricultural Costs and Prices (CACP). The SMP refers to the cane delivered at factory gate on the basis of corresponding sugar recovery of 8.5%. The price constitutes a floor price that sugar mills are required to pay for cane. In addition to this. State Governments also fix cane price which is known as State Advised Prices (SAP). The SAP is a "mark up" over the SMP, and reflects dice power of sugarcane grower lobby in any given State. Thus being strongly motivated by political considerations,SAP has considerably inflated the prices of sugarcane and, therefore, has introduced serious distortion in the production of sugarcane (Mann 1995). In recent years there has been large scale diversion of areas from food grains and other valuable cash crop to sugarcane because of exceedingly high SAP of sugarcane. One of the inherent weakness of the present cane pricing system is that cane growers are not adequately rewarded for quality of cane supplied to the sugar mills because payment is on cane weight. The sucrose- or quality-linked payment procedure is difficult to introduce as ^ie farmers supplying cane to sugar factory are so numerous. This poses practical difficulties in the way of introducing such a system in India.

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SUGAR PRODUCTION IN INDIA BY 2000 AD. 2. CONSTRAINTS AND STRATEGIES FOR REMOVING PROCESSING, INFRASTRUCTURE AND ECONOMIC LIMITATIONS

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ABSTRACT

The Indian Sugar industry is required to produce 320 Mt of sugarcane for producing white sugar for domestic consumption, export and enough quantity of jaggery and khandsari to meet the requirement of 1000 million people by 2000 AD. The national sugar policy is,therefore, structured to meet the target set for 2000 AD., with thrust on the following programmes:
(a) Judicious utilization of wide base of germplasm for the development of high sugar content and disease tolerant cane varieties, and varieties for specific abiotic stress conditions: varietal planning and maturity-wise harvesting; biotechnological approaches in varietal improvement.
(b) Increased ratio of cane supplied to sugar factories by reducing diversion to the decentralized production sector of jaggery and khandsari.
(c) Emphasis on integrated disease, pest and nutrient management,efficient use of water, and increasing economic viability of
(d) Improvement in yield and recovery in sub-tropics and prevention of after harvest losses (pre and post-processing).
(e) Development of an efficient marketing system and appropriate decisions at government level in respect to import-export regulations, domestic sugar pricing, maintenance of buffer-stock and licensing and decontrol of industry.
(f) Establishment of modern energy-efficient sugar processing units with thrust on suitable diversification programme.

INTRODUCTION

Sugar is an essential item of mass consumption and. therefore, is a politically sensitive commodity, in India. The consumption of sugar is rising fast at an annual rate of 5%, and therefore 18.5 Mt of white sugar and 13.8 Mt of jaggery and *khandsari* will be required by 2000 AD. The per capita consumption of sugar has increased from 6.2 kg in 1972-73 to 13.6 kg in 1992-93. which indicates a sharp increase of about 119%. The level of other sugarcane-based sweeteners .especially jaggery consumption, is however relatively stable at around 12 kg per capita per annum. The Indian Sugar Industry, therefore, has to plan for sugar production accordingly and not rely on imports which have proved to be costly. In recent past, farmers and sugar industry have displayed tile potential to meet requirements of sugar within the country and also for export. In India, maximum yield of about 255 t/ha has been recorded in sugarcane, however, the average yields in the farmers' field are still very low. It is thus possible that record yield of cane and sugar could be attained in the country through technological improvements in sugarcane cultivation and removing processing infrastructure,management and economic constraints, to meet the sugar production target set for 21st century. This paper briefly highlights the various strategies to meet the demands for sugar in the country by 2000 AD and beyond.

Development of improved varieties capable of giving higher yield of cane and sugar along with good field stability, resistance to important diseases and pests, and good ratooning ability should be the main goal of an effective breeding programme in sugarcane. In future, some new or modern approaches will have to be taken up to supplement the conventional breeding programme. These are outlined below:

(a) Evaluation and exploration of superior clones at species level, suitable for red rot resistance and abiotic stresses, especially drought, waterlogging and salinity.
(b) Exploitation of better parents in the breeding programme particularly for the development of varieties for the sub-tropical region.
(c) Use of biotechnology and genetic engineering, like somaclonal variations, cell and anther culture, recombinant DNA techniques and use of RAPD and RFLP techniques for generating basic information to be used in breeding programme.
(d) Production of transgenic plants for specific attributes in sugarcane.

The recommended package of practices, viz., planting time, varieties, seed rate, fertilizer dosage and number of irrigations should be followed strictly to get a healthy crop.

In cane cultivation, varietal planning is an important factor. This should be done as indicated in Table 1.

Table 1 *The recommended proportion of cane area which should be allocated to different maturity varieties.*

	North Central Zone	North Western Zone	Southern Zone
		(% area)	
Early	20	33	33
Mid-late	50	34	34
Late	30	33	33

In recent times, varietal planning during the late-crushing period has become very crucial due to extension of milling season. Cane varieties which have a thermo-insensitive invertase system and are able to maintain sucrose plateau for a longer period need to be identified to obtain higher sucrose recovery during the decline phase. The Indian Sugar Industry needs varieties which mature fast early in the season (October/November) and deteriorate less late in the season (summer months).

The nature and duration of the preceding crop causes considerable variation in planting time, fertility status of soil, and crop productivity. In some areas sugarcane is planted after a wheat crop, i.e. in April and May. This results in poor tillering and low yield. An appropriate cropping system needs to be devised for such regions to improve cane yield and sucrose recovery.

There is a great scope to develop the area under sugarbeet cultivation in India. The possibility of beet cultivation in marginal and sub-marginal lands needs to be explored.

With the escalating cost of chemicals and fertilizers, it is imperative to search for alternative sources of nutrients, such as Biological Nitrogen Fixation and Biological Potash Fixation in sugarcane . Crop residue recycling (trash, bagasse, press mud.distillery waste) has to be popularised to make the cane production system more economical. This should be supplemented with proper crop rotation practices with leguminous crops.

In India, flood irrigation systems are commonly used which result in enormous wastage of water. About 6-10 irrigations are usually given in sub-tropics as compared to 30 or more in tropics and, therefore, this input has to be used judiciously. For effective use of irrigation water, feasibility of micro-irrigation system like drip irrigation and sprinklers have to be worked out. The practice of trash mulching needs to be encouraged to conserve soil moisture and suppress weed growth.

The ratoon crop is an important component of the cane production system in India. To save the cost of cultivation and to get higher return

in terms of sucrose and yield, the practice of multiple ratooning needs to be promoted. This is possible only through an effective ratoon management programme. Under Indian conditions, the following scientific recommendations (Anon. 1991) are to be followed:

(a) Ratoons should be kept of those crops which are harvested in February/March or early October/ November,
(b) Stubble shaving or harvesting at ground level and trash burning after harvest,
(c) Irrigation within 24 h after trash burning,
(d) Dismantling of ridge and gap filling,
(e) Proper fertilizer dosage as prescribed for different regions,
(f) Effective disease and pest management practices to be followed.

Harvesting of plant cane of early maturing varieties during winter months (November- January) in sub-tropics results in poor and gappy ratoons. However, observations (S Solomon unpublished data) showed that pre-harvest foliar application of ethephon at 500-1000 mg/L, 7-10 days before harvest may result in better sprouting of underground stubble buds of winter-started ratoons. This treatment along with other recommended practices of ratoon management could improve the sprouting and yield of winter-started ratoon crops.

To get maximum recovery in the early part of the milling season (November-January) ratoons of early varieties are preferred. The harvesting of plant crop of early maturing varieties is not desirable in the colder months in the interest of sprouting of subsequent ratoon crop. Table 2 shows a suggested schedule of varietal harvesting and supply of cane to get maximum sugar from different varieties.

Table 2 *Schedule of varietal and crop type for different harvest time.*

Milling period	Time of year	Crop type	Varietal maturity
Early	October/November	Ratoon	Early
	December	Ratoon	Early,mid-late
	January	Ratoon	Mid-late
	February	Plant	Early
		Ratoon	Mid-late
Late	March	Plant	Early,mid-late
	April/May/June	Plant	Mid-late,late

Supply of cane to the sugar factories should be done maturity-wise after measuring sucrose content with a hand refractometer. All factories should make such arrangements to get higher recovery. Government incentives to cultivators who grow early varieties should be continued.

To sustain quality during late-milling period, application of chemicals such as dinitrosocifrol (2.5 kg/ ha) and glyphosate (0.15 kg ai/ ha) are found to be useful (Solomon & Madan 1995).

Although several methods have been suggested to minimize the incidences of diseases and pests, an integrated approach is found to be the most effective (combination of physical, cultural, biological and biotechnological) to combat their onslaught. Biological control should be promoted as far as possible. In this, use of natural parasites is adopted, viz., for shoot borer, *Trichogramma* is very useful. This will also avoid pollution hazards created due to indiscriminate use of chemicals. Similarly, many fungi have now been identified which could be used as bioagents against diseases. In addition to these preventive methods, available models of disease and pest forecasting should be adopted.

Seed programme in sugarcane husbandry should be given top priority. The development agencies and factory management must ensure availability of quality seed to the farmers when needed for planting. In this regard, due consideration should be given to three tier seed programme using Moist Hot Air Therapy (MHAT). This will prove to be beneficial in producing disease-free seed. For rapid multiplication of seed cane, pre-germinated single bud planting, STP and tissue culture methods would be useful. The Government sponsored schemes, viz. Sugarcane Adaptive Research Programme (SARP) need to be

popularised to demonstrate the advantages of a healthy seed programme and other aspects of cane production.

Sugarcane undergoes quick deterioration in yield and quality after harvest. This results in monetary loss to growers as well as sugar factories. Solomon & Madan (1995) reported that farmers lose around A$ 250 per 100 tonne of cane supplied to the sugar factories if the time lag between harvesting and milling exceeds 72 h during late-milling period. The sugar factory (2500 t cane/day) loses around A$ 15,000 because of low sugar recovery from stale cane. There is no substitute for quick and efficient transport of harvested cane from field to factory. However, if deterioration is unavoidable an integrated approach using both physical (water spraying and trash covering) and chemical methods (use of biocides) should be followed to minimize sugar losses (Anon. 1995). A direct linkage between the cane growers and sugar factory should be established for supply of cane. The time lag between harvesting and milling should not exceed more than 24 h to avoid deterioration. To ensure this, infrastructure facilities such as link roads, culverts, drainage system, etc., should be created by the respective State Governments.

Most of the Indian sugar factories use outmoded machinery and technology of sugar processing. It is imperative that the latest technology and know-how which are now being followed in countries like the USA, Australia and Mauritius be introduced in the Indian Sugar industry. The accurate assessment of cane quality supplied to sugar mills and losses taking place at various sections of plants need to be studied, and suitable measures should be taken to minimize these losses. Methods for quick-on-line analysis of intermediate products needs to be introduced. There is an urgent need to promote the use of NTR analyser system, microprocessor pH control, and double filtration processes in the mills.

Remunerative cane prices should be given to cane growers to promote sugarcane production. In this regard, both Central and State Governments should fix cane prices which may attract farmers to grow sugarcane. The payment should be released to farmers within fifteen days as per the statutes of the Government. The development of an efficient system of marketing will act as a catalyst for increasing sugarcane production, especially in sub-tropics.

Timely credits to farmers for investments towards irrigation, fertilizers, pesticides, seeds, and other necessary inputs be given by the Government or sugar factories. Poor financial condition of the farmers is also responsible for the slow adoption of modern technology.

There is an urgent need to strengthen the linkage that exist between the sugarcane growers and sugar factories in each of the factory zone. The sugar mills should take up the primary responsibility of cane development in their command area.

As already established, cane diversion has a serious impact on total sugar production. Thus planning the share of sugarcane for jaggery and khandsari units and vacuum pan mill at 40:60 ratio could lead to higher sugar production. However, drawal ratio has to be increased gradually if net sugar output is to be raised. The crushing efficiency of local crushers engaged in the manufacture of jaggery and *khandsari* is around 60% only, resulting in a loss of 20-25% juice. This can be improved if crushing is carried out by high efficiency crushers or in the sugar mills and then juice is supplied to the jaggery manufacturers. If this procedure is adopted by the sugar industry, uncontrollable diversion of cane into the decentralized sector could be checked.

Sugarcane is regarded as multi-product crop and, therefore, more avenues for use of its by-products and co-products have to be explored. Suitable diversification programmes.viz., paper, newsprint and boards from bagasse, chemicals from alcohol, animal feed from ligno-cellulosic residues, etc., will help in boosting the production of sugarcane as many large and medium size industries could be established using sugarcane as raw material (Singh & Solomon 1995 ; Solomon & Singh 1995).

Timely policy decisions at government level with respect to import-export regulations, buffer/surplus stock, domestic sugar prices, control of sugar and co-products, licencing and deregulation of industry etc., will help in

sustaining cane production. Furthermore, establishment of new factories should be based on the projections of cane availability and in this regard, crushing capacity in the sub-tropics needs to be increased.

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VIETNAM SUGARCANE IN 2000

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ABSTRACT.

Sugarcane has been cultivated and produced in Vietnam for a long time and traditional sugar processing has also been well developed. Presently, one can see in many parts of Vietnam some primitive species of sugarcane such as Saccharum officinarum, S. sinense and S. spontaneum. Production of sugar per hectare has been at a low level, especially because in some regions there is no irrigation in the dry season. Sugar processing in some regions remains manual or semi-mechanised with unsophisticated technology. Therefore, the sugar recovery percentage is very low. Because of increasing demand for sugar, the Vietnamese government is trying to increase sugarcane production by 2- 2.5 times between now and year 2000.

CURRENT SITUATION

Vietnam is predominantly an agricultural country with an abundance of agricultural resources. Sugarcane which is one of the most important crops in the country has been cultivated for a long time and is well adapted to Vietnam conditions. Perhaps, Vietnam is one of the centres of origin of sugarcane? Old, traditional methods of processing sugar have largely been used so far. Presently, Vietnam has about 140,000 ha under sugarcane (1992-3 data). Intensification for high production per ha has been at a low level.

Natural resources

Sugarcane genetic resources
Presently, one can see in many parts of Vietnam some primitive species of sugarcane. The origins for the sugarcane of the world are summarised as follows.

> *Saccharum sinense:* Small stem, hard rind, intermediate sugar content. It grows mainly in middle land and up-land of the north of Vietnam. The varieties which belong to the species are Gie Phu tho, Gie Tuyen quang, Gie Tau nay, Gie Lang son.

• *Saccharum officinarum* (Noble cane): Large, soft stem, high contents of juice and sugar. The species is widely grown in the Red River delta. Well-known sugarcane genotypes such as: Mia Voi (big stem), Mia Tim, Mia Do, etc have been selected and evaluated. Other varieties such as Thanh dieu and Mung are widely grown in Phu yen, Khanh hoa, Ninh thuan districts.

• *Saccharum spontaneum:* This is a primitive species in Vietnam and it grows widely in many provinces. In south Vietnam one can find 3-4 varieties. They are being paid more attention by geneticists for use in crossing programs.

Hundreds of sugarcane clones have been introduced from Cuba, India, China, USA. Australia, and elsewhere. These have been conserved and used in crossing programs to improve commercial varieties. At the present time, the leading varieties are F. 156, Comus, My5514, Ja 60-5, Co715, NCo 310 and VN84.

Physical resources
Soil: Sugarcane is grown on two major soil types, viz. degraded red-yellow podzolics and grey soils, and acid sulphate soils. Large areas of land suitable for sugarcane have not been exploited as yet, e.g. in the midlands of the North, in the highlands of Central Vietnam, and grey podzolic soils in southeastern provinces.

Rainfall: Rainfed management systems predominate, with annual rainfall in the range of 1400-1700mm. In southeastern and southwestern regions there is a 6 month dry season, with irrigation needed to obtain satisfactory production.

Climate: Vietnam is a tropical country; in the North, average annual temperature is 27-30°C with minimum of 10°C and maximum of 37°C; comparable data for the South are 27, 25 and 30°C, respectively. Typhoons occur in the Northern and Central regions. Solar radiation averages 450 cal/cm^2/day, annual sunshine hours average 2500h, with a minimum of 2200h.

Usual cultural practices for sugarcane

Cane is planted manually and ratooned on average for about 3 years. Harvesting is manual with transport off farm by vehicles and barges. Some areas are serviced by canals for irrigation but in many areas there is no irrigation available for the 6 month dry season. Cultivation is by traditional manual techniques or in some areas it is mechanised. Fertilisation commonly comprises 120-140 kg N/ha, 60-70 kg P_2O_5 /ha and 120-140 kg K_2O/ha.

On-farm household production

Since 1988, the state has considered the farm household as an independent, self-directed economic unit, that has the right to plan and perform its own production and business, and to enjoy its products. Therefore cane production has been well supported, with currently 140000 ha under cultivation over five major regions (Table 1). Because of market demand and good economic return from sugarcane, there are opportunities and incentives for rapid expansion of the industry. However, cane yields/ha in the different regions, ranging from 34.6 to 50.2 t/ha are low (Table 1) and offer considerable scope for intensification in all regions.

Table 1 *Current situation for sugarcane production in different parts of Vietnam (1993-94)*

REGION	Area ('000 ha)	Production ('000 tons)	Yield (t/ha)
North	14.0	520	37.1
Central	27.1	969	35.7
Highland	6.6	228	34.6
Southeastern	28.4	1320	46.5
Mekong delta (SW)	64.0	3211	50.2
Total	**140.1**	**6248**	
Average			44.6

Processing facilities

The sugar industry in Vietnam is small. There are only 12 factories, with a processing capacity ranging between 300 and 2000 t cane/d (Table 2). They can process only c. 20% of the sugarcane currently produced. The remaining sugarcane harvested is processed by smallholder units with capacities of only 5-50 t cane/d. They use old. traditional practices to produce raw sugar for domestic consumption, and recovery efficiency is low.

MEETING THE CHALLENGE UP TO YEAR 2000

To meet demand, the total sugarcane area must be increased from 140000 ha to 250000 ha with an average yield of 50-60 t/ha.

Table 2 *Sugar factories in Vietnam (1994).*

No.		Designation	Capacity (t cane/day)	Location
1.		*Sugarcane factory*		
1		VIETTRI	350	Vinh phu
2		VAN DIEM	1,000	Hatay
3		VINHTRU	500	Nam ha
4	LAM SON		1,500	Thanh hoa
5		SONG LAM	350	Nghe an
6		QUANG NGAI	1,500	Quang ngai
7		PHAN RANG	300	Ninh thuan
8	LA NGA		2,000	Dong nai
9		BINH DUONG	1,500	Song be
10		HIEPHOA	1,500	Long an
11		TAY NINH	500	Tay ninh
12		DONG BO	1,200	Phu yen
2.		*Sugar refinery factory*		
1		KHANH HOI		HoChi Minh city
2		BIEN HOA		Dong nai

The focal areas will be Thant Hoa (north). Quang Ngai (central) and Tay Ninh (south). Because, in general, production of sugarcane per hectare in Vietnam is lower than this projected average level in most regions, there will have to be a major intensification of production. Greater effort must be expended to research and apply advances in technology, with emphasis on new varieties, fertiliser management, and pest and disease control, to increase yield/ha and farmers' incomes.

Attention should be paid to achieving sustainability of resources. Being situated in an ecologically diverse region that has hundreds of years experience in sugarcane growing, Vietnam has a great resource of sugarcane genes. Particular focus should be on preserving and efficiently using these plant genetic resources which are considered a valuable national asset. Strong emphasis will be placed on maintaining soil fertility, and managing the resource of the rainfed ecosystem.

Besides industry area expansion and yield production intensification, emphasis must also be placed on increasing processing efficiency to meet the domestic demand of 12-16 kg sugar per capita. The most important issue in coming years will be to enlarge and improve sugar processing facilities. Investment will be needed to construct new factories with higher capacities of 1000 - 6000 t cane/day. Improvements will be sought to increase the speed of transport of harvested cane to processing factories. To achieve this goal requires good logistical arrangements between farmer and factory. Improvements in sugar recovery efficiency will be sought.

CONCLUSIONS

To meet the projected domestic market demand of 12-16 kg of sugar per capita in year 2000, the Vietnamese government seeks to increase both the area and yield of sugar /ha to achieve a 2-2.5 times higher sugar production than at present. Improved processing capacity will be achieved with new and larger factories.

2. Climatic, biological, economic and social limits

THE SUGARBAG DATABASE SYSTEM: ENHANCING THE ABILITY OF FIELD EXPERIMENTATION TO OVERCOME CONSTRAINTS TO SUGARCANE PRODUCTION

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ABSTRACT

Databases collating crop, soil, climate and management information can be valuable adjuncts to field experimentation. We have developed a database, called SUGARBAG, which stores data from a particular experiment on sugarcane in a systematic format for ready use by crop simulation models or for comparative data analysis. The database can also be used as an electronic "crop diary" for experiments in progress. The development of SUGARBAG has facilitated the consolidation of experimental datasets from diverse production environments, encouraged consistency between experimenters in the definition of minimum datasets and aided the development and testing of sugarcane crop-soil-management simulation models.

Currently, SUGARBAG contains 41 sugarcane experiments from Australia, South Africa and Hawaii with imposed treatment factors of nitrogen fertiliser rate, cultivar, irrigation regime, crop duration, crop class, crop start date, and soil fumigation. The database system is freely available to other researchers, and new contributions are invited from interested users.

INTRODUCTION

Traditional field experimentation has provided empirical knowledge of the impact of climatic, soil, management, and varietal factors and their interactions on sugarcane productivity. Comparison of experimental data across environments can allow the identification of production constraints. However, often it is difficult to compare across experiments as essential data (e.g. daily climate data) is usually not collected routinely. Moreover, by its very nature experimentation cannot "sample" the infinite number of combinations of factors that impact upon productivity. Hence, there is a need to quantify the importance of different physiological processes, and soil type and climatic elements in their contribution to yield variation, and to incorporate these relationships into mechanistic growth models. These models enable researchers to integrate the underlying processes in a quantitative way to help understand the behaviour of complex agricultural systems.

There are relatively few comprehensive datasets available for field experimentation on sugarcane. These data were expensive and time-consuming to obtain, so it is important to gain the maximum information from them. SUGARBAG is a database system, which facilitates the consolidation and standardisation of the fragmented data from these field experiments, which have examined the climatic, water and nitrogen constraints to production. It also provides a framework into which data from new experiments can be entered and readily collated, and compared with past work. SUGARBAG is aimed primarily at sugarcane field researchers, and came about from the recognition of the need for standardised climate-crop-soil-management data from widely contrasting environments to; (i) allow comparison of productivity across environments to identify production constraints, (ii) enhance research efficiency by specification of minimum data to be collected in field experimentation, and (iii) aid sugarcane model development and testing.

The database contains:

Fixed Information on experimental locations (viz. latitude, longitude, slope) and soil characteristics (e.g. soil classification, soil physical properties for each layer, land use history, potential maximum rooting depth, depth to permanent water table).

Experimental Data to include information on the design of the experiment (viz. design of treatments, researchers involved, plot size, climate station used).

Operations were carried out on the experiment (viz. planting, harvesting and harvest dates, amounts and dates of fertiliser, biocides and irrigation applied, tillage operations, occurrences of crop damage).

Measurements collected (**crop measurements:** e.g. cane yield, sucrose concentration of the stalks, nitrogen accumulation in the leaves; **soil measurements:** e.g. soil nitrate concentration, soil water content; **climate measurements:** daily minimum and maximum temperature, solar radiation, rainfall).

SUGARBAG allows electronic input of measurement data and keyboard entry, via forms, of experimental and operational information. A data hierarchy and glossary of terms used in SUGARBAG, as detailed by Prestwidge et al (1994), has been designed to ensure efficient storage of data, ease of searching the database, and consistency in the naming of terms between experiments. The SUGARBAG database system was developed in Microsoft Access, a relational database management system for Windows, is available in an executable form, and is operated entirely via forms. SUGARBAG has been described in detail elsewhere (Prestwidge et al 1994; Prestwidge & Robertson 1995).

The aims of this paper are to describe progress on data inputs into SUGARBAG, examine current and potential outputs to deliver benefits to sugarcane researchers.

THE SUGARBAG DATABASE SYSTEM: DATA INPUT

Minimum dataset specifications

SUGARBAG defines data collection requirements in a manner that will eventually allow their comparison of productivity across environments, or for crop model development or testing. The system also allows a systematic "filing" of all the information associated with an experiment, and as such can also be used as a "crop diary" for work in progress.

Nix (1984) introduced the concept of a minimum dataset for modelling purposes, and suggested different classes of datasets appropriate to different applications. Three classes of datasets, classified on frequency of measurement and whether the data are applicable to model development or testing, have been adopted for SUGARBAG. Most current crop growth simulation models running with a daily time-step, have certain similar data input requirements (Ritchie 1991). Generally, these are: daily climate data (minimum and maximum temperature, solar radiation and rainfall): amounts and dates of irrigation and nitrogen inputs; information on soil water and nitrogen properties; crop class, and the variety grown. The minimum requirements vary with the conditions under which the crop is grown. Detailed specification for potential crop growth conditions, water-limiting conditions, and nitrogen-limiting conditions is given in Prestwidge et al (1994). Class 1 datasets comprise detailed time-courses of crop growth and are primarily used for model development. Class 2 datasets include limited time-course measurements on major variables describing the processes of interest, that should always include final biomass, cane and sugar data. These data are used to test different processes (or modules) (e.g. leaf development) described in the growth simulation model. Class 3 datasets are the least intensive, and are to be used for testing overall model performance.

Current datasets from diverse production environments

The database has been a collaborative exercise involving research organisations in Australia. South Africa and Hawaii. Currently, datasets

originate from Australia (24.5 to 15.5°S). South Africa and Hawaii, with imposed treatment factors of fertiliser nitrogen rate, cultivar, irrigation regime, crop duration, crop class, crop start date, and soil fumigation (Table 1). A number of the datasets are unpublished, and a number of the Hawaiian datasets were compiled from reports published in the 1930s and 40s. Hence, a benefit of the database has been the consolidation of data from various sources that otherwise may not have occurred. While the database is aimed at experiments examining the climatic, water and nitrogen constraints to production, there is currently a lack of datasets with soil water supply as an experimental factor (Table 1). Experimental work is currently underway in Australia and South Africa to remedy this deficiency.

THE SUGARBAG DATABASE SYSTEM: DATA OUTPUT

Assessment of production constraints

Output from SUGARBAG can be used to assess the extent of production constraints across diverse environments. An example of the use of SUGARBAG for this purpose was the comparison of yield accumulation by high-yielding crops from North Queensland and Hawaii to identify the occurrence of an early yield plateau before scheduled crop harvest (Muchow et al 1995). SUGARBAG facilitated the entry of the previously-published Hawaiian study into standardised electronic form for re-analysis against the recently collected data from North Queensland.

Output to assist modelling

One of the uses of SUGARBAG is the collation of datasets that can be used for the development and testing of crop growth simulation models. Hence, the database is designed to produce file outputs to be used as input files for model runs. Typically, a user would produce the following for a given experiment:

- input files of daily climate data for the course of the experiment (Fig. 1)
- irrigation dates and amounts (Fig. 2)
- nitrogen fertiliser dates and amounts
- tillage dates and types

The database contains soil physical and chemical properties by depth increment down the soil profile that can be used to construct model input files of the soil type for the particular experiment. SUGARBAG also exports to file the selected observed crop and soil variables of interest against which the performance of the model would be tested.

The SUGARBAG database has facilitated the objective comparison of three sugarcane crop-growth models (QCANE. APSIM-Sugar and CANEGRO) using datasets from North Queensland, South Queensland and Hawaii (Keating et al 1995). The exercise showed areas of agreement between the three models, challenged some assumptions in modelling sugarcane growth and posed some questions that should lead to more focused research and better models in all three instances.

Fig. 1 Example of model input file from SUGARBAG for daily climate data. Variables listed in order are: the experiment number, the date in day/month/year format, year, day of year, solar radiation (MJ/m²), maximum and minimum air temperature, rainfall.

Fig. 2 Example of model input file from SUGARBAG for irrigation dates and amounts. Variables listed in order are: the experiment number, the treatment to which the irrigation is applied, the date in day/month/year format, year, day of year, amount of irrigation and units, nitrate concentration of irrigation water in mg/l.

Potential future applications

The primary focus to date has been to produce output files for model runs. In the future, SUGARBAG has the potential to be used by researchers not interested in modelling, but for data exploration, data interpretation and presentation to analyse production constraints from diverse environments, or to analyse control of growth and soil processes. We welcome contributions of sugarcane experimental datasets from other researchers interested in using the database system, which is freely available for use.

ACKNOWLEDGEMENTS

Table 1   Details of the datasets currently in SUGARBAG.

Country	Location	Irrigated/ rainfed	Treatment factors	No. experiments	No of treatments x sampling times x variables measured
Australia	Macknade	Irrigated	Cultivar, crop class, N rate	4	4332
	Ayr	Irrigated	Soil fumigation, Crop class, N rate	4	8588
	Bundaberg	Irrigated	Cultivar, N rate, irrigation, Crop duration	6	5500
	Kununurra	Irrigated	N rate, cultivar	6	261
South Africa	La Mercy	Rainfed	Cultivar	11	2360
	Pongola	Irrigated	Cultivar	3	58
Hawaii	Kunia	Irrigated	Cultivar	2	320
	Waipio	Irrigated	N rate, Planting date	2	576
	Makiki	Irrigated	N rate	3	648

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BUNDABERG FARM BLOCK RECORDING SCHEME - AN INFORMATION SYSTEM TO ASSIST FARM MANAGEMENT

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ABSTRACT

The Bundaberg Cane Productivity Committee provides an extensive range of information to cane growers through a Farm Block Recording Scheme (FBRS) operated by Bundaberg Sugar Limited. This information includes yield data by farm, variety and crop class as well as yield responses to irrigation. Such information enables growers to compare the performance of individual blocks on their farms and their overall farm performance with other farms in their zone. Information is provided on the yield and commercial cane sugar (CCS) content of varieties and comparisons made using the parameter net $ return per hectare. An indication of water use efficiency is provided by relating yield and irrigation water use. The FBRS is also used to select winners of productivity-awards. It has evolved into a comprehensive information system for Bundaberg cane growers and their advisers by providing information to assist decision-making as well as performance indicators for farm managers.

INTRODUCTION

The Farm Block Recording Scheme (FBRS) commenced in 1987 following a request to Bundaberg Sugar Company from the Bundaberg Cane Productivity Committee (BCPC). Ledger (1991) described a pilot project with 36 growers in the Bingera mill area. Similar pilot projects were also set up in the Millaquin and Fairymead areas. This was expanded in 1988 and by 1989 all growers were included in the scheme.

Chappell et al (1991) reported the objective of the FBRS was to help reverse a 24% drop in productivity which occurred in the Bundaberg district in the 1980s. The information provided by FBRS would assist growers to improve production by identifying high yielding varieties, suitable cropping cycles and high producing farms. To make inter-farm comparisons more meaningful, farms were organised into zones based on locality and soil type, and comparisons presented as net $ return per hectare. Net $ return per hectare is the gross return calculated for the yield and CCS minus harvesting costs. Information was presented on both a zone and mill area basis and a ranking made on a zone basis.

Locality-based grower discussion groups, termed cell groups, were established to provide a forum to present and discuss the information flowing from the FBRS. The booklet "Sugar Cane Yields & Varietal Performance in the Bundaberg District" produced each year by Bundaberg Sugar Limited was used as the basis of discussion.

In 1989, BCPC introduced productivity awards. Award-winning farms were selected using information from the FBRS. The awards have proved to be a popular method of identifying farms with high levels of production and have encouraged other growers to adopt the farming practices of the award winners.

The FBRS was expanded in 1994 and 1995 to provide information relating crop yield to water use. This followed a survey undertaken by Queensland Department of Primary Industries Economic and Financial Services for the BCPC. The survey showed that the major factors affecting cane farm productivity at Bundaberg were related to irrigation (Smith et al 1994). Factors such as irrigation water use, days between irrigations and irrigation method had significant effects on crop yield. It was therefore appropriate to provide information showing average yield for various levels of water use as an additional benchmark. The FBRS has evolved into a comprehensive information system for Bundaberg cane growers and their advisers.

METHOD

Information required to operate the FBRS is drawn from assignment information, production history, cane officers' field books, harvest information and water use information.

The information is collated and sorted to produce several reports:
(i) Mid-season variety performance
(ii) Annual variety performance
(iii) Inter-farm comparisons by locality
(iv) Crop yield and water use

Each year, these reports are compiled into a booklet "Sugar Cane Yields & Varietal Performance in the Bundaberg District" published for the BCPC by Bundaberg Sugar Limited. This booklet contains:
(i) Crop yield and water use information (See Fig. 1)
(ii) Variety performance information
(iii) Variety performance Tables and Figures (See Fig. 2)
(iv) Weekly CCS Figures by variety for each mill area (See Fig. 3)
(v) Climatic data
(vi) Variety productivity by zone (See Fig. 4)

FBRS also provides information to select the winners of the Bundaberg Cane Productivity Committee's awards. Awards made using FBRS are:
(i) Highest t sugar/assigned ha (average of past 3 seasons)
(ii) Highest t sugar/harvested ha (improvement from previous year)
(iii) Highest t sugar/harvested ha
(iv) Highest individual farm average CCS

RESULTS

Some selected examples of data output from the FBRS are reproduced in Figs. 1 to 4 in the same form as they are published in the booklet "Sugar Cane Yield & Variety Performance in the Bundaberg District" each year. Fig. 1 illustrates wide variation in farm cane yields for equivalent water usage and also shows the average increase in cane yield per megalitre of water applied.
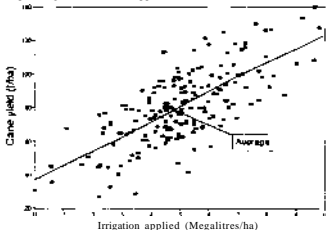
Fig. 1 *Cane yield and water use for individual farms in the Bingera mill area. 1994-95.*
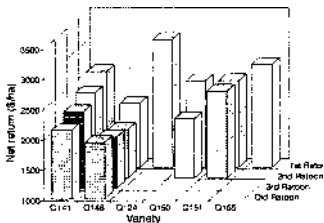
Fig. 2   *Net financial return for different varieties and ratoon crop classes in the Bingera mill area in 1995.*

Fig. 2 shows the average net return ($/ha) by variety for the Bingera mill area for the 1995 crop and demonstrates the superior performance of the major variety Q141 in ratoons and the outstanding yields of the new varieties Q124 and Q155 as first ratoon. Fig. 3 shows average weekly CCS for the varieties Q154 and Q155 in the Bingera mill area in 1995 and indicates that harvest of Q155 before week 18 optimises CCS relative to the mill average.

Fig. 3   *CCS levels of two varieties, Q154 and Q155, compared to the average of all cane throughout the 1995 harvest season in the Bingera mill area.*

Fig. 4 presents the net return by variety and crop class for all cane harvested in the Currajong/St Kilda zone of the Bingera mill area in 1995 and shows that Q155, Q151, Q141 and Q124 give superior returns to Q154 and Q146 in this zone.

Fig. 4   *Net financial return for different varieties and crop classes in the Currajong/St Kilda zone of the Bingera mill area in 1995.*

CONCLUSION

The Bundaberg Cane Productivity Committee views the FBRS as a valuable information system. The information helps cane growers with decisions on varieties and cropping cycles and provides a performance indicator for their farms.

The FBRS has contributed to the productivity gains made at Bundaberg (Cox & Hansen 1995) since implementation of the scheme. Early identification of high-yielding varieties, supported by an extension forum to hasten their adoption, resulted in rapid uptake of superior

The scheme has also provided information on which to base productivity awards which give positive feedback to high achievers and has established benchmark yields.

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ANALYSIS OF LARGE COMMERCIAL DATA BASES FOR DECISION MAKING

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ABSTRACT

The Colombian sugar industry is unique in that all cane produced is harvested by the mills. This has allowed the development of a large data base of more than 12,000 fields per year for 6 years with complete data sets on cane production, recovered sugar %, date of planting and harvest, and variety: and partial data sets on other characteristics such as soil type, management practices, and time from burning to milling have been collected.

The data base is used to analyze the commercial results and develop models to assist rational decision making in the industry. Examples are given of analysis of optimum age for harvesting, and the relationship between sugar yield and cane tonnage.

Future integration of the data base with Geographical Information Systems will enable more in-depth analysis of spatial variation and also rapid validation of new technology for different ecological zones using commercial results.

INTRODUCTION

The Colombian sugar industry is peculiar in that all cane, whether produced by the mills or the independent producers, is harvested by the mills which maintain records on the production from each of the more than 12,000 fields that are harvested annually. As each field is harvested over a short time period (a maximum of 2-3 days) it is possible to obtain good information on the productivity of each individual field. The cane growing area has traditionally been considered to be relatively homogeneous in terms of physical characteristics, however production of individual fields was highly variable. The existence of a large potential data base offered the opportunity to relate production to factors such as crop management and others such as soil type and climate. These relationships can be used to assist in management decisions.

MATERIALS AND METHODS

The Department of Statistics and Economic Analysis of Cenicaña (the Colombian Sugarcane Research Centre) obtained from mills' data sets for 12,000 individual fields harvested each year over the period 1990-1995. For each mill, cane production, sugar yield, date of planting and harvest, and variety are recorded. Partial data sets exist for other characteristics such as soil type, management practices, and time from burning to milling. Data sets were put into a data base and analyzed using the statistical packages tools in SAS (The SAS Institute, North Carolina, USA). This software package is an integrated system of software providing complete control over data access, management, analysis and presentation. Many different aspects of production were analyzed. In this paper a few examples are used to illustrate the system

In the Mayaguez mill area the variety MZC 74-275 predominates, and soil type varies little. Data from this mill offered an opportunity to analyze the relationship between cane production and recoverable sugar. A data set of more than 900 entries was extracted from the mill data set in which the age of harvest was between 12 and 14 months, and the variety was MZC 74-275. The relation between cane production and recovered sugar (%) was then determined by fitting a linear function of production against recovered sugar % (the quadratic term was not significant at p=0.05). From this the relationsip between total recovered sugar per ha and recovered sugar % could be calculated.

The data for 1994 were also separated into sets for each variety and then subdivided into different ages at the time of harvest (note: cane is harvested year round and cane age depends on the capacity of the mills and the supply of cane in the field). The cane production and recoverd sugar per ha and per kg per month, and recovered sugar % were then estimated for different age groups for each of the varieties.

RESULTS AND DISCUSSION

The most common cane payment system in Colombia is based on tonnage of cane with no incentive for high sucrose content. The industry is aware that a cane quality payment system is necessary to improve the efficiency of the sugar sector. However, independent producers have resisted a change to a system that pays for sugar rather than cane, as they traditionally believe that as cane production increases, then sugar content declines and hence the total sugar production declines. Under these circumstances a cane payment system for total sugar production would induce fanners to produce more cane at low sugar contents as this would increase their total returns. On the other hand the mills would face high costs for cutting, lifting and transporting cane with low sucrose content.

Mayaguez mill data, indicate that cane tonnage is indeed reduced when the cane is managed in such a manner as to obtain higher recovered sugar % (Fig. 1). Nevertheless total sugar production per ha increased as recovered sugar % increased (Fig. 1). Hence the commercial results indicate that it is possible to provide the mills with high sucrose cane, which is to their advantage, and at the same time increase total sugar production per unit area which is to the advantage of die independent
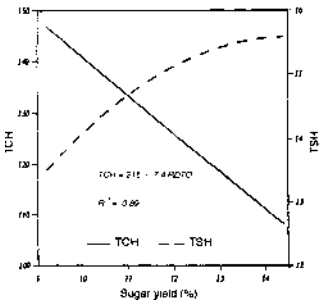
Fig. 1   *Cane and sugar production of the variety MZC 74-275 in the Mayaguez Mill in relation to recoverable sugar (%). Age at harvest restricted to 12-14 months with 941 data points.*
[TCH = total cane yield (t/ha); TSH = total sugar yield (t/ha); RDTO = sugar yield%]

Table 1 *Productivity and rates of yield accumulation at various ages of harvest; commercial data, Colombia 1994.*

Age at harvest (months)	Recovered sugar (%)	Cane yield t/ha	Sugar yield t/ha	Cane t/ha/mo	Sugar t/ha/mo
<11	10.5	96	10.0	9.4	0.99
11-13	11.4	106	12.1	8.7	0.98
13-15	11.4	124	14.2	8.8	1.01
15-17	11.1	138	15.3	8.7	0.96
17-19	10.7	148	15.8	8.3	0.89
>19	10.2	162	16.4	7.9	0.81

cane producers under a cane payment system based on cane quality. This analysis permits the producers and the mills to assess new payment schemes using solid commercial data as the basis for negotiations.

Given that the Colombian sugar industry harvests cane all year round, age at harvest fluctuates widely. When cane production is greater than expected the age rises as the mills are not able to harvest all the cane that is mature, and *vice versa*. The industry has in the past not been able to quantify how costly it is to manage the harvest in this manner in terms of the inefficiencies inherent in harvesting cane at sub-optimal ages. Various varieties were analyzed and representative data from only one variety, viz. MZC 74-275, are given (Table 1). As age increased above 15 months, the critical parameters for profitability, viz. t sugar/ha/month and recoverable sugar % decreased rapidly. These data sets can be used to estimate the costs of always maintaining mature cane in the field in order to maximize cane milled throughout the year.

Careful interpretation of the analysis of the data sets is required. The Mayaguez mill data, with the age of harvest covering a small range of between 12-14 months (Fig. 1), indicate that as cane production increases recoverable sugar % decreases due to differences in management practices. On the other hand the complete data set for 1994 (Table 1), indicates that recoverable sugar % increased with cane production and then declined when age was the variable being analysed. There is no real conflict between the two interpretations: in the first case if age was kept constant then recovered sugar % decreased as cane production increased due to differences in management of the crop. In the second case, die relationship between recovered sugar % and cane production is confounded with the effect of age on maturity and

recovered sugar %. However, erroneous conclusions can readily be drawn if one is not aware of confounding effects: from the 1994 data set, the incorrect conclusion that recovered sugar % increased with cane production, *ceteris paribus*, up to the level of about 120 t cane per ha could easily be deduced.

This type of problem frequently occurrs. In other analyses that compare varietal performance, certain varieties, which are well adapted to excellent conditions, appear to be vastly superior to others that tend to be grown on poor soils. However, when they are compared under similar growth conditions the apparently superior variety turns out to be inferior.

CONCLUSIONS AND FUTURE DEVELOPMENTS

The examples indicate that data base analysis offers the opportunity to use commercial data as a powerful tool in the analysis of relationships to assist organizational decisions (such as negotiation on cane payment systems) and management decisions (such as the benefit to production of increasing harvesting and milling capacity). The use of this type of analysis requires care to avoid erroneous conclusions resulting from confounding effects of variables that are not analysed. Other analysis of the data shows that the top quintile in Colombia produces more than twice as much sugar per hectare per year as the bottom quintile. We believe that by combining the large data base on production and management with climatic data in Geographical Information System, management practices for the different ecological niches can be developed. For example these techniques are now being developed to determine varietal performance in precise ecological niches. These techniques also offer the opportunity to move towards more intensive agricultural management practices.

RAINFALL RISK AND SCHEDULING THE HARVEST OF SUGARCANE

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ABSTRACT

Knowledge of the probability of wet weather disrupting mechanical harvesting operations can assist in decision-making on scheduling the harvest of sugarcane. Daily rainfall databases were developed to calculate the risk, expressed as cumulative probability, of rainfall within and outside of the normal harvest period for three regions (Ord, Burdekin and Herbert) in the Australian sugar industry. The conclusions from the analysis were:

(i) Considerable differences in rainfall exist between the three regions with the Herbert having the highest risk and the Ord the

(ii) In all three regions, the risk of rainfall occurring is far greater at the end of the harvest season than at the beginning. An earlier start to the season would cause less disruption to harvest than a late finish.

(iii) Geographical variation in rainfall risk exists within the Herbert region which may allow harvesting operations to commence earlier in some parts of the region.

This information together with knowledge on cane yield and sugar content profiles from current and subsequent ratoon crops from different times of planting, ratooning and harvest, can be used to optimise the scheduling of sugarcane harvest.

INTRODUCTION

Climate has a major impact on agricultural production, and both temporal and spatial variability in climate has been analysed for different agricultural systems (Muchow & Bellamy 1991). In sugarcane production, whilst climate has a major impact on the productivity of individual fields in terms of cane yield and sugar content, climate and in particular rainfall also has a major influence on harvesting operations.

Wet weather can cause considerable disruption to harvest operations particularly in regions such as the Burdekin where harvesting equipment is not designed to operate in wet field conditions. Mechanical harvesting under wet conditions can cause loss of cane stool, soil structural damage, compaction and rutting in paddocks. This impacts on Industry profitability by reducing the yield of the following years ratoon crop. It is therefore important to ensure that harvest operations are not scheduled at times when there is a high risk of rainfall and that the risk of extreme rainfall events is considered when planning harvest schedules so mat periods of extended wet weather can be accommodated.

The scheduling of harvest is important to the profitability of the Australian sugar industry as it impacts on net farm income and the utilisation of milling capacity and industry assets including bulk shipping terminals. The crush start date and the harvest season length are negotiated by growers and millers by taking account of many factors including cane yield and sugar content profiles over time, the likely yields of subsequent ratoons, wet weather interruptions to harvest, transport and milling capacity and costs. These decisions have been made based on knowledge accumulated over many decades by the Australian sugar industry. Currently, the Australian sugar industry is expanding in terms of land area under sugarcane in existing mill districts and in new sugarcane growing regions (eg. Atherton Tablelands and Ord Irrigation Area). Knowledge of rainfall impacts on harvest operations in different mill areas and districts, particularly beyond the current season length, can assist in decision-making on optimising the scheduling of harvest.

Economists distinguish between risk and uncertainty: risk refers to a probability that can be estimated from prior information; uncertainty applies to situations in which probability cannot be estimated (Heady 1952). The interpretation of risk from any rainfall event is subjective and ultimately depends on the values of the individuals affected. Our purpose in this paper is to use historical rainfall records to present the rainfall risk at different times of the sugarcane harvest as clearly and objectively as possible, so as to enable industry to make its own decisions. Two case studies are presented: (i) regional differences between the Herbert and Burdekin in north Queensland and the new sugar growing area in the Ord

in NW Western Australia; and (ii) geographical variation within the Herbert

MATERIALS AND METHODS

The availability of long-term complete climatic records is a major constraint to this method of analysis. Databases of daily rainfall were developed from the climatic records for Kununurra in the Ord Irrigation Area(1907- 1989), Ayr in the Burdekin region (1887- 1989), and Ingham in the Herbert region (1896 - 1987). Within the Herbert region, daily rainfall databases were developed for Bambaroo (1920 - 1993) and Halifax (1924 - 1993). For each year, the amounts of rainfall in each standard week (Table 1) and in consecutive three-weekly periods were calculated. These data for the 70 to 102 years (depending on location) were sorted in ascending order, and the cumulative probability associated with different amounts of rainfall was estimated. Selected probabilities are presented. The 50% probability value is the median; and the 100% probability value is the highest rainfall for the long-term record. The 80% probability means that there is a 80% chance of receiving less than that amount in a given period or in 20% of years that amount or more can occur. The selection of probability level is dependent on the attitude to risk of the decision-maker.

Table 1   *Commencement dates for standard weeks*

Week	Date	Week	Date	Week	Date
15	Apr 09	27	Jul 02	39	Sep 25
16	Apr 16	28	Jul 09	40	Oct 02
17	Apr 23	29	Jul 16	41	Oct 09
18	Apr 30	30	Jul 23	42	Oct 16
19	May 07	31	Jul 31	43	Oct 23
20	May 14	32	Aug 07	44	Oct 30
21	May 21	33	Aug 14	45	Nov 06
22	May 28	34	Aug 21	46	Nov 13
23	Jun 04	35	Aug 28	47	Nov 20
24	Jun 11	36	Sep 04	48	Nov 27
25	Jun 18	37	Sep 11	49	Dec 04
26	Jun 25	38	Sep 18		Dec 11

RESULTS AND DISCUSSION

The amount of rainfall occurring in each week at different probability levels is shown for three regions in Fig. 1. The harvest season in the Burdekin usually starts in week 24 and aims to finish in week 46, whilst
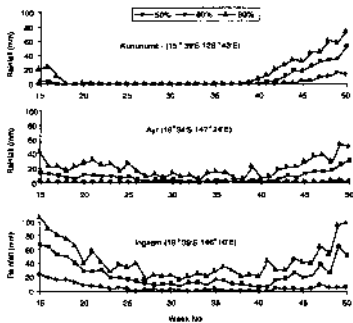
Fig. 1 *Weekly amount of rainfall at the 50, 80 and 90 % cumulative probability level for three regions: Ord (Kununurraj, Burdekin (Ayr) and Herbert (Ingham).*

in the Herbert the harvest season usually starts in week 25 and aims to finish by week 46. The new sugar industry in the Ord aims to start in week 18 and finish in week 47. At the beginning, and during the middle, of the harvest season at the three locations, the rainfall risk is lowest in the Ord and highest in the Herbert. At all locations, the rainfall risk towards the end of the harvest season is much higher than at the beginning of the harvest season. In terms of rainfall risk, if harvesting was extended outside the current season length, an earlier start rather than a later finish would cause less interruption to harvest.

Geographical variation in rainfall risk in the Herbert region is examined in Table 2. Bambaroo, south of Ingham, has lower rainfall early in the harvest season and higher rainfall later in the harvest season compared to Halifax, north of Ingham. The period from 18 June to 8 July is normally the first three weeks of the harvest in the Herbert region. The rainfall amount in the three weeks prior to this start date (28/5 - 17/6) at Bambaroo is lower than that at Halifax for the same time period and is also lower than the rainfall amount for the first three weeks of the normal harvest season (18/6 - 8/7) at Halifax. This suggests that harvesting could commence earlier at Bambaroo. For the last three weeks of the harvest season (23/10 - 12/11) and for the following 3 weeks (13/11 - 2/12), the risk of rainfall is greater at Bambaroo than at Halifax. This suggests that an earlier finish to the season may be a good strategy at Bambaroo. The 100% probability values shown in Table 2 indicate that it is possible, albeit rarely, for substantial rainfall to interrupt the harvest, particularly late in the season.

This analysis can be extended by developing rules for assessing the number of wet days for interruption of the harvest. A further elaboration would be the use of a soil water balance and crop simulation model which takes account of crop water use and soil water losses, linked with rules on trafficability. Differences in rainfall intensity and duration between early and late in the harvest season could also be included. These elaborations require more input data, and an important issue is the benefit/ cost ratio of further elaboration beyond the current simple rainfall analysis.

Table 2 *Rainfall amount (mm) during 3 week periods at different cumulative probability levels for 2 locations within the Herbert region.*

Location	Period	Cumulative Probability			
		50%	80%	90%	100%
Bambaroo	28/5 -17/6	12.5	40.1	59.4	125
(18°52'S)	18/6 - 8/7	8.4	31.8	51.8	187
	23/10-12/11	**31.0**	**53.2**	**105.0**	**225**
	13/11 - 3/12	**63.4**	**74.4**	**186.0**	**652**
Halifax	28/5 -17/6	42.9	66.5	81.4	276
(18°35'S)	18/6 - 8/7	17.5	52.8	95.4	248
	23/10-12/11	**27.2**	**39.7**	**122.0**	**224**
	13/11-3/12	**43.5**	**71.1**	**149.0**	**623**

The rainfall risk to harvest interruption is only one aspect to be considered in optimising harvest schedules. The cane yield and sugar content of current and subsequent ratoon crops associated with different times of harvest is an important component, as is the capacity of transport systems and milling operations. Evidence suggests yield losses of subsequent ratoons by late harvest (Chapman & Leverington 1976; Leverington et al 1978). In the Ord, Albertson et al (1981) showed that sugar content was low with early harvest increasing to a maximum in the September period, and there were varietal differences in sugar content profiles. This is similar to the experience in the Queensland sugar industry. However, few data sets are available for the yield consequences as modified by crop age and crop class for modern varieties, from harvest outside the currently accepted harvest season. Further research to obtain these data is warranted to establish the relative magnitudes of the potential benefits and costs from changing season length. On that basis, options might then be developed for optimising crop schedules that offer opportunities for further productivity improvement in the Australian sugar indistry.

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RAINFALL VARIABILITY AND THE NEW SOUTH WALES SUGAR INDUSTRY

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ABSTRACT

Knowledge of rainfall variability can give insights into the possible consequences of drainage and irrigation as well as impacts on the scheduling of farm operations including harvesting. Daily rainfall databases were developed from long term records (1889 - 1993) to calculate weekly and three weekly rainfall probabilities for five locations (Murwillumbah, Ballina, Coraki, Maclean and Grafton). These locations cover the north-south and east-west axes of the sugarcane areas of NSW. The conclusions drawn were:
(1) Little difference between locations in variability of annual totals but considerable differences in total rainfall and in annual distribution pattern.
(2) The risk of high rainfall affecting crop production, via reduced germination and establishment or by reduced growth due to waterlogging effects, or via crop management through interference with weed control or cane harvesting, occurs over all the NSW area. However the degree of risk increases and extends for longer periods further north and east.
(3) The risk of water deficit increases during the period from July to December and is highest in the southern and western sectors of the region.

INTRODUCTION

The NSW sugar industry is at the southern extreme of the Australian sugarcane area and so is strongly influenced by climate (temperature, rainfall and solar radiation). The main production areas are located on the flood plains of the three main rivers on the North Coast: the Tweed, the Richmond and the Clarence. Soils are mainly alluvial and are derived from basaltic sources in the Tweed and the Richmond and from sandstones and shales in the Clarence. The climate is subtropical with a summer dominant rainfall that is highest in the Tweed valley (1800 mm) and declines to the south and away from the coast (980 mm at Grafton).

High rainfall variability dominates the climatic impacts on agricultural production in Australia (Angus 1991) but there have been few attempts to analyse this variability on the North Coast of NSW. Hall (1972) presented the lowest, highest and 10. 50 and 90 percentile values for annual rainfall plus monthly means for selected locations while The Bureau of Meteorology (1988) have published monthly rainfall means, medians and number of raindays. Edwards (1979) estimated rainfall variability by analysing monthly and annual records to give median, first and third quartiles and the median deviation from the median. He showed that, for three locations in or near the NSW sugarcane growing areas, monthly deviations were highest for those periods when monthly totals were lowest, i.e. from May to September. Murtagh (1982) calculated the co-efficients of variation (CV) of annual and monthly rainfall for 35 meteorological stations on the east coast of Australia. Although the latter approach provides a good measure of rainfall variability its focus is too wide to be of use within the NSW sugarcane.

The NSW industry is expanding and much of this is into areas of lower rainfall. This expansion combined with the recent drought has resulted in considerable interest in irrigation. Conversely, intense and repeated summer rainfall events can result in periods when soils are waterlogged. The consequences to yield and management can be considerable. Germination and establishment can be reduced resulting in either crops with low yield potential or a costly replanting (Parsons, personal communication), and growth of established crops can be restricted. Cultural operations can be hindered resulting in poor weed control, while unavoidable operations such as harvesting can result in damage to soil structure and the loss of cane stool. As both drainage and irrigation schemes vary in complexity, efficiency and cost it is important to know how variable the rainfall is both temporally and spatially.

The aim of this paper is to quantify rainfall variability from long-term rainfall records for key locations and times of the year to gain insights into the possible consequences of both drainage and irrigation.

Table I *Commencement dates for standard weeks and three weekly periods.*

Week	3 week period	Date
1	1	Jan 01
4	2	Jan 22
7	3	Feb 12
10	4	Mar 05
13	5	Mar 26
16	6	Apr 16
19	7	May 07
22	8	May 28
25	9	Jun 18
28	10	Jul 09
31	11	Jul 30
34	12	Aug 21
37	13	Sep 11
40	14	Oct 02
43	15	Oct 23
46	16	Nov 1
49	17	Dec 04

MATERIALS AND METHODS

Databases of daily rainfall were developed from climatic records to cover the north - south and east - west axes of the NSW sugarcane area. We were able to obtain reliable and long term (> 95yrs) records for the following 5 locations: Murwillumbah (1890-1993) in the Tweed valley, Ballina (1893-1993) and Coraki (1896-1993) in the Richmond valley, and Maclean (1889-1993) and Grafton (1897-1993) in the Clarence valley, (note we have already covered the Ballina by Coraki. etc, issue in "the north - south and east - west axes of the NSW sugarcane area.") The amounts of rainfall in each standard week and in consecutive three weekly periods for each year were calculated (Table 1). These data were sorted in ascending order, and the cumulative probability associated with different amounts of rainfall was estimated. Probabilities of 20%, 50% and 80% are presented. The 50% probability is the median; the 80% probability indicates that there is a 80% chance of receiving less than that amount in a given period or in 20% of years that amount or more can occur. These data are from a first pass interactive analysis on key locations in NSW and are not comprehensive.

RESULTS AND DISCUSSION

Maximum and minimum yearly rainfall, mean rainfall and its coefficient of variation for the five sites are shown in Table 2. Murwillumbah and

Ballina had similar maximum, minimum and total rainfall as did Coraki and Maclean while Grafton was the driest of the five sites. The CV's of the means over the five sites was similar indicating that, despite differences in total rainfall, the variability of annual rainfall was similar across the region.

Table 2 *Annual rainfall maximum, minimum, mean and the CVofthe mean for 5 locations in NSW*

Location	Maximum (mm)	Minumum (mm)	Mean (mm)	CV of mean (%)
Murwillumbah	2984	742	1700	28 2
Ballina	2601	712	1772	25 6
Coraki	2323	466	1269	28.2
Maclean	2457	468	1259	29.2
Grafton	1817	371	983	27.9

The pattern of rainfall variability throughout the year is presented in Fig. 1 as three weekly totals. The pattern for Ballina was clearly different to the other four locations. Median rainfall fell sharply from period 3 to period 6 in all centres except for Ballina where the decline was much more gradual. At Ballina the median was greater than 50 mm/3 week period for the first 10 periods and there is an 80% probability of receiving more than 25 mm for the first 8 periods. The median rainfall at Murwillumbah was higher than that at Coraki, Maclean and Grafton for the first 7 and the last 6 periods. It was clear that the probability of waterlogging occurring in the Tweed (Murwillumbah) and close to the coast (Ballina) was high but proper drainage remains a necessity in all areas to ensure crop survival in the few years when high and frequent rainfall events can occur even in those locations with relatively low mean rainfall.

The data in Fig. 1 indicate that rainfall was considerably lower in the second half of the year than in the first six months. Weekly median rainfall amounts were examined for weeks 27-52 to assess the likely response to irrigation (Fig. 2). Small rainfall events (<5 mm), particularly if isolated, are generally considered to be ineffective as much of this water evaporates rapidly. Weekly median rainfall amounts for Ballina only fell below 5 mm/week on 2 occasions and at both Ballina and Murwillumbah there is a 20% probability of receiving 25 mm or more for most of this period. At the other times weekly median rainfall amounts were less than 5 mm for 12-14 weeks (Coraki - weeks 27-39; Maclean and Grafton - weeks 27-41) while the 20% probability of receiving 25 mm or more only occured for 8-10 weeks (Coraki - weeks 42-52; Maclean and Grafton - weeks 44-52). Responses to irrigation at both Ballina and Murwillumbah are only likely to occur on freely draining soils and even then costs and returns need to be carefully checked. At Coraki, Maclean and Grafton responses to irrigation merit further investigation even on soils with high water holding capacity.

These data could also be used to improve scheduling of mechanical harvesting within and between mill areas (Muchow & Wood 1996). The start and finish of the harvesting period vary depending on numerous factors including crop size for the mill in the defined season, changes in cane yield and sugar content over time, effects on subsequent ratoons and any delays due to wet weather. Starting dates over the last 5 seasons ranged from weeks 24 to 29 while harvest completion dates ranged from weeks 47 to the first week of the new year. The data in Fig. 1 indicate an increase in both median rainfall and risk of high rainfall late in the season especially from period 16 (week 46) on at Murwillumbah suggesting that an early finish to harvesting at Condong Mill would be a good strategy. Median rainfall and the risk of high rainfall are considerably higher for Ballina than for Coraki suggesting that consideration should be given starting the harvest later in the eastern sector than in the western sector of the Broadwater Mill area. In the Clarence River district median rainfall and the risk of high rainfall events are similar for Grafton and Maclean during the harvesting season implying that there are limited opportunities to avoid wet weather harvesting by changing harvesting schedules.
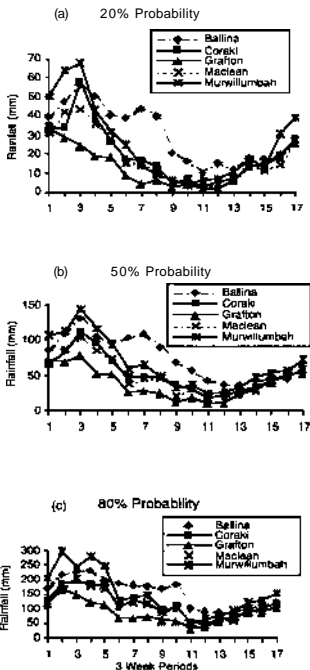
Fig. 1 *Rainfall amount during 3 week periods at (a) 20% (b)50% and (c) 80% cumulative probability levels for 5 locations in NSW.*

This analysis has clearly shown differences in rainfall totals and distribution along north-south and east-west axes and the impacts of these differences on drainage, irrigation and harvesting schedules have been discussed. The analysis could be extended (developing rules for defining effective rainfall, adding in a soil water budget model and including a crop simulation model) but some of the required data on infiltration rates, hydraulic conductivity and effective rooting depth on different soils are not available. A more beneficial approach would be to extend the number of locations and to calculate CV's for monthly data to give a better picture of variability across the region. A similar analysis for temperature data would provide insights into yield limitations due to low temperature and frosting.

ACKNOWLEDGMENTS
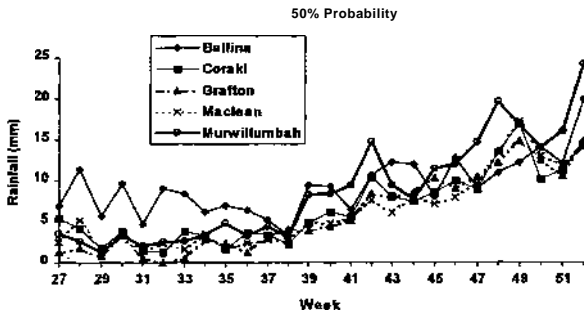
Fig. 2 *Weekly rainfall amount at 50% cumulative probability levels for 5 locations in NSW.*

interactively calculating cumulative rainfall probability over selected periods for different locations.

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RISK ASSESSMENT OF ACID SULFATE SOILS IN SOUTH QUEENSLAND CANE LANDS

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ABSTRACT

This assessment involves soil sampling and mapping of south Queensland cane lands located on flood plain alluvia, low lying coastal land and adjacent areas to determine the extent and potential risk of acid sulfate soils. Field soil description and laboratory analyses are being used to identify potential and actual acid sulfate soils. Digital elevation models, satellite imagery airphoto interpretation and field survey assessments are being utilised to produce risk maps. Boundaries are being digitised and recorded using ARC-INFO software on a geographic information system (GIS).

Preliminary desktop assessments show an estimated 20114ha of cane land is at potential risk from acid sulfate soils. This represents 21% of the total area of cane lands in southern Queensland. Although Bundaberg appears to have the greatest total area at risk (6940ha), this represents only 10% of the local region. By contrast other southern cane lands at potential risk are Maryborough district 2134ha (19%), Moreton district 6139ha (78%) and Rocky Point 4901 ha (72%). All of these areas are in a sensitive coastal environment dissected by fresh water streams and tidal inlets.

INTRODUCTION

Cane lands in southern Queensland located on low lying coastal areas may be associated with acid sulfate soils. Mapping to establish probable locations of acid sulfate soils has been conducted along the coastline of New South Wales (Naylor et al 1995) and many cane areas in the north of that state were found to be associated with acid sulfate soils.

Acid sulfate soils contain pyrite or iron sulfides (mainly FeS_2) which, when drained or disturbed and aerated, oxidise to sulfuric acid. The released acid can not only limit cane production but also leak into adjacent drains and waterways, degrading the aquatic environment, and on some occasions causing fish kills. White et al 1995) provide a recent review of the subject.

Cane industry practices in these sensitive areas may in some cases contribute to the release of sulfuric acid - by lowering water tables through the installation of drains, dumping pyritic materials excavated from drains or water storages onto field, or as stockpiles, laser levelling land and cultivating deeply. Deep cultivation can bring acidic material to the surface damaging cane roots, making plant nutrients unavailable, causing aluminium toxicity, and resulting in acidic runoff water. This necessitates the application of large amounts of lime to reclaim the land for cane. Therefore, the further development of these areas for cane with our current knowledge must be highly questionable. Cane expansion can now only be justified on the basis of detailed investigations that determine the distribution and abundance of pyrite, and the avoidance of high risk areas.

For existing cane areas, production systems which minimise acid generation should be considered. Such systems focus on the drainage of surface water rather than the ground water and involve the use of shallow drains and minimal disturbance of the potentially acid subsoils. Shallow cultivation and accurate assessment of the lime requirement through soil analysis are also recommended. Lime applications should be well mixed to maximise neutralisation. There is some evidence (I.White, personal communication) that the liming of existing deep drains may help neutralise acid drainage waters for a time before they leave the property. See NSW EPA (1995) for a range of management options.

The Queensland Department of Natural Resources has commenced a program to assess and map the severity and distribution of acid sulfate soils in four key sugar producing areas in southern Queensland: Rocky Point, Moreton, Maryborough and Bundaberg. This information will allow the sugar industry, state agencies and local government to better plan for the future, and develop production systems which sustain and conserve the soil and the community's natural resources.

MATERIALS AND METHODS

An understanding of the processes which lead to the accumulation of pyrite is a valuable tool in mapping the distribution of pyritic sediments.

Pyrite forms over geological time under conditions of tidal waterlogging in the presence of iron and easily decomposed organic matter (Dent 1986). Anaerobic bacteria break down the organic matter and reduce sulfate from sea water to sulfide (Pons et al 1982). Such environments are typically found in tidal flats, salt marshes, mangrove swamps and the bottom sediments of tidal water bodies. Consequently pyritic sediments can be expected to be found where these conditions occur now or occurred in the past (White et al 1995).

In common with much of eastern and northern Australia, conditions on the southern Queensland coastline over the last 10 000 years (the Holocene) have been very conducive to the formation of pyrite (White et al 1995). Prior to the onset of the Holocene. 20000 years ago, sea levels, which were about 100m below present, began to rise. By 6000 years before present, sea level stabilised at close to or slightly above present sea level. As a result, all the ancient coastal valleys were gradually infilled with sea water and fine sediments, creating vast tidal swamps such as mangrove flats (Chappell 1990). Under these conditions pyrite has accumulated within the sediments to form potential acid sulphate soils. The pyritic sediments in many cases are buried by various thicknesses of river alluvium or coastal aeolian sand. Upstream the alluvium is thicker, but close to the coast overlying alluvium is often <1m thick before pyrite is encountered.

As demonstrated by Naylor et al (1995) in New South Wales, this knowledge can be used to great effect in determining the distribution and likely maximum extent of acid sulfate soils. This is because the conditions conducive to coastal pyrite formation are likely to occur at elevations no higher than a few metres above sea level. Hence the value of digital elevation data derived from contour maps. At 10m Australian Height Datum (AHD) on coastal sediments, pyrite would be expected to be covered by up to 9m of overburden alluvia or aeolian sand.

A combination of digital elevation model, satellite imagery and air photo interpretation is being used with fixed grid and free survey techniques to produce risk maps. In some areas existing soil maps, which do not identify acid sulfate soil materials, can be reinterpreted to aid in the risk assessment. Map boundaries are digitised and stored using ARC-INFO software on a geographic information system.

Both potential and actual acid sulfate soils are identified during field mapping and sampling by landscape features, soil morphological attributes and subsequent laboratory analysis of soil and water samples. The laboratory analysis provides a quantitative assessment of the acid sulfate soil hazard.

Soil analyses to assess potential and actual acid sulfate soils may include pH (1:5 water), pH after oxidation with hydrogen peroxide, total sulfur (S) using Xxay fluorescence, water soluble sulfate, peroxide oxidisable sulfuric acid or POSA (Lin & Melville 1993), total actual acidity (TAA)

and total potential acidity (TPA) (Dent et al submitted). Total sulfidic acidity (TSA) is calculated by (TPA- TAA),

RESULT

The cane areas with potential for acid sulfate soil materials occur at low elevations (<10m AHD) and as a preliminary exercise, these areas have been mapped for southern Queensland [Fig. 1(a), (b). (c), (d)]. This is probably an overestimate as some areas of hard rock may be included. A lower elevation cut off would have been preferred but was not uniformly available across the region. In this case the digital data is based on 1 :100 000 scale topographic data with 20m contour intervals for which the 10m contour is interpolated.

The data derived from the map (Table 1) show that the area where there is a risk of encountering acid sulfate soils in southern Queensland cane fields is proportionally smaller for Bundaberg (10%) and Maryborough (19%) but is more significant for Moreton (78%) and Rocky Point (72%). Overall, about 20% of cane lands carry a risk of encountering acid sulfate soils within the profile. These results are preliminary estimates which give a broad indication of the potential extent of acid sulfate soils.

Table 1 *The area of current sugar lands at risk of acid sulfate soils in south Queensland (% of total area in brackets).*

Sugar region	High risk areas <10mAHD (ha)	Low risk areas >10mAHD (ha)	Total Area (ha)
Bundaberg	6 940(10)	63 816	70 756
Maryborough	2 134(19)	9 328	11 462
Moreton	6 139(78)	1 745	7 884
Rocky Point	4 901(72)	1 840	6 741
Total SE Qld	20 114(21)	76 729	96 843

Where semi-detailed soil maps at 1:50000 scale and published soil analysis are already available, as at Rocky Point (Holz 1979), soil units are being reinterpreted to produce more accurate maps of risk. Soil sulfur levels and soil pH together with descriptions of land and soil morphology strongly suggest that the soils at highest risk of encountering significant pyrite at shallow depth (<1.2m) are heavy textured humic gleys, peaty gleys (formerly marine couch - *Sporobolis virginicus* grasslands) and saline gleys (reclaimed salt pans and samphire flats). The soils with lower risk of pyrite presence within 1.2m of the surface were podzols and siliceous sands, medium textured humic gleys and acid grey gleys. It was noted that the high risk soils were generally at <1.5m AHD and represented 8570ha (70%) whereas lower risk soils were found at elevations >1.5m AHD and covered 3760ha. The data from the Holz study represent both cane and non-cane lands in the district and use different elevation cut offs which cannot strictly be compared with the broader data above.

To make maximum use of soil analysis, sampling is required at regular depth intervals down the profile. An example from the Rocky Point Mill area is presented in Table 2. This example shows the presence of high levels of oxidisable sulfur (pyrite).

The presence of significant pyrite is indicated in clayey soils when values of 0.05% oxidisable sulfur are recorded (Bowman 1993, NSW EPA 1995). Bowman also suggests that values in excess of 0.2% indicate high levels of pyrite. Results in Table 2 show that the profile from 0.3m to 1.5m has significant accumulations of pyrite. The pH values of 3.4 to 3.7 indicate that the top 1.2m of this soil is an acid sulfate soil. Below 0.6m levels of oxidisable S are higher with a maximum of 2.3% oxidisable S present. It is quite likely that pyrite extends well below the depth of sampling (1.5m).

The amount of net acid which would be generated by pyrite oxidation alone has been calculated for each soil depth (Table 2), as has the lime requirement to bring these soils up to a pH of 5.5. If the pyrite in

Table 2 *Selected chemical analysis of an acid sulfate soil at Hotham Creek, Rocky Point Mill region (near Brisbane).*
[POSA =peroxide oxidisable sulfuric acid; TSA = total sufide activity]

Depth (m)	pH (1:5 H$_2$0)	pH (Oxid.H$_2$0$_2$)	Total S (%O.D.)	Oxid. S (%O.D.) POSA	TSA (mol H\Vt)
0-0.1	3.7	3.28	0.54	0.043	42
0.1-0.2	3.6		0.56		
0.2-0.3	3.6	3.31	0.25	0.038	42
0.3-0.4	3.6	2.89	0.28	0.095	91
0.4-0.5	3.5		0.81		
0.5-0.6	3.4	2.94	1.25	0.056	56
0.6-0.7	3.4	2.71	1.33	0.109	134
0.7-0.8	3.4		1.20		
0.8-0.9		1.99	2.24	0.85	678
0.9-1.2	3.5	1.85	2.13	2.33	1378
1.2-1.5	4.7	1.96	1.52	1.54	925

the top 0.3m (the plough layer) were oxidised, 27 t/ha of lime would be needed to neutralise the acidity produced. If the entire profile were drained and oxidised to a depth of 1.5m, then 514 t/ha of lime would be required. Clearly, deep drainage of this soil should be avoided on both economic and environmental grounds.

Soil morphology

Acid sulfate soils used to grow cane display some characteristic features, not all of which will necessarily be present in the same profile. A horizons (topsoils) are typically dark, deep and initially, strongly structured clay loams and clays. Below the A horizon, a grey clay B horizon (subsoil) with brown ironstained mottles is found which grades with depth to a moist grey clay with a straw yellow mottle. This yellow material is jarosite, a mineral which forms only under extremely acid conditions (pH < 4). The mottle patterns often follow the shape of roots and mangrove pneumatophores.

Below this horizon, below the water table, grey to bluish grey sulfidic sands or muds occur. Shells may be present and in some circumstances, the presence of H$_2$S may be detected (the smell of rotten eggs). The presence of pyrite can be confirmed by a violent reaction with 30% or 100 volumes hydrogen peroxide (H$_2$0$_2$) and a substantial reduction in pH. The field pH of the grey sulfidic layer in the anaerobic conditions experienced below the water table is commonly neutral rather than acidic. Acidification only results following exposure to air, which will happen when water tables are lowered in dry seasons or by drains, or soils are excavated and dumped on the surface.

In some cases, jarosite may occur just below the A horizon, and material is brought to the surface by cultivation. In others, jarosite is not observed, but the A or B horizons directly overlie grey sulfidic clays. Although the A and upper B horizons are usually quite acidic (pH <4). these layers have already strongly oxidised and are less likely to contain significant amounts of pyrite.

Water - symptoms and analysis

Symptoms in drains and waterways that indicate the presence of acid sulfate soils include red/orange ferric iron staining on the banks, an oily looking scum on the surface of the water, unusually clear water and areas of red brown flocculated iron in the water, and low pH in the surface water. The presence of red iron compounds can also mean the water will be low in dissolved oxygen and hostile for aquatic animals. Crystal clear water is caused by the release of soluble aluminium from clay under extremely acid conditions, causing the clay to flocculate and fall to the bottom of the waterway.

CONCLUSION

Information on the severity and distribution of acid sulfate soils in southern Queensland cane lands will allow the sugar industry and local shires to better plan for their future by avoiding high risk areas. This information, combined with the implementation of production systems
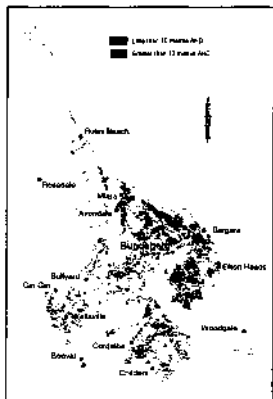
(a) **Bundaberg region**
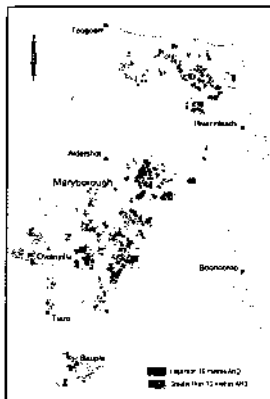
(b) **Maryborough region**
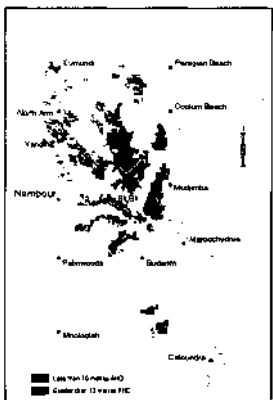
(c) **Moreton region**
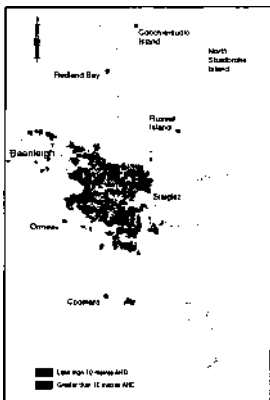
(d) **Rocky Point region**

Fig. 1   *Sugar lands currently above and below 10m AHD in south Queensland*

which minimise acid generation, will enable the industry to maintain yields, conserve the soil and avoid offsite impacts on the environment. It will enable the sugar industry to reply in an informed way to possible environmental concerns and provide a basis for research and development. Furthermore, there is an urgent need to develop and adopt techniques to manage acid sulfate soils in such away that there are minimal offsite effects in drains and waterways.

ACKNOWLEDGMENTS

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3. Opportunities for improved plant performance

3.1 Breeding/breeding efficiency

INCREASE IN SUGAR YIELD FROM PLANT BREEDING FROM 1946 TO 1994

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ABSTRACT

Sugar yield was increased by 0.12 and 0.15 t/ha/yr rainfed and irrigated sugarcane culture respectively, by the release of new varieties over the period 1946 to 1994. This increase in yield gave a 135 % return on investment to the Australian Sugar Industry from plant breeding expenditure, in 1994.

These estimates were calculated from the results of two variety trials grown at the Sugar Experiment Station, Mackay over 8 seasons, 1987-95.

The six varieties used in the experiments were the dominant commercial varieties for the period in central Queensland, thus enhancing the reliability of the predicted gain in yield.

INTRODUCTION

The Bureau of Sugar Experiment Stations is the main provider of new sugarcane varieties for the Australian Sugar Industry. The majority of these varieties are bred at Sugar Experiment Stations located at Meringa, Ayr, Mackay and Bundaberg along the Queensland east coast. There is an exchange of varieties with other sugarcane breeding countries and some foreign varieties are grown. Currently, 90% of sugar production in Australia is from locally bred varieties. When new varieties are introduced they have superior attributes which may be associated with specific disease and insect resistance, improved sugar quality, and favourable agronomic attributes. Usually, they are also selected to have a higher yield than the varieties they replace.

The trend in sugar yield over time associated with the introduction of new varieties is confounded with changes in cultural and management practices. Consequently, it is usually impossible to isolate the magnitude of the individual effects. Examples of change in cultural and management practices over the last 48 years include increased irrigation and fertiliser use, the adoption of mechanical harvesting, the use of herbicides and reduced cultivation, to name but a few.

This paper used data from recent experiments in the Mackay district to assess the gain in yield and industry benefit from important commercial varieties released over the last 48 years. The experiments were a series of ratoon trials on a range of varieties, released between 1946 and 1994, which were grown under common cultural and environmental conditions. The six varieties were grown in rainfed and irrigated experiments for eight crops on the Sugar Experiment Station, Mackay. The benefit to the Australian Sugar Industry on expenditure on plant breeding was estimated by extending these results to the whole industry.

METHODS

Full details of the field experiments were published by Chapman et al (1992). Briefly, the varieties Q50, Q68, Q87, Q124,Q138 and NCo310 were grown in two experiments on the Sugar Experiment Station, Mackay between 1987-95. One experiment was rainfed and the other furrow irrigated. Scheduling of irrigation was based on estimating soil water deficit from 'Class A' pan evaporation. Plots were irrigated at a soil water deficit of 64 mm, calculated by using pan evaporation, canopy development and a pan factor of 0.8 (McGuire 1991). Experiments had three replicates, and a plant crop and seven ratoons were grown.

Notable features of the seasonal conditions were good moisture for plant, first, fifth, sixth and seventh ratoons, dry conditions for early growth of second, third and fourth ratoons and late growth of third ratoons. Plant and third ratoon growth was adversely affected by high cyclonic rainfall. Cane yield was measured in whole plots by weighing harvested-cut-billets with a truck-mounted scale. CCS content was measured by the standard method (Anon 1984) in juice crushed from 10-stalk samples from each plot at harvest.

The yield level of varieties was calculated as the mean production over 8 crops for cane yield, CCS and sugar yield. These were then regressed over the year that the varieties were first grown commercially in central Queensland: Q50, 1946; NCo310, 1958; Q68, 1957; Q87, 1968; Q124, 1984; Q138, 1994.

RESULTS

The newer varieties Q124 and Q138 consistently had higher cane yield than the older varieties (Table 1). Cane yield generally declined from first to third ratoon, increased again from fourth to sixth ratoon and declined in seventh ratoon. Irrigation increased cane yield, but there was a seasonal variation, even in the irrigated yields, not entirely related to water stress effects on crop growth. Season variation also occurred in CCS with NCo310 and Q124 having high levels in most seasons, and Q87 having high levels under irrigation (data not presented).

Table 1 *Yield of cane (t/ha) six varieties grown to seventh ratoons in rainfed and irrigated experiments.*

Crop Class	Variety					
	Q50	NCo310	Q68	Q87	Q124	Q138
Rainfed						
P	86	88	89	85	114	105
1R	92	117	108	109	112	121
2R	58	86	65	86	81	89
3R	48	73	46	75	77	74
4R	65	90	61	96	100	99
5R	57	91	49	100	109	104
6R	53	94	49	110	128	123
7R	51	63	43	77	80	91
Mean	64	88	64	92	100	101
Irrigated						
P	86	98	94	92	119	110
1R	109	132	123	124	115	150
2R	68	106	96	100	105	127
3R	55	64	41	75	87	89
4R	87	119	93	118	140	148
5R	58	104	73	127	130	139
6R	85	117	75	115	145	140
7R	70	84	72	90	110	120
Mean	75	106	83	105	119	128

Regressions of cane and sugar yield on time of first commercial planting were significant for both the rainfed and irrigated crops (Fig. 1). These regressions indicated that introducing new varieties increased cane yield by 0.75 and 1.00 t/ha/yr, or 0.12 and 0.15 t sugar/ha/yr for rainfed and irrigated situations respectively. There were no significant trends for CCS. This contrasts with the results of Cox & Hansen (1995) who
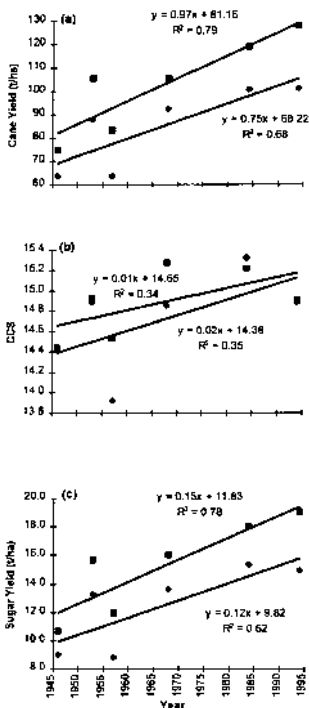
Fig. 1 *Regressions of mean (a) cane yield, (b) CCS and (C) sugar yield on year variety was first planted commercially in central Queensland. for rainfed (♦) and irrigated (■) sugarcane.*

attributed recent high CCS to new varieties in the central and southern Queensland regions.

DISCUSSION

In assessing the reliability of these estimates of the yield benefit from plant breeding there are a number of positive features of this study. The positive attributes are the cultivars selected for the experiments have been or will in the near future be significant for the central Queensland area. Q50 and NCo310 have both, in their time, produced over 90% of the crop in one year. Q68 and Q87 were also widely popular and successful varieties. Q124 contributed over 60% of the crop in 1994 and is increasing in popularity. Q138 is not yet widely grown, because it was released in 1994. The experimental data were not confounded with management or cultural effects, as the varieties were all grown together at the same site in a properly randomised experiment and all received the same treatments under rainfed

and irrigated conditions. Current commercial practice is to use a crop cycle of plant and four ratoons. However, older ratoons are regularly grown. Provided yields can be maintained, profitability of cane growing is favoured by having a longer ratooning cycle, as the high cost of planting can be amortised over a longer crop cycle. Mean yield of sugar for varieties was therefore calculated over plant and seven ratoons rather than plant and four ratoons.

However, in contradiction to these various positive features, the data are somewhat limited in scope because they are only from two experiments at one site over one eight year period. Notwithstanding this above limitation, a cost/benefit analysis was conducted to compare the return to the Australian Sugar Industry from improved varieties against the cost of plant breeding, using data for the 1994 season. Several assumptions were made: (1) a crop cycle of plant and seven ratoons; (2) the increase in sugar yield of 0.12 and 0.15 t/ha/yr were applied across all rainfed and irrigated canegrowing areas respectively; (3) the irrigated area was 0.4 of the total area and (4) the sugar price was $350/t.

The returns to the Australian Sugar Industry from the use of new varieties, both imported and locally bred, was calculated as: 365 000 ha x 0.4 irrigated x 0.15 t/ha of sugar x $350/t = $7.7M; plus 365 000 ha x 0.6 rainfed x 0.12 t/ha of sugar x $350A = $9.2M; giving a total of $16.9M.

The cost of plant breeding activities in 1994 was estimated at $7.2M. These costs include $5.3M by BSES (BSES 1994), $0.5M by CSR (A Wood, personal communication). $1.4M from SRDC (SRDC. 1994/ 95).

CONCLUSIONS

Plant breeding in 1994. delivered a return of 135% on the year's investment using this simple cost/benefit analysis. This return was reduced to 100% if a crop cycle of plant and 4 ratoons was assumed. There is a 10-12 year delay from the time of original crossing until new varieties are delivered. As investment on plant breeding is ongoing, this analysis is adequate to indicate yield increases are occurring and that the high costs are justified. The cost input included not just the funds used for direct crossing, selection and production of new varieties, but also those used for research into plant breeding technologies such as disease resistance, molecular markers, flowering control and so on. Not included in the returns from new varieties are benefits to the industry of disease and insect control, and of sugar quality, which are often associated with new varieties.

Investing in plant breeding is profitable for the Australian Sugar Industry as plant breeders are producing higher yielding varieties. The investment in research into new technologies are likely to enhance returns from plant breeding in the future.

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Sugar Research and Development Corporation (1994/5) Annual Research and Development Program

BEST LINEAR UNBIASED PREDICTION AS A METHOD OF ESTIMATING BREEDING VALUE IN SUGARCANE

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ABSTRACT

Bureau of Sugar Experiment Stations (BSES) currently assess the breeding potential of sugarcane parents by combining several years of agronomic performance data, breeding information and disease ratings into an index. Although this method is comprehensive, it takes many years to estimate reliably the breeding value of a parent.

Family selection trials are typically highly unbalanced and data analysis cannot be undertaken by ordinary least squares approaches. Statistical techniques such as Best Linear Unbiased Prediction (BLUP) were specifically designed to allow prediction of breeding values from unbalanced animal data sets. Although the theory could be adapted to other breeding programs, there have been few applications in plants.

BLUP analyses undertaken on family selection data provided BSES plant breeders with a simple and rapid means of combining data from a wide range of sources to identify superior parents. Preliminary results suggest that BLUP is as effective as the current BSES method for identifying superior parents and these parents are rapidly identified with fewer years of information. The use of BLUPs is likely to increase the rate of population improvement by better choice of parents and crosses.

INTRODUCTION

Identification of superior genotypes for use as parents is a key component of any plant breeding program. Sugarcane breeders from BSES use a formula for assessing the breeding value of a parental clone which combines several years of agronomic performance data, breeding information and disease ratings into an index. Although this method is comprehensive, it takes many years to estimate reliably the breeding value of a parent.

Each year about 300 new clones enter the parental collection. Crossing is expensive and only a limited number of crosses can be made. A method is needed which can be applied to early stage family selection trials, so mat inferior parents can be rapidly identified by a progeny test and removed from the collection. This would result in a more efficient breeding program and thus increase the rate of population improvement.

Data from early stage family selection trials are typically highly unbalanced and so classical statistical approaches such as ordinary least squares are inappropriate. Best Linear Unbiased Prediction (BLUP) is a proven technique in animal breeding for obtaining precise estimates of breeding value from highly unbalanced data sets. BLUP allows data from a diverse range of mating designs, relatives, traits and precisions to be combined into a single breeding value for each trait and genotype (White & Hodge 1989).

BLUP has been used extensively in animal breeding but there are few applications in plants. There are only two published applications of BLUP to sugarcane data. Using a balanced data set, Chang & Milligan (1992a,b) evaluated crosses at one location and so family by environment interactions were not considered. It is when data are highly unbalanced that BLUP usually exhibits superiority over other techniques and is of relevance to BSES.

The objectives of this study were to determine the effectiveness of BLUP as a method for estimating the breeding potential of sugarcane parental clones. Comparisons were made between BLUP and the current BSES method of estimating breeding value.

CURRENT BSES METHOD OF ESTIMATING BREEDING VALUE

The traditional method used by BSES in Australia to estimate the breeding potential of parental clones incorporates the following information.

Agronomic performance

Clones are initially planted in the parental collection if they exhibit superior performance in yield trials conducted over a number of years and locations. Clones are assessed relative to commercial standards and the results are combined in a selection index called net merit grade (NMG) (Skinner 1965).

Disease resistance status

Disease ratings for several major diseases are assigned to a clone on a 1-9 scale where 1 is resistant and 9 is susceptible. In the empirical formula, disease ratings are in the form of an adjustment factor. The form of the adjustment depends on the region from which the parental clone comes and thus more important diseases in a particular area are given greater weighting.

Breeding performance

Several hundred experimental crosses are evaluated each year at five Sugar Experiment Stations spread across the major sugar growing regions. The aim is to identify those crosses with specific combining ability (SCA) coupled with high general combining ability (GCA). These superior crosses are called proven which means more seedlings from that cross should be planted. The system for identifying proven parents

(i)   Selection rate - the percentage of original seedlings that are selected and replanted in later stages of testing (Hogarth & Skinner 1986).

(ii)   Family selection - whole families are rejected or selected based on mean performance (Falconer 1960).

Inbreeding

Before a cross is made, the level of inbreeding (F) is determined. Depending on the level of inbreeding, a proposed cross may not be made or it may be penalised. Four levels of inbreeding are recognised:

(i)   Selfs - Female and male clones are identical, F = 1/2

(ii)   Line breeding or parent/offspring - Female clone is same as either parent of male clone or vice versa, F =1/4

(iii)   Half sibs - Female and male clones have one parent in common, F= 1/8

(iv)   Full sibs - Female and male parents of both clones are identical, F= 1/4

For selfs, line breeding and full sibs the crosses are avoided and half sibs are penalised.

The agronomic performance data, breeding information and disease ratings are used to calculate a breeding value estimate of a clone for a particular breeding program. This procedure is detailed in Hogarth & Skinner 1986. The breeding values range from 0-10.

Difficulties in making improvements

BSES plant breeders have been dissatisfied with the current method of calculating breeding value but have found it difficult to make improvements. A highly unbalanced mating design caused by unreliable and sparse flowering at the main breeding station in North Queensland (Berding & Skinner 1987) coupled with visual assessment of yield for families had precluded the use of a statistical approach. Since the introduction of mobile weighing machines in the late 1980s an objective evaluation of families can be obtained. These data facilitated an investigation into statistical techniques such as BLUP.

MATERIALS AND METHODS

Trial details

In the current assessment of the usefulness of BLUP, plant crop (first harvest) NMG data for the 1988-1994 series seedlings from the Mackay and Bundaberg breeding programs were used for analysis. Each family plot contained 20 clones planted as single seedlings in 12-13m plots with each clone 0.6m apart. Families were planted in several blocks with varying replication on the Mackay and Bundaberg Sugar Experiment Stations.

Model

BSES family selection data have an incomplete diallel mating design (Griffing 1956). The linear model is:

$$Y_{ijk} = \mu + g_j + g_k + s_{jk} + e_{ijk}$$

where

Y_{ijk} is the phenotypic observation for progeny in the ith replicate from the jkth cross.

μ is the population mean

g_j is the random variable associated with the GCA of the jth female $\sim NID (0, \sigma^2_{g_j})$

g_k is the random variable associated with the GCA of the kth male $\sim NID (0, \sigma^2_{g_k})$

s_{jk} is the random variable associated with the SCA of parents j and k $\sim NID (0, \sigma^2_{s_{jk}})$

e_{ijk} is the random plot error associated with the ith replicate from the jkth cross. $\sim NID (0, \sigma^2)$.

Analysis

The statistical program, GAREML (Huber 1993), was used to obtain BLUP estimates of parental general and specific combining abilities. GAREML applies the algorithm developed by Giesbrecht (1983) to estimate REML variance components (Patterson & Thompson 1971) and uses the theory developed by Henderson (1973) to obtain BLUP estimates of breeding value.

Although GAREML is computationally efficient, the inversion of matrices in the BLUP procedure is memory intensive especially with large data sets. To simplify the analysis due to memory and time constraints, the year effect was removed from the NMG family selection data prior to analysis. For each year in the data sets, this involved subtracting the mean and divided by the variance.

As the BLUP analyses were undertaken on standardised NMG data, the resulting breeding values ranged from -1 to 1. A data transformation was applied so the breeding values ranged from 0 - 10.

RESULTS AND DISCUSSION

In the 1994 and 1995 crossing seasons, GAREML was used to determine BLUP estimates of breeding value for parental clones from the Central and Southern Queensland breeding programs. This provided plant breeders with a rapid method of identifying superior parents based on family information. BSES plant breeders noted that BLUP could identify many similar superior parents based on fewer years of data (maximum of 6 years) in comparison to the current method (10 years).

Changes were made to computer programs to enable a detailed examination of the crosses made in the 1995 season. Of 1140 crosses made. 127 were designated as proven crosses. Approximately 60% of these provens had BLUP ratings for both parents (73 out of 127). Of these 73. 54 had average BLUP breeding values above 5 (good crosses) while 19 were designated as inferior (3 or less). This indicated that BLUP is effective for identifying superior parents.

The most accurate method to compare breeding value estimates based on the current BSES method and BLUPs is to contrast the agronomic performance of crosses selected from parents chosen by the two techniques in a progeny test. Research to make this comparison commenced in 1994 when 40 BLUP, 40 empirical and 20 random crosses for the Central and Southern Queensland breeding programs were made. Progeny performance data will be available for assessment in late 1996.

The power of BLUP as a predictive tool was determined using family selection data from Central and Southern Queensland. BLUP estimates based on all available family NMG information up to 1993 were calculated. Predicted performance from the BLUPs based on some 200-400 clones was correlated with actual trial performance in 1994. Predicted performance using the current method based on the 1993 series breeding clones was also correlated with actual NMG in 1994. Breeding values were updated using 1994 information and predicted performance for the BLUPs and current method were correlated with actual performance in 1995. The results are given in Table 1.

Table 1 *Correlation coefficients (r) to allow a comparison of the predictive power of BLUP with that of the current BSES method using family selection NMG data from Southern and Central Queensland.* [n is the sample size of the data set used to calculate each correlation coefficient.]

		Year	
		1994	1995
Southern Queensland	BLUP vs NMG	0.62 (n = 81)	0.63 (n = 97)
	Empirical formula vs NMG	0.45 (n = 81)	0.50 (n = 97)
Central Queensland	BLUP vs NMG	0.64 (n = 71)	0.38 (n = 125)
	Empirical formula vs NMG	0.49 (n = 71)	0.12 (n = 125)

The correlation coefficient for BLUP vs NMG was always greater than for the current method vs NMG. These results are encouraging as the current method incorporates information for up to ten years whereas BLUP results are based on a maximum of 6 years. Both sets of correlations were low for Central Queensland in 1995. This may be due to problems experienced with moisture stress in the propagation of me seedlings. On the benches, all seedlings from one family are planted together. Given that the irrigation system only failed on one part of the bench, then some families would have been suffered severely from moisture stress and shown uncharacteristic results.

CONCLUSIONS

Preliminary results suggest that the BLUP method is as effective as the current BSES method for identifying superior parents. As the BLUP method becomes more refined by including information on relatives its superiority will increase. Economic savings associated with using BLUP and a reduced generation interval make it a potentially effective method for estimating the breeding value of parental clones.

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FAMILY SELECTION IMPROVES THE EFFICIENCY AND EFFECTIVENESS OF A SUGARCANE IMPROVEMENT PROGRAM

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ABSTRACT

Mass selection of individuals in seedling or early clonal stage trials is routinely used in most sugarcane improvement programs throughout the world. It is, however, inefficient as the heritability of cane yield on a single plant basis is low. In Australia, the introduction of mobile truck-mounted weighing equipment offered the opportunity to implement family selection utilising weighed family data. Family selection has been used in some Bureau of Sugar Experiment Stations' (BSES) selection programs since 1986 and is now routinely used in all regional selection programs. Several research projects have shown that a combination of family and mass selection in the early stages of selection will result in larger genetic gains and a higher frequency of superior clones in later stages than mass or family selection alone. This combination allows improved efficiency since fewer resources are required to select only within superior families in the first ratoon crop than to mass select individuals across the entire population. A liberal family selection rate (about 40%) balances genetic gain and the need to maintain a broad genetic base. Differential selection rates within families are used so that more clones are selected out of the best families. The availability of objective family data also allows more accurate estimation of the breeding value of parents utilising best linear unbiased predictors (BLUP). This results in better genetic combinations through crossing and provides more objective information on new parents.

INTRODUCTION

Selection in early stages of a sugarcane breeding program has been described as being very inefficient (Skinner 1971). Selection for cane yield on an individual plant basis (mass selection) is likely to be confounded by environmental plot effects. So genotypic values are difficult to assess and heritability is low. Consequently, selection intensity in seedling stages of Australian cane breeding programs was low (10-30%) to reduce the possibility of discarding superior clones.

Hogarth (1971) recognised the value of family selection as an alternative to individual mass selection in sugarcane. Generally, family selection is considered superior to mass selection when heritability on an individual basis is less than 0.5 and is appropriate to the early stages of selection programs where heritability on an individual basis is usually low. The development of mobile weighing equipment in the early 1980s (Hogarth & Mullins 1989) provided the opportunity to mechanically harvest and weigh large numbers of plots of cane. This resulted in the ability to assess families in early stages of selection (plantcrop) to identify superior families. Mass selection may be used subsequently to select individuals within these superior families in the first ratoon crop.

Anodier factor contributing to the difficulty of mass selection of individual plants is lodging of cane which occurs frequently in heavy yielding environments such as the Burdekin irrigation area in north Queensland. This problem was recognised as a major factor limiting genetic progress in the Burdekin selection program (Pollock 1982).

Initial family selection trials were conducted in the Burdekin and reported by Hogarth et al (1990) and McRae et al (1993). They found that selection based on weighed family plots of seedlings was effective. In addition, field operations were simpler and less expensive. Mass selection within families was also effective in a poorly grown plant crop of seedlings. The number of elite clones (NMG 10) in stage 3 was significantly higher for combined family and mass selection than for mass selection alone. NMG (net merit grade) is a measure of economic worth, incorporating cane yield and CCS, relative to a group of standard clones which are adjusted to 10.

This paper reports the results of family selection experiments conducted at Bundaberg where lodging is not as great a problem as in the Burdekin.

MATERIAL AND METHODS

In Bundaberg about 200 families were evaluated in stage 2 family trials in two series of family selection experiments harvested in 1989 and 1990. Family plots consisted of 10 clones, each clone within a family plot being planted as a 2 m plot with a small gap (0.2 m) between

clones. The number of rreplications of each family varied. Family plots were sampled for CCS by taking a single stalk out of each of five clones taken at random. These were crushed through a small mill and CCS (commercial cane sugar) determined using standard procedures (BSES 1984). Family plots were mechanically harvested and weighed.

From each of these stage 2 family trials, 36 families were selected based on family mean NMG, with six families being randomly chosen from each of six NMG categories (low to high). Twelve clones were randomly sampled, and 12 were visually selected, from the first ratoon crop of each of these families and the 864 clones were evaluated in stage 3 trials in plant and first ratoon crops. A split-plot design was used with whole-plots allocated to families represented by 8 clones, and sub-plots consisting of 4 random or 4 selected clones. Families but not clones were replicated (3) and plot length was 10 m with 1.5 m between rows. NMG was derived from the measurement of cane yield (TCH) and CCS. Different selection strategies were compared relative to the performance of all randomly chosen clones.

RESULTS AND DISCUSSION

Analyses of variance of the Bundaberg experiments showed that differences among families were highly significant for all traits (TCH, CCS, NMG) in both crops. The effect of selection type (random or visual) was significant for TCH but, as expected, not for CCS. The results clearly showed that the average performance of clones was higher when they were selected from superior families in stage 2. The percent gain in NMG over the random population for mass selection, family selection, and combined family and mass selection is shown in Table 1. The percentage of elite clones in stage 3 resulting from different selection strategies was evaluated for the combined results of the two experiments (Table 2). Family selection level was set at NMG 8, which included the top 16.7% of families in experiment 1 and the top 33.3% of families in experiment 2.

These results showed that gains in NMG of the order of 10-13% resulted from a combination of family and mass selection. More importantly, the percentage of elite clones (based on plant and first ratoon stage 3 results) was about 50% greater than for either random or mass selection alone. The implications for a selection program where 2000 clones progress into stage 3 testing are shown in Figure 1.

This shows that an extra 118 elite clones would be selected through combined family and mass selection compared with random (or indeed mass) selection.

As part of the core selection program in Bundaberg in 1988, 419 clones were mass selected out of plant crop stage 2 families considered (subjectively) by breeders to be superior. These families were subsequently

weighed and NMG assessed. The stage 3 performance (mean of plant and first ratoon crops) showed that over 40% of clones from families with NMG 8 were elite (NMG 11), while only 30%, 23%, and 16% of clones from families with NMG 7-8, 6-7, and <6 were elite.

Table 1 *Percentage gain in net merit grade (NMG) for different selection strategies over the NMG of randomly selected stage 3 clones - Bundaberg experiments [proportion selected in parentheses]*

Stage 2 selection		Gain (%) over random stage 3 clones	
Family	Mass	Exp 1	Exp 2
No	Yes	3.4	5.3
Yes (0.17)	No	8.5	5.7
Yes (0.33)	Yes	9.7	12.9

Table 2 *Percentage of elite stage 3 clones [NMG 11] from random choice, mass selection, family selection (NMG 8), and combined family and mass selection in stage 2 - Bundaberg experiments [combined results of two experiments]*

Stage 2 selection		% of elite stage 3 clones
Family	Mass	
No	No	12.2
No	Yes	11.9
Yes	No	13.9
Yes	Yes	18.1

In terms of selection programs, the higher population mean and higher frequency of superior clones resulting from family/mass selection is important as it demonstrates that selection has been effective in shifting the population mean. This is the major objective in early stages of a selection program. Another major practical benefit of family selection is that it allows genetic material (families) to be evaluated across locations at a very early stage of selection, whereas individual clones cannot. This will be important if family x location (F x L) interactions are large relative to family genetic variance. Evidence of important F x L interactions is conflicting (Jackson et al 1993). Testing of two series of families at two locations in the Bundaberg area indicated that F x L interactions were either not significant or unimportant (MC Cox, unpublished data). However previous results for families grown at three south Queensland locations with differing soil types indicated that F x L interaction was significant and important (Bull et al 1992).
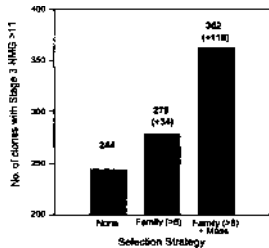
Fig. 1 *Number of elite clones (stage 3 NMG 11) expected from a population of 2000 clones*

Further family selection work is currently being conducted in the Burdekin, central and northern regions and in New South Wales (TA McRae and JK Bull, *personal communication*). In addition to evaluating family selection in original seedlings, these experiments will also determine the effect of differential rates of mass selection within families. Under the system currently being used by BSES breeders, more clones are selected out of the best families (the top 10%), with more stringent visual selection being applied to medium performing families (30-40% category).

CONCLUSIONS

The practical utility and effectiveness of family selection combined with mass selection within selected families has been demonstrated for heavily lodged seedling crops typical of the Burdekin and for early stages of the Bundaberg program. The research has resulted in a change in all BSES selection programs to use family selection in the plant crop of either original seedlings or early clonal stages. Visual mass selection is then conducted within selected families in first ratoon crops. The large increase in efficiency (time and resources) together with apparent increases in genetic gains has resulted in more cost effective selection programs. Evidence to date indicates that family selection should be fairly liberal (about 40%) but further research is aimed at quantifying this better, as well as investigating the effectiveness of differential selection rates within families. The advantages of better estimates of breeding value (BLUPs) as a result of family selection are dealt with elsewhere in these proceedings (Stringer et al 1996)

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TOWARDS OPTIMIZED INDUCTION OF FLOWERING IN SUGARCANE

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ABSTRACT

Variable and generally low to moderate flowering of parental sugarcane germplasm (Saccharum spp. hybrids) is the most serious impediment to genetic improvement of the Australian crop. Our research sought to verify the efficacy of plant management and photoperiod techniques developed in nine experiments that aimed to optimize floral initiation. Flowering of potted plants of 192 clones, classified into three response classes, induced in a controlled regime in the Bureau of Sugar Experiment Station's (BSES) Meringa photoperiod facility was compared with the flowering of similarly managed plants induced under natural photoperiod in external, ambient conditions. Flowering in these regimes was 67.0 and 5.4%, respectively, on a stalk basis, and 86.9 and 16.2%, respectively, on a clonal basis. Use of the controlled photoperiod regime and delaying induction to avoid days with a maximum temperature >32 °C are believed to explain this success. Pollen fertility in the optimized regime was excellent. The impact that controlled photoperiodic induction will have on production of higher quality parental combinations will be substantial.

INTRODUCTION

Artificial photoinduction of flowering in sugarcane is old technology in temperate and subtropical sugar industries. Photoperiod facilities are essential tools for crop improvement in Argentine, China, Florida, Louisiana, South Africa, and Taiwan. More recently, such facilities have become operational in tropical industries in Australia, Colombia, and Cuba. The Australian facility is located at BSES Meringa, on the lowland tropical coast, at 17°04' S lat. The primary *Saccharum* spp. hybrid germplasm collection for the Australian industry is located here. Variable and generally low to moderate field flowering at Meringa has been identified as a major limitation to genetic improvement of the Australian crop. Many of the most desirable parental clones are unavailable for cross pollination, and the most desirable parental combinations are rarely possible. Berding (1995) has summarized recent flowering research in field and photoperiod facility experiments at Meringa.

Research to optimize the induction of flowering of sugarcane in the Meringa photoperiod facility has been undertaken for some years. Early research using regimes from overseas facilities, perhaps confounded with inappropriate plant management, failed to achieve consistently high levels of induction. Eleven experiments have been completed in more recent research to optimize induction. The first eight were summarized by Berding (1995). In this paper, we present results from Experiment 10. which sought to verify the effectiveness of the controlled regime developed from the nine previous experiments. Flowering in clones subjected to a controlled inductive regime in the photoperiod facility was compared to flowering in similarly established potted plants subjected to a natural inductive regime in external ambient conditions.

MATERIALS AND METHODS

The 192 clones used in the experiment were from the active, commercial parental collection at BSES Meringa in 1994. Flowering data for the period 1983 to 1994 were considered. Data for years with flowering below the long term average from 1978 to 1994 for BSES Meringa (37.7%) were removed. The years, and the average flowering, were: 1983 (18%). 1986 (33%), 1987 (31%), 1990 (23%), 1992 (17%), and 1993 (16%). This excluded clones that flowered in harsher years. Clones that flowered in any of the remaining years were classified as "flowering" (n = 599). Clones with no record of flowering were classified as "non-flowering" (n = 784). Random sets of 91 and 92 clones were drawn from these, respectively. Nine clones used throughout the nine earlier experiments were included as "standard" clones.

One-eye setts of the 192 clones were germinated commencing 7 September, 1994. From 17-25 November, germinated setts were planted to two sets of 2 x 192 pots, of 33 L capacity, located in an external growth area. One set of pots, destined for the controlled regime in the photoperiod facility, was filled with a mixed compost (coarse river sand, #1 vermiculite, and a local peat, 400 L each; dolomite, hydrated lime, and superphosphate, 1 kg each). The pH was 6.0. The set of pots destined for the external, natural regime was filled with a similar mix, except loam replaced the vermiculite because of cost constraints. The pH of this mix was also adjusted to 6.0.

All pots received 250 mL of diluted Wuxal liquid foliar nutrient concentrate (Schering; 300 mL to 20 L) weekly for six weeks from 7 December, and every two weeks for 10 weeks from 25 January 1995. Pots were maintained in 100 mm of water while in the external growth area and on the photoperiod facility trolleys. The water was replaced weekly. Pots were irrigated three times daily using under-tree micro sprinklers located on the top of the pots. Meteorological data for the controlled and natural regimes were collected using Campbell Scientific (UT) 21X data logger systems. Sensors were scanned every minute, with average hourly data being stored.

All plants were exposed to a 14.5 h photoperiod from the commencement of the growth phase on the external growth area, on 26 November, until the start of the respective inductive regimes. One set of two replicates of the 192 clones in the external growth area received a natural photoperiod, instead of the 14.5 h photoperiod, from 1 February 1995. The second set of two replicates of the 192 clones remained in the external growth area until 23 March 1995. and continued to receive a photoperiod of 14.5 h. This set was relocated to trolleys in the Meringa photoperiod facility on 23 March 1995. immediately before receiving die controlled regime. A randomized complete block design was used in each inductive treatment. The commencing night length in the controlled regime was 11 h 15 min, increasing by 30 s/d. Plants received the controlled regime by entering lit chambers from external conditions at day's end to receive an instant sunset at almanac sunset plus 10 min. After the required dark period, plants received an instant sunrise before exiting to external conditions after almanac sunrise plus 30 min. and when external temperature was >21°C. Days with a maximum temperature >32°C dominated early induction in the controlled regime (8 of the first 11 days), and induction was stopped. Plants were given a night break of 1 h of light from 3 - 10 April. Induction recommenced on 11 April, at a night length of 11 h 15 min. The night length increase of 30 s/d in the controlled regime contrasted with an increase of about 60 s/d in the natural photoperiod during the inductive window, 14 February to 10 March. The physical aspects of the Meringa photoperiod facility, and improvements effected, have been detailed (Berding 1995).

In both regimes, panicles were scored as emerged when spikelets first opened. Panicles from the controlled regime had pollen availability determined using a standard I/KI test. Panicle samples were rated for pollen abundance (abundant, average, or sparse) and percent stained grains. Crosses were made among clones flowering in the facility when possible. Meristems of unflowered stalks in the controlled regime were dissected and rated from the 9 - 11 October 1995. Stalks remaining on

Table 1 *Summary of individual and combined analyses of variance for traits determined in replicated clonal experiments subjected to natural and controlled inductive regimes at BSES Meringa.*

Statistic[1]	Natural (n = 180)		Controlled (n = 191)		
	No. stalks/pot	% flowering[2]	No. stalks/pot	% flowering[2]	% initiation[2]
Mean	3.51	5.36	3.35	66.96	72.59
MS (reps)	0.64	236.81	0.94	52.07	3.48
MS (clones)	1.61**	473.45**	2.95**	2482.39**	2,180.87**
CV%	20.9	228.3	28.9	39.9	35.2

	Combined (n = 189)		
MS (regimes)	20.87**	851064.56**	
MS (clones)	3.58**	1723.40**	
MS (T x C)	1.03**	1237.87**	
CV%	25.4	56.1	

*P<0.05 **P<0.01

[1] MS = mean square. [2] % of total stalks number.

plants in the natural regime were cut and rated for flowering on 31 October 1995.

Data available from the controlled regime were number of emerged panicles, number of meristems initiated but unemerged, number of uninitiated meristems. number of stalks per pot, and pollen fertility. Percent flowering (%F; emerged panicles) and percent initiation (%I; emerged panicles + meristems initiated but unemerged) were calculated on a pot basis. A 1 g sub-sample of dried, processed panicle from each of 98 crosses made with panicles from the controlled regime was germinated using standard techniques, and scored for number of seedlings. A simpler data set for the natural regime consisted of the number of emerged panicles and the number of stalks per pot. Percent flowering was calculated. Data were analyzed using analyses of variance models for a single randomized complete block design, or a combined design over regimes. Routine statistics from these are presented.

RESULTS

Clones in the flowering group had been in the Meringa parental collection for 1 to 12 y. Average residence was 4.9 y. The clones in the non-flowering group had been resident from 1 to 9 y, with an average of 4.5 y. Clones in the standard set were resident from 7 to 12 y, and averaged 10.1 y.

Flowering in the natural regime (5.4% of stalks) compared poorly to that obtained in the controlled regime (67.0%, Table 1). An additional 5.6% of stalks initiated but did not emerge in the latter. Differences among clones were highly significant for number of stalks per pot and %F in both regimes, and for %I in the controlled regime. Flowering in the natural regime was variable, as indicated by the high CV% value (Table 1). Thirty one clones (16.3%) flowered in both the natural and controlled regimes, giving 32.2 and 86.8% flowered stalks, respectively (data not shown). An additional 135 clones flowered only in the controlled regime, giving a total of 86.9% of clones flowered in this regime. Combined analyses over regimes revealed significant differences between regimes for number of stalks per pot, and highly

Table 2 *Analyses of variance for three traits, measured on clones subjected to a controlled inductive regime, with sums of squares for clones partitioned into 'among' and 'within' clonal groups classified as flowering (F), non-flowering (NF), and standard (S), and tested against the respective components of similarly partitioned error terms.*

Mean square	d.f.	No. stalks/pot	% flowering[1]	% initiation[1]
Clones	190	2.95**	2482.39**	2160.87**
Groups	2	1.97	24907.54	20929.69
Clones/F	89	2.35**	2008.35**	1610.12**
Clones/NF	91	3.61**	2605.75**	2451.67**
Clones/S	8	2.43	746.58	753.92*

[1] % of total stalks number.

significant differences between regimes for %F (Table 1). Differences among clones were highly significant for both traits. There were highly significant regimes x clones interactions for number of stalks per pot and %F.

The partition of variation among clones into components, for the controlled regime data, revealed highly significant differences among clones for the flowering and non-flowering groups, for number of stalks per pot, %F, and %I (Table 2). The differences among standard clones were significant for %I only. Interestingly, differences among groups were almost significant (P = 0.068 and 0.062) for %F and %I (Table 2). This was reflected in the mean values (Table 3). For %F, for example, the values for the set of standard clones (90.3% of stalks; 100% of clones) were higher than those for the flowering clones (72.4%; 91.1%). These, in turn, were higher than those for the non-flowering set (52.4%; 83.3%). Data for %I showed a similar trend. Impressively, 52.4% of stalks of clones classified as non-flowering on entry to this experiment produced panicles.

Maximum temperatures during the natural induction period at Meringa from 14 February to 10 March 1995 exceeded the 32°C threshold from 16-24 February, inclusive, and from 7-10 March, inclusive. The maximum temperature in the controlled regime exceeded 32°C on 12 and 17 April, and also on 16 July and 26 September. Research in both the field and the photoperiod facility at Meringa has led to a working hypothesis that days during the inductive window with a maximum temperature >32°C are detrimental to induction. These results are not at variance with this hypothesis. However, some caution in interpretation is required because of confounded differences between the regimes, but these are regarded as inconsequential.

The first panicles from the natural regime were available on 15 May. Panicles from the controlled regime were available from 27 July until 10 October. This ranged from 107 to 182 days after recommencement of induction. A total of 684 panicles were cut from the controlled regime in 544 events. Pollen tests were available for 673 panicle samples from 536 events. Panicle samples with a pollen test rated abundant and >80% stained grains accounted for 32.1% of samples. Another 33.6% was rated abundant with staining >20% and <80%. These classes were very strong and strong males, respectively. The remainder was classified as female, 30.8% being rated abundant with <20% stained, 2.4% rated average with no staining, and 1.1% rated sparse. Overall, pollen tests from panicles produced in the controlled regime were excellent, and far exceeded that expected from field-produced panicles at Meringa.

Germination tests for the 98 crosses made revealed 45 crosses (46%) with <10 seedlings/g, 43 crosses (44%) with 10 to <100 seedlings/g, and 10 crosses (10%) with >100 seedlings/g. Germination of crosses produced was disappointing, and contrasted with the excellent maleness. However, because of resource restraints, maintenance of crosses was less rigorous than applied in the core cross-pollination program, and this may account for poor seed germination.

Table 3 *Range and mean values for three traits measured on groups of clones classified as flowering (F), non-flowering (NF), and standard (S), subjected to a controlled inductive regime in the BSES Meringa photoperiod facility.*

| | | | % flowering | | | | % initiation | | | |
| Clonal group | No. stalks/pot | | Stalks | | Clones | | Stalks | | Clones | |
	Range	Mean	Range	Mean	No.	%	Range	Mean	No.	%
NF (n = 90)	1-6	3.2	0-100	72.4	82	91.1	0-100	77.4	85	94.4
S (n = 92)	1-7.5	3.1	0-100	52.4	75	81.5	0-100	58.6	79	85.9
(n = 9)	2-6	3.6	42-100	90.3	9	100.0	42-100	91.7	9	100.0

CONCLUSIONS

Flowering obtained in the controlled regime was excellent for percent flowered stalks and percent flowered clones, and verified the efficacy of the controlled induction regime developed in the nine earlier experiments. The flowering of 52% of stalks in clones that entered the experiment classified as non-flowering was an additional indication of the success of the controlled regime. Clones that flowered in both regimes flowered more profusely in the controlled regime. The delayed start of the controlled regime relative to the natural regime largely avoided days with a maximum temperature >32°C during induction. This, coupled with the nature of the controlled regime, is believed responsible for the successful result. Results from the controlled regime suggested that the regime developed using the standard clones actually may have favoured the standard clones over those in the flowering and non-flowering groups. Further progress may be made by developing regimes specific to such groups. Overall, results of this experiment indicate the excellent potential of the Meringa photoperiod facility to generate genetic variation of a range and quality previously unavailable to the Australian sugar industry.

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HOW MUCH SELFING OCCURS IN SUGARCANE BREEDING PROGRAMS?

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ABSTRACT

Family selection is widely used in Australian sugarcane breeding programs to select both promising progeny and parents of future crosses. The occurrence of selfing in the program may affect the reliability of family means for assessing the breeding value of parents and for identifying promising crosses. While the possibility of selfing and its associated problems have long been recognised, there is no accurate data about the level to which it is occurring.

Three sugarcane crosses which exhibited variable levels of performance were selected. Their progeny were analysed with 6-8 RAPD markers to determine the number of selfed progeny in each cross. The percentage of selfing ranged from 5.6 % -17.6%, with the better performing cross containing the least number of selfed progeny and the poorest cross containing the most selfed progeny. These preliminary data suggest that a significant level of selfing is occurring in some sugarcane crosses.

INTRODUCTION

The crossing procedure in sugarcane breeding programs in Australia involves placing a variety producing relatively low levels of viable pollen (the "female") just below a variety producing higher levels of viable pollen (the "male"). Selfing may occur if some pollen from the "female" fertilises its own stigmas. Emasculation is not done routinely, as is the case in many other crops and in some overseas sugarcane breeding programs (e.g. Copersucar, Brazil). Sugarcane is not self-incompatible; selfing has been done deliberately in the past for research projects by omitting a male parent from die cross. In routine crosses made in breeding programs, where flowering is unpredictable and variable, crosses often involve females with at least some pollen. Thus, some level of selfing would seem likely.

Low precision in estimating breeding values of parents, and in predicting superior crosses, has been recognised as a major constraint to the rate of genetic gain in sugarcane breeding programs. The current method of crossing in breeding programs means that self-pollination in the "female" used in crosses could easily occur and be widespread. If selfing occurs even to a fairly limited degree, the value of family performance data for assessing parent breeding values would be questionable. This is because seedlings produced by selfing are usually markedly inferior and would "pull down" the average of the seedlings in the same cross. In addition, current methods of assessing breeding values of parents (eg. BLUP analysis or other approaches relying on mean seedling performance in crosses) may need to be reassessed if the level of selfing is significant. Alternatively, techniques such as hot water emasculation of clones used as females in crosses (as routinely performed in Copersucar, Brazil) may need to be adopted.

However, the degree of selfing in crosses in sugarcane breeding programs has not been widely assessed. Levels of selfing were assessed visually, using reduced vigour as the criterion, in a 5x5 diallel cross of sugarcane and found to vary from 0-80%, depending on the cross and direction of the cross (Hogarth 1980). Molecular marker technology now makes it possible to easily and accurately determine the extent of selfing. If significant selfing is occurring in sugarcane crosses, this could have a large and detrimental impact on the conduct of sugarcane breeding programs.

The present paper presents the results of a pilot study to assess the level of selfing in 3 crosses in the CSR sugarcane breeding program using molecular markers.

MATERIALS AND METHODS

Three crosses from the CSR sugarcane breeding program were selected on the basis of their variable performance (Table 1). Cross 92-153(Q96xQ142) performed well, cross 92-123 (MQ81-71 1xQl 15) had an intermediate level of performance and cross 92-224 (Ql 17xMQ66-1399) performed poorly. Thirty-six, 37 and 34 progeny were available for analysis from each cross respectively.

Table 1 *CCS, sugar yield (TSH) and cane yield (TCH) for the three crosses studied*

Cross	CCS	TSH (t/ha)	TCH (t/ha)
92-153	15.3	148	22.7
92-123	12.3	129	16.0
92-224	13.6	81	11.3

DNA Isolation

Fresh young leaves were collected in the field and transported on ice overnight to die laboratory where they were stored at -20°C until lyophilised. After lyophilisation, die material was ground to a fine powder and DNA extracted using a modification of die method of Saghai-Maroof et al (1984), as described in the UMC Maize Genetics Laboratory Manual.

Identification and scoring of male- and female- specific bands

The six parents of the 3 crosses were screened with approximately 40 10-mer primers (Operon) to identify both male- and female- specific bands. PCR was performed as described in Tao et al (1993). Five to eight male-specific bands and 5 female-specific bands were identified for each cross. The progeny of each cross were scored for the presence or absence of each male- and female- specific band. In the case of die male-specific bands, the presence of a male-specific band in die progeny indicated that the progeny was a hybrid between the two parents and not a self. Female-specific bands were also scored to check the number of bands required to determine the female contribution to the progeny, given that RAPD markers are dominant (i.e. only half the progeny on average will receive a specific parental marker) and that occasional chromosome loss (and the markers located thereon) is not unusual in sugarcane.

RESULTS

After scoring 8 male-specific RAPD bands in cross 92-153. 34 of the 36 progeny could be identified as hybrids (94.4%)(Table 2). After analysing 5 male-specific RAPD bands in cross 92-123 and 6 male-

specific RAPD bands in cross 92-224. 24 of the 28 progeny (90.2%) and 26 of the 34 progeny (82.4%), respectively, could identified as hybrids (Table 2). In all 3 crosses, 5 female-specific bands confirmed the female contribution to the progeny.

Table 2 *Percentage of selfed progeny in each cross*

Cross	No. progeny	No. male-specific bands	% Selfing
92-153	36	8	5.6
92-123	28	5	10.8
92-224	34	6	17.6

CONCLUSIONS

These preliminary results suggest that a significant level of selfing is occurring in some sugarcane crosses. In the three crosses analysed in die present study, the cross with the highest level of selfing is also the cross with the poorest performance, while the cross with the lowest level of selfing is die best performing cross. Such a level of selfing may seriously affect die current method of selection utilised in sugarcane breeding and of determining parental breeding values. It has been suggested, however, that hybrid seedlings which are near the selfs may be able to compensate for the poor performance of selfed progeny in a cross by above average performance. If hybrid seedlings can partially or completely compensate for the poorly performing selfs, the overall performance of the cross may not be greatly affected. Such compensation would depend on the level of selfing occurring in the cross. Thus, it is important that more crosses are studied to assess the extent to which selfing is occurring in sugarcane breeding programs and its possible correlation with performance.

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THE IMPORTANCE OF WATER AND NITROGEN IN GENERATING CLONE BY ENVIRONMENT INTERACTION IN SUGARCANE: A PRELIMINARY INVESTIGATION BASED ON PLANT CROP RESULTS

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ABSTRACT

Clone x environment interaction (CxE) has been found to be a major factor limiting the gains from selection of sugarcane clones in southern Queensland. The specific contribution of water and nitrogen availability to CxE effects was examined in managed-environments (MEs) and used to establish techniques for investigating factors leading to CxE. Water and N were considered important because their availability varies significantly among selection sites both within and between crop seasons.

High and low regimes for water and N were applied to a range of clones (selected and unselected) grown at Bundaberg. Patterns of yield accumulation were investigated throughout the season and preliminary results from the plant crop indicated that the differential availability of water and N can cause substantial CxE. These findings have important implications for regional selection programs and highlight the possibility of selecting specifically adapted niche cultivars.

INTRODUCTION

The magnitude of genotype x environment interaction (GxE) for yield can complicate selection in many crops (DeLacy et al 1990J. This is also the case in sugarcane selection programs where clone x environment interaction (CxE) may be as large as the main effect for clone (Jackson etal 1991; Bull etal 1992a; Mirzawan etal 1993). Consequently, it is necessary to test clones across a range of environments (Hogarth & Mullins 1989; Bull et al 1992) which is time consuming and costly.

If the major environmental factors giving rise to CxE could be identified, they could either be obviated or exploited by using managed-environments (MEs) in the selection programs. These MEs allow the factor of interest to be assessed while controlling or accommodating the influence of other factors (Cooper et al 1995). They also offer the opportunity to use an experiment station as a selection site for targeting repeatable management factors during the multi-environment testing stage, and may ultimately enable the number of off-station sites to be decreased and costs to be reduced.

This paper focuses on the contribution of two factors, water and N, to CxE for cane yield throughout a plant crop in southern Queensland and on the ME methods necessary for investigating CxE. Both water and N are economically important factors in crop management and their availability varies substantially within the region.

MATERIALS AND METHODS

Treatments

A trial using ten unselected clones and two commercial cultivars (CP44-101 and Q150) was planted at the Bundaberg Experiment Station in southern Queensland. These clones were chosen from previous experiments to represent a range in clonal response. Each clone was planted to a nine-row plot 10m long with inter-rows of 1.5m. Two levels of water application, rainfed (low, L) and irrigated approximately weekly (high, H) until 279 days after planting (DAP); and two levels of N application, 20kg/ha (low, L) and 220kg/ha (high, H), were combined factorially in four treatments with two randomised replicate blocks replicates.

Cane yield (t/ha) for the first seven harvests (DAPs: 103, 132, 160, 187, 221, 263, 320) was determined from a sample of 20 contiguous stalks (plus immature tillers) and stalk counts were taken over a measured 4m length within each plot. For the eighth harvest (DAP 376) cane yield was determined from a sample of eight contiguous millable stalks and stalk counts were taken as above. Cane yield for the ninth harvest (DAP 382) was determined by harvesting green (using a mechanical Toft 7000 harvester), and weighing (using an in-field weigh truck) the cane from two rows (measured for length) within each plot.

Data analysis

An analysis of variance was conducted over all harvests and for each harvest individually.Harvest time for each treatment were considered to be different environments and the data from each harvest were centred by removing the environment effect and standardised by dividing by the environment's phenotypic standard deviation (Fox & Rosielle 1982). Environments were grouped using hierarchical agglomerative cluster analysis following the method of Ward (1963) for the fusion strategy and squared Euclidean distance as the dissimilarity measure (Wishart 1969; Burr 1970).

A principal component analysis (PCA) was performed on the CxE matrix with each harvest again considered to be a different environment. The PCA biplots provide a graphic illustration of the relationships among treatments at different harvest times based on their discrimination among clones (Chatfield & Collins 1980).

RESULTS AND DISCUSSION

Pattern of yield accumulation

Cane yield for the high water/high N treatment (HH) was significantly higher than for other treatments at every harvest time, except the first (Fig. 1). Low water precluded an overall response to N (LH and LL), and low N (HL) precluded most of the response to water. Treatment responses at harvest 9 are not presented as they are not directly comparable because the mechanical harvester had a lower precision in separating stalk component from sheath and leaf than hand harvesting.
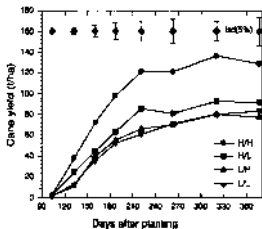
Fig. 1. *Cane yield (t/ha) at each harvest (except harvest 9) during the season for the treatments: high water/high N (HH), high water/low N (HL), low water/high N (LH), low water/low N (LL).*

Analyses

The analysis of variance over all harvests gave significant (P<0.05) effects for treatment, clone, clone x treatment interaction (here referred to as CxE) and clone x harvest time interaction. The data used for this analysis and for the analyses conducted at each harvest were transformed by taking the natural log of yield plus one to adjust for the association between mean yield and variance.

The ratio of CxE to clonal variation (Fig. 2) was low for the first harvest (3%) before the treatments were initiated and rose to 110% at harvest 3 during the peak growth period immediately after the treatments were first applied. The ratio subsequently tailed off particularly after harvest 6 when the irrigation treatment ceased. Within the normal sugar industry harvest time the ratio of CxE to clone ranged between 14% and 32%. Clearly these MEs allowed significant CxE effects to be generated at a single location.
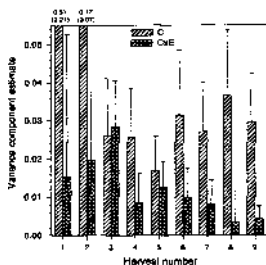
Fig. 2. *Estimated variance components and associated standard errors during the season for clone (C) and clone x environment interaction (CxE).*

These ratios for CxE variation to clonal variation are smaller than those found in core selection trials, conducted in farmers' fields over some 250km along the Queensland coastline, where the ratios range from 66% to 177% and average around 98% (Mirzawan 1994). Traditionally, only the clone x site component of this interaction would be thought of as repeatable and it is on average 56% of the total interaction found in core trials (Mirzawan 1994). The amount of interaction generated in the MEs compares favourably with the 'repeatable' portion of the interaction found in core selection trials, and may explain from 25% up to 57% of the repeatable interaction. The amount of interaction in core selection trials that can be explained by MEs is the focus of ongoing research.

Pattern analysis

The dendrogram presented in Figure 3 was based on the classification of the 36 treatment/harvest combinations (four treatments harvested nine times) and was formed from the six- to one-group level. There was a strong separation of the harvests from differing treatment at the four-group level (which accounted for 43% of the CxE sum of squares). Beyond the four-group level there was separation of the LH treatment into early and late harvests. Likewise at the six-group level there was separation of the LL treatment into early and late harvests. All four treatments from the first harvest (before the treatments were applied) grouped together indicating little or no pre-treatment interaction. The HL and LH treatments at harvest 9 were grouped with the LL treatmentsfrom harvest 2 to 9 at the six-group level, whereas for hand-sampling harvest 8, conducted one week earlier, the treatments were placed with other harvests of their respective treatment. This difference
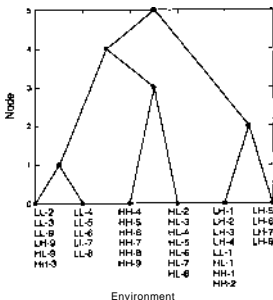
Fig. 3. *Dendrogram showing the similarity among the four treatments at each harvest (environment code = level of irrigation (low, L or high, H); level ofN (low, L or high, H); harvest (1 to 9)).*

in grouping may reflect the lower precision of separating stalks from other crop components and extraneous matter when using the mechanical harvester. As such the difference could have important implications in relating sampling to harvester derived yield assessments and may require further investigation.

Since at the four-group level, groups mostly consisted of harvests of the same particular treatment it may be concluded that treatments were the major source of interaction followed by time of harvest.

The first three vectors from PCA cumulatively accounted for 25%, 44% and 60% of the CxE sum of squares. The scores of each harvest of each treatment were plotted and indicative boundaries that principally separated harvests of differing treatments were superimposed on these plots (Fig. 4a,b,c). From the plot of vector 1 versus vector 2 and vector 1 versus vector 3 (Fig. 4a.c) the nine harvests of each particular treatment were placed in relatively close proximity. Vector 1 separated the harvests from the low- and high-N treatments at low water, and placed the low- and high-N treatments at high water close together. Vector 2, however, did the reverse and separated harvests from the low- and high-N treatments at high water, and placed the low- and high-N treatments at low water in close proximity. Vector 3 gave partial separation of the H/H treatment harvests from the other treatment harvests (Fig. 4b,c). From Figure 4c there was a broad differentiation between rainfed and irrigated treatments (harvests). In accordance with what was found from for the cluster analysis the HL and LH treatments at harvest 9 were placed in close proximity to the LL treatment harvests, while the sampling harvest 8 for these treatments was placed closer to other harvests of their respective treatment. Again this may reflect the decreased precision in separating stalks from other crop components and extraneous matter when using the mechanical harvester.

These analyses indicate that the main source of variability was generated by N at low water availability followed by N at high water availability. Some interaction was also generated by water availability and time of harvest.

CONCLUSIONS

The MEs allowed substantial and interpretable CxE effects to be generated at a single location. The interaction that was generated was consistent across a number of harvests but was strongest immediately after the management treatments were initiated and weakened **with time**

after irrigation ceased. Both water and N could contribute to CxE in core multi-environment selection trials where their availability varies significantly. MEs offer an efficient method of screening clonal material at a central experiment station.

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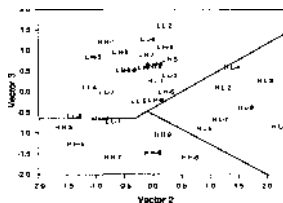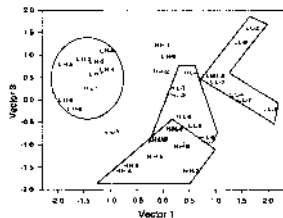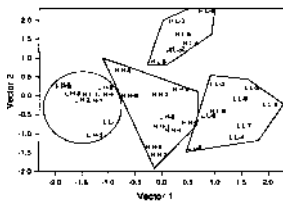
Fig. 4. *Principal component plot for vector 1 versus 2 (top), vector 1 versus 3 (middle), and vector 2 versus 3 (bottom) for cane yield for each treatment at each harvest (codes as in Fig. 3 caption)*

ROLE OF VARIABLE SOIL NUTRIENT LEVELS IN CAUSING GENOTYPE X SITE INTERACTIONS IN SUGARCANE

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ABSTRACT

Genotype x site interactions are often large in sugarcane selection programs in Australia. Resolution of the factors responsible for causing these interactions would lead to more focused and effective approaches to sugarcane improvement. Results from a recent research project suggested that differences in soil fertility could be a major factor contributing to genotype x site interactions. The work reported here aimed to test this hypothesis in two further data sets. These data were obtained from two series of trials previously conducted within a core breeding program in the Herbert region of North Queensland, Australia. The two trial series contained 34 and 57 genotypes respectively, each trial involving all genotypes evaluated across four sites and two crop-years.

The results supported, but could not confirm, the suggestion that some soil fertility factors, particularly calcium, zinc and manganese levels, play a major role in causing genotype x site interactions. However, further evidence is needed to confirm this role. On average, sites which were relatively similar in levels of these nutrients tended to discriminate similarly among genotypes. In particular, there is a need to conduct controlled trials where nutrient levels are changed while other factors (eg. weather) are held constant.

INTRODUCTION

Genotype x environment (GE) interactions in sugarcane have been studied in several regions in Australia. In the Southern and Northern regions genotype x site interactions were found to be large relative to die genotype main effects (Hogarth & Bull 1990; Jackson & Hogarth 1992; Mirzawan et al 1993). By contrast, in the Burdekin region, GE interactions appear to be small and of little importance (McRae & Jackson 1994). In most studies where GE interactions have been large, genotype x site interactions have constituted a major proportion of the total interaction variance, and have usually been larger man genotype x crop-year interactions.

Recently, in a study conducted in the Herbert region of North Queensland, family x site interactions were examined in detail (Jackson et al 1995). In this study, trials were grown on well managed commercial farms, following conventional commercial cultivation and fertilization practices. There were very large differences in mean cane yield levels between the trials (30t/ha to 105t/ha), which were caused mostly by variation in rainfall amount and distribution. However, there was no association between similarity among environments for final yield and their similarity for family rankings. Likewise, among environments, there was no association between similarity in any weather variables (rainfall, temperature, solar radiation) and similarity for genotypic response. For example, yield at the wettest site had a high genetic correlation with yield at the driest site, while genetic correlations between some pairs of environments with similar yield/rainfall levels were low. Soil chemical analysis revealed large differences in nutrient levels between sites, with some sites having levels of some nutrients mat would be considered marginal. It was found that sites which had relatively similar levels of a number of soil nutrients, including calcium and zinc, tended to produce relatively similar ranking of families. Sites with contrasting levels of these nutrients tended to be relatively dissimilar for ranking of the families.

These results, while not conclusive, suggested that marginal soil fertility in relation to some nutrients could be causing genotype x site interactions in selection trials in the Herbert. This hypothesis is also consistent with the occurrence of large genotype x site interactions in the Herbert region where there is large variability in soil fertility, and the absence of large genotype x site interactions in the Burdekin region where soil nutrient levels are very high throughout. Confirmation of this hypothesis would have a major impact on how selection is conducted in sugarcane, and perhaps on how soils are managed commercially for realising the potential of genotypes susceptible to marginal soil fertility.

In this paper, two further series of selection trials in the Herbert were examined. These trials had been previously conducted within the CSR

core breeding program targeting this region. The aim of the study reported here was to test the hypothesis that sites with similar levels of soil nutrients (particularly Ca, Zn) give similar genotypic responses, while those with contrasting levels give relatively dissimilar responses.

MATERIALS AND METHODS.

Two separate series of advanced stage selection trials were examined. These constituted the final stage of selection in the Herbert region in the CSR breeding program. The first series consisted of 34 genotypes planted at four sites in 1991, while the second series consisted of 57 genotypes planted at four sites in 1992. In each series both plant and first ratoon crops were harvested at all sites. The trial design at each site consisted of a randomised complete block design with two replicates. The plot size was 4 rows x 10m with a spacing of approximately 1.5m between rows. All plots were harvested mechanically and the cane from the harvester weighed using a tractor drawn bin with electronic load cells.

In 1994, soil was sampled from all sites for chemical analysis. A composite sample of soil was taken from each replicate at each site. Samples representing each replicate were sent to INCITEC Ltd. (P.O. Box 140, Morningside. Qld 4170) for measurement of levels of all essential nutrients and pH.

The data for cane yield from the trials was subjected to pooled analysis of variance across environments using the model given by Jackson & Hogarth (1992), and principal component analysis (PCA), both using the SAS for Windows (v. 6.08) statistical package. For PCA, the correlation matrix among environments for cane yield was used as input.

RESULTS

The mean cane yield, CCS and sugar yields at each site, along with levels of some nutrients are summarised in Table 1. The nutrients shown were suggested as being of importance in previous work (Jackson et al 1995).

Results from pooled analyses of variance are shown in Table 2. In both series of trials, the genetic component of variance (c^2) and all interactions involving genotypes were significant ($P<0.01$). The genotype x site (a_{gs}^2) effects were between 40% and 50% of the size of genotype main effects. This relatively high level is consistent with the importance of genotype x site interactions in previous studies. However, compared with other analyses of GE interactions in the Herbert, the genotype x site component was of smaller size relative to the other interaction effect components, while the genotype x crop-year (o^2) interaction was of larger magnitude. The larger magnitude of genotype

Table 1 *Mean cane yield, CCS and sugar yield, and levels of some nutrients at each site in each series of trials.*

1991 series

	Site							
	Cantamessa (CAN)		Castorina (CAS)		Cavallo (CAV)		Sera (SER)	
	Plant	1st ratoon	Plant	1st ratoon	Plant	1st ratoon	Plant	1st ratoon
Cane yield (t/ha)	63	75	105	90	83	89	70	76
CCS	14.4	13.6	14.4	13.1	16.5	13.6	16.5	15.8
Sugar yield (t/ha)	9.0	10.2	15.2	11.8	13.8	12.3	11.6	11.9
PH	5.25		5.15		5.0		4.6	
Ca (meq/100g)	4.54		4.56		1.27		0.14	
Zn (mg/kg)	1.15		2.45		1.30		0.20	
Mn (mg/kg)	15.5		14.0		26.5		25.5	
Mg (meq/100g)	3.60		2.25		0.41		0.12	

1992 series

	Site							
	Cantamessa (CAN)		Castorina (CAS)		Mackee (MAK)		Pace (PAC)	
	Plant	1st ratoon	Plant	1st ratoon	Plant	1st ratoon	Plant	1st ratoon
Cane yield (t/ha)	68	66	70	47	81	72	98	65
CCS	13.7	15.0	13.1	13.9	13.3	15.1	11.6	13.4
Sugar yield (t/ha)	9.3	9.8	9.2	6.4	10.6	10.9	11.3	8.6
pH	5.0		5.5		4.75		5.5	
Ca (meq/100g)	4.19		2.48		1.69		5.61	
Zn (mg/kg)	2.70		0.40		1.35		2.10	
Mn (mg/kg)	20.5		18.0		40.5		18.5	
Mg (meq/100g)	2.78		0.90		0.56		2.90	

x crop-year interactions may be at least partly associated with the four row plots used in this study. Most previous studies have used trials with single row or two row plots which would increase competition effects. Competition effects would tend to be correlated across crop-years within each location, thus increasing genotype effects and genotype x site interaction effects.

Table 2. *Variance components (± standard errors) for pooled analyst of variance of cane yield (t/ha) for the 1991 and 1992 series trials.*

	1991 series	1992 series
a^2	89.4±8.7	36.1±5.9
$o_{g_2}^2$	34.3±9.7	20.1±6.9
o_{gc}^2	31.4±8.8	13.5±7.1
$a_g^2 J$	19.9±8.2	16.1±5.5

For the 1991 series of trials, the first two principal components from PCA of environments accounted for about 70% of the variance. For the 1992 series, three components were needed to account for this level of variance. In both analyses, the first component was highly correlated (r>0.97) with mean yield of genotypes across all environments.

Figure 1 shows the loadings of the environments in relation to the first two components for the 1991 series (Fig. 1a) and three components for the 1992 series (Figs, 1b, 1c). In these figures, environments in close proximity in relation to a component had discriminated similarly among the genotypes for the pattern of variation explained by that component.

Figure 1a shows that all sites in the 1991 series had a similar loading for the first component. There were larger differences among sites for the second component, with the Cavallo and Sera environments having zero or positive loadings, and Cantamessa and Castorina environments being negative. This separation roughly corresponded to differences among the sites for levels of a number of nutrients. Cavallo and Sera

had lower levels of calcium and magnesium and slightly lower pH, and higher levels for manganese (which is usually negatively correlated with Ca and pH) than the Castorina and Cantamessa sites (Table 1). There was a significant (P<0.05) correlation between levels of these nutrients and loadings of the environments on the second principal components (r = -0.80 for Ca, 0.88 for Mn, -0.79 for Mg).

For the 1992 series, the Mackee site was depicted as being the most dissimilar in relation to the first and second components. This site had the lowest calcium level and pH and highest manganese level of the four sites in this series (Table 1). However, the correlation between calcium level and loadings of the environments for the first (r = 0.67) and second (r = -0.55) components in this case were not statistically significant (P<0.05). There was a significant (P<0.01) correlation between loadings for the second component and manganese levels.

For the 1992 series, there was a significant correlation (r = -0.89) between zinc level and loading for the third component, with the Castorina site having the lowest level of zinc, and a high loading, and the Cantamessa site having the highest level and a negative loading (Fig. 1c, Table 1).

DISCUSSION

The findings of this study are consistent with the hypothesis that soil fertility factors may play an important role in causing genotype x site interactions in sugarcane. Generally, sites that were similar for levels of calcium, manganese and zinc tended to discriminate more similarly among genotypes than those environments that differ for these nutrients.

However, this study was limited in a number of aspects, and this precluded clearer relationships being shown. Firstly, other factors (eg. weather, soil pathogens) would also have been expected to contribute to some degree in causing GE interactions and these factors were confounded with effects due to soil fertility. These other effects would have "distorted" the relationships among environments caused by soil
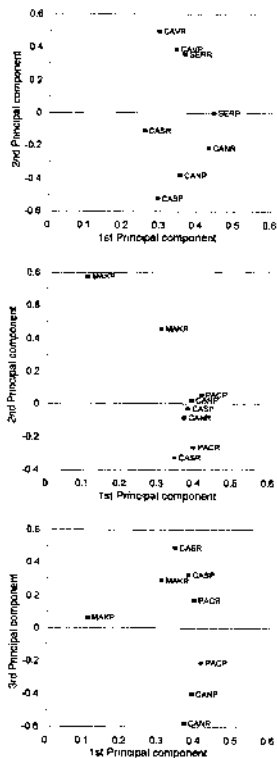
Fig. 1 *Loadings of environments from principal component analysis.*
la. First two components for the 1991 series of trials.
lb. First two components for the 1992 series of trials.
1c. First and third components for the 1992 series of trials.
[Codes for sites (first three letters of each point label) are given in Table 1. P and R refer to plant and 1st ratoon crops, respectively.]

fertility effects. Secondly, levels of soil nutrients may be unreliable indicators of nutrient availability or effects on the plant, as there may be complex interactions with pH, other nutrients and other factors.

The results reported here therefore support but cannot confirm the role of soil fertility levels in eliciting large variation in response among sugarcane genotypes. This positive but inconclusive result, together with the importance of gaining some understanding of the biological causes of GE interactions in sugarcane, strongly supports further investigation of this issue. This could be best accomplished by evaluating unselected genetic populations under controlled environments where only soil fertility factors are varied. The results from this study support those of Jackson *et al* (1995) which suggested that the effects of calcium and zinc should be investigated.

If it is confirmed that soil fertility differences play an important role in causing GE interactions there would be important implications for selection in sugarcane breeding programs, and for management of released cultivars. If the low fertility factors could be readily ameliorated it would be important to ensure that selection trials were only conducted in soil with high fertility. This may involve soil with higher levels of nutrients than critical levels estimated for existing commercial cultivars growing in relatively infertile areas. Such cultivars may have been (unknowingly) selected for some tolerance to reduced fertility conditions, and may be less sensitive than many unselected clones. Testing under only very high fertility conditions would allow the potential of all genotypes under examination to be realised. Furthermore, such an approach would reduce the magnitude of genotype x site interactions in sugarcane selection trials, and facilitate greater gains from breeding.

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MEASURING SUGAR CONTENT IN VARIETY TRIALS

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ABSTRACT

Sugarcane breeding and other research programs use hand cut, sound whole-stalk samples for determination of Commercial Cane Sugar (CCS) and other quality components. There is a potential bias associated with the processing of whole-stalk samples of sugarcane through a small-roller mill with low levels of juice extraction. This bias was quantified in selection trials in Australia at Tully, Ayr, Bundaberg and Broadwater. The influence of extraneous matter in samples of cane for determination of CCS was also evaluated. Results show that processing of whole-stalk samples through a small-mill with low levels of juice extraction seriously biased the accuracy of estimation of yield of cane sugar from sugarcane. The inclusion of extraneous matter in samples of shredded cane markedly decreased mean CCS. Despite these biases, the current practice of estimating sugar content from the juice of whole-stalks crushed through a small-mill is adequate for ranking of clones for selection purposes. However, to improve labour use efficiency and workplace safety, and to increase the accuracy of estimating sugar content, an automated method of sampling harvested cane from trial plots is required.

INTRODUCTION

The efficient and accurate determination of sugar content of harvested cane from small experimental plots is essential for estimating the relative yield potential of a new clone, and to predict the responses of commercial varieties to various agronomic treatments. Mechanical harvesting of the Australian sugarcane crop produces billets of cane for factory processing. In contrast, sugarcane breeding and other research programs use hand cut and lightly topped sound whole-stalk samples for determination of Commercial Cane Sugar (CCS) and other quality components. The use of whole-stalk samples may provide a biased sample of the material harvested from trial plots (Skinner, 1976), because usually only sound stalks which are free from extraneous matter (any solid material delivered with cane stalk, including dead and dried out stalks, dirt, roots, trash and tops) and inferior quality cane are sampled. The collection of samples is also highly labour intensive and ground staff are at risk of injury while working in close proximity to harvesters, weighing trucks and haulout equipment.

The small laboratory roller mills presently used to extract juice from whole-stalk samples for analysis have very low efficiency compared to a commercial sugar mill, extracting only 30-40% of the absolute juice. Juice quality depends on the extent of juice extraction, and also on the part of the sugarcane plant from which it is derived. Consequently, whole-stalk samples as used in variety selection may provide a doubly biased result compared with a truly heterogeneous sample of billets and extraneous matter as processed through a commercial mill. In a study in North Queensland, Skinner (1976) indicated that taking billet samples from selection trials should provide a direct measure of the harvested material with less systematic bias and fewer assumptions. However, a lower heritability associated with billet samples, as a result of increased sampling error, made this method inferior to whole-stalk sampling for selection purposes. Unfortunately, the billet samples were also processed through the small-mill with relatively low levels of juice extraction, and this may have led to an underestimate of the bias.

While the existing whole-stalk sampling method is rapid, it is likely to be biased and not an accurate predictor of sugar content of commercially harvested material. In this study, the bias associated with the processing of whole-stalk samples of sugarcane through a small laboratory roller mill is quantified. The influence of extraneous matter on the determination of CCS is also evaluated.

MATERIALS AND METHODS

Samples for determination of CCS were taken from replicated plant breeding trials located at Bundaberg (25°S,152°E), Ayr (20°S,147°E), Tully (18°S,146°E) and Broadwater (29°S,153°E). Each trial was a randomised complete block experiment with two replicates. In Bundaberg, 25 unselected clones grown in single-row 10 m plots were sampled green as plant cane in October, 1994. In Ayr, 40 clones grown in three-row 10 m plots were sampled as burnt third ratoon cane in June, 1995. In Tully, 40 clones grown in four-row 8.6 m plots were sampled green as first ratoon cane in June, 1995. In Broadwater, 33 clones grown in two-row 14.5 m plots were sampled as burnt first ratoon 2-year cane in August, 1995. Clones sampled in Ayr, Tully and Broadwater had previously undergone selection, and included the major commercial varieties for that district.

Two samples of six sound whole-stalks were hand cut from each clonal plot, lightly topped and stripped of trash and leaves. Juice was extracted from one sample by crushing the stalks through a small laboratory roller-mill (*Mill*). The second sample was shredded using a Dedini or Jeffco cutter-grinder and a 1 kg sub-sample hydraulically pressed at 25 MPa for 1 minute to extract juice (*Press*). To study the influence of extraneous matter on level of juice extraction and CCS, an additional 6-stalk sample including tops and trash was sampled in Bundaberg. In Tully and Broadwater tops, leaves, suckers and damaged stalks, were sampled from each plot, respectively for comparison with the whole-stalks. The samples were shredded, sub-sampled and juice extracted using the hydraulic press (*Press+EM*). Fibre content was estimated on a clonal basis for whole-stalk samples, with and without extraneous matter, using the bag-fibre method (Skinner 1969). Brix and pol of juice were measured, and CCS (Bureau of Sugar Experiment Stations 1991) calculated.

Statistical Analysis

The data were subjected to analyses of variance and covariance. Heritability and genetic, phenotypic and environmental correlations between traits were calculated. Direct and correlated response to selection were estimated using standard formulae (Falconer 1981). It was assumed 15% of the population would advance to the next selection stage, giving a standardised selection differential of 1.55.

RESULTS AND DISCUSSION

Juice quality depends on the extent of juice extraction. For each location, estimates of CCS (Table 1) for juice extracted from whole-stalks using the small-mill (CCS$_{SM-H}$) were higher than estimates based on juice extracted by the hydraulic press method (CCS$_{Pr:j}$). Mean levels of juice extraction for the small-mill and press were 26.4 and 82.7% for Tully, 39.4 and 86.0% for Ayr, 44.2 and 81.9% for Bundaberg, and 49.5 and 79.9% for Broadwater, respectively. In general, estimates of broad sense heritability on a clonal basis for CCS$_{SMILL}$, CCS$_{Para}$ and CCS$_{Press+EM}$ were medium to high as expected for CCS.

The genetic correlation (r_g) between estimates of CCS using the press and the small-mill (CCS$_{pm:}$ and CCS$_{Jwa:}$) were high and not significantly different from unity at each location (Table 1). This suggests, that despite an absolute difference in level of juice extraction between the press and small-mill for samples of whole-stalks were relatively unimportant for selection purposes. At Tully, Bundaberg and Broadwater, correlated response (CR) for press CCS, based on

Table 1 *Influence of extraction levels and extraneous matter on CCS at four locations. Means, heritabilities (h^2), genetic correlations (r_g + SE), direct (R) and correlated response (CR) to selection for whole-stalks processed through small-mill (CCS_{SMill}) and hydraulic press (CCS'J, and whole-stalks with extraneous matter and pressed ($CCS_{Press+EM}$).*

Statistic	Character(s)	Tully	Location Ayr	Bundaberg	Broadwater
Mean	CCS_{SMill}	12.57	13.44	16.83	12.60
	CCS_{press}	12.02	12.72	16.13	12.48
	$CCS_{Press+eM}$	9.37		12.90	11.49
h^2	CCS_{SMill}	0.84	0.30	0.87	0.41
	CCS_{press}	0.68	0.65	0.70	0.42
	$CCS_{press+EM}$	0.68		0.75	0.37
r_g	CCS_{press} and CCS_{SMill}	1.09 + 0.10	0.86 + 0.33	1.07 + 0.07	0.97 + 0.36
	$CCS_{Press+EM}$ and CCS_{SMill}	0.94 + 0.12		0.86 + 0.09	0.79 ± 0.32
	$CCS_{press+EM}$ and CCS_{Press}	1.09 ± 0.14		1.04 + 0.13	1.41 +0.57
R		1.94	0.74	1.55	0.96
		1.22	1.33	1.09	0.70
		1.24		1.03	0.68
CR (Character selected)	$CCS_{Press}(CCS_{SM_n})$	1.36	0.77	1.21	0.67
	$CCS_{Press+EM}(CCS_{SMill})$	1.30		0.96	0.57
	$CCS_{press+EM}(CCS_{Press})$	1.24		1.00	0.68

indirect selection using small-mill CCS. resulted in greater or similar predicted gains compared with direct selection (R) for press CCS (Table 1). In contrast, indirect selection using small-mill estimates was less effective than direct selection for press CCS at Ayr. resulting in lower predicted gains. Therefore at Ayr, selection based on small-mill estimates would slow genetic gain for sugar content. Although this result is of concern, it may be a product of sampling error as there was a lack of significant differentiation among clones for small-mill CCS and heritability was low. The genetic correlation between small-mill and press CCS was also subject to a large standard error (Table 1).

When comparing the efficiency of the two extraction methods, relative costs must also be considered. It is more expensive in terms of cost/ sample to extract juice using the current hydraulic press method, and this would impact on selection efficiency. Substantial improvements in the press method would be needed if it was to be used for routine screening of clones.

Extraneous matter in samples of shredded cane and processed through a hydraulic press resulted in a decrease in mean $CCS_{Press+EM}$ (Table 1). As expected, the difference from press CCS of whole-stalks (CCS_{Press}) was greater for the trials sampled green at Tully and Bundaberg. The Broadwater trial was burnt prior to sampling, and this would have resulted in the removal of some leaves and trash. The trial at Ayr was only sampled for whole-stalks with few suckers and little extraneous matter were evident following burning. Despite a marked reduction in mean CCS due to the inclusion of extraneous matter, the genetic correlations of $CCS_{Press+EM}$ with CCS_{SMill} and CCS_{Press} were high and not significantly different from unity at Tully. Bundaberg and Broadwater (Table 1).

At each location, predicted gains from indirect selection for $CCS_{Press+EM}$ based on selection for CCS_{SMill} were similar to gains from direct selection for $CCS_{Press+EM}$. The correlated response for $CCS_{press+EM}$ based on selection for CCS_{Press} also resulted in similar gains to direct selection for $CCS_{Press+EM}$ (Table 1). Extraneous matter in samples did not significantly alter predicted gains from selection. Therefore, the current practice of estimating sugar content from the juice of whole-stalks crushed through the small-mill would seem adequate for selection purposes. The levels of extraneous matter included in these hand-harvested whole biomass samples were set at a maximum for each clone, and overall levels were higher than would be expected if these trials were harvested with a commercial harvester.

In contrast, differential removal of extraneous matter by commercial harvesters may occur among varieties and this may affect the ranking of clones for sugar content. Further studies are needed to compare samples of whole-stalks with samples of harvested cane.

The ultimate objective of this work remains to develop an automated cane billet sampler to collect a random sample of harvestable material during the routine harvest of selection trial plots. The acquisition of an automated billet sampler would improve labour use efficiency and workplace safety. The use of unbiased samples should improve the accuracy of CCS assessment and provide a direct measure of the commercial value of harvested material.

CONCLUSIONS

The processing of whole-stalk samples through a small-mill with low levels of juice extraction seriously biases the accuracy of estimation of yield of cane sugar from sugarcane. In general, however, the method does not significantly alter the ranking of clones for selection purposes. Whole-stalk samples are adequate for screening of clones for sugar content. The inclusion of extraneous matter in samples of shredded whole-stalks markedly decreased mean CCS, but did not alter predicted gain from selection. There is a need for an automated method of sampling harvested cane from trial plots for a more accurate and more efficient estimation of sugar content. However, the current practice of estimating sugar content from the juice of whole-stalks crushed through a small-mill is adequate for selection purposes in a cane breeding and selection program.

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ANALYSIS OF SAMPLES FROM SUGARCANE EVALUATION TRIALS BY NEAR INFRA-RED SPECTROSCOPY USING A NEW AT-LINE, LARGE CASSETTE PRESENTATION MODULE

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ABSTRACT

Near infra-red spectroscopic (NIS) analysis of samples from sugarcane evaluation trials promises reductions in costs, immediacy of results, and minimal heavy metal use. This research aimed to establish the efficacy of presenting samples to a spectrophotometer using a specifically built at-line, large cassette module. Spectra were collected from fibrated samples from about 1,000 clonal plots, from nine replicated trials in the Mossman to Tully region. Parallel routine laboratory analyses were conducted. Excellent calibrations were developed from these data for five major quality components. These calibrations were applied to spectra from a single trial in the set to determine the relative precision and ranking of predicted data. Near infra-red prediction was as precise as routine analyses for Brix and fibre, but less precise for moisture and pol. Residuals for some components had a genetical basis. This was of little consequence as clonal ranking was altered minimally. Analysis with NIS using the large cassette module offers a significant development to replace routine laboratory analyses of samples from sugarcane improvement programs.

INTRODUCTION

Near infra-red spectroscopy (**NIS**) increasingly has been adopted to provide precise, rapid analyses of a diverse range of materials in agriculture and industry. Application of **NIS** until recently has been confined largely to dried or low moisture products. Problems unique to NIS analysis of high moisture materials, such as millable sugarcane stalks, have been considered (Berding & Brotherton 1996). Recent research at the Bureau of Experiment Stations' (BSES) Meringa Experiment Station (Berding & Brotherton 1995, 1996; Brotherton & Berding 1995) has demonstrated the successful application of **NIS** to the analysis of clean, fibrated sugarcane, a sawdust-like material of 65 - 70% moisture. In that research, samples were presented to the spectrophotometer for scanning in relatively small cells, with multiple cells required because of sample heterogeneity. This was a labour intensive operation unsuited to extensive plant improvement operations. In this paper, we present preliminary results from research that demonstrates the efficacy of **NIS** analysis of clean, fibrated samples from sugarcane evaluation trials by presentation of a much larger sample in a large cassette module, a specifically developed at-line device.

MATERIALS AND METHODS

Samples

Routine plot samples for quality component analyses were taken at harvest from clonal evaluation trials in the Mossman to Tully region in the period 2 October - 20 November 1995. The trials were Stage 4 yield observation trials at BSES Meringa (3) and Tully (1), a Stage 5 sub-station yield trial from the Mulgrave Mill area, and Stage 6 replicated variety trials from the Mossman (2) and Babinda (2) Mill areas. The trials sampled a diverse range of environments, crop classes, and crop conditions. Samples were prepared, fibrated, and handled as described previously (Berding & Brotherton 1996).

Routine laboratory analyses (**RLA**) were conducted essentially as described in Brotherton & Berding (1995). Fibre and moisture analyses were duplicated for most plot samples. Duplicate juice samples, obtained by hydraulically pressing two sub-samples of fibrated cane from each plot, were analyzed for Brix and polarization reading. Duplicates for Brix, fibre, and pol reading were averaged for calculation of a single commercial cane sugar (CCS) value per plot. In this report, only Brix, CCS, fibre, moisture, and polarization reading are discussed.

Instrumentation

The large cassette module consists of three stations arranged along an aluminium beam: loading, scanning, and unloading. A bottomless cassette 80 x 80 x 1000 mm is placed on horizontal tracks positioned above the beam, with the cassette's lower edge just clearing the beam. Fibrated cane is loaded into the cassette via a hopper. The upper surface of the fibrated cane in the cassette is roughly hand-smoothed, and the hopper cleaned and removed from above the cassette. The contents of the cassette are compressed by an automatically operated, pneumatically loaded plate 80 x 1000 mm exerting a surface pressure of 14 kPa. The cassette is moved from the loading to the scanning station by endless chain. At the scanning station, the cassette stops with the forward portion of the cassette positioned in a light proof shroud. This protects a NIRSystems Inc. (MD) remote reflectance probe, mounted underneath the beam, and scanning upwards. This probe receives diffracted radiation (400 - 2500 nm) from a NIRSystems 6500 spectrophotometer via a fibre-optic link, and is protected underneath a NIS-grade quartz window mounted flush with the top of the beam. Once sample identification is entered into software controlling the spectrophotometer operation, an endless chain moves the cassette of fibrated cane across the sensor at a specified speed. This is determined by the number of spectral scans required within the 1000 mm sample. The cassette is automatically unloaded by bottom dumping on exit from the sensor shroud. Scanning of a ceramic spectral reference built into the probe follows, and when complete, the next prepared cassette waiting at the loading station automatically advances to the scanning station.

The NIRS 3 software package (InfraSoft International, PA) was used for instrument maintenance, spectral data collection and management, and calibration development. The SCAN module of this was configured so that initially the only usable format was 47 sample scans followed by 47 reference scans. Software modification allowed use of a 96:32 format for the last three trials. Processing each cassette required 2.3 min, giving a throughput of 25/h. Duplicate spectra from 400 - 2200 nm were obtained by scanning two sub-samples for most samples. A prediction using a preliminary calibration was made as each spectrum was processed. An additional spectrum was collected for any sample giving a significant global 'H' statistic for one or both spectra.

Statistical analyses

Samples from the 1097 clonal plots resulted in data from 2038 **RLA** for each component and 1874 spectra. Elimination of aberrant **RLA** and/or spectral data produced a final data set of 1796 spectra with complete **RLA** data. Calibrations were performed in the regions 1100 -1414 nm and 1500 - 1874 nm, using data collected at a 2 nm gap. Calibration development used modified partial least squares regression and cross validation techniques. Spectral data of 4 nm gap and 4 nm smoothing were subjected to first derivative math treatment, i.e. 1,4,4 data were used. Standard normal variate and detrend scatter corrections were used. Equations from this were applied to duplicate spectral data from the Stage 4 trial from BSES Tully for a test of prediction. This trial was the most diverse in environment and crop condition of the nine used. Any statistical analyses performed outside NIRS 3, e.g. sub-sampling analyses of variance, were performed with MSTAT-C (MSU, MN).

RESULTS AND DISCUSSION

Ranges and means for all components in the combined **RLA** data set were acceptable for the lateness in the season when this research was

conducted (Table 1). All RLA data were highly precise (data not shown). Calibration equations contained 11-14 terms, but cannot be considered exhaustively optimized, as this is beyond this preliminary report. Standard error of calibration and standard error of cross validation values were close. The R^2 values (0.96 - 0.99) were very high (Table 1).

Table 1 *Summary statistics from routine laboratory analyses for five components of samples of clean, fibrated sugarcane (n = 1796) from a combined population drawn from nine clonal evaluation trials, and cross-validation statistics from calibration development using modified partial least squares regression techniques on near infra-red spectral data (1100 -1414 nm and 1500 - 1897 nm) from these samples.*

Measure[1]	Brix (g/kg)	CCS (g/kg)	Fibre (g/kg)	Moisture (g/kg)	Pol reading (°Z)
No. samples	1721	1723	1736	1737	1726
Mean	221.3	157.4	131.6	680.2	85.4
Minimum	103.9	37.1	85.7	622.7	25.5
Maximum	261.6	186.8	182.1	824.8	101.0
SD	16.97	16.70	15.45	23.16	8.74
No. terms	11		13	13	13
SEC	1.75	3.16	3.24	2.18	1.09
R^2	0.99	0.96	0.96	0.99	0.99
SECV	1.78	3.25	3.31	2.24	1.11

[1] SD = standard deviation; SEC = standard error of calibration; R^2 = multiple coefficient of determination; SECV = standard error of cross validation.

Prediction statistics for application of the calibration equations to Tully spectral data (Table 2) showed minor bias for all components, but none was of concern. Skew, indicated by deviation of the slope (b) from 1.0, for Brix, CCS, and fibre approached a level of concern. Again, R^2 values (0.96 - 0.99) were very high for all components except fibre (0.90).

Table 2 *Prediction statistics, from 336 spectra from the Tully trial, for five components of clean, fibrated sugarcane using near infra-red spectroscopic (NIS) calibration equations developed from spectra (n = 1,796) from a combined population of samples from nine clonal evaluation trials.*

Measure[1]	Brix (g/kg)	CCS (g/kg)	Fibre (g/kg)	Moisture (g/kg)	Pol reading (°Z)
RLA mean	211.9	149.3	125.6	692.2	80.6
NIS mean	212.2	151.0	123.8	693.1	81.3
SEP	2.12	4.07	4.78	2.54	1.58
Bias (a)	-0.26	-1.68	1.84	-0.90	-0.69
Slope (b)	0.95	1.03	0.95	1.00	0.98
R^2	0.99	0.96	0.90	0.99	0.97

[1] RLA = routine laboratory analysis; SEP = standard error of prediction; R^2 = multiple coefficient of determination.

Table 3 *Minimum and maximum values for duplicate determinations by routine laboratory analysis (RLA) and duplicate predictions by near infra-red spectroscopy (NIS) for five components of clean, fibrated samples from 58 replicated, sugarcane clones, together with estimates of sub-sampling standard deviation (d_i-precision) from sub-sampling analyses of variance of data from both analytical techniques, and their ratio.*

Analytical technique	Measure	Brix (g/kg)	CCS (g/kg)	Fibre (g/kg)	Moisture (g/kg)	Pol reading (°Z)
RLA determined	Minimum	101.0	37.1	84.4	646.9	24.5
	Maximum	238.0	177.5	171.0	825.3	94.7
	σ_i	1.397		2.009	0.847	0.387
NIS predicted	Minimum	101.5	37.7	83.4	647.1	23.8
	Maximum	239.4	178.1	170.5	823.6	94.5
	σ_i	1.068	1.927	1.989	1.727	0.675
	$\sigma_{NIS}/\sigma_{RLA}$	0.764	-	0.990	2.039	1.744

The minimum and maximum component values for the data sub-set from the Tully trial for which duplicate RLA and duplicate NIS prediction data were available, again showed minor bias for all components (Table 3). The precision for prediction of Brix and fibre by NIS was about equal to that obtainable by RLA determination, but was reduced by a factor of about 2.0 for moisture and pol reading (Table 3). This value is comparable to the error multiplier for NIS prediction of components of clean, fibrated cane determined by Brotherton & Berding (1995).

Analyses of variance of residual values (RLA determined minus NIS predicted) revealed highly significant clones terms for CCS and pol reading (Table 4). This supports earlier observations (Berding & Brotherton 1995) that some residual values for all components of clean, fibrated sugarcane except moisture are genetically based. Spearman rank order correlations between RLA-determined and NIS-predicted values for clones for Brix, CCS, fibre, moisture, and pol reading for the data used in analyses reported in Table 4 (n = 232) were 0.98, 0.95, 0.94, 0.99, and 0.97, respectively.

Table 4 *Mean squares for clones and error from analyses of variance of residual values (routine laboratory analysis minus near infra-red spectroscopic predicted) for five components of clean, fibrated samples from 58 replicated sugarcane clones. [**P<0.01]*

Mean square	Brix (g/kg)	CCS (g/kg)	Fibre (g/kg)	Moisture (g/kg)	Pol reading (°z)
Clones	9.86	31.26*	36.75	11.01	4.54*
Error	8.30	11.68	29.85	7.82	2.129

CONCLUSIONS

Results from NIS calibrations for components of clean, fibrated sugarcane developed from spectra collected with the large cassette module, an at-line sample presentation device, were excellent. This development takes NIS analysis of clean, fibrated sugarcane from a R&D basis to a high-throughput routine laboratory application for clonal evaluation. Precision of NIS prediction of moisture and pol reading was half that obtainable with traditional RLA, but is sufficient to allow detection of outliers in NIS-predicted values that have a genetical basis. This loss of accuracy does not detract from the use of NIS-prediction values for clonal evaluation as Spearman rank correlations between RLA-determined and NIS-predicted data were very high. Additionally, NIS analysis significantly reduces the number and qualification of personnel required, gives immediate availability of results, and eliminates minimal heavy metal waste from juice clarification used in

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MOLECULAR MARKERS: USEFUL TOOLS FOR SUGARCANE BREEDERS

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ABSTRACT

Simple diagnostic molecular markers were developed for sugarcane in order to enable efficient technology transfer to sugarcane breeders. A simple DNA extraction procedure was adapted to sugarcane, allowing processing of numerous samples when bulk analysis is required. An example of the efficiency of RAPD markers to fingerprint sugarcane varieties is shown, confirming the identity of a widely grown unknown clone ("Abergowrie" seedling). Simple molecular markers were also developed in order to allow a rapid screening of Saccharum x Erianthus hybrids in introgression programs. A list of RAPD primers that can be potentially used for such a screening is given.

INTRODUCTION

As in many crop species, sugarcane breeders have improved sugarcane varieties by performing selection on the basis of their phenotypes. However, the expression of a phenotype, although directly dependent on the genetic composition of the plant, is highly subject to environmental effects. Since the discovery of molecular marker technology (Beckman & Soller 1983), breeders have been provided with a means of assessing directly the genotype of their plants, as a complement to agronomical and morphological characterisation. Fingerprinting genotypes is one of the major issues breeders have to deal with. As demonstrated by their increasing use in human forensics, molecular markers have rapidly proven indispensable to ascertain identity and parentage. Molecular marker technology can be an expensive and complex process that is accessible only to highly skilled molecular biologists. However, with the advent of techniques such as the polymerase chain reaction (PCR), a range of simple tools has been made available that can be used routinely "in the field" with a minimum of molecular skills and lab equipment. As part of CSIRO's effort to develop molecular markers for sugarcane, we have adapted and simplified protocols and identified molecular markers that could be used as simple and reliable fingerprinting tools. In this paper, we describe results obtained by fingerprinting sugarcane clones and assessing the legitimacy of progeny in crosses by using simple diagnostic molecular markers.

RAPID TOOLS FOR SUGARCANE BREEDERS

A rapid DNA extraction protocol for sugarcane

Background
Classic DNA extraction procedures are time consuming and allow only a limited number of plant samples to be processed on a daily basis. A simplified DNA extraction protocol was needed, fulfilling the following requirements: extraction of DNA of good quality suitable for PCR analysis, from fresh or freeze-dried leaf material.

Methodology
A rapid DNA extraction protocol was developed for fresh and freeze-dried leaves of sugarcane, based on the method of Chalmers et al (1992). A small amount of leaf material (10 mg) was ground using a disposable Eppendorf ™ grinder in an Eppendorf tube containing 200ul of extraction buffer (200mM Tris HCl pH 7, 250 raM NaCl, 25mM EDTA, 0.5% SDS, 1OmM Mercaptoethanol). After vortexing for 5 seconds, the sample was centrifuged at 10,000 rpm for 1 minute. The supernatant was incubated at room temperature for 30 minutes in the presence of 40 (ig of RNase A. After dilution by adding 300 UL of distilled H2O, the sample was extracted with 500 uL of PCIA A (Phenol : Chloroform: Isoamyl alcohol 25:24:1). The supernatant was retained and extracted with 500 uL of C1AA (Chloroform: Isoamyl alcohol 24:1). The DNA was then precipitated by adding 1 ml of absolute Ethanol, centrifuged at 10,000 rpm for 6 minutes, and the resulting DNA pellet vacuum dried and resuspended in 50 UL of TE buffer.

Results
DNA yields ranging from 2 to 10 ng/ul were obtained. The quality of the DNA was checked on an agarose gel, and found to be suitable for PCR analysis. For PCR reactions, 2.5 to 4 uL of the DNA solution can be used. The 5s primers (D'Hont et al 1995) were used to assess the quality of the DNA for PCR amplification. Of the 200 individuals extracted with this method, 97% of the samples gave a PCR amplification using these primers (Burner et al 1995). This method allows a rapid and bulk (100 individuals can be processed in a day) extraction of good quality DNA that can be used for PCR analysis, from small amounts of fresh or freeze-dried sugarcane leaf material.

Fingerprinting sugarcane clones with RAPD markers

Background
The RAPD method (Welsh & McClelland 1990, Williams et al 1990) combines the PCR technique with the use of primers of arbitrary sequences in order to amplify different random loci from any genome. The amplification pattern resulting from RAPD analysis reveals a high number of bands, allowing this technique to be used as a powerful fingerprinting tool. RAPDs were used to determine the identity of the "Abergowrie" seedling, an unknown clone widely grown in the Herbert region in Australia.

Methodology
Leaf material from the "Abergowrie" seedling and Q96 were supplied by Dr M. Christopher and Dr N. Berding (BSES - Australia). DNA extraction was performed according to Hoisington (1992), as the rapid DNA extraction protocol described above was not developed at the time of the study, and RAPD analysis as described by Tao et al (1993) using Operon primers.

Results
A previous diversity study on 42 sugarcane varieties (McIntyre et al 1995) from a CSR collection used 40 RAPD primers. It can be shown that 15 randomly selected RAPD markers (e.g. "bands") are sufficient to give 100% fingerprinting of the 42 clones studied, corresponding to 4-5 RAPD primers (CL McIntyre, unpublished data). A similar resolution was described by Harvey et al (1994). On the basis of the previously mentioned results, 9 different RAPD primers (AA3, AN1, AN5, AN7, AN14, AN15, AN18, C12) were used, giving 78 different markers, to show that the "Abergowrie" seedling patterns were always identical to the Q96 sugarcane variety. Figure 1 shows an example of the patterns obtained using primer C12. This result confirms morphological evidence suggesting that the "Abergowrie" seedling is Q 96 or closely related to it.

RAPD markers to identify hybrids in Saccharum X Erianthus crosses

Background
Modern breeding programs in Australia are incorporating genetic material from a related genus (Erianthus sect. Ripidium) into sugarcane, to introduce improved ratooning and vigour, drought and flooding tolerance, and Pachymetra chaunoriza resistance. However, these intergeneric crosses are difficult to perform and are characterised by high levels of selfing of the female Saccharum parent. In order to detect true hybrids from selfs at an early stage, molecular markers were developed that were specific for the Erianthus genus. Our aim was to develop markers that could be used by breeders themselves to perform an easy screening of the hybrids.

RFLP markers specific for the Erianthus genus have previously been identified (D'Hont et al 1995, Besse et al 1996). However, RFLP

AS   Q96   Q117

Fig. 1 *RAPD primer C12 showing pattern identity between "Abergowrie" seedling (AS) and the variety Q96, as opposed to Q1 17.*

technology is time consuming and requires large amounts of DNA and leaf material. PCR markers, particularly the amplification of 5s spacer gene, have already proved successful in assessing hybrids in *Saccharum* x *Erianthus* population (D'Hont et al 1995). However, this marker only tags a limited number of *Erianthus* chromosomes. More numerous markers are thus needed in order to assess accurately full *Saccharum* x *Erianthus* hybrids, as chromosome elimination has been noticed in such crosses (D'Hont etal 1995).

Methodology

DNA samples from the parental clones Bamboo Cristalina (*S. officinarum*) and IJ76-422 (*E. arundinaceus*), and from 1011 progeny of a Bamboo Cristalina x IJ76-422 cross were kindly supplied by Drs. W. Burnquist and E. Ulian (Copersucar, Brazil). PCR amplification of the 5s ribosomal DNA spacer was performed as described by D'Hont et al (1995) and RAPD analysis as described by Tao et al (1993).

Results

Amplification of the 5s spacer gene (Fig. 2A) revealed a band of approximately 230 bp in Bamboo Cristalina and one of approximately 400 bp in IK 76-422. as expected from previous work involving S. *officinarum* and *E. arundinaceus* genotypes (D'Hont et al 1995). Hybrid patterns, exhibiting both bands, were revealed in 6 of 10 progeny (number 1, 4. 5. 8, 9, 10) (Fig.2A).

As part of a preliminary study, twenty RAPD primers (Operon primers AN 1-20) were screened between *E. elephantinus* (SES 305) and the sugarcane variety Q117. Ten of them revealed *Erianthus* diagnostic bands (AN 1, 5, 6, 7, 8, 10, 15. 16, 18 and 19) that can be used to assess true hybrids in *Saccharum* x *Erianthus* crosses (CL Mclntyre, unpublished data). Operon primers C12, C13. and C19, subsequently screened, also revealed *Erianthus* specific patterns. Although these primers were screened on two particular genotypes, they have proven to be useful for assessing hybrids from any *Saccharum* x *Erianthus* cross (Fig. 2A). Primers C12, C13, C19, and AN15 were used to screen the Bamboo Cristalina x IK 76-422 cross. Results (Fig. 2B, primer C19) showed that 6 hybrid individuals were present in the 10 progeny. These individuals are identical to the ones revealed using the 5 s spacer PCR marker.

As chromosome loss is suspected during intergeneric crosses (D'Hont et al 1995) many markers are needed to ensure that an individual detected as non hybrid using one marker does not reflect the loss of a chromosome carrying the particular marker or allele. Complementary to the PCR amplification of 5s ribosomal RNA spacer, RAPD markers can provide breeders with a more complete set of PCR-based markers for the assessment of hybrids in *Saccharum* x *Erianthus* introgression populations.

CONCLUSION

The present results demonstrate the usefulness of molecular markers as simple molecular tools for breeders. A range of markers is now available to allow fingerprinting of varieties to be performed. This enables various issues such as mislabelling identification, classification of unknown accessions, and cross verification to be investigated in sugarcane.
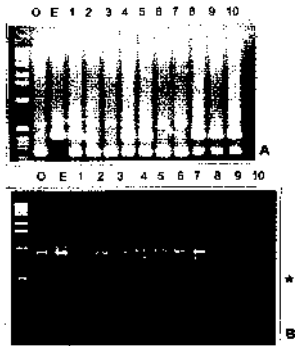
Fig. 2 *Analysis of 10 11 progeny of a cross between S. officinarum (O) Bamboo-Christalina x E. arundinaceus (E) IK 76-422*
A: with 5s PCR primers, B: with RAPD primer CI9
* indicates E. arundinaceus specific RAPD band

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ISOLATION OF AN *ERIANTHUS* SECT. *RIPIDIUM* SPECIFIC RIBOSOMAL DNA SPACER FRAGMENT AND ITS USEFULNESS FOR STUDYING *SACCHARUM* X *ERIANTHUS* INTROGRESSION POPULATIONS

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ABSTRACT

As part of an effort to isolate repetitive sequences specific for Erianthus *sect* Ripidium *that would assist the characterisation of the genetic composition of* Saccharum *x* Erianthus *hybrids, a 2 kb fragment was isolated which corresponds to part of the ribosomal DNA spacer. The occurrence of this fragment in different* Erianthus *sect.* Ripidium *species, and its absence from* Saccharum *species was demonstrated by Southern hybridisation experiments. Using this fragment as a probe, a dot blot technique enabled a rapid and reliable screening of true hybrids in a* S. officinarum *(Bamboo Cristalina) x* E. arundinaceus *(IK-76-422) cross. As the current status of the occurrence of chromosome loss in such intergeneric crosses is not clear, specific chromosome tagging using repetitive sequences will be useful in assessing such cytogenetic changes. The present results show that at least one chromosome set carrying the rDNA genes is transferred to the hybrids. The potential usefulness of* Erianthus - *specific repetitive sequences to introgression programs is discussed.*

INTRODUCTION

Introgression of related species (such as *S. spontaneum*) into noble sugarcane *(S. officinarum)* has been largely used as a way of creating new sugarcane varieties. Attempts have also been made to cross sugarcane with other genera of the *"Saccharum* complex", a rich and variable primary genetic resource pool (Harlan & De Wet 1971). In addition to the genus *Saccharum*, this complex includes four other genera *(Erianthus* sect. *Ripidium*, *Miscanthus* sect. *Diandra*, *Narenga*, *Sclerostachya)* believed to have contributed to the origin of sugarcane (Daniels & Roach 1987). Wider crosses using secondary and tertiary (sorghum, maize) genetic pools have been attempted (reviewed by Sreenivasan et al 1987). Most of these intergeneric crosses were performed as a means of unravelling sugarcane's complex cytogenetic features. Recently, considerable progress has been made towards the incorporation of genetic material from the genus *Erianthus* sect. *Ripidium* into sugarcane for improvement and breeding purposes. In Australia, *Erianthus* sect. *Ripidium* species, and *E. arundinaceus* in particular, are being used as a source of variation to improve ratooning ability and vigour, drought and flooding resistance, and introduce *Pachymetra* resistance. Sugarcane's complex cytogenetic structure is a major obstacle to the success of these introgression programs. Irregular meioses and aneuploidy are frequent in interspecific *Saccharum* crosses (Sreenivasan et al 1987). Similarly, intergeneric crosses between *Saccharum* and *Erianthus* may involve chromosome losses.

A project was initiated to isolate repetitive sequences that would provide breeders with markers to characterise the cytogenetic structure of intergeneric hybrids. Repetitive sequences are highly abundant as they represent an average of 80% of the total genome content in plant species (Flavell et al 1974). Repetitive sequences specific to a species, combined with *in situ* hybridisation techniques, have been largely used to identify chromosome fragments of one species introgressed into the genome of a related species. Examples include the detection of rye chromatin in wheat-rye addition lines (Lapitan et al 1986, McIntyre et al 1990) or *Lophopyrum* chromosomes in *Lophopyrum* substitution lines in wheat (Zhang & Dvorak 1990). Genomic *in situ* hybridisation (GISH) can also be used for the same purpose, as shown in potato (+) tomato fusion hybrid backcross progeny (Jacobsen et al 1986) or in a natural grass intergeneric hybrid (Bailey et al 1993). GISH technology has been successfully applied to study *Saccharum* x *Erianthus* hybrids (D'Hont et al 1995). However, a significant advantage of the use of repetitive sequences as compared with the GISH technique is the possibility of chromosome identification (Rayburn & Gill 1987). Such sequences could prove useful in determining possible chromosome losses in intergeneric hybrids.

In this paper, we describe the isolation of a repetitive sequence specific for the *Erianthus* sect. *Ripidium* genus, its preliminary characterisation as part of the spacer region of the ribosomal DNA genes, and its use to assist the characterisation of *Saccharum* x *Erianthus* hybrids.

PLANT MATERIAL

DNA samples from the two parents and 1011 progeny of a cross between *S. officinarum* Bamboo Cristalina (2n=80) and *E. arundinaceus* IK76-422 (2n=60) were kindly provided by W. Burnquist and E. Ulian (Copersucar Brazil). The clones IK 76-48 (*E. arundinaceus*), Black Cheribon (*S. officinarum*) and Mandalay (*S. spontaneum*) came from the BSES collection (courtesy of N. Berding. BSES Meringa. Australia). The *Erianthus* and *Saccharum* collections used for Southern analysis were those previously described (Besse et al 1996).

METHODS

DNA was extracted from the clones IK76-422. Black Cheribon, and Mandalay, and digested using *Mbo*I as previously described (Besse et al 1996). Comparison of the digestion smears showed the occurrence of a prominent band for *Erianthus* at approximately 2kb not visible in either *S. officinarum* or *S. spontaneum* species. This band was excised from the gel and the DNA fragments purified (Bresa-clean™.Bresatec, Australia). An aliquot was retained for further cloning into the BamHl sites of the pUC18 plasmid. The remainder of the DNA solution was used for hybridisation experiments. Although it represents a mixture of DNA fragments, the purified DNA solution contains predominantly the 2 kb repetitive fragment and will subsequently be referred to as such. Under stringent conditions and with short exposure times (4 hours), only bands homologous to this repetitive sequence will be evident by hybridisation, this enabling preliminary characterisation.

Southern blots analysis was performed on DNA isolated from representatives of the *Erianthus* and *Saccharum* genera digested with *Scal*, *Sacl* and *Bam*Hl (Besse et al 1996). Dot blots consisted of 2ug of DNA (2 uL) from the progeny and the two parents of the Bamboo Cristalina x IK76-422 cross. The hybridisation conditions used in these experiments have been described previously (Besse et al 1996)

RESULTS

Characterisation of the fragment

Figure 1 demonstrates that, under the high stringency conditions used in the analysis, the 2kb fragment is *Erianthus*-specific as it does not hybridise significantly to any *Saccharum* individuals. However, this fragment does hybridise to many different *Erianthus* sect *Ripidium* species, including *E. arundinaceus*, *E. elephantinus*, *E. ravennae*, *E. procerus*, and to the two New World *Erianthus* species. *E. longisetosus* and *E. rufipilus*, already demonstrated to be part of sect. *Ripidium* (Besse et al 1996). No significant hybridisation of the 2kb fragment was

Fig. 1 DNA samples from various clones digested with Sca/ and probed with the 2kb purified fragment.

[Lanes 1 to 15 are individual clones from the species: (1-3) E. arundinaceus, (4) E. elephantinus, (5) Saccharum sp., (6) E. procerus, (7) E. ravennae. (8) E. longisetosus, (9) E. rufipilus. (10) E. trinii, (11) E. brevibardis. (12-13) S. spontaneum. (14-15) S. officinarum.]

Fig. 3 Restriction map of an Erianthus sect. Ripidium ribosomal DNA unit (from Besse et al. 1996) showing the possible locations of the cloned 2kb fragment.

[Restriction sites are BglII (Bg). BstEII (Bs), BamHI (B), EcoRI (E), EcoRV (V), MboI (M), SacI (S), XbaI (X).]

detected to DNA isolated from the New World species E. brevibardis and E. trinii. nor with S. spontaneum and S. officinarum. This isolated 2kb fragment is thus Erianthus sect. Ripidium-specific.

Subsequent hybridisation of the 2kb fragment to DNA isolated from Erianthus sect. Ripidium representatives and digested with BamHI, SacI, and BamHI x SacI, showed that the size of the hybridising fragments were identical to that obtained using a wheat ribosomal DNA probe (Besse et al 1996. Fig. 2). This 2kb fragment thus corresponds to part of the ribosomal DNA unit. Moreover, using the Erianthus ribosomal DNA restriction map previously developed (Besse et al 1996). die 2kb fragment can be located between the two BamHI sites surrounding a 2.7kb spacer fragment. As the MboI restriction site sequence (GATC) is included within the BamHI restriction site sequence (GGATCC), the three possible deduced locations for the isolated fragment are depicted

Species and genus specificity of ribosomal spacer fragments has already been demonstrated in a large number of plant species (Delcasso Tremoussaygue et al 1988; Torres et al 1989) due to their high sequence variation rates. It appears that Erianthus is not an exception to this rule.

The 2kb ribosomal DNA fragment isolated is currently being cloned, and will be subsequently be sequenced.

Use in Saccharum x Erianthus introgression population study

Based on the Erianthus specificity of the fragment, and its repetitive nature, a dot blot procedure was developed that allows a rapid screening

of Saccharum x Erianthus hybrid progeny versus Saccharum selfs. Results obtained (Fig. 4) show that hybrid clones can be identified using this method, confirming previous result obtained using different markers (Besse & McIntyre 1996). The other clones, most probably resulting from selfing of the female Saccharum parent (Besse & McIntyre 1996) do not hybridise with the Erianthus sect Ripidium rDNA spacer fragment.

Previous work using ribosomal DNA as a probe for in situ hybridisation revealed that 6 sites of hybridisation were present in E. arundinaceus (2n=60) (D'Hont et al 1995; Jenkin et al 1995), suggesting a basic number of 10 in this species. The results described above demonstrate that at least one of the 6 homologous chromosomes carrying the ribosomal DNA genes is being transmitted to the hybrid progeny.

DISCUSSION

Chromosome loss is suspected during intergeneric crosses. Analysis of one hybrid from a cross between BNS 3066 (S. offvinarum, 2n=80) and IK76-48 (E. arundinaceus 2n=60) showed that only 25 to 26 E. arundinaceus chromosomes, rather than 30, were transmitted in this particular hybrid (D'Hont et al 1995). This shows that chromosome loss is occurring. Similarly, Rumke (1934) reported that only 61-69 chromosomes were transmitted in a S. officinarum x E. arundinaceus cross. However, many authors report that the expected number of chromosomes were detected, particularly in the present cross (Burnquist. personal communication), as well as in a S. spontaneum (2n= 112) x E. ravennae (2n=20) cross (Janaki Ammal 1941) which gave Fl hybrids with 66 chromosomes, and in a S. officinarum (2n=80) x Erianthus

Fig. 2 BamHI digests of DNA isolated from Erianthus sect. Ripidium and Saccharum clones, hybridised with (A) the 2kb fragment (B) pTA71 wheat ribosomal DNA probe (B from Besse et al 1996)

E O 1 2 3 4 5 6 7 8 9 10

Fig. 4   *Dot blot hybridised with the 2kb fragment Individuals are (E)
IK 76-422, (O) Bamboo-Cristalina, (1-10) 11 progeny from a Bamboo-
Cristalina x IJ 76-422 cross (Copersucar, Brazil). Hybrid clones are
number 1, 4, 5, 8, 9, 10.*

(2n=40) cross (Rao et al 1963) which produced 2n=60 hybrids. The
situation regarding *Saccharum* x *Erianthus* crosses is therefore not clear,
demonstrating the need for sequences that will enable the tagging of
specific groups of homologous chromosomes in order to enable the
characterisation of possible chromosome losses.

The isolation of this *Erianthus*-specific ribosomal DNA spacer fragment
represents a first step in the isolation of repetitive sequences specific to
particular chromosome sets. As shown in rice, where a chromosome 5-
specific repetitive DNA sequence was isolated (Wang et al 1995), it
should be possible to isolate chromosome-specific repetitive DNA for
each chromosome in *Erianthus*. We are currently in the process of
isolating and characterising such sequences. Ultimately, these sequences
will be placed on linkage groups of the *Erianthus* linkage map, providing
a first bridge between physical and genetic maps in the *Saccharineae*
sub-tribe.

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PRELIMINARY GENOMIC MAPPING AND PHENOTYPIC ASSESSMENT OF A COMMERCIAL SUGARCANE CROSS (AA40)

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ABSTRACT

Genomic maps provide opportunities for determining linkage of DNA markers to phenotypic characteristics of interest. By following the inheritance of the markers, researchers are able to trace the inheritance ofparticular phenotypes. In sugarcane, segregating populations, suitable for DMA mapping are scarce, and when available, limited phenotypic information on the progeny exists. At the South African Sugar Association Experiment Station, researchers have been presented with an opportunity to map a commercial sugarcane cross AA40. This is being undertaken using various DNA-based methodologies such as PCR-RAPDs, RFLPs, AFLPs and microsatellite and telomere sequences. At the same time, extensive phenotypic screening is underway and as information becomes available, linkage between these data and the markers will be established. Preliminary investigations have shown that AA40 is suitable for use in such a mapping attempt as it already shows good segregation for various important traits and the parents of the cross are reasonably diverse in terms of their PCR-RAPD banding profiles.

INTRODUCTION

Genetic maps have been developed for numerous crop plants such as maize (Ajmone-Marsan el al 1994), eucalypts (Grattapaglia & Sederoff 1994), tomato (Foolad el al 1993). and sugarcane (da Silva el al 1995). in an attempt to identify as many DNA markers as possible and determine linkage between them and important phenotypic traits. The aim is to use these markers to follow the inheritance of particular characters and select new varieties with improved agronomic potential. One of the most important requirements for successful genomic mapping is the availability of accurate and extensive phenotypic data on the population being mapped (Landry 1993). However, in sugarcane, this kind of phenotypic information is lacking, with the result that only a few putative markers, linked to agronomically important characters, have been found (Msomi & Botha 1994).

In the Biotechnology Department at the South African Sugar Association Experiment Station, researchers have been presented with the unique opportunity to map a commercial sugarcane cross, AA40. This population has shown promise in preliminary observations where the progeny appear to segregate for a number of important phenotypic characteristics. As a result, in collaboration with the Plant Breeding Department, a project has been undertaken to map the entire, segregating population of AA40. including the parents N18 and CP57-614 and 150 progeny from the cross. All individuals have been planted out and will be carried through to variety trial stage in an effort to obtain extensive phenotypic data on the population. Concurrently, with the screening efforts by various departments, genetic mapping is under way based on PCR-RAPDs, RFLPs, AFLPs and microsatellite and telomere sequences.

MATERIALS AND METHODS

Phenotypic evaluation of the population

From the bulking programme conducted by the Plant Breeding Department, stalks from the single line stage have been harvested and assessed for measurable millroom characteristics such as sucrose, brix and fibre levels (Anonymous 1987). These results are being used to establish a database of phenotypic information on AA40.

DNA analysis

Extraction of genomic DNA

DNA was bulk-extracted from leaf roll tissue obtained from 6 stalks from 6 separate stools of each clone, according to the method of Dellaporta et al (1983). After spectrophotometric quantification of DNA, the concentration and purity of the samples was confirmed by electrophoresis through 1 % (w/v) agarose gels and staining with ethidium bromide.

PCR-RAPD analysis

Diluted DNA stocks (3 ng/µL) were used diluted in 15 - 24µL PCR reaction mixtures 'total DNA 15 - 24 ng), which were set up as described previously (Harvey et al 1994). Amplification conditions were as reported by Harvey et al. (1994). On completion of amplification, PCR products were electrophoresed through 2 % (w/v) agarose gels, stained with ethidium bromide and the gels scored for presence or absence of the amplified fragments.

RFLP studies

Probes have been obtained from the University of Missouri-Columbia and from the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD) in France (Table 1). These have been shown to map various sugarcane linkage groups (Lu et al 1994a,b) and, therefore, were considered ideal for use in preliminary screening of AA40.

These probes were random-primer labelled with [^{32}P]-dNTP using the Prime-It II kit (Stratagene) and hybridised to nylon membranes onto which 10-15µg of restricted genomic DNA had been blotted (Chomczynski & Mackey 1994). Use was made of various restriction enzymes for digestion of DNA (Lu et al 1994a,b). After hybridisation and washing of membranes (Rapid-Hyb system, Amersham), bands were visualised by autoradiography at -80°C.

Table 1 *Probes obtained for preliminary screening of AA40 from the University of Missouri-Columbia (UMC) and from the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD)*

Probe	Source	Sugarcane Linkage Group
umc44	UMC	*
umc107	UMC	*
umc4	UMC	*
umc85	UMC	*
umc49	UMC	*
umc39	UMC	*
umc108	UMC	*
umc54	UMC	*
SSCIR73	CIRAD	1
SSCIR251.172	CI RAD	2
SSCIR119.69	CIRAD	3
SSCIR101.77	CIRAD	4
SSCIR217	CIRAD	5
SSCIR230	CIRAD	6
SSCIR194	CIRAD	7
SSCIR92,105,86	CIRAD	8
SSCIR76	CIRAD	9
SSCIR83,190,188	CIRAD	10

* To be determined

AFLP investigations

An AFLP kit (AFLP Analysis System I. Gibco BRL) was used as per the manufacturers' instructions. In preliminary experiments 4 sets of

primer pairs were tested, namely E (EcoRI primer)-AGG with the following M (MseI) primers, M-CAC, M-CAT, M-CTA and M-CTG.

Microsatellite and Telomere PCR amplification

Previous studies have shown that various microsatellite and telomere sequences, used in specific PCR reactions, generate complex and highly polymorphic banding profiles in sugarcane (Harvey & Botha 1996). These sequences (Microsatellite - SAT1 (GATA)$_2$ (GACA), ,Telomere-TELFOR (TTTAGGG)$_2$ and TELREV (CCCTAAA)$_2$) were used, as described by Harvey and Botha (1996), in the mapping of AA40.

Data analysis

Polymorphisms within the mapping population of AA40 were scored for presence or absence across all the progeny and the parental varieties. This information was analysed for linkage to phenotypic traits using MAPMAKER software (Lander et al 1987).

RESULTS AND DISCUSSION

Phenotypic assessment of AA40

Preliminary screening of AA40 has shown good segregation within the population for numerous important phenotypic traits. As shown by Fig. 1 there are individuals clustering at both the low and high extremes for the fibre (Fig. 1a), brix % cane (Fig. 1b) and pol % cane (Fig. 1c) traits. This is an important pre-requisite for any mapping population in order to map polymorphisms between individuals at the extremes of a characteristic.

The phenotypic assessment of AA40 is a continual and on-going process and as more and more data become available they will be used in linkage analysis studies.

DNA markers

Thus far, DNA has been extracted from the parents and progeny of AA40. Initial PCR screening of the parental varieties N18 and CP57-614, with 7 random decamer primers, has shown a 22.5 % level of diversity in their genomes (Fig 2) This result was echoed closely by preliminary AFLP analysis, where 3 sets of primers generated 228 loci, of which 59 (25.9 %) were polymorphic (Fig 3)
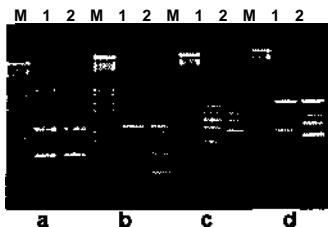
Fig. 2 *Agarose gels showing the RAPD banding profiles obtained for the parents of AA40, namely N18 (1) and CP57-614 (2). Lambda DNA - HindIII / EcoRI molecular weight markers are shown in lane M. [Panels a-d represent the banding profiles obtained with different random 10-mer primers]*

Fig. 1 *Bar chart showing the phenotypic ratings within AA40 for various important characteristics, (a) fibre, (b) brix % cane and (c) pol % cane. There are clones clustering at both the low and high extremes for all these traits.*

Fig. 3 *Representative portion of a silver-stained, denaturing polyacrylamide gel showing the AFLP bands resolved for CP57-614 (1) and N18 (2). [Panels a-c represent the profiles resolved with 3 sets of AFLP primers]*

The microsatellite and telomere primers similarly showed approximately 20 % genome diversity between N18 and CP57-614 (results not shown). Such a level of DNA diversity between parents of a mapping population is highly favourable as it increases the likelihood that progeny will segregate for important traits (Anderson et al 1992).

Preliminary screening of restriction enzymes for digestion of sugarcane genomic DNA has shown that HindIII and XbaI produce a reasonable smear of DNA fragments from high to low molecular weight (results not shown). It is likely that these enzymes will be used to screen the RFLP probes mentioned previously (Table **1**).

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APPLICATION OF THE PCR - RAPD METHODOLOGY TO SUGARCANE BREEDING AT THE SOUTH AFRICAN SUGAR ASSOCIATION EXPERIMENT STATION

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ABSTRACT

The biotechnology programme at the South African Sugar Association Experiment Station (SASEX) contributes to the production of new, improved sugarcane varieties indirectly, by developing technologies to aid conventional breeding and directly, by incorporating novel genes into existing varieties by genetic engineering. In both these areas use has been made of the Polymerase Chain Reaction Random Amplification of Polymorphic DNAs (PCR-RAPDs). PCR-RAPDs have been used to elucidate putative markers linked to phenotypic traits via a Bulk Segregation Analysis (BSA) and genealogical approach. In addition, this technology has been used to determine DNA diversity between various sugarcane varieties. In the production of plants by genetic engineering, PCR-RAPDs have shown that no somaclonal variation occurs in callus tissue maintained in culture over extended periods of time. Also, this methodology is being used to identify stem-preferential promoter sequences in an attempt to target transgene expression to this area of the sugarcane plant.

INTRODUCTION

In the 1994/95 growing season the sugar industry in South Africa produced over 1.6 Mt of cane which earned in excess of 2 billion Rand. In addition to making a significant contribution to the country's economy, this industry provides employment for almost 45000 growers, 96 % of whom are smallholder growers (producing up to 1600 t per

In several cane growing regions in the world, sugarcane growth is hampered by certain diseases and pests that severely reduce yield. These pathogens are often specific to different geographic locations, for example, the sugarcane borer *Eldana saccharina*, which in South Africa resulted in a 60 M Rand loss for the industry in 1994. Therefore, the need exists to develop sugarcane varieties that are resistant to these pests and diseases and able to thrive under local growing conditions.

The production of new, improved sugarcane varieties is the task of the South African Sugar Association Experiment Station (SASEX). Since 1993, SASEX has channelled resources and research into biotechnology in order to supplement the conventional breeding programme. The approach of the biotechnology programme is two-fold, firstly, to assist plant breeding by developing technologies which will aid and speed up existing breeding practices and secondly, to produce new varieties directly by genetic engineering. Progress has been made towards achieving these aims, particularly due to the application of the Polymerase Chain Reaction (PCR), Random Amplification of Polymorphic DNAs (RAPDs) methodology (Welsh & McClelland 1990; Williams etal 1990). This technique involves random amplification of genomic DNA in the presence of arbitrary oligonucleotide primers and requires no prior knowledge of genetic structure (Welsh & McClelland 1990; Williams et al 1990), making it ideal for use in a crop such as sugarcane, where relatively little is known about the genome.

This paper describes the adaptation of the PCR-RAPD methodology for use in our laboratories and its subsequent application in various research projects.

MATERIALS AND METHODS

DNA and RNA extraction

For PCR-RAPD analysis of mature sugarcane plants, DNA was extracted from the leaf roll using a protocol modified from Honeycutt et al (1992) and Harvey et al (1994).

DNA isolation from callus was achieved using the protocol of Dellaporta et al (1985).

DNA concentrations and purities were determined spectrophotometrically. In addition, aliquots of all DNA samples were electrophoresed on 1% (w/v) agarose gels at 5.6 V/cm, stained with ethidium bromide (0.5μg/mL), destained and the DNA concentration confirmed by comparing the intensity of band staining with that of known standards. Dilutions of the concentrated stocks (3 ng/uL) were then prepared for use in PCR reactions.

For RNA extraction, tissue (approximately 5 g) was ground in liquid nitrogen and transferred to a 50 mL tube. On addition to 10 mL denaturing solution (Chomczynski & Sacchi 1987 - plus 50μM aurin tricarboxylic acid [ATA]). After addition of 2 mL sodium acetate (pH 4.0), 10 mL phenol and 2 mL chloroform:isoamylalcohol (49:1), the mixture was homogenised for 2-3 min using an ultra turrax and stored on ice for 15 min. The sample was then centrifuged at 10000 g for 20 min at 4°C, the aqueous phase transferred to a fresh tube and RNA precipitated by the addition of 10 mL isopropanol. Following incubation of the extract at -20°C for 2 hours, RNA was pelleted by centrifugation at 10000 g for 20 to 30 min at 0°C. The pellet was resuspended in 2 mL 4 M LiCl, vortexed to remove polysaccharides and centrifuged again at 3000 g for 15 min at 4°C. After discarding the supernatant, the pellet was resuspended in 2 mL of DEPC treated water, to which 400μl 1 M sodium acetate (pH 4.0) was added together with 2.4 mL isopropanol. Samples were placed at -70°C for 15 min and then at -20°C for 2 hours, before being centrifuged at 10000 g for 20 min at 0°C. The pellet was dissolved in 1 mL of DEPC water, after which 100μL of 3 M sodium acetate and 2.75 mL of ice-cold 100 % ethanol were added. Extracts were incubated at -20°C and centrifuged at 10000 g for 30 min at 0°C. After washing with 70 % ethanol, samples were dried and dissolved in 500u M ATA. Following incubation at 65°C for 20 min samples were stored at -80°C. RNA was quantified spectrophotometrically and these results confirmed by electrophoresis of aliquots of the sample through 1.5 %(w/v), formaldehyde containing, agarose gels.

PCR amplification and visualisation of products

PCR amplification of genomic DNA was carried out in 15-24μ volumes containing 15-24 ng DNA, as described previously (Harvey et al 1994). Reverse Transcription PCR (RT-PCR) reaction mixtures (15μL) contained purified reverse transcription mixture (Maniatis et al 1989) in a 1 : 100 dilution, 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 3.8 mMMgCl₂, 0.13 mM each of dATP. dCTP, dTTP and dGTP, 0.2μM primer and 1 0 of Taq polymerase Stoffel fragment (Perkin Elmer).

For all samples the PCR amplification profile was as described by Harvey et al (1994). PCR products were visualised by electrophoresis through 2 % (w/v) agarose gels at 5.6 V/cm. After staining in ethidium bromide (1μg/mL) for 30 - 40 min and destaining for a further 20 - 30 min bands were viewed under UV light (300 nm) and results recorded using a gel documentation system.

% Fibre content

| 7-9.8 % | 9.9-10.6 % | 10.7-11.8 % | 11.9-15.6% |
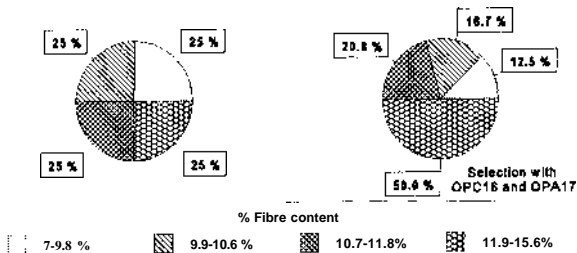
Fig 1 *Bar chart showing a simulated marker-based selection, on population AA157, with the RAPD markers OPA 17.1 and OPC16.1. Use of these markers resulted in carriage of fewer of the low fibre individuals and an overall enrichment for high fibre clones.*

Scoring of results

Samples were scored manually for the presence / absence of bands. These data were used as is, or entered into computer software packages for further analysis. For example, genetic diversity between various varieties was determined using the programme NTSYS-pc (Rohlf 1993) and linkage analysis was performed with Mapmaker software (Lander et al 1987).

RESULTS AND DISCUSSION

Development of PCR-RAPD technologies to aid conventional breeding

Identifying putative DNA markers

Linkage of RAPD markers to phenotypic traits of interest has been determined most successfully using pairs of backcross-derived near isogenic lines (NILs) (Martin et al 1991; Paran et al 1991). However, in sugarcane, the lack of NILs and complexity of the genome has necessitated the use of alternative approaches for identifying markers, such as use of Bulk Segregation Analysis or BSA (Michelmore et al 1991).

For the purpose of this study, the presence or absence of various RAPD polymorphisms was investigated in 80 progeny from a commercial sugarcane cross (AA157). This population was selected as it showed a normal distribution for the fibre phenotype, allowing 10 low and 10 high fibre individuals to be bulked for analysis (Msomi & Botha 1994). These bulks were amplified with 60 random primers. To date two fragments, found to be polymorphic between the bulks, have been linked to fibre at the 99 % confidence level (Mapmaker software, Lander et al 1987). In simulated marker-based selection with the AA157 population, use of these two markers (OPA-17.1 and OPC-16.1) in tandem, led to carriage of less than 10 % of the low fibre individuals and an overall enrichment for clones with a high fibre content (Fig.1).

A second approach for identifying markers has examined the potential for using a genealogy to trace the inheritance of putative RAPD markers. For this purpose two closely related commercial varieties, shown to differ in their response to sugarcane mosaic virus (SCMV), were chosen for analysis, namely N1 1 (resistant) and NCo376 (susceptible). These varieties were screened for polymorphisms using the PCR-RAPD technique .

A total of 100 random primers produced 1159 loci of which 10 were polymorphic. The primers producing these polymorphisms were used to amplify DNA from 19 varieties in the common genealogy of Ni1 and NCo376. Results indicated that certain of these PCR-RAPD fragments could be traced back across all seven generations screened, indicating that they are extremely stable (Fig. 2). In addition, these
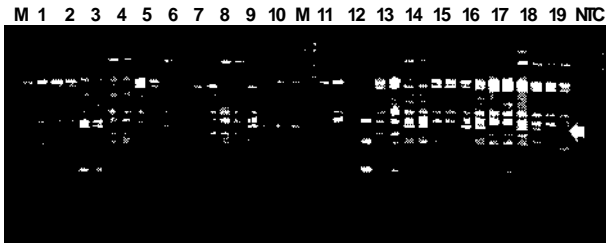
Fig 2 *Expression of a stable polymorphic RAPD fragment across the genealogy: result of screening for OC06₈₅₆ . Each variety was represented by two PCR-RAPD reactions conducted using template concentrations differing by an order of magnitude (24 and 2.4 ng DMA per reaction) and the respective products run in adjacent gel lanes (1-19). The varieties were then scored for the presence / absence of each polymorphic fragment. Fragment OC06₈₅₆ (arrow) was found to be clearly and unambiguously present or absent in all the varieties screened, independently of the template concentration (100% fidelity). Of the ten polymorphisms screened, two showed 100% fidelity and five showed 89% or greater fidelity. M =l- Eco RI + Hind III molecular weight markers. NTC = no template control.*

fragments could be used to trace parentage (Huckett & Botha 1995) and may be used in linkage analysis. At present the 19 varieties used for PCR analysis are being subjected to extensive glasshouse and field trials to determine their SCMV resistance phenotype and once these data are available, they will be analysed for possible co-segregation with the polymorphic RAPD fragments / putative markers.

Determination of DNA diversity between varieties
To improve varieties using classical breeding techniques, detailed characterisation of the germplasm is necessary to allow exploitation of available potential and prevent reduction in DNA diversity.

To determine the extent of DNA diversity within the South African sugarcane germplasm, PCR-RAPD analysis was conducted on a total of twenty six varieties. This included two S. *spontaneum* varieties, a single variety of S. *officinarum* and 23 S. *spontaneum* x S. *officinarum* hybrids (some of which are commercial varieties and others, ancestral varieties from early in the sugarcane genealogy). DNA extracted from these varieties was amplified in the presence of 15 random decamer primers, the varieties scored for the presence / absence of 160 loci (63 % polymorphic) and the data used to generate phenograms (Harvey & Botha 1996).

Results indicate that there has been a gradual decline in DNA diversity (84 % reduction) from the early inter-specific crosses to the commercial hybrids, probably as a result of the backcrossing and in-breeding strategies used in the previous 5 to 6 generations of sugarcane breeding. Based on these findings, a recommendation might be made to introgress more wild-type varieties into the breeding germplasm.

Use of PCR-RAPDs in the development of resources for genetic engineering
Minimising somaclonal variation in tissue culture systems
Efficient tissue culture procedures which ensure reliable production of large numbers of plants are a prerequisite for successful transformation studies. In addition, it is important that all regenerated plantlets are true clones of the donor plant. As a result, spontaneous genetic changes which have been shown to occur in culture systems (somaclonal variation) (Larkin & Scowcroft 1981) must be minimised.

In the Biotechnology programme at SASEX, the PCR-RAPD technology has been used to determine the extent of genetic change which occurs in callus over an extended maintenance period.

Sugarcane callus was cultured on a callus induction medium containing various levels of the hormone 2,4 -dichlorophenoxyacetic acid (1 mg/L, 3 mg/L or 5 mg/L 2,4-D). Callus samples for RAPD analysis were removed from each hormone treatment at monthly subculture intervals, for a period of 12 months and DNA extracted. PCR-RAPD analysis was carried out using 15 random decamer primers. Visual comparison of banding profiles suggests that somaclonal variation, in callus maintained for 12 months in culture, is relatively low (less than 10 % polymorphisms in the 147 bands scored), regardless of the concentration of growth hormone in the medium. In addition, preliminary phenotypic assessment of plantlets regenerated from callus over the 12 month period has shown no significant change in measurable millroom characteristics (fibre, brix and sucrose levels) or gross plant morphology, corresponding favourably with the RAPD results.

Identifying promoters for targeted expression of inserted transgenes
In sugarcane one of the most important areas for transgene expression is the stem, because it is there that sucrose is accumulated and the stalk borer *Eldana saccharina* makes its entry. The isolation of tissue-specific

promoters requires identification of gene sequences that are expressed in a tissue-specific manner. An RT-PCR approach was used to compare expressed sequences in 4 sugarcane tissues (leaf, leafroll, young and mature stem). mRNA isolated from these tissues was reverse transcribed and the cDNA amplified with random decamer primers (PCR-RAPDs). After separation of the amplified products on agarose gels the banding profiles obtained from the various tissues were compared. Fragments which appeared to be unique to the stem were isolated and characterised. So far, out of a total of 1767 fragments amplified using 120 primers, 4 appear to be stem-preferential. Further characterisation of these fragments is in progress.

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3.2 Growth physiology and sucrose metabolism

AEROPONIC CULTURE AS A TECHNIQUE TO STUDY SUGARCANE ROOT GROWTH AND ACTIVITY

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ABSTRACT

There currently is a lack of information on the important relationship between root function and tops growth for sugarcane. To rectify this a simple aeroponic facility was constructed and tested using three sugarcane cultivars known to have different shoot:root ratios. Significant cultivar effects on plant shoot and root parameters in aeroponic culture were similar to those observed for field-grown plants. Advantages of aeroponic culture include an ability to observe and control root size and activity and to directly determine root effects on above ground productivity.

INTRODUCTION

Few detailed studies have been conducted into sugarcane root growth and activity, or into the relationship between roots and above ground productivity. Some reasons for the lack of research on sugarcane root systems include difficulties in observing or sampling root systems over time, and inability in determining the activity of the observed roots. Due to root system variability, large numbers of samples are required to describe full profile root distribution for crops (Upchurch 1987). For a fourth ratoon Q122 cultivar, sample numbers for root description were found to exceed practical limits (Reghenzani 1993b), and a sub-sampling strategy was suggested. While both approaches above provide an estimate of root system distribution or relative size, neither was entirely satisfactory. Cost was high, no data were provided on root system activity and limited information was provided on the relationship between roots and above ground productivity.

An effective root system is required for the absorption of water and nutrients. Particularly for sugarcane, due to large crop mass and associated leverage, an extensive root system is required for the anchorage of plants in the soil. Sugarcane has a much greater above ground biomass than wheat, but its root length of almost 34000 km/ha is much less than the 60000 - 100000 km/ha commonly found for wheat (Reghenzani 1993). It has been suggested that large areas of sugarcane are suffering loss of productivity directly attributed to debilitated root systems (Egan et al. 1984). While soil factors influencing root growth and health such as microbiology (Magarey 1996), nutrition (Reghenzani 1993a) and compaction (Braunack et al. 1993) are being investigated, there is a need to establish the relationship of root systems with above ground growth. An aeroponic technique for growing, manipulating and non-destructively observing sugarcane root systems described in this paper is suggested as a means of establishing the above relationship. Data on growth of three cultivars with different shoot:root ratios are presented.

MATERIALS AND METHODS

Aeroponics is defined as the culture of whole plants whose roots are suspended in and fed by nutrient solution spray. Weathers & Zobel (1992) have suggested aeroponics as the optimum soil-less culture system, because root temperature, nutrition, moisture and gaseous phase can be controlled. Previous aeroponic systems (Smucker & Erickson 1976; Zobel et al 1976) were more complicated than the design reported in this paper.

The initial aeroponic facility reported here consisted of ten circular 55 L black, food grade polyethylene vats, 555 mm in height and 490 mm in diameter. Lids were painted white to reduce heat load and were modified by the addition of a second lip to eliminate light and prevent nutrient solution leakage. Three, evenly spaced 67 mm diameter holes were drilled in each lid for plant access. Nutrient solution was sprayed onto roots through twin foggers each rated at 28 L/h (at 405 kPa), situated at the base of each vat. Nutrient solution drained from the base of vats to a common graduated reservoir holding 40 L. A timer set to 15 min on, 15 min off, operated a 0.6 kW pump which supplied nutrient solution under pressure to the foggers. The system including pump, reservoir tank and vats were enclosed in an air-conditioned bench, similar to that used for glasshouse pot trials (Reghenzani 1984).

Three sugarcane cultivars with a wide range of shoot:root ratios were chosen for a preliminary evaluation trial. The cultivars were Q78 (small ratio) Q138 (mid ratio) and Q162 (large ratio). Single-eye setts were germinated in 76 mm plater pots filled with black, high density polyethylene beads, under 200 mM $Ca(NO_3)_2.4H_2O$ mist. The mist was applied for 15 min periods on a 33% duty cycle during the day and on a 11% duty cycle during the night. The germination solution was replaced at two to three day intervals. Plants at the 2-3 leaf stage and approximately 50 mm in height were graded on size and transferred to the glasshouse aeroponic system two weeks after planting. As far as possible, plants of similar size were placed within each of the ten replicate groups.

Observations were made at two week intervals until the twelfth week, when the trial was harvested. An additional observation was made on the eleventh week due to rapid plant growth. The aeroponic nutrient solution was changed every week until the final week, when it was necessary to change mid-week due to high plant usage. Commercial hydroponic twin pack powder (HydroLogic) supplied by Growth Technology, South Fremantle, was used to make the nutrient solution. Elements and their nominal concentration (mg/L), when made up according to directions were: N(220), P(31), K(280), Ca(160), Mg(50). S(66), Fe(3), Mn(1). B(0.35), Zn(0.20), Cu(0.15) and Mo(0.05). When made up, the solution contained 2.5 g/L total dissolved solids, with an electrical conductivity of 2.25 mS/cm. Solution pH was adjusted to 6.0 using 1M KOH.

RESULTS AND DISCUSSION

This initial experiment was conducted to identify and solve problems with the technique, to determine if cultivars reacted as they did in the field with respect to shoot:root ratio, and to observe plant growth and if restricted, correct factors which may have caused the problem. Within the first week of transfer to the aeroponic system Q78, which is known to be susceptible to iron deficiency, showed severe iron chlorosis. A single foliar spray with 1% iron sulfate solution overcame the problem. There was difficulty in differentiating between sett and shoot roots for the cultivar Q78. otherwise ease of observation of developing root systems was excellent (Fig. 1).

Shoot: root ratio

At the conclusion of the experiment the three cultivars ranked according to shoot:root ratio in the same order as for previous pot and field trials i.e. Q162, Q138>Q78 (P<0.05): ratios were 3.39, 3.24 and 2.59 respectively. The above finding shows that in aeroponic culture, sugarcane cultivars reacted as expected, and this fact encouraged confidence in results from future trials in the facility.

Water use

Progressive water usage was monitored during the trial. There was no appreciable usage until week seven (Fig. 2). Use over weeks 9-11 was depressed due to overcast conditions, while the increase in week 12 was consistent with fine, hot conditions, rapid growth and tillering of plants. Although not reported here, rate of nutrient uptake was determined by analysis of the reservoir solution. Both water and nutrient uptake can be used as progressive, non-destructive indicators of root system activity.

Fig. 1 *Top removed from aeroponic vat, exposing root systems of 12 week old Q162 (left), Q138(centre) and Q78(right).*
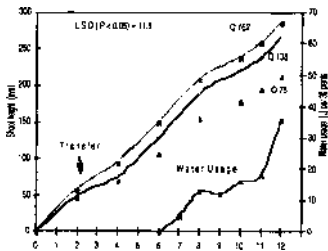
Fig. 2 *Primary shoot height and water use for three sugarcane cultivars grown in aeroponic culture.*

Primary shoot height

There was a near linear increase in primary shoot height with time for all three cultivars (Fig. 2), indicating no restriction to growth, except from slight slowing on transfer from the germinating facility and during the period of overcast conditions. Analysis of shoot height data indicated highly significant (P<0.001) effects due to cultivar (Q 162>Q 138>Q78) and time (week 12>11>10>8>6>4>2>0). Observed primary shoot growth compared well with plants in the field and differences between cultivars were consistent with known genetic characteristics.

Total primary root length

Only sett roots were apparent until week four (Fig. 3). By week six, shoot roots emerged and their length then increased at an exponential rate. Analysis of progressive weekly data showed a very significant (P<0.01) root length difference due to cultivar, (Q138>Q 162>Q78) and a highly significant (P<0.001) effect due to time (week 12>11>10>8>6=4=2=0). As for shoot growth, root length appeared to be increasing at a satisfactory rate, with significant cultivar differences probably due to differences in genetic potential.
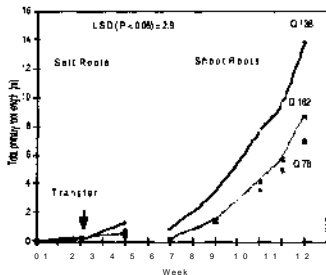
Fig. 3 *Total primary root length for three sugarcane cultivars grown in aeroponic culture.*

CONCLUSIONS

The initial trial has shown that the early growth of sugarcane shoots and roots, in the absence of imposed constraints, was satisfactory using the aeroponic technique. As highly significant cultivar differences in shoot and root growth reported in this paper were similar to expected field responses, it is suggested that the facility is suitable for the study of the relationship between root growth and activity, and above ground productivity. Future trials will investigate the effect of imposed root constraints on early shoot growth. There is a need for additional study of root growth and activity conducted on larger and more mature plants.

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SUGARCANE GROWTH IN A CONTROLLED ENVIRONMENT I: TECHNICAL SPECIFICATIONS AND CULTURAL REQUIREMENTS

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ABSTRACT

This paper describes the specifications of the CSIRO Division of Tropical Crops and Pastures' controlled environment facility (CEF) at St. Lucia. Particular mention is made of the need to regulate light quality in controlled environments, and the means by which this is achieved in the CEF is described. Cultural practices (including irrigation, fertiliser application and plant support) for sugarcane developed over two years are also described. Representative data for growth of sugarcane variety QI 17 in the CEF are presented which show that it is only in 'tall' rooms that studies of stalk development and hence sucrose accumulation can be achieved under controlled conditions.

INTRODUCTION

Controlled environment facilities (phytotrons) are useful tools in the study of plant physiology and biochemistry. They enable the identification of factors, often discrete environmental parameters, which limit plant growth, development or productivity. Such limits, once defined, can potentially be resolved by altering management practices, by specific breeding or by molecular manipulation. Modern controlled environment facilities allow tight regulation of environmental parameters such as temperature, light, daylength, humidity and CO_2.

Sugarcane is a vigorous C_4 grass which grows 3-7 m tall, has a high nutrient requirement, and has a life cycle of 8-12 months to maturity. These characteristics make it an especially difficult plant to grow in controlled environment facilities. The CSIRO Division of Tropical Crops and Pastures at St. Lucia has designed and built a controlled environment facility (CEF) specifically to study tropical crop and pasture species. Special (tall) rooms which could accomodate sugarcane and horticultural species were included in the design of the facility. Sensitive to the high light conditions in which many tropical plants grow, special attention was paid to control of light levels, uniformity and quality. This paper presents detailed technical information about the new facility.

TECHNICAL SPECIFICATIONS OF THE CEF

Physical Specifications

There are 14 growth rooms, six 'standard' rooms 3 x 2.7 x 3m high, four 'tall rooms' 3 x 2.7 x 8m high with hydraulic moveable floors, and four 'small' rooms 3 x 1.5 x 3m high. Each growth room has a plant (air conditioning) room at the side and a lamp loft above. Photosynthetically active radiation (PAR) is supplied by six, 1 kW high pressure metal halide lamps (Sylvania) and six, 1 kW tungsten halogen lamps (Phillips). Photoperiod lighting is provided by six, 150 W tungsten lamps (Phillips) in each room. There is a 40 mm deep temperature-controlled water bath and 6 mm of toughened plate glass to reduce the heat load from the lights on the plants and equipment. All rooms have full microprocessor control, programming and recording capabilities and operate to the precise specifications given below.

Radiation Specifications

Photon irradiance can be controlled in the range of 300 to 700 umol/m^2/s at the standard plant height of 1200 mm from the floor by the use of a PAR light sensor coupled to a computer-controlled dimming system. This range of PAR levels is consistent with recently published recommendations for lighting in controlled environments (Dietzer et al. 1994).

Cook & Russell (1983) reported that the yearly mean of short wave solar radiation intensity at Townsville (dry tropics) was 19.7 MJ/m^2/day. In the wet tropics, the yearly mean is even lower 17.2 MJ/m^2/day (Wilson & Ludlow 1991). Szeicz (1974) determined that PAR is ~50% of short wave solar radiation. As 1 mole of natural daylight is

approximately 0.23 MJ of PAR (Charles-Edwards 1982), this means that yearly average PAR values for dry and wet tropics are 996 and 865 pmol/m^2/s. Given these data and the observation that plants in controlled environments receive much more indirect (reflected) radiation than plants in the field (Bugbee 1994), we believe the range of PARs in the CEF to be appropriate for plant growth. Figure 1 shows a representative plot of light distribution at 1200 mm from the floor for a 'small' room set to deliver 500 umol/m^2/s PAR at that height. Similar trends have been observed for the 'tall' rooms used for sugarcane growth. In sugarcane trials plant rows are never closer than 450 mm to the side walls, where radiation flux is lowest.
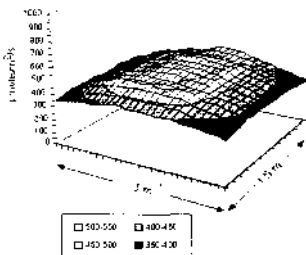
Fig. 1 *Light distribution at 1200 mm in a CEF 'small' room set to deliver 500 umol/m^2/s PAR at that height.*

It has been reported that the quality of light in controlled environments can significantly alter plant growth and development in some species (Bugbee 1994). Warrington & Mitchell (1976) reported that blue- or red-biased lighting in controlled environments effected significant changes to the protein (blue-biased) and carbohydrate (red-biased) content of *Sorghum bicolor*. The spectral composition achieved in the CEF minimises such a potential problem, as the blue (400-500 nm) light to red (600-700 nm) light ratio of the rooms (0.502) is very similar to that of sunlight (0.564) at 0920 h. Figure 2 shows the spectral distribution comparison of CEF irradiance against solar irradiance at 0920 h.

Another parameter identified as altering the phenological and physiological development of plants in controlled environments is the ratio of red to far red radiation (R:FR). The R:FR ratio has been linked, through phytochrome activity, to variations in the rate of growth and

the pattern of development of plants growing in controlled environments (Smith 1994). The R:FR ratio (660 nm/ 730 nm) in the CEF is 1.13, which is within 10% of the observed daylight range R:FR (1.05-1.25).

The CEF has the facility of being able to control photoperiod from 1 to 24 hours, and can provide daylength extension using low wattage tungsten lamps.
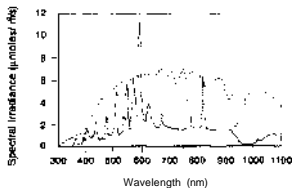
Fig. 2 *Spectral distribution comparison of irradiance (uynol/m²/s) in a CEF room (no dimming) (-) and solar radiation at 0920 h in Brisbane (—). Photosynthetically active radiation is 400-700 nm.*

Non-radiation Specifications

To investigate the discrete effects of temperature on sugarcane physiology, the CEF rooms can maintain day or night temperatures from 10 to 50°C (± 0.2°C). A special 'frost room' can operate down to -10°C.

In the CEF. dew points in the range of 10°C to 48°C are achieved using steam injection and coil dehumidification. Dew points down to 0°C can be achieved in the 'small' rooms using chemical dehumidifiers. Control of night time humidity to above 85% has been found to be critically important for normal leaf development in a number of tropical grasses (JR Wilson personal communication). At night time humidities below this threshold, a 'leaf-tip withering' symptom has been observed.

The concentration of CO_2 is monitored in each room with a capability to enrich to 1000 ppm. Air flow through each room in the CEF is from top to bottom with wind speed of either 0.5 or 1.0 m/s. Fresh air is added at the rate of 35 L/min to help maintain ambient CO_2 levels.

SUGARCANE CULTURE CONDITIONS

Plants are germinated from setts in seedling trays (300 x 300 x 40 mm), and are transplanted to plastic pots (200 mm internal diameter, 300 mm high) when plant height is approximately 200 mm. The potting mixture for both germination and mature plant growth is the same; 33% coarse sand, 33% vermiculite (Grade 1) and 33% coir fibre peat. Per 6 m³ of this mix. 5 kg each of hydrated lime, dolomite and superphosphate are also added. The potting mixture recipe was originally provided by Dr. Nils Berding of BSES. The potting mixture is pasteurised at 80°C for 20 min prior to use.

Potted plants are irrigated by individual trickle irrigation 'drippers'. Pots receive three waterings of 500 mL of tap water each day. Saucers beneath the pots hold a reservoir of water which is used by the plant. Under this irrigation regime visual signs of water stress such as leaf tip drying or leaf wilting have not been observed.

Fertiliser (Wuxal™ liquid foliar nutrient) is applied fortnightly to all pots. Wuxal™ contains 9.9 % w/w N (as urea), 4.3% w/v P (as P_2O_5), 6.2 % w/v K (as K_2O), 0.15% w/v Mg (as $MgSO_4$) and the trace nutrients; B, Co, Cu, Fe, Mn, Mo and Zn. Wuxal™ is diluted 15 mL in 1 L, and 150 mL is applied directly to each pot. Slow release fertiliser (Osmocote™, 14 % N, 6.1% P, 11.6% K) is applied every 2 months at the rate of 50 g per pot. Librel® Fe-lo chelated iron is applied every 2 months at the rate of 5 g per pot.

Plants are grown as two, two-pot rows, within CEF rooms. This arrangement allows a maximum of 40 plants per room. Plants are supported by an extendable trellis, which varies from 2 to 3.5 m in height. Individual stalks are attached to the trellis by loose-fitting wire nooses. Earlier trials allowed experimental randomisation by growing plants on movable trolleys, however it was difficult to support tall stalks with this system and sampling caused considerable canopy damage. Growing plants supported by a fixed trellis limits experimental randomisation. To minimise the variation of plants within a row, twice the number of plants needed for a trial are grown to the small plant (< 200 mm) stage. The heights of the plants are then measured, and the most uniform plants are selected for the trials. This procedure yields data with low levels of variation, essential given the limitations to replication and randomisation within the rooms (Campbell & Bonnett 1996).

Experiments with sugarcane variety Q117 growing under 14 hour days, (PAR of 500 umol/m², 30°C, 65% humidity) and 10 hour nights (20°C, 95% humidity) yielded plants of 2.7 m (base to apex) at 80 days after planting (DAP) and 5.8 m at 160 DAP. Growth rates under such conditions evidently limit the duration of experiments, as plants become too tall for the rooms after approximately 200 days. The meristem to apex length remained constant after 60 DAP at 1.8 m. Given the meristem to apex length, it is clear that only a facility with tall rooms such as the CEF can maintain controlled conditions during the cane-producing phase of sugarcane growdi. A detailed study of sugarcane growth in the CEF compared to field growth is presented in the second paper of this series (Campbell & Bonnett 1996).

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SUGARCANE GROWTH IN A CONTROLLED ENVIRONMENT II: COMPARISON WITH GROWTH IN FIELD ENVIRONMENTS

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ABSTRACT

Comparisons of the growth of two commercial varieties of sugarcane (Q117 and Q138) grown in a controlled environment facility (CEF) and in the field were made to evaluate the suitability of the CEF for physiological experiments from which data are to be extrapolated to the field. The CEF was demonstrated to provide a constant environment with very little variation between the growth and partitioning of individual plants. Internode lengths, though longer in the CEF, showed similar patterns up the stem in both environments. Dry matter accumulation was shown to occur at the same rate (relative to thermal time) in both the CEF and the field. As a function of dry matter content, fresh weight accumulation occurred faster in the CEF than in the field. We have shown that the CEF is a valuable tool for studies on the effects of environmental parameters on yield accumulation, but that caution must be applied when undertaking studies of water relations using our current protocols.

INTRODUCTION

The scope for investigating the effects of discrete environmental parameters on plant growth, development and productivity has been enhanced by the development of controlled environment facilities (phytotrons). Modern controlled environment facilities allow the control of temperature, light, daylength, humidity and CO_2, concentrations within tightly set limits. Another advantage of such systems is the low level of experimental variability due to plant-to-plant variation. There is however a need for caution when extrapolating results from controlled environments to field situations, as controlled environments do not normally imitate the full range of external conditions (gradual fluctuations of radiation intensity or temperature, air movements, etc) (Bugbee 1994).

This study determines the experimental variation of internode lengths and of internodal sucrose, glucose and fructose contents of plants grown in the CSIRO Division of Tropical Crops and Pastures' controlled environment facility (CEF), and makes a preliminary comparison of sugarcane growth in the CEF and in the field. This comparison was made using commercial varieties Q117 and Q138 grown either in the CEF, outdoors but under similar cultural conditions to the CEF (referred to as 'pot-grown'), or as a field-grown crop. Comparisons of internode length were made between CEF-grown and pot-grown sugarcane. Internode length is an important architectural aspect of potential crop yield as it imposes a physical constraint on sink volume, hence comparative strength. Comparisons between CEF-grown and field-grown sugarcane were made for fresh weight and dry matter (biomass) accumulation and percentage dry matter for millable stalks.

MATERIALS AND METHODS

Plants grown in the CEF

Setts of sugarcane varieties Q117 and Q138 were planted on 27 June 1995 and grown in the CEF according to established protocols (Campbell et al 1996a). Plants were grown under 14 h days. PAR of 500 umol/m^{2}/s, and 10 h nights. Day/night temperatures (30°C/20°C) and humidities (65%/95%) were constant during the trial. Emerging tillers were removed to ensure single stalk plants. Four plants of each variety were destructively harvested 84, 108, 138 and 165 days after planting (DAP). Expanded leaves and leaf sheaths and rolled leaves above the first node shorter than 10mm were removed. Mass of the resultant 'millable stalk' was determined and the stalk was cut into constituent internodes. Internode lengths were measured, and two, 2 mm disks were removed for extraction of sugars. The rest of the internodal tissue was weighed fresh, dried at 70°C for 5 days, and weighed to determine percentage dry matter and biomass accumulation. For sugar extraction, the tissue disks were incubated three times for 2 h in 10 mL of 80% ethanol in water (v/v) at 75°C, the pooled e:

were vacuum-dried then reconstituted in water to a x10
The concentrated extracts were analysed for sucrose, glucose and fructose (Hansen et al 1996; Campbell et al 1996b). Only data from 108 DAP for variety Q117 are presented.

Plants grown outdoors in pots

Cultivars Q117 and Q138 were grown in the open at the CSIRO Davies Laboratory in Townsville (19° 15' S, 146° 46' E), in pots (400 mm diameter x 300 mm depth) filled with a mixture of course sand, fine sand and peat 2:1:2 (v/v/v). Two 2-eye setts were planted in each pot on 3 August 1995 and the pots placed in rows centred 1.5 m apart with pots 450 mm apart in the row. Plants were fertilised with die same nutrient products and frequency as those grown in the CEF (Campbell et al 1996a), and were watered to field capacity three times daily. Stems were supported by wire along the sides of the plants and between the pots. Three or four plants per pot germinated and the main tiller was marked and used for the measurement of internode lengths at 236 DAP. The uppermost internodes enclosed by leaf sheaths were not measured. Internode lengths from 4 plants of each variety (one main stem selected randomly from each of four pots) were compared with internode lengths of plants grown in the field at 108 DAP.

Plants grown in the field

Previously published results for stalk mass accumulation of crops of sugarcane varieties Q117 and Q138 growing in 'non-limiting' conditions at the Macknade Research Station (18° 44' S, 146° 12' E) (Muchow et al 1993) were accessed from the SUGARBAG database system (Prestwidge & Robertson 1994). Data were converted from determinations based on area to determinations on a per stalk basis, to enable comparison of dry weight and biomass accumulation and the stalk percentage dry matter between plants grown in the field and CEF. Comparisons were made using both time (DAP) and thermal time (base 14°C) from planting (Robertson et al 1994).

RESULTS AND DISCUSSION

Figure 1 shows sucrose, glucose and fructose content (% dry matter) of internodes of Q117 growing in the CEF. Consistent with previous findings (Fernandes & Benda 1985), the oldest internodes had the greatest % sucrose, and sucrose content declined with immaturity. Interestingly, the two youngest (still expanding) internodes contained significantly greater amounts of sucrose (c. 6%) titan the youngest fully expanded internodes (c. 3%), presumably reflecting the large change in cell wall content as tissue ages (Wilson 1976). Levels of the reducing sugars glucose and fructose were inversely proportional to those of sucrose for all but the youngest two internodes, in which they too increased (from - 7% to - 9%). The levels of variation for sugar determinations between plants were < 5% in all instances. In older plants grown in the CEF internodal sucrose contents have attained 46% of DM (data not shown).
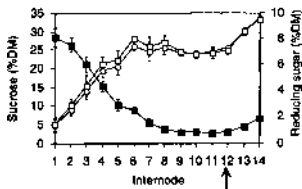
Fig. 1 Sucrose (■), glucose (□) and fructose (○) as a % of dry matter for internodes of sugarcane variety QI17 grown in the CEF.

Fig. 1 Sucrose (W), glucose (3) and fructose (O) as a % of dry' matter for internodes of sugarcane variety QI 17 grown in the CEF.

[Internode 1 is the oldest internode, (?) indicates the last fully-expanded internode. Means of 4 plants sampled at 108 DAP ± standard error.]

Having demonstrated that plants grown in the CEF show little experimental variation in their content of carbohydrate metabolites, it was necessary to show that these plants were similar to field-grown plants. This would allow more confident extrapolation of results from metabolic studies in the CEF to field situations. The variation of internode length with age for QI 17 and Q138 grown in the CEF and grown in pots are shown in Fig. 2. A comparison of the standard errors revealed that for QI 17 variation was smallest in the CEF-grown plants, although it averaged < 5% of the mean for both pot- and CEF-grown plants. For Q138, standard errors were smaller in the CEF-grown plants (5-10% of mean), than in the pot-grown plants (10-15% of mean). This level of variation in the data was consistent with that observed in sugar concentrations.

Lengths of the oldest 4 internodes in QI 17 were the same in both environments (Fig. 2a), but subsequent internodes were up to 35 mm longer in the CEF. Maximum internode length was observed at internode 8 for both CEF- (170 mm) and pot-grown (145 mm) plants. Maximum internode length was observed in older internodes of Q138 (Fig. 2b) compared with Q117; internode 10 for the CEF-grown (245 mm) and internode 11 for the pot-grown plants (140 mm). Whilst the internodes of CEF-grown plants, growing in a constant environment attained a plateau beyond which they did not expand further, internodes of pot-grown plants decreased in length with age past a certain internode number. It is possible that the highly conserved nature of internode lengths reflect environmental or anatomical constraints to growth of sink tissues and hence potential yield. If the observed decrease in internode length in the pot-grown plants is a function of seasonal change (decreasing mean daily temperature or radiation input), it appears that Q117 was more sensitive to such influences than Q138.

Comparisons of fresh and dry matter accumulation and percentage dry matter between the CEF and a field environment were made using both time and thermal time, only the latter being presented in (Fig. 3). Thermal time was calculated according to Robertson et al (1994), using a base temperature of 14°C, an optimum temperature of 32°C and a maximum temperature of 45°C. Millable stalk fresh weight accumulation for both varieties was faster in the CEF than in the field (Fig. 3a), although these differences can be explained by the greater water content of CEF-grown plants of both varieties (Fig. 3b). Figure 3c shows that the rate of biomass dry matter accumulation was the same for CEF- and field-grown plants. In isolation, this observation suggests that temperature has the predominant influence on total crop yield, overriding the marked difference in light intensities between CEF and field. However, the integral of photosynthetically active radiation received by spaced plants with
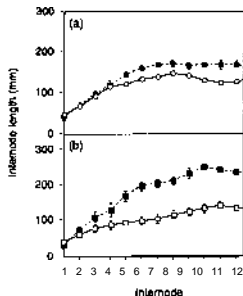
Fig. 2 Internode lengths (mm) of CEF- (■) and pot- (○ □) grown sugarcane for (a) Q117 and (b) Q138.

[Internode 1 is the oldest internode. Means of 4 plants ± standard error.]
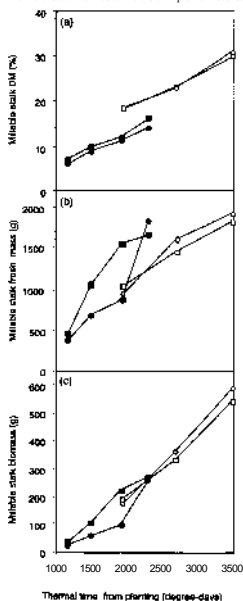
Fig. 3 Trends in millable stalk (a) % dry matter, (b) fresh weight accumulation, and (c) biomass (dry matter) accumulation for sugarcane varieties Q117 (○ ●) and Q138 (□ ■) grown in the CEF (● ■) and in the field (● ■) plotted against thermal time (base temperature 14°C).

single stems in the CEF may be equivalent to that received by stalks growing in a crop. This is currently under investigation.

Data indicate that sugarcane grown in the CEF shares some important growth trends with that grown in the field. Whilst fresh matter accumulation of CEF-grown sugarcane occurred at a greater rate than in the field, this clearly reflected a greater water content rather than an increased capacity to fix carbon. That the rate of dry matter accumulation was the same for sugarcane growing in the CEF and in the field suggests that despite the differences in water content, other aspects of the physiology of sugarcane grown in the CEF are unaffected. Further work in the CEF will attempt to resolve the problem of unusually high tissue water contents by decreasing the daytime humidity levels.

CONCLUSION

This paper shows that the variation (as standard errors) of representative data sets obtained in the CEF (internode length and sugar content) was <10%, reinforcing the value of this tool for future research. Consistent trends were noted in the development of internodes of the sugarcane varieties Ql 17 and Q138 grown in the CEF and in pots. It was shown that in the CEF the highly controlled conditions produced constant maximum internode lengths (170 mm for Q117 and 260 mm for Q138) after the laying down of a given number of initial internodes. Pot-grown plants exhibited similar trends in internode length with age, although internodes were smaller and affected by seasonal variations. CEF-grown plants of Ql 17 and Q138, exhibited the same dry matter accumulation rates as plants growing in the field. However CEF-grown plants had much higher rates of fresh matter accumulation, as percentage dry matter for a given thermal time differed between the two environments. The CEF is a valuable tool for studies of the effects of environmental parameters on yield accumulation, but that caution must be applied when undertaking studies of water relations.

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MEASUREMENTS OF SUGARCANE (*SACCHARUM* SP) RESPIRATION RATES USING DETACHED STEM AND LEAF TISSUE AND *IN SITU* STEM TISSUE

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ABSTRACT

Up to 50% of carbon fixed by sugarcane over a 2 year cropping period is lost to respiration. This loss of photosynthate equates to a considerable reduction in harvestable product. A considerable proportion of total sugarcane respiration occurs in the stem, even though the mature stem respiration rate is approximately one tenth of leaf respiration rate on a dry weight basis. This is due to the stem containing approximately 90% of the total dry matter in a mature sugarcane plant. When sugarcane internodes along the stem were compared similar respiration rates were recorded for non-expanding tissue, except for the basal stem internode which gave values similar to leaf tissue due to the development of buttress roots. This paper presents destructive and non-destructive techniques for measuring mature stem respiration and discusses preliminary results obtained using these techniques.

INTRODUCTION

Moore (1995) has recently reviewed the extensive work carried out to determine the physiological processes of sucrose accumulation in sugarcane (*Saccharum sp*). Respiration processes in sugarcane have received little research attention. The only substantial report on sugarcane stem respiration is by Glover (1973) who measured respiration of whole plants at intervals over the life of an 18 month crop in South Africa. During the early stages of sugarcane development, leaf respiration accounts for the majority of plant respiration (Glover 1973). However, as the plant develops, the increasing proportion of stem mass as a contributor of total plant mass, approximately 80 - 90% by harvest time (Glover 1973; Robertson et al 1996), means that stem respiration is likely to become a major contributor to total plant dark respiration.

Respiration, the oxidative breakdown of glucose and fructose, has been identified as a target for down regulation by breeding or molecular manipulation (Wilson 1992). Over an 18 month crop cycle in South Africa, Glover (1973) found that up to 50% of the carbon fixed by photosynthesis was subsequently catabolised by respiration. Research on the C3 monocot ryegrass (*Lolium perenne*) has shown that varieties selected for low respiration rates had a higher dry matter yield than high respiring varieties (Wilson & Jones 1982). The observed higher dry matter yields were due to more carbon being retained in the plant. A similar pattern has been shown in a C₄ plant, maize (Heichel 1971), suggesting scope for variation in dry matter yield within varieties. In sugarcane, if respiration load could be decreased then this could result in increased dry matter yield or increased sucrose levels. Identification of low respiring varieties could thus augment selection in existing breeding programs for commercial varieties. Wilson & Jones (1982) reported that the gene or small group of genes associated with low respiring ryegrass varieties was conserved in offspring.

Respiration of stem tissue is difficult to measure and usually achieved destructively by using detached stem pieces sealed in perspex tubes which may not provide accurate *in vivo* levels. Glover (1973) used whole plant chambers, a technique quite impractical for determining variation between genotypes, particularly with mature plants.

This paper presents preliminary results on dark respiration rates of sugarcane stem using a specifically constructed chamber for measuring intact stem carbon fluxes *in situ*.

METHODS

Plant material

Sugarcane variety Q117 was used for all respiration measurements. For the detached stem method, plants were grown under controlled environment conditions (Campbell et al 1996) with a 12h day/night regime at 330ppm CO_2, 500 mmol/m²/s photon flux and relative humidity of 65% (day) and 95% (night). For the intact method, plants were grown under a 14h day and 10h night regime, with all other

conditions being the same as for the detached stem method. For both methodologies, the leaf sheath was removed from the stem prior to determination of respiration. Internodes were labelled according to their age, thus internode 1, the oldest internode was the first internode visible above the soil level. For the detached stem method, respiration determinations were performed on internodes 2 (oldest), 4, 6, 8, 10, 12 and 14 (youngest). It was not possible to assay internodes above internode 14, as removal of the sheath from the younger internodes damaged the stem tissue. The intact stem measurements contained internodes 3 and 4 within the measuring chamber. The leaf measurements of dark respiration presented in this paper were made on blade material from the central 300mm of the last fully emerged leaf from the same plants used for stem respiration; this leaf equated to internode 12.

Detached stem and leaf respiration

Stem sections included one internode with its subtending node. The 300mm leaf portion was cut into 100mm lengths and sealed in individual perspex tubes under a constant air supply. Respiration rate was determined by the differential between CO_2 concentration entering the tube and the CO_2 concentration exiting the tube, using a Horiba® infra-red gas analyser.

Intact stem respiration

Clear perspex tube of 50mm diameter and 250mm length was fitted at both ends with a cap with a central hole of 25mm diameter sufficient to accommodate a sugarcane stem. The tube was then split in two and holes at the top and base of the tube were fitted with gas inlet and outlet. All the joining surfaces were lined with high density foam gasket to give an air tight seal when the two halves were clamped around the stem. The gas inlet tube was connected to an ADC portable pump which is specifically designed for constant flow rates in gas exchange measurements. The outlet tube was connected to an ADC portable infra-red gas analyser which also had a tube connected from the pump. The CO_2 differential between the air flow directly from the pump and the airflow through the respiration tube gave the CO_2 given off by the section of sugarcane mature stem in ppm. This value along with the flow rate, the volume of the tube, and the dry weight of the stem sections (determined destructively at the end of measurements) gave the mature stem respiration rate. To reduce differences in respiration rate due to the surrounding environment all measurements were made in controlled environment rooms, and the RH through the pump was maintained at 70%. Respiration rates were measured beginning 2h before, and finishing 3h after, the daily dark period.

Respiration values for detached stem, intact stem and leaf section were determined on a dry weight basis, and are the mean of three replicates for the detached stem and four replicates for the intact tissue.

RESULTS AND DISCUSSION

Detached stem and leaf respiration

Internodes 4 through 12 gave respiration rates over the 48h period which were not significantly different. These values were designated mature

stem respiration (Fig. 1). The youngest measured internode, internode 14, was shorter than mature internodes in all 3 replicate plants (data not shown). Its mean respiration rate was approximately 3 times greater than the stem respiration rate of the mature internodes 4 -12. This observation is consistent with the fact that internode 14 was immature and still expanding, and thus required high catabolic energy to sustain cell expansion.
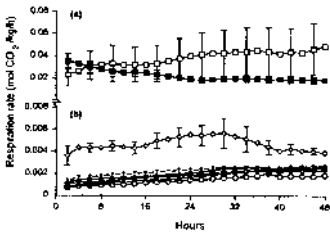
Fig. 1 *Detached tissue: dark respiration rates over a 48h period for sugarcane stem and leaf sections. (a) Basal stem internode 2 (-□-) and leaf section (-■-); (b) Internode 4 (-○-), internode 6 (-△-), internode 8 (-▽-), internode 10 (-▲-), (internode 12 (-+-) and internode 14 (-◇-). (Internode 4 (oldest) - internode 14 (youngest). Standard errors for internodes 4 - 12 were approximately the size of the symbols. [Note: The scale for (a) and (b) are different.]*

The oldest stem internode measured (internode 2) gave abnormally high respiration rate (Fig. 1a) of up to 10 times greater than internodes 4 - 12. We believe that the abnormally higher levels of respiration in internode 2 are explained by the development of "buttress" roots from root primordia of the basal internode. The high variability obtained between replicates of internode 2 could be due to observed variability in buttress root development between replicates. Meristematic tissue (in the root tips) can have a much higher respiration rate than fully expanded tissue (Day et al 1985), therefore a large contribution of internode 2 respiration could be equated to root respiration. Detached leaf respiration rate (Fig.1a) was 10 times greater than mature stem respiration rates of the mature internodes (4-14) and 5 times greater than for immature stem, viz. internode 14.

The mature stem tissue's respiration rate gradually increased over the 48h period (Fig. 1a,b) while the leaf respiration rate declined for 24 hours before plateauing (Fig 1a). We believe that the mature stem respiration rate increases due to the gradual breakdown of cellular structures releasing stored sucrose . The detached leaf respiration rate declines due to the low levels of stored sucrose and therefore reserves are quickly depleted during the first 24 hours. After this period, catabolic structural breakdown starts to occur and the respiration rate is sustained through the breakdown of proteins and fats.

Intact stem respiration

The trend in respiration of intact stems over the 15h period (Fig. 2) showed a small rise in respiration when the lights were turned off. A similar increase has been noted in intact sugarcane leaf measurements (data not shown). Thereafter, respiration declined to a relatively constant level for the following 6h with a slight up trend in the last 4h of the dark cycle. A sharp rise in the respiration rate occurred when the plants were illuminated. This reflects a higher level of metabolic activity occurring during the light cycle. The dark respiration rates for the intact stem tissue were of a similar order to those of detached stem tissue (Fig. 1).

Glover (1973) and Robertson et al (1996) found that the stem constituted approximately 80 - 90% of above-ground dry matter. Even though the current results indicated that leaf respiration was ten times greater than
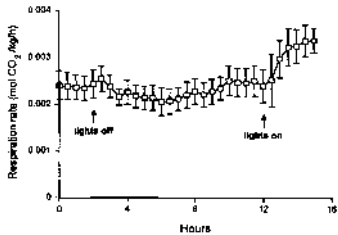
Fig. 2 *Intact tissue: sugarcane stem respiration (internodes 3 and 4) over a 15h period on a dry weight basis.*

stem respiration, the much larger biomass of the stem compared to the leaf means that the stem and leaf would contribute approximately equally to total respiration of mature plants. Therefore both leaf and stem are important targets for reduced respiratory activity

The stem chamber technique allows respiration rates of numerous plants to be determined *in situ*. With an automatic sampler which can periodically switch between several chambers, a large number of plants can be analysed over a short period of time. Therefore this technique is a faster and more practical method for determination of stem respiration than whole plant chamber technique used by Glover (1973). By clamping the chamber onto the mature stem where the leaf sheath has naturally detached, wounding effects are reduced compared to cutting stem sections and Glover's technique of cutting off all the leaf blades to obtain a stem respiration value. Therefore misleading respiration values should be minimised.

CONCLUSIONS

The *in vivo* mature stem respiration method allows faster, more practical and realistic measurements. The very basal internodes with adventitious roots should be avoided for comparative measurements.

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COMPARATIVE GROWTH ANALYSIS AND LEAF CHARACTERS OF A HIGH-SUCROSE AND MEDIUM-SUCROSE INDIAN CULTIVAR

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ABSTRACT

A growth analysis study was conducted in Peninsular India with an objective of identifying growth analysis and leaf characters which might distinguish high-sucrose, high-yielding cultivars. Two early-maturing cultivars CoC671 (high-sucrose, low leaf area index (LAI) and CoC 772 (medium-sucrose, high LAI) were studied. Significant differences in LAI, dry matter (DM) solar energy utilisation coefficient (Eu), dry matter accumulation rate (AR), specific leaf weight (SLW), leaf vein frequency (LVF) and mean interveinal width (IVW) were observed between these two cultivars.

The higher yielding and higher sucrose cultivar CoC 671 had higher LVF and lower IVW. The relation of these two parameters to genotypic differences in yield needs to be examined in future trials with a wide range of genotypes. LAI at 4th month was significantly related to DM yield of cane at harvest. Lower LAI of CoC671 at later growth stages was offset by a higher AR than CoC 772.

INTRODUCTION

Dry matter (DM) accumulation over time and economic sugar yield is a function of leaf area index (LAI), its intrinsic capacity for carbon exchange rate (CER) and translocation of photosynthates from source to sink. The productivity efficiency of cane cultivars can also be evaluated by taking the ratio of the solar energy utilised by leaves through its quantitative and qualitative characters to die total available solar energy. Sugarcane intercepts more than 60% of incident solar radiation due to high leaf area duration (LAD), unlike other crops, which have hardly 30-35% total light utilisation (Udaya Kumar & Devendra 1984). The present productivity of cane in many regions of India leaves scope for further increase in cane yield and sucrose content in view of the high availability of solar energy and other inputs including irrigation.

Literature shows the importance of light interception for DM accumulation. A positive association of LAI with DM was established by Irvine (1967:1975) and Perumal (1989). However, it has not always been possible to establish a positive relationship between CER in leaves and DM accumulation although there is significant variation in CER among varieties. Besides LAI and LAD, several leaf anatomical characters, namely, leaf vein frequency (LVF) and mean interveinal width (IVW) have been reported to have positive association with CER and rate of translocation of assimilates from leaves (Wardlaw 1976). In 42 genotypes of finger-millet, variation in CER and rate of translocation of assimilates was reported to be associated with variation in LVF and IVW (Perumal 1980). In sugarcane, studies indicated significant difference in IVW and LVF among 6 cultivars (Perumal 1981). These two characters proved to be stable across environments.

In view of the importance of leaf characters in influencing the productivity of sugarcane, growth analysis studies were initiated to identify specific leaf characters which could serve as easily measurable potential markers to select cultivars with high production potential.

MATERIALS AND METHODS

Field experiments were conducted in six locations (replications) in the reserved area of Kothari Sugar Mill in Tamilnadu, southern part of India during 1980-81. CoC 671, a high-sucrose cultivar with low LAI, and CoC 772, a medium-sucrose cultivar with high LAI were planted in January, February, March, April and May with 900 mm between rows and 330 mm between plants. Nutrients applied in kg/ha were: 225N, 112.5 P_2O5 and 112.5 $K_2 O$. Growth analysis was conducted by collecting 3 complete stools from each treatment at 4, 6, 8, 10 and 12 mondis. The areas of leaves 3, 4, 5 and 6 as calculated at these times were expressed as the total area per stool. Since 80% of total photosynthesis has been reported to take place in the first six leaves (Udaya Kumar & Devendra 1984), total LAI for leaves 3, 4, 5 and 6 was used throughout. Moisture content of the leaf sheath (SM) was determined by oven-drying at 70°C.

The LAD in months 4, 6, 8, 10 and 12 was determined. Specific leaf weight (SLW) was determined for the four sampled leaves. The stools

collected from the field, hand-cut at ground level, were used to record cane fresh weight. The height of 10 primary shoots up to the top of the last fully-expanded internode was measured and the average height determined. The sucrose % juice was determined from the shoots collected, after extracting the juice in a crusher.

Leaf 3 collected from primary shoots at each harvest in months of 4, 6, 8, 10 and 12 was used for anatomical studies. The procedure described by Crookston & Moss (1974) was followed for anatomical studies. LVF and IVW were computed as below :

$$LVF = \frac{\text{Total number of veins per leaf}}{\text{Total width}}$$

$$IVW = \frac{\text{Leaf width-Total veinal width}}{\text{Total number of veins}}$$

Each cane crop was harvested 12 months from planting date and the yields of cane recorded. Total DM yield was calculated by determining the stalk moisture from a sample of 10 stools per replication collected from the field. Total DM included only cane weight, dried leaves and trash were excluded due to practical problems.

The mean values of the daily solar radiation, recorded in this region were collected for computing the solar energy received over months 4, 6, 8, 10 and 12. Solar energy utilisation coefficient (Eu) was computed by applying the formula developed by Kanda (1975). It is total solar energy stored to the total solar energy received during the crop growth period and expressed as percentage. Assimilation rates was calculated

$$AR = \frac{DM(g)}{LAD \text{ (LAI days)}}$$

Experimental data were subjected to statistical analysis using a factorial randomised block design. Regression analysis was applied to relate LAI and SLW with DM and sucrose content respectively (Draper & Smith 1969).

RESULTS

Cane height and weight

Cane height increased near linearly over the growth period in both cultivars with height at 12 months of CoC 671 (2.8m) being significantly greater (P<0.05) than CoC 772 (2.4m). Significant difference was observed in cane weight between cultivars at month 4 with a parallel linear rate of increase in both cultivars between 4 and 12 months; final cane weight/ stool was 5.7 kg for CoC 671 and 4.6kg for CoC 772 (Fig.la).

Leaf sheath moisture

SM was high (>78%) in early growth but declined sharply between 10 and 12 months. SM of CoC671 was consistently higher than CoC 772 at all harvests (Fig. 1b). CoC 772 recorded a low SM of 69% at final harvest well below the generally considered optimum SM of 73% (Perumal 1995).
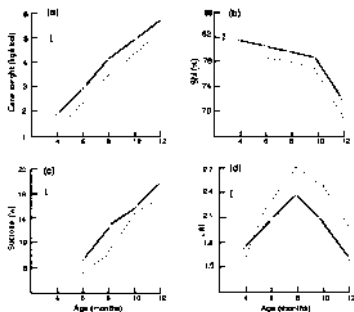
Fig. 1 Comparison of time trends in (a) cane weight/stool (b) moisture content, (c) sucrose % juice, (d) leaf area index (LAI) for two cultivars CoC 671 (——·) and CoC 772 (.....).
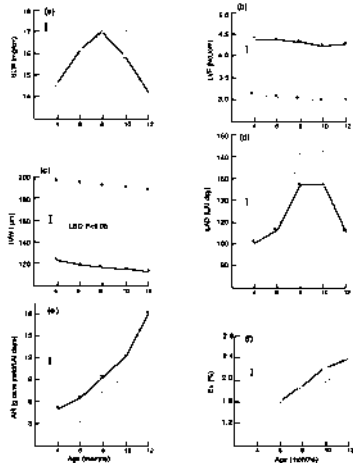
Fig. 2 Comparison of time trends in (a) specific leaf weight (SLW), (b) leaf vein frequency LVF, (c) interveinal width (IVW), (d) leaf area duration (LAD), (e) assimilation rate AR (cane dry matter yield/LAI days), and (f) solar energy utilisation (Eu)for two cultivars CoC 671 (·——.) and CoC 772 (•....).

Sucrose (% juice)

CoC671 has higher rate of sucrose accumulation than CoC 772 up to month 6 (Fig. 1c). At month 8, the sucrose content in CoC 671 was 5% higher than CoC 772. The rate of increase in sucrose % from 8 to 12 months was approximately similar for both cultivars. CoC 671 showed an ability to start with high sucrose accumulation much early in growth than CoC 772.

Leaf characters

CoC 772 had a higher leaf weight in months 6-12 than CoC 671 (Fig. 1d). LW (data not shown) showed a similar trend except that the higher levels in CoC772 were not evident until 8 months. SLW was significantly higher in CoC 772 than CoC 671 (Fig. 2a).

Anatomical characters

LVF of CoC 671 (4.2-4.5) was consistently higher over all ages than for CoC 772 (3.2.-3.4) (Fig. 2b). Conversely, IVW of CoC 772 (170-180 nm) was consistently greater than for CoC 671 (120-125 nm) (Fig. 2c).

Growth analysis parameters

Total LAD was consistently higher at all ages in CoC 772 than in CoC 671 (Fig.2d). Despite low total LAD CoC 671 had consistently high AR (Fig. 2e) and Eu than CoC 772 at all growth phases (Fig. 2f).

DISCUSSION

LAI at 4th month was significantly correlated with DM yield of cane at harvest irrespective of cultivars. Even in CoC772, LAI at peak was positively associated with DM. This observation suggests the scope to further increase DM yield through irrigation to increase LAI in early growth and even at the stage of peak LAI. Similar results were reported by earlier workers (Irvine 1975: Perumal 1989). An increase in LAI from 1.5 to 2.4 at month 6 would result in a quantum jump in cane yield.

The significant higher AR and sucrose content for CoC 671 might be due to its higher LVF and lower IVW. These parameters were reported to be significantly related to AR and sucrose content (Perumal 1981). The higher AR of CoC 671 offset its lower LAI compared to CoC 772.

CONCLUSIONS

LAI at 4th month appeared to have significance in crop productivity. Cultivars with high LAI at this stage would record high Eu, due to high interception of solar energy. Therefore, efforts to evolve varieties with high LAI at month 4 and maintain optimum LAI at peak would help to increase productivity under irrigated conditions. The consistent leaf characters of high density of vein and narrow interveinal width in early growth phase was critical in establishing die difference between the two cultivars.

Highly-yielding, higher sucrose cultivar COC 671 is interesting. Comparison of these leaf anatomical characters over a much wider range of genotypes is needed to establish whether they could be useful markers for selecting high-yielding, high-sucrose cultivars.

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RELATIONSHIP BETWEEN BIOMASS AND SUCROSE ACCUMULATION BY SUGARCANE

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ABSTRACT

In the absence of biotic constraints, accumulation of stalk sucrose yield in sugarcane is affected by climate, variety, water and nitrogen supply. Models can integrate the combined effects of these various constraints on productivity in quantitative terms, and can be used to explore the scope for improvement in yield through management or genetic improvement. In this paper we analyse the utility of modelling stalk sucrose accumulation as a simple function of stalk biomass. We analyse the linear relationship between stalk sucrose (g/m²) and stalk biomass (g/m²) for a number of sugarcane crops varying in variety, crop age, and location under irrigated tropical conditions in Australia, and compare these with previously published Australian and international studies where variety, water and nitrogen supply also varied. For the majority of varieties and under variable water and N supply, stalk sucrose dry weight concentration is around 0.48 g/g at moderate-to-high levels of stalk biomass. Sucrose accumulation in stalks is a continuous process as stalks grow and biomass increases and does not require promotion by low temperature or water deficit. Varietal differences can be encapsulated in the two coefficients of the linear relationship.

INTRODUCTION

In Australia, commercial cane yield and sucrose concentration are commonly measured and reported on a fresh weight basis, and are used as a basis for cane payment to growers. Since dry weight and sucrose accumulation are 'driven' directly by resource capture and utilisation, it is difficult to compare productivity across treatments, locations or climates using such fresh weight measures. Hence it is important to establish relationships between crop dry weight and sucrose accumulation. This will assist the analysis of production constraints and the scope for overcoming them, the efficiency of crop resource use (e.g. solar radiation, nitrogen and water) and the scope for genetic improvement of sucrose storage by sugarcane stalks.

Muchow et al (1996b) and Robertson et al (1996) have related stalk sucrose accumulation to stalk biomass accumulation using a simple linear relationship. In this paper, we extend the analyses by considering the stability of the relationship between sucrose accumulation in the stalk and stalk biomass, and the concentration of sucrose in the stalk on a dry weight basis. Data for this analysis are taken from a number of intensively-sampled crops in Australia and South Africa, which vary in crop duration, crop class, variety, nitrogen and water supply. The relationship, if proven stable, will have an important application in modelling crop productivity.

METHODS

Table 1 lists the datasets from experiments conducted under high nitrogen supply, tropical, irrigated conditions in Australia, that were analysed for the relationship between stalk sucrose (g/m²) and stalk biomass (g/m²). Crops differed in variety, location, season and crop age. Crops were sampled at 4-6 week intervals and stalk sucrose (determined by High Performance Liquid Chromatography) and stalk biomass were measured following the procedures described by Muchow et al (1993).

Data from a study of Inman-Bamber (1994), of eight ratoon crops of varieties N12 and NCo376, grown at La Mercy, South Africa, under rainfed conditions, were re-analysed, to examine the relationship between stalk sucrose and stalk biomass under variable water supply.

Linear regressions were fitted to the relationship between stalk sucrose (g/m²) and stalk biomass (g/m²). These provided a value for the slope and the effective start to the linear relationship (equal to the negative value of the intercept divided by the slope). The coefficients from the slope. The effective start represents the minimum amount of stalk biomass (g/m²) that needs to be present before sucrose accumulation commences. The resulting

coefficients were compared with published relationships derived in other studies, to determine the degree of variation in the relationship with variety, crop age, water and nitrogen supply regime, and climate. While logistic curves could probably provide slightly better fits to the data over the linear approach, the simplicity of linear equation was favoured because it gives easily-interpretable coefficients, and is a commonly-used approximation in plant growth analysis.

Table 1 *Datasets from irrigated experiments in tropical Australia analysed for the relationship between stalk sucrose and stalk biomass.*

Dataset	Variety	Location	Crop class	Reference
1	Q96	Ayr	Plant, 1st & 3rd ratoon	Muchow etal 1994
2	Q96	Ayr	1st Ratoon	Muchow etal (unpublished)
3	Q138	Macknade	Plant	Muchow etal 1993
4	Q138	Macknade	Plant, 1st ratoon	Muchow etal 1996a
5	Q117	Macknade	Plant	Muchow etal 1993
6	Q117	Macknade	Plant, 1st ratoon	Muchow etal 1996a
7	Q117	Ayr	Plant	Muchow et al 1994
8	Q117	Ayr	1st ratoon	Muchow etal (unpublished)

RESULTS AND DISCUSSION

The three varieties in the Australian studies formed two distinctly different relationships for stalk sucrose versus stalk biomass (Fig. 1). Ql 17 and Q96 had similar slopes and effective start values, while Q138 had an effective start that was almost twice that of Ql 17 and Q96, and a higher slope (0.61 versus 0.55). In the study of Inman-Bamber (1994) in South Africa, varieties N12 and NCo376 formed almost identical relationships, which were similar to those of Ql 17 and Q96. In this study there was little evidence that the variable occurrence of water deficit produced outliers to the fitted relationship. This suggests that water deficit in the South African study had a negligible effect on sucrose partitioning. Muchow et al (1996b) found that nitrogen deficit, while having large impacts on stalk biomass accumulation, also had little impact on the relationship between stalk sucrose and stalk biomass.

The coefficients fitted to the data in Fig. 1 are compared in Table 2 with coefficients fitted to data from other published studies. There was consistency in the coefficients of the relationship for the same variety.
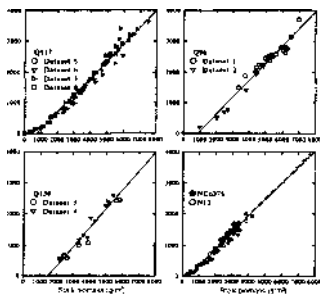
Fig. 1 *Fitted linear relationships for stalk sucrose (Y) versus stalk biomass (X)for varieties*
[Q1 17 (Y = 0.549±0.070 * X − 389+43.2, R^2=0.976, n=72), Q96 (Y = 0.554±0.022 * X − 472±107, R^2=0.957, n=31), Q138 (Y = 0.606±0.038 *X−888±157,R^2=0.946,n=17), N12(Y = 0.527±0.018*X−176±42.5, R^2=0.970,n=41), NCo376 (Y = 0.544±0.020 * X − 225+53.6, R^2=0.950, n=38). Details of datasets are given in Tables 1 and 2.]

Across all studies, the slope coefficient varied less (0.49 to 0.61) than the effective start to sucrose accumulation (239 to 1152 g/m^{2}of stalk biomass). There was a negative association between the steepness of the slope and the intercept value (Fig. 2a), indicating that a later effective start to stalk sucrose accumulation was generally associated with a subsequently greater slope. This means that curves differing in effective start and / or slope tend to converge as stalk biomass increases.

The tendency for relationships differing in slope and effective start to converge as stalk biomass increases implies that stalk sucrose concentration on a dry weight (DW) basis will tend towards a similar value at moderate-to-high levels of stalk biomass. Fig 2b shows curves of stalk sucrose DW concentration as a function of stalk biomass for values of the slope and intercept of (0.50, -100), (0.55, -400), and (0.60, -700). which span the range of the negative association shown in Fig. 2a. As predicted, stalk sucrose DW concentration for the three curves tends towards a common value of 0.48 g/g at levels of stalk biomass above 4000 g/m². Apart from those reported here, a number of further studies have reported maximal stalk sucrose DW concentrations of 0.48-0.50 g/g (Das 1936; Muchow et al 1996b).

Fig. 2 *(a) Association between fitted intercept and slope value for datasets in Table 2. Fitted relationship is Y = -6732 * X + 3317, R^2 = 0.834, (b) Derived curves of stalk sucrose DW concentration (g/g) as a function of stalk biomass, using fitted slope and intercepts of A (0.50, • 100), B (0.55, -400), C (0.60, -700).*

CONCLUSIONS

The findings of this study have a number of important implications:

i)   for the varieties tested to date, and under variable water and N supply, stalk sucrose DW concentration tends towards a predictable value of 0.48 g/g,

ii) sucrose accumulation in stalks appears to be a continuous process as stalks grow and biomass increases, rather than being due to a trigger such as low temperature or water deficit,

iii) biomass accumulation is the dominant component of stalk sucrose accumulation rather than increase in sucrose concentration,

iv) stalk sucrose can be modelled as a simple function of stalk biomass, with variety differences encapsulated in the two coefficients of the linear relationship,

v)   this is a simple framework to explore potential genetic differences and opportunities for improvement in sucrose storage in stalks.

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Table 2 *Coefficients of the fitted linear relationship between stalk sucrose and stalk biomass for the three varieties analysed in the present study, and for previously published studies. Unless otherwise stated crops were grown under irrigated, well-fertilised conditions.*
[standard error in brackets; effective start = stalk biomass in g/m² at start of sucrose accumulation]

Variety	Treatments	Slope	Intercept	Effective start	Reference
Q138	Crop class	0.606 (±0.038)	-888 (±157)	1465	Present study
Q117	Crop class	0.549 (±0.070)	-398 (±43.2)	725	Present study
Q96	Crop class	0.554 (±0.022)	-472 (±107)	852	Present study
Q117	3 N rates	0.489 (±0.014)	-117 (±40.8)	239	Muchow, 1996b
Q138	Ratoon	0.594 (±0.033)	-684 (±131.7)	1152	Robertson et al., 1996
Q117	Ratoon	0.549 (±0.033)	-370 (±137.7)	674	Robertson et al., 1996
NCO376	Rainfed, ratooning date	0.544 (±0.020)	-225 (±53.6)	414	Inman-Bamber, 1994
N12	Rainfed, ratooning date	0.527 (±0.038)	-176 (±42.5)	334	Inman-Bamber, 1994
N14	Plant& 1st ratoon	0.554 (±0.040)	-247 (±40.9)	446	Thompson,1988 renalysed by Robertson et al 1996

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RAPID ENZYMATIC ASSAY TECHNIQUE FOR DETERMINATION OF SUCROSE IN EXTRACTS OF SUGARCANE TISSUES

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ABSTRACT

We have adapted to microplate scale an existing enzymatic assay procedure for the quantification of sucrose in sugarcane juice. Sample preparation involved appropriate dilution of previously filtered samples with water. Aliquots of diluted sample were transferred to 96-well microplates and an aliquot of assay reagent containing buffers and the required enzymes was added to each well and mixed. After incubation the plates were read at 340 nm in a microplate reader. A set of prepared sucrose standards were processed with each batch of juice samples permitting a calibration curve to be plotted. Absorbances of samples were read against this calibration curve, allowing calculation of sample sucrose concentrations.

This technique was used for analysis of sucrose on juice samples from stalk, cabbage and leaf tissues of sugarcane. Up to 80 samples were analysed per day, a dramatic improvement on sample throughput using gas-liquid chromatography (glc), high performance liquid chromatography (hplc) or ion chromatography (ic). Data are presented which show that this technique, as well as being fast and cost effective, gives sucrose determinations of a similar reliability to data achieved by hplc.

INTRODUCTION

Estimations of sucrose in sugarcane by the industry are conveniently made on juice samples using a density or refractive index measurement (brix), an optical rotation measurement (pol) and an estimation of cane fibre to calculate commercial cane sugar (CCS). The inclusion of fibre as a component of CCS determination limits the applicability of this unit to trials investigating sucrose accumulation in sugarcane. For more exacting estimations of sucrose, a number of chromatographic methods have been used including gas-liquid chromatography (glc), high performance liquid chromatography (hplc) or ion chromatography (ic) (Vercellotti & Clarke 1994).

Each of these employs a separation medium to separate mixtures of component sugars and a detector to quantify the separated sugars. While such techniques can determine the major sugars of interest (sucrose, glucose and fructose) simultaneously, typical run times are from 8 to 30 min per sample, excluding sample preparation. Sample preparation usually involves dilution followed by one or more filtration steps and may involve removal of interfering ions. Typically, 12 to 20 samples can be prepared and analysed per day.

Large numbers of sugarcane juice and tissue samples are assayed for sucrose content at the Analytical Laboratory of the CSIRO Division of Tropical Crops and Pastures. Because some experiments produced up to 150 samples per week over a period of 6 months, novel techniques were needed for rapid, specific and sensitive analysis of sucrose. Enzymatic techniques offer not only specificity and sensitivity of analysis for constituent sugars but also reduce volumes of sample and reagents needed, and the time and cost of analysis. The most convenient enzymatic assay for sucrose is that of Birnberg & Brenner (1984), which has the advantage of a common pH for the breakdown of sucrose into its constituent monosaccharides and measurement of those constituent sugars. The reactions involved are shown below.

Sucrose + Pi—> Glc- I-P + fructose   (by sucrose phosphorylase)

Glc- 1-P -> Glc-6-P   (by phosphoglucomutase)

Glc-6-P + NAD -> 6-P-GlcU + NADH + H^{+} (by glucose-6-phosphate dehydrogenase)

NADH, the reduced form of NAD is stoichiometrically balanced with the initial concentration of sucrose and is the measured end product of direct colorimetric assay at 340 nm.

This paper describes how we have modified the existing enzymatic technique for sucrose analysis for use with a microplate reader to permit preparation and analysis of up to 80 samples per day.

MATERIALS AND METHODS

As part of a series of field trials, juice samples of three different sugarcane tissues (stalk, leaf and cabbage/tops) were prepared according to the method of Muchow et al (1993) and stored at -20°C until analysis. Duplicate series of vials of the extracted juice were analysed by hplc as described by Muchow et al (1993) and by enzymatic assay. The hplc analyses were performed at the analytical laboratory of the CSR Ltd. Technical Field Department, Ingham.

For the enzymatic assay a buffer is prepared by mixing two separate buffer solutions (A and B) to achieve pH 7.0. Solution A contains 10 mM K$_2$HP0$_4$ and 1 mM MgS0$_4$ while Solution B contains 10 mM KH$_2$P0$_4$ and 1 mM MgS0$_4$. The reagent mixture, sufficient for one full plate of 96 wells, is prepared by dissolving 8.3 mg nicotinamide adenine dinucleotide (NAD) (Sigma N-7004) in 25 mL of assay buffer. Enzymes are added to this mixture as 80 uL (32 U) phosphoglucomutase (EC 5.4.2.2) (Boehringer Mannheim 108 375), 73 uL (75 U) glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (Boehringer Mannheim 165 875) and 53 pL (5 U) sucrose phosphorylase (EC 2.4.1.7) (Sigma S-7660).

Assays were performed in "U" type 96-well microplates. Two duplicated water blanks were included on each microplate, one as a zero point for the calibration curve and the other as a reagent blank, the absorbance of which was subtracted from all samples and standards. Standards were prepared from analytical grade sucrose at five concentrations (0.1, 0.2, 0.35, 0.5 and 0.75 mg/mL). Sugarcane juice samples were thawed to room temperature and diluted with water using an automatic pipette (Digiflex, ICN Micromedic Systems) to achieve a sucrose concentration of between 0.1 and 10 mg/mL. 13.5 uL of appropriately diluted sample or sucrose standard was added to each assay well, followed by 250 uL of the reagent mixture. All samples and standards were determined as the mean value of two replicate assay wells. The combined solutions were mixed well and incubated at room temperature for 70 min before determining the production of NADH at 340 nm. To compensate for any optical imperfections in the plastic microplates, absorbances were corrected against a second wavelength (562 nm).

Sucrose determinations achieved by hplc and enzymatic assay were compared against determinations of juice CCS for 72 different samples. Both techniques (hplc and enzymatic assay) were tested against CCS determinations and against each-other by linear correlation analysis.

RESULTS

Mean results of six different standard sucrose solution calibration curves are shown in Fig. 1. Whilst the standard errors of the mean cannot be resolved in the figure, they ranged between 0.003 and 0.005 absorbance units. It can be seen that the determination of sucrose concentrations by the enzymatic assay technique is linear over the experimental range indicated (r^2 = 0.998).
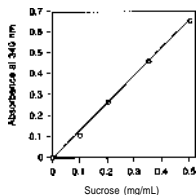
Fig. 1 *Calibration curve for standard sucrose solutions derived from 6 different data sets. Standard errors of the mean are too small to be resolved in this representation.*

Fig. 2 shows % sucrose in juice determined by both hplc and enzymatic assay against CCS values for 72 samples from various plant components giving a wide range of sucrose levels. Over the range of the complete data set, the linearity of determinations by both techniques, when compared to CCS values, were almost identical ($r^2 = 0.987$ for enzymatic assay, $r^2 = 0.986$ for hplc) (Fig. 2a). Figs. 2b and 2c show limited sub-sets of the complete data set, indicating determinations by hplc and enzymatic assay for samples of< 13% CCS and > 13% CCS respectively. The values obtained by the two techniques were in high agreement for values lower than 13% sucrose ($r^2 = 0.980$ for enzymatic assay, $r^2 = 0.995$ for hplc) (Fig. 2B). However, at CCS values of>13% correlation coefficients decreased ($r^2 = 0.888$ for enzymatic assay, $r^2 = 0.823$ for hplc) and there was a greater spread of residuals for sucrose concentrations determined by both techniques. It is possible that this increased scatter for sucrose determinations by either hplc or enzymatic assay reflects inconsistencies with determinations of CCS rather than problems with the two distinct chemistries of determination (separation and enzymatic breakdown). This argument is supported by the linear correlation of sucrose determinations achieved by hplc against enzymatic assay ($r^2 = 0.986$) for the 72 samples analysed (Fig. 3).

CONCLUSION

The Birnberg & Brenner (1984) enzymatic assay method for the quantification of sucrose has been modified for application with micro-plate techniques without loss of assay sensitivity. This technique has been used to quantify sucrose in juice samples of various sugarcane tissues, and was found to be as sensitive and reliable as quantification by hplc. It is stressed that, at this time, neither method of sucrose determination (hplc or enzymatic assay) should be used as a precise predictor of CCS. Nor, as the data indicate, is CCS a precise estimate of sucrose concentration in sugarcane juice samples. High correlation between determinations by hplc and enzymatic assay suggest that both methods are equally reliable for sucrose determination in sugarcane juice. Given the large savings in analysis time (and hence cost) afforded by the enzymatic assay we recommend that laboratories dealing with large numbers of sucrose determinations consider utilising this technique.

ACKNOWLEDGMENTS

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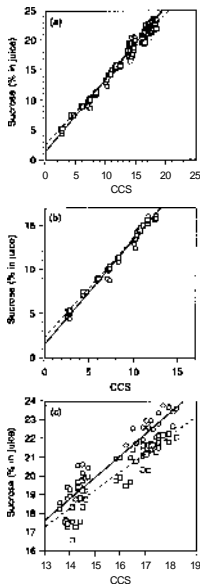
Fig. 2 *Comparison of % sucrose determined by enzymatic assay (O) (—) and hplc (a) (- - -) against measured CCS for (a) full CCS range (n = 72), (b) CCS <13 (n=28) and (c) CCS>13 (n = 44).*
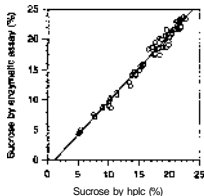
Fig. 3 *Comparison of % sucrose in juice determined by hplc and by enzymatic assay (n = 72).*

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RELATIONSHIP OF SUCROSE METABOLISM ENZYMES WITH SUCROSE STORAGE IN SUGARCANE

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ABSTRACT

Sucrose storage in sugarcane is the composite result of a cycle of sucrose synthesis and sucrose hydrolysis, and thus involves the coordinated activity of several enzymes. A better understanding of how the activities of critical enzymes are regulated to enhance sucrose storage is expected to suggest strategies for breeding and selection for superior sucrose storing cultivars. We have investigated the biochemical aspects of the trait of sucrose accumulation among siblings in a family segregating for differences in ability to store sucrose. The activity of soluble acid invertase appears to be the critical factor regulating sucrose accumulation. This investigation found a significant correlation between the activities of soluble acid invertase and sucrose accumulation. We have cloned several sugarcane acid invertase genes, which will be used to characterize the expression of these genes, and to discover how they relate to sucrose accumulation.

INTRODUCTION

Cultivated sugarcane, which is noted for storing high levels of sucrose, is an interspecific hybrid of two or more species of tropical grasses belonging to the genus *Saccharum*. The ability of cultivars to store sucrose is derived primarily from the species *S. officinarum* which may store up to 18% (fw) as sucrose (Bull & Glasziou 1963). Other species of *Saccharum* contribute the agronomic characters of high vigor and disease and pest resistance but characteristically accumulate less than 5% (fw) of sucrose. In hybrid cultivars that store high quantities of sucrose, the level and rate of accumulation of sucrose is a function of the environment and crop development throughout the growing season. Early in the crop cycle when plant growth is rapid, the transported sucrose is largely transformed into reducing sugars for metabolism and structural components of the plant. At this stage there is relatively little sucrose stored. Later in the crop cycle less of the translocated sucrose is partitioned into structural components and a larger fraction is stored as sucrose (Fernandes & Benda 1985).

The metabolic pathways by which sucrose is synthesized from smaller carbohydrates or is broken down to provide substrates and energy for growth and structural elements are well known. However, our understanding of which of these pathways are critical to sucrose storage and how these pathways are regulated is incomplete. A better understanding of molecular regulation of the level and timing of sucrose accumulation could lead to improved cultivars with higher production and to agronomic practices for better timed production of sucrose.

Different investigations over the past several decades into the factors which determine sucrose accumulation in sugarcane have not always been in agreement. Studies on whole plants, tissue slices or cell suspension cultures have often identified different factors as being primary determinants of sucrose storage. Some of the studies suggested that there are no "major" determinants, only marginal changes in a number of enzyme activities which result in substantial differences in sucrose accumulation.

We investigated progeny of a cross between a high sucrose-storing clone and a low sucrose-storing clone to resolve whether determinants of sucrose storage could be identified in closely related genotypes. Stalks from progeny which segregated for sucrose accumulation were analysed for sugar (sucrose, fructose and glucose) content and the activities of several enzymes involved in sucrose metabolism: soluble acid invertase, neutral invertase, sucrose phosphate synthase and sucrose synthase.

Both sugar and enzyme assays were performed on the same tissue samples to avoid problems due to environmental or developmental variables. This investigation found a significant correlation between the activities of soluble acid invertase and sucrose accumulation. We have cloned several sugarcane acid invertase genes, which will be used

to characterize the expression of these genes, and to discover how they relate to sucrose accumulation.

MATERIALS & METHODS

Plant material

Experiments reported were conducted on the parents and progeny of a cross between the female clone Louisiana Purple (LA Purple, *S. officinarum*, 2n=80) and the male clone Molokai 5829 (Mol 5829, 5. robustum, 2n=80). The siblings of the family were recognized as hybrids since LA Purple did not produce viable pollen and all progeny exhibited hairy leaf sheaths characteristic of Mol 5829 but lacking from LA Purple. The parents and approximately 110 progeny were grown in replicated plots in two different environments. Eight clones, identified from a previous study as either high-sucrose or low-sucrose types, were selected for analysis of stored carbohydrates and enzyme activities.

Sugar analyses

Both sugar and enzyme activity assays were performed on the same tissue samples to avoid problems due to environmental or developmental variables. Tissue samples were homogenized in 70% ethanol then boiled for 2 h. Aliquots were taken for measurements of reducing sugars and sucrose. Reducing sugars were determined by the Somogyi-Nelson method (Nelson 1944). Sucrose was determined by anthrone method (van Handel 1968).

Enzyme assays

The enzyme extraction method was described by Goldner et al (1991). Frozen tissue was ground to a fine power in a chilled mortar and pestle. Quartz sand (Sigma) was added to facilitate cell disruption. Then extraction buffer was added to the mortar and the slurry was ground to thoroughly mix powder and buffer. The extraction buffer contained 50 mM Hepes (pH, 7.5), 12 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 10 mM DTT, 2 mM Benzamidine, 2 mM N-aminocaproate, and 10 mM Diethyldithiocarbamate (DIECA). When the extract started to melt, it was ground again to achieve full homogeneity. The homogenate was passed through two layers of Miracloth and the filtrate was centrifuge at 15,000 g for 10 min at 4°C. The supernatant was desalted immediately on a Sephadex G-25 (Pharmacia PD-10) column.

All enzyme assays were carried out at 37°C. For the assay of acid invertase activity, 50 μL of desalted extract was added to 50 μL of 1 M sodium acetate (pH 4.5). The enzyme reaction was started by the addition of 100 uL of a 120 mM sucrose solution. The reaction was stopped at 30 min or 60 min by addition of 30 uL Tris base and boiled for 3 min. For neutral invertase activity, the reaction was carried out at pH 7.5 and no Tris base was added. The concentration of glucose was then determined with the glucose test kit from Sigma. Sucrose phosphate synthase (SPS) and sucrose synthase (SS) activity assays were carried out in die direction of synthesis, pH 7.5 (Hubbard et al 1989). Protein

content was determined by the method of Bradford (1976). All the enzyme activities were expressed as mmol product formed/g protein/min.

RESULTS

The sucrose concentrations of the parents differed by one order of magnitude from <25 to >300 umol/g fw (Fig. 1). The sucrose concentrations of the progeny ranged between those of the parents. Phenotype (thin, fibrous, light-weight stalks versus thick, heavy-weight stalks) was not a good indicator of the sucrose concentration in the stem, i.e. sugar-high-sucrose clones were found among light-weight stalked clones as well as among the heavy-weight stalked clones (Fig. 1). The fresh weight of stalks of the progeny ranged from lighter than Mol 5829 to heavier than LA Purple.
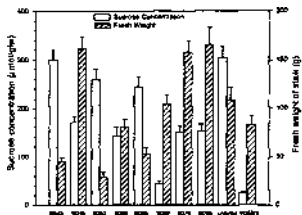
Fig. 1 *Sucrose concentrations and fresh weights of the whole stalk of six hybrid progeny and their* S. officinarum *(LA Purple) x* S. robustum *(Mol. 5829) parents.*

On a whole stalk basis there was an inverse relationship between the soluble acid invertase (SAI) activity and the concentration of sucrose. Neutral invertase (NI) and sucrose phosphate synthase (SPS) activities were relatively low and there was no significant relationship between these enzyme activities and sucrose concentration (Table 1). Although the relationship between SPS activity and sucrose concentration was weak ($r^2 0.075$), the difference between SPS and SAI was the most strongly correlated of all enzyme activities ($r^2=0.86$).

Table 1 *Correlation coefficient (r^2) of enzyme specific activities with sucrose concentration in the stalks of the high-sucrose storing* S. officinarum *cv LA Purple and the low-sucrose storing* S. robustum *clone Molokai 5829 and six hybrid progeny of a cross between the parents.*

Enzyme	Correlation coefficient with sucrose concentration
Acid invertase (SAI)	0.52
Neutral invertase (NI)	0.15
Sucrose synthase (SS)	0.32
Sucrose phosphate synthase (SPS)	0.075
SPS-SAI	0.86
SPS-(SAI+NI)	0.77

Young internodes of all clones were low in sucrose while the older internodes accumulated sucrose to varying levels depending on whether the clones were high-sucrose or low-sucrose storing types. The difference between high-sucrose and low-sucrose clones was not only the final sucrose concentration reached in the old internodes but also the internode developmental stage at which the sucrose storage started. High-sucrose clones usually started storage of sucrose three to four internodes earlier than did the low-sucrose clones.

SAI of internodes decreased during internode maturation and sucrose accumulation. The decrease of SAI activity may be required for sucrose accumulation in sugarcane. A significant non-linear negative relationship between SAI activity and sucrose accumulation of the individual internodes was observed. Whenever SAI was high, the sucrose content was low; when SAI was low, the sucrose content could be either low or high. Thus, the data fit a hyperbolic curve. The cluster of points which do not fit onto the curve inflection were the intermediate-aged internodes (e.g. internodes 5 to 7). We postulate that in these internodes there is a time interval between the decline in activity of SAI and the increased accumulation of sucrose.

DISCUSSION

Sucrose storage in sugarcane is the composite result of a rapid cycle of sucrose synthesis and sucrose hydrolysis (Wendler et al 1990). Synthesis is achieved in parallel pathways by two enzymes, SS and SPS. Intracellular sucrose hydrolysis is accomplished by three enzymes - NI and SS located in the cytosol, and SAI located in the vacuole (for reviews see Glasziou & Gayler 1972; Hawker 1985; Hawker etal 1991;Moore 1995). In recent years it has become increasingly clear how these enzymes contribute to the storage of sucrose. However, the question remains whether any one of these enzymes plays a master role in determining the final sucrose level reached in the mature internode. That is, can we expect to identify genes encoding a particular sucrose metabolizing enzyme which accounts for the differences among sugarcane clones in their ability to store high levels of sucrose?

Early research identified SAI as the principle enzyme regulating sucrose storage (Hatch & Glasziou 1963; Slack 1965; Gayler & Glasziou 1972). Later research identified other enzymes such as SS (Goldner et al 1991) or the balance of activities of several enzymes (Veith & Komor 1993; Komor 1994) as responsible for regulation of sucrose storage. Neither sets of studies can be considered conclusive since comparisons were made among extremely divergent environmental conditions, plant types (even to comparing different *Saccharum* species), and different plant

To avoid these problems we chose to study enzyme activities among the progeny of a cross between a high-sucrose storing S. officinarum and a low-sucrose clone S. robustum clone. Analyses were made on a whole stalk basis and on internodes of different stages of maturity along the stalk. Both analyses confirmed the critical role of SAI in regulating sucrose accumulation. Our results indicated that high-sucrose clones differ from low-sucrose strains in the timing of the onset of the storage process and in the final sugar level reached in the old internodes.

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CYCLES OF SUGAR TRANSPORT AND SUCROSE METABOLISM IN SUGARCANE TISSUE: QUANTITATIVE DETERMINATION

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ABSTRACT

Experiments were performed on tissue slices of internodes of different developmental state from field-grown sugarcane plants. Slices were incubated with labelled sugars of different kinds and concentration, and the uptake and synthesis of sucrose, and the label distribution into sucrose were determined. There was a rapid cycle of sugar uptake and release through the plasmalemma, which was always faster than the rate of sucrose synthesis. In young and maturing internodes the uptake of hexoses was always much faster than the uptake of sucrose, in mature internodes sucrose fluxes are predominant because of lack of hexoses in these

The cycle of sucrose metabolism decreased in rate with maturation of the parenchyma. Sucrose synthesis was equally accomplished by sucrose synthase (SS) and sucrose phosphate synthase (SPS) in young internodes, but the contribution of SPS increased with maturation. SS participated in sucrose hydrolysis in mature tissues.

In all tissues the sucrose cycle was consistently faster than the net flow of sugar into storage, and into growth and energy metabolism

INTRODUCTION

Since the basic work of Australian plant physiologists it is known that there is uptake and release of sugar (hexoses mainly) through the plasma membrane of sugarcane cells and a cycle of sucrose synthesis and breakdown in the cells (Glasziou & Gayler 1972). These data have been confirmed and quantified with sugarcane suspension cultures (Wendler et al 1991). Recently, however, several new findings question this old concept or ask at least for modification or re-evaluation. Lingle (1989) and Thorn & Maretzki (1992) claimed that sucrose can be taken up by sugarcane cells without prior cleavage to hexoses, so that sucrose synthesis from hexoses in the storage cells may not be a key step for the level of sucrose storage. Jacobsen et al (1992) showed that cell walls of storage parenchyma lignify with tissue maturation, and that a suberin lamella between bundle sheath and parenchyma tissue developed with the consequence that the apoplast of the storage parenchyma is isolated from the apoplast of the bundles. Consequently sucrose supply from the phloem has to occur symplastically, probably in the form of sucrose. Welbaum & Meinzer (1990) found that the apoplastic concentration of sucrose and hexoses (and some other compounds) is close to that of the symplast and therefore usually rather high, confirming data of Hawker (1965). These newer findings suggest that the cleavage of sucrose by cell wall-bound invertase, the uptake of hexoses by active transport systems (which saturate at low sugar concentrations), and the synthesis of sucrose in the cell from hexoses are not important for sucrose storage.

To re-evaluate the above issues, we have quantified the rates of the various transport and metabolic reactions under discussion, i.e. measured the rates of uptake of hexoses and sucrose, the rates of sucrose synthesis and breakdown, and the enzymes involved in these. In this paper the rates are compared and evaluated to assess their contribution in the overall process of sucrose storage.

MATERIAL AND METHODS

Plant material

Variety L 62-96 was grown in the field at CTCAS/ORMVAG research station, Kenitra, Moroc, and internodes of different developmental state, e.g. of number 0 (dewlap just visible), 3 and 13 from top visible dewlap were harvested.

Uptake measurements

Tissue slices of 1mm thickness and c. 75mm^2 were rapidly cut with a razor blade from storage parenchyma of sugarcane internodes. The slices were rinsed for 15 min in 200mM sorbitol, 25mM MES-KOH pH5.5 and 1mM CaCl$_2$ in ice to remove adhering solutes from cut cells without

provoking expression of wound responses, and then incubated in different concentrations of radioactively labelled sugar in buffer as above, keeping the osmotic strength constant at 250mM with sorbitol. Usually at 15 or 30 min intervals slices were removed quickly (a few seconds) in the same way as above and then either directly counted in scintillation fluid or extracted in 80% ethanol at 70°C. The extract was separated by TLC, the labelled compounds quantified and, in case of sucrose, eluted and hydrolyzed with invertase to determine by TLC the distribution of label between glucose and fructose.

In vitro enzyme determination

The tissue was frozen in liquid nitrogen after harvest, ground in extraction buffer (50mM Hepes-KOH pH7.4, 12mM CaCl$_2$, 1mM EDTA, 1mM EGTA, 10 mM dithiothreitol, 2mM benzamidin, 2 mM N-amino-caproate, 10 mM diethyldithiocarbamat) in liquid nitrogen, desalted and concentrated through sephadex G25 columns and Centricon 30 membranes. The enzymes were measured by standard procedures followed by colorimetric tests (Dancer et al 1990).

RESULTS

Uptake of sugar by young internodes was very dependent on the sugar species, hexoses were taken up rapidly at low concentrations of 5-20 mM, whereas sucrose uptake was slow. At high sugar concentrations above 50 mM a diffusion-like linear uptake phase became prominent for all sugars, but sucrose uptake was still slow (Fig.l). As internodes
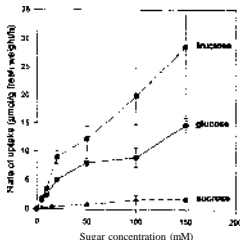
Fig. 1 *Sugar uptake by slices of internodal parenchyma from immature internode number 0 (last visible dewlap). Data are means of 3-5 independent preparations, bars indicate standard deviation.*
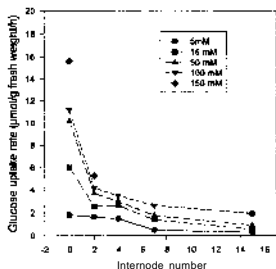
Fig. 2 *Glucose uptake rate at different glucose concentrations by tissue slices from internodes of different age, all derived from the same stalk.*

Fig. 4 *Labelling of sugars in tissue slices during chase 30 minutes after a pulse labelling with* ^{14}C *glucose.*

matured the uptake rate for hexoses decreased strongly, for both low and high sugar concentrations (see Fig.2 for glucose, fructose rates showed similar trends). As the active component of sugar uptake decreased the diffusion-like component became relatively more important, although it did not increase in absolute activity. When expressed in terms of permeation coefficient sucrose "diffusion" is not higher than hexose "diffusion" (data not shown). When the natural apoplastic concentrations of sugar were considered according Welbaum & Meintzer (1990), then calculations showed that hexose uptake decreased *in situ* from 10 to 1 umol/g/h, the sucrose uptake increased *in situ* from 0.5 to 1 umol/g/h. So only when hardly any hexose was around, and in presence of very high sucrose concentrations in the apoplast as in old, ripe internodes, would uptake of sucrose versus hexose play a significant role.

Rates of synthesis and degradation of sucrose were determined by incubation of tissue slices in a 1:1 mixture of labelled glucose and unlabelled fructose. After uptake of hexoses, the pathway of sucrose synthesis and its rate could be determined by the label distribution of the hexose moieties in sucrose. The measured synthesis rates were c. 3.5 umol/g/h in young internodes with approximately equal contribution

by sucrose synthase and sucrose phosphate synthase. During internode maturation the synthesis path via sucrose synthase faded out and the overall synthesis rate of sucrose fell to near zero, being supported only by sucrose phosphate synthase. The different development of SS and SPS was reflected by *in vitro* enzyme activity (Fig. 3).

The cyclic breakdown of internal sucrose was measured by a pulse-chase experiment, in which labelling of internal sucrose during incubation with 1:1 labelled glucose and unlabelled fructose was switched to a 1:1 mixture of unlabelled glucose and fructose. The label in sucrose decayed at a rate of c. 4 umol/g/h in young internode tissue, and labelled glucose and fructose were detected (Fig. 4).

Comparison of cycle rates is especially interesting in internode 3, where net accumulation of sucrose occurs. *In situ* the rate of net sucrose storage is c. 0.4 to 1.8 umol/g/h, the growth and energy requirement (measured as respiration) is c. 2.8 umol/g/h, the sucrose synthesis rate c. 4.6 umol/g/h, and the sugar uptake rates (mostly as hexose uptake) is c. 10 umol/g/h (all numbers in hexose units for easier comparison). That means that sucrose synthesis is fivefold faster than the storage rate and uptake tenfold faster. In the event that the import of sucrose from the phloem

Fig. 3 *Activity of (a) sucrose synthase and (b) sucrose phosphate synthase in tissue samples from internodes of different age, means of 3 independent preparations, bars indicate standard deviation.*
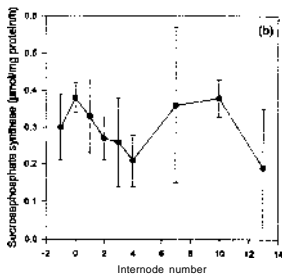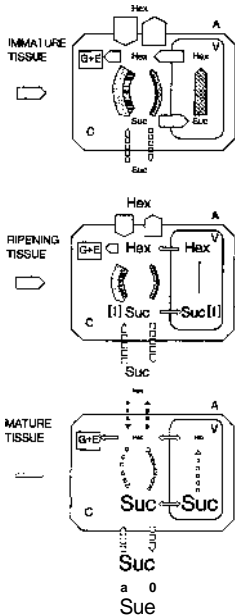
Fig. 5 *Schematic diagram of sugar uptake cycle and sucrose synthesis cycle in immature internode (number 0), ripening internode (number 3) and mature internode (number 13). The width of the arrows represent the rate of uptake or metabolism. The data summarize the rates obtained by incubation of tissue slices at sugar concentrations, which are found in the apoplastic space of storage parenchyma. The arrows at the far left indicate the net entry of sugar from the phloem. The participation of the different enzymes in sucrose synthesis and hydrolysis is indicated by different shadings, light shading (left) is SPS, strong shading SS and netted shading is invertase (neutral invertase in cytosol and acid invertase in vacuole). Abbreviations:A=apoplast, V=vacuole, C=cytosol, G+E=growth and energy use.*

proceeds symplastically, then it is at most identical to the overall gain in sucrose, namely 2.8 umol/g/h. The apoplastic cyclic transport steps and the cyclic synthesis and degradation of sucrose in the cytosol are obviously faster and cause a rapid mixture and equilibration of hexoses and sucrose in symplast and apoplast. The fluxes and their relative size in a ripening (number 3) internode are summarized in Fig. 5 and can be compared with the situation in an immature internode, where the metabolic and transport cycling is even faster but there is no sucrose storage because of high invertase activity, and an old, ripe internode , where all cycles have nearly come to a standstill.

CONCLUSIONS

Hexose uptake is *in situ* more important than sucrose uptake, except for old, ripe internodes, where all fluxes are small. The fluxes through the plasmalemma, and the sucrose synthesis rates, were found to be higher than the net import rate of sugar from the phloem, so that the pathway of sugar delivery whether from apoplast or symplast does not seem of great importance for the regulation of sugar storage in the parenchyma. The uptake rates of glucose and fructose apparently do not limit sucrose synthesis. In the maturing internode, SPS is responsible for net sucrose synthesis, whereas SS is equally involved in synthesis and hydrolysis. The transfer rates into and out of the vacuole, the presumed site of acid invertase, await determination.

ACKNOWLEDGEMENT

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RATES OF SUGAR ACCUMULATION IN SUGARCANE IN RELATION TO SUCROSE SYNTHASE ACTIVITY

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ABSTRACT

Sugarcane cultivars vary in rates of sugar accumulation, but the biochemical mechanisms for the differences are unknown. Sugar accumulation rates and sucrose synthase activities were determined in developing internodes of contrasting sugarcane cultivars. The highest rates of total sugar accumulation were observed in internodes which had just reached maximum elongation. In general, internodes with higher sugar accumulation rates had higher activities of sucrose synthase in the cleavage direction than internodes with lower sugar accumulation rates. This suggests that sucrose synthase plays a role in sugar import into sugarcane storage

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrid) cultivars vary considerably in the rates of growth and accumulation of total sugars and sucrose. Improvement in juice sucrose concentration is the desirable endpoint of sugarcane breeding programs, and identification of genes that influence rate and capacity of sucrose accumulation would aid in that effort. Juice sucrose concentration is a quantitative trait, determined by the interaction of several physiological processes: 1) how much sucrose is synthesized and exported from the leaves; 2) how much sucrose is unloaded from the phloem in the stem; 3) how much sucrose the parenchyma cells of the storage tissue can accumulate; and 4) what happens to the sucrose in the storage tissue.

Metabolic activity of the internode may be one determinant of sugar accumulation. The sucrose cleavage activity of sucrose synthase (SS) is considered important in determining carbohydrate allocation and metabolism in sink tissues (Sung et al 1989). In an earlier study (Lingle & Irvine 1994), SS activity was not associated with sucrose accumulation in the sugarcane cultivars studied. However, in another study there appeared to be an increase in SS activity in mature internodes during the ripening period, when sucrose concentration increases in juice (SE Lingle, unpublished data). The purpose of the current study was to compare sugar accumulation and sucrose metabolism in internodes of an early- and a late-ripening cultivar as they developed during the growing season.

MATERIALS AND METHODS

An early-ripening cultivar (CP70-324 (CP70)) and a late-ripening cultivar (TCP81-3058 (TCP81)) were used for the experiment. Plants were sampled from a 10 m long row in field plots at the Texas Agricultural Experiment Station Annex near Mercedes, Texas. At the start of this experiment, plants were in the first ratoon crop. Plots were fertilized at planting as recommended for local conditions, and irrigated every two weeks during the growing season if there was less than 25 mm of rain during that period. On 4 August 1993, 50 randomly selected stalks of each cultivar were flagged. The first internode from the top (TVD 1) was defined as that below the point of attachment of the leaf with the most recently exposed dewlap. On six dates at 3 to 4 week intervals TVD 3 was marked on each flagged stalk, and five of these marked stalks per cultivar were sampled by cutting at ground level. After determining total number of aboveground internodes and stalk length from the cut end to the topmost dewlap, the marked internode(s) were removed to the lab on ice. The experiment was repeated in 1994, with initial tagging, internode marking and stalk sampling occurring on 12 July. After the first sampling, TVD 2 was marked rather than TVD 3.

In the laboratory, the length and fresh weight of each section (internode + subtending node) were determined. The rind and node were discarded, and remaining storage tissue split into at least 3 lengthwise wedges of c 6 g fresh weight. One was stored at -80°C for later enzyme extraction, one was stored at -20°C for later sugar extraction, and one was weighed and freeze-dried to determine percent moisture.

Full details of enzyme extraction and assay procedures are available from the author. Soluble enzymes were extracted from thawed, diced sections by homogenizing the tissue for 1 min in about 15 mL extraction buffer (50 mM Hepes-KOH (pH 7.5), 0.05% Triton X-100 (w/v), 5 mM Na-EDTA, 10 mM MgCl₂, 20% glycerol (v/v), and 5 mM DTT). Extracts were filtered through cheesecloth, and 4 mL of the filtrate was centrifuged for 10 min at 4500 g. Three mL of supernatant was desalted through Pharmacia PD10 (Pharmacia Biotech, Inc., Piscataway, NJ, USA) columns preequilibrated with desalting buffer (25 mM Hepes-KOH (pH 7.5), 1 mM Na-EDTA, 10 mM MgCl₂, and 5 mM DTT). Sucrose synthase was assayed the same day.

Sucrose synthase activity in the cleavage direction was assayed at 34°C as described in Lingle & Irvine (1994), but the final UDP concentration was 2 mM, since this concentration gave maximum SS activity in preliminary tests. Protein was determined using the microplate Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA) protein assay, which is based on the dye-binding method of Bradford (Bradford 1976). Microplates were read at 620nm.

Sugars were extracted from thawed, diced tissue in 80% (v/v) aqueous ethanol. Total sugars in extracts were determined using a Dionex ion chromatograph (Dionex Corp., Houston, TX, USA) with pulsed amperometric detection as previously described (Lingle & Irvine 1994). Sucrose, glucose and fructose concentrations were determined by comparison of peak areas with those of external standards.

Data were analysed using PC-SAS (version 6.04, SAS Institute, Cary, NC). Nonlinear curves were fitted using the software program SigmaPlot for Windows (version 2, Jandel Scientific Software, San Rafael, CA). Total sugar accumulation over time was fitted to the growth curve

$$\text{total sugar} = a[1 - e^{b \cdot days}] + c$$

(Coale et al 1993). Sugar accumulation rate was calculated by subtracting the mean total sugar concentration for a particular internode on a particular date with the mean concentration on the next sampling date. These data were then fitted to an exponential decay equation:

$$\text{sugar accumulation rate} = a[e^{-b \cdot days}].$$

SS activity data were also fitted to an exponential decay equation.

RESULTS

There were few differences in stalk growth between the early (CP70) and late (TCP81) cultivars (Fig. 1a,b). On most sampling dates the two cultivars had the same number of internodes (Fig. 1a), but stalks of CP70 were usually longer than stalks of TCP81 (Fig. 1b). This suggests that internodes of CP70 were longer than those of TCP81. Stalks of both varieties elongated at approximately the same rate after the initial sampling dates in both years. Stalks sampled in 1994 were usually longer, and had slightly more internodes than those sampled the same period in 1993. The difference between years in numbers of internodes per stalk is probably due to differences in accumulated heat units between years (data not shown); 1994 was slightly warmer than 1993. However, heat units did not explain the differences in stalk length between years.
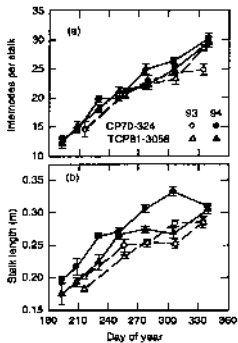
Fig. 1 *Number of internodes and length of stalks of an early cultivar, 'CP70-321', and a late cultivar 'TCP81-3058' sampled at intervals during 1993 and 1994. Vertical bars represent ±standard errors*

Internodes of CP70 initially accumulated total sugar faster than internodes of TCP81 (Fig. 2a,b), but the rate slowed earlier, and the concentration on the final sampling date in both years was lower in CP70 than TCP81 (Fig. 2a). The youngest internodes sampled had the highest sugar accumulation rate (Fig. 2b). These internodes had just ceased elongating (TVD 3). or had achieved about 80% of their final length (TVD 2; data not shown). The sugar accumulation pattern was
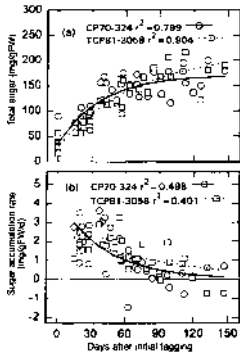
Fig. 2 *(a) Total sugar concentrations and (b) estimated sugar accumulation rates in sampled internodes of an early cultivar, 'CP70-321', and a late cultivar 'TCP81-3058' sampled during two years. Internodes were initially tagged as number 3 from top (1993) or number 2 from top (1994). Exponential growth curves were fitted for total sugar concentration data, and exponential decay curves were fitted to sugar accumulation rate data for both cultivars using data from both years combined.*

consistent with the ripening characterization of the two cultivars. There was no difference between cultivars in the change in sucrose/reducing sugar ratio with development (data not shown).

Specific activities of SS (Fig. 3) were highest in the most immature internodes sampled. Sucrose synthase activity was initially higher in CP70 than TCP81, but the activity declined more rapidly in the former cultivar. Although these differences were not statistically significant in either year, they were consistent between years. Sucrose synthase activity declined to a minimum about 30 days after initial sampling. The minimum was greater in TCP81 than in CP70. The large variability in SS activity at the initial sampling stage is due to the change in first sampling stage. In 1993 and the first sampling of 1994, internodes were first sampled at the TVD 3 stage, while in subsequent samplings, internodes were first sampled at the TVD 2 stage. TVD 2 internodes had higher SS activities than TVD 3 internodes.
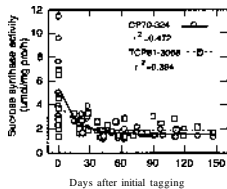
Fig. 3 *Sucrose synthase activity in sampled internodes of an early cultivar, 'CP70-321', and a late cultivar 'TCP81-3058' during 1993 and 1994. Exponential decay curves were fitted for both cultivars using data from both years combined.*

There were significant regressions between SS activity and estimated sugar accumulation rate (Fig. 4) in both years. Because the TVD 2 internodes sampled in 1994 were still elongating, and had significantly higher SS activities than the other internodes, the regression for TVD 2 was done separately. In TVD 2. although the regression was highly significant, a wide range of SS activity was associated with a small range of sugar accumulation rates. The relation between sugar accumulation rate and SS activity in older internodes was weaker. A narrow range of SS activities was associated with a wide range of sugar accumulation rates.
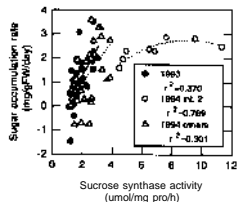
Fig. . *Relationship of sugar accumulation rate with sucrose synthase (SS) activity of internodes of an early cultivar, 'CP70-324', and a late cultivar, 'TCP81-3058', sampled during two years. Internodes were initially sampled as internode 3 (1993) or internode 2 (1994) from the top. Because of much higher SS activity in internode 2, this regression was done separately from the others in 1994. Regressions are linear (1993 and 1994 other) or quadratic (1994 internode 2).*

SUCROSE ACCUMULATION RATE, CARBON PARTITIONING AND EXPRESSION OF KEY ENZYME ACTIVITIES IN SUGARCANE STEM TISSUE

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ABSTRACT

There is a gradient of total sugar down the stalk of sugarcane, with the younger top internodes containing the lowest levels. The increase in total sugar to more than 50% of the dry matter was at the expense of the water insoluble fraction, and most importantly at the cost of the other water soluble components. Investigating the metabolic activity of thin sections off stem parenchyma *in vitro*, it was observed that the increase in sucrose content was as a result of an increased rate of sucrose deposition. Radiolabelling studies clearly indicated that the pattern of carbon allocation changed during maturation. The total cellular respiration increased between internodes 2 and 7. The highest rate of respiration therefore occurred in those tissues where the highest rate of sucrose accumulation was evident. An increase in respiration coincided with an increase in glycolytic enzyme activities. The measured sucrose hydrolytic activities (acid and neutral invertase) and cleavage activity (sucrose synthase) were higher than the maximum sucrose accumulation rates.

INTRODUCTION

In sugarcane, a gradient of sucrose content exists between the young and older internodes. In the immature top internodes sucrose content is low and a substantial pool of hexoses, predominantly glucose and fructose, is present. However, mature internodes can contain up to 50% sucrose on a dry mass basis, and at this stage very low levels of hexoses remain (Glasziou & Gaylor 1972; Celestine-Myrtil-Marlin & Ouensanga 1988; Lingle & Smith 1991).

Despite the many studies of sucrose accumulation in sugarcane, the biochemical basis for the regulation of sucrose accumulation is poorly understood (see review Moore 1995). A major factor contributing to this uncertainty is that the existing information is highly fragmented. It is not evident from the literature whether the increase in sucrose content on a dry mass basis occurs as a result of an increased accumulation rate of sucrose, or a decreased accumulation rate of other cellular constituents. Similarly, data on the expression levels of key enzymes are highly variable (Lingle & Smith 1991), and depending on whether activity is expressed on a tissue mass or protein content basis very different patterns are evident (Hatch et al 1962; Gaylor & Glasziou 1972; Lingle & Smith 1991).

Using radiolabelled hexoses, we have previously shown that maturation coincides with a redirection of carbon from water-insolubles and respiration to sucrose (Botha & Whittaker 1995). Consistent with this apparent decrease in flux of carbon towards respiration, the activities of most of the measured glycolytic enzymes expressed on a dry mass basis decreased from the young to older internodes (Botha & Whittaker 1995). This is apparently in contrast to the situation in sugarcane cell suspension cultures where between sucrose accumulation and respiration was found (Wendler et al 1990).

The apparent reduction in total respiration could be due to a change in regulation of a key reaction step in glycolysis, increased gluconeogenesis, inhibition of the TCA-cycle, altered gene expression or substrate availability. The mass-action ratios of PFK (ATP-dependent phosphofructokinase), FBPase (fructose-1,6-bisphosphatase), PK (pyruvate kinase) and HPI (hexose phosphate isomerase) in internodes 3 to 7 (Botha & Whittaker 1995) are consistent with those published for other tissues (Leegood & ap Rees 1978; Turner & Turner 1980). As in other tissues, the reaction steps of PFK, FBPase and PK are tightly regulated (Stitt 1989; Stitt 1990; Podesta & Plaxton 1994). No major changes in the regulation of any of these reactions occur in the sugarcane stalk as the tissue matures (Botha & Whittaker 1995).

However, the redistribution of the C^{14} within an internode, and a decrease in enzyme activity on a dry mass basis does not necessarily imply that

the total respiration rate decreases. A complication with evaluating metabolism and gene expression in sugarcane, is the large increase in the contribution of sucrose to the total dry mass, a large part of which is stored in the vacuole and apoplast. As a result, the contribution of the cytosolic compartment to total mass decreases as the internodes get older. Expression of cytosolic constituents on a dry mass basis might therefore give a very skewed picture. In preliminary trials, we found that the number of cells per internode reached a maximum early in internode development and that elongation of the internodes was predominantly as a result of cell elongation. Expression of metabolism on a internodal basis will therefore represent a measure of changes per cell. We investigated this possibility in the present study.

In this paper we report on the sucrose accumulation rate and expression of some key enzymes in primary carbon metabolism in the top ten internodes of sugarcane.

MATERIALS AND METHODS

All coupling enzymes, cofactors and substrates used for metabolite studies and enzyme assays were from either Boehringer Mannheim or the Sigma Chemical Company. The [U -^{14}C] glucose (2.07 G Bq/mmol) and fU - ^{14}C] fructose (2.0 G Bq/mmol) were from Amersham International. All other biochemicals and solvents were of analytical grade.

Mature, field-grown, non-flowering stalks, containing approximately 25 above ground internodes, of the variety, NCo376, were randomly selected and cut in the field during mid-summer. Twelve stalks were separated into internodes 1 to 10, and the material from each internode bulked for analysis. The internode attached to the leaf with the uppermost visible dewlap was defined as internode 1. Due to the limited material in internodes 1 and 2, enzymes were only extracted from internodes 3 to 10 as described by Lingle & Smith (1991).

Enzyme activity was assayed as described previously: PFP (pyrophosphate: fructose-6-phosphate phosphotransferase, Botha & Botha, 1990), PFK (Botha et al 1988), aldolase and PK (Moorhead & Plaxton 1988), UDPGlc pyrophosphorylase, Sowokinos et al 1993), and hexokinase (Nakamura et al 1992). Sucrose synthase (SS) was measured in the cleavage direction according to Hampp et al (1994) with the following modifications: 50mM Tris, 25 mM sucrose, 1mM UDP, and 0.03 IU UDPGlc dehydrogenase. For invertase, reducing sugars were assayed according to Huber & Akazawa (1986) after incubating extract in 125 mM sucrose and 25 mM Hepes (pH 7.1) for 3 h. The presence of possible enzyme activators and inhibitors was determined by preparing series of extracts each containing at least two different tissues (Botha & Small 1987). The measured enzyme activity

in these combined tissue extracts was 108± 14.0% (PFP), 109± 18.2% (PFK), 97.5 ± 7.7% (aldolase), 98.7 ± 25.5% (PK), 91.8 ± 1.8% (UDPGPPase), 95.5 ± 2.1% (hexokinase), 108 ±10% (SS), 93.5 ± 4.67% (neutral invertase), 108 ± 6.6% (soluble acid invertase) and 97.4 ± 8.6% (cell-wall bound invertase) of that when the tissues were extracted separately.

Sucrose and fibre contents were measured according to Bergmeyer & Bernt (1974) and Anon (1987) respectively.

Internodal tissue was excised with a cork borer with a diameter which removed all tissue except the rind. The tissue cylinder was then sliced into transverse sections of less than 1mm. The sliced tissue was washed for 1 h as described by Lingle (1989). Two grams of tissue per treatment were incubated in 2 mL 25 mM K-Mes buffer containing 225 mM mannitol and either 5uCi [U- ^{14}C] glucose or [U-^{14}C] fructose in 500 mL flasks with a centre well. Carbon dioxide was collected in 500uL of 12% (m/v) KOH. Incubation was terminated after 5 h, the **KOH** removed and the tissues washed for 30 min according to Lingle (1989). The tissues were frozen in liquid nitrogen, extracted and the chemical components analysed (Dickson 1979). Preliminary trials indicated that uptake of label is linear over the incubation period.

RESULTS AND DISCUSSION

Sucrose increased from the young to the older internodes (Fig. 1a). This occurred at the expense of both the total water-insoluble component (predominantly fibre) and the non-sucrose water-soluble fraction, which probably largely represented reducing sugars, amino acids and organic acids (Fig. 1a). In the immature internodes (2 and 3), the water-insoluble component (fibre) and the non-sucrose component represented almost 90% of total dry matter. It is the contribution of the latter to the total mass which decreased most. The rate of dry mass accumulation (total content/total age) increased linearly between internodes 2 and 10 (Fig. 1b). An increase in both the rate of sucrose and fibre accumulation was evident.

Accumulation rates of sucrose, fibre and dry mass were also calculated by using the change in content during the period in which a new internode was produced (dContent/dTime). From the data (Fig. 2), actual rates were evidently under estimated compared to when total age was used for calculation (Fig. 1b). Both the rates of total dry mass and sucrose accumulation increased exponentially (Fig. 2a,b). The contribution of sucrose to the increase in dry mass increased from 10% in internode 1 to more than 50% in internode 10 (Fig. 2c).

The above results confirm the well described gradient in sucrose down the stalk of sugarcane (Glaszriou & Gaylor 1972; Lingle & Smith 1991). However, contrary to the general belief that sucrose accumulation is at
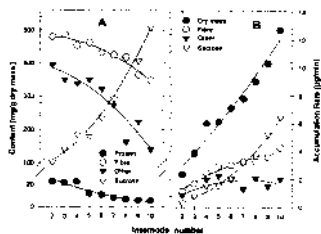
Fig. 1 Changes in (a) the concentration of the four main components of total dry matter and (b) the accumulation rates of dry mass, fibre, sucrose and other water soluble components, for internodes 2 (youngest) to 10.

the expense of fibre (the water-insoluble component), our data indicated that a redirection of carbon from other water-soluble components significantly contributed to sucrose accumulation.

The distribution of the ^{14}C from labelled glucose into fibre and sucrose (Table 1) was consistent with the calculated accumulation rates (Fig. 1b). Evidently, the more mature internodes had a higher capacity to mobilize glucose and to synthesise sucrose. The data also suggest that the total respiration per cell, between internodes 3 to 7, was fairly constant whilst sucrose labelling doubled (Table 1). Using the specific activity of the endogenous glucose pool, we calculated a potential flux through the glycolytic pathway. For this, we assumed that all the released CO_2 was coming from glucose, that it represented complete oxidation of the substrate, and that all the carbon skeletons were mobilised through glycolysis. These values for glucose flux ranged from 14 to 28 nmoles/min/internode. When the total radioactivity in all the water soluble components, excluding sucrose and fructose, are used in the calculation, the observed flux ranges between 210 and 489 nmoles glucose min^{-1} internod^{-1}.

Some of the glycolytic enzymes were measured and total enzyme activity per internode was calculated. This also reflected the total enzyme activity per cell. Total hexokinase, PFP, PFK, PK, UDPGPPase, aldolase, cell wall bound active invertase and neutral invertase activities increased between internodes 3 and 10 (Fig. 3). Fructokinase and sucrose synthase activities
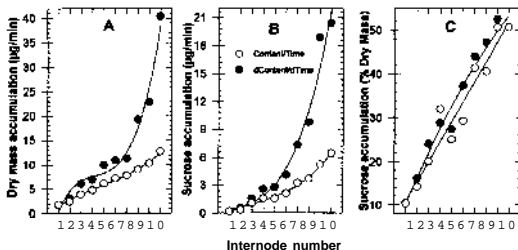
Fig. 2 Changes in (a) dry mass accumulation rate, (b) sucrose accumulation rate and (c) contribution of sucrose accumulation to total dry mass accumulation for internodes 2 (youngest) to 10. [Accumulation rates were calculated by mass accumulated per time (open symbols) and as the change in content during the period in which the last new internode was produced (closed symbols)].

Table 1 *Distribution of ^{14}C labelled carbon recovered from sugarcane internodes 2 (youngest) to 10. Tissue slices were fed with [U-^{114}C]glucose for 5 H (see text for details).*

Fraction	Label distribution in internodes (kBq per internode)			
	2	3	5	7
Water insoluble (fibre)	44.1	85.3	120.1	60.3
Water solubles				
-sucrose	36.9	143.3	225.0	302.0
-glucose	3.8	13.4	21.4	28.8
-fructose	2.8	5.3	6.9	7.7
-amino acids, sugar-P organic acids	21.7	18.2	67.1	41.3
Pigments and oils	0.9	3.3	6.8	9.4
CO_2	10.3	16.4	20.9	18.1

remained constant with the latter showing a slight decrease in internode 10. Others have also reported significant levels of SS activity (Lingle & Smith 1991: Buczynski et al 1993; Lingle & Irvine 1994) and neutral invertase (Hatch et al 1962; Gaylor & Glasziou 1972; Lingle & Smith 1991).

Internode number
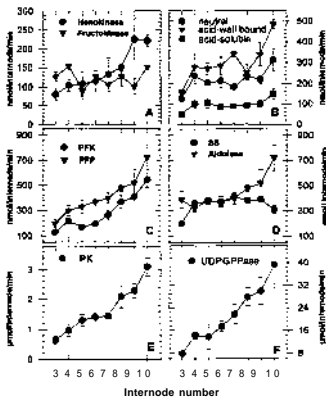
Fig. 3 *Changes in the total activity of (a) hexokinase and fructokinase, (b) acid and neutral invertases, (c) PFK and PFP, (d) SS and aldolase, (e) PK and (f) UDPGPPase for internodes 2 (youngest).*

The measured invertase activities (acid and neutral), and sucrose synthase activity, exceed the maximum rate of sucrose accumulation in the internodes. This was surprising because very low levels of glucose and fructose were present in the mature tissue. Both SS and neutral invertase activities might be exclusively in the cytosol, a compartment which might contain very little sucrose. However, the soluble acid invertase would be present in the vacuole where most of the sucrose is stored and the observed activity was adequate to hydrolyse most of the sucrose. This phenomenon is currently under investigation.

The apparent increase in the activity of the glycolytic enzymes (PFK, PFP, aldolase) and the two hexokinases in the older internodes (Fig. 3) might be necessary as the calculated flux indicates that enzyme levels might be limiting.

CONCLUSIONS

Collectively, these results indicate an increase in sucrose content in the more mature (older) internodes at the expense of fibre and other water-soluble components. Although proportionally less carbon was allocated to respiration in the mature internodes, total cellular respiration increased from the immature to mature internodes. This might be required to provide energy for the accelerated sucrose accumulation. How sucrose accumulation is maintained in an environment where high hydrolytic and cleavage activities are present is unclear. At least this might imply that high rates of cycling occur between sucrose and the hexose-phosphate pools as is suggested to occur in sugarcane cell suspension cultures (Wendler et al 1990).

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STRUCTURE AND EXPRESSION OF SOLUBLE ACID INVERTASE GENES IN THE STEM OF HIGH- AND LOW-SUCROSE ACCUMULATING *SACCHARUM* SPECIES AND HYBRIDS

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ABSTRACT

Partial cDNA clones encoding acid invertase have been isolated from Molokai 5829 (Mol 5829), a low sucrose accumulating Saccharum robustum and H65-7052, a high sucrose accumulating sugarcane hybrid cultivar. Comparisons of these sequences to other plant invertase sequences indicate that these genes encode soluble acid invertase (SAI) isoforms. Peptide sequences deduced from these partial nucleotide sequences do not identify obvious areas of structural difference between the cDNA clones. RNA gel blot analysis of Mol 5829 and Louisiana Purple (LAP), a high sucrose accumulating S. officinarum reveals both developmental and genotype specific patterns of SAI expression. Both genotypes have maximum acid invertase mRNA pools in stem apices which diminish as internodes mature. In all internodes compared, acid invertase mRNA pools of the low sucrose Mol 5829 are substantially larger than those in the high sucrose LAP. A comparison of SAI activity and acid invertase mRNA pools in the stem apices of the parents and seven progeny of an LAP X Mol 5829 cross show a positive correlation. This data suggests that higher levels of SAI activity found in stem internodes of low sucrose genotypes may result from higher levels of SAI gene expression, particularly in the stem apex and younger internodes.

INTRODUCTION

Like most plants, sugarcane exports photosynthates as sucrose from source leaves to growth or storage sinks. Many plants convert the transported sucrose to starch in the storage sinks for long-term storage. In contrast, sugarcane (the sucrose-storing hybrids of *Saccharum*) uses sucrose not only for transport, but also for storage. This sucrose does not always move unaltered from phloem to storage parenchyma cells, but rather may undergo considerable metabolic processing, which differs among internodes (storage sinks) of different age. The wild species of *Saccharum* do not accumulate high levels of sucrose, but synthesize a higher percentage of fresh weight as fiber (Bull & Glasziou 1963) so metabolic processing of sucrose in these genotypes differs from both starch-storing plants, and from high sucrose-storing sugarcanes.

In the young growing internodes of sugarcane the sucrose transported from source leaves is cleaved to provide hexoses for biosynthetic processes, respiration and metabolism. Simultaneously, sucrose is resynthesized from the residual hexoses and recycled through the storage compartment. In old mature stem internodes there is an overall decrease in enzyme activities. However, there is a greater decrease in the activity of sucrose-hydrolysis enzymes than of sucrose-synthesis enzymes and this allows sucrose to remain in storage. In an intermediate aged internode, one which has just achieved full expansion and is rapidly accumulating sucrose, both sucrose-synthesizing and -hydrolyzing enzymes are active. In one model of this process, photoassimilates are continuously cycled as sucrose is cleaved only to be synthesized again (Wendler et al 1990). The net difference between synthetic and hydrolytic activities is reflected in the rate of sucrose accumulation. Therefore, if the rate of sucrose turnover or cycling is high, then small differences in the activity of one or more enzymes could result in substantial differences in sucrose accumulation. Determining whether sucrose accumulation is the result of small differences in enzyme activity is difficult, in part because enzyme activities vary significantly among genotypes and for the same genotype growing under different environmental conditions. Despite these difficulties, recent work (Zhu et al 1996) has identified SAI as having a key role in sucrose accumulation in the sugarcane stem. In this study, sucrose concentrations of sugarcane internode tissue in high or low-sucrose clones, and the activities of enzymes involved in sucrose metabolism, including SAI, neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) were determined. A significant negative relationship between SAI activity and sucrose accumulation was observed.

Most researchers agree that plant cells contain soluble acid invertase isozyme(s) in the vacuole, and insoluble, or cell wall-associated invertase isozyme(s) (CWI) in the apoplast. Some workers (Moore 1995 and references therein) argue there is additionally a soluble acid invertase in the apoplast. If a soluble apoplastic invertase exists, this activity and that of vacuolar soluble acid invertase would be combined in most enzyme studies such as Zhu et al (1996), where invertase activity assays are performed only on soluble and insoluble fractions of tissue homogenates. This is a possible source of confusion in understanding the role of invertase isozymes in sucrose accumulation, as vacuolar and apoplastic SAI activities may change in very different ways during plant development. Furthermore, in hybridization studies of invertase mRNA pools, it is likely that moderately long cloned sequences used as probes would hybridize to both SAI and CWI mRNAs, as many regions of these genes may be highly similar. For example, nucleotide sequence of a maize SAI (Xu et al 1995) is 65% identical to a maize CWI (Shanker et al 1995) over 1600 bp, and some 100 bp regions exceed 80% identity. In previous work (Sacher et al. 1963)and in the population studied above (Y Zhu & E Komor, unpublished results). CWI activity in internodes was very low compared to SAI. If this low CWI activity is a reflection of low CWI mRNA pools, then it may be that trends observed for this combined (SAI and CWI) acid invertase mRNA pool are essentially correct for SAI mRNA. Nucleotide sequences for neutral (or "alkaline") invertases have not yet been published, however partial peptide sequence from a carrot neutral invertase indicates these enzymes are unrelated to acid invertases (Sturm et al 1996). Cross hybridization of SAI and NI nucleic acids is unlikely.

Within the limitations imposed by these possible sources of confusion, we intend to determine whether the different levels of SAI enzyme activity seen in high- and low-sucrose accumulating sugarcane genotypes are the result of differential expression of essentially identical genes, or whether there are structural differences among these genes which may account for substantial differences in their specific activity. We have cloned several SAI partial cDNAs from high and low- sucrose sugarcane genotypes and are sequencing these to identify potentially important structural differences. We are also using these clones as probes in RNA gel blot analysis to estimate the SAI mRNA pool size in different internodes of the same lines used for the enzyme activity study described in Zhu et al (1996).

MATERIALS AND METHODS

Plant material

Experiments reported were conducted on the parents and progeny of a cross between the female parent LAP (*S. officinarum*, 2n=80) and the male parent Mol 5829 (*S. robustum*, 2n=80). The siblings of the family were recognized as cross progeny since all progeny exhibited hairy leaf sheaths characteristic of Mol 5829 and lacking from LAP.

Approximately 110 progeny and parents were grown in replicated plots in two different environments. RNA was extracted from the parents and seven progeny lines which had been analyzed for sucrose accumulation and enzyme activities (Zhu et al 1996), and also from H65-7052, a high-sucrose hybrid cultivar. Internode numbering was done as described in (Moore 1987), such that numbers increase down the stem, with the internode subtending the top visible dewlap designated 1.

RNA extraction and cloning of invertase cDNAs

Internode tissue samples were ground to a fine powder with a mortar and pestle under liquid nitrogen. Total RNA was extracted by the method of Bugos et al (1995). cDNA was made from total RNA with Superscript II (Life Technologies) reverse transcriptase at 42° or 50° per suppliers instructions. 1/20 of the cDNA product was used as template for PCR amplification. Initially, a 0.6kb fragment (SCINVH, see Fig. 1) within the mature peptide coding region which starts at the conserved NWMNDP sequence and ends at the putative active site TGMWECVDF, was isolated. New primers were synthesized based on the sequence of the initial clone, and these were used to produce 3' RACE clones (SCINVH3'2, Fig. 1) starting immediately downstream of the active site, and 5' RACE clones (not full-length) which extend upstream of the putative amino terminus of the mature peptide (SCINVM5'33, Fig. 1). Clone SCINVM30 (Fig. 1) contains the entire coding sequence of the mature protein.

mRNA ~2.5kb
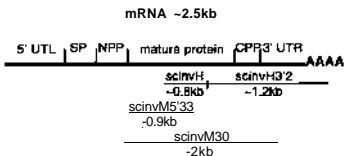
Fig. 1. Sucargane SAI mRNA and cDNA clones.
Schematic diagram of a plant soluble acid invertase mRNA and cDNAs which have been isolated, showing their relative positions (not to scale). UTL=untranslated leader, SP=signal peptide, NPP=amino terminus pro-peptide, CPP=carboxy terminus propeptide, UTR=untranslated

RNA gel blots

20 (mg total RNA per lane was size fractionated on 1.2% agarose formaldehyde denaturing gels (Fourney et al. 1988) and transferred to positively charged nylon membranes (Amersham). Probes were ^{32}P labeled by the random primed method (Feinberg & Vogelstein 1983). Hybridization and washes to high stringency were carried out by the method of (Virca et al 1990). The cDNA clone SCINVH3'2 (Fig.1) was used as a probe template; this clone contains numerous regions wherein the nucleotide sequence of both SAI and CWI genes from other species are highly similar. It is likely that this probe hybridizes to both SAI and CWI mRNA.

Quantitation of SAI mRNA pools involved several steps: OD[260] was used to calculate total RNA concentration and equal amounts (20 *mg*) of each sample were loaded. Gels were stained with ethidium bromide and visually inspected for equal fluorescence of ribosomal RNA bands. Kodak X-Omat RP XRP-5 film was preflashed to an OD[M0]to maximize linearity of response in autoradiography. Image analysis of autoradiograms to integrate pixel OD values was performed using Image-Pro Plus II software.

RESULTS AND DISCUSSION

Partial sequence analysis of the cDNA clones which have been isolated show that the sugarcane genes are highly similar to other plant invertases which have been sequenced. For example, sequence from the 5' end of SCINVH3'2 (Fig. 1) is 75% identical to amaize gene encoding a soluble acid invertase (Xu et al 1995), and 65% identical to a maize cell wall invertase (Shanker et al 1995). Comparison of peptide sequences deduced from plant invertase genes reveals regions of specificity for soluble acid invertases vs cell wall invertases. For example, plant soluble acid invertases share the consensus sequence WSNAMLQWQ near the amino terminus of the mature protein (Fig. 2). Cell wall invertases do not share this consensus. Similarity analysis of this type indicates that the cDNA clones isolated so far probably encode soluble acid invertases.'

RNA gel blots show acid invertase mRNA pools in apices of both *Saccharum* parents, which decline with internode maturation. Mol 5829 mRNA pools were substantially larger than LAP pools in all tissues tested, but particularly in the apex and internode 2 (Fig. 3). Visual inspection of acid invertase RNA gel blot autodiagrams and acid invertase activity from the apices of the two parents and seven progeny suggests a positive correlation (Fig. 4). Relative SAI mRNA pool sizes were estimated by integrating pixel optical density values from the RNA gel blot autoradiogram. Regression analysis of the estimated SAI mRNA pool sizes and enzyme activities from this single experiment yield a correlation coefficient of approximately 0.8. Experiments are underway

Fig. 2. Peptide consensus sequence specific for SAI.
Pileup (Genetics Computer Group) alignment of plant acid invertase deduced peptide sequences (partial). CW=cell wall, S= soluble. Sequences above the dotted line are cell wall (acid) invertases, below the dotted line are soluble acid invertases. The consensus sequence present in SAI but not in CWI is underlined with a solid line.

to replicate these results, analyze commercial hybrids, expand the progeny analysis to other internodes, and to more precisely quantitate RNA gel loading.
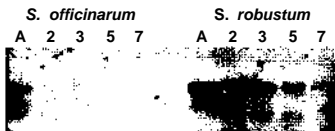
Fig. 3. *SAI RNA gel blot.*
RNA gel blot hybridized to scinvH3"2 cDNA probe. A =apex, numbers indicate internode number, with internode subtending the top visible dewlap = 1.
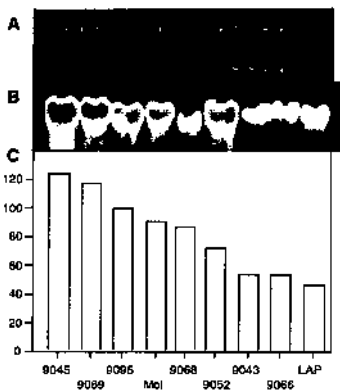
Fig. 4. *Acid invertase mRNA pools and SAI activity in stem apices of parents and seven progeny of S. officinarum (LAP) X S.robustum(Mol 5829) cross.*

A) *Elhidium bromide staining of ribosomal RNA bands in formaldehyde denaturing gel showing approximately equal loading.* B) *Autoradiogram of ~2.5kb region of blot from above gel following hybridization with SCINVH3'2 probe.* C) *Soluble acid invertase activity (jl moles/min/g protein)*

CONCLUSIONS

Analysis of enzyme activity and sugar content in parents and progeny of a high sucrose-accumulating by low sucrose-accumulating *Saccharum* cross have indicated a strong negative correlation between SAI activity and sucrose accumulation in stem internodes (Zhu et al 1996). Differences in SAI activity could result from structural (hence specific activity) differences in the proteins, or from differences in the quantity of SAI present. SAI quantity differences could reflect differences in protein turnover rates, or differences in SAI gene expression. Our work here follows on that above with a study of sugarcane SAI gene structure and expression. Several partial SAI

cDNAs from Mol 5829, the low sucrose parent in the study population, and from H65-7052, a high sucrose accumulating hybrid cultivar have been isolated. In most of the regions which have been sequenced, the SAI genes of both varieties are highly similar; no obvious differences which might account for differences in specific activity have been identified. RNA gel blot analysis of Mol 5829 and LAP shows that while both the high- and low- sucrose variety have similar developmental patterns, the mRNA pools in a given internode differ substantially. Both parents have relatively high levels of SAI mRNA in the apex, which declines with internode maturation. The SAI mRNA pool in the low sucrose parent, however, starts significantly higher than in the high sucrose LAP, starts to decline later, and stabilizes at a higher level. The developmental time course of acid invertase mRNA accumulation in two commercial hybrids grown in Australia differs, with little accumulation in the stem apex and youngest internodes, increasing to a maxima in internodes 3 or 4 (Peters et al 1996). Whether this reflects a difference between the varieties tested, or a difference in Hawaiian versus Australian growing conditions has not yet been determined.

Preliminary analysis of acid invertase mRNA pools in apices of LAP and Mol 5829 and several progeny plants reveals a positive correlation between mRNA pools and acid invertase enzyme activity. Together this data indicates that the higher levels of SAI activity in low sucrose varieties is due, at least in part, to higher levels of SAI gene expression.

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PATHWAY OF SUCROSE UNLOADING FROM THE PHLOEM IN SUGARCANE STALK

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ABSTRACT

The physical path of sucrose unloading in the sugarcane stalk is described. About 50% of the vascular bundles in the internodes were located within 3 mm of the outside of the stalk. These bundles were inactive in long distance sucrose transport, as assessed by dye tracers of phloem flow. A sheath of fibres isolated the phloem apoplast from that of the storage parenchyma. In those bundles associated with long distance transport (i.e. in the central region), the fibre sheath was narrowest to either side of the phloem fibre cap, and consisted of living cells with plasmodesmata within pits in the secondary wall. The flux of sucrose into an internode was calculated to be consistent with a symplastic unloading path through this narrow region of the fibre sheath, within vascular bundles of the central portion of the stalk. Plasmodesmata were arranged into pit fields between cells of the storage parenchyma. Sucrose unloading within the storage parenchyma is suggested to follow a symplastic route, with unloading into the apoplast of the storage parenchyma occurring as part of a turgor mechanism to increase sink strength.

INTRODUCTION

Understanding the pathway of sucrose movement from phloem to storage tissue in the sugarcane stem is integral to the study of control, and subsequent genetic modification, of sucrose unloading and storage in the sugarcane stem (e.g. J.W. Patrick, in Wilson 1992). This pathway may be through the symplast and/or apoplast. The symplastic pathway involves movement directly from cell to cell via plasmodesmata. The apoplastic pathway involves movement via the cell wall space, widi carriers on the cell membrane responsible for loading and unloading between the cell and the apoplast. Sugarcane stem contains high levels of sugars in the apoplast, leading Hawker & Hatch (1965) to suggest that sucrose was unloaded from the phloem complex directly into the apoplast, with uptake of inverted sugars from the apoplast by the storage parenchyma cells. However, the vascular bundles (VB) of the cane stalk are surrounded by a sheath of fibre cells, which serve to isolate the xylem water from the apoplast of stalk storage tissues (Jacobsen et al 1992; Welbaum et al 1992). Welbaum et al (1992) observed plasmodesmata between all cell types in the pathway from phloem to storage parenchyma and suggest that sucrose must follow a symplastic path, with subsequent release into the apoplast of storage parenchyma.

In this report, we quantify the flux rate of sucrose through the plasmodesmata of the fibre sheath of the VB, and compare this rate to other published reports of flux through plasmodesmata to assess the potential of the symplastic pathway to support observed rates of sugar accumulation in the cane stalk.

MATERIALS AND METHODS

Saccharum officinarum L. (var. Q124) plants were maintained on the CQU Rockhampton campus, fertilized monthly with Greenland 44 (equivalent of 300 kg N/ha/yr). Stalk material (three stems) was harvested at monthly intervals from March to August. 1994. At each harvest, internodes were juiced, and soluble sugar content estimated in a 1:5000 dilution by the anthrone method (Yem & Willis 1954), using sucrose as a standard. Phloem transport was assessed by monitoring movement of the phloem-mobile, membrane-impermeant dye, 5,6 carboxyfluorescein (CF), applied in the membrane-permeant diacetate form. Dye was applied to an abraded area (15 cm²) of the leaf, and covered with parafilm to limit evaporation. Feeds (n = 4) were undertaken to the fourth leaf (counting from the top visible dewlap) using separate stalks. After 2 h, each stalk was harvested and transverse hand sections of the leaf lamina, sheathing base and internodes were prepared for observation by epifluorescence microscopy, using blue excitation wavelengths. To allow counts of VB within the stalk, transverse hand sections of all internodes were stained with 1% (w/v) phloroglucinol.

Tissue (c. 1mm³) containing VB was dissected from a depth of at least 5 mm within internode 4 (stem diameter 32 mm) and fixed for 2 h at 4°C, in phosphate buffered 2.5% glutaraldehyde containing 0.6 M sucrose (pH 7.2), post-fixed in 2% osmium tetroxide for 2 h, then dehydrated in an acetone series, embedded in LR White resin, and sectioned. Serial 2 pm thick sections were stained for suberin and lignin (Bundrett et al 1988). For electron microscopy (EM), gold coloured sections were cut using glass knives, collected on Formvar-coated copper grids, and stained with 2% uranyl acetate for 30 min. then 0.2% lead citrate for 5 min. Electron micrographs were taken with a Jeol 100C transmission electron microscope.

RESULTS

Stalk sugar content increased as winter progressed, with first the lower internodes, and then upper internodes, accumulating a maximum of 14 % soluble sugar (sucrose equivalents, FW basis). The maximum rate of sugar accumulation occurred in the fourth internode below the node of the first visible dewlap leaf during the month of June (internode 4 became internode 7 during this period) (Table Items 1-4).

Table 1   *Calculation of rate of sucrose flux through plasmodesmata (pd) of the phloem fibre flank of the bundle sheath of vascular bundles of* Saccharum officinarum *L. var. Q124.*
[Data are presented of the fourth internode below the top visible dewlap leaf. This internode was estimated to have the highest rate of sucrose accumulation. Data presented as mean with associated s.e.m., with n = 3 (separate canes) for Items 1-5, and n = 4 (vascular bundles, VB) for Item 6. Calculation rationale is presented in text.]

Item	Value	Unit
1. Sucrose accumulation	2.71 ±0.18	mg.g⁻¹FW.d⁻¹
2. Internode fresh weigh	29.1 +3.2	g
3. Sucrose accumulation per internode	79 ± 9	mg.internode⁻¹.d⁻¹
4. Internode length	50.5 ±4.3	mm
5. Number of VB in the stalk central region	421 ± 80	internode⁻¹
6. Phloem fibre shoulder perimeter	120	mm. vascular bundle⁻¹
7. Surface area of fibre shoulder perimeter	2551	mm².internode⁻¹
8. Pit frequency	0.008	pits.urn⁻²
9. Plasmodesmata (pd) per pit field	42	pd.pit⁻¹
10. Bundle sheath pd per internode	8.6 × 10⁸	pd. internode⁻¹
11. Sucrose flux through bundle sheath pd	0.011	pmol.pd⁻¹h"
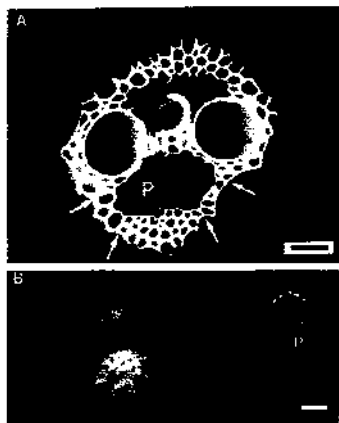
Fig. 1 *Transverse hand-section of vascular bundles in fresh internode 4 tissue of sugarcane stalk. (A) Section was stained with berberine sulphate and aniline blue, mounted under ferric chloride in glycerine, and viewed under blue excitation, epifluoresence microscopy. Berberine sulphate is a stain of suberin and lignin. There is only a single layer of fibre cells in the phloem 'shoulder' region (between arrows). Sucrose movement from phloem (P) to storage parenchyma is suggested to follow a symplastic path through this region. (B) Following a carboxyfluorescein feed to the leaf, tracer was present in the phloem (P) of scattered vascular bundles in the central region of the stalk. Sections were not stained, with autofluorescence of fibres and xylem tissue. In both panels, scale bar represents 50 m/m.*

About one-half of VBs (i.e. about 400) noted in internode cross section were tightly packed around the periphery (outer 3 mm) of the stem, with the remainder relatively widely scattered in the remaining 'central'region (Table 1, Item5; also Jacobsenetal 1992). In the central region where VB density was lower, about 10 storage parenchyma cells separated the bundles. The density of VB per unit area, and number of VB per internode, did not vary greatly with internode number (data not shown). All VBs possessed a complete sheath of thick-walled, living (as judged from the presence of cytoplasmic contents) fibre cells (Fig. 1A), although in VBs of the peripheral region this sheath was much more extensive. Following feeding of CF to leaf 4, tracer was noted in leaf VB, and in central VB of internodes subtending the leaf (Fig. 1B). The fibre cell walls were about 2 urn thick and were lignified or suberised, as indicated by berberine sulphate staining. In the VBs of the central region the sheath was characterised by a fibre cap directly 'above' the phloem (ie. towards the epidermis), but was reduced to a layer only about two cells deep on the two flanks of the phloem fibre cap (Fig. 1A). The perimeter of VB fibre sheath in the phloem flank position was measured off SEM visualised transverse sections and multiplied by internode length to give area (Table 1, Item 6,7).

The VB fibre cells were linked to adjacent cells through plasmodesmata located within large pits (up to 1 mm diameter) (Fig.

2A,B). Pit frequency in the fibre cells of the phloem shoulder - storage parenchyma interface was measured of resin sections using
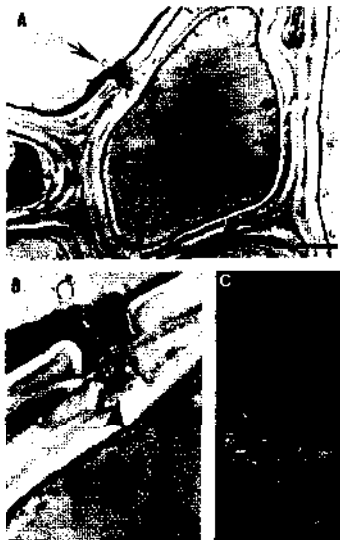
Fig. 2 *TEM images of plasmodesmata in sugarcane stalk tissue. (A) Resin embedded material. Sclereid cell in the phloem 'shoulder' region of a vascular bundle, showing a pit in the secondary wall (arrow). Scale bar represents 2 mm. (B) Detail of pit as shown in (A), illustrating plasmodesmata through the primary cell wall. The section is oblique to the direction of the pit-pair, such that the connecting pit in the secondary wall of the lower cell (arrowhead) is not completely illustrated. (C) Freeze fracture replica of cell wall surface between two storage parenchyma cells. Plasmodesmata are grouped into pit fields. Scale bars in panels B and C represent 500 nm.*

light microscopy (Table 1, Item 8). Plasmodesmata were observed at all cell interfaces in the phloem to storage parenchyma pathway, and were clustered into pit fields of about 50 plasmodesmata between storage parenchyma cells (e.g. Fig. 2C; Table 1, Item 9). Plasmodesmata were typically constricted in the region of the connection to the cell membrane (diameter 32 ± 2.7 nm, n= 10), and dilated within the cell wall region (diameter 64 ± 1.4 nm. n= 12) (cf. 37 nm diameter of straight channelled plasmodesmata in soybean nodule vascular endodermis. Brown et al 1995. As it was not possible to reliably section through the pit fields of the VB fibres, it was assumed that the plasmodesmatal frequency in these pits was as measured of the storage parenchyma pit fields. The number of plasmodesmata between the phloem flank fibres and the storage parenchyma for internode 4 (Table 1, item 10) was calculated by multiplying surface area of the phloem flank fibres, pit frequency in these fibres, and the estimate of plasmodesmata per pit (Table 1, Items 7,8,9).

Finally, the rate of sucrose flux through the plasmodesmata of the phloem fibre flank was estimated by dividing the assessed maximum

rate of sucrose accumulation per internode, adjusted from mass to molarity, by the estimated number of plasmodesmata per internode (Table 1, Item 11 = Item 3 / Item 10).

DISCUSSION

We conclude that the VBs in the peripheral region of the stem strengthened the stalk (Wilson 1990), but were not active in long distance phloem transport. This transport role was served by VBs of the central region. VBs were surrounded by a fibre sheath with lignified and/or suberised cell walls which should isolate the phloem apoplast from that of both the xylem and storage cell parenchyma. This observation is consistent with the report of Jacobsen et al (1992) and Welbaum et al (1992) that xylem water was isolated from the apoplast of storage tissue. Thus, sucrose cannot travel a strictly apoplastic path between the phloem complex and storage parenchyma.

All cells in the pathway from phloem to storage parenchyma in VBs of the central region were alive and connected by plasmodesmata, as observed by Welbaum et al (1992). Sucrose may thus move symplastically from the phloem, through the fibre sheath, and throughout the storage parenchyma. Storage parenchyma cells were well interconnected by plasmodesmata, such that the rate of sucrose flux per plasmodesmata in this tissue would be much lower than that in the fibre sheath. The fibre sheath around each VB of the central region was reduced to only 1-2 cells adjacent to the phloem fibre cap. We surmise that the majority of sucrose flux from the phloem to the storage parenchyma will pass through this section of the fibre sheath, as a path of least resistance. The maximum flux of sucrose through the plasmodesmata (pd) of these fibre cells was estimated at only 0.011 pmol/pd/h (Table 1). This rate is well within the range of rates reported in the literature for movement through plasmodesmata (e.g. Brown et al 1995 report a flux of 1.06 pmol sucrose/pd/h through the endodermis of soybean nodule VB). This calculation suggests that the 'plumbing connection' as represented by the symplastic pathway is not rate limiting to the storage process. However, evidence is accumulating that plasmodesmata are not simple channels between cells. For example, viral movement proteins, hypothesized to be homologues of a class of plant proteins, act to dilate plasmodesmata (Lucas et al 1993). The plasmodesmata observed in cane material were characterized by a narrow neck at each end, which may represent a control point for flux.

The control of the storage process is ill-defined, but given a symplastic path of unloading, two approaches to increased sucrose storage have merit. The incorporation of a viral movement protein into tobacco altered carbohydrate partitioning (Lucas et al 1993), and this strategy could be applied to sugarcane. Alternatively, Patrick (1990) has proposed that the sink cell regulates its turgor, and thus the pressure gradient driving phloem import, by controlling unloading to the apoplast. Indeed, Moore & Cosgrove (1991) document constant turgor in cane storage cells during sugar accumulation. Partitioning to storage may thus be improved by increasing sucrose unloading to the parenchyma apoplast by elevating the levels of transport proteins on the storage cell membrane. The gene for a sucrose carrier has been identified, with antisense expression achieved in potato leaves (Riesmeier et al 1994).

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3.3 Molecular modification of metabolic processes

POTENTIAL FOR INCREASING SUGAR PRODUCTIVITY THROUGH BIOTECHNOLOGY IN MAURITIUS

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ABSTRACT

During the last decade, sugar production in Mauritius has faced constraints such as unfavourable climatic conditions, increasing cost of labour, loss of land under sugarcane to urbanization, and competition from new industries. To meet the requirement of some 0.65Mt sugar, the country has to adopt new technologies to increase production efficiency per unit area. Biotechnology is expected to play a major role in increasing this efficiency. New techniques of disease diagnosis, such as monoclonal antibodies, DNA probes and the polymerase chain reaction should enable increased sensitivity, speed and reliability of pathogen detection. Monoclonal antibodies and DNA probes have been produced for the diagnosis of the gumming disease pathogen (Xanthomonas campestris pv. vasculorum). These techniques are also being used to study leaf scald bacterium (Xanthomonas albilineans), to differentiate the African and Mascarene serotypes that exist in Mauritius, and their use will be extended to other pathogens. These new diagnostic tools will be important in the safe international movement of germtpasm, in the characterization of pathogen variants and in epidemiological studies to allow more efficient control measures to be formulated. Another advance is expected through the in vitro culture of sugarcane and the micropropagalion of new varieties using this method on a large scale. Coupled with new diagnostic techniques, tissue culture will speed the release of varieties free from diseases to the planting community. Genetic fingerprinting of varieties, using restriction fragment length polymorphism (RFLP) analysis and random amplified polymorphic DNA (RAPD), is being carried out. This should allow a better choice of parents for crossing and hence make the breeding programme more efficient. Molecular markers associated with two important fungal diseases, rust (Puccinia melanocephala) and yellow spot (Mycovellosiella koepkei) by the RAPD technique are being sought. This approach will aid the rapid screening of varieties at an early stage in the selection programme, with the aim of producing varieties specifically adapted to the wet uplands of Mauritius, where resistance to both rust and yellow spot is essential.

INTRODUCTION

Sugarcane is the most important crop in Mauritius, contributing about 30% of the island's gross export earnings and providing more employment than any other industry. Sugarcane is well adapted to the local soil and climatic conditions and tolerates cyclonic winds. Such characteristics have brought about its extensive cultivation for more than a century, and Mauritius is one of the world's most efficient producers. Presently, the sugar industry is facing several difficulties : a rapid loss of cane lands to urbanization; scarcity of labour; increases in production costs; low prices on the world market; and competition with new industries. Despite these difficulties, the industry has the will to increase its sugar productivity. Several measures have been proposed to meet this objective. Biotechnology will play an important role in providing better varieties, and biotechnology has been identified as one means that could contribute to increased sugar production. Various aspects of biotechnology can be applied to sugarcane. These include tissue culture, genetic transformation, improved disease diagnostic tools and application of molecular markers to plant breeding. This paper reports some of the biotechnology approaches being followed by the Mauritius Sugar Industry Research Institute (MSIRI).

TISSUE CULTURE

At least one new improved variety is released annually in Mauritius. At release, the material available in nurseries is often inadequate to supply all planters willing to exploit the variety immediately. Micropropagation by *in vitro* culture of new varieties would help to obtain plantlets rapidly for large scale cultivation and also ensure clean, disease-free material (De Boer & Rao 1991). Hot water treatment of setts prior to the establishment of nurseries will also not be required. After identifying promising varieties, cultures are established *in vitro* from apical buds and meristems and at the final stages of selection, they can be rapidly bulked. At MSIRI with two laminar flow cabinets, one growth room, glasshouse space, two technicians and one scientist, we can produce 150,000 plantlets of a new variety in 8 months, according to the scheme described in Fig. 1. This material will be sufficient to plant 10 ha of nurseries. These facilities may be expanded to produce more material. Further research is needed on the conditioning and transplanting of plantlets into commercial fields.

Through tissue culture techniques, sugarcane calli can be produced for genetic transformation by bombardment with a biolistic gun (Bower &
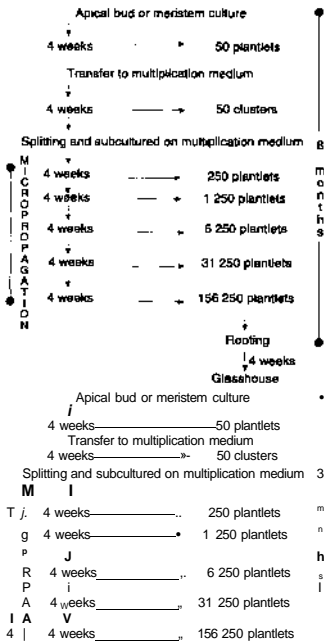
Apical bud or meristem culture

4 weeks	→	50 plantlets

Transfer to multiplication medium

4 weeks	→	50 clusters

Splitting and subcultured on multiplication medium

MICROPROPAGATION

4 weeks	→	250 plantlets
4 weeks	→	1 250 plantlets
4 weeks	→	6 250 plantlets
4 weeks	→	31 250 plantlets
4 weeks	→	156 250 plantlets

Rooting

4 weeks

Glasshouse

8 months

detection methods for these diseases with biotechnology techniques. New identification methods would increase speed and sensitivity of diagnosis, and generate information that would provide a better understanding of the epidemiology of the pathogen and hence the most suitable control measures to be adopted.

Gumming disease (*Xanthomonas campestris* pv *vasculorum*) is a dangerous threat to Mauritius and the Mascarene region. Three races of the bacterium occur in Mauritius and different entities of the pathogen are suspected to exist in the Mascarene and Southern African region (Qhobela & Claflin 1992). In 1964 and 1980, compulsory uprooting of two major varieties was enforced by law as they had succumbed to new races of the bacterium. The development of monoclonal antibodies in Mauritius provided the necessary tool to investigate variation in the pathogen. Isolates from South Africa, Zimbabwe, Madagascar and Mauritius were shown to be distinct using monoclonals (Dookun 1993). Moreover, in Mauritius, race 1 proved to be serologically different from races 2 and 3. A genetic study of the same isolates by RFLP analysis confirmed this heterogeneity of the gumming pathogen on a local and regional basis (S Saumtally, unpublished data). These studies demonstrate the high variability in the population of the bacterium, the possible emergence of new races and the danger of introducing new strains from neighbouring regions. Specific genetic differences among the isolates, such as the presence of a ubiquitous plasmid in race 1, is being exploited for detection by the polymerase chain reaction (PCR).

Outbreaks of leaf scald *(Xanthomonas albilineans)* have occurred recently in several countries, including Mauritius. These epidemics has led to the rejection of several commercial clones and variation in the pathogen has been suggested as an explanation for the outbreaks. In Mauritius, two serotypes (Mascarene & African) of the bacterium have been detected (Autrey et al 1995). To investigate the epidemiology of the disease, RFLP analysis of the isolates has been conducted using DNA probes produced by genomic subtraction. The two serotypes were found to be genetically different (Y Fakim & A Dookun, unpublished data) and DNA sequencing of specific DNA fragments is being carried out for the eventual development of primers for detection by PCR. This will allow the distribution of the two variants to be determined as well as producing a rapid test to ensure that planting material is disease-free.

The exchange of sugarcane plant material between countries needs to be tightly regulated owing to several systemic diseases. Disease diagnosis techniques based on molecular detection technology will be valuable tools for the early detection of diseases during quarantine. A reliable technique is essential because glasshouse conditions (high humidity, temperature fluctuations, lack of sunshine) often mask disease symptoms. Biotechnology tools such as PCR offer a high level of detection in the absence of symptoms. The necessity for extreme caution in germplasm exchange has been amplified in recent years with the discovery of several new diseases.

MOLECULAR MARKERS

Plant breeders and plant pathologists require accurate screening methods to help them release varieties with specific phenotypic characters. Varietal screening against major diseases has often proved difficult as factors such as climatic conditions, physiological status of the plant and fluctuations in disease pressure are involved. Variety x year interactions have been observed, necessitating a lengthy screening program. Molecular markers would help to identify the genes responsible for a specific phenotype to a region of the genome. Provided that the markers were not cross-specific, and depending on how closely they were associated, the association of such markers in the progeny would enhance the efficiency of the selection process.

For the two important fungal diseases, rust *(Puccinia melanocephala)* and yellow spot *(Mycovellosiella koepkei)*, the search for molecular markers has been initiated using the random amplified polymorphic DNA (RAPD) technique. Progenies derived from several biparental crosses and selfing of parents with known rust resistance, have been evaluated in the field and in the laboratory. DNA extracts from the different populations are being studied in the search for markers that

could be associated with the disease resistance gene(s). Recent findings revealed that three to four genes are involved in the resistance to yellow spot disease in a dominant way (Ramdoyal et al 1996, Table 1). Fingerprinting of varieties would also allow genetic diversity to be examined and might provide a method for clonal classification.

Table 1 *Probable genetic constitution of different phenotypes in relation to yellow spot disease : (a) 3 gene pair; and (b) 4 gene pair. [R = resistant, SS = slightly susceptible, S = susceptible, HS = highly susceptible]*

(a)

Phenotype		Genotype		
	Gene 1	Gene 2	Gene 3	
R =	R_1	R_2	R_3	(At least 3 dominant genes, one at each locus)
SS =	r_1	R_2	R_3	(At least 2 dominant genes, one at any two loci)
S =	R_1	r_2	r_3	(At least 1 dominant gene, at only one locus)
HS =	r_1	r_2	r_3	(Absence of dominant genes)

(b)

Phenotype			Genotype		
	Gene1	Gene 2	Gene 3	Gene 4	
R =	R_1	R_1	R_3	R_3	(At least 3 dominant genes, one at any two loci)
SS =	r_1	r_2	R_3	R_3	(At least 2 dominant genes, one at any two loci)
S =	R_1	r_2	r_3	r_3	(At least 1 dominant gene, at only one locus)
HS =	r_1	r_2	r_3	r_3	(Absence of dominant genes)

PERSPECTIVES

The application of biotechnology to sugarcane is still in its embryonic stage, but our knowledge and technical capabilities are rapidly increasing. Thus, the genetic transformation which was believed to be successful only in the long term is now a reality. It is therefore important to explore the various avenues offered by biotechnology in order to reap the full benefits. With this objective, the MSIRI launched its biotechnology program in 1993. Tissue culture and disease diagnosis techniques are already meeting the desired objectives. *In vitro* micropropagation is now enabling the early exploitation of newly released varieties to growers. Tools for disease detection are allowing unequivocal identification and detection of low levels of infection of several diseases, and are already generating information on pathogen variation and a better understanding of the epidemiology of diseases. Mid-term perspectives include the study of the genome of Mauritian sugarcane clones to help the improvement of varieties in a more efficient manner. Linking of molecular markers to agronomic traits relevant to the country is also a major goal of the program. However, in order to make significant progress over a short period, it is imperative to establish collaborative relationships with other institutions to strengthen our research capacities.

ACKNOWLEDGEMENTS

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BIOTECHNOLOGY IN THE SUGAR INDUSTRY: SOCIO-ECONOMIC ASPECTS OF PROBLEMS AND PROSPECTS FOR DEVELOPING COUNTRIES

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ABSTRACT

Within the last decade, the emergence of biotechnology and related developments have spawned fertile grounds which now offer and will no doubt continue to offer inexpensive and speedy solutions to fundamental agricultural and agro-industrial problems. These solutions can be applied in all latitudes with equal effectiveness and possibly with equal benefits. Results to date are indicative of the potential available to broaden the base and accelerate development strategies leading to sustained agricultural, industrial

The unlimited panorama of opportunities in the uses and applications of bio-technological developments has only just begun to emerge and conventional wisdom suggests that the panacea for increasing global food production and improvements in the quality of life has been found.

The majority of the developing world however lacks the financial resources to meet both capital and recurrent costs in exploiting such opportunities.

THE ISSUES IN PERSPECTIVE

The principal agricultural policy objectives of most of the developing countries of the world can be briefly summarized:

(i)   Continuous and sustained increases in agricultural production and agricultural productivity under production systems which are environmentally friendly and sustainable,

(ii)   Increase in opportunities in the rural sector,

(iii) Increase in household earnings and household savings from agricultural-sourced incomes,

(iv)   Improvements in the nutritional and welfare status of these households.

(v)   Increase in agricultural export earnings.

The economic and agricultural transformation processes which have already occurred in some developing countries and the status of agricultural research in other countries confirm significant opportunities for the future.

Most developed countries have been involved to various degrees in the basic sciences which gave rise to biotechnology. Successful commercialization of laboratory findings in the years ahead will offer many opportunities in agriculture, horticulture and forestry. Beneficial applications will also be found in the areas of environmental hygiene, pollution control and the recycling of wastes. These biotechnological developments will certainly impact upon third world economies in the following way:

(i)   Induce changes in production systems, structures and costs.

(ii)   Change demand/supply relationships of traditional inputs.

(iii) Substitution of traditional products with new products.

(iv)   Increase the opportunities for agricultural pursuit.

(v)   Impact on employment, income and consumption patterns.

(vi)   Alter the existing market structures.

What the developments cannot guarantee is equity and efficiency in the distribution of benefits in order to maximize social welfare goals.

DEFINITION AND SCOPE

Biotechnology

Very simply, biotechnology refers to any technique that uses living organisms to make or modify any products to improve plants or animals or to develop microorganisms for specific uses. These techniques include the use of new technologies such as recombinant DNA, cell fusion or other new processes.

The Overseas Development Institute (UK) (September 1988) defines the concept as follows:

"Biotechnology applies scientific and engineering skills, disciplines and principles to enable nationals to be processed by biological agents resulting in faster and more accurate breeding programmes for plants, animals and micro-organisms".

Of the several definitions available, it is evident that biotechnology integrates several recent advances in basic molecular biological research and encompasses many facets of management and manipulation of biological systems. In this regard, the very nature of biotechnology is related to several disciplines:

(i)   In Natural Sciences - genetics, biotechnology, physiology and microbiology,

(ii)   In Engineering - fermentation technology, production engineering, industrial chemistry, microbiology, etc.

BIOTECHNOLOGY IN SUGARCANE - OVERVIEW

The principal objective of cane growing countries is to continuously increase production efficiencies at lower costs. This objective now seems mandatory given the present economics of world sugar production. By-products from sugarcane production and processing also lend themselves to tremendous value-added opportunities.

The production of non-sucrose sweeteners by biotechnological processes may impact negatively on the current glut of beet and cane sugar stocks on the world market. Sugar prices continue to be low because of the inflexibility of the industry to structurally adjust downwards in the short run given the nature of the cane growing cycle. Further, intensification of competition from fructose syrups, semi-synthetic sugars (aspartame) and artificial sweeteners such as acesulfame K continues unabated.

Modern biotechnology and related developments will impact on the sugarcane industry over the next 5-10 years in the following areas.

(i)   Seed Material/Planting Material - New varieties of sugarcane plants carrying novel genetic traits such as pest or disease resistance, improved yield and quality attributes, new technologies, new production systems, etc.

(ii)   Agricultural (Sugarcane) Microbiology - This will become a real possibility through the use of genetically engineered microorganisms as biological control agents for pests and diseases or as inoculants to stimulate plant growth and reduce fertilizer

(iii) Sugarcane Diagnostics - Biotechnology will assist in the control of sugarcane diseases by providing rapid diagnosis on which to base decisions on fungicide applications and other control measures. Further, results will assist in the identification of diseases of quarantine significance.

(iv) Research Programmes - These will develop insect and pest-resistant commercial varieties, high-yielding commercial varieties, varieties with high sucrose content and varieties with other specific attributes, e.g. ratooning ability, minimum lodging, maturity times,

REVIEW OF BIOTECHNOLOGICAL DEVELOPMENTS IN THE GENETIC IMPROVEMENT OF SUGARCANE

Genetic improvement of sugarcane varieties in different countries has been taking place at varying levels of intensity and with different levels of resource commitment. Research programmes have been financed both by the public and private sector and are of critical importance in countries that depend on the export of sugar and related by-products to continuously generate export earnings.

The principal focus of such research programme can be briefly summarized:
(i) To develop insect and pest-resistant commercial varieties
(ii) To develop high-yielding commercial varieties
(iii) To develop varieties with high sucrose content
(iv) To develop varieties with other specific attributes, e.g. ratooning ability, minimum lodging, maturity times, etc.

The principal difficulty with sugarcane breeding programmes emanates from the genetic concern that the sugarcane plant is octo or decaploid and is highly heterozygous. Given these conditions it normally takes about 10-15 years to commercialize a particular variety for selected traits. Any research technique which can circumvent these inherent difficulties will therefore provide enticing opportunities for the cane growing economies of the world.

Micro propagation of the sugarcane plant is possible from cultures of auxiliary buds and from calli. Such a technique enables the rapid propagation of virus free plants and can therefore shorten the quarantine period by two to three years.

On the other hand in-vitro micro propagation also offers pragmatic opportunities in effective storage facility requirements which are necessary for sugarcane propagation under traditional breeding programmes.

Within recent times, sugarcane researchers in Hawaii, Taiwan, Fiji, Cuba and Argentina have been successful in regenerating plants from calli obtained from stem or leaf cuttings. Similarly, and simultaneously, the IRAT Institute in France has developed a technique to inoculate pathogens into young plantlets regenerated from calli. Cuba has also made encouraging advances in this regard.

Protoplast fusion could also stimulate interest in the years ahead. Roque (1984) reported that it could be of great importance in the propagation of several varieties which are sterile. Work of this nature was initiated in 1981 at Piracicaba Campus, University of Sao Paulo, Brazil.

SOCIO ECONOMIC DIMENSIONS OF BIOTECHNOLOGICAL DEVELOPMENT

Generally, there appears to be some degree of concurrence among biological researchers, policy makers and planners that quantitative estimates of the likely impact of biotechnology on agriculture are difficult to obtain. The explanation resides in paucity of solid and reliable information to develop meaningful cost/benefit analyses. Further, the reluctance of independent scientists, private farms and industrial concerns to divulge financial information compounds this problem. Notwithstanding, there are positive socio-economic benefits.
(i) Intensification of agricultural production across the globe.
(ii) Sustaining and assuring high levels of agricultural productivity, per unit land area of tropical and subtropical crops.
(iii) Bringing into the productive stream agricultural lands formerly classified as marginal.
(iv) Reduction in recurrent expenditures for production, e.g. reduction in cost of pesticides.

The negative aspects will be amplified in a later section.

Barker (1989) in his assessment of the potential economic impact of biotechnology in the third world explained and emphasized all the salient factors which can be briefly summarized as follows:
(i) Biotechnology will contribute modestly to increases in agricultural productivity in the area of crop production (1.5% - 2.2% per annum). He argues that yield plateaus have been achieved in some major crops and that even these modest increases will not be forthcoming in the absence of biotechnology.
(ii) Biotechnology is knowledge-intensive and often location-specific. Biotechnology is potentially a "scale-neutral" technology but its application could contribute to serious, long term negative consequences for global trade and development.

SUMMARY OF ADVANTAGES AND DISADVANTAGES TO DEVELOPING COUNTRIES

Advantages
One school of thought argues that early participation in new technologies is axiomatic for maintaining a leadership and thereby creating future wealth and employment. Atkinson & Mavituna (1983) have outlined three major factors in which the current interest in biotechnology and belief in its expansion are founded:
(i) Biotechnology utilize raw materials obtained from renewable resources, i.e., cereal crops, celluloses and lignocelluloses.
(ii) Biotechnological processes appear to have advantages over the chemical processing of vegetable materials. Improvements in process technology can improve the efficiency of biotechnological industry.
(iii) A wide range of product appears possible both as a result of traditional and raw biological methods. Genetic engineering offers the promise of new products, increasing yields of existing products and modification of existing products.

Specific advantages clearly discernible for sugarcane are as follows:
(i) Rapid propagation of planting material.
(ii) High yielding cultivars will be produced at low cost,
(iii) Planting material will be free from pathogens and contaminants,
(iv) Expectation of increased agricultural productivity.
(v) Value-added opportunities from fermentation technologies and associated development of down stream industries.

Disadvantages
(i) The primary agricultural, agro-industrial and food processing sector may suffer commercialization of biotechnological products through substitution effects and changes in processes and products. Multinational Corporations will continue to play a dynamic and technological leading role in these developments.
(ii) The existing trading pattern between the developed and developing world will be significantly altered to the detriment of the developing countries. Because new products will emerge primarily from the developed world and commercialization will make traditional production systems and processes obsolete. Undesirable effects will include unemployment, loss of income and possibly poverty and malnutrition. New production processes and systems may not produce sizeable economic benefits since they are more than likely to be capital intensive.
(iii) Effects on farming systems. Sugarcane production in most countries shows a dualistic production pattern - large commercial plantations side by side with small farmers. Biotechnological improvements in varieties with desirable agronomic traits will clearly strengthen the opportunities for large commercial plantations vis-a-vis small farmers. Large plantations have highly developed infrastructure, management and operational skills, investment in research and development facilities and stable organized marketing and financing arrangements. On the other hand, the small farmers lacking these will not be able to adopt the new technologies.
(iv) Reduction in genetic diversity. Special arrangements and facilities will be required to safeguard genetic material with desirable traits.
(v) Some countries will not have the technical, financial and institutional resources to optimize the benefits from the new technologies and may lag behind in the biotech revolution.

POLICY PERSPECTIVES

National policies in respect of biotechnology are at various stages of development. In this regard the following classification has been suggested in the literature:
(i)   Countries with interest but no direct involvement at present.
(ii)   Countries with a biotechnological policy centered around traditional biotechnology. They have established collaborative links with industrialized countries for training and transfer of results.
(iii)   Countries which have a national biotechnology policy, and in-country program in modern technology, and vibrant public and private sector with strong intra-country linkages.

In this regard, Singapore has a comprehensive national programme for biotechnology and its main features are as follows:
(i)   Tax free status to deserving new companies over the first five
(ii)   Tax deduction on the investment (up to 50%) in the new equipment or renovation of plant.
(iii)   Provision of cheap loans and equity investment, through a S\$ 100 M venture capital fund to assist local companies to acquire new technologies, to diversify their activities and to attract foreign firms.
(iv)   Small Industries Technical Assistance scheme to finance the provision of outside consultants.
(v)   Product Development Assistance Grants (up to S\$ 2 M) to contribute to first biotechnology investment and to help in the commercialization of the products.
(vi)   Training of local staff, through a Skills Development Fund which covers visits to company headquarters abroad (S\$ 5 M).
(vii)   Investment in foreign companies with spin-offs for Singapore, through a S\$ 78 M Venture Capital Fund. Investments of up to S50 M have been pumped into biotechnology projects since 1987.

From a wider perspective, developing countries now wishing to embark upon the biotechnology bandwagon will need to fulfill a number of prerequisites such as the creation of commitment and willingness, etc., improvement in institutional infrastructure, improvements in university teaching, creation of an information pool, significant investment in personnel development, strengthening of basic research, communication and interaction with the developed world, sources of supplies of new biochemicals, establishment of modern and well equipped laboratories, development of repair and maintenance capability for equipment, rapid acquisition and adaptation of new technologies, comprehensive national policy, creation of intra-country linkages and special funding arrangements.

FINANCIAL IMPLICATIONS FOR DEVELOPING COUNTRIES

Indigenous development of new biotech firms in developing countries must focus on research, development, demonstration and commercialization of biotechnological systems and processes from the most cost-effective standpoint.

Given the current economic and financial circumstances in most of these countries particularly:
(i)   Onerous external debt burdens.
(ii)   Failing export earnings from traditional and primary export commodities,
(iii)   Slow agricultural and economic transformation, and,
(iv) Superimposition of economic adjustment programmes on weak economic structures.

The net result will be difficulty in sourcing capital funds for investment in biotechnology programmes and projects. Interested investors and companies in these countries will have to explore all the possibilities and will probably intensify competition for limited capital funds in the financial market place. The likely sources of funds will include the venture and equity capital, research contracts, joint ventures/partnership arrangements, public sector funding, research grants and self owned capital.

These new companies in developing countries will have advantages in respect of their size, flexibility and growth with the indigenous market. However, undercapitalization, cash-flow problems and successful development of a marketing and distributive network will pose real threats to their growth, development and survival.

CONCLUDING OBSERVATIONS

Research and development efforts with concurrent investments in biotechnology are now increasing at a rapid pace. These developments will undoubtedly offer exciting and innovative possibilities for increasing crop and livestock production and for the overall improvement of human welfare.

The financial, economic and social benefits which are likely to accrue at the turn of the century, will possibly induce academic dormancy on critics at the turn of the century. Colossal quantities of energies, efforts and resources are currently invested in the areas of medicine, industry and agriculture. The industrial countries are in the forefront with major transnational corporations being the major actors.

For third world countries to benefit, certain critical prerequisites must be fulfilled. Because of the inherent physical, financial, managerial and institutional shortcomings, these developing countries will always lag behind as beneficiaries of these exciting advances. The transnational corporations in the developed world will continue to have a comparative advantage in the following areas:
(i)   Sufficient cash flow to initiate new investment and support existing investments in high-risk, high profit yielding areas.
(ii)   International marketing network.
(iii)   Information and communications.
(iv)   Control and release of products in the market place.
(v)   Regulatory control mechanisms.

In the years ahead development and successful commercialization of the new technologies will assume prominent importance over the globe. Biotechnology in my own view will make important and long lasting contributions in agriculture, biological products, chemicals, energy, enzymes, food, health care, pollution management and waste treatment. The vast panorama of opportunities has only just begun to emerge on the horizon.

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REGULATION OF EXPRESSION OF p-GLUCURONIDASE IN TRANSGENIC SUGARCANE BY PROMOTERS OF RUBISCO SMALL SUBUNIT GENES

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ABSTRACT

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, E.C.4.1.1.39), one of the key enzymes in photosynthesis, is encoded by chloroplast and nuclear genes. Genome reconstruction experiments indicate that sugarcane hybrid cultivars contain approximately 16 rubisco small subunit (scrbcs) genes. The role of such a large family of genes is not known. Experiments on other plants show that products of individual rbsc genes are expressed in specific tissues at precise stages of leaf development. We investigated regulation of expression of two scrbcs genes by fusing individual scrbcs gene promoters with the uidA gene as a reporter with a NOS terminator. Transient analysis of these chimeric gene constructs in sugarcane leaves showed that compared to the constitutively expressed polyubiquitin promoter the scrbcs promoters gave a lower level of GUS expression. However, the scrbcs promoters directed relatively high expression in photo synthetic tissues. Stable expression in transgenic callus lines and regenerated plants showed that the scrbcs-1 gene promoter directed GUS expression in leaves of young plants in vitro but not in calli. Experiments in progress to determine if this tissue-specific expression pattern is maintained in mature plants.

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrid) is one of the most efficient crop converters of solar energy into stored chemical energy (Hunsigi 1993). This efficiency is due in part to distinct biochemical characteristics of C_4 photosynthesis which is associated with tissue differentiation within the leaf. This anatomical feature is known as "Kranz" anatomy because of the "halo" or "wreath" arrangement of the vascular bundle encircled by a single layer of bundle sheath cells (BSC) which is in turn surrounded by a concentric sheath of mesophyll cells (MC). Data reviewed by Furbank and Taylor (1995) supports the hypothesis (Hatch & Slack 1970) that Kranz anatomy allows for the compartmentation of enzymes, substrates and products which enables the increased photosynthetic efficiency of C_4 plants.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, E.C.4.1.1.39) is the most abundant protein in the leaves of light-grown plants and catalyses the first step in carbon fixation. In mature leaves of C_4 plants, Rubisco occurs in the BSC but not the MC, nor in any non-photosynthetic tissues of the plant. In immature leaves of C_4 plants, before Kranz anatomy differentiates, Rubisco appears in the MC. The spatial separation of C fixation versus C reduction activities in mature leaves of C_4 plants acts to concentrate CO_2 in the BSC for reduction by Rubisco. Concentrating CO_2 in the vicinity of Rubisco allows high rates of C fixation while conserving water, reducing the inhibitory effect of O_2 on photosynthesis, and reducing photoassimilate losses due to photorespiration.

The holoenzyme of Rubisco is a hetero-16-mer composed of eight 52-kD large subunits plus eight 15-kD small subunits. The large subunits have the catalytic function of the enzyme and are encoded by chloroplast DNA. The small subunits have the regulatory function of the enzyme and are encoded by a family of nuclear genes (*rbcs*). The number of *rbcs* genes encoding the Rubisco small subunits varies by plant species and ranges from 4 in diploid *Arabidopsis* to 12 in hexaploid wheat. Although extensively studied, the function of the various members of the *rbcs* family of genes is largely unknown. Nevertheless, products of specific *rbcs* genes are expressed in specific tissues at different stages of leaf development (Bansal et al 1992). Therefore, the *rbcs* genes are potential sources for promoter elements to regulate expression of transformed heterologous genes in a leaf tissue-specific or developmentally-specific manner. Precise targeting of heterologous gene expression is one of the important goals of genetic transformation.

In sugarcane, c.16 *rbcs* genes encode the Rubisco small subunits; we have isolated and partially characterized seven of these. They are very highly conserved, not only in the coding regions but also in the 5' and 3' untranslated regions (UTR). The high conservation of the 3' UTRs of the *rbcs* gene sequence is apparently unique to sugarcane. We hypothesize that the 5' region might be involved in differential expression of *scrbcs*. To test this hypothesis we studied the activity of promoters of the two *scrbcs* gene members having the most divergent nucleotide sequences among the seven cloned genes. The goal of this research is to identify promoter elements with potential application to genetic transformation.

MATERIALS AND METHODS

Chimeric Gene Constructs

A *scrbcs-1* promoter-b -glucuronidase (GUS) chimeric gene was constructed by subcloning nucleotide (nt) sequence #1 through #1174 of *scrbcs-1* into the complimentary *HindIII* and *NcoI* restriction sites of a promoterless plasmid, pB 1101 (Clontech), containing the *uidA* gene which encodes b -glucuronidase (GUS), the nopaline synthase II (NOS) untranslated region, and polyadenylation signals. This fusion construct was termed pWD-1. A *scrbcs-3* promoter-GUS fusion gene was similarly constructed from the partial genomic clone *scrbcs-3* and termed pWD-3. A negative control expression plasmid was constructed by shortening *scrbcs-1* by 30 nt and eliminating the TATA box region. This construct was termed pWD-2. The positive control expression plasmid consisted of the maize polyubiquitin promoter (Ubi) fused with the GUS gene and NOS terminator (pAHC27 of Christensen et al 1992). This construct was termed pUbiGUS. The selection plasmid construct, pHA9, consisted of the maize ubiquitin gene promoter with the neomycin phosphotransferase II gene (*npt*II) and NOS terminator (H Albert, unpublished data).

Transient expression in sugarcane leaves

Leaves were collected from 3 month old greenhouse-grown sugarcane plants (Cultivar H32-8560). A subset of the plants was placed under continuous darkness for 10 d prior to sampling. Leaves were excised, surface-sterilized, cut into 20 x 10 mm segments, and placed, lower epidermis upward, on petri plates containing MS medium (Murashige & Skoog 1962). The leaf segments were cultured for 3 d under laboratory ambient illumination then bombarded with DNA-coated particles using the Biolistics Particle Delivery System-1000He (BioRad). The bombarded leaf segments were cultured at room temperature under continuous illumination for 24 h then stained for GUS activity (Jefferson 1987). The tissue was infused with the substrate for visualizing GUS activity by vacuum infiltration (twice for 15 min) followed by incubation at 37 °C for 24 h.

GUS positive leaf segments were embedded in Tissue-TekÒ OCT. compound (Miles Inc., Elkhart, Indiana) and sectioned into 50mm-thick sections at -20°C, using a cryostat (Cryocut 1800, Reichert-Jung,

Cambridge Instruments GmbH, Germany). The sections were thawed, mounted on room temperature slides with CryoSeal™ 280 (Stephens Scientific Cornwell Corporation, NJ), and examined microscopically to determine cellular localization.

GUS activity was quantified by the 4-methylumbelliferyl glucuronide (MUG) assay, modified from Gallagher (1992). Briefly, 100 mg leaf pieces were ground with 100 mg PVPP (Polyvinyl poly-pyrrolidone) in 0.5 mL lysis buffer (1 mM EDTA, 0.1% Triton X-100, 10 mM 2-mercapto ethanol. and 0.1% Sarkosyl in 50 mM NaHPO₄ buffer pH 7). The extract was centrifuged for 10 min and the supernatant was filtered through a Sephadex G-25 Spin Column. The crude protein extract was incubated with MUG at 37°C. GUS activity was calculated by measuring the production of 4-methylumbelliferone (MU).

Transformation of sugarcane plants

Embryogenic callus of sugarcane, *Saccharum* spp. hybrid cultivar H62-4671, was initiated on MS3 (MS medium containing 3 mg/L 2,4D) and subcultured on MS1 (MS medium containing 1 mg/L 2,4D) (Fitch & Moore 1993). Embryogenic calli were bombarded as above, following the optimized conditions of Sun et al. (1993). The test plasmid and selection plasmid constructs were co-transformed. Each experimental treatment consisted of bombarding 15 plates per combination.

Selection for transformed calli was based on methods modified from Bower & Birch (1992). Bombarded cultures were placed for 1 wk on fresh MSI plates without selection. They were then placed on MSI with 20 mg/L geneticin (G418) for one month. Calli were then transferred to MSI plates with 50 mg/L G418 for three 1-month subcultures. The vigorous-looking calli were transferred to MSI without selection. When sufficiently large, calli were transferred to MS medium without 2,4-D for plant regeneration. Regenerated plantlets were transferred to soil at about 2 months of age.

Detection of NPTI1 and GUS enzyme in transgenic plants

Protein was isolated from embryogenic calli and leaves. 100 mg of tissue were ground in liquid N₂ and suspended in 1 mL PBST-PVP (PBST: 8 g/L NaCl, 0.2 g/L KH₂PO₄, 1.15 g/L Na₂HPO₄, 0.2 g/L KCl, 0.2 g/L NaN₃, pH7.4, with 0.5 mi/l Tween-20; PBST-PVP: 2% PVP-40 in PBST). The sample was vortexed briefly, then microfuged at 16,000 g for 10 min. Total protein concentration of the resulting supernatant was determined by the Bradford method. The amount of NPTII enzyme was measured by NPTII-ELISA (5 Prime-> 3 Prime, Inc.). GUS expression was evaluated by a histochemical assay modified from Jefferson (1987).

RESULTS

Transient expression in bombarded leaves

No GUS foci were found after bombardment with the negative control, pWD2 (Table 1). For the other constructs, the young unexpanded leaf lamina (-3) produced about 10 times more foci than did the fully expanded green leaf (+1). Although the initial number of foci was relatively low, expression increased 10-fold after bombarded leaves were cultured for 3 days. The positive control, pUbiGUS, produced about 10 times more GUS blue foci in bombarded leaves than each of the tested *scrbcs* promoters.

Table 1 *Number of blue foci per bombardment of sugarcane (Cultivar H32-8560) leaf segments with gene constructs containing the uidA (GUS gene driven by various promoter sequences). Values given are the means ± SD per leaf segment.*

| Construct | Cultured for 0 day | | Cultured for 3 days | |
	-3 leaf	+1 leaf	-3 leaf	+1 leaf
pUbiGUS	32 ±8	4 ± 1	342 ±111	31 ±12
pWD1	5	±2	45 ±17	3 ±1
pWD3	6 ±3	0	57 ±23	2 ±2
pWD2	0	0	0	0

The sectioned leaf segments showed that most GUS expression was in the epidermis (EP) of the leaf segment regardless of the promoter construct (Table 2). Few blue-stain spots were located in the MC and BSC. The scrbcs promoters produced 10-fold greater expression in the BSC than did the ubiquitin constitutive promoter.

Table 2 *Number and distribution (% of total in brackets) of GUS foci among sugarcane leaf cells when leaf segments were bombarded with gene constructs driven with promoters from ubiquitin or scrbcs genes. C BSC=bundle sheath cells; MC=mesophyll cells; EC=epidermis cells)*

| Construct | Total no. foci | Distribution between cell types | | |
		BSC	MC	EC
pUbiGUS	771(100)	17(2)	23(3)	731(95)
pWD1	178(100)	42(24)	16(9)	118(66)
pWD3	339(100)	74(22)	22(7)	243(72)

Stable expression in plants from embryogenic callus

Kanamycin-resistant callus lines were obtained after selection of bombarded callus cultures growing on MS 1 medium containing 20 mg/L G418 for 1 month followed by selection on 50 mg/L G418 for 2.5 months. Samples of the selected calli, co-bombarded with pWD1 and pHA9, expressed the NPTI1 enzyme in excess of 75 ng NPTI1 protein per mg total protein.

Plants were regenerated from calli bombarded with pUbiGUS and pWD-1 and grown in the presence of G418. About 30 plants were obtained from a single callus culture surviving after bombardment with pUbiGUS. Twenty plants were regenerated from three isolates surviving after bombardment with pWD-1. Histochemical stain of pUbiGUS-bombarded isolates showed strong GUS expression in calli and leaves, but low expression in the roots. pWD-1 bombarded calli did not show GUS expression, but leaves regenerated from these calli were GUS positive. No stably transformed cultures were obtained with the pWD-

DISCUSSION

The primary goal of this research was to compare the activity of the promoters of two *scrbcs* genes. This goal was not achieved because the low efficiency of transformation failed to produce stable transformation for pWD-3, one of the two constructs. Nevertheless, the transient expression experiments with each of the constructs and the stable transformation with one of the *scrbcs* promoters, indicate the potential for obtaining tissue-specific expression of transgenes in sugarcane.

In transient expression experiments, the promoter from the constitutively expressed maize ubiquitin gene directed higher levels of expression in sugarcane leaves than did promoters isolated from the sugarcane *scrbcs* genes. However, 95% of the GUS expression driven by the ubiquitin promoter was limited to the epidermal cells of the leaf and only 5% was divided between the photosynthetic BSC and MC tissues. The level of expression by the ubiquitin promoter in the MC and BSC may be too low to draw definitive conclusions, nevertheless, equal expression of pUbiGUS in these two tissues indicates that the parameters set for particle bombardment introduces an approximately equal number of DNA-coated particles into the MC and BSC of sugarcane leaves.

Transient expression of GUS driven by the *scrbcs* promoters gave an average of 23% expression in the BSC, 8% in the MC, and 69% in the epidermis. This result is in stark contrast with expression patterns from pUbiGUS. The *scrbcs* data show that these sugarcane photosynthetic gene promoters are functional and that they appear to direct expression in a tissue-specific manner. Since the bombardment conditions did not favour transient expression in a particular photosynthetic tissue, the 3 to 1 preferential expression of *scrbcs* promoters in the BSC over the MC is consistent with differential regulation of *scrbcs* expression in the two tissues. One result which may cast doubt on the meaning of these transient assays is that the *scrbcs* promoters elicited fairly high transient expression in the leaf epidermis.

The stable transformation experiments confirm that the *scrbcs-1* promoter could be functionally integrated into a sugarcane chromosome where its expression is tissue specific. Southern analysis would confirm the apparent integration. The *scrbcs-1* promoter did not express GUS in long-term callus cultures but it was active in leaves of plants regenerated from that callus culture. It is probable that the *scrbcs* promoters include light-regulated sequences whose trans-acting factors were limited to photosynthetic tissues. This has been shown to be true for maize (Bansal *et al* 1992) and is consistent with our observation that expression levels increased in sugarcane after bombarded leaf segments were incubated in the light. Dark controls and detailed deletion analyses are needed to test this hypothesis. The development of functional sugarcane photosynthetic gene promoters increases our potential for successfully transforming sugarcane to alter aspects of photosynthesis or to increase resistance to leaf pests or diseases.

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DD7FERENTIAL EXPRESSION OF B-GLUCURONIDASE IN SUGARCANE UNDER THE CONTROL OF THE RICE RUBISCO SMALL SUBUNIT PROMOTER

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ABSTRACT

The differences between C, and C_4 species are clearly manifest in the different anatomies of their leaves. Similarly, there are differences in the localisation within the leaf of key metabolic enzymes. Ribulose bisphosphate carboxylase (Rubisco) occurs in the mesophyll of C_3 plants but is found predominantly within the bundle sheath cells of C_4 plants. Given the sequence similarity of C_3 and C_4 Rubisco small sub-unit (rbcS) genes such differences in localisation prompt questions about the means by which these differences arise. This work constitutes the first attempt to determine the expression pattern in a C_4 monocot (sugarcane) of a marker protein (B-glucuronidase (GUS)) gene linked to a C_3 monocot (rice) rbcS promoter. GUS was expressed in all leaf cell types in young leaves, but was restricted to bundle sheath cells in older leaves. This developmental change may have significance in relation to mooted positive or negative transacting factors.

INTRODUCTION

Plants possessing the C_4 pathway of photosynthesis are characterised by a ring of bundle sheath cells encircling the vascular tissue (Krantz anatomy). The primary step of CO_2 fixation in the Calvin cycle is confined to the bundle sheath and hence genes encoding photosynthetic enzymes (such as ribulose bisphosphate carboxylase (Rubisco)) are expressed predominantly in the bundle sheath cells. In C_3 plants, which lack this dimorphic cell structure, photosynthetic CO_2 fixation takes place in the mesophyll cells. The small subunit of Rubisco encoded by *rbcS* correspondingly differs in specificity of cell expression, being expressed in mesophyll cells of C_3 leaves and bundle sheath cells of mature C_4 leaves.

C_4 species are believed to have evolved independently several times from C_3 species (Edwards & Walker 1983). A comparison of the *rbcS* genes from C. and C_4 species revealed a high level of similarity in the structure of the coding region (Matsuoka & Yamamoto 1989). Furthermore, sequence motifs in the *rbcS* promoter of maize (a C_4 monocot) which are essential for gene expression have very similar counterparts in C_3 species (Schaffner & Sheen 1991). Given this similarity in C_3 and C_4 *rbcS* promoter regions, it seems likely that other factors affect the observed differential expression patterns.

The B-glucuronidase (GUS) gene has been widely used as a convenient tool for investigating plant transformation systems and promoter activities (Jefferson 1987). Fusion of GUS to specific C_3 or C_4 promoters can elucidate the observed differential expression patterns of chimeric genes. The transformation of rice with a chimeric gene consisting of the maize *rbcS* promoter fused to GUS revealed mesophyll specific expression in leaf blades and leaf sheaths (Matsuoka et al 1994). That is, the promoter which effects bundle sheath-specific expression of Rubisco in maize directed GUS expression to the mesophyll in rice.

A complementary study to that of Matsuoka et al (1994) would be to determine the expression pattern in a C_4 monocot of GUS linked to a C_3 monocot *rbcS* promoter. A promoter from a putatively constitutive gene such as maize ubiquitin (*ubi*) fused to GUS provides a suitable control for such an investigation. This study sought to compare the expression patterns in transformed sugarcane of GUS linked to a rice *rbcS* promoter against that of GUS linked to a maize *ubi* promoter.

METHODS

Sugarcane embryogenic callus was generated from tissue pieces 2-3 mm in thickness taken from the unemerged leaf and sheath roll of mature stems as described by Franks & Birch (1991). The callus was co-transformed by bombardment with pEmuKN (Last et al 1991) which conferred resistance to geneticin based on expression of the *aphA* gene and either rice *rbcS-GUS* or maize *ubi-GUS*. The callus was selected and grown to young plantlet stage following the methods of Bower &

Birch (1992) with only minor modification. Histochemical GUS staining was carried out following the method of Jefferson (1987). Plant organs were then fixed and sectioned.

RESULTS AND DISCUSSION

GUS activity under the control of the rice *rbcS* promoter was detected in both the mesophyll and bundle sheath cells of very young leaves (Fig 1a) and at a very low level in roots, but not in stems (data not shown). This type of expression is consistent with the observations of Wang et al (1992) that during early developmental stages of *Amaranthus*, both the large and small subunits of Rubisco were expressed in bundle sheath and mesophyll cells in a C, type pattern. Histochemical GUS staining of more mature tissue taken from a *rbcS* transformant exhibited predominantly bundle sheath-specific expression in the leaf blade (Figs 1b & 1c). In contrast to GUS activity under the control of *rbcS* . it was found that GUS activity controlled by the maize *ubi* promoter was evident in all leaf cell types (Fig. 1d), with only low level GUS activity in the stem and roots (data not shown).

Two explanations have been invoked to explain the differential expression of *rbcS* between C_3 and C_4 species. As the *cis* elements are believed to be unchanged functionally in C_4 species, Matsuoka et al (1994) proposed the differential localisation of a positive transacting factor(s) which enhances *rbcS* expression exclusively in the bundle sheath cells of C_4 species and exclusively in mesophyll cells of C, species. Alternatively, Langdale & Nelson (1991) proposed the presence of a "factor X" which suppresses the expression of *rbcS* in mesophyll cells adjacent to the bundle sheath. These preliminary observations are consistent with the presence of either a positive or negative transacting factor, but suggest that there are developmental influences on the expression or efficacy of any such factors.

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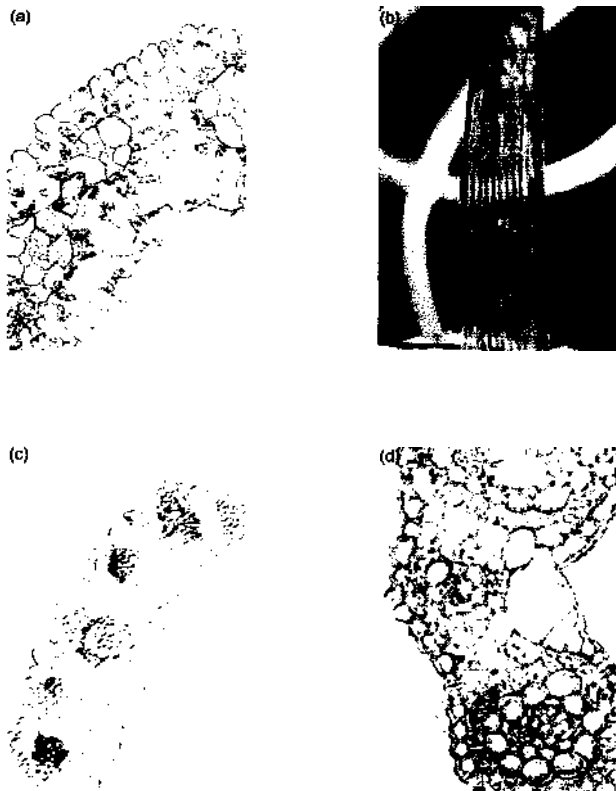
Fig. 1   *Histochemical GUS staining in leaf tissue of sugarcane transformants:*
(a) Cross section, young leaf, rbcS-GUS (x 100);
(b) Adaxial view, mature leaf, rbcS-GUS (x 10);
(c) Cross section, mature leaf, rbcS-GUS (x 100);
(d) Cross section, mature leaf, ubi-GUS (x 100).

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MOLECULAR MANIPULATION OF SUCROSE PHOSPHATE SYNTHASE IN SUGARCANE

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ABSTRACT

Three targets for molecular manipulation of sugarcane for increased sucrose accumulation have been identified; the sucrose synthetic process, the expression or activity of proton-sucrose transporters, and the apparently futile activity of acid invertase in the stem parenchyma. Seeking to increase the amount of sucrose produced in the leaves of sugarcane by over-expression of sucrose phosphate synthase (SPS), we have demonstrated the insertion of the spinach SPS transgene into the genome of sugarcane variety Q] 17. Western blot analysis showed expression of detectable quantities of spinach SPS in four transgenic lines, and gene expression was supported by RT-PCR. Ongoing work in our laboratory will assay the SPS activity of wild type and the most promising transgenic lines, and compare their sucrose accumulation performance.

INTRODUCTION

Sugarcane differs markedly from most other C[4] crop plants in that its economic product, sucrose, undergoes little metabolic metabolism after being synthesised in the leaves. Once produced, sucrose is loaded into the phloem and translocated from the leaves to the stem where it is stored in the parenchyma. There are three evident targets for molecular manipulation towards increasing the concentration of sucrose stored in the stem. The first is manipulation of the sucrose synthetic process, to increase the amount of sucrose produced in the leaves. The second is to increase the expression or activity of proton-sucrose transporters located either at the phloem interface or in stem parenchyma. The third target is to decrease expression or activity of acid invertase, the principal enzyme involved in the hydrolysis of sucrose in the vacuoles of stem parenchyma. Our laboratory is currently undertaking research in each of these areas, with the most advanced work being in the manipulation of source (leaf) sucrose synthesis.

A key target enzyme which has been identified as potentially rate-limiting to the formation of sucrose in the leaf is sucrose phosphate synthase (SPS) (Stitt & Quick 1989). The enzyme SPS catalyses the production of sucrose phosphate from the monosaccharides UDP-glucose and fructose-6-phosphate. This penultimate step of sucrose production is essentially irreversible, because the activity of sucrose phosphate phosphatase which catalyses the final step producing sucrose, is extremely high and maintains a very low concentration of sucrose phosphate, the product of the SPS reaction (Krause & Stitt 1992).

To increase the activity of SPS in sugarcane leaves, we are attempting to over-express the spinach SPS protein. Three different expression vectors have been constructed, spinach SPS driven by either the rice actin promoter or the maize ubiquitin promoter, and a mutated form of the spinach SPS gene driven by the maize ubiquitin promoter. The mutated SPS gene encodes a protein which is enzymatically active, but lacks the phosphorylation site needed for regulation of the enzyme's activity *in vivo*. As SPS is inactivated by phosphorylation, the mutant SPS gene effectively encodes an 'ever-active' form of the enzyme.

This paper describes the successful transformation of the commercial sugarcane variety Q117, and preliminary screening for foreign SPS protein in the transformants.

MATERIALS AND METHODS

Embryogenic callus preparation and tissue culture

Unemerged leaf and sheath roll of mature stems of sugarcane variety Q] 17 were cut into 2-3 mm thick slices and used to initiate embryogenic callus according to Franks & Birch (1991). Callus was co-transformed by bombardment with one of the three described SPS expression vectors and pEmuKN-*aph*A (Last et al 1991) for resistance to geneticin. Callus was selected and grown to young plantlet stage according to Grof et al (1996). To date, more than 200 independent lines of transgenic cane have been produced using the SPS expression vectors.

Genomic Southern blot analysis

Total DNA was isolated from sugarcane using the method of Doyle & Doyle (1991) with minor modification. Approximately 20ug of DNA was digested with the appropriate restriction enzyme and the fragments separated on a 1% (w/v) agarose gel. The DNA was then transferred to Hybond -N membrane (Amersham). DNA probes were labelled using the random-priming method (Feinberg & Vogelstein 1983). Prehybridisation was carried out at 68°C overnight in 6X SSC, 5X Denhardt's solution, 1% (w/v) SDS and 100 g/mL denatured salmon sperm DNA. Hybridisation was carried out overnight in a solution of the same composition with the addition of dextran sulfate to a final concentration of 10% (w/v). Filters were washed twice in 2x SSC for 30 min, then 2x SSC, 0.1% (w/v) SDS for 30 min and 0.lx SSC, 0.1% (w/v) SDS, for a further 30 min. All washes were carried out at 68°C. The filters were autoradiographed at -80°C for 7 days.

Western blot analysis

Protein was extracted from 150mg of leaf tissue. 20uL aliquots of the protein extracts were separated on 10% (w/v) SDS polyacrylamide gels (Laemmli 1970). The proteins were transferred to nitrocellulose membrane in electrophoresis buffer containing 20% (v/v) methanol. The membrane was blocked overnight at 4°C in buffer (10mM Tris-HCl, pH 8.0, 0.9% (w/v) NaCl, 0.5% (w/v) Tween 20) containing 5% (w/v) nonfat dried milk. After blocking, the membrane was washed 5 times, each for 10 min, in buffer containing 1% (w/v) nonfat dried milk. The membrane was then incubated for 3 h with primary antibody in the 1% milk buffer at a dilution of 1:2000. The primary antibody was a polyclonal antisera raised against purified spinach SPS protein (Sonnewald et al 1993; Weiner 1995). The membrane was washed 5 times, each for 5 min, in buffer containing 1 % (w/v) nonfat dried milk, then incubated for 3h with secondary antibody diluted in the 1% milk buffer (1:3000 dilution) and washed again 5 times, each for 5 min, in buffer containing no milk. The membrane was incubated in alkaline phosphatase buffer (100 mM Tris HCl pH 9.5, 100 mM NaCl, 5 mM MgCl₂) for 15min, to which p-nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt (BCIP) were added to final concentrations of 400 nM and 380 nM respectively.

RNA isolation and RT-PCR

Total RNA for the RT-PCR procedure was extracted in the presence of guanidinium thiocyanate followed by caesium chloride centrifugation (Ausubel et al 1987). Superscript II (Gibco BRL) reverse transcriptase was used for RT-PCR in accordance with the manufacturer's instructions, to synthesise first strand cDNA from total RNA. An aliquot of first strand cDNA thus produced was amplified using the EXPAND PCR system (Boehringer Mannheim)

using specific primers designed to produce a 1.7 kb fragment of the spinach SPS.

RESULTS

Genomic Southern blot analysis revealed that multiple copies of the SPS transgene were incorporated into the genome of some transgenic lines (Fig. 1). As expression of the gene is a key criteria for success of this work, an initial screening strategy to detect the presence of the foreign spinach protein using western blot analysis was followed. Protein extracts from approximately 200 putatively transgenic plants were screened for the presence of the spinach SPS protein. A small subset of these are shown in Figure 2. Examination of the blot revealed common non-specific binding of Rubisco in wild type and transgenic QI117 sugarcane. Bands indicating this non-specific binding appeared after 20-30 min. There was no binding to wild type QI 17 SPS, emphasising the specific antigenicity of the primary antisera raised against the spinach SPS protein. This was not a function of low expression of SPS in wild type sugarcane, as previous tests using antisera raised against maize SPS had shown significant amounts of native sugarcane SPS (data not shown). Sequence of partial cDNA clones of sugarcane compared with maize SPS have shown high (>80%) homology at nucleotide and peptide level (data not shown). Binding of the primary antisera to spinach SPS was fast (within 10 s) and specific. Bands corresponding to the spinach SPS appeared in the extracts from three of the transgenic lines (306, 65 and 73) a short time after the band had developed in the spinach control.

A less distinct band corresponding to spinach SPS appeared in the extract of transgenic line 125 after several minutes.

The positive results were reinforced by the detection of the spinach SPS messenger RNA transcripts by RT-PCR in the four putative transgenic lines expressing spinach SPS (Fig. 3). Frame A shows the amplified PCR products of an aliquot of the RT mix used to produce the first strand DNA. A band of 1.7 kb was evident in all lanes, although that amplified from wild type QI117 was significantly weaker, suggesting minimal binding of the spinach-specific primers to the sugarcane SPS gene. In order to ensure that the expected band of 1.7 kb was derived from mRNA of the spinach SPS an aliquot of the isolated RNA was treated with DNase to remove possible genomic DNA contamination prior to RT-PCR (Frame B). That there was no amplification product from the wild type QI 17 confirmed that the wild type band in Frame A arose from genomic DNA rather than mRNA. Frame C shows the amplification products of an aliquot of the same RNA not reverse
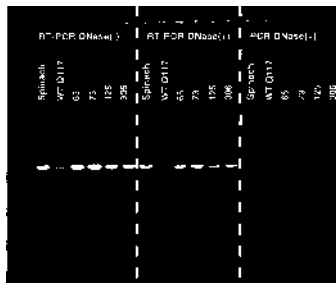
Fig. 3 *RT-PCR with labelled spinach SPS gene of a range of putative transgenic sugarcane lines (125, 306, 65 and 73), wildtype QI117 sugarcane and spinach. Frame A shows PCR amplification products of reverse transcribed mRNA. Frame B shows the PCR amplification products of reverse transcribed mRNA from isolated total RNA treated with DNase. Frame C shows the PCR amplification products of total RNA treated with DNase.*

Fig. 1 *DNA extracted from sugarcane leaves subject to genomic Southern blot analysis. The labelled probe was a fragment of the actin-SPS construct which included a portion of the promoter and the 5' region of the spinach SPS gene. A range of putative transgenic sugarcane lines (1-15) and wildtype QI 17 sugarcane (C) were analysed.*

CONCLUSION

We have demonstrated the insertion of multiple copies of the spinach SPS transgene into the genome of sugarcane variety QI 17. Having established incorporation, several hundred putative transgenic plants were screened for expression of spinach SPS protein by western blot. The four lines which exhibited detectable spinach SPS protein expression were found to express the spinach SPS gene by RT-PCR. Ongoing work in our laboratory will assay the SPS activity of wild type and the four most promising transgenic lines, and compare their sucrose accumulation performance.

ACKNOWLEDGMENTS

Fig. 2 *Western blot of a 10% SDS-PAGE gel of a variety of plant extracts visualised with anti-spinach SPS antisera. Lanes contained leaf proteins from wildtype QI 17 sugarcane, spinach, and a range of putative transgenic sugarcane lines (125, 306, 65, 73, 72, 33, 24, 23, 8, 57, 22 and 29).*

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ISOLATION AND GENETIC MANIPULATION OF INVERTASE GENES IN SUGARCANE

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ABSTRACT

Invertase hydrolyses sucrose to glucose and fructose. Such activity is thought to be important in the regulation of sucrose accumulation because invertase activity decreases as sugarcane reaches maturity. Ongoing research aims to develop transgenic sugarcane plants with reduced vacuolar and/or cytosolic invertase activities, and to determine the effects of such reduction on rate and extent of sucrose accumulation and remobilisation to monosaccharides. Antisense technology will be used to achieve down-regulation of invertase(s) and consequently the gene(s) encoding these enzymes must be isolated and sequenced. Current progress on this research is presented.

INTRODUCTION

Invertase (B-fructofuranosidase; 3.2.1.26) hydrolyses sucrose into the component moieties glucose and fructose (Sturm & Chrispeels 1990). Invertase is present in several isoforms in the plant cell. These isoforms are characterised by solubility, subcellular location and pH optima (Sturm & Chrispeels 1990). Insoluble acid invertase is localised in the apoplast, ionically linked to the cell wall. Soluble invertases with pH optima ranging from pH 4.5 to 7.5 are localised in the vacuole although there is some activity from this isoform in the apoplast as well. Both cell wall bound invertases and soluble vacuolar forms are glycoproteins. The possibility of a cytosolic neutral (or alkaline) invertase in sugarcane was proposed by Hatch & Glasziou (1963). An invertase with a neutral pH optimum and apparent cytoplasmic location (suggested by lack of glycosylation) has been purified from chicory roots (Van den Ende & Van Laere 1995). However, a gene encoding this isoform has yet to be isolated from any plant species.

Vacuolar acid invertase (VAI) activity is high in immature, actively growing stem tissue and declines with increasing maturity of the stem (Hatch & Glasziou 1963; Moore 1995). VAI is also believed to be involved in the remobilisation of sucrose during periods of stress for maintenance of cellular processes or growth. Factors such as unseasonal rainfall or warmth, carbon stress or delayed harvest may trigger remobilisation (Bull & Glasziou 1975).

The model for sucrose accumulation in sugarcane proposed by Glasziou & Gayler (1972) still best describes our understanding of this process. Sucrose, produced in the leaves is loaded into the phloem by a proton co-transporter and then is translocated to the stem for storage (Gahrtz et al 1994). At the stem parenchyma, sucrose is cleaved by cell wall invertase and the constituent monosaccharides cross the cell membrane. In the cell, sucrose is re-synthesised and stored within vacuoles. If stored sucrose is required for growth at a later stage it is hydrolysed by vacuolar

Many studies have been conducted on invertase in sugarcane and these have been extensively reviewed (Glasziou & Gayler 1972; Moore 1995), however very little is known about mRNA expression of the genes. Enzyme studies have produced conflicting results primarily due to the range of experimental material used, being either cultured cells, whole plants or tissue slices (Moore 1995). Consequently, the sink regulatory mechanisms of the sucrose accumulation and storage processes remain largely unknown.

Our initial target is to modify the expression and hence the activity of invertase localised in the vacuole of stem tissue by producing antisense constructs specifically aimed at down-regulating this isoform. By reducing the capability to remobilise sucrose we hope to be able to increase the level of sucrose storage of transgenic sugarcane plants.

A preliminary investigation of invertase expression in stem tissue and the isolation of a full length cDNA clone for vacuolar acid invertase from sugarcane are in progress.

MATERIALS AND METHODS

Plant material

This investigation used tissue from *Saccharum* hybrid varieties Q117 and Q145. The plant crop was field-grown at the CSIRO Division of Tropical Crops and Pastures, Samford Research Station (27° 22' South, 152° 53' East), with surface irrigation as needed. At planting fertiliser was applied as urea at 100 kg / ha. Overall, both these varieties are high yielding, however Q117 is a high sucrose accumulator while Q145 stores lower amounts of sucrose, as determined by CCS measurement (JA Campbell personal communication). Internodes were numbered following the system of Moore (1987) where the node to which the top visible dewlap leaf attaches is designated +1. The internode above is therefore numbered 0 and the internode below +1. In this study each stem sample contained the respective node and internode tissue. The 14 month old plants were harvested on 7 December 1995.

Plasmid construction

The vector pU3Z is derived from pGEM 4Z and contains the ubiquitin promoter from maize *(ubi)* and the NOS terminator separated by several cloning sites. The vector was digested with *Sma* I (NEB) and then de-phosphorylated. The insert, a 3' end fragment of VAI (scinv3'2) cloned by RACE PCR (Albert et al 1996) was cut and blunt-ended prior to ligation into pU3Z using the T4 DNA ligase Ready-To-Go kit (Pharmacia).

Northern analysis

Total RNA was extracted from the internode tissue by guanadinium extraction followed by caesium chloride purification (Ausubel et al 1987). 15 ug of total RNA from internodes 0 and +1 combined, +2, +3, +4, +5 and +6 were separated on a 1% agarose formaldehyde gel (Ausubel et al 1987) and transferred to Hybond N+ membranes (Amersham) in accordance with the manufacturers instructions. 5 ug of total RNA from internode 0 of NCo310 was run on both gels as a positive control for invertase. A partial invertase PCR product (scinv) was ^{32}P labelled using the Rediprime kit (Amersham) and used as a probe. Scinv is a fragment from the centre of VAI which was cloned by PCR using degenerate primers. The relationship of the partial invertase clones from sugarcane is described in Albert et al (1996). Pre-hybridisation was carried out at 65°C in 6x SSPE, 1% SDS, 5x Denhardts' solution, 200 ug/ml denatured salmon sperm. Hybridisation was carried out in a solution of the same composition with 10% dextran sulphate. The membranes were washed in 6x SSPE, 0.1% SDS twice for 30 minutes at 65°C followed by 2x SSPE, 0.1 % SDS twice for 30 minutes at 65°C, then autoradiographed. Such washing conditions are moderately stringent. Both membranes were hybridised simultaneously with the same probe and were autoradiographed for a common period of time.

RESULTS

Plasmid construction

Sense and antisense constructs were made for transformation of sugarcane by particle bombardment. The fragment used for these

constructs was the partial invertase clone, scinv3'2 (Albert et al 1996), and extends from the active site as designated by Sturm & Chrispeels (1990) to the poly A+ tail. This region of the invertase polypeptide was compared for sequence homology and the resulting tree is shown in Fig. 1.
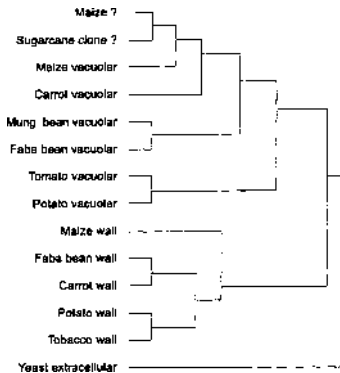
Fig 1 *Tree of the carboxy terminal ends of the deduced polypeptide sequence of a selection of invertase clones (from Genbank database). The sequence region compared extends from the amino acid residue after the active site to the end of the coding sequence. The sugarcane clone came from the Saccharum variety H6S-7052 (Albert et al 1996). Sequences were aligned using ClustalW multiple alignment algorithm on ANGIS (Australian National Genomic Information Service, University of Sydney). The tree was drawn using the Phylip program and is an unrooted tree with "yeast extracellular" as the outgroup. For clarity the tree is presented as a phenogram. From the top down Genbank accession numbers are: U31451, Sugarcane clone not on database, U16123, X67163, D10265, Z49831, Z12025, L29099, U17695, Z35162, X69321, Z22645, X81834, V01311.*

Northern analysis

Fig. 2 shows the autoradiographs resulting from northern analysis of RNA from Q117 and Q145. The level of expression of invertase varies in different internodes of the two varieties. The intensity of signal from internodes +2 to +6 of Q145 is higher than that for the corresponding internodes of QI 17. A strong signal is seen in internode +6 from Q145 whereas in QI 17 transcript level declines dramatically from +5 to +6.

DISCUSSION

Based on sequence comparisons (Fig. 1) the invertase fragment scinv3'2 most likely represents the vacuolar form of the enzyme. The maize clone (U31451) was isolated from root tips and is 93% homologous at the peptide level to scinv3'2, however the function of this clone is not known. In carrot, several genes encoding different soluble forms of the enzyme have been cloned and these are regulated both spatially and temporally (Sturm et al 1995). It is likely that multiple vacuolar invertase genes also exist in sugarcane.

The preliminary Northern analyses show that invertase transcripts were present in all the internodes tested in the varieties QI 17 and Q145. Overall invertase expression was higher in Q145 in all internodes except the combined 0& +1 sample. Furthermore, the peak of expression in Q117 appeared to be much lower and expression dropped more dramatically in internode +6 of Q117 compared with Q145. The level of invertase expression in internodes 0& +1 appeared to be low in both

varieties, which suggests that the high requirement for carbon in this area of rapid growth is fuelled directly by photosynthesis rather than stored sucrose hydrolysed by VAI.
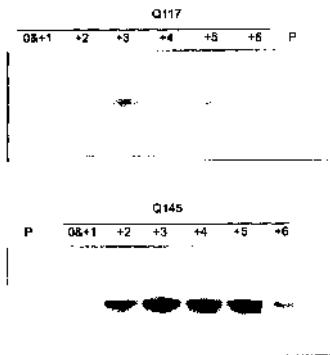
Fig. 2 *Northern analysis oj stem samples of Q117 and Q145 hybridised with the invertase PCR product scinv Internodes were numbered as described in the text. All lanes loaded with 15 ug of RNA except lanes designated P. These lanes were loaded with 5 ug of an internode 0 sample from variety NCo310, as a positive control.*

The work described represents a preliminary investigation of invertase expression in mature sugarcane stems. The environmental conditions at the time of tissue harvest, high temperature following a period of rainfall, may be expected to favour increased VAI expression and activity (Bull & Glasziou 1975). The probe used to assess the level of expression (scinv), is a highly conserved region of the invertase gene and in conjunction with the moderate conditions of hybridisation used, complete discrimination of isoforms might not have been possible, meaning that the signal detected may represent net invertase expression. Dissection of invertase expression and activity, and possible direct correlation with patterns of sucrose accumulation in different cultivars will require measurement under controlled conditions and the use of a specific VAI probe, such as scinv3'2. The role of VAI in relation to remobilisation of carbohydrate in setts will also be investigated.

A number of strategies will be pursued in order to produce transgenic sugarcane with specific down regulation of the vacuolar isoform of invertase. It has been shown that different regions of a gene can have different effects on the down regulation of that gene by antisense RNA (Sandler et al 1988). Antisense and sense constructs using the scinv3'2 DNA fragment have been made with which to bombard sugarcane callus in order to determine the effect of this putative 3' portion of the VAI gene. Antisense and sense constructs using a full length cDNA clone of VAI will be made as soon as it has been isolated.

In order to obtain a full length clone of invertase we are currently optimising the PCR-based technique RACE (Random Amplification of cDNA Ends) (Frohman et al 1988) for sugarcane mRNA. We hope to obtain a clone from tissues expressing high amounts of invertase such as internodes +2 to +6 of Q145 (Fig. 2).

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GENETIC ENGINEERING OF SUGARCANE FOR LOW COLOUR RAW SUGAR

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ABSTRACT

The activity of polyphenol oxidase (PPO) contributes significantly to the enzymic browning processes which occur during the early stages of raw sugar extraction. Antisense expression of PPO has been shown to abolish discolouration after bruising in potatoes with no apparent side effects. A similar approach is being taken in sugarcane in an attempt to down regulate PPO activity to reduce enzymic browning and produce a lower colour raw sugar.

Two PPO cDNAs have been isolated from sugarcane. Both clones have been sequenced and found to be 91% conserved at the nucleotide level, suggesting that the clones represent two distinct members of the sugarcane PPO family. The more abundant cDNA, pSugPPO1, has been selected for use in sense and antisense constructs to transform sugarcane using particle bombardment. A genomic clone of pSugPPO1 is being sought with the aim of isolating the native pSugPPO1 promoter sequences for use in transformation constructs. Transgenic plants will be screened for transgene expression and enzyme activity. The effects of reduced PPO activity on disease and herbivore resistance will be investigated in the long term.

INTRODUCTION

Export markets for Australian raw sugar are increasingly quality conscious and maintenance of Australia's pre-eminent position as a producer of high quality sugar will depend, in part, on the production of cost effective solutions to intractable quality problems. Removal of colour from sugar during refining is time consuming and expensive (currently estimated at SA5 million per annum in Australia). The process necessitates the use of extra purification processes, including the addition of sulfiting compounds, the safety of which regarding human consumption has recently been questioned by the Federal Register published in the US. Browning in raw sugar is minimised by use of low temperature storage and transport facilities. By tackling the problem of enzymic browning using genetic engineering, this project seeks a permanent solution to the problem at the source.

Colour formation in sugarcane processing results from oxidative reaction of phenolic compounds (an enzymic process), thermal degradation and condensation of sugars (caramelisation, a non-enzymic process),

Fig. 1 *Effect of 90°C heat treatment and subsequent addition of PPO on colour formation in cane extracts. Partially purified PPO was added to heat-treated extracts made from different internodes of sugarcane. The same quantity of PPO was added to each extract. [The different A420 absorbances reflect varying availability of phenolic substrate in each of the extracts] (C. Bucheli & S. Robinson, unpublished data).*

alkaline degradation and condensation of sugars (non-enzymic) and the formation of melanoids via the Maillard reaction (also non-enzymic) (Kort 1979). Previous studies by Smith (1976) and Tu (1977) have shown that enzymic browning contributes significantly to colour formation. In laboratory trials. Smith (1976) found that heating cane to 80-90°C prior to crushing resulted in a 47% reduction in average juice colour, a result confirmed by C. Bucheli & S. Robinson (unpublished data) whose results are shown in Fig. 1. Tu (1977) observed a similar reduction in both cane juice and raw sugar colour when the juice was treated with lime to inhibit enzyme activity.

The enzyme responsible for browning in cane juice is polyphenol oxidase (PPO) (Bucheli & Robinson 1994). PPOs constitute a family of nuclear encoded genes, the products of which are localised in the plastids of photosynthetic and non-photosynthetic tissues (reviewed by Steffens et al 1994). During and after crushing of plant material, the PPOs are released from their storage location and catalyse the oxidation of phenolics which are released from the cell vacuole. This reaction produces highly reactive O-quinones, which then polymerise to form high molecular weight black, red and brown coloured melanins (Mayer & Harel 1979). Experiments indicate that removal or inhibition of PPO from cane juice greatly reduces colour formation (Smith 1976), in particular the formation of high molecular weight coloured polymeric compounds, during raw sugar extraction. Comparison of the deduced amino acid sequence of sugarcane PPO with PPOs from other plants shows a low degree of homology (C. Bucheli & S. Robinson, unpublished data). This may be because sugarcane is the only monocot plant in the comparison. The sugarcane sequence does, however, contain the two Cu-binding domains which are characteristic of PPOs (Steffens et al 1994). Comparison of the Cu-binding domain sequences are shown in Fig. 2.

The approach being used to tackle the problem of PPO in sugarcane makes use of antisense technology to reduce the activity of PPO genes. The feasibility of this approach has been demonstrated in potatoes by Bachem et al (1994) and CSIRO, Division of Horticulture. Adelaide. Tubers from these transgenic plants have very low PPO activity and show greatly reduced browning in response to mechanical damage. Data obtained by Bachem et al (1994) is shown in Fig. 3. A similar approach applied to sugarcane should allow the specific inhibition of PPO production.

This paper describes the experimental approach being used to achieve reduced colour formation in raw sugar production, and reports on current progress.

MATERIALS AND METHODS.

Plant material and Plant Transformation

Apical meristems of sugarcane variety Q117 were provided by Dr Nils Berding Bureau of Sugar Experiment Stations, Meringa, Qld.

Fig. 2 *Homology between the putative Cu-binding domains (CuA and CuB) of PPO amino acid sequences from different plants. Histidine residues believed to be involved with Cu-binding are shown in bold (C. Bucheli & S. Robinson, unpublished data).*

Embryogenic callus was prepared according to the methods of Franks & Birch (1991).

Transformation was carried out using the micro-projectile bombardment system, as described by Bower & Birch (1991). All PPO constructs were co-bombarded with the plasmid pEmuKN (Last et al 1991) containing the NPTII kanamycin resistance gene under the control of the Emu promoter and NOS terminator (Depicker et al 1982).

Sugarcane PPO cDNA

The sugarcane PPO cDNA clone was isolated from a sugarcane cDNA library constructed in the vector lZAPII (Stratagene, La Jolla, Ca. USA) from raRNA isolated from the growing tip (10-15mm above apical meristem) (C. Bucheli & S. Robinson, unpublished data). The clone had been manipulated according the *in vivo* excision procedure described by the manufacturer to produce the clone pSugPPO1. This comprised a 2.2Kb *EcoRI* restriction fragment, containing the PPO sequence, cloned into the *EcoRI* site of the vector pBluescript (SK-).

Plasmid Construction

All DNA manipulations were carried out according to Sambrook et al (1989). The PPO1 cDNA was excised from pBluescript (SK-) (Stratagene) as a *DraI-EcoRV* restriction fragment and cloned into the *SmaI* site of the vector pU3Z (a gift from Dr Lianhui Zhang, University of Queensland) which contains the maize ubiquitin promoter (Christensen et al 1992) and the NOS terminator (Depicker et al 1982). Sense and antisense constructs were obtained and termed pSPPO1 and pAPPO1 respectively. A second pair of constructs, pSPPO2 and pAPPO2, were produced by removing the redundant *BamHI* and *SacI* fragments of pBluescript (SK-). Constructs used are shown in Fig. 4.

Fig. 4 *Expression constructs used for sugarcane transformation. All PPO constructs were co-bombarded with pEmuKN. ( ) indicates redundant pBluescript sequences.*

Library Screening

A sugarcane genomic library in lEMBL3 was provided by Henrik Albert, USDA, Hawaii. The library was constructed using a partial *MboI* digest of genomic DNA from the sugarcane variety H32-8560 (Albert 1991). Screening was carried out using a full length (2.2Kb) fragment of pSugPPO1.

Positive clones were isolated and analysed by restriction digestion and Southern blotting (Southern 1975) in order to identify fragments containing PPO sequences for subcloning and further analysis.

Fig. 3 *Mean units of microtuber PPO activity in transgenic lines of potato varieties Diamant and Van Gogh expressing antisense PPO genes are significantly reduced when the constitutive CaMV 35S and tuber specific GBSS-G28 promoters are used to drive the antisense PPO gene, when compared to tuber specific patatin promoter and untransformed control. /Data represent means of 50 replicate lines, 4 different PPO genes per promoter per variety. Standard errors of the means are indicated/ (after Bachem et al 1994).*

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RESULTS AND DISCUSSION

Sugarcane embryogenic callus was co-bombarded with pEmuKN and pSPPO1, pSPPO2, pAPPO1 and pAPPO2. The callus is currently undergoing regeneration and putative transgenic shoots have been obtained. Plants arising from these transformations will be analysed for target gene transcripts, PPO activity, and browning of juice. We anticipate that a degree of inhibition will be obtained with both sense and antisense constructs by co-suppression of native PPO genes and conventional antisense suppression, respectively.

Once plants with low juice colour have been produced, further work will be undertaken to establish whether this leads to a corresponding reduction in crystal colour. Also, since PPO is thought to be involved in disease and herbivore resistance, it will be important in the longer term to demonstrate that advantages gained through improved refinability of low PPO sugar will not be counteracted by increased susceptibility to disease and herbivores.

The PPO constructs used are all under the control of the maize ubiquitin promoter which is a constitutive promoter and the most active promoter characterised to date for use in sugarcane transformation (A. Elliott, personal communication). We plan to identify native sugarcane promoters, in particular PPO promoters, for use in future constructs. Use of native PPO promoters to direct the expression of antisense PPO transcripts will provide a number of advantages over the use of the ubiquitin promoter. Under the PPO promoter, antisense transcripts will be subject to the same spatial and temporal control as the PPO gene itself. Antisense transcripts will also be produced in quantities comparable to those of the native gene. With the aim of identifying such promoters, we have screened a sugarcane genomic library and identified nine clones which contain sequences closely related to pSugPPO1. We are currently subcloning the relevant fragments of the genomic clones for characterisation at the nucleotide level. Putative promoters will be characterised using promoter-reporter gene fusion constructs in transient assays and used to produce PPO constructs for stable transformation.

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3.4 Disease and insect resistance

ANTIMICROBIAL PROTEINS: NEW OPTIONS FOR DISEASE CONTROL IN SUGARCANE

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ABSTRACT

An increasing number of proteins that exhibit anti-microbial activity are being isolated from plants. The genes encoding these anti-microbial proteins have potential for the manipulation of heterologous plant systems for enhanced resistance to plant pathogenic microbes. These proteins are categorised into classes or families based on sequence homology or mode of action. In a screen of Australian plant accessions significant levels of antimicrobial activity were discovered when soluble protein extracts were bioassayed against the growth of commercially important microbial pathogens in vitro. Two of these extracts were further purified to reveal two low molecular weight cysteine-rich peptides with potent antimicrobial activity toward a panel of phytopathogens including several pathogens of sugarcane. These might be used to increase the resistance of sugarcane to pathogenic organisms.

INTRODUCTION

Current breeding techniques for disease control in plants rely on the existence of natural resistance genes to specific microbial pathogens. For many of the commercially important microbial pathogens resistance genes are limited or non-existent within the host germplasm. The use of plant transformation technology to introduce a gene or genes encoding resistance determinants will increase the options available to plant breeders for the production of disease-resistant plants. In recent years, many different plant proteins with antimicrobial and/or antifungal activity have been identified and described. These proteins have been categorised into several classes according to either their presumed mode of action and/or their amino acid sequence homologies. These classes include chitinases, b-1,3-glucanases, permatins, ribosome-inactivating proteins, plant defensins, thionins, chitin binding proteins, thaumatin-like, or osmotin-like proteins, PR1-type proteins and the non-specific lipid transfer proteins. There are also other anti-microbial proteins from plants which have not been categorised for example, the anti-microbial proteins of *Mirabilis jalapa.*

There is already evidence that the expression of genes encoding proteins that have *in vitro* anti-microbial activity in transgenic plants can result in increased resistance to microbial pathogens. Examples of this engineered resistance include transgenic plants expressing genes encoding: a plant chitinase. either alone (Broglie et al 1991) or in combination with a b-1,3-glucanase (Melchers et al 1993); a plant defensin (Terras et al 1995); an osmotin-like protein (Liu et al 1994) and a ribosome inactivating protein (Logemann et al 1992). The results obtained *in vivo* for most of these proteins are consistent with the *in vitro* inhibition experiments performed on the pathogen with purified protein.

The cysteine-rich, basic, low molecular weight antimicrobial proteins are the most potent plant antimicrobial proteins isolated so far. The thionins, whose members have been separated into three classes based on their sequence homology and spectrum of activity are the best characterised of these peptides. The thionins are prevalent in the endosperm of many plant seeds and are believed to have a storage (sulphur) as well as a defensive role. Analogues have been observed in many plant organs including leaf specific isoforms which are stress induced, especially in response to microbial challenge. The **a** and **b** thionins are the most toxic of the thionins isolated thus far. They inhibit the growth of gram negative and gram positive bacteria, fungi, insect cells, mammalian cells and some members of these two classes have been shown to inhibit the growth of plant cells (Bohlman et al 1994). This general toxicity may limit the usefulness of these proteins in transformation work. The third class of thionins are the g-thionins, which also falls under the classification of plant defensins. Like the other members of the thionin family, the g-thionins and plant defensins were initially isolated from seeds. They show inhibitory activity towards fungi and gram positive bacteria but show no inhibition towards the growth of either plant or animal cells (Moreno et al 1994, Osborn et al 1995). Other cysteine-rich low molecular weight antimicrobial peptides include

the two novel peptides isolated from *Mirabilis jalapa* and *Amaranthus caudatus.* These two peptides show no homology to the thionins but show a similar spectrum of inhibitory activity to that observed from the Y-thionins.

Fungal and bacterial pathogens cause considerable losses in sugarcane production each year in Australia. The major diseases include poor root syndrome associated with *Pachymetra chaunorrhiza* and *Pythium* spp. and ratoon stunting disease caused by *Clavibacter xyli* subsp. *xyli.* Other diseases of either sporadic or lesser importance include pineapple disease caused by *Ceratocystis paradoxa,* red rot caused by *Colletotrichum falcatum* and sugarcane common rust caused by *Puccinia melanocephala.* Sugarcane breeding is a slow and difficult process due to its complex genetics. Durable methods of disease control are desirable to provide protection against the wide range of pathogens which infect sugarcane. Current plant breeding strategies for disease control generally address one disease at a time and are dependent on sources of natural resistance which are limited for many of these diseases. Antimicrobial proteins with broad range activity, that can be expressed directly from suitable constructs in transgenic plants, may be used for the production of disease-resistant sugarcane. This paper describes the screening of seed protein extracts from Australian native plants for antimicrobial activity and the isolation of two novel antimicrobial proteins which inhibit the growth of some sugarcane pathogens.

MATERIALS AND METHODS

Extraction of basic proteins of seeds

Australian native seeds were ground and extracted for 4 hrs at 4°C in 2 volumes of cold extraction buffer (Terras et al 1992). The resulting homogenates were strained through cheese cloth to remove particulate matter and centrifuged at 3,000 x g for 30 min to clarify the solutions. Solid ammonium sulphate was added to the supernatants to obtain 30% relative saturation and the precipitate allowed to form overnight while gently stirring at 4°C. Following centrifugation at 3,000 x g for 30 min, die supernatants were taken and ammonium sulphate added to achieve 80% relative saturation. The solutions were allowed to precipitate overnight and then centrifuged at 3,000 x g for 30 min in order to collect the precipitated fraction. The 30%-80% fractions were then resuspended in a minimal volume of 20 mM Tris-HCl pH 9 and dialysed overnight at 4°C in the presence of protease inhibitors. After dialysis the protein solutions were passed through anion-exchange columns equilibrated at pH 9 with 20 mM Tris-HCl. The collected flow-through from this column represents the basic (pi >9) protein fraction of the seeds.

Bioassay of protein extracts

All bioassays were carried out in 96-well microtitre plates. Typically, the test organism was suspended in a synthetic growth medium (Terras et al 1992). The test organism consisted of bacterial cells, fungal spores (50,000 spores/mL) or fungal mycelial fragments (produced by blending a hyphal mass from a culture of the fungus to be tested and then filtering through a fine mesh to remove larger hyphal masses). Fifty microlitres

of the test organism suspended in medium was placed into each well of the microtitre plate. A further 50 uL of the test anti-microbial solution was added to appropriate wells. To deal with well-to-well variability in the bioassay, 4 replicates of each test solution were performed. Sixteen wells from each 96-well plate were used as controls for comparison with the test solutions. All fungi were grown at 25°C. *Clavibacter* spp. was grown at 28°C and *E. coli* was grown at 37°C. Percent inhibition was measured using an optical density measurement following the change in absorbance at 600 nm. The time intervals between measurements were dependent on the pathogen being assayed. Growth inhibition is defined as 100 times the ratio of the change in absorbance of the average growth in the control wells minus the change in absorbance in the test well over the change in absorbance at 600 nm for the mean of the control wells. The IC50 value (concentration of which growth was inhibited 50%) was used to compare the activity of protein. Percent inhibition levels used in the calculation of IC50 values were taken from the second time period (usually 24-48h) in the time course.

RESULTS

Screening for antimicrobial activity

Extracts of soluble basic protein were obtained from the seeds of 200 indigenous Australian plant species. These extracts were screened to assess antimicrobial activity against a panel of important fungal phytopathogens representing the major classes of fungi. The panel included; *Phytophthora cryptogea, Fusarium oxysporum* f.sp. *cubense, Sclerolinia sclerotiorum, Sclerotium rolfsii* and *Colletotrichum gloeosporioides*. Screening revealed 20 extracts to show significant inhibitory activity to various members of this panel. The 20 extracts were then screened against a wider range of phytopathogens including several of the major pathogens of sugarcane. Several of the extracts exhibited promising activity against *Pythium* spp. and *Clavibacter* spp., and two of these extracts were further characterised. Further purification from the basic extracts of *Macadamia integrifolia* and *Hardenbergia violacea* isolated MiAMPl and HvAMPl two novel antimicrobial proteins. Mass spectrometric analysis of MiAMPl and Z/vAMPl revealed low molecular weight proteins of 8.1kDa and 5.3kDa respectively.

Difficulties were experienced in assaying the antimicrobial proteins against *C. xyli* subsp. *xyli* (Cxx) and *Pachymetra chaunorhiza*. The nutritionally fastidious Cxx was subject to contamination in the microplate assay but MiAMPl had significant activity against a closely related bacteria *C. xyli* subsp. *cynodontis*. Current work is aimed at developing a viability assay based on light emission using *C. xyli* subsp. *xyli* transformed with luciferase genes. The fungus *Pachymetra chaunorhiza* would not grow in microtitre plates. Tests using antimicrobial peptides applied to wells cut out from agar plates did not indicate any inhibition by the two peptides but more work is needed to develop a better assay system for this fungus.

Antimicrobial activity of ffvAMPl and AfiAMPl

The results of bioassays on *HvAMVl* and AfiAMPl are presented in Table 1. Examples of growth inhibition plots for the proteins against *Ceratocystisparadoxa* are shown in Fig. 1. Both AfiAMPl and/vAMPl are potent inhibitors of the *in vitro* growth of many of the major pathogens of sugarcane as well as other crops. The anti-microbial activity is greatly reduced in the presence of the divalent cation (Ca²+). Similar reductions in potency in the presence of 1 mM Ca²⁺ have been seen with other anti-microbial proteins (Terras et al 1992). Significant morphological changes were observed when several of the ascomycetes were treated HvAMPl and AfiAMPl. Increased branching and swelling of the hyphae was typically seen in the presence of the proteins.

DISCUSSION

Through the screening of 200 basic protein extracts we have isolated 20 extracts with antimicrobial activity against many commercially important phytopathogens. Two of these extracts have been further purified to reveal two novel antimicrobial proteins designated //vAMPl and AfiAMPl. Both peptides have been demonstrated to be potent inhibitors of the growth of many microbial phytopathogens *in vitro*.

Table 1 *The IC50 value (mg/mL) of HvAMPl and MiAMPl against various fungal and bacterial pathogens of sugarcane, as well as several other commercially important fungal pathogens.*

[The results for the fungal pathogens presented in the first column under "A", were obtained using the synthetic low ionic strength growth medium (Terras *et al.* 1992). The antimicrobial protein was also tested in the presence of 1 mM Ca²+ and 50mM KCl in the test medium. The results for these experiments are presented in the column headed "B". The bacterial pathogen was growth in S8 media ("A") and S8 media supplemented with 1 mM Ca²+ and 50mM KCl ("B"). *Clavibacter xyli* subsp. *cynodontis* was used in preference to *C. xyli* subsp. *xyli* because of its faster growth rate. ND means the experiment was not done.]

Pythium graminicola	50	5	>100	>100
Ceratocystis paradoxa	10	20	>100	75
Colletotrichum falcatum	>100	>100	>100	>100
Clavibacter xyli subsp. *cynodontis*	ND	10	ND	10
Alternaria helianthi	5-10	2-5	75	>100
Sclerotinia sclerotiorum	20	5	100	>100
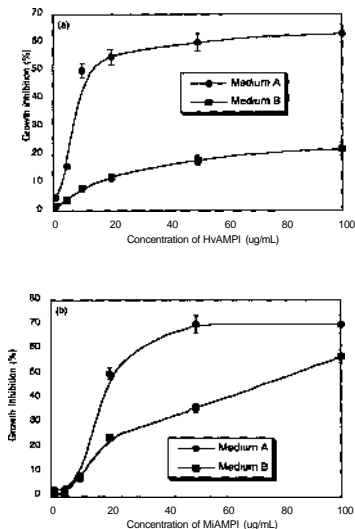
Fig. 1 *Growth inhibition % of* Ceratocystis paradoxa *treated with proteins* (a) *HvAMPl and* (b) *MiAMPl over a 12 to 24 h period in medium A and B.*

[Medium A is a synthetic low ionic strength growth medium (Terras et al 1992), medium B is medium A supplemented with 1 mM Ca²⁺ and 50mM KCl.]

These results identify *MiAMP1* and HvAMP1 as possible candidates for the production of transgenic plants expressing these two proteins.

Amino acid sequences have been determined for both proteins and attempts are currently underway to clone cDNAs corresponding to the MiAMP1 and HvAMP1 proteins. Once cloned, plant expression constructs can be made to examine the activity of these proteins *in vivo*. Initial experiments will involve their expression in model plant systems such as tobacco or *Arabidopsis*. Their ability to enhance resistance to phytopathogens will be assessed after transforming commercially important plant species. The ultimate aim of the work is to express genes corresponding to these proteins in sugarcane. Plant expression constructs will be produced under the control of monocot organ-specific promoters dependent on the target disease eg. vascular-expressing promoter for *Clavibacter xyli* subsp.xyli, leaf-expressing promoter for *Puccinia melanocephala* or root-expressing promoter for *Pachymetra chaunorhiza*.

A number of basic protein extracts which exhibited potent antimicrobial activity are yet to be investigated. Attempts are currently underway to purify the active components of these protein extracts with the aim of broadening the arsenal of antimicrobial proteins. If proteins could be isolated with either a broader range or higher level of activity they could be important in producing cassettes containing numerous peptides, that could be concurrently expressed to provide a broader range of resistance.

At present most antimicrobial proteins under development have been identified by overseas multinational corporations. The use of these genes/proteins in Australian agriculture will be under terms dictated by these off-shore companies. The identification of new proteins in an Australian institution will permit less encumbered applications.

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GENETICALLY ENGINEERING RESISTANCE TO SUGARCANE MOSAIC AND FIJI DISEASE VIRUSES IN SUGARCANE

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ABSTRACT

Two important pathogens of sugarcane are sugarcane mosaic and Fiji disease viruses. These viruses can cause significant yield losses in susceptible crops, while their potential presence can restrict the extent of cultivation of sugarcane clones or affect the choice of parents in plant improvement programs. Backcrossing to introduce resistance genes is not practical in sugarcane due to the complex polyploidy and heterozygosity of the genome. Pathogen-derived resistance (PDR) is being exploited successfully in other crops to produce transgenic virus resistant clones. The coat protein coding region of sugarcane mosaic virus (SCMV) has been cloned and developed into a gene suitable for sugarcane transformation. Transgenic sugarcane plants containing this coat protein are being evaluated. Further PDR genes for resistance to SCMV are being developed, which will allow full exploitation of potential transgenes and the possibility of pyramiding genes into transgenic plants; similar work is underway to develop PDR genes from the Fiji disease virus genome. Novel genes for resistance to these viruses will produce significant gains for the sugar industry.

INTRODUCTION

Commercial cultivation of sugarcane (*Saccharum* L. spp. hybrids) is affected by a wide range of viral, fungal and bacterial pathogens. The main viral pathogens of sugarcane present in Australia include sugarcane mosaic potyvirus (SCMV), Fiji disease reovirus (FDV) and sugarcane bacilliform badnavirus (SCBV). The recently reported yellow leaf syndrome is probably caused by a luteovirus, while chlorotic streak is probably of viral or viroid aetiology. In general, there is resistance to these pathogens within the '*Saccharum* complex' gene pool, but introducing resistance into agronomically elite clones has proved difficult because of the complex genetics of sugarcane. Backcrossing to introduce specific genes is virtually impossible due to the uncertain chromosome composition of commercial clones and the aneuploid nature of the progeny.

Resistance against viruses from a wide range of families, including the potyviridae (Clough & Hamm 1995) and the luteoviridae (Wilson 1993), is being successfully engineered into many crop plants. Work is currently underway to engineer resistance to reoviruses such as rice dwarf and rice ragged stunt (Matsummura & Tabayashi 1995). These resistance genes originate from the pathogen itself, and are usually referred to as pathogen-derived resistance (PDR) genes (Sanford & Johnstone 1985). Following the first practical demonstration of the concept by Powell Abel et al (1986), transgenic plants have been produced carrying full length, truncated, untranslatable or modified versions of coat proteins, replicases, movement proteins, and other sequences of viral origin. A genetic construct based on the full length coat protein of one Australian isolate of SCMV strain A (SCMV-A) was developed and expression of this gene demonstrated in sugarcane protoplasts (Smith et al 1992). However, there are questions about the basic underlying principle of coat protein mediated resistance (Wilson 1993; Smith et al 1995b). There is evidence that some transgene-mediated resistance acts at the RNA and not the protein level, and that resistance results from the activation of natural plant defence mechanisms. Genetic variability in the pathogen also can significantly influence the effectiveness of transgenic resistance. In some instances, very small differences in the nucleic acid sequence between the transgene and the infecting virus can result in no resistance to the pathogen (Wilson 1993). However, there are also examples where transgenic resistance holds even though there are considerable differences between the nucleic acid sequences (Wilson 1993).

Meaningful exploitation of PDR genes for sugarcane genetic engineering requires knowledge and clones of the pathogen nucleic acid so that informed decisions can be made about the type of resistance transgene that could be developed. Here, we present progress on the development of SCMV-resistant transgenic sugarcane, the selection of further genes for SCMV resistance, analysis of pathogen variability, and the development of PDR genes for resistance to FDV.

MATERIALS AND METHODS

SCMV transgenics

The coat protein (CP) coding region of SCMV-A was cloned by Frenkel et al. (1991) and subsequently modified into a gene and constructed into an expression vector driven by the *Emu* promoter (Smith et al 1992). This gene subsequently was placed under control of the maize ubiquitin promoter (*Ubi*) in both sense and antisense directions. Induction of embryogenic callus, microprojectile transformation, and selection/regeneration of plantlets were essentially as described by Bower & Birch (1992). Analysis of regenerated plants by PCR, western and Southern blots were essentially as described by Smith & Gambley (1993).

Analysis of SCMV field isolates

Leaves infected with SCMV were collected from the BSES Pathology Farm (Brisbane) and near Bundaberg. Childers (Isis Mill area) and Nambour in south east Queensland. Total nucleic acids were extracted, and viral specific RNA was amplified essentially by the reverse transcription-polymerase chain reaction (RT-PCR) protocol described by Smith & Van de Velde (1994). The amplified products were cloned using the pGEM-T Vector System (Promega) and three independent clones from each sample were cycle-sequenced to eliminate errors due to PCR amplification. The RT-PCR and cycle-sequencing conditions and generation of sequence data were essentially as described by Smith etal (1995a).

Generation and analysis of FDV clones

Galls were cut from FDV-infected leaves of sugarcane maintained at the BSES Pathology Farm, Eight Mile Plains. Double-stranded RNA (dsRNA) was extracted and then primed for reverse transcription by boiling the dsRNA and hexanucleotide primers together for 8-10 min followed by quenching in dry ice/ethanol or liquid nitrogen. Second strand synthesis was by a combination of DNA polymerase I and RNaseH, and after the ends were polished, the cDNA was cloned into the SmaI site of pUC18 or pGEM-3Zf+. The ligation reaction was transformed into competent *E. coli* JM109 cells and clones containing recombinant plasmids selected by blue/white differentiation on IPTG/X-gal supplemented media. Plasmids were prepared from selected clones, labelled with ^{32}P by random priming and used as hybridisation probes against northern blots of FDV dsRNA to identify the segment from which the cloned cDNA originated. Clones unique to individual FDV segments were cycle-sequenced as described above, and the sequences aligned and compared using programs maintained on the

Australian National Genetic Information Service (ANGIS) computer

RESULTS AND DISCUSSION

SCMV transgenics

Transgenic plants, transformed with the *pEmuCP* constructs and co-bombarded with pEmu/NPTII as the selectable marker, from cultivars Q95, Q124, Q137, Q153 and Q155 were selected and regenerated on geniticin-supplemented media and are now growing in a PC2 standard glasshouse. Initial screening using PCR indicated the presence of the CP-gene in many of the selected plants. Subsequent analysis by Southern hybridisation revealed CP specific genomic bands. The banding pattern suggested that some plants were clones derived from the same transformation event, while other plants were from independent transformation events. Genomic blots probed with the NPTII gene indicated several copies of this gene were present in transformed sugarcane. Preliminary Western blots failed to detect any coat protein in the transformants that were tested.

Putative transformants containing the CP gene (in both sense and antisense forms) behind the *Ubi* promoter have been regenerated. These plants were selected on geniticin at 60 mg/L and are being transferred to the glasshouse. The *Ubi* promoter is being investigated as we have been unable to detect expression of SCMVCP in mature plants transformed with Emu-based constructs. However, these plants may be resistant to SCMV by RNA-mediated resistance. Analysis of mRNA by northern blotting and nuclear transcript run-off is underway as well as establishing glasshouse pathology trials to characterise these plants. We anticipate testing the plants transformed with ubi'-based constructs for protein (sense) and mRNA (sense and antisense) in the near future.

Table 1 *(a) Nucleic acid and (b) translated amino acid homology similarities (percent) between the full coat protein coding region of eight SCMV field isolates and the transgene sequence. Origin of isolates: nam - Nambour, isis - Isis, bund - Bundaberg, bris - Brisbane.*

(a)

	nam2	nam7	isis5	bund	isis3	isis7	bris	isis2	transgene
					Nucleic acid homology (%)				
nam2	100	99.6	98.4	98.2	96.1	96.2	96.7	98.5	95.3
nam7		100	98.9	96.4	96.3	96.5	96.8	96.7	95.5
isis5			100	98.8	96.5	96.6	96.3	97.2	95.5
bund				100	99.7	99.4	99.2	95.8	97.5
isis3					100	99.6	99.5	96.6	97.3
isis7						100	98.9	95.5	97.5
bris							100	95.2	97.5
isis2								100	93.7
transgene									100

(b)

	nam2	nam7	isis5	bund	isis3	isis7	bris	isis2	transgene
					Translated amino acid homology (%)				
nam2	100	100	100	97.1	96.8	98.8	96.8	97.4	95.5
nam7		100	100	97.1	96.8	96.8	96.8	97.4	95.5
isis5			100	97.1	96.8	96.8	96.8	97.4	95.5
bund				100	99.7	99.4	99.7	95.8	97.8
isis3					100	99.7	99.4	95.5	97.4
isis7						100	99.4	95.5	97.8
bris							100	95.5	97.4
isis2								100	94.2
transgene									100

Analysis of SCMV field isolates

The nucleic acid sequence encoding the coat protein of field isolates of SCMV-A showed a number of differences to the sequence of the transgene being used for sugarcane transformation (Table 1). The lowest homology between the field isolates and the transgene was 93.7% (the *isis2* sequence). *Isis2* also had the lowest homology at the amino acid level to the transgene (94.2%) (Table 1). While some of the isolates such as *bund* and *isis3* were within 3% of the transgene sequence, others such as *nam2* and *nam7* show more than 4% difference to the transgene sequence. This value is of concern as recent results with transgenic

resistance to papaya ringspot potyvirus indicated that transgenic resistance was not conferred when the difference between the nucleic acid sequence of the transgene and the infecting virus was only 4% (Bateson et al 1994; Tennant et al 1994). Constructs based on *isis3* are being prepared. These constructs include both full-length translatable and untranslatable versions of the coat protein gene for both protein and RNA-mediated transgene protection against Australian field isolates of SCMV-A.

Generation and analysis of FDV clones

Approximately 70% of the FDV genome is represented in clones which have been generated and mapped to a specific segment. Emphasis has been placed on segments 1 and 7. These segments potentially encode the replicase and an outer coat protein spike, two targets proposed for the development of PDR transgenes for control of FDV. We have sequenced and mapped 88% of the 4.4 kbp segment 1 and 94% of the 2.2 kbp segment 7 (Fig. 1). All RNA-dependent RNA polymerases contain a glycine-aspartic acid-aspartic acid (GDD) motif in the translated sequence that is essential for enzymatic activity. This motif is a potential target for mutagenesis: changing one of the aspartic acid (D) residues to glutamic acid (E) should delete polymerisation activity but not template (RNA) binding. Expression of defective FDV-replicase in transgenic plants should result in specific binding of FDV-specific RNA but no replication would occur, and hence the infection cycle would be inhibited leading to FDV resistance.
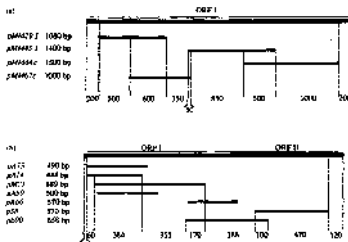
Fig. 1 Clone maps of Fiji disease virus (a) segment 1 (4.4 kbp) and (b) segment 7 (2.2 kbp)

[not drawn to scale, ORF: open reading frame]

Segment 7 contains two open reading frames (ORFs) encoding proteins of approximately 37 and 41 kD. Usually each dsRNA segment in the reoviruses has only one ORF and encodes one protein. Interestingly, segment 7 of another fijivirus, rice black-streaked dwarf also encodes two proteins (Azuhata et al 1993). The proteins encoded by FDV segment 7 should be outer coat proteins and expression of one or both of these proteins may inhibit viral uncoating and hence lead to resistance. Both of the mechanisms proposed lead to protein mediated resistance. Untranslatable versions of these and other FDV genes will result in RNA-mediated transgenic resistance. We also have partial clone maps to most of the other ten FDV segments. For example, almost 1.2 kbp of the 2.8 kbp FDV segment 6 has been sequenced and a number of other segment 6 specific clones have been identified and await sequencing.

CONCLUSIONS

PDR genes initially will be deployed to provide virus resistance in existing agronomically elite but pathogen susceptible clones. Incorporation of these new transgenes into potent parents for wide exploitation in crossing programs must be carefully considered to avoid genetic uniformity and over reliance on a single resistance gene. While

there is no field evidence to support the hypothesis that PDR genes will provide more robust resistance than natural genes (Sanford & Johnstone 1985), there is cautious optimism for their long term durability. However, given the evolutionary flexibility shown by pathogens reliance on one control strategy is dangerous (Michelmore 1995). Variability between Australian isolates of SCMV-A suggests that RNA-mediated resistance may not provide field resistance, although the same transgene may provide adequate field resistance if coat protein is expressed, as protein-mediated resistance appears to be less susceptible to sequence variation than RNA-mediated resistance (Lomonossoff 1995). Variation between FDV isolates in virulence has been reported once (Hayes 1974), although the significance of this variation for transgene mediated resistance is uncertain. Targeting a number of viral functions by developing a range of PDR genes should minimise any potential for isolate or sequence variation to negate the long term durability of genetically engineered resistance to Fiji disease virus.

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INCREASING THE RESISTANCE OF SUGARCANE TO ATTACK FROM WHITEGRUBS BY INTRODUCING NOVEL INSECTICIDAL GENES

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ABSTRACT

Whitegrubs (Coleoptera: Melolonthini) are the Australian sugar industry's most important pests. BSES is developing a system of integrated management for whitegrubs using several control strategies. An important component of this system is to increase the resistance of sugarcane to whitegrub attack by developing transgenic sugarcane plants expressing novel insecticidal genes. We are investigating antimetabolic compounds to determine their potential for improving plant resistance. Several proteinase inhibitors are effective in reducing proteinase activity in whitegrub guts. Feeding bioassays have confirmed that proteinase inhibitors, incorporated into artificial diets, have a significant detrimental effect on whitegrub growth. Lectins from snowdrop and wheatgerm also have a detrimental effect on whitegrubs, slowing development and increasing mortality. Genes coding for proteinase inhibitors from potato and ornamental tobacco and for a lectin from snowdrop are being genetically engineered into sugarcane. Transgenic plants are being characterised before assessing the effectiveness of the introduced genes on whitegrub development and survival.

INTRODUCTION

Whitegrubs, larvae of melolonthine scarabs, are the most important insect pests of Australian sugarcane (Allsopp et al 1993). Larvae damage the roots and underground parts of the stems of sugarcane, reducing plant growth and in severe cases killing the plant. Root damage also makes the plant more susceptible to lodging and during harvest removal of the below-ground parts of the stem from which the following year's crop grows. Nineteen endemic species damage sugarcane in different areas of eastern Australia. In 1994, whitegrubs cost the Queensland industry AS10.5M in lost production and insecticides (Anon 1995). Experience before the arrival of organochlorine insecticides in the late 1940s suggests that, without effective controls, whitegrubs have the potential to damage thousands of hectares of Australian sugarcane and cause annual losses of at least AS150M (Robertson et al 1995).

Since the late 1940s, control of whitegrubs in Australian sugarcane has relied heavily on synthetic insecticides, initially organochlorines, now organophosphates. Efficient use of insecticides is complicated by the long-term nature of the crop (c. 5 yr between replanting), inaccessibility of the crop for much of the year, difficulties in sampling and treating subterranean insects, and by the 1 - or 2-yr life cycles of different species (Robertson et al 1995).

Exploitation of plant resistance already present in sugarcane germplasm is one option to improve the integrated management for whitegrubs. Resistant cultivars offer an important low-input alternative to insecticides, as Australian canegrowers regularly adopt new higher-yielding cultivars. Tolerance and antibiosis effects are known in sugarcane cultivars (Allsopp et al 1995), but selection for these characters is long term and must compete with selection for a myriad of other attributes. A second option is to develop transgenic sugarcane cultivars with enhanced resistance to whitegrubs through the introduction of novel insecticidal genes. Here, we review progress towards achieving this option.

SELECTION, ASSESSMENT AND USE OF NEW GENES

The evaluation and selection of effective genes are key prerequisites before the introduction of antimetabolic genes into sugarcane. We are concentrating on two groups of antimetabolic compounds namely, proteinase inhibitors and lectins. These compounds are known to affect the development and survival of insects. Many also have low mammalian toxicity, which make their use in a food crop more acceptable.

Proteinase inhibitors

Proteinase inhibitors affect proteinase activity, regulation or production in the insect gut. This limits the availability of essential dietary amino acids and proteins, and results in poor development and increased mortality. The midgut proteinases of three species of whitegrubs were characterised, and the effect of a range of proteinase inhibitors on *in vitro* proteinase activity was assessed.

Serine-type, trypsin proteinases predominate in the midguts of the sugarcane whitegrub species *Lepidiota negatoria*, *L. noxia* and *Antitrogus consanguineus* (McGhie et al 1995). Evidence includes: extracts from the midguts show greatest proteinase activity at high pH, indicative of serine proteinases; inhibitors specific to serine proteinases reduced proteolytic activity by about 80%; and there was significant hydrolysis of specific trypsin, chymotrypsin and elastase synthetic substances. Analysis by gel electrophoresis indicated that the three whitegrub species have different proteinases, but the proteinase activity of all the midgut extracts was reduced by the same inhibitors and to approximately the same extent. From this work three proteinase inhibitors were identified for further testing, viz. potato inhibitor II (*pin-2*), soybean trypsin inhibitor type I *{sbti}* and wheatgerm trypsin inhibitors 1 and 2 (*wgi-1, wgi-2*).

The *in vitro* results were validated in a feeding bioassay with soybean trypsin inhibitor type I *{sbti}* incorporated into an artificial diet. *Sbti* at a rate of 10 mg per gram of diet significantly reduced the growth of third instars of A. *consanguineus* and also reduced survival of the larvae (Allsopp 1995; Fig. 1). *Thepin-2, wgi-1* and *wgi-2* proteinase inhibitors are being purified and will be tested in future bioassays, to confirm the

The gene for *pin-2* was available from the Horticulture and Food Research Institute of New Zealand and genetic constructs assembled with both the *Emu* and *Ubi* promoters and the *Nos* terminator. Transient expression of *pin-2* was detected following electroporation into sugarcane protoplasts using the method of Smith et al (1992), confirming that these constructs are active in sugarcane plant cells. These constructs are being introduced into cultivars Q95, Q117, Q124, Q137, Q153 and Q155 using microprojectile bombardment of sugarcane embryogenic callus, and plants regenerated by the method of Bower and Birch (1992). The first regenerated plants of Q95 and Q124 have not grown as quickly as the untransformed plants.

We suspect that expression of *pin-2* is disrupting plant metabolism, possibly because the proteinase inhibitor is not being directed to, and
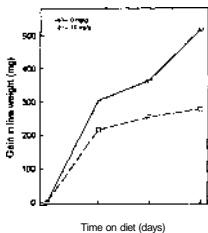
Fig. 1 *Cumulative weight gain of larvae of A.* consanguineus *on semi-artificial diet with added soybean trypsin inhibitor (from Allsopp 1995).*

stored in, the cell vacuole. Further research on the biochemical fate and cellular compartmentalisation of *pin-2* and the other proteinase inhibitors appears necessary to fully exploit this approach. Transgenic plants will then need to be tested to determine if the degree of expression of inhibitors in the roots is sufficient to affect whitegrub development and survival, and therefore provide some measure of resistance to white grubs.

We have also obtained the *NaPI* gene for the proteinase inhibitor from ornamental tobacco, *Nicotiana alata*, isolated at the University of Melbourne and have assembled genetic constructs for expression in sugarcane. These constructs are currently being transformed into sugarcane plants, and regenerated transgenics will be characterised and assessed as described above. Effects of the transformation on plant metabolism also need to be addressed.

Lectins

Lectins are proteins or glycoproteins of nonimmune origin that agglutinate cells and/or precipitate complex carbohydrates. They have been isolated from many natural sources, including plants, fungi, bacteria, invertebrates and mammalian cell membranes. Plant lectins probably function as a defence mechanism against a variety of fungal, bacterial and viral pathogens and against animal herbivores including insects. Many of these purified lectins have been screened against insects in an attempt to identify insecticidal proteins and therefore isolate the genes encoding them for subsequent plant transformation. Genes encoding for pea lectin and snowdrop (*Galanthus nivalis*) lectin have been expressed at high levels in transgenic plants and have conferred some resistance to the appropriate insect pest. Lectins are generally more toxic to insects than are proteinase inhibitors. Thus, the genes encoding such lectins are ideal candidates for transformation into food crops.

We have bioassayed snowdrop lectin and wheatgerm lectin against third-instar *A. parvulus* (Allsopp and McGhie 1996). Both lectins caused 40-93% mortality after 28 days, and lowered weight gains at levels of 1 and 5 mg/g of sucrose-free diet (Fig. 2). Snowdrop lectin appeared more toxic than the wheatgerm lectin (93% and 47% mortality, respectively, at 5 mg/g of diet). Further bioassays, to test other lectins such as the pea lectin are planned. Constructs with the snowdrop-lectin gene (*gna*) have been assembled and introduced into sugarcane tissue. Wheatgerm lectin shows higher mammalian toxicity than does snowdrop lectin. Hence, it is not being developed further in sugarcane, although amounts likely to get through to manufactured sugar are likely to be minuscule.

CONCLUSIONS

The introduction of specific insecticidal genes into plants to produce whitegrub resistance has several advantages. First, the development of
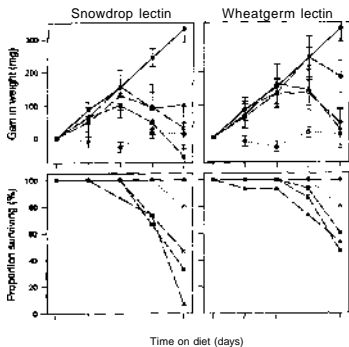
Fig. 2 *Cumulative weight gain and survival of larvae of A. parvulus on semi-artificial diet with added snowdrop or wheatgerm lectins (from Allsopp & McGhie 1996). Lectin levels in mg/g of diet: 0 (•); 0.5 (m); 1 (D); 5 (A.); no food (O).*

resistant transgenic plants independently of the breeding process does not complicate selection in the normal breeding programs, and therefore does not slow general genetic improvement of the crop, especially for traits such as sugar yield. Furthermore, transgenes are alternative sources of resistance, and, if properly exploited by techniques such as gene pyramiding, could provide durable long-term resistance, or a useful alternative to natural plant resistance to minimise the development of resistance in the insect population. Technology is becoming available which should allow fine control of single genes, limiting expression to specific tissues such as roots and/or after particular events such as wounding. This should limit the insecticidal compound to specific parts of the plant, reducing the metabolic cost of extra protein production on the plant, limiting the impact on non-target organisms such as insect predators and reducing the likelihood of contaminating the sugar. Transgenes for resistance to whitegrub would have immediate application to provide resistance in current commercial cultivars such as Q96 and Ql 17 in the Burdekin region, and in the medium to long term to provide alternative resistance genes for exploitation in conventional breeding programs.

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SUGARCANE BACILLIFORM VIRUS RESTRICTS ACCESS TO *SACCHARUM* GERMPLASM

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ABSTRACT

Sugarcane bacilliform virus (SCBV) is perceived as a quarantine problem and a limitation to the continued importation of foreign germplasm. Considerable isolate variation has been observed and a high security quarantine system is required to prevent the possible introduction of aggressive isolates into the country. This variation is a concern for the accurate screening of quarantine germplasm and several diagnostic methods are available. Major differences between SCBV isolates have been detected by DNA probe hybridisations and restriction fragment length polymorphisms within PCR products. The PCR primer pair SCBVF5 and SCBVR5, selected to amplify a presumed conserved region of the viral genome, are able to amplify SCBV DNA from a wide range of Saccharum germplasm.

INTRODUCTION

Importing foreign germplasm has enormous benefits for the Australian sugar industry. High yielding, disease resistant *Saccharum* spp. hybrid clones are introduced for commercial production, while hybrid clones with particular attributes are introduced for breeding. Basic germplasm from the *'Saccharum* complex' is also introduced for use in introgression programs.

Access to foreign germplasm depends on the continued operation of a high security quarantine. The Bureau of Sugar Experiment Stations (BSES) is the only organisation authorised by the Australian Quarantine and Inspection Service (AQIS) to import and quarantine sugarcane in Australia. In recent years, the release of foreign germplasm to the Australian sugar industry was severely reduced because several issues became major concerns for the operation of quarantine. These included: the presence of new, unidentified viruses in vegetatively propagated grasses: symptoms resembling yellow leaf syndrome in commercial canes in quarantine in 1994: symptoms resembling red leaf mottle in noble canes in quarantine in 1994: and the recent identification of two new viral pathogens of sugarcane, viz. sugarcane mild mosaic closterovirus and sugarcane bacilliform badnavirus.

SCBV is widely distributed throughout the major geographical regions in which sugarcane is grown and infects both noble canes (*Saccharum officinarum* L.) and commercial cultivars (Autrey et al 1992). Serological and transmission studies indicate that SCBV is closely related to banana streak virus (BSV) (Lockhart 1986), and SCBV and BSV can be considered as strains of the same virus (Lockhart & Olszewski 1993). SCBV and BSV are members of the badnavirus group (Lockhart 1990). Virions in this group are bacilliform in shape and contain a double-stranded DNA genome. The genome of one strain of SCBV has been cloned and sequenced (Bouhida et al 1993). The largest open reading frame is capable of encoding a protein with regions of similarity to the RNA-binding domains, aspartic proteases and replicases of retroelements. The presence of these coding regions plus a tRNA^{met} binding site suggests that SCBV, like Commelina yellow mottle virus (CoYMV), the type member of the badnavirus group, is a pararetrovirus (Medberry et al 1990).

SCBV is already present in Australia but BSES regards it as a quarantinable pathogen for several reasons. The major reason is the considerable variation between isolates. The wide host range of SCBV is another concern. SCBV infects a wide range of germplasm including commercial hybrids and noble canes, as well as other basic germplasm. In addition, SCBV is closely related to BSV and some very aggressive isolates of BSV have been identified. This paper compares the methods available to detect SCBV and discusses the consequences of genetic variation for accurate detection.

MATERIALS AND METHODS

Mechanical inoculation was performed using the method of Lockhart & Autrey (1988), while vector transmission using the pink sugarcane mealybug (*Saccharicoccus sacchari* (Cockerell)) was performed following the method of Lockhart et al (1992). Leaf dips followed by examination under the electron microscope (EM), enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM) were performed essentially as described by Lockhart & Autrey (1988). An antiserum was prepared against an Australian isolate of SCBV purified from the noble cane IJ76-465, while an antiserum prepared from a mixture of seven SCBV isolates was a kind gift from Ben Lockhart (University of Minnesota). Polymerase chain reaction (PCR) amplification of SCBV DNA was essentially as described in Braithwaite et al (1995) using the primer pair SCBVF5/SCBVR5, except that the reaction cycle began with a hot start, and an annealing temperature of 60° C was used for all cycles. Restriction enzyme analysis of PCR products and Southern hybridisations were also as described in Braithwaite etal (1995).

RESULTS

Diagnostic methods

A number of viral detection methods has been tested for their usefulness in detecting SCBV in sugarcane germplasm. All methods had certain limitations as summarised in Table 1. Diagnosis by symptomatology is unreliable because the symptoms of infection are variable or absent but may include flecks or freckles on the leaves. Mechanical > indicator hosts occurs only at low levels. Both

Table 1 *A comparison of the methods used to detect SCBV.*

Diagnostic method	Advantages	Disadvantages
Symptomatology	Fast	Unreliable
Transmission to indicator hosts	Can obtain biological/pathological information	Difficult, occurs only at low levels
Electron microscopy	Can observe virions	Virus levels generally too low
ELISA	Detects virions, quantitative	Limited by isolate variation, lacks sensitivity
IEM	Detects virions, reliable, semi-quantitative	Slow, operator dependent
	Detects DNA, very sensitive	Influenced by template constraints

mechanical and mealybug transmission of SCBV to sweet corn, sorghum, panic grass and cereals has so far been unsuccessful when assayed by IEM, and PCR amplification has not yet detected SCBV DNA within mealybugs. Generally, the viral titre in hybrid canes is too low to detect SCBV by direct amplification under EM (leaf dips), although examination of partially purified preparations can be more reliable.

n prepared against an Australian isolate of SCBV has been used to develop an ELISA test. The test does not appear to be as sensitive as IEM when the virus is in low concentration. However, the technique is useful for the initial screening of samples and for quantifying virus concentrations. Overseas workers have found diagnosis by ELISA unreliable except for heavily infected noble canes (Autrey et al 1990). This is believed to be due to strain variation because more reliable results were obtained after substituting a BSV antiserum for the SCBV antiserum (Autrey et al 1992). IEM, although slow and tedious, is the most reliable method for detecting virions. Mixed antisera raised to a large range of isolates are now used to overcome isolate variation. PCR is a rapid and sensitive method for detecting SCBV DNA. Primers selected to amplify conserved regions of the viral genome are used to overcome isolate variation. This is described in more detail

Genetic variation in SCBV

Investigations into the genetic variability between SCBV isolates have used a combination of PCR, restriction enzyme digestion, and Southern blotting. Cloning and sequencing of PCR products has also recently begun. In order to detect as many isolates as possible, a conserved region of the genome, the reverse transcriptase coding region, has been targeted. Some sites within this coding region are very conserved and the primer pair SCBVF5/SCBVR5 was selected from these regions. These primers allow SCBV DNA to be amplified from a wide range of *Saccharum* germplasm including *Saccharum* spp. hybrids, *S. officinarum, S. robustum, S. spontaneum, S. barberi, S. sinense* and *Erianthus arundinaceus* (Braithwaite et al 1995). Although the primers target conserved sites, it appears that the DNA between the recognition sites is extremely variable amongst isolates. This v detected by digesting the PCR product with o enzymes (*Alu*I, *Asp*IOO, *Bam*HI, *Nco*I, *Sau96*), and *Sty*X) or by using the PCR products as labelled hybridisation probes to assess the degree of homology to other PCR products. To illustrate this variation, SCBV DNA amplified from total nucleic acid extracts from sugarcane leaves was compared to DNA amplified from a clone of the viral genome (pSCBV20) and a purified virion preparation from IJ76-465. An example of the variation observed between isolates from four canes, EK28 and NG57-99 (both *S. officinarum*), IM76-229 (*S. robustum*) and IJ76-407 (*E. arundinaceus*) is shown in Table 2. The results suggest that variation is not host species dependent, that more than one isolate may be present in a particular plant, and that some unique isolates may be present in the viral population.

CONCLUSION

le the possibility of introducing an aggressive strain of SCBV to Australia, it is essential to design a diagnostic test which accounts for as much isolate variation as possible. Of the detection methods available, IEM is the most reliable for detecting SCBV virions and PCR is the most reliable for detecting SCBV DNA. IEM detects many isolates with a polyclonal antiserum raised against a mixture of virions purified from different sources. However, more isolates will need to be included in the mixture as variation is further characterised. PCR also detects many isolates using primers targeted to conserved regions of the viral genome. Sequencing will allow further definition of the genetic variation and may lead to the design of better primers. A combination of serological and molecular techniques may in the future provide a highly sensitive method of identifying a wide range of SCBV isolates.

Variation in any pathogen complicates detection and diagnosis and at present the implications of the variation in SCBV on the operation of

Table 2 *Variation observed in SCBVF5/SCBVR5 DNA amplified from four sugarcane clones. Results expressed as V or P if the restriction pattern or hybridisation pattern is the same as that observed for IJ76-465 virions or the cloned viral genome pSCBV20, respectively. Other patterns are recorded as unique.*

	NG57-99	IM76-229		IJ76-407
Restriction enzyme:				
*Alu*I	V	P	V	Unique
*Asp*700	V	P	V	V/P
Sam*HI	V	P	V	P
*Nco*I	V	P	V	Unique
Sat/96I	V	P	V	V
*Sty*I	V	P	V	Unique
Hybridisation	V	P	V	P

the quarantine system are uncertain. Until the extent of variation in SCBV can be defined and conserved epitopes or DNA sequences identified, no definitive SCBV test will be available. A definitive test, when developed, will be used to verify the significance of SCBV to the Australian sugar industry, and allow appropriate informed decisions to be made about the importance of this virus in imported germplasm.

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METHODS FOR SUGARCANE SMUT CONTROL IN EGYPT

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ABSTRACT

The reliability of different methods to artificially inoculate sugarcane clones with smut were compared. Differences in varital reaction to smut were noted. Hot-water and fungicides treatments were also assessed to smut control.

Both the dip and wound-paste method were satisfactory for testing smut resistance. The dip method (dipping one budded cane setts for 30 minutes in a spore suspension of 5x10 teliospores/mL water) was preferred as this method is less time consuming than the wound-paste method.

Of the main promising sugarcane varieties tested, G.68/88, F 153, and G.47/84 were found to be highly resistant to smut; G. 37/ 85 and G. 68/84 resistant; G. 74/96, G. T. 54/9 (C9) and G. 368/75 moderately resistant, while NCo 310, E. 68/18 and F 151 were highly susceptible. Hot water treatment at 52° C for 20 min. and 50° C for 2 h were found to be effective as control treatments.

Preliminary results showed that smut is successfully controlled by Bayleton and Benlate. Treating seedcane in hot water containing Bayleton at 50°C for 2 h was more effective than either treatment applied separately. An integrated approach for controlling sugarcane smut is recommended.

INTRODUCTION

Culmicolous smut of sugarcane caused by *Ustilago scitaminea* Sydow, is widely distributed and has had a significant effect on productivity in most sugarcane producing countries of the world. The disease was observed in Natal, South Africa in 1877 by McMartin (1945). Since then the disease has been reported in countries that lie between 20°N and 20°S of the equator, (Martin et al 1961). Sugarcane smut has been reviewed by Lee-Lovick (1978).

In Egypt, the total cultivated area of sugarcane is approximately 140000 ha and only one variety, G.T.54/9 (C9), is in production. Culmicolous smut was first encoated in Egypt in 1930. The latest outbreak was observed at EL-Sebaeiia in 1974 and 1975. During 1981-1983 it appeared on the variety NCo 310 in Minya, Kena and Aswan Govemorate. This variety was replaced by G.T. 54/9 (C 9) according to ministerial decree in 1983 due to the severe effect of smut on NCo 310.

Planting resistant varieties is the most practical and economical way to control the disease; thus screening for smut resistance is a pre requisite in breeding programmes (Flores 1981; Waraitch 1982; Perez & Mauri 1983;AbdelFattah 1989).

Varietal reactions to smut can be evaluated using several inoculation techniques. The most widely used is the dip inoculation method (Byther & Steiner 1974). Another is the wound-paste technique (Leu et al 1976) and the brushing technique (Luthra et al 1938).

The first attempt to cure smut disease of sugarcane by hot water treatment was in 1889. Continuous use of the hot water treatment has not apparently changed the heat resistance of the disease-causing organism. Hot water treatment (**HWT**: 2 h/50°C) is well known for eliminating smut from infected seedcane (Benda 1981; Bailey 1983 & Farias 1985).

Chemical control with Agallol at 0.5 % and Dithane Z-78 at 0.3 % concentration has been found effective against surface contamination (Muthusamy & Subba Raja 1972). Recently, Bailey (1979b) suggested triadimefon (Bayleton) with hot water treatment for controlling both smut and ratoon stunting disease.

The present study was conducted to optimize sugarcane smut disease control procedures. Trials were carried out at Giza Research Station, Protection Section, Sugar Crops Research Institute, Agriculture Research Center, Giza, Egypt.

MATERIALS AND METHODS

Resistance testing methods

Experiment 1 The dip, wound- paste and brushing methods were compared using the variety NCo 310 planted in pots 50 cm diam. with 10 replication. Each pot contained five - bud setts. For the dip inoculation method (Ferreira et al 1980), the cane setts were dipping in a spore suspension of 5 xlO teliospores/mL of water for 30 minutes with 1 drop oftween 20/100 mL as recommended. In the wound - paste method (Leu et al 1976), the buds were pricked 6 times at the periphery with a fine needle and then a spore suspension of 5.0 g teliospores/L of water was brushed on the wound. The brushing technique (Luthra et al 1938) was accomplished by atomizing a spore suspension (spray) using the same rate of teliospores.

Varietal resistance testing: Two years (1990 -1991)

Experiment 2 Varietal resistance testing using the dip method, was done according to randomized block design with 10 replicates. Eleven varieties were tested, viz, G 68/88, NCo 310, G.T. 54/9 (C9),G.74/96, E 68/18. G.368/75, F153, F151, G. 37 /85, G. 47/84 and G. 86/84.

Seed treatment

Experiment 3 Hot-water treatment (HWT) is well known for eliminating smut from infected seed cane. The sugarcane setts were treated as shown in Table 1.

Table 1 *Hot water treatments examined for smut control in experiment*

50°C 1 h	52°C 10 min	54°C 5 min
50°C 2 h	52°C 20 min	54°C 10 min
50°C 4 h	52°C 30 min	54°C 15 min

Inoculated single - bud cuttings of the variety NCo310 were planted in 10 replicated pots (5 buds/pot). Untreated checks were simultaneously planted.

Experiment 4 The trial was carried out as a randomized block design with 10 replicates using the variety NCo 310. Three treatments were compared: (1) control (2) dip method (see experiment 1), (3) immersion of the cane setts for two h in systemic fungicide solutions, viz., Benlate 50% lg /L, Bayleton 5% 2.5 g /L, or protective fungicides, viz. Dithane M 45% 2.5 g /L., Dithane Z 78% 2.5 g/L and preventative fungicide Daconil 2.5 g/L of water prior to inoculation by the dip method.

Experiment 5 Combined hot water and fungicide treatments were as described in experiment 3 and 4. The following conditions were adhered to in all experiments.

Smut spores of 95 percent viability were collected from smut - infected cane The seedcane used in all experiments obtained from special nurseries at Giza and EL- Mataana (Kena governerate). The treated cane setts were incubation for 24 h before planting. All experiments were conducted in the greenhouse with planting in the spring and summer monhs. Infection was expressed as % infected stools. Stools showing mih symptoms were destroyed. Cumulative disease incidence was calculated 3 - 4 months after planting using a numerical rating of 0-9 (Table 2) as proposed by Hutchinson (1969).

Table 2 *Disease incidence, resistance classification and rating system used in these studies of smut (after Hutchinson 1969).*

% of diseased stools	Classification	Reaction rating
< 1		0
1 - 2	Highly resistant (HR)	1
2.1 - 3		2
3.1 - 5	Resistant (R)	3
5.1 - 8		4
8.1 -11	Moderately resistant (MR)	5
11.1 -15		6
15.1 - 22		7
22.1 - 30	Susceptible(S)	8
> 30.1	Highly susceptible(HS)	9

RESULTS AND DISCUSSION

The disease development associated with the different inoculation techniques is illustrated in Table 3.

Table 3 *Smut disease development associated with the different inoculation techniques examined.*

Treatments	% Diseased stools
Control	0
Dip method	34
Wound-paste method	72
Atomizing spore suspension spray method	12
L. S. D. at 0.01	10.5

The wound-paste method led to significantly higher disease level than all other treatments. The dip method led to moderate levels while the atomizing spore suspension spray method gave poor results. The dip method is preferable for testing the reaction of cane varieties to smut disease since the wound-paste method is very severe and more time consuming than the other methods. The dip technique has been recommended by various authors (Byther & Steiner 1974, Ferreira et al 1980).

In experiment 2: the reaction of 11 commercial and promising varieties, viz., G. 68/88, NCo 310, G.T. 54/9 (C9), E 68/18, G. 368/75, F153, F 151, G. 37/85, G. 47/84, and G. 68/84 were tested using the dip method. As the disease cannot be completely controlled by fungicides and cultural practices, the use of resistant varieties is the only alternative method. Differential reactions to smut were observed. The results are recorded in Table 4. It was evident that three clones, namely G.68/88, F153 and G. 47/84 were highly resistant to smut. Of the remaining clones two varieties were rated as resistant, three as moderately resistant, and three as highly susceptible.

Such variation indicates the presence of a genetical source of resistance among the tested cane varieties which could be utilized in a breeding programme for smut control.

Hot water treatment against smutted setts showed that hot water treatment at 52°C for 20 minutes or 50°C for 2 h was more effective than other treatments (Table 5). Benda (1981) found an intermediate length of time (52°C for 30 - 45 min.) gave adequate smut control.

Table 4 *Reaction of different sugarcane varieties to artificial inoculation with U. scitaminea.*

Varieties	% Stool infected	Rank	Reaction	Rating
G. 68 / 88	0	D	HR	0
NCo 310	44	B	HS	9
G.74 / 96	12	C	MR	5
G.T. 54 / 9 (C9)	11	CD	MR	5
E 68 / 18	72	A	HS	9
G 368 / 75	11	CD	MR	5
F 151	43	B	HS	9
F 153	0	D	HR	0
G. 37 / 85	11	CD	R	4
G. 68 / 84	8	CD	R	4
G. 47 / 84	0	D	HR	0

L.S.D. at 0.01 level   10

Some treatments led to reduced germination rates. Sett viability is a critical factor limiting the use of higher temperature treatments.

Table 5 *Effect of hot- water treatment of sugarcane setts on smut disease levels percent.*

Water Temperature	Dipping Time	% diseased stools
50°C	1 h	12
	2 h	6
	3 h	0
52°C	10 min	8
	20 min	8
	30 min	2
54°C	5 min	0
	10 min	0
	15 min	0
Control (dip method)		30

LS.D.at 0.01   N.S'

*N.S. between the two treatments 50°C and 52°C.

Smut incidence as affected by fungicide treatment is detailed in Table 6. Dip treatment of setts with the fungicides Bayleton and Benlate was highly effective in reducing smut infection in the highly susceptible sugarcane variety NCo 310 (Table 6).

Bayleton resulted in the lowest incidence (6.0 %) followed by Benlate (8.0%).Dithane Z 78 or Daconil, were also effective in reducing disease incidence. Handojo & Legow (1984) found that immersing two-budded setts of Poj 3016 in Bayleton 250 EC 0.5 g. a.i. /L water for 2 h prior to dipping in a spore suspension of 5x10 teliospores/mL. water for 10 min. protected the treated setts against smut infection. Also, Natarajan & Muthusamy (1981) stated that sugarcane smut can be controlled by pre-treating the setts in Bayleton at 1 mL/L or Doconail at 2.5 g/L for 5

Table 6 *Effect of fungicides on smut disease development when setts were immersed in fungicide solution prior to a dip inoculation with*

Treatment	% Diseased stools
Bavistin	18
Bayleton	6
Daconil	10
Dithane M 45	12
Dithane Z 78	10
Dip method	30
L.S.D. at 0.01	N.S.

A combination of hot water and fungicide treatments provided the most effective control of sugarcane smut (Table 7). Differences between treatment were significant however.

Table 7 *Effect of combined hot water and fungicide treatment on sugarcane smut disease development.*

Treatment	% diseased stools
50°C 2 h + Bayleton	0
52°C 20 min. + Bayleton	2
Dip method (control)	30
L.S.D. at 0.01 N.S.	

Bailey(1979 b) suggest a tridefon plus hot water treatment for controlling both smut and ratoon stunting disease. Benda (1981) developed a short hot water treatment (52°C for 20 min.) combined with fungicide which can be used as preventive treatment, Bailey (1983) found that treating sugarcane for 2 h in hot water at 50°C containing 250 mg/mL triadimefon was more effective than a similar treatment in cold water when smut

CONCLUSIONS

No single method alone gave adequate control. Hot water treatment combined with Bayleton as an eradicative and prophylatic treatment appears to give the best results for controlling sugarcane smut.

ACKNOWLEDGMENT

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SELECTION FOR SMUT RESISTANCE IN TWO SUGARCANE POPULATIONS

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ABSTRACT

Sugarcane populations were created by making crosses between cultivars US 65-4 X UThong 1 and CP 52-68 X Chai Nat 1. US 65-4 and CP 52-68 are smut resistant in the growing conditions of Thailand. The 238 and 32 seedlings obtained from each cross, respectively, were used in 2 stages of selection; seedling selection and the first clonal selection. Both stages aimed to select resistant clones which also have good agronomic characters. The results showed that resistant clones could be selected in the seedling stage. Together with agronomic data, 27 and 19 clones from the crosses, respectively, were selected. Results from the first clonal stage selection showed that some selected clones from the seedling stage were susceptible. This suggested that additional screening for smut resistance at this stage was necessary to assure that only resistant clones will be carried forward. Again, together with agronomic data, some clones have been selected for further yield and quality evaluation.

INTRODUCTION

Smut caused by *Ustilago scitaminea* Syd. has been found in cane growing areas of Thailand since 1963 (Jarupat et al 1983). Cane yield and CCS (Commercial Cane Sugar) are reduced 8-18 % and 7-13 %, respectively, in a susceptible cultivar, Chai Nat 1 (Ouvanich et al 1985). The best method of controlling smut is the growing of resistant cultivars (Ferriera & Comstock 1989). A selection program for smut resistance was initiated by Suphan Buri Field Crops Research Center in 1983. The objective of the program was to select resistant clones which also have good agronomic characters. The studies presented here involve two stages of selection, seedling stage and the first clonal stage, of two sugarcane populations derived from US 65-4 X UThong 1 and CP 52-68 X Chai Nat 1.

MATERIALS AND METHODS

Selection in the seedling stage

Seedling inoculation

Two months old seedlings from two crosses (238 seedlings from US 65-4 X UThong 1 and 32 seedlings from CP 52-68 X Chai Nat 1) were inoculated by wound prick method at the lowest bud with a spore suspension of 5×10^6 spores/ml. They were transplanted into the field in an unreplicated trial in June 1992. The row and plant spacing were 1.30 and 0.5 m, respectively, and using one seedling per hill. A susceptible Eheaw which is more susceptible than Chai Nat 1 and resistant UThong 1 check were planted as a single plant/hill in 8 replicates. Irrigation was applied immediately after planting and herbicide was sprayed at the rate of 6.25 kg/ha on moist soil. Fertilizer was applied 1.5 and 2.5 months after planting with 15-15-15 of $N-P_2O_5-K_2O$ at the rate of 312.5 kg/ha. During the growing season, irrigation and hand weeding were practised as necessary.

Smut evaluation

Two methods of smut detection were conducted:
1. Detection of smut hyphae by staining technique (Sinha et al 1982). Growing points of 2 months old seedlings after transplanting were cut and stained by tryphan blue and observed under microscope for smut hyphae.
2. Field detection. The transplanted seedlings from 2 month-old were inspected for whip formation at about 1 month intervals.

Selection in the first clonal stage

27 clones from UT 65-4 X UThong 1 and 19 clones from CP 52-68 X Chai Nat 1 were planted in April 1993 in a randomized complete block design with two replicates including the standards, UThong 1, Chai Nat 1 and Eheaw. A plot contained one 8 m row of each clone. One replicate was inoculated with smut using the dipping technique. Two cane-setts with two buds each were planted using row and plant spacings at 1.30 and 0.50 metres, respectively. Management was as in the seedling selection trial. The number of infected stools in each plot was recorded including the number of whips-like symptom at 5 months after planting. Disease reaction was determined according to % of infected stools and the number of whips.

Agronomic data

In both trials, agronomic data were recorded on stalks/stool, stalk diameter (cm), height (cm), Brix (%) (using hand refractometer, only in the seedling selection), 10 random stalks weight and CCS. Data were analysed using MSTATC software.

RESULTS AND DISCUSSION

Seedling selection

The results indicate that the number of infected seedlings detected increased with plant age (Table 1). It means that the fungus needs time to develop whip-like symptoms and the time will differ depending on clones or genotypes. The difference in time for symptom appearance may be accounted for the physiological resistance (Rampersad & Brathwaite 1985). The results of detection of fungal hyphae by the staining technique showed that more than 75 % and 90 % of samples taken from each tested clone of US 65-4 X U-Thong 1 and CP 52-68 X Chai Nat 1, respectively, were positive (Table 2). This suggests that even though the fungus mycelium was found in most tested clones, the whip-like symptom may or may not develop during the growing season. The time required for symptom development may refer to a latent period which is one type of quantitative resistance (Parlevliet 1979). Selection for resistant clones may be practised at the seedling stage by considering the clones which have a long latent period (Momol et al 1990).

Table 1 *Number of seedlings (percentage in brackets) of two crosses which showed whip-like symptom measured at different months after transplanting in 1992.*

Cross	Total	Month after transplanted				
		Aug	Nov	Dec	Jan	Feb
1[1]	238	36(15)	62(26)	71(30)	81(34)	95(40)
2	32	3(9)	6(19)	6(19)	7(22)	11(34)

[1]1 = US 65-4 X UThong 1 ; 2 = CP 52-68 X Chai Nat 1
Susceptible check Eheaw showed 50 % infected stool
Resistant check UThong 1 showed 12 % infected stool

Table 2 *Number of seedlings of two crosses in which smut hyphae were found in the growing point at 2 months after transplanting compared with field observation of whips in 1992 (percentage in brackets).*

Item	1[1]	2
No. clones tested	209	32
No. clones with positive hyphae detection	159(76)	30(94)
No. clones with positive hyphae detection and showing whip-like symptom in the field	70(44)	11(37)

[1]1 = US 65-4 X UThong 1 ; 2 = CP 52-68 X Chai Nat 1

The means of the agronomic characters for the two populations were close to each other (Table 3). However, the mean number of stalks/stool of US 65-4 X UThong 1 was higher than that of CP 58-62 X Chai Nat 1, whilst Brix (%) was higher in CP 58-62 X Chai Nat 1. This may reflect the parents, US 65-4 which has a large number of elongated stalks/stool and Chai Nat 1 which has a high Brix (%). Based on smut and agronomic performances, only 27 and 19 clones of US 65-4 x UThong 1 and CP 52-68 x Chai Nat 1, respectively, were selected for further testing.

Table 3 *Seedling stage: Means, standard deviations (SD), minimum and maximum of four agronomic characters studied in a number of clones derived from two crosses tested in 1992.*

Character	US 65-4 x UThong 1				CP 52-68 X Chai Nat 1			
	Mean	SD	Min	Max	Mean	SD	Min.	Max.
Stalks/stool	7.7	3.92	1	22	5.9	2.72	1	13
Stalk dia.(cm)	2.2	0.37	1.3	3.0	2.3	0.38	1.7	3.0
Height (cm)	196	43.5	79	302	209	53.2	110	295
Brix (%)	13.9	4.01	3.0	22.4	18.3	4.19	5.0	24.4

First clonal stage selection

The results showed that the tested clones from both populations could be grouped into 4 groups based on their smut reactions (Table 4).

Table 4 *Number of clones of two crosses classified by smut reaction in 1993.*

Smut Reaction	Number of Clones	
	US 65-4 X UThong 1	CP 52-68 X Chai Nat 1
Resistant	8	6
Moderately resistant	2	7
Moderately susceptible	14	5
Susceptible	3	1

Susceptible check Eheaw showed S reaction
Resistant check UThong 1 showed MS reaction

For the cross US 65-4 X UThong 1, 8/27 were rated resistant (R) and 2/27 moderately resistant (MR), and for the cross CP 52-68 X Chai Nat 1, 6/19 were rated R and 7/19 MR. The rest of the clones were rated moderately susceptible and susceptible. Data from seedling selection and the first clonal selection suggested that transgressive segregation may cause the segregation of susceptible clones from US 65-4 X UThong 1 (Skinner 1981). However, no evidence suggests that different races of smut pathogen are found in the tested area. Due to the negative correlation usually found between disease rating and yield, selection for highly resistant may cause unacceptable loss in cane yield. Thus, moderately resistant clones may have to be selected to obtain clones with good cane yield. However screening for smut resistance in the first clonal selection is necessary to ensure that only resistant clones are selected. Variations in agronomic traits were observed (Table 5) which indicate that selection for these traits can be made. From though the stalks/stool, stalk diameter and height are related to cane yield, the 10 random stalk weight may be the best selection character for the first clonal trial. The 10 random stalk weight is directly related to cane yield

(Iamsupasit 1993). For quality, CCS can be computed and used directly to identify high sucrose clones. From smut resistance ratings and agronomic characteristics some clones have been selected and used for yield and quality tests in the 1994 season.

Table 5 *First clonal stage: Means, standard deviations (SD), minimum and maximum of five agronomic characters studied in 1993 on clones derived from the two crosses.*

Character	US 65-4 X UThong 1				CP 52-68 X Chai Nat 1			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Stalks/stool	7.9	1.99	4.2	11.1	5.5	1.30	3.9	8.9
Stalk dia.(cm)	2.4	0.26	1.9	3.1	2.8	0.23	2.3	3.1
Height (cm)	198	24.89	154	251	200	26.05	154	254
10 Stalk wt.(kg)	9.1	3.18	4.8	19.1	11.4	2.08	8.2	14.8
CCS	9.3	1.23	7.1	12.2	11.2	1.51	7.9	13.5

CONCLUSION

In two populations, smut resistant clones could be selected in the seedling stage. However, further screening for smut resistance in the first clonal stage is neccessary to guarantee mat only truly resistant clones are selected. Variation in some agronomic traits could be observed in both stages which indicated that selection for good agronomic traits could also be made.

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PACHYMETRA ROOT ROT : INCIDENCE AND POTENTIAL SOLUTIONS TO MINIMISE ITS INFLUENCE ON YIELD DECLINE IN QUEENSLAND

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ABSTRACT

Glasshouse resistance screening of members of the Saccharum complex showed that S. spontaneum and Erianthus arundinaceus are highly resistant to Pachymetra root rot. There was a range of resistance ratings between clones of the same Saccharum spp. District surveys for Pachymetra root rot showed that Fairymead mill area in the Bundaberg district, and some parts of the Herbert River district, had moderate levels of the disease. Survey data are being used in the BSES breeding program to aid in the selection of parent clones for each district. Green cane trash blanketing, compared to a burnt cane management regime, does not appear to lead to higher levels of Pachymetra root rot.

INTRODUCTION

Poor root growth resulting in restricted yields and poor anchorage of plants in the soil was reported in Babinda as early as 1968. Research was unable to identify a cause of the problem. In 1970, cultivar Q90 yielding 20% more than existing commercial cultivars in yield experiments was released in northern Queensland and farmers rapidly increased the area planted to this cultivar. Reports of root disease in Q90 at Babinda were first noted in 1972, and by the late 1970s yields of Q90 were declining. Serious problems with ratooning of Q90 and increases in dirt in cane being supplied to mills were also causing concern. The problem was named poor root syndrome (Egan et al 1984) and similar symptoms were subsequently identified in nearly all canegrowing regions of Queensland.

Pachymetra root rot caused by the oomycete fungus, *Pachymetra chaunorhiza* Croft & Dick (Dick et al 1989) was identified as a major cause of poor root syndrome (Croft & Magarey 1984). The cultivar Q90, was found to be highly susceptible to Pachymetra root rot with yield losses of 30-40% (Magarey 1994). Cultivars with resistance to Pachymetra root rot have been identified (Croft 1989) and have successfully reduced die populations of *P.chaunorhiza* in cane fields (Magarey & Mewing 1994). Although the distribution of *P. chaunorhiza* within Queensland has been established, soil inoculum densities have not previously been quantified. On a statewide basis this information is needed to guide plant breeders and farmers in the need for Pachymetra root rot control measures. A popular sugarcane management practice, particularly in northern Queensland, is the harvesting of non-burnt sugarcane crops and the retention of trash as a surface mulch. The effect of this strategy on *P. chaunorhiza* inoculum densities has not previously been reported. This paper reports on research in each of the areas of cultivar resistance, soil inoculum density (by district), and on the effect of trash retention on inoculum density.

MATERIALS AND METHODS

Survey

A previous survey (Magarey et al 1987) based on Pachymetra incidence but not severity, suggested that Pachymetra root rot was widely distributed in Queensland. A further survey, described here, was to assess severity of the disease in each district. Soil samples (0-250mm) collected from sugarcane fields from Ingham to the New South Wales border were assayed for *P. chaunorhiza* using the technique described by Magarey (1989). Soil was wet sieved through a nest of sieves and oospores collected on a 38 |im aperture sieve. The 38 u.m sieve fraction was then bleached and stained with lactophenol cotton blue. The characteristically ornamented oospores were then directly counted in a nematode counting chamber ("Hawksley", England). Data was calculated as oospores/g of soil.

Effect of cropping systems

The effect on Pachymetra soil inoculum density of a trash retention system called green cane trash blanketing (GCTB), was compared to the traditional system of burnt cane in two experiments, one in northern Queensland (Highleigh) and the other in central Queensland (Te Kowai). In each experiment, sugarcane cultivars were planted in a randomised complete block design and subjected to two management strategies: green cane harvest/trash retained, and green cane harvest/trash raked and burnt. The Highleigh experiment included 25 cultivars while the Te Kowai experiment included six cultivars, with two replicates in each experiment. In the Te Kowai trial, early and late season harvests were included as treatments. Soil samples were taken after a plant and one ratoon crop as described elsewhere (Magarey & Mewing 1994).

Cultivar resistance

Clones were screened for resistance to Pachymetra root rot using a pot technique described by Croft (1989). Briefly, pre-germinated test plants were transferred into pots containing a sand peat potting mixture infested with oospores of the fungus. Plants were grown for six weeks before soil was washed from the root systems and the number of infected and the total number of primary roots recorded. Clones were rated relative to a set of 10 standard clones which covered the range highly susceptible (9) to resistant (1) (Croft 1989).

RESULTS

Distribution of Pachymetra

Quantification of field inoculum densities in this survey showed that the disease was generally at low levels in the central district (14 sp/g Mackay, 8 sp/g Proserpine), but at moderate-high levels in the Herbert River mill areas (28 sp/g Macknade, 53 sp/g Victoria and in some parts of the Bundaberg districts (82 sp/g Fairymead mill area). Contrary to expectations, forest loam soil types in the Bundaberg district had very high inoculum densities.

Effect of cropping systems

Average inoculum density for the 25 cultivars grown under green cane-trash blanket (GCTB) compared to the burnt cane cultivation system at Highleigh (NQ) was 150 vs 258 sp/g respectively. GCTB led to a significantly lower inoculum density (P<0.005, LSD (0.05) = 49.5). Results from Te Kowai (central Queensland) are detailed in Table 1. At Te Kowai, in the early harvested comparison, GCTB led to slightly higher inoculum densities while in the late-harvested comparison, GCTB led to lower spore levels than the burnt cane management strategy - however, differences were not significant (P = 0.05). At this stage in the central district trial, no clear trends are evident.

Cultivar resistance

Clones from plant improvement programs from around the world varied in resistance (Table 2). All species of the *Saccharum* complex evaluated had a range of resistance to Pachymetra root rot (Table 2). *S.spontaneum* clones were the most resistant of the *Saccharum* species tested. However, *Erianthus arundinaceus*, a closely related species, was also highly resistant. *E.arundinaceus* is the focus of a large introgression project in Australia. Glagah, which was a parent in many of the first hybrid cultivars of sugarcane was the most susceptible *S.spontaneum* clone tested.

Table 1 *Pachymetra spore counts as influenced by trash blanketing in an experiment at Te Kowai, Mackay.*
[Soil samples were collected after a plant and one ratoon crop]

Variety	Early harvest		Late harvest		Mean	Resistance rating
	Trash blanket (sp/g soil)	Burnt cane (sp/g soil)	Trash blanket (sp g soil)	Burnt cane (sp/g soil)		
Q138	7	13	9	5	8.5	2
H56-752	4	10	14	8	9.0	5
Q136	23	34	11	11	19.8	8
Q124	52	45	10	7	28.5	5
Q135	89	8	33	57	46.8	6
Q159	70	71	19	41	50.3	9

[1] differences not significant (P=0.05)

Table 2 *Pachymetra root rot resistance of clones from different sugarcane breeding programs around the world.*

Country/Organisation /Region	Mean rating
Australia, BSES, commercial	6.1
Australia, BSES, northern	5.4
Australia, BSES, Burdekin	6.1
Australia, BSES, central	6.1
Australia, BSES, southern	6.1
Australia, CSR	5.2
Australia, NSW	5.9
India, Coimbatore	5.9
USA, Canal Point	6.2
USA, Hawaii	4.7
Taiwan	5.0
Fiji	5.7
Reunion	3.8
Brazil, Sao Paulo	6.8
Sth. Africa	6.2
Other	4.5
Total Germ plasm	5.6

Table 3 *Pachymetra root rot resistance rating (1= resistant, 9=highly susceptible) of basic germplasm of* Saccharum *species and* Erianthus arundinaceus.

Species	Mean Rating	Range
S.officinarum		
S.spontaneum		
S.robustum		
E.arundinaceus		

DISCUSSION

After the identification of the disease, breeding for Pachymetra root rot resistance was introduced as a component of the BSES plant improvement program in the mid 1980s. The range of resistance available in basic germplasm of *Saccharum* and the related *E.arundinaceus* should allow for improvement in the level of disease resistance through directed introgression. The parent clone 66N2008 has produced a number of important resistant cultivars (eg. Q138 and Q158) and this clone was derived from a cross involving the *S.spontaneum* clone Mandalay, rated as highly resistant to Pachymetra root rot. Although there is resistance to Pachymetra root rot in Australian germplasm collections, the inability to make crosses between susceptible clones continues to restrict advances in breeding for other characters.

Pachymetra surveys have highlighted districts and regions within the Queensland sugar industry where control measures for Pachymetra root rot require careful implementation. These include parts of Fairymead mill area in the Bundaberg district and the Ingham Line area in the Herbert River. The survey has given a clear indication of the areas in Queensland requiring resistant cultivars and therefore has provided better focus to the BSES plant improvement program. The resistance of parent cultivars for each district is considered before crosses are made.

GCTB is a management option which has favourable economic and environmental qualities and is becoming an increasingly popular cultivation system. Agronomic research suggests that soil moisture levels are maintained at higher levels by GCTB compared to a burnt cane cultivation system. Magarey & Soper (1992) correlated higher rainfall with increased levels of Pachymetra root rot, and therefore it was thought that the severity of Pachymetra root rot might be increased through GCTB. This does not appear to be the case in the present study, but longer term studies are required to confidently predict the effect of GCTB on Pachymetra root rot severity.

Pachymetra root rot remains an important consideration for the Australian sugar industry. The disease is being successfully controlled by enhancing varietal resistance in commercial germplasm.

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HISTOPATHOLOGY OF *PACHYMETRA CHAUNORHIZA* AND *PYTHIUM ARRHENOMANES*

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ABSTRACT

Pachymetra chaunorhiza and Pythium arrhenomanes are two oomycete fungi responsible for root rot diseases in sugarcane. These soil-borne pathogens contribute significantly to the problem of yield decline in Australian cane production. To better understand the host-pathogen interaction of these diseases, various stages in disease development were examined. Plants were grown in specially designed pots with removable sides to facilitate access to the roots and observation of symptoms. Diseased roots were collected over time and the processes of infection and colonisation studied using light and electron microscopy. With P.arrhenomanes, macroscopic symptom development occurred within three days of inoculation. Hyphal swellings were observed on the root surface and after colonisation, vesicles were formed in the cortex. Roots inoculated with P. chaunorhiza produced oospores in both the cortex and vascular tissue just behind the root tip where infection is thought to occur. Techniques developed within this project will have application in the rapid screening of sugarcane cultivars for resistance to these pathogens.

INTRODUCTION

Pachymetra chaunorhiza Croft & Dick (Dick et al 1989) and *Pythium arrhenomanes* Drechs. (Rands & Dropp 1938) are two oomycete fungi responsible for root rot diseases in sugarcane. These soil-borne pathogens contribute significantly to the problem of yield decline in Australian cane production causing reduced yields and agronomic problems such as crop lodging, stool tipping and harvesting damage, leaving gaps in the ratoon crop and interfering with the milling process (Egan et al 1984).

The processes of infection and colonisation of sugarcane roots by both *P.chaunorhiza* and *P.arrhenomanes* remain largely undescribed. A glasshouse technique for screening sugarcane cultivars for resistance to Pachymetra root rot was developed by Croft (1989); this technique however is not useful in studying the initial stages of host invasion by the pathogen. Simple root squash techniques have been used to observe oospores and sporangia within rotted tissue (Magarey 1991), but no detailed studies on the histopathology have been made. To better understand the host-pathogen interaction of these diseases, the stages of infection and colonisation were examined.

MATERIALS AND METHODS

A bioassay system was developed where a flat-sided pot was made using a plastic conduit and a perspex side plate which enabled roots to be observed and sampled throughout the infection process. Single node setts of the susceptible sugarcane cultivar Q90 were pre-germinated in autoclaved vermiculite and then transferred to the specially designed pots for experimentation. The pots were made from 100mm diameter plastic tubing cut to 300mm and split in half lengthways. The base and open face were covered with clear perspex and the pots placed on a 60 degree angle (Fig. 1). Plants were grown in autoclaved sand in a controlled environment growth cabinet at 28°C and watered with sterile distilled water.

After five days when the roots had grown down against the perspex cover, the cover was removed and the plants inoculated with a mycelial suspension of *P.chaunorhiza* or *P.arrhenomanes*. Inoculum was prepared by blending five day old cultures grown in potato sucrose broth (200g fresh potato, 20g sucrose, and 1L water) at 28°C to form a mycelial suspension. Roots were collected at early stages of infection and prepared for microscopic observation.

Light microscopy

The root tissue was either stained with trypan blue and observed as root squashes (Magarey 1991), or fixed in glutaraldehyde, dehydrated in a graded alcohol series, and embedded in paraffin wax. Embedded samples were sectioned using a rotary microtome, and the sections mounted on microscope slides and stained using Pianeze III.

Fig. 1 *Plants were grown in specially designed pots with removable sides to facilitate access to the roots and observation of symptoms. Pots were laid on a frame at an angle of 60 degrees to force root growth along the perspex plates.*

Scanning electron microscopy

Infected root tissue fixed in glutaraldehyde was dehydrated in a graded acetone series, critical point dried, mounted on stubs and sputter coated with gold. The prepared samples were then examined by scanning electron microscopy.

RESULTS AND DISCUSSION

Pachymetra chaunorhiza

Disease symptoms such as watersoaking and rotting of primary roots have been observed in both pot trials and in the bioassay system. In the

susceptible cultivar Q90, root growth slowed after inoculation when compared with uninoculated control plants. When inoculated, healthy roots progressed in appearance from white to off-white before becoming translucent and finally watersoaked. In some cases infection sites had red-coloured margins. Infection apparently occurred behind the root tip which initially appeared healthy.

Examination of sections of infected roots seven days after inoculation indicated that hyphae and oospores were produced initially throughout the cortex and at later stages of colonisation, in the vascular tissue (Fig. 2).

Pythium   arrhenomanes

Roots infected by *P.arrhenomanes* were collected 5h after inoculation and then at 12h time intervals and the processes of infection and colonisation studied. Using the scanning electron microscope, hyphal fragments were seen on the root tip 5h after inoculation with extensive growth over the root tip by 12h. Hyphal swellings appeared at the tips of many of the fragments after 12h.

Fungal colonisation of the cortex occurred as early as 5h after inoculation. Mycelium was observed in the parenchyma cells with the hyphae growing intracellularly, constricting to pass between the cells. Seven days after inoculation, vesicles were formed in the outer layers of the cortex, confined by the cell walls.

CONCLUSION

The symptoms produced by *P.chaunorhiza* and *P.arrhenomanes* on sugarcane plants in the specialised pot system were typical of those observed in the field and in pot trials. The technique has proven beneficial in histology studies allowing access to the roots for inoculation, observation and sampling with minimal disturbance. This system may form the basis for a more efficient method of screening cultivars for disease resistance if a range of varieties can be classified in the same way as the existing glasshouse pot trial.

ACKNOWLEDGMENTS

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Fig. 2 *Longitudinal section of an infected sugarcane root with P.chaunorhiza oospores (arrow) present in the cortex cells (bar = 20mm).*

4. Opportunities for improved crop management

4.1 Crop agronomy and yield improvement

YIELD MAPPING FOR THE CANE INDUSTRY

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ABSTRACT

Yield mapping has a range of potential applications in the cane industry, but there are as yet no convenient means for measuring yield as a function of distance along the row. A spatial resolution of 5-10m can be achieved with the simple weigh truck, but it is time consuming and inconvenient to weigh short sections of the cane row. The issues involved in yield mapping are discussed, and preliminary experiments to establish the basic technology required are reported.

A simple calibration has been established, which defines average hydraulic motor power as a function of overall yield as measured by a weigh truck. This calibration has been used to create a yield map for a limited area of a field, which revealed a substantial degree of yield variation both along and between rows. It appears to be possible to develop this approach further, and in conjunction with GPS (Global Positioning System) and GIS (Geographic Information System) inputs, to derive a yield mapping system.

INTRODUCTION

Currently most growers apply chemicals to their crops at a uniform rate calculated from historical field data. An average yield is predicted for individual fields and application rates of fertilisers and chemicals are tailored to the assumed field output (Massey Ferguson Group Ltd 1993). In practice, crop yields vary not only from year to year, between farms and between fields, but significant yield variations also appear within individual fields (Vansichen & de Baerdemaeker 1993; Kirchner & Lee-Lovick 1991). Variable soil fertility, varieties used, management practices, use of fertilisers, irrigation, control of weeds, pests and diseases, and many other factors explain the spatial variation in sugarcane yields (Humbert 1968, p.4). These variations in field characteristics suggest that the proper placement of the inputs such as fertilisers and chemicals according to spatial location would help reduce production costs. This type of 'site specific' crop management involves the application of crop management practices which are tailored to a specific area in a field (Clark et al 1987).

McBratney & Whelan (1995) proposed that site-specific farming should be investigated for use in the Australian cotton industry. They discussed the use of spatially measured soil attributes as inputs to managerial intervention such as soil tillage, fertiliser application, nitrification inhibitor application, gypsum application, seeding rates, crop variety, pesticide application and the application of irrigation. Measurement of soil attributes involves the development of suitable sensors for nitrogen, organic matter, soil salinity, soil moisture content and soil compaction.

The crop itself shows the overall effect of the management regime and the soil status, and so the measurement of yield over small areas within the crop will provide information on the effects of these variables.

Yield maps provide essential information on crop performance within and between fields, for spatial analysis and evaluation of crop management. This information can be an input to decision making for field operations during the next growing season (Vansichen & Baerdemaeker 1993). Detailed measurement of crop yield, which integrates the influence of many variables, is considered to be the most practical method of assessing management techniques for site specific farming practices.

Yield maps can also greatly improve information available for making longer term strategic management decisions and will also highlight problems with drainage, disease or weed infestation (Clark et al 1987). Accurate yield maps give a clear indication of good and poor areas of the field, and a farm manager can then investigate the many possible reasons for these yield variations. Some reasons for yield variations are relatively easy to rectify, for example, by subsoiling compacted areas. Other reasons can be established by soil analysis, where the yield map allows this task to be performed more selectively than traditional 'random sample' methods (Massey Ferguson Group Ltd 1993).

'The sugarcane plant, *Saccharum officinarum* L., has been described as the most efficient of all storers of the suns energy' (Humbert 1968, p. 16). If its maximum yield potential is to be approached, the soil-plant relationship must be at an optimum. The many factors controlling growth must be integrated into an optimum environment. The fact that sugar production in Australia ranges from 160 to 60 tonnes per hectare highlights the range in productivity that can occur due to different soils, climatic conditions and crop management. Even within ten adjacent rows of a crop we have measured a yield variation of this order, which illustrates the potential for maximising yield by improving crop management. Kingston & Hyde (1995) have also reported high intra-field variation of commercial cane sugar content (CCS), which has consequences for both the growing and milling sectors of the industry, and will compound the task of maximising overall return.

These considerations justify support for the application of yield mapping technology to sugarcane agriculture. It will allow the fine tuning of resource inputs and management, and improve the sustainability of sugarcane production.

DEVELOPING YIELD MAPS

There are two major approaches to measuring the spatial variability of yield in cane. The first of these is based on remote sensing technology, and the second is based on measuring mass flow rate through the harvester, with this information being spatially referenced using a Global Positioning System (GPS). For this application, we consider that spatial location with a sub metre accuracy is realistic and achievable.

Remote sensing

Remote sensing is a technique using light reflected from earth. A recording instrument, such as a camera, can record the spectral data from the plant reflected back to the receiver, and this information can be interpreted in many ways. The extension of this method to separately measure and record different sections of the light spectrum is known as multispectral sensing, which can provide additional data on crop condition. Specifically, information about crop biomass can be obtained and from this information crop yield can be devised. However, Colwell (1983) has stated that spectral biomass techniques have been found to be accurate for low to medium biomass quantities, but are of little value at yields over 5t/ha. Lee-Lovick & Kirchner (1991) reported on the assessment of remote sensing technology capable of resolution of an area 30m×30m square. For sugarcane, they found that "crop canopy moisture levels appear(ed) to dominate the spectral signature, masking long term stalk development trends", so that CCS or yield at harvest could not be predicted. They concluded that the technology was inappropriate for the Australian sugar industry.

Global Positioning System

The GPS is a satellite based radio navigation system developed and operated by the U.S. Department of Defence. There are two modes of

operation, Differential GPS (DGPS) and Absolute GPS (AGPS). AGPS requires the use of only one receiver and is also known as "stand-alone" GPS, and delivers an accuracy varying from two meters to over 100 metres (Harrison 1992). DGPS is designed to improve the accuracy of GPS-derived positioning information. A stationary receiver at a known location (the "base station") receives signals from the satellites, and calculates its own position. Since the actual position of the base station is known, the errors in the satellite signals can be accurately calculated. This error information can be recorded in a computer data file for later use (post processing) and/or transmitted to a mobile receiver (the "rover") over a radio link in real time (Shropshire 1993). For yield mapping, post processing would be acceptable because the real time location of the harvester is not required. Position accuracy for a simple system is presently at the sub-metre level.

GPS location hardware could easily be incorporated into yield mapping. Installation would be a matter of mounting an antenna on the cabin of the harvester and securing the receiver in the cabin. Location data would be interfaced with the necessary data logging equipment, which would simultaneously log yield measurements. Post processing of the location data would simply involve software application on a personal computer.

Measuring cane mass flow rate

Because GPS and data acquisition technology are available in a variety of forms, we have focussed our attention on the development of a cane mass flow rate sensor. In defining its functional and performance requirements, we have assessed recommendations made in the literature for grain mass flow sensors, and adapted them to the needs of the cane industry.

A measurement technique for yield mapping of corn silage by measuring chopper power was devised by Vansichen & de Baerdemaeker (1993). Although the harvesting of silage is notably different from that of sugarcane, the similarity lies in the fact that both methods involve the removal and billeting of a whole crop. The rotary drum chopper (chopper system) of a sugarcane harvester uses seven cuts per second to billet sugarcane at a rate of up to 50 kg/s. Measuring chopper power should therefore provide an approach to measuring mass flow rate.

Another component of a sugarcane harvester, whose power consumption may be related to the material flow rates, is the elevator. This system is driven by two hydraulic motors, coupled at the top of the elevator. Billeted cane is lifted some 2 to 3 vertical metres over the length of the elevator, and obviously energy is required to overcome gravity and the effect of friction on the elevator floor as the sugarcane is dragged up the elevator. The power required to elevate the cane should be proportional to the mass flow rate.

FIELD TRIALS

Field trials were set up to assess the potential for measuring chopper and elevator power as indicators of cane mass flow rate.

Instrumentation

Power for an hydraulic motor can be calculated as the product of pressure drop and flow through the motor. For these preliminary experiments we measured supply pressure only, and not the pressure drop, and inferred the oil flow rate by measuring the speed of the motor, knowing the displacement per revolution of the motor.

Pressure transmitters were installed in the chopper and elevator supply lines. The chopper hydraulic system also supplies the feed train rollers, but it was not convenient to separate these systems. Only one of the two elevator motors was instrumented.

Chopper speed was measure directly with a speed sensor, and the elevator motor speed was inferred from the speed of an idler sprocket at the base of the elevator. We also measured engine speed and ground speed of the harvester using similar sensors.

Data from the pressure transmitters and speed sensors were recorded on a seven track analogue tape recorder (AMPEX FR 1300) mounted in the cab and powered by a generator tied to the pump box. Each reel of tape had a capacity for fifteen minutes of recording.

Measurements

The Bureau of Sugar Experiment Station (BSES) Bundaberg provided all personnel and harvesting equipment, which included an AUSTOFT 7000 harvester and a weigh truck.

The three days of testing included different weather conditions and changes in crop variety and quality. Day one was fine and dry, harvesting a crop of Q146-2R, which yielded heavily at 120 t/ha and was harvested 'green'. Day two was carried out on a different field with variety Q146-3R, which was harvested 'burnt' and also yielded approximately 120 t/ha. Day two was wet and drizzly, and day three

The hypothesis being tested was whether the mass flow rate of sugarcane through the harvester was related to the power required to process it. A range of mass flow rates was achieved by driving the harvester at a different speed for each test run. Assuming that the crop yield was somewhat uniform over the field, each run would produce a mass flow rate roughly proportional to the ground speed of the harvester.

Test runs were over approximately 100 m of row, when the weigh truck would stop to measure and record the mass harvested in that run. This mass was expressed as an average mass flow rate over that run, and compared with the average pressures and powers recorded during the run to give a calibration for mass flow rate in terms of chopper and elevator pressures and powers. This calibration process was repeated for a number of runs.

We found a highly significant linear correlation between both average pressures and both average powers, and the average mass flow rates. This finding supported the hypothesis that processing power is proportional to mass flow rate.

A typical relationship for chopper power as a function of mass flow rate is that established on the first day of testing, namely

$$\text{chopper power(W)} = 6239 + 173.5 \text{ flowrate(kg/s)}$$

This correlation has $R^2 = 0.96$ and an average absolute error of 1.4% full scale, or MOW. It was used to calculate the variation of mass flow rate during the run, which was then combined with the ground speed data for that run to give the mass of cane per metre and the yield map.
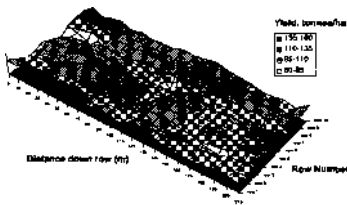
Fig. 1 *Yield map produced using the chopper power data.*

Figure 1 shows a typical yield map derived from the chopper data. The yield depression around 105m is an artefact of the testing technique, where the harvester was stopped and restarted to allow the weigh truck to measure the accumulated cane.

CONCLUSIONS

We have discussed an approach to a yield mapping system in cane. Our conclusions are that differential GPS is suitable and sufficiently accurate for spatial location, and that it appears possible to measure cane mass flow rate through the harvester by monitoring pressures and powers. From a preliminary series of measurements under real conditions, we have derived yield maps for a section of a field.

These yield maps immediately raise the question of what is causing the yield variation, but the answers to that question await further investigation. The data that we have presented suggest that the technique for measuring mass flow rate shows promise, given that the effects of other variables such as chopper sharpness and the elevator/cane friction interaction have not yet been resolved.

A complete yield map system will involve integration of spatial information, mass flow rate and ground speed data. We suggest that this processing should take place post-harvest on a daily basis, and be combined with GIS software to produce yield maps keyed to the field and the known inputs. These maps would then be the main source of information for crop management.

ACKNOWLEDGMENTS

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PLANTING IN CANE HOLES WITH SINGLE-EYE TRANSPLANTS IN POLYETHYLENE BAGS

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ABSTRACT

A planting system called the hole planting method (HPM) has been developed to resolve the problem of lack of planting material and reduce the cost of seedcane. The technique involves germination of single-bud cuttings in 130 mm x 200 mm polyethylene bags before transplanting 10,000 40-day old seedlings/ha at the onset of the first heavy monsoon rains.

Although there were 8% less millable stalks at harvest 9-10 months after transplanting at 1 m row and hole spacings, individual stalks in HPM were 13% heavier compared with stalks in the conventional method (CM) using 40,000 three-node seedpieces/ha. Cane and sugar yields with HPM were 5% higher than those of CM using six cultivars for a crop cycle (a plant crop and one ratoon).

In addition to the 85% saving in planting material and excellent establishment, other cultural advantages which derive mostly from the spatial arrangement of the millable stalks are cited. It may be possible to adapt the system to machine operations.

INTRODUCTION

The cost of planting material, which can reach from 20% to 40% of total production cost in the Philippines, is one of the major items of expense. For early-planted cane at the start of the milling season, tops as planting material are readily available. When milling ends before the start of the rainy season, seedpieces become scarce and expensive. Maintaining seed farms is one solution but most planters are hesitant to cut back vigorously-growing plant cane.

The quantity of seedcane required for planting is generally dictated by practical considerations and is determined by furrow width and spacing of seedpieces in the row. To a large extent, the amount of planting material used does not appear to greatly affect yield because sugarcane has a big capacity to compensate for differences in the number and weight of millable stalks produced. This capability has been confirmed by local experiments on planting rate and furrow width (Villarico & Panol 1972).

Cane used to be planted in holes in Barbados, Mauritius, the drier parts of Jamaica, and on sloping land in Antigua (Blackburn 1984). A system was devised to markedly reduce the amount of planting material required by planting or dibbling in holes using a row spacing of 1 m, and spacing holes 1 m apart within rows. The requirements of 4-5 t (tonnes) of planting material (assuming 10,000 seedpieces weigh 1.3 t) can be reduced to only 0.5 t/ha with the HPM method. The remaining 3.5-4.5 t of cane can be crushed. The savings in cash flow can be substantial even after considering the materials and labor to produce the bagged seedlings (Tianco & Ocampo 1992).

A different version of HPM was tried using bare-root seedlings from bud chips weighing 300 kg instead of 8 t used normally (Ramaiah et al 1977). The polyethylene bag transplanting technique (similar to HPM) is used in Punjab, India to accelerate the multiplication of promising varieties to eventually reduce the time of release from 10 years to 5 years, and to replant ratoons (Kanwar 1991).

MATERIALS AND METHODS

The study was conducted from 16 June 1993 to 15 April 1994 in the plant crop and to 24 Jan 1995 in the first ratoon in Nasugbu, Batangas. A split-plot design in four replications was used with commercial varieties Phil 56226, Phil 6607, Phil 6723, Phil 7544, Phil 8013, and VMC 7139 as main plots. Methods of planting as subplots were: 1) conventional method (CM) with 40,000 three-budded seedpieces/ha using a row spacing of 1 m ; and 2) hole planting (HPM) with 10,000 40-day old bagged seedlings/ha with holes 1 m apart within rows . Plots were 6 rows at 1 m spacing x 8 m long. Seedlings were grown from one-node seedpieces 50 mm long. Ammonium sulfate as basal fertilizer was applied at the base of each hole at 42 g/hill equivalent to 90 kg N/ha during transplanting for HPM, while the same rate was applied evenly along the row under the seedpieces in CM. A second dose of fertilizer as urea was applied about 2 months after planting/ratooning at 20 g/hill in HPM or at the same rate spread along the row in CM, making the total fertilizer applied equivalent to 180 kg N/ha in each treatment. Similar rates and forms of fertilizer were used in the ratoon. Usual practices of weeding and cultivation were followed.

Yield components were measured at harvest time on each plot. Millable stalks were counted while cane yield was measured by weighing the cane from the two middle rows for the plant crop, and calculated from 10-stalk sample weight and number of stalks for the first ratoon. The 10 stalks per hole were also crushed for juice analysis.

RESULTS AND DISCUSSION

Number of millable stalks

CM produced more millable stalks/m^2 at harvest in plant cane, except for cultivar Phil 8013 (Table 1). This method also produced more millable stalks in the first ratoon, except for cultivar VMC 7139. Although there was some varietal variation, HPM produced 8% fewer millable stalks/m^2

Table 1 *Effect of hole planting (HPM) and conventional planting (CM) methods with six varieties on stalk population (millable stalks/m^2)*

Varieties	Plant cane			First ratoon		
	HPM	CM	Difference	HPM	CM	Difference
Phil 56226	8.83 a	9.11 ab	-0.28[ns]	5.97 b	6.75b	-0.78[ns]
Phil 6607	7.97 a	8.82 abc	-0.85[ns]	7.13 ab	7.78 ab	-0.65[ns]
Phil 6723	8.17a	10.38 a	-2.21**[ ]	6.11 b	8.29 a	-2.18**
Phil 7544	7.91 a	8.52 bc	-0.61[ns]	7.83 a	8.08 ab	-0.25[ns]
Phil 8013	7.59 a	7.41 c	0.18[ns]	6.43 ab	6.58 b	-0.15[ns]
VMC 7139	7.28 a	7.87 bc	-0.59[ns]	7.84 a	7.21 ab	0.63[ns]
Mean	7.96	8.68	-0.72*	6.88	7.45	-0.57*

Average of four replications   Mean separation in a column by DMRT at 5% level.   * = significant at 1% level, * = significant at 5% level, [ns] = not significant

Table 2 *Effect of hole planting (HPM) and conventional planting (CM) methods with six lvarieties on individual stalk weight (kg)*[1]

Varieties	Plant cane			First ratoon		
	HPM	CM	Difference	HPM	CM	Difference
Phil 56226	1.04 b	0.97 bc	0.07[ns]	1.37 ab	1.28 ab	0.09 [ns]
Phil 6607	1.14 ab	0.99 bc	0.15 [ns]	1.22 b	0.98 c	0.24[**]
Phil 6723	1.05 b	0.91 c	0.14 [ns]	1.46 ab	1.06 be	0.40[**]
Phil 7544	1.18 ab	1.02 abc	0.16[*]	1.52 a	1.36 a	0.16[*]
Phil 8013	1.25 a	1.10 ab	0.15 [ns]	1.43 ab	1.30 ab	0.13[ns]
VMC 7139	1.24 a	1.14a	0.10 [ns]	1.30 ab	1.24 ab	0.06[ns]
Mean	1.15	1.02	0.13[*]	1.38	1.20	0.18[*]

[1] Average of four replications. Mean separation in a column by DMRT at 5% level. ** = significant at 1 % level, * = significant at 5% level, [ns] = not significant.

(P<0.05) compared to CM in both plant and ratoon crops. Phil 6723 performed best under CM in plant and first ratoon, with 21 and 26% more millable stalks, compared to HPM (P<0.01). There was no statistically significant method x variety interaction in either plant or ratoon cane.

Weight per millable stalk

Individual stalks from HPM were heavier than those from CM by 7-16% in the plant crop and 5-38% in first ratoon depending on the variety (Table 2), with an overall average of 13-15% (P<0.01). Individual stalks of Phil 7544 were 16% heavier (P<0.05) than single stalks of Phil 6607 and PhD 6723 were 24 and 38% heavier, respectively, in the first ratoon (P<0.01). This result is not surprising considering that fewer number of stalks will have lesser competition for nutrients, sunlight, and water. HPM cane also had a 40-day longer growing period in the plant crop and received more fertilizer per stool, although the total amount per hectare was the same. Owing to the great variation in the magnitude of response to HPM of the different varieties in the first ratoon, there was a significant method x variety interaction (LSD$_{0.5}$ = 0.23 kg/stalk).

Tonnes cane per hectare

Cane yield was uniform for all six varieties in the plant crop, a good demonstration of sugarcane's ability to compensate for fewer stalks/ha with more weight per stalk. Mean results showed no differences between planting methods in t/ha at harvest for a crop cycle (a plant and one ratoon). There was no significant difference due to planting method for the different varieties although the trend appeared to be for similar or better yield with HPM, except for Phil 6723 in plant cane and Phil 56226 in first ratoon (Table 3). There was no significant method x variety interaction for either plant or ratoon cane yield.

Sucrose % cane was not affected by planting method in either plant or first ratoon (not shown). Differences in cane quality may be largely attributed to the differences in the inherent sweetness of the varieties (Ocampo & Tianco 1994), considering age of crop and season of planting. VMC 7139 was the sweetest, followed by Phil 8013, Phil 7544, Phil 56226, Phil 6723, and Phil 6607 in descending order.

CONCLUSIONS

In addition to the 85% saving in planting material and excellent establishment, there are other advantages of hole planting. One is the

reduction in the amount of chemical possibly required to control soil insects like white grubs, since less soil needs protection, and to control seed-borne pathogens like downy mildew, since less volume of seedpiece will be treated (Ocampo & Tianco 1994). Another is the opportunity to intercrop the vacant spaces between-the-row and in-the-row with peanut, mungbean, or some other non-competitive cash crop.

Advanced cultural practices that have not been adopted because of impracticality under Philippine conditions may be worth re-evaluating if hole planting becomes acceptable. Manual application of chemical ripener with knapsack sprayer will be easier because penetrating the inter-rows will be less difficult. Hot-water treatment of seedpieces for disease control especially of ratoon stunting disease can be looked into with a new perspective because there will much less planting material to treat, making it easier to maintain the purity of the variety and its freedom from disease.

Commercial acceptance of **HPM** has been slow mainly because of the opinion that the method is laborious and the impression that cane yield will be reduced. Further research may involve investigating the age of transplants, spacing of seedlings, and the availability of moisture at transplanting. Attention to the amount and timing of fertilizer to be applied when using HPM may be an important consideration to encourage early tillering. It may also be possible to adopt the system to machine operations.

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Table 3 *Effect of hole planting (HPM) and conventional planting (CM) methods with six varieties on cane yield (tonnes/ha)*[1]

Varieties	Plant cane			First ratoon		
	HPM	CM	Difference	HPM	CM	Difference
Phil 56226	91.8 a	88.2 a	3.6[ns]	81.9 b	86.0 b	-4.1[ns]
Phil 6607	90.7 a	87.9 a	2.8[ns]	86.8 b	76.0 b	10.8[ns]
Phil 6723	86.3 a	94.2 a	-7.9[ns]	90.5 b	87.1 ab	3.4[ns]
Phil 7544	93.7 a	86.3 a	7.4[ns]	119.2 a	109.5 a	9.7[*s]
Phil 8013	94.2 a	82.0 a	12.2[ns]	92.6 b	85.6 b	6.8[ns]
VMC 7139	90.4 a	90.2 a	0.2[ns]	101.9 ab	90.2 ab	11.7[ns]
Mean	91.2	88.1	3.1 [ns]	95.4	89.1	6.3 [ns]

[1] Average of four replications. Mean separation in a column by DMRT at 5% level. [ns] not significant.

OPPORTUNITIES FOR INCREASED RADIATION UTILISATION BY SUGARCANE IN SOUTH AFRICA

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ABSTRACT

Sugarcane grown in South Africa can take 250 days to form a complete leaf canopy. This can result in a substantial wastage of solar radiation and a protracted period for weed control. This paper considers options regarding planting and ratooning dates, harvest age and row spacing which could be exercised to improve light interception and yields. Fractional light interception (LI) was defined as the amount of radiation intercepted by leaves and stems relative to the amount received at the crop or soil surface. Five varieties were planted on three dates during the summer of 92/93 at La Mercy near Durban. LI was derived from 2-weely measurements of photo synthetically active radiation (PAR, 400 to 700 nm) taken above and below the green canopy. Twelve months after planting, cumulative LI (CLI) for both January and March crops was 20% more than for the December crop. Reasonable agreement ($r^2 = 0.83$) was obtained between measured PAR interception and that computed using the CANEGRO model. Further simulations indicated that CLI could be increased by extending both the milling period and the harvest age. However, increased CLI would lead to increased biomass yield only where there was sufficient water to supply the increased demand. Reworking the results of row spacing experiments led to a similar conclusion that increased CLI from reduced row spacing would result in increased yields only in conditions favourable enough to produce 15 t sucrose/ha/annum or more.

INTRODUCTION

The high productivity of sugarcane compared to an annual crop such as sugar beet depends more on the proportion of annual radiation that is intercepted than on the proportion of intercepted radiation that is converted to dry matter (Austin et al 1978). This is true even though leaf area develops more rapidly in annuals than in sugarcane (Bull & Glaziou, 1975). The slow early leaf area production in sugarcane could be improved by selecting for leaf size and angle or simply to select varieties which intercept the most light (Irvine 1973). Irvine and Benda (1980) reviewed experiments on sugarcane row spacing and noted that the effect of row spacing on yield was more pronounced at high latitudes than in the tropics. This was attributed to increased importance of the period of incomplete canopy where growing seasons were shortened by frost at these latitudes. In South Africa frost is seldom a reason for a short growth period but the occurrence of Eldana borer has forced earlier harvesting resulting in a decrease in the proportion of land covered entirely by leaves. Growers are concerned about loss of productivity and increased costs of weed control resulting from this change. The aim of this study was to consider how planting date, harvest season, harvest age and row spacing could be managed to increase the fraction of light intercepted by leaves (LI) and thereby to increase biomass and

MATERIALS AND METHODS

The data in this study were obtained from i) planting date experiments conducted at La Mercy by the author, ii) simulations with the CANEGRO model and iii) a number of row spacing experiments conducted by various scientists at the South African Sugar Experiment Station (SASEX) in the 1960s and 1970s.

i) The planting date experiment was conducted on a sandy clay Swartland soil (Alfisol Rhodoxeralf) at La Mercy, South Africa (29° 34' S, 30° 8' E) with NCo376 and four other varieties which were not considered in this paper. Two plots, 25m long and 10 rows 1.2 m apart, were planted to each variety on 2 December 1992, 27 January and 24 March 1993. Fertiliser was applied in the furrow and later as a top dressing to provide 90 kg N, 48 kg P and 150 kg K/ ha . No irrigation was applied. A Sunflek Ceptometer (Decagon Services. Inc., Pullman, Washington) was used to record photosynthetically active radiation (PAR , 400 to 700 nm) above and below the canopy 3 to 4 times on a cloudless day every two weeks. The fraction of light intercepted (LI.) on day (D.) between measurement dates (D1 and D2) was computed as $LI_i = LI1 + (LI_2 - LI_1)(D_i - D_1)/(D_2 - D_1)$ where LI_1 and LI_2 are fractional light intercepts on dates 1 and 2 respectively. LI measured at midday was increased by as much as 30 % using a correction function based on the data of Inman-Bamber (1994a); $LI_d = 1.39 - 1.05LI_m + 0.73LI_m^2$ where

subscripts d and m refer to diurnal and midday LI respectively. Treatments were compared in terms of cumulative light intercepted (CLI) relative to the amounts of incoming radiation accumulated since planting. The energy equivalent of PAR was assumed to be half that of solar radiation.

ii) Details of the CANEGRO model have been published (Inman-Bamber 1991, 1994b). The water balance is similar to the WATBAL subroutine used in CERES-Maize (Jones & Kiniry 1986). The soil properties used in the simulation were those of a Swartland soil similar to that of the La Mercy experiments. Eight ratoon crops (Inman-Bamber 1994a) and the three plant crops described in (i) were simulated to allow their results to be applied more widely. At least 20 years of daily records of rainfall, temperature, sunshine duration, wind speed and humidity were obtained from eight meteorological stations selected to represent the range of climates in the industry. Malelane (25° 28'S, 31° 32'E, 309m) and Pongola (27° 24'S, 31° 35'E, 308m) are stations located in irrigated regions and Umfolozi (28° 45'S, 31° 54'E, 74m), Mtunzini (28° 56'S, 31° 32'E, 36m), Tongaat (29° 34'S, 31° 08'E, 72m), Esperanza (30° 18'S. 30° 38'E, 195m), Powers Court (29° 58'S, 30° 36'E, 631m) and Seven Oaks (29° 14'S, 30° 36'E, 1,067m) are in rainfed regions. A commonly used irrigation regime of 45 mm irrigation on a 10 d minimum cycle was assumed for the two irrigated sites. The effect of planting date on LI and yield was simulated by starting crop simulations in 1970 on the 15th of each alternate month (February to December). The simulated cropping schedule involved crops harvested at 12 months and replanted after seven ratoon crops. Nominal harvest ages of 14, 16, 18, 20 and 22 months were also simulated by adjusting harvest dates to adhere as often as possible to these target ages without 'harvesting' outside the traditional milling period (May to December). The results of simulations spanning 1970 to 1995 were meaned.

iii) Yield responses to a reduction in row spacing from 1.37 to 0.91 m in seven experiments conducted by Thompson & du Toit (1965) were compared to the trial mean sucrose yield in order to determine the conditions under which reduced row spacing could be expected to enhance yields. Unpublished data obtained from row spacing experiments conduced by Gosnell (1967) were analysed graphically.

RESULTS AND DISCUSSION

Planting date experiment

Canopy development was most rapid in the January crop and slowest in the March crop (Fig.1). This was due, in part, to differences in mean soil temperature during the 21 d period after germination (29.2, 27.9 and 27.1° C for December, January and March plantings respectively). The December crop germinated when radiant intensity was greatest (Fig.1) so that when about 1000 MJ/m² PAR had accumulated from
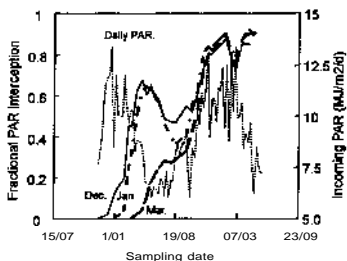
Fig. 1 *Fractional PAR intercepted by NCo376 planted on 2 December 1992, 27 January or 24 March 1993 and weekly mean incoming PAR*
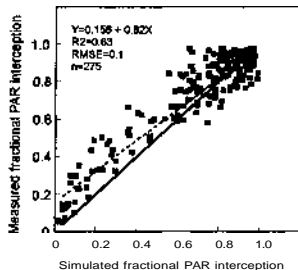
Fig. 2 *Measured and simulated fractional PAR interception by eight ratoon crops and three plant crops ofNCo376 starting on various dates between 1989 and 1993 at La Mercy.*
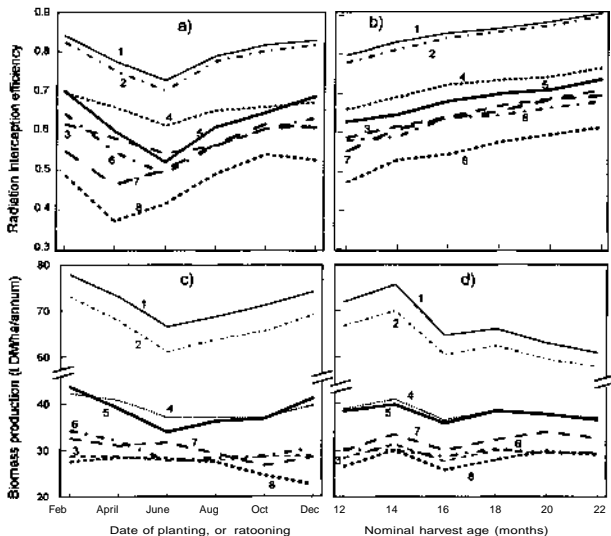
Fig. 3 *Simulated CLI (a) and dry biomass yield (c) for 12 month crops starting in alternate months of the year, and simulated CLI (b) and dry biomass yield (d) for crops harvested at nominal ages from 12 to 22 months. Sites 1.Malelane, 2.Pongola, 3.Umfolozi, 4.Mtunzini, 5. Tongaat, 6.Esperanza, 7.Powers Court, 8. Seven Oaks.*
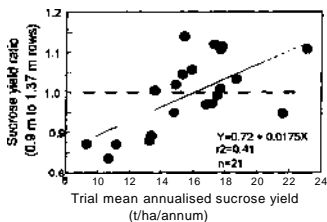
Fig. 4 *Sucrose yield of NCo376 planted in 0.91 m rows relative to that in 1.37 m rows collated from trials with different potentials for sugar yield (some data reported by Thompson & du Toit 1965).*

Fig. 5 *Tiller density and sucrose yield during the growth of rainfed NCo376 planted in 0.45 m rows (broken line) or 1.37 m rows (solid line) [after Gosnell (1967)].*

planting, this crop had intercepted only about 110 MJ/m² (CL1=0.11). Comparative CLI for January and March crops was 0.28 and 0.17 respectively. Twelve months after planting when approximately 3300 MJ/m² PAR had accumulated, CLI for the successive plantings was 0.46, 0.55 and 0.55 respectively. The December crop thus failed to recover from the radiation wasted during the early stages of growth and this is likely to occur in most years. Ratoon crops intercepted more of the annual incoming radiation when harvested in summer than when harvested in winter (Inman-Bamber 1994a). This was because the winter crops took up to 2.5 times longer than summer crops to reach full canopy and winter radiation was only about 40% lower than summer radiation. The initiation, growth and death of the main components of the canopy (leaves and tillers) are influenced by temperature and soil water conditions (Inman-Bamber 1994a). CLI will therefore vary in relation to these factors and incoming radiation. The effect of planting and ratooning date on annual CLI is determined best by model simulation of these processes.

Simulation

CANEGRO simulated light interception with a the root mean square error for prediction of 0.10 LI (Fig.2). The residual variation was not significantly correlated with planting or ratooning (starting) date. The model underestimated radiation interception in the early stages of the crop but it was considered the best method of applying the results of the La Mercy trials to the rest of the industry. Comparisons between measured and simulated yields of a large number of crops (Inman-Bamber et al 1993) supported this assumption.

Simulated CLI over 12 months was generally lowest for crops planted or ratooned in June and was greatest for crops starting in December or February (Fig. 3a). CLI changed as much as 0.16 in response to changes in starting date and as much as 0.13 in response to changes in harvest age. The effect of harvest age and season was greatest at the high altitude site, Seven Oaks, (Fig.3a, 3b) because of low temperatures and the protracted canopy formation consequently predicted for this region.

According to these simulations the annual production of biomass would be greater with increased CLI only in annual irrigated crops, viz. at Malelane and Pongola (Fig. 3c). The reduction in annual biomass production of irrigated crops after 14 months (Fig. 3d) was due to respiration losses assumed in the CANEGRO model. For rainfed areas the model predicted that water stress would increase if CLI was increased. The association between CLI and annualised stress days (unpublished data) was significant (P =0.05). Water stress was most pronounced at Seven Oaks where the effect of starting date on CLI was greatest; the model predicted 52 stress-days for the February crop and 144 stress-days for the December crop.

Row spacing

Cane and sucrose yields of three varieties planted in 1.37 m rows exceeded yields obtained in 0.45 m rows even though canopy closure
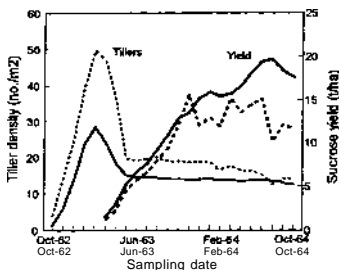
was achieved after 14 weeks in the narrow rows and not at all in the wide rows (Thompson & du Toit 1965). Thompson (1962) noted that cane in narrow rows often showed more water stress when than when in wide rows. The results of all trials in which 0.91 m and 1.37 m spacings were compared indicated that yield increases from reduced row spacing could be expected only in conditions favourable enough to produce > 15 t sucrose/ha/annum (Fig. 4). For NCo376 planted in 0.45 m rows, tiller density was almost twice that recorded in 1.37 m rows but sucrose accumulation started at approximately the same time and rate in both row spacings (Fig. 5) despite the presumed difference in the amount of radiation intercepted (Gosnell 1967). It is likely that stalk death in the dry conditions that prevailed in the second year of growth was responsible for the poor performance of the narrowly spaced crop. A likely explanation for this is that increased light interception resulted in increased water use leading to greater water deficits in the second year.

CONCLUSION

December planting is likely to result in reduced CLI in most years and March planting would be preferable. Planting in January or February is not advocated because of the high intensity rains occurring at that time. Simulations indicated that CLI could be increased up to 13 % by extending harvest age from 12 to 22 month and up to 16 % by ratooning in summer instead of winter. The yield benefit arising from increased CLI is likely to be limited by increased water stress or increased respiration. The option that deserves most attention is summer ratooning of irrigated crops. A reduction in row spacing failed to hasten sucrose accumulation in NCo376 and it resulted in a yield increase only in favourable moisture conditions. The data presented indicated that a delay in canopy development is often necessary for water conservation in the South African Industry. Efforts to reduce radiation wastage will improve yields only where there is sufficient rainfall or irrigation to supply increased demand for water.

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INCREASING SUGARCANE YIELDS THROUGH HIGHER PLANTING DENSITY - PRELIMINARY RESULTS

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ABSTRACT

Small replicated plant crop trials have strongly supported earlier results that row close spacings can increase cane yield in responsive clones by over 50% when compared with conventional row spacing (1.5m). In addition, unselected clones grown in close rows outyielded commercial cultivars grown at 1.5m or 0.5m. These results followed from the use of unselected clones, a purpose built multi-row planter, adequate irrigation and fertilizer application rates based on a per length of row rather than a per unit area basis. Close row spacing reduced tillering and allowed a more efficient harvesting of available light, water and nutrients, particularly during the early stage of rapid crop growth when these resources were most available.

INTRODUCTION

Sugarcane row spacings have steadily increased to accommodate increasing levels of mechanization and sizes of tractors and harvesters. The predominant row spacing in Australia is about 1.5m but spacings up to 1.8 metres or more are under examination in order to minimize damage to ratoon crops and reduce soil compaction problems (Ridge & Hurney 1994). However, wider row spacings reduce crop yield potential because they limit interception of light energy during the early stages of growth (Irvine et al 1980). Commercial varieties have been selected to tiller rapidly and minimize the loss in light interception. However, tiller initiation requires a diversion of photosynthate away from the primary stalks, leads to increased competition for light, water and nutrients amongst stalks of the same stool and leads to a marked loss of young tillers at canopy close-in (Bull 1975).

One way to overcome this potential loss in yield is to look beyond the current sugarcane selection and agronomic practices which have been imposed by traction and harvest equipment design. Crops grown at high planting density tend to be composed mostly of primary stalks which grow rapidly, compete actively with weeds, avoid death of tillers near canopy closure and exploit soil water and nutrient reserves more efficiently than conventionally planted crops. Irvine & Benda (1980 a,b) reviewed row spacing studies in sugarcane. They predicted significant yield increases by reducing row spacing to 0.6m, or less, and recorded two-to three- fold increases in yield of cane and sugar from close spaced short season crops (7 to 8 months) in Louisiana. These results were confirmed in further field trials where crops grown at row spacings of 0.3m and 0.6m yielded twice the average yield from the standard (1.5m) spacing (Irvine et al 1980). Singh & Singh (1963) concluded that a 0.75m row spacing was superior to 0.6m or 0.9m for recently released varieties in India. However, a review of sugarcane row spacing research in Australia indicated that the results were disappointing, with little or no yield advantage from closer row spacing (0.6m or 0.9m) or from dual row planting (Ridge & Hurney 1994). Such results are not surprising given that the trials used commercial varieties, the result of over 15 years of intensive selection for high performance at the prevailing commercial row spacing, and rates of irrigation and fertilizer application which were based on crop area rather than on length of row.

However, an alternative approach was proposed and tested in small plots over 20 years ago by Bull (1975). He suggested using unselected clones (i.e. original seedlings or clones from the first stage of selection) with irrigation and fertilization supplied on a per row basis, designed to avoid the above constraints. His results indicated that there was potential to improve crop yields by up to 100% at higher planting densities. The current paper reports on recent trials to investigate the genetic and physiological potential of unselected clones to respond to high planting densities.

MATERIALS AND METHODS

Yield potential

A trial using eight unselected clones and one commercial cultivar (Q150) with two replicates was planted at three row spacings (1.5m,

1.0m and 0.5m) on 1 December 1994 using a prototype multi-row planter designed for studies on high planting density. Plots were 7m long and 4.5m wide. Each row received 3.75g/m N, 3.9 g/m P and 19.5 g/m K at planting followed by 30 g/m of urea twelve weeks later. Light interception was monitored using a linear quantum sensor and datalogger (Li Cor) in each row spacing on 16 March, 12 April and 24 May 1995. Stalk fresh weights were determined on 20 stalk samples on 28 June and 6 stalk samples harvested from each plot on 11 October 1995. Whole plot weights were also obtained at harvest using a commercial harvester (Toft 7000) and weigh truck on 18 October 1995.

Fertilizer response

A trial using one unselected clone (87S9262) and one commercial cultivar (Q154) with two replicates was planted at two row spacings (1.5m and 0.5m) using the prototype multi-row planter on 6 December 1994. Plots were 10m long and 4.5m wide. Four rates of N, P and K were applied as shown in Table 1. Plot weights were also obtained at harvest using a commercial harvester (Toft 7000) and weigh truck on 18 October 1995.

Both trials plots were irrigated weekly by overhead spray until 5 July 1995.

Table 1 *Rates of nitrogen (N), phosphorus (P) and potassium (K) applied at row spacings of 0.5m and 1.5m*

Row Spacing	N	P	K	N	P	K
	(kg/ha)			(g/m row)		
	20	22	108	1.0	1.1	5.4
0.5 m	60	66	325	3.0	3.3	16.2
	101	22	108	5.0	1.1	5.4
	300	66	325	15.0	3.3	16.2
	20	22	108	3.0	3.3	16.2
1.5 m	60	66	325	9.0	9.9	48.7
	101	22	108	15.1	3.3	16.2
	300	66	325	45.0	9.9	48.7

RESULTS

Yield potential

The leaf canopy of close rows (0.5m) closed-in about 50 days earlier than at wider spacings (Fig. 1) and cane yield increased, on average, by more than 50% (Table 2) over the standard row spacing of 1.5m. The increase in cane yield was strongly associated with the retention of a higher number of millable stalks at harvest (Table 3). Calculations based on final stalk numbers indicate that final stalk numbers per stool were almost halved in close rows, viz. 2.1/stool for 0.5m rows and 3.8/stool for 1.5m rows. Clonal responsiveness to close rows, reflected by cane yield increase, ranged from 34% to 112% and suggests that there is

ample genotypic variation available to justify further trials to identify and select suitably responsive clones. Interestingly, the commercial clone (Q150) also responded to close row spacing but was below average in its level of response. The interaction term for clone x row spacing was significant (P<0.01) at the 300 day harvest, indicating that there is variation in the pattern of clonal response to row spacing.

Harvesting with a mechanical cane harvester gave average cane yields of 110, 89 and 83 t/ha at 0.5, 1.0 and 1.5m row spacing respectively. The difference between these figures and the sample yields reflects the difficulty faced with harvesting small plots, particularly in lodged crops at close row spacing. However, the results still indicated an average yield increase of over 30% due to close rows. Final crop yields recorded in this trial were relatively low because late planting restricted the period of high light intensity available for crop growth.
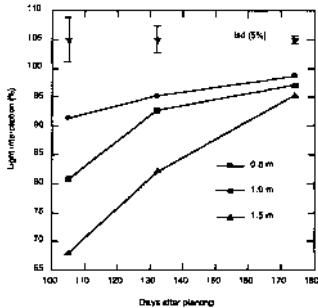
Fig. 1   *Average light interception by nine clones at three different row spacings*

Table 2   *Effect of three row spacings on the cane yield (tonnes /ha) of nine different clones at 300 days after planting   in December 1994*

Clone	Cane yield (t/ha)			% Increase over 1.5m	
	0.5m	1.0m	1.5m	0.5m	1.0m
8110	116.3	100.5	87.0	34	15
8124	157.8	99.5	98.2	61	1
8214	142.2	98.7	67.1	112	47
8428	143.0	81.4	96.9	48	-16
9086	151.1	97.5	96.9	56	1
9234	155.8	106.0	110.7	41	-4
9262	129.6	127.7	85.5	52	49
9475	123.9	107.7	75.6	64	42
Q150	129.6	115.9	96.5	34	20
MEAN	138.8	103.9	90.5	55.6	17.4

LSD (P<0.05) for means = 11.5 t/ha

Table 3   *Effect of three row spacings on the stalk number per ha of nine different clones   at 300 days after planting   in December 1994*

Clone	Stalk numbers (,000's/ha)			% Increase over 1.5m	
	0.5m	1.0m	1.5m	0.5m	1.0m
8110	118	89	73	62	22
8124	118	76	71	66	8
8214	138	116	82	68	42
8428	130	73	73	79	0
9086	140	114	89	57	28
9234	103	70	62	66	14
9262	145	120	91	60	32
9475	133	104	82	62	27
Q150	105	108	67	58	61
MEAN	125	97	76	64	26

LSD (P<0.05) for means = 24.9

Fertilizer response

Fig. 2 suggests that the closer row spacing was more efficient at extracting and utilizing fertilizer, particularly at rates of 100 kg/ha nitrogen and higher. Close rows produced significantly higher yields than the standard row spacing from the same fertilizer rate per row, indicating that the close rows captured or extracted more nutrients. These results suggest that the cost of additional fertilizer (and possibly irrigation) might be minimal when assessing the incremental costs of production at high planting density.
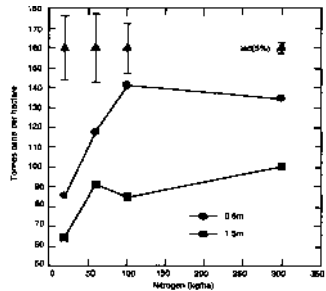
Fig. 2   *Average yield response of two clones (87S9262, Q154) to nitrogen (at non-limiting rates of P and K) for 0.5m and 1.5m row spacings,   300 days after planting   in December 1994*

DISCUSSION AND CONCLUSIONS

This trial confirmed earlier results (Bull 1975) on the potential to increase yield by using closer row spacing and responsive genotypes. The trial results also suggest that it may be unnecessary to apply fertilizer at the full "per row" rate to obtain improved yields at higher planting densities

The main conclusions are :

(i)   higher planting densities can increase the cane yields of unselected clones by over 50%

(ii)   unselected or rejected clones grown at close row spacing can outyield commercial cultivars grown at either 1.5m (80% at 200 days after planting (DAP) or 64% at 300 DAP) or at 0.5m (18% at 200 DAP or 21% at 300 DAP)

(iii)   genetic variability appears to exists for the capacity to respond to high planting densities

(iv)   the efficiency of light, water and nutrient use (units per tonne cane) appears to be higher at close row spacings

ACKNOWLEDGMENTS

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ECONOMICS OF RATOON CYCLE LENGTH IN SUGARCANE

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ABSTRACT

Profitability of growing sugarcane is enhanced if long ratooning cycles are used, for high costs of the plant crop can be amortised over a number of lower cost ratoon crops. A crop cycle of plant and six ratoons was the most profitable management procedure for cultivars which maintained stool populations. However, cultivars which became gappy, due to mechanical harvester damage, were less profitable. Gappy cultivars were three times less profitable, when grown under either rainfed or irrigated conditions.

No decline in sugar per stool occurred in any cultivar as a result of long ratooning crop cycles.

Yield data were analysed by a technique which minimised the confounding effects of cultivar by year interactions with the cultivar by ratoon number interactions. Mean cane yield from neighbouring farms was used as an indication of year effects. Year effects were also eliminated by comparing plant and first ratoon crops to sixth and seventh ratoon crops grown in the same year. Good ratooning cultivars yielded as high in late ratoons as in plant and early ratoon crops.

INTRODUCTION

Management practices which can achieve longer, more profitable ratoons are a high priority for the Australian sugar industry. The growing of good ratooning cultivars is a necessary component of these practices in order to contain costs, since operating costs are three times higher for plant than ratoon cane. Also, the proportion of farm in production is usually increased with longer ratooning cycles.

Ratoon yields in Australia are usually believed to decline later in the cropping cycle, but in other countries this is not always the case. For example, in Swaziland there was no yield decline for crop cycles up to 24 ratoons (Todd G M, personal communication).

The decision to discontinue ratooning and replant is ideally taken when the cumulative lost production, and therefore returns from the past ratoons, plus the forward estimate of the next ratoon crop, exceeds the cost of replanting (Simms 1982).

Six cultivars of sugarcane, grown commercially in central Queensland ranging from 50 years ago to the present, were compared in rainfed and irrigated experiments. Yield was compared over a succession of 7 ratoons, and also with new plant and first ratoon crops coinciding with sixth and seventh ratoons. The profitability of longer ratooning cycles was analysed by comparing the estimated lost value of production in later ratoons with the higher costs of replanting after a plant crop or after a varying numbers of ratoons.

METHOD

Six cultivars of sugar cane, Q50, Q68, Q87, Q124, Q138 and NCo310, were planted into three experiments on the Sugar Experiment Station, Mackay (149.21°E, 21.46°S). Experiment 1 and Experiment 2 were grown under rainfed and irrigated conditions respectively for a plant crop and 7 ratoons. Experiment 3, also irrigated, was planted so that plant and first ratoon crops could be compared with sixth and seventh ratoon crops in Experiment 2. Plots were 4 rows wide and had three replicates. Harvesting dates were between 21 August and 6 October so that the average age of plant crops was 59 weeks (range 54 to 64 weeks) and ratoon crops was 51 weeks (range 44 to 59 weeks). No yield adjustment for crop age was made.

Cane yield was measured in whole plots by weighing billets cut by a harvester into a truck-mounted weighing bin. Sugar concentration was measured, in juice extracted from 10-stalk samples of cane, by the CCS method (Anon 1984).

Adjusted yield

Measured yields include the effects of true yields over ratoons and variations due to year, principally climatic, effects. These latter effects were excluded from the measured yields of cane to give an adjusted yield which was used for the economic analysis in this paper. Adjusted yields for seventh ratoon are not yet available. This was done by a statistical procedure which used yields from neighbouring farms, five irrigated and five rainfed, which provided an index of seasonal variation (Jones et al 1993). Adjusted sugar yield was calculated as the product of measured CCS and adjusted cane yield.

Gaps and production per stool

Single stools of sugarcane cannot be readily identified in machine planted fields as a continuous row of setts is planted and stools intergrow. Stools were assumed to occupy 0.5m of cane row in this paper. When stubble pieces failed to ratoon, gaps occurred along the cane row. Gaps, greater than 0.5m, were measured when each crop was established and total gaps were calculated as a percentage of the row length. Sugar production per stool was calculated as the adjusted sugar yield divided by the total stools, adjusted for gaps.

Economic considerations

Adjusted sugar yields for each cultivar and year were used to calculate mean production for seven crop cycles of plant/fallow, plant/one ratoon /fallow, up to plant/six ratoons/fallow. There is one year lost production in a fallow as the stubble is ploughed out in summer and cane replanted in autumn.

Annual returns to canegrowers for crop cycles were calculated as adjusted sugar yield multiplied by the value of sugar ($350/tonne) multiplied by 0.66, minus fixed, operating and harvesting costs. The remainder of returns goes to millers.

Costs of production included: fixed (administration, electricity, mill deductions, rates and repairs); operating (cultivation, planting, fertiliser, crop protection and irrigation) and harvesting (fuel, harvester and transport). Wages were not included.

Annual costs per hectare were: fixed ($600), operating ($1100, $400 and $100 for plant, ratoon and fallow respectively) and irrigation ($250). Harvesting costs were $40/tonne of sugar. These estimates are based on financial records from 83 canegrowers in central Queensland (Graham 1995).

RESULTS AND DISCUSSION

Yield

The highest producing cultivars were Q124 and Q138 for both cane and sugar yield. In measured yields, there were large seasonal effects for both the rainfed and irrigated experiments. Extremes were, low yields for ratoon three which was affected by cyclonic rain and prolonged waterlogging, and high yields for sixth ratoon and plant crop of

Experiment 3, which resulted from a well distributed but less than average rainfall. Irrigation increased average cane yield by 18 ± 3 t/ha/yr.

The adjusted cane yields (Fig. 1a) showed an increase to first ratoon, a decline to second ratoon followed by a plateauing from third ratoon to sixth ratoon for the four best cultivars Q124, Q138, Q87 and NCo310. The low yielding cultivars Q68 and Q50 had a drop in yield to third ratoon followed by slightly increased yields. The patterns for adjusted cane yield were similar but more variable for the irrigated crops. A feature of these data is that the high and low yields caused by seasonal variation have been moderated by the statistical manipulation. Adjusted sugar yields (Fig. 1b) followed a similar pattern to the adjusted cane yields, with Q124 and Q138 consistently being the highest yielding cultivars.
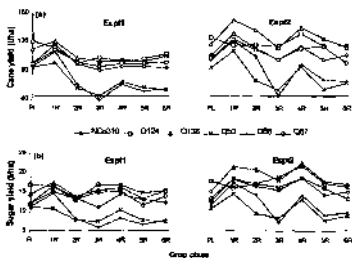
Fig. 1 *Adjusted yields of (a) cane and (b) sugar for cultivars by crop class for rainfed Experiment 1 and irrigated Experiment 2.*

Gaps and production per stool

When crops lodged, some stools levered the stubble out of or higher in the soil in a process called tipping. Stool tipping led to harvester damage as the base cutter sliced under the stubble including stool pieces with the harvested crop. If stubble was damaged or removed, gaps within the cane rows occurred in the next crop. For Experiment 2, gaps generally increased in older ratoons and were highest in Q50 and Q68, with Q124 and Q87 having fewer gaps and NCo310 and Q138 virtually no gaps (Fig. 2a).
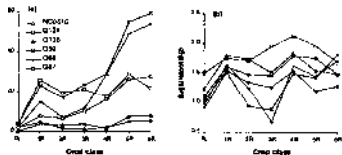
Fig. 2 *Effect on (a) loss of stools (gaps as % of row length) and (b) sugar yield (kg) per stool for cultivars by crop class, under irrigation.*

Q124 developed a full canopy even though it had 25% gaps by sixth ratoon in the irrigated experiment. It could sustain yield because 80% of gaps were only 0.50 - 0.75m long. By comparison, Q50 and Q68 had 50 - 60% gaps by sixth ratoon with most gaps 1 - 1.5m, and 12% >2m. Q50 and Q68 could not compensate for this gappiness and therefore

lost yield. Gaps in the irrigated and rainfed experiments were similar, with most gaps developing after the plant and fourth ratoon harvests.

The perceived decline in production in older ratoons has generally been attributed to lower productivity per stool due to disease, insect attack, soil physical and chemical properties or unknown factors. The analysis of adjusted sugar yield when calculated on an individual stool basis (i.e. taking account of gaps) showed no decline in production for most cultivars with successive ratoons as shown for Experiment 2, the irrigated experiment (Fig. 2b). Q50 and Q68 showed some yield decline per stool for second and third ratoon but recovered stool productivity for fourth to sixth ratoon. These latter increases are probably due to the larger gaps reducing competition for light and water between stools. The same pattern of maintenance of productivity per stool in older ratoons was also evident in the rainfed experiment.

Economic considerations

Adjusted sugar yield for the plant/fallow cycles ranged from 6.8 to 9.1 t/ha/y and increased to 14.0 to 16.8 t/ha/y for plant/sixth ratoon/fallow for cultivars Q124, Q138, Q87 and NCo310 in Experiment 2 (data not shown). The lower producing cultivars Q50 and Q68 had maximum yields of 9.5 t/ha/y for plant/fourth ratoon/fallow rotation and 11.1 t/ ha/y for plant/second ratoon/fallow cycle respectively. The effect of cycle on adjusted sugar yield was similar in general trend, but with lower yields, for the rainfed experiment. The operating costs per tonne of sugar decreased with increasing number of ratoons for all cultivars in both the rainfed and irrigated experiments, as the high planting costs were amortised over a larger number of crops, for example, 48,41,38,35,33.32 $/tonne sugar for ratoons of 1 to 6 ratoons respectively for Q124 in the rainfed experiment..

The combined effect of increased production and reduced operating costs per tonne of sugar had a dramatic effect on returns per hectare for various length of crop cycle (Fig. 3). Returns increased from a loss of $ 128/ha for growing Q50 for a plant crop only to $ 1603/ha for growing Q124 for a 6 ratoon rotation under rainfed conditions. Extremes for the irrigated experiment were a loss of $270/ha for a plant crop only for Q50 to a return of $1946/ha for a 6 ratoon crop cycle for Q138. A 6 ratoon crop cycle is unquestionably the most profitable for cultivars Q124, Q138, Q87 and NCo310 for both rainfed and irrigated cane. There was a general plateauing of returns after first ratoon for cultivars Q50 and Q68. There was no cultivar by irrigation interaction in these experiments, but this result may not extend to all districts in central Queensland. Many ratoons, including Q124, failed under rainfed conditions from the disastrous drought which existed for some periods of these experiments.
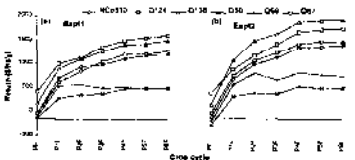
Fig. 3 *Economic return for crop cycle by cultivars for (a) rainfed (Experiment 1) and (b) irrigated (Experiment 2) treatments.*
[PF=plant crop then fallow; P1F=plant crop, first ratoon then fallow].

Yields for early vs late crop

Further evidence of the comparable yields from older ratoons was obtained by comparing sixth and seventh ratoon in Experiment 2, with plant and first ratoon in Experiment 3 grown in the same years. Both experiments were fully irrigated. There was no decline in actual yield in older ratoons compared to plant and first ratoon crops for cultivars Q124, Ql38 and NCo310 (Table 1). All three of these cultivars have a record of successful commercial production in older ratoons compared to cultivars Q87, Q50 and Q68 which were generally grown

for shorter crop cycles. These results confirm that the estimates of sugar production, excluding year effects, by the use of neighbouring farm yields, gave realistic predictions.

Table 1 *Sugar yields (t/ha) of early (Experiment 3, plant and first ratoon) and late (Experiment 2, sixth and seventh ratoons) crop classes for irrigated sugarcane cultivars grown in the same years(mean yields and standard errors).*

Cultivar	Early Plant/first ratoon	Late Sixth/seventh ratoon
Q124	18.5+1.9	19.0 + 1.7
Q138	18.5 + 2.0	19.3±1.7
Q87	18.6 ±0.8	15.3+1.6
NCo310	15.8 ±2.4	15.3 ±2.0
Q68	17.1 ±2.3	10.9 ±0.3
Q50	16.6 ±2.2	10.1 ±0.8

CONCLUSIONS

The most profitable crop cycle was a plant crop/six ratoons/fallow for cultivars which maintained stool populations. An earlier plough-out would be the most profitable for cultivars which became gappy due to harvester damage. Gappy cultivars were three times less profitable when grown under either rainfed or irrigated conditions. Sugar yield per individual stool did not decline in any cultivar, due to long ratooning effects. Good ratooning cultivars yielded as high in sixth and seventh ratoons as in the plant and first ratoon crops.

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CHEMICAL RIPENING OF SUGARCANE IN SWAZILAND

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ABSTRACT

Four field experiments on variety N 19 confirm that immature, actively growing sugarcane responds well to ethephon (2-choroethane-phosphonic acid) and Fusilade Super (fluazifop-p-butyl). A combination treatment of ethephon, followed by Fusilade 4-6 weeks later produced the best improvements in cane quality and the highest increases in sucrose yield. There was little difference between Fusilade rates of 37.5 and 56 g ai/ha but yields were reduced if harvest was delayed too long. Both chemicals increased sucrose content as a percentage of stalk dry weight, indicating true ripening effects. Fusilade reduced cane moisture content and cane yield per hectare

Improvements in cane quality and sucrose yields in Swaziland have coincided with increasing use of ripener treatments. The cost/benefit ratio from using ripeners ranged from 1:6 to 1:15, depending on the chemicals used. It is estimated that chemical ripening increased the net return to Swaziland growers in 1994 by E9.7 m ($A6.64 m). Ripeners could increase profits of a 150,000 ton sucrose mill by E0.38 m ($A0.14 m) per year.

INTRODUCTION

Sugarcane is grown under irrigation in the semi-arid, low altitude, region of Swaziland Lat. 27°S Long. 31°E). Annual rainfall of 600-750 mm/y falls mainly in the hot summer months of October to March. This causes poor cane quality at the beginning (April/May) and end (October/ November) of the 32-34 week milling season, reducing cane throughput and sucrose recovery in the factory. These conditions are ideal for chemical ripeners, which restrict stalk elongation during periods of rapid crop growth, diverting photosynthate from growth to storage processes. This improves cane sucrose content, sucrose yield and factory

Most sugarcane in Swaziland is grown on large estates with good, progressive management and many fields are large (20-50 ha) and ideal for aerial spraying. High yielding crops are cut annually and the main variety, NCo376, has a fairly low natural sucrose content. Approximately 60% of the 38,000 hectares of sugarcane is ripened annually, some fields receiving more than one ripener application. The objectives of this paper are to report on the response of variety N19 to ripening with two chemicals and to assess the value of ripeners to both sugarcane growers and millers.

Chemicals currently used as ripeners are ethephon (2-choroethane-phosphonic acid, 480 g ai/L) (Rostron 1975) and Fusilade Super (fluazifop-p-butyl, 12.5 g ai/L) (Rostron 1985). Other chemicals such as glyphosate (Clowes 1980) do not compete economically and may damage subsequent ratoons. All chemicals produce similar increases in sucrose yield under suitable conditions but sucrose percent cane fresh weight (% f.wt.) often varies because of differing effects on cane

Juice purity (purity) reflects the effect of growing conditions on the crop up to the time of spraying and is a measure of a crop's suitability for ripening (Rostron 1975). The critical value above which there is no response to ethephon (E), confirmed recently by Kingston et al (1991), is about 75%. Very immature sugarcane ripened with E at the start of the milling season can be successfully re-sprayed with Fusilade (F) 4-6 weeks later, providing that purity does not exceed about 85%. This combination treatment (E+F) usually improves sucrose % f.wt. and increases sucrose yield more than either chemical alone (Rostron 1985). Variety NCo376, which is about 65% of the Swaziland crop, is particularly responsive to ripeners and new varieties must at least equal the yield of ripened NCo376.

MATERIALS AND METHODS

There were four replicated (x5) experiments in three years on ratoon crops of variety N19 in irrigated commercial sugarcane fields at the start of the milling season. Fertilisation, based on soil analyses, was sufficient to produce maximum yields and crops were harvested between 11 and 12 months of age. Ripeners E and F were applied in about 60 L water/ha with a constant pressure sprayer and an overhead boom. Harvest dates, treatments and times of treatment are shown in Table 1. Rates of F and times of treatment were combined factorially in all experiments. Ethephon and E+F treatments of E 720 g ai/ha followed by F 56 g ai/ha, applied at the first spray date only, were also included. There were E+F treatments for all rates and all spray dates in expt. 4. Plots were 108 m² and the centre two of the four rows were sampled randomly during the experiments and harvested for yield on termination. Samples of 20 whole stalks, topped at the natural breaking point, were chaffed, sub-sampled and disintegrated prior to analysis for moisture and pol (sucrose) content. An estimate of recoverable sugar was obtained from the formula:

$$Ers = (S \times 0.9804) - ((brix - S) \times 0.5149) - (fibre \times 0.0203)$$

Where : Ers is weight of estimated recoverable sugar, S is weight of sucrose and brix is weight of total dissolved solids in juice

Table 1   *Rates and times of application (weeks before harvest) of two ripeners in four experiments.*

| Expt. | Year | Ethephon | | Fusilade Super | | Harvest |
		g ai/ha	weeks	g ai/ha	weeks	date
1	1992	720	13.5	37.5,56,75	9.5,7.5,5.5	11/6/92
2	1993	720	18	37.5,56,75	12.9, 8	29/6/93
3	1993	720	17	37.5,56,75	12,10,8	7/7/93
4	1994	720	13	37.5,56	11, 9, 7a	3/5/94

*   No 37.5 g ai/ha rate at 7 weeks

RESULTS

Crop growth **and** ripener response

Ripener response was related to cane condition when sprayed and stalk growth rate during the experiment (Table 2). There was little or no response to either E or F in expt. 1, where purity was 75% when E was sprayed and 84% when F treatments were applied. Similarly, there was little response in expt. 2 where initial purities at spraying did not exceed critical levels but unfavorable growing conditions during the experiment resulted in small increases in stalk weight and poor cane yields. Purities were below critical levels when treatments were applied in expts. 3 and 4, stalk growth was good and there were statistically significant improvements in estimated recoverable sugar % f.wt. for most treatments.

Table 2 *Ethephon spray details, increase in stalk fresh weight (F.wt.) between spraying and harvest and estimated recoverable sugar response*

Experiment:	1	2	3	4
Ethephon applied :	10/3/92	23/2/93	10/2/93	2/2/94
Purity when sprayed :	75.0	63.9	66.5	65.0
Control :				
Cane yield (t/ha)	116	88	98	107
Recoverable sugar (% cane)	13.7	13.6	14.4	13.2
Recoverable sugar (t/ha)	15.8	11.9	14.1	14.1
F.wt. increase (g/stalk)	438	156	250	388
Mean response to all treatments :				
Recoverable sugar (% cane)	0.0	0.5	1.1"	1.4"
Recoverable sugar (t/ha)¹	-0.3	0.1	0.9	1.4

- Best response : Expt. 3 - 2.3 t/ha   Expt. 4 - 2.6 t/ha
" Statistically significant (P<0.01)

Ethephon

The ripening response of N19 to E was poorer than the response to either F or E+F (Fig. 1) and there were few statistically significant improvements in recoverable sugar % f.wt. Fig. 2 illustrates how these effects were mostly lost after 12 weeks and well before harvest.
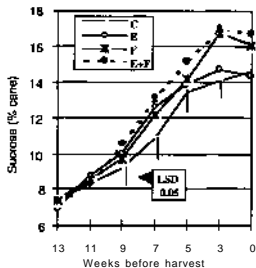
Fig. 1 *Expt. 4: Sucrose % cane for control, ethephon, Fusilade and E+F treatments applied 9 weeks before harvest.*
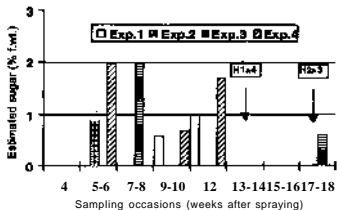
Fig. 2 *Expts. 1-4: Statistically significant increases in recoverable sugar following ethephon treatment (H 1 etc. = harvest times of expts.).*

Fusilade Super

There were consistent, statistically significant, increases in recoverable sugar % f.wt. from 2 to 4 weeks after treatment with F in expts. 3 (Fig. 3) and 4 following statistically significant improvements in sucrose % f.wt. (Fig. 1) and purity. These beneficial effects remained until harvest for most treatments and there were consistent, but statistically non-significant increases in yield of estimated recoverable sugar in both experiments, despite some reductions in tons cane per hectare.

The higher the rate of F applied and the longer interval between spraying and harvest, the better the effect on cane quality (Fig. 3). However, treatment with F 75 g ai/ha, or too long an interval between spraying and harvest, reduced improvements in recoverable sugar yield in expt. 3. Fusilade rates of 37.5 and 56 g ai/ha increased recoverable sugar by between 0.5 and 2.3 t/ha in expt. 3 and by 0.8 to 2.1 t/ha in expt. 4.
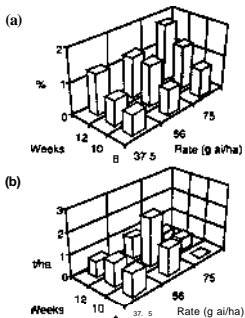
Fig. 3 *Expt. 3: Increase in recoverable sugar % cane (a) and t/ha (b) for rates of Fusilade and weeks applied before harvest.*

Improvements in cane quality in expt. 3 were associated with improvements in sucrose percent cane dry matter (% dm) (Fig. 4) as treatments had little effect on moisture content.

Cane quality quality improvements in expt. 4 were due to increases in both sucrose % dm and reductions in moisture content (Fig. 5).

In both experiments, E + F improved cane quality more than either chemical alone (Fig. 1) by increasing sucrose % dm (Fig. 4) and prolonging the response. Fig. 6 illustrates how E+F produced better quality and maintained the yield response longer than F applied at 12 weeks in expt. 3. Combination treatments increased recoverable sugar by between 1.2 and 2.6 t/ha in expt. 4.

DISCUSSION

Results of experiments

The results demonstrate that chemical ripeners work only if sugarcane is in the correct (immature) condition when sprayed and has the ability to grow actively after treatment. They also confirm varietal differences in response to E (Rostron 1973; Kingston et al 1991). The response of N19 to E was less than that of NCo376 and the beneficial effects were lost after 12 weeks, compared with up to 16 weeks for NCo376 (Rostron et al 1976).

Fusilade ripening results on N19 were similar to those on NCo376 (Rostron 1985). Best improvements in cane quality were obtained from
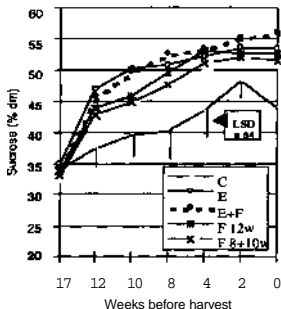
Fig. 4 *Expt. 3: Sucrose % cane dry matter for control, ethephon and E+F treatments and for mean of 37.5 and 56 g ai/ha Fusilade rates applied 12 weeks and mean of 8 and 10 weeks before harvest.*
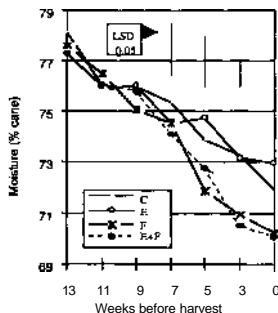
Fig. 5 *Expt. 4: Moisture content for control, ethephon, Fusilade and E+F treatments applied 9 weeks before harvest.*

high rates and a long interval between spraying and harvest. There was little difference between 37.5 and 56 g ai/ha rates of F. The response to the higher rate was quicker but it reduced cane yield sooner and increases in recoverable sugar yield were smaller. The optimum harvest time was 8 to 10 weeks for both rates. Although not statistically significant, improvements in yield of recoverable sugar confirmed results of sample data and were accompanied by increases in sucrose % dm, indicating true gains in sucrose accumulation.

Ethephon in the E+F treatment apparently reduced the adverse effect of F on cane yield. E+F was the most successful treatment producing

more recoverable sugar per hectare and extending the ripening effect beyond that of each chemical individually. These results also confirm those obtained previously with NCo376 and other varieties (unpublished data).
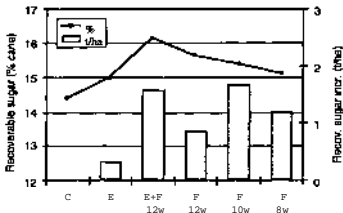
Fig. 6 *Expt. 3: Recoverable sugar % cane and yield increase for ethephon, Fusilade and E+F treatments for 8, 10 and 12 week times of application.*

Long term benefits of chemical ripening

Until results of experiments translate into economic benefits they are of only academic interest. There has been a dramatic improvement in sucrose % cane and sucrose yield per hectare in Swaziland since 1985 (Fig. 7), coinciding with the introduction of F and the E+F treatments. These industry trends indicate real and economically valuable benefits from chemical ripening, confirming results of mill-scale trials undertaken to confirm the findings of small-scale experiments (Rostron 1975; Rostron et al 1976). There has also been a gradual improvement in the purity of mixed juice and overall sucrose recovery in all Swaziland factories since 1985
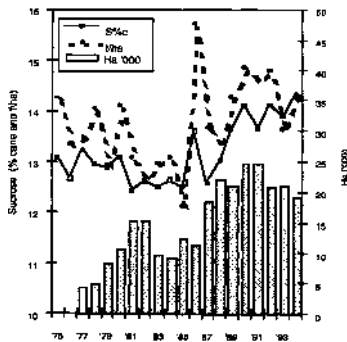
Fig. 7 *Hectares ripened, industry sucrose % cane and sucrose yield (t/ha) in Swaziland 1975-1994 (Source: Swaziland Sugar Industry Extension Services).*

Economic assessment of chemical ripening

Assuming an average increase in sucrose yield of 0.75 t/ha from either E, or F and 1 t/ha for E+F, as shown in trials and from commercial data, it is estimated that the net profit per hectare in 1994 was between E483 and E635 (Table 3). The impressive cost/benefit ratios of 1:6 to 1:15 are achieved within a 3-4 month period. It is difficult to convert profit per hectare to a value for the industry because the area sprayed with each treatment is not known. If an average cost of E100 ($A37.5) and a sucrose response of 0.8 t/ha is assumed for the industry then the net agricultural value is E9.7 mill ($A3.6 mill), based on an estimated 19,300 hectares ripened in 1994. The net return over the past ten years is estimated to be 9.9 mill per year ($A 3.7 mill) in 1994 values.

Table 3 *Actual ripening costs, estimated sucrose response and estimated profit per hectare in 1994 in Swaziland emalangeni (E)*

Treatment	Cost[a] (E/ha)	Sucrose response (t/ha)	'Net profit' (E/ha)	Cost/benefit[b] ratio
Ethephon	80	+0.75	483	1: 6.0
Fusilade Super	35	+0.75	528	1:15.1
Eth. + Fusil.	115	+1.00	635	1:5.5

[a] Including spraying & labour costs
[b] Sucrose price to the grower E750/t

Note : Exchange rate $A1 - E2.665

Comparison of mill runs of ripened and unripened sugarcane over periods of one week in Malawi (LR Pilot, personal communication) showed that ripening improved sucrose % cane, purity of the first expressed juice and boiling house recovery. Overall recovery increased by 0.9%. If a mill produces 150,000 t sucrose per year, receives 36% of the industry sucrose price and ripening improves overall recovery by 0.9% on two thirds of the crop, then based on the 1994 Swaziland sucrose price this would increase milling profit by E0.38 mill (SAO. 14 mill).

Chemical ripeners have been of tremendous benefit in Swaziland. They have increased the proportion of photosynthate stored as sucrose, so improving cane quality, sucrose yields and growing and milling profits. It should be possible to obtain similar results wherever immature sugarcane is harvested at the beginning and at the end of the milling season.

ACKNOWLEDGMENTS

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MANAGEMENT OF GREEN CANE HARVESTING IN HIGH YIELDING CROPS

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ABSTRACT

In Colombia, cane yields average more than 130 t cane/ha, with individual fields often yielding 200 t/ha. There is no specific season for maturing cane; and consequently the quantity of tops and dry leaves produced is high, yielding 50 to 100 t/ha. These quantities of cane trash, left in the field after green cane harvesting, reduce germination in wet periods, and create problems for traditional agricultural practices. The problems associated with harvesting cane and managing crop residues are currently overcome by pre- and post-harvest burning. However, all cane will have to be harvested green by the year 2005 due to environmental

Cenicana (The Colombian Sugarcane Research Centre) in conjunction with the sugar industry is developing an integrated production package for green cane harvesting. This package involves evaluation of erect self-trashing varieties, with less top, and high sugar content to improve cane harvestability. To overcome the problem of poor germination under wet conditions, trash residues will be finely chopped and the cane grown on ridges, so that the chopped residues fall into the inter row.

INTRODUCTION

Sugarcane is grown in a densely populated area of Colombia, and is harvested all year round. Almost all the cane is harvested by the sugar mills, even though slightly more than half the total area is managed by independent producers. Burning of cane was introduced by the mills in the 1970s to facilitate harvesting operations, and reduce extraneous matter arriving at the mills. Recently, the local population has pressured the sugar sector to reduce the practice of burning for environmental reasons. Consequently, Cenicana and the sugar industry are trying to develop new technology for green cane harvesting. The Colombian conditions of heavy soils, erratic rainfall patterns, year round harvesting and high cane production (130 t/ha with some fields 150-200 t/ha) require the development of technology specially adapted to the region. These heavy cane crops lead to two major obstacles to green cane harvesting: (i) lodged crops that are difficult to harvest mechanically and (ii) large quantities of residues in the field after harvest that complicate management of the ratoon crops and replanting.

HARVESTING

Currently, almost all cane is harvested by hand with average cutting rates for burnt cane of 6-8 t/man-day with burnt cane. Rates in green cane drop drastically to 2.5 t/man day when cane cutters are required to produce low trash levels in the cut cane. At this level of efficiency and with present labour pay rates it is not economically viable to harvest green cane manually. In order to harvest green cane the industry is moving towards mechanical harvesting.

In Colombia cane is harvested all year round with only minor fluctuations in estimated recoverable sugar (% cane), (Fig 1). Hence the industry maximizes the use of capital invested in the mills, however this results in harvesting both in the wet and dry seasons. Average rainfall patterns are presented in Fig. 1. In the wet season, milling rate is often reduced due to the difficult conditions. However, during wet periods the mills attempt to maintain sugar production by maintaining stocks of cut cane in the factory, and in the field, to ensure a steady supply for milling. As a result the period between burning (or cutting in the case of green cane) and milling frequently exceeds 72 hours.

The only commercial cane harvesters in Colombia that have up to now been successful are the single row chopper harvesters which work at a rate of c. 20-25 t/hr in the heavy cane crops that prevail. Although these machines will work under wet conditions the problems of in-field transport make it almost impossible to guarantee a continuous supply of cane to the mill without maintaining stocks in the mills. The possibility of using tracked in-field transport should be studied, however under extreme conditions this is unlikely to be effective as damage to ratoons is still likely to be severe. In the case of chopper harvesters the
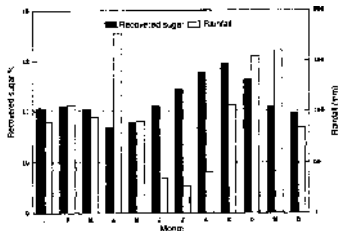
Fig. 1 *Monthly average of recovered sugar (%) during the year in 11 mills in Colombia and rainfall patterns in Cenicana.*

rate of deterioration of the chopped cane prevents stockpiling cut cane. Consequently the Colombian sugar industry has commissioned the construction of a prototype long billet harvester, which should enable the storage of cane in the mill during wet periods without excessive loss of sugar due to cane deterioration. The prototype harvester in initial trials has given indications that it may prove capable of commercial rates of 70-100 t/hr and its novel integrated cleaning system permits very low trash levels even in lodged cane.

The Colombian cane industry averages 12 t sugar/ha/yr, and vies with the Australian industry for the prize of being the most productive sugar producer per ha. The Australian industry achieves this level through high cane sugar contents, while the Colombian industry has moderate sugar contents and high crop yields. This results in the cost of cutting, loading and transporting cane per unit of sugar, being higher in Colombia than in Australia. Furthermore, the heavy cane crops often lodge, complicating mechanical harvesting and cleaning of cane. The move to green cane harvesting will aggravate this situation, by increasing the costs and difficulty of cleaning cane. The Colombian research program at Cenicana is developing high sugar content, erect, self-trashing varieties with less tops to obviate these problems. In preliminary commercial trials the variety CC 85-68 has a commercial sugar yield 0.5 percentage points higher than the traditional standards, slightly lower cane yields but similar production of sugar per ha. Commercial chopper harvesters, working in green cane, have achieved rates of close to 40 t/hr hour in this variety. However, some lodging in cane is almost bound to occur, and the topper mechanisms of commercial harvesters are ineffective under these conditions. Also, tops and cane cannot be effectively separated in the cleaning mechanisms. Consequently, it may

be necessary to search for varieties which produce less tops. Preliminary observations in field trials with promising varieties indicate that genetic variability exists for this character.

RESIDUES

In Colombia, green cane harvesting results in 50-100 t trash/ha left in the field. Trials in commercial fields indicate that ratoon crops germinating under dry conditions are not seriously affected by the trash blanket: however, under wet conditions germination and development are seriously retarded. When cane setts in pots were watered with the leachate obtained from fresh cane trash their germination was severely reduced (Table 1). These results indicate that the negative effects of the trash blanket under wet conditions are not only due to physical impediments to growth, but also to phytotoxic effects of the leachate from residues.

Table 1 *Germination (%) of cultivar MZC 74-275 in pots irrigated with tap water and leachate from trash residues.*

Treatment	Number of days after planting				
	10	15	20	30	45
Tap water	13	33	53ab	77a	87a
Leachate from dry residues	10	40	67a	83a	83a
Leachate from fresh residues	10	20	23b	23b	23b

Numbers followed by different letters in the same column are significantly different (P=0.05).

In field trials where the young cane is ridged (200-250 mm high ridges) and the residues are chopped, with a forage harvester immediately after harvest, the chopped residues fall into the inter-row or after rains wash into the inter-row. In this manner the stools germinate well even under very wet conditions and cane and sugar yields are similar to those with ratoons from burnt cane (Table 2). Furthermore, under the hot humid conditions the chopped residues dehydrate and decompose rapidly and all traditional cultural practices can be performed satisfactorily.

The current commercially available forage harvesters do not effectively handle the heavy residues load, their work rate is less than 0.3 ha/hr,

Table 2 *Cane and estimated recoverable sugar yields of a ratoon crop with wet and dry conditions in the early growth phase. Soil type: Inceptisol; variety MZC 74-275.*

	Dry conditions, first ratoon			Wet conditions, second ratoon		
	Cane yield (t/ha)	Sugar % cane	Sugar yield (t/ha)	Cane yield (t/ha)	Sugar % cane	Sugar yield (t/ha)
Burnt cane	168	11.6	19.5	104	14.7	15.3
Residue blanket	152	11.9	18.1	95	14.5	13.8
Chopped residues	171	11.9	20.4	121	14.2	17.2

and costs are excessively high. At present the Colombian sugar industry is developing a cane residue chopper/harvester specifically designed to handle large quantities of residues. The successful development of this residue chopper/harvester would open the possibility of collecting part of the residues and using them as an energy source either for combustion in boilers or for gasification to cogenerate electricity using steam or gas turbines. For each tonne of dry residue consumed it is estimated that 1 MWh of electricity could be produced. With a production of 20 t dry residue/ha and 180,000 ha of sugar cane the potential is 400 MW continuous supply of electricity.

CONCLUSIONS

In order to move towards green cane harvesting under heavy residue conditions, the Colombian sugar sector is developing erect, high sucrose, self stripping varieties with low top weight suitable for mechanical harvesting. In order to maintain supply of cane to the mills under wet conditions a long billet cane harvester is being developed to permit storage of cut cane for short periods when field operations are not possible because of high rainfall. Residues after harvesting are a major obstacle to implementing green cane harvesting systems. The industry is developing technology based on ridging and chopping of residues that permits standard field operations with no adverse effects on cane or sugar yields. In the future this technology may permit collection of residues for the cogeneration of electricity.

LONG TERM EFFECTS OF GREEN CANE TRASH RETENTION ON HERBERT RIVER SOILS

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ABSTRACT

Long term trials established in the Herbert Valley near Ingham, Queensland in the early 1980s demonstrated consistently higher cane yields where trash from green cane harvest was retained as a surface blanket (TB) compared with raked and burnt trash treatments (RB). Microplot experiments set up within these trials in 1992 to compare seasonal and depth variation in the soil microbial biomass showed levels in TB were significantly and consistently higher than those in RB. Soil N-mineralisation, total-N and C and soil temperature were compared between treatments and related to microbial biomass levels. The results confirm a positive influence on the soil micro-biota due to the retention of cane trash as a blanket and suggest an association between the higher productivity of this system and soil organic matter turnover and the storage and release of nutrients.

INTRODUCTION

In response to increasing farm production costs and difficulties associated with harvesting of cane in wet weather, farmers in tropical north Queensland experimented with harvesting the crop "green" and the retention of the substantial crop residues as a surface mulch or "trash blanket". A period of depressed world sugar prices from 1983-1985 provided a catalyst for the rapid adoption of this system in the far north of the State. Advantages commonly cited include reduced cultivation time and cost due to weed suppression; reduced soil erosion; increased moisture conservation, soil organic matter, soil fertility and structure; greater yields; longer ratoons; less fertiliser requirement; more flexibility in harvesting and a more continuous and fresher supply of cane to the mill.

Long-term trials have demonstrated consistently higher cane yields where trash from green cane harvest has been retained as a surface blanket when compared with burnt trash treatments (Wood 1991). The trash blanket returned on average about 16.8 t/ha of dry cane residues on to the soil surface (Wood 1990).

Any management practice that increases total-C accumulation should also increase the size of the soil microbial biomass (Cochran et al 1994). The soil microbial biomass has been suggested as a sensitive indicator of early changes in soil organic matter turnover (Saffigna et al 1989). Microbial biomass constitutes the active fraction of soil organic matter whose fast turnover makes it important as a potential source of nutrients, especially nitrogen. It is responsible for both the decomposition and accumulation of organic matter as well as nutrient and mineral transformations in soil (Pankhurst & Lynch 1994). Soil microbial biomass has been the subject of intensive research in relation to a variety of cropping systems, but the literature shows no reference to microbial biomass in a sugarcane environment.

This paper therefore aims to : 1) compare seasonal variation in levels of soil microbial biomass of a long-term field comparison of green and burnt trash management systems, and 2) compare various chemical and physical parameters of soils associated with these two trash management systems.

MATERIALS AND METHODS

Site and field treatments

The Abergowrie experimental site used is in the Herbert Valley 30km NW of Ingham, Queensland. The area has a humid tropical climate with an annual rainfall of 2100mm, 80% of which falls between December and April. Mean monthly maximum and minimum temperatures in Ingham are 32° and 23° C, respectively, in January and 24° and 14° C in July. The soil is an alluvial silty loam (moderately well drained Dystropept).

Trials were initiated after harvest of a fourth ratoon crop in November 1992 within a field with burnt trash (RB) and green trash (TB) retention treatments, which had been in place for 10 years. In both treatments 32 microplots were established comprising 100mm diameter PVC tubes inserted 200mm into the ground with a 50mm section above-ground. Half of the tubes had lime applied at the start of the trial at 2t/ha.The equivalent of 15t/ha of chopped, dry cane trash was inserted in the above-ground portion of each microplot. Both trash treatments had the benefit of a trash cover in each microplot for the duration of the experiment. This was necessary for a concurrent experiment dealing with the relative trash decomposition rates between the trash treatments. This design ensured results were, as much as possible, due to long-term treatment effects on the soil rather than more obvious short-term environmental effects associated with a comparison of mulched and unmulched soils.Soil temperature was measured at a single point adjacent to the microplots within each trash treatment at 50mm depth. In the case of the RB treatment the temperatures thus measured give an indication of the soil environment immediately surrounding the PVC microplots. Effects on soil temperature within the microplots due to the covering of chopped cane trash were not measured.

Microplots were retrieved at four sampling times at approximately 90 day intervals. Soil was sampled from four depths within each microplot viz. 0-25,25-50, 50-100, and 100-200mm for analysis. The following properties were assessed : i) microbial biomass by Ninhydrin-N reaction (Amato & Ladd 1988); ii) N-mineralisation (Waring & Bremner 1964); iii) total N & C (Dumas Combustion); iv) soil temperature (50mm depth).

Treatment means were compared by completely randomised, fixed effect, two factor ANOVA and LSD. No significant differences between means for the limed/no-lime treatments were found, hence replicates were bulked to give 8 reps/treatment for the trash management factor.

RESULTS

Soil microbial biomass

Soil microbial biomass in TB was as significantly higher than that in the RB treatment in all but three depth comparisons (Fig. 1). The greatest absolute and relative differences occurred in the surface (0-25mm) horizon, with both generally decreasing with depth.

Soil anaerobic N-mineralisation and total N

Nitrogen mineralisation in the surface horizons was consistently and significantly greater (50%) in TB than RB (Table 1). Differences in the lower 50-100mm horizon were in the same direction but were much smaller and not significant.Total N was generally higher under TB with the difference achieving significance in the upper soil layers at the last two samplings (Table 2).

Soil total carbon

Soil total C followed a similar trend to total N in the upper 25mm though at greater depths this was reversed with most comparisons revealing RB>TB (Table 2).

Soil temperature

In the period up to canopy closure in March/April, TB reduced maximum temperatures by 10-20°C compared to RB (Fig.2). This trend continued
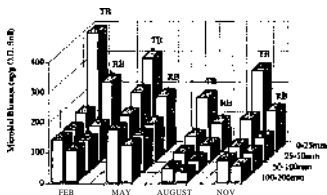
Fig. 1 *Comparison of the effect of green trash blanketing (TB) and raked and burnt (RB) management systems on soil microbial biomass C (mg/g O.D> Soil) at Abergowrie measured in 1993 at different soil depths and times*

[Paired treatment means at the same depth with the same lettering are not significantly different at P=0.05 (LSD)]

Table 1 *Comparison of soil anaerobic N-mineralisation (mg NH₄-N/g dry wt. soil) under green trash blanketing (TB) and raked and burnt (RB) cane management systems at Abergowrie in different seasons and at two soil depths.*

[Paired treatment means at each sampling time/depth with the same lettering are not significantly different at P=0.05 (LSD)]

Soil depth (mm)	February TB	RB	May TB	RB	August TB	RB	November TB	RB
0-25	24.1a	15.0b	24.8a	15.6b	22.3a	12.9b	20.6a	14.3b
50-100	12.1a	11.6a	16.4a	14.4a	9.8a	9.0a	11.9a	10.9a

Table 2 *Comparison of (a) soil total N (%) and (b) soil total C (%) under green trash blanketing (TB) and raked and burnt (RB) cane management systems at Abergowrie in different seasons and at four soil depths*

[Paired treatement means at each sampling time/depth with the same lettering are not significantly different at P=0.05 (LSD)]

Soil depth (mm)	February TB	RB	May TB	RB	August TB	RB	November TB	RB
(a) Soil total N (%)								
0-25	0.012a	0.077a	0.091a	0.080a	0.091a	0.077b	0.100a	0.077b
25-50	0.082a	0.071b	0.076a	0.074a	0.073a	0.069b	0.074a	0.073a
50-100	0.074a	0.069a	0.067a	0.066a	0.066a	0.070a	0.066a	0.066a
100-200	0.071a	0.066a	0.067a	0.066a	0.067a	0.066a	0.065a	0.063a
(b) Soil total C (%)								
0-25	1.88a	1.51a	1.55a	1.63a	1.51a	1.45a	1.72a	1.43a
25-50	1.39a	1.35a	1.22a	1.45a	1.14a	1.28b	1.22a	1.35b
50-100	1.24a	1.28a	1.11a	1.35b	1.05a	1.23b	1.10a	1.28b
100-200	1.18a	1.22a	1.05a	1.21b	1.00a	1.11b	1.04a	1.13b

to a much lesser degree during the rest of the growing season. Temperature minimums during the cooler months (May-August) in TB were generally higher by 1-3°C than in RB. These findings support the results of Wood (1991).

DISCUSSION

The TB treatment had greater absolute and relative levels of microbial biomass than RB at all sampling times in the upper 0-25mm stratum. In this stratum, the soil total N and C, and N-mineralisation were also generally higher in TB than RB. Because of their insulating properties, surface mulches reduce temperature
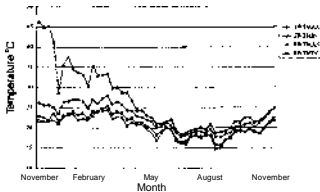
Fig. 2 Comparison of the effect of green trash blanketing (TB) and raked and burnt (RB) cane management systems on soil temperature (°C) at Abergowrie measured at 50mm depth for J year

fluctuations so that daily extremes are not as pronounced in mulch-covered soils as they are in bare soils (Horton et al 1994). Soil temperature differences of 10-20°C were measured here at 50mm depth, and it is expected that the difference between TB and RB at the soil surface would be greater. Absolute microbial biomass levels in both trash treatments follow a similar pattern to soil temperature ie. levels are greatest in summer months and least in the cooler, winter months.

With increasing soil depth, absolute biomass levels decreased as is usually found, and the relative treatment advantage due to TB also decreased. A corresponding absolute decrease in soil total C occured at depth, though the trend was for greater levels in RB than TB. The latter suggests a slower turnover rate of soil organic matter due to a decrease in microbial population/activity in RB. It also suggests that the soil total C in terms of substrate availability for microbial growth is not a limiting factor. Similarly, temperature fluctuations at depth do not explain the relative differences in microbial biomass between the two treatments. If these differences in microbial biomass are to be ascribed to some effect of the trash blanket then it appears that either an interaction of those parameters measured is responsible or that another unmeasured parameter is active at these depths. The possibility of an effect of a soluble C fraction on the microbial biomass below the surface stratum has been considered (Nelson et al 1991; M.Amato, *pers. comm.*), though not measured in this experiment.

CONCLUSIONS

The experiment demonstrated higher soil microbial population and activity under green trash blanketing than under burnt cane in tropical Queensland, especially in the surface 0-25mm horizon. Higher microbial biomass in the surface stratum was associated with increased N and N-mineralisation under the trash blanket without significantly higher soil C content. Absolute biomass levels in both treatments were higher in the hotter months with TB relatively higher at the surface under lower maximum temperatures. Interpretation of responses lower in the profile is difficult due to either an interactive effect among the measured parameters or perhaps that of an unmeasured term such as soluble C. Direct contribution of higher microbial biomass to the sugarcane crop has not been assessed though grounds for a positive effect is supported in the literature. Tangible benefits of an increased microbial biomass population / activity are evidenced by the greater N-mineralisation capacity of the TB soils. Implicit in this finding is the suggestion of savings in N-fertiliser on soils with a history of trash blanketing.

ACKNOWLEDGEMENTS

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PRELIMINARY INVESTIGATION OF THE EFFECTS OF FOLIAR APPLICATION OF GLYCINE BETAINE ON THE SUCROSE CONTENT OF SUGARCANE

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ABSTRACT

Many plant species accumulate a range of osmoprotectant substances when subject to water, temperature or salt stress. Glycine betaine is one such naturally-occurring osmoprotectant which, when sprayed on foliage, has been found to increase yields of various crops growing under various conditions. Whilst not the major osmoprotectant of sugarcane, we tested 6 varieties of field-growing sugarcane under conditions of mild cold-stress in northern NSW for response to glycine betaine. The preliminary trial found some positive and negative effects on stalk dry matter content and stalk sucrose content for some varieties. In calculated sucrose yield some varieties showed increases of up to 40%. We believe that these results are sufficiently encouraging to warrant further trials, particularly in relation to potential benefits in areas suffering from cold, water and sodic/saline stresses.

INTRODUCTION

One of the factors limiting yield and expansion of sugarcane (*Saccharum* L. spp.) cropping land in Australia is the stress tolerance of the crop. Water and/or sodic/saline stresses can limit yield in many of the cane growing regions, and in southern Queensland and New South Wales an additional yield-limiting factor is cold stress (Weaich et al 1993). Strategies which help overcome problems of stress are potentially attractive to the Australian sugarcane industry.

Recent experiments demonstrate that plants accumulate a variety of osmoprotectant compounds (Stewart 1995). Betaines are a group of such naturally-occurring osmoprotectants which enhance plants' ability to tolerate water and saline stresses (Wyn Jones & Storey 1981). Various C$_4$ tropical grasses accumulate glycine betaine under water (Ford & Wilson 1981) and salt (Marcum & Murdoch 1994) stress. Sugarcane, which is also a C$_4$ tropical grass, has a relatively low capacity to accumulate glycine betaine, but instead accumulates 3-dimethylsulfoniopropionate (DMSP) when stressed (Paquet et al 1994). DMSP, a sulfur analogue of glycine betaine, has been shown to be as effective as glycine betaine as a protein/membrane protecting compatible solute (Mason & Blunden 1989; Grone & Kirst 1991). Unlike glycine betaine which is stable once formed, DMSP is short-lived *in vivo*, being catabolised to the so-called greenhouse gas dimethylsulfide (Paquet et al 1994). The mechanism by which glycine betaine (and presumably DMSP) protects plant cells from damage under water, salt or temperature stress has been linked to protection of membrane integrity (Zhao et al 1992) and hence enzyme activity (Paleg et al 1985). Recent improvements in the industrial preparation of glycine betaine have lowered its price, and made possible the use of this substance as an osmoprotectant in agriculture and horticulture. It is anticipated that exogenously applied betaines might enhance plant ability to withstand cold, water and sodic/saline stresses. Recent work suggests that foliar application of glycine betaine at doses of only a few kg per hectare increases the yield of a variety of crops species growing under conditions of cold stress (by 10 to 51%) (BP Naidu, unpublished data), and helps raise crop yield towards its 'biological potential' (Naidu et al 1996).

The aim of this preliminary study was to determine whether foliar application of glycine betaine enhanced the sucrose yield of a plant crop of six varieties of field-grown sugarcane in northern NSW, near the southern limit of the Australian sugarcane industry.

MATERIALS AND METHODS

The field site was near Broadwater, NSW (153° 25' East, 29° 2' South). The non-irrigated trial was an incomplete randomised block design, consisting of 72 field blocks. Each 20 m long block consisted of 5 rows of plant-cropped varieties BN78-8301, CP44-101, CP57-526, CP63-588, Q68 and TS65-28. Plant material was provided by the New South Wales Sugar Milling Co-Operative. The inter-row distance was 1.5 m. Urea was applied during planting at 240 kg N/ha in September 1993. Glycine betaine was applied in March 1994 when mean plant height (defined as the distance from the ground to the base of the last fully expanded leaf) was 1.5 m. Glycine betaine (Finn Sugar Bioproducts, Helsinki, Finland) was dissolved in water with 0.2% (v/v) detergent (as non-ionic wetter), and sprayed onto the plant canopy at effective application doses of 0, 2, 4 and 8 kg/ha. The control dose of 0 kg/ha glycine betaine was applied as tap water with 0.2% (v/v) detergent. Absolute control plots which received no treatment were also included. There was no significant rainfall (> 3 mm) within 5 days of the foliar application. There were three randomised replicate blocks for each of the 24 (6 varieties x 4 doses) treatments investigated.

Plants were harvested by hand in October 1994. From the inner three rows of each replicate block five stalks were chosen at random and cut at ground level. Leaf, leaf sheath, trash and cabbage were removed and the total mass of the five millable stalks was recorded. Stalks were then fibrated in a Jeffco cutter/grinder. Sub-samples of the fibrated stalk matter were weighed, dehydrated (70° C for 5 days) and re-weighed to determine dry matter content. Other sub-samples were pressed to extract stalk juice as described by Muchow et al (1993). Juice was analysed for sucrose, glucose and fructose by enzymic assay. Sucrose was quantified according to the technique of Hansen et al (1996), whilst reducing sugars were determined by a microplate adaption of manufacturers' instructions for the Boehringer Mannheim kit 139 106 (enzymatic determination of D-glucose and D-fructose).

Data were analysed by two tailed Student's t tests, based on the incomplete randomised block design of the trial. Experimental analysis compared mean values of the three randomised blocks for each variety x dose against a 0 kg/ha glycine betaine control.

RESULTS AND DISCUSSION

Application of 0.2% detergent in water, when compared to the absolute control plots, elicited no significant differences for dry matter content or stalk concentrations of sucrose, glucose or fructose. All statistical analysis of effects elicited by glycine betaine application were consequently based on comparison with the 0.2% detergent in water control treatment.

Table 1 shows that glycine betaine had no significant effect on the dry matter content of CP57-526, Q68 and TS65-28. At 2 kg/ha glycine betaine, dry matter was significantly decreased by approximately 5% in CP44-101. Varieties BN78-8031 and CP63-588 both showed significant increases in dry matter content with glycine betaine applied at 4 kg/ha, also at 8 kg/ha for BN78-8031. Increased stalk dry matter content should equate to increased relative sucrose yield, making these observations of some significance to the industry. Clearly though, varietal differences in response were variable and need to be further elucidated.

Table 1 *Effect of foliar application of glycine betaine at different rates on stalk dry matter content relative to the detergent in water control (%) of 6 varieties of sugarcane.*

Mean control dry matter contents were: BN78-8031, 29.3%; CP44-101, 30.3%; CP57-526, 31.8%; CP63-588, 30.1%; Q68, 29.4% and TS65-28 30.6%. Significant differences from control values (P < 0.05) are indicated *.

Cultivar		Glycine betaine (kg/ha)		
BN78-8031	100	100.8	104.1*	105.9*
CP44-101	100	94.4*	99.5	98.8
CP57-526	100	98.0	98.1	99.7
CP63-588	100	103.2	107.3*	104.4
Q68	100	106.2	107.3	106.1
TS65-28	100	100.9	102.6	100.2

The effect of glycine betaine application on stalk sucrose concentration (g sucrose/ kg fresh weight of stalk) showed varietal specificity (Table 2). None of the varieties CP57-526, CP63-588, Q68 and TS65-28 showed a significant response to any of the doses applied. There was an apparent (but not statistically significant) dose-dependent increase in stalk sucrose content in BN78-8031, and a similar, significant response in sucrose content of CP44-101.

Table 2 *Effect of foliar application of glycine betaine at different rates on stalk sucrose content (g sucrose per kg FW) relative to the detergent in water control (%) of 6 varieties of sugarcane.*

Mean control sucrose contents as g/kg FW were: BN78-8031, 132.1; CP44-101, 138.3; CP57-526, 130.2; CP63-588, 159.7; Q68, 147.6 and TS65-28 163.0. Significant differences from control values (P < 0.05) are indicated *.

Cultivar	Glycine betaine (kg/ha)		
BN78-8031	103.6	105.3	107.9
CP44-101	104.9*	92.7	108.2*
CP57-526	98.9	92.0	99.1
CP63-588	100.6	101.7	103.3
Q68	98.6	97.5	91.9
TS65-28	100.2	102.5	103.1

Glycine betaine application increased mean stalk mass by up to 35% in some treatments, although the differences were not statistically significant (data not shown). Based on these stalk mass data and the sucrose concentrations given in Table 2, calculated sucrose yield increased by 41% for CP44-101 and 20% for Q68 as a result of glycine betaine application, although again these responses were not statistically significant with a coefficient of variation of 20.6%. Application of glycine betaine did not significantly affect the stalk reducing sugar content (as g/kg FW) nor did glycine betaine affect the ratio of sucrose to reducing sugars (data not shown).

CONCLUSION

This preliminary investigation indicated possible benefit to sucrose yield arising from foliar application of glycine betaine to sugarcane. The results at this stage must be regarded with caution because of variability in response between varieties and doses. However, in view of more consistent positive responses on sugarcane growth obtained in four other growth trials (JA Campbell, unpublished data), and on growth of buckwheat in Tasmania (Naidu et al 1992) and cotton in Queensland (BP Naidu, unpublished data), further experimentation is warranted and will be undertaken, particularly in areas of water and sodic/saline stresses. Future research will attempt to optimise the dose rate and time of application for glycine betaine, and to clarify varietal response. Foliar application of glycine betaine could offer new opportunities for yield improvement in the Australian sugarcane industry.

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REAPING THE BENEFITS OF NEAR INFRA-RED SPECTROSCOPY IN THE SOUTH AFRICAN SUGAR INDUSTRY

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ABSTRACT

Sugar industries world wide are showing increasing interest in the potential applications of near infra-red (NIR) analysis as research and management tools in the fields of soil fertility, cane nutrition, cane quality testing, and in the possibility of screening for resistance to certain pests and diseases. During the past decade both filter and scanning NIR reflectance spectrophotometers have been used to improve nitrogen use efficiency of sugarcane by matching the crop's N requirement to soil N mineralising potential and plant N status, both properties determined by NIR. Calibrations were developed and validated for N in leaf as well as total N, organic matter, N mineralization potential, and texture of soil samples. Well over 70000 leaf and 140000 soil samples submitted by cane growers have been routinely tested by our Fertilizer Advisory Service using NIR in conjunction with other instrumental techniques. Recent developments have centred on comparing the suitability of both filter and scanning instruments for the rapid determination of various constituents in cane juice, shredded cane, baggase, raw sugar, and molasses. Possible new applications of NIR that are discussed in the paper include partitioning the N pool in the cane plant, estimating photosynthesis, predicting yield potential and screening for pest and disease resistance.

During the past two decades near infra-red (NIR) spectroscopy has gained wide acceptance in the food sciences, chemistry and chemical engineering, biochemical, environmental, pharmaceutical and medical fields. The success of NIR may largely be attributed to the ability to conduct rapid quantitative and qualitative analysis of multicomponents in single samples using minimal sample preparation. Despite advances in applying NIR at the research and process levels for cereals, oilseeds and forage assessment, relatively little progress has been made in adopting this exciting technology for routine use in the cane industry. Research has mainly been confined to the USA, Australia and South Africa, and includes using both filter and scanning instruments for foliar diagnosis (Meyer 1983), N fertilizer management (Meyer et al 1986), cane juice analysis (Meyer & Wood 1988; Edye & Clarke 1993), shredded cane analysis (Sverzut et al 1987 ; Berding et al. 1989; Brotherton & Berding 1995; Clarke et al 1995; Schaffler & Meyer 1996), assessing soil properties (Meyer 1989), analysis of sugar related products (Schaffler et al 1993), and predicting resistance to the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Rutherford et al 1993). This paper summarises some past and current research in South Africa as well as indicating potential new applications. A comparison of NIR spectra for a range of products tested is shown in Fig. 1, while Table 1 summarises the constituents for which successful calibrations have been developed.

Table 1 *Summary of working calibrations developed for a range of constituents in various products.*

Cane leaf	Soil	Cane juice	Shredded cane	Raw sugar/ molasses
N, P, S, Si	Total N	Pol	Moist.	Pol
Photosynthesis	Org.C	Brix	Pol	Brix
Yield	Min. N	Sucrose	Brix	Sucrose
Eldana rating	Clay	Glucose	Fibre	Fructose
Mosaic rating	Silt	Fructose	Tannin	Glucose
	Sand	Alcohol	Lignin	Ash
	CEC	Total N	Waxes	Starch
				Invert

FOLIAR DIAGNOSIS

Determination of the N requirement of sugarcane is an important activity undertaken by SASEX. In 1983, a Technicon 300 Bran filter instrument was first calibrated and validated for leaf N analysis (Meyer 1983). Fifty leaf samples with N content ranging from 0.80-3.0% were used to
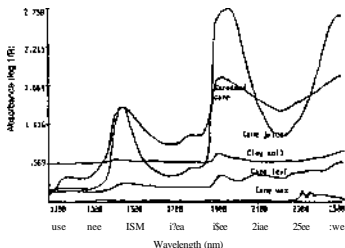
Fig. 1 *Comparison of NIR spectra for various materials.*

calibrate the instrument. A further 125 samples analysed by standard Kjeldahl steam distillation were used to validate the N calibration (see Table 2). The accuracy and precision of the NIR method was further evaluated by repeatedly analysing 13 reference samples over a period of five days. The small difference between the mean results (0.07%), and the small variation in the N values obtained (CV range 0.8-4.5%), suggested that the NIR method was sufficiently reliable. In practise, a level of accuracy of +/- 0.1% and reproducibility of below 5%, is considered to be an acceptable level in assessing the N status of sugar cane from leaf analysis. The method also was about ten times faster man the Kjeldahl procedure.

Some of the more important applications of NIR in leaf analysis concerns its use in controlling whole crop cycle fertilizer recommendations and in nutrient survey programs. Since 1983, >65000 leaf samples have been analysed for N content by NIR. The data set is updated regularly and used to determine comparative changes in nutrient availability in the sugar industry (Meyer et al 1989).

N USE EFFICIENCY STUDIES

NIR has provided a rapid means of detecting, through leaf analysis, the relative efficacy of timing, placement and the use of different N carriers in various trials (Meyer & Wood 1994). Recently leaf NIR analyses have proved invaluable in assessing N requirement of different cane varieties. For many years, fertilizer recommendations in the South African sugar industry have been based on die variety NCo376. Analyses of the standard third leaf, covering thousands of samples from variety trials, have shown significant varietal differences in N composition. Current trials indicate that the optimum N requirement of important

Table 2 *Examples of calibrations developed for selected constituents.*

Product/constituent	Range	Wavelength (nm)	Calibration n	R	SEC	Validation n	R	SEP
Cane Leaf			98			125		
N	0.8-3.0	2186,1240		0.98	0.11		0.96	0.15
Ash	1.0-9.0	1900,1860,1968	61	0.97	0.15	94	0.93	0.23
Silicon	0.5-4.0	1900,1682,2448	27	0.89	0.05	27	0.77	0.07
Photosynthesis	15-40mmol/m²/s	2100,2139,2190	42	0.90	0.84	30	0.65	2.90
Cane yield	85-210 t cane/ha	2384,2238,2448	51	0.91	13.00	191	0.86	14.00
Eldana	20-220	2332,1754,2320	51	0.75	17.00	191	0.69	18.00
Soil			200			74		
Nmin. pot.	1-4	2236,2230		0.86	0.30		0.83	16.00
Total N	0.03-0.60	2050, 1870		0.90	0.01		0.84	19.00
Organic C	0.3-7.0	2050,1744		0.92	0.46		0.83	19.00
Clay	5-75	1956, 1920		0.94	3.80		0.81	15.00
Cane Juice			90			35		
Pol	0.7-13.0	2274		0.96	0.25		0.91	3.20
Brix	1.7-14.0	1366,2160		0.98	0.15		0.92	3.20
Sucrose	0.7-13.0	2322		0.94	0.29		0.90	3.40
Glucose	0.15-0.80	2342		0.65	0.04		nd	
Fructose	0.15-0.80	2292		0.68	0.05		nd	
Shredded cane			26			136		
Brix	6-21	1434,2082		0.94	0.24		0.85	3.80
Pol	5-19	1198,2282		0.93	0.42		0.88	3.90
Dry matter	16-38	2224,1838		0.88	0.86		0.82	4.70
Fibre	7-14	1376		0.85	0.41		0.80	6.00
Cane stalk			32					
Wax	Qualitative	1940,1194,2072		0.65	2.10	Still to be		
Bud scale flavonoids	Qualitative	2180,1734,1680		0.75	1.70	determined		

n = number of samples, R = regression correlation coefficient,
[SEC = standard error of calibration, SER = standard error of prediction, nd = not determined]

varieties such as N12 and N14 can differ from NCo376 by up to 75 kg/ha. Limited field evidence also suggests that a less N-use efficient variety such as N12 may also respond more effectively to split N applications compared with an N-use efficient variety such as NCo376. Threshold levels for interpreting leaf analyses may require adjustment for less N-use efficient varieties.

Quantifying the distribution of plant N pools in sugarcane is another new research area for NIR. Pools include nitrate-N, amino-N. soluble-protein (mainly Rubisco) and structural forms of protein. Total N is often used to assess N sufficiency but it includes stored N in the form of Rubisco which is not very mobile. Possibly, as in other crops, nitrate may be a more sensitive indicator of N sufficiency than total nitrogen. Knowledge of the transformation of proteins in the cane plant from structural to soluble proteins could assist in predicting the effects of N on cane quality as well as explaining the tolerance of plants to certain pests and diseases. Proline is protein that has been linked to moisture stress (Rutherford 1989) and as such could be a useful diagnostic indicator when interpreting foliar analyses. High proline indicates the crop was stressed at sampling and that caution is needed when interpreting leaf analysis.

SOIL NITROGEN MINERALISATION POTENTIAL

Attention was given in 1986 to assessing the merits of NIR for soil testing. Previous work had shown that the N requirement of sugarcane could be estimated more reliably from soil properties such as N mineralisation potential, texture, colour and organic matter. For advisory purposes a system was developed for placing soils into low, moderate, high and very high mineralizing categories (Meyer et al. 1986). Two hundred air-dried ground soil samples (0.25 mm sieve), of known organic matter, total N, clay and mineralizing potential were used to calibrate a Technicon InfraAlyzer 450 instrument. Comparative statistical information obtained for these different constituents (Table 2) suggests that most of these constituents could be satisfactorily

estimated by NIR. The reliability decreased in the order: clay, organic matter, total nitrogen and N mineralization rating (Meyer 1989). Coded soil samples from 21 N trials showed that predicted N mineralization ratings were correct in 17 of the trials.

SUGAR PRODUCTS

Analysis of pol and brix in sugarcane is an important analytical service rendered by laboratories in the sugar industry. The standard procedure based on filtration and clarification of expressed cane juice is tedious and labour intensive. In 1987, the suitability of NIR for rapidly estimating cane juice quality components was assessed (Meyer & Wood 1988). Mixed cane juice samples of known pol, Brix, sucrose, fructose and glucose content were used to calibrate a Technicon InfraAlyzer 450. Regression analyses indicated that Brix, pol and sucrose values by NIR were closely correlated with those obtained by the standard conventional methods of analysis.

In 1992, an NIRSystems 6500 spectrometer was used to study analytes in bagasse, shredded cane, direct analysis of cane (DAC) extracts, mixed juice, molasses and raw sugar (Schaffler et al 1993). Analytes included pol, Brix, dry solids, moisture, sucrose, glucose, fructose, invert, ethanol, colour, ash and starch. NIR produced a surprisingly good estimate for many of the analytes tested (Table 2). More recently an intensive collaborative investigation between the Sugar Milling Research Institute and SASEX, used >500 shredded cane samples to calibrate and validate a NIRSystem 5000 spectrophotometer for pol, Brix and moisture readings (Schaffler & Meyer 1996). Calibration R values based on partial least squares regression analysis were better than 0.95 for Brix, pol and dry matter, but lower for fibre (0.89). The NIR predictions for pol, Brix and dry matter were very acceptable with R values of 0.88-0.94, and standard errors of performance from 0.24-0.42%. It was concluded that the technique was sufficiently reliable for rapid analysis of sugarcane in plant breeding and agronomy variety trials. NIR is currently under evaluation as an alternative to DAC for rapidly assessing cane quality in growers' cane consignments in the millyard.

YIELD POTENTIAL PREDICTIONS

NIR has the potential to detect key constituents such as starches, sugar, cellulose, lignin, proteins, water, amides and also certain constituents linked with S, Mg, Ca and K. As part of a project to evaluate the merit of NIR scanning of cane leaves for assessing crop performance, 400 top visible dewlap leaf samples collected from 20 regional variety trials, scattered throughout the cane regions, were used to determine possible relationships between yield parameters, leaf composition and NIR reflectance measurements. Results from irrigated trials at Pongola showed that NIR absorption spectra of leaf samples from 4-5 month old cane, in the 2238-2500 nm range, were positively correlated with cane yield (R=0.91) and negatively correlated with pol% (R=0.82) at 12 months. Accuracy of the calibration equations was tested on five independent data sets, and although the correlation coefficients for validation were lower (0.60-0.86), the results were sufficiently promising to continue this field of study.

Of additional interest is that much of the variation in pol could be accounted for by the N and ash content of stalk and leaf. Previous studies have also shown that high N levels in the leaf could be associated with reduced sucrose values (Wood 1979; Gascho et al 1986) and high ash levels in cane juice were negatively correlated with sucrose content (Mullins & Roach 1985). NIR research in Australia indicated that the ratio of total N to non-structural carbohydrate in whole shoots of wheat may be a key indicator of yield potential (Blakeney et al 1995). It is possible that this ratio in irrigated young cane also may have a role as a diagnostic indicator of crop potential.

ESTIMATING PHOTOSYNTHETIC RATES

Measuring photosynthesis in the field is time consuming, weather dependent and requires considerable skill. Photosynthetic rates were measured on 70 leaf samples from a variety trial using a portable infrared gas analyser (Inman-Bamber 1995). The samples were then scanned by NIR, both in the fresh and dried state, in the 1100-2400 nm region. Step-wise regression analysis showed that photosynthetic rate and internal CO_2 determined concentrations were highly correlated with NIR absorption values (R >0.95). The wavelengths that were selected for the calibration equation (2594 nm, 2100 nm and 2190 nm) were consistent with the third overtone stretching vibrations of C-O, O-H and C-H bonds associated with carbohydrate compounds as well as second overtone N-H bending modes found in proteins. Various investigators have also demonstrated positive correlations between leaf photosynthetic rate, chlorophyll and soluble protein content (Dornhoff & Shibles 1976; Hesketh et al 1981). Recently, leaf photosynthesis in soya beans was positively correlated with leaf greenness, as non-destructively measured by a hand held portable chlorophyll meter (SPAD-502)(Ma et al 1995).

Examination of the NIR data showed that 30% of the variation in photosynthetic rate could be accounted for by variation in leaf N. Photosynthetic response to increasing light intensity is strongly dependent on leaf N (Ludlow et al 1991; Allison & Haslam 1993). Inherent differences in yield potential between varieties in many crops may be due to differences in N use, which in turn determine radiation use efficiency (Muchow et al 1994). Surprisingly, the other element that accounted for a significant variation (37%) in photosynthesis was leaf Si content. It has been shown, under normal light, that silica deposited in silica cells and stomatal guard cells could serve as 'windows' allowing more light to pass through the epidermal to the photosynthetic mesophyll tissue (Lau et al 1978), thus enabling higher rates of photosynthesis and more tillers per plant. This could partly account for the significant relationship that was obtained between cane yield and leaf ash content in the Pongola data set (R=0.68), as silica comprises about 70% of the ash in sugarcane.

PREDICTING HOST PLANT RESISTANCE TO PEST AND DISEASES

There is the exciting prospect that NIR may prove suitable for screening breeding and wild germplasm for resistance to pests and diseases. NIR was recently evaluated for predicting flavonoid characteristics associated with *Eldana saccharina* resistance (Rutherford et al 1993). This stalk borer is endemic in South Africa and causes costly damage to cane each year. Multiple regression predictive models based on NIR data from 30 clones of known *Eldana* resistance suggested that stalk bud scale and wax components accounted for up to 55% of the variation in resistance. Current work is validating these NIR bud scale and wax resistance models.

Leaf NIR scanning is also under investigation as a means of predicting host *Eldana*, mosaic and smut resistance in cane. Preliminary results using 230 leaf samples from trials on 12 commercial varieties suggested that up to 60% of the variation in eldana resistance could be accounted for by absorption of constituents in the NIR region. Further investigation into likely cause and effect relationships suggests that some of the resistance was linked to leaf silicon (R=0.60) and nitrogen content (R=0.39).

Leaf Si is a useful indicator of the silicon status of sugarcane (Clements 1967). Although not yet proven, it is possible that Si is an important element that has been overlooked in stalk borer resistance in sugarcane. In Florida, high Si uptake in sugarcane following treatment with a silicate slag served as a deterrent to the stem borer *Diatraea saccharalis* (Elawad et al 1985). Pot trials are currently investigating the association between host plant Si and N and infestation by *Eldana*. NIR leaf calibrations for Si have been established. Another possible new application of NIR is for detecting resistance to diseases such as mosaic and smut. Preliminary results with 15 cane varieties have shown that the standard ratings of mosaic and smut were significantly correlated with leaf spectral absorbance in the NIR region.

CONCLUSIONS

With analytical applications as diverse as soil, plant tissue, shredded cane, cane juice, bagasse and molasses, to mention a few, there is currently no other analytical technique that can lay claim to being as versatile and as fast as NIR. It is envisaged that, with the rapid advances being made with portable handheld NIR units, cane producers may be able to use this technology in checking crop N status, monitoring crop maturity, when to apply chemical ripeners and planning field harvesting programs. Handheld NIR units with limited spectral ranges are already being used in various agricultural applications in the USA and Australia. Staff at the Yanco Agricultural Institute in New South Wales are currently evaluating a portable system for monitoring rice quality. NIR monitoring of crops also could have inputs into crop modelling, through monitoring N, photosynthesis and crop maturity status, thereby improving the accuracy of crop forecasting. Ultimately, NIR remote sensing from NASA's Airborne Imaging Spectrophotometer to determine yield and quality of sugarcane crops, using NIR calibrations of the crop canopy, needs to be researched.

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4.2 Fertiliser use and soil nutrient problems

SOIL SURVEY - A TOOL FOR BETTER FERTILIZER MANAGEMENT IN THE AUSTRALIAN SUGAR INDUSTRY

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ABSTRACT

Current fertilizer recommendations for the Australian sugar industry are not soil specific. Consequently they do not take into account important soil properties such as the buffering capacity of soils for added nutrients, the rate of reaction between added nutrients and soils, the rate of biological turnover of nutrients, and interactions between nutrients. Fertilizer application may therefore result in excessive, adequate or insufficient application of nutrients. Excessive application results in inefficient fertilizer use and may also lead to possible detrimental environmental impacts. Conversely, insufficient application will result in sub-optimal crop yields. Thus, both excessive and insufficient fertilizer application may be costly to the industry.

Considerable scope exists for improving the applicability of fertilizer recommendations by taking account of soil characteristics. This paper describes the results from a detailed soil survey of sugarcane soils in the Herbert River District. Associated physical and chemical analytical data provide the basis for the delivery of soil-specific fertilizer recommendations. This information offers growers the opportunity to improve the precision of their crop nutrition.

INTRODUCTION

The Australian sugar industry currently uses fertilizer recommendations that are industry wide, with no specific recommendations, apart from N, being made for different regions, climatic conditions or soil types (Calcino 1994). Consequently, the recommendations are imprecise as they do not take into account important soil factors such as the ability of soils to retain added nutrients, the rate or magnitude of the reaction between added nutrients and soils, nutrient interactions in different soils, the effects of soil biological activity on nutrient release, and the effects of different rainfall regimes on nutrient movement in soils. Instead, the philosophy has been to base fertilizer recommendations largely on nutrient replacement for optimum crop yields, soil test values or in some cases on sugar price. Critical levels have been derived for most nutrients from the relationship between soil test values and crop response. These are based on an aggregation of trial results from many different regions and soil types.

With increasing emphasis now being placed on the possible detrimental environmental impacts of excessive fertilizer application, a more precise approach to developing fertilizer recommendations for each district based on soil properties is required. Furthermore a recent survey of the behaviour of sugarcane farmers in the Herbert River catchment indicated that many growers were dissatisfied with current fertilizer recommendations and that 87% of growers were in favour of using soil-specific recommendations (Johnson 1995). These are therefore strong reasons for basing soil management recommendations on the distribution of different soil types in the Herbert.

This paper describes the results from a detailed soil survey which is being conducted in the Herbert River District of north Queensland, and gives examples of ways in which more precise, soil-specific fertilizer recommendations can be developed for the Australian sugar industry.

THE HERBERT RIVER SOIL SURVEY

A detailed survey of soils used for sugarcane production in the Herbert River district commenced in 1981. Mapping is based on numerous soil observations in every sugarcane field and on soil patterns visible on 1:20,000 colour aerial photographs. Soil maps at a scale of 1:8,000 are then produced (Fig.1). The main criteria used for separating soil types are colour, texture, drainage of top and subsoils and position in the landscape. The purpose has been to convey information to cane farmers, who are the main users of the soil survey information, rather than on correlation with an existing pedological soil classification such as that of Isbell (1996). Hence, the names of the mapping units are based on simple terms using colour and texture, (eg.

red loam and black organic clay) since these are understandable and recognisable to growers. To date, 24 soils have been delineated and the survey has covered about 35,000 ha, which is approximately 60% of the sugarcane area.

An additional feature of the soil survey is the acquisition of chemical and physical analytical data for each soil type. Samples are taken from the upper cultivated layer (0-100 mm) and from the subsoil below the layer of soil mixing at a number of locations within each mapping unit. Samples from 720 locations have been analysed for physical and chemical characteristics, using laboratory procedures described by Wood (1986). The data, which are stored in an analytical database, have helped indicate research priorities and areas where further detailed soil characterisation is required.

A major concern when using results from any soil survey is whether the soil mapping units are meaningful in terms of delineating areas which differ in their soil properties and management requirements. Preliminary results from statistical analysis of soil physical and chemical analyses using discriminant analysis and non-hierarchical cluster analysis support the use of the mapping units as delineating areas of different soils (P. Toscas, CSIRO IPPP Biometrics Unit, personal communication). These results also indicated that it is possible to reduce the number of soil mapping units from 24 to around 5, by clustering soils with similar physical and chemical properties. This has important implications for the development of soil-specific fertilizer recommendations and management practices for growers in the Herbert River District. Instead of having one all-encompassing and possibly imprecise fertilizer recommendation, growers would be able to select fertilizer recommendations which are specific to the soils they are using for sugarcane production.

FERTILIZER MANAGEMENT

Selected mean soil properties from the soil survey database for 3 contrasting soils which occur throughout the district and occupy significant areas are shown in Table 1. All three soils are highly acidic, with mean pH values of 5 or less. Soils with a high clay content in the Herbert are typically high in organic matter, total N, exchangeable Ca and Mg, exchange acidity, cation exchange capacity and extractable Cu and Zn. Conversely, soils with a sandy texture are generally low in organic matter, CEC and both macro and micro nutrients. These differences have important implications for fertilizer management.

Sugar industry N recommendations are based on average response curves for different regions (Chapman 1994). Recommendations are the same for all regions apart from those under full irrigation where

Table 1 *Selected mean topsoil properties (0-100 mm) for 3 of the main mapping units in the Herbert River sugarcane area*

Soil type	No. of samples	Clay (%)	pH water	Exch.Ca (cmol(+)/kg)	Exch.Mg (cmol(+)/kg)	Exch. acidity (cmol(+)/kg)	CEC (cmol(+)/kg)	Organic C (%)	Total N (%)	DTPA extr.Cu (mg/kg)	DTPA extr.Zn (mg/kg)
Clay loam	16	34.9	4.97	2.94	1.70	2.41	7.26	0.92	0.094	1.69	2.14
Red loam	34	20.6	4.83	1.16	0.35	1.65	3.38	0.68	0.058	0.56	1.10
Coarse sandy loam	23	14.4	5.01	0.89	0.26	0.69	2.01	0.47	0.043	0.27	0.99

higher cane yields are expected. This is in contrast to N fertilizer recommendations in the South African sugar industry which are based on the capacity of each soil type to mineralise N (Meyer & Wood 1994). Whilst the range of soil organic matter levels in Herbert River soils is narrower than that in South Africa, soil-specific N recommendations based on soil total N (Table 1) can be developed for the Herbert (Wood 1986).

Industry P recommendations are currently based on a soil test which involves dilute acid extraction. However, this test is not a precise indicator of the differing P requirement of cane grown on contrasting soils, as it does not differentiate between soils in terms of their P sorption characteristics as well as less extractive (ie. more sensitive) test such as those based on ion-exchange (Bramley et al, 1995). To increase the precision of P fertilization management so that it takes account of the capacity of the soil to supply P to plants, it has been suggested that P sorption characteristics need to be considered and that the use of one of the less extractive tests for soil P may be appropriate (Bramley et al, 1995). P sorption characteristics have been described for all the main soil types (Wood, 1986) in the Herbert and these have been used to develop soil-specific P fertilizer recommendations (Wood 1988).

Industry recommendations for lime are made only where soil exchangeable Ca is considered deficient (Calcino 1994). Lime at 5 t/ha is recommended for soils with exchangeable Ca <0.55 cmol(+)/kg, and lime at 2.5 t/ha is recommended for soils with 0.55-1.25 cmol(+)/kg exchangeable Ca. Thus, for the soils in Table 1, lime at 2.5 t/ha would be recommended for the coarse sandy loam and red loam soil types but none for the clay loam, even though mean soil pH is less than 5 and mean exchange acidity amounts to over 30% of CEC. Using these criteria, most of the heavier textured soils in the Herbert River District with higher CEC and exchangeable Ca >1.25 cmol(+)/kg, would never be treated with lime.

The emphasis on soil Ca means that lime recommendations do not address the problem of continuing soil acidification which occurs largely as a consequence of the application of N fertilizer and through the removal of nutrients in harvested cane. Furthermore, the recommendations fail to account for differences in the nature and rate of reaction between lime and different soil types. To increase precision, it is suggested that lime recommendations need to take into account soil pH, exchangeable aluminium, cation exchange capacity (Table 1) and pH buffering capacity.

Apart from assisting decisions on the amount and frequency of lime applications needed on different soils, a knowledge of soil electrochemical properties and cation and anion exchange capacity is essential for effective fertilizer management especially for soils having a significant amount of pH-dependent charge. Gillman & Sinclair (1987) have shown that it is possible to group soils in north Queensland having similar charge properties and that each group requires different nutrient management. Soil charge characteristics determine the ability of soils to hold onto cations such as Ca, Mg and K and anions such as NO_3 and SO_4. It has also been demonstrated that the extent of leaching of cations from different sugarcane soils and thus the potential for nutrient loss, can be explained through a knowledge of soil hydraulic and electrochemical properties (Gillman et al, 1989), which could also be measured as part of the soil survey.

Where soils have a low CEC, such as the coarse sandy loams (Table 1), careful nutrient management is essential. If soils become too acidic then acidic cations (H + Al) dominate the exchange complex leaving insufficient exchange capacity for essential nutrients like Ca, Mg and K. In cases of extremely low CEC, it may not be possible to achieve the Industry "critical levels" for exchangeable cations on which current recommendations for lime, Mg and K application are based. With industry recommended levels for Ca. Mg, and K being 1.25, 0.25 and 0.24 cmol(+)/kg respectively (Calcino 1994), the mean CEC of the coarse sandy loams (Table 1) is only slightly higher than the sum of these levels. It may be more appropriate to base recommendations on the proportion of the CEC occupied by each nutrient so that imbalances of one nutrient over another are avoided and differences in charge characteristics between soil types are taken into account. Fertilizer management strategies should also acknowledge that large nutrient applications are not appropriate on low CEC soils. If they do not, then wastage of fertilizer and off-farm environmental impacts are the likely consequences.

Industry recommendations for minor element nutrition are not well developed apart from Zn which is based on an acid extraction soil test. Reghenzani (1993) has noted that it is possible to group soil types in north Queensland on the basis of their potential for Zn deficiency. However, in view of the lack of precision in relating crop response to soil test data and the limited knowledge of the role of other minor elements such as Cu, B and Mo in sugarcane nutrition, it is sensible to apply small maintenance quantities of minor elements as part of all fertilizer programmes and to ensure that these elements are applied in situations where soil micronutrient levels are low (as on most sandy soils in the Herbert) and where lime is to be used, as an increase in soil pH will further restrict the availability of some micronutrients.

CONCLUSIONS

The basis on which fertilizer recommendations are made for Australian sugarcane producers has not changed much over the last 50 years. With increasing concern being shown by the Industry for the minimisation of off-farm impacts of fertilizer use, a different approach is needed for fertilizer management. A regional approach to nutrient management based on soil properties should maximise long-term profitability whilst minimising nutrient losses from the farm.

The existence of a detailed soil survey in the Herbert River district coupled with a comprehensive soil analytical database should enable soil-specific fertilizer management strategies to be developed which can be progressively refined as more information about nutrient availability and retention is obtained.

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Detail from the CSR Soil Survey for the area around Macnade Mill.

LEGEND

Digital Cadastral Database

c   Clay
cl   Clay Loam
csl   Coarse Sandy Loam
fsl   Fine Sandy Loam
rl   Red Loam
rb   River Bank
ro   River Overflow
rs   River Sand
sc   Silty Clay
tz   Terrace Silt
     Unassigned/Riparian/Urban

N
W   E
S

0.5   0   0.5   Kilometers

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SPLITTING N FERTILISER APPLICATION - DOES IT INCREASE PRODUCTION EFFICIENCY OF SUGARCANE?

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ABSTRACT

Sugar yield was not increased by applying sulfate of ammonia in two or three applications compared to a single application, on ratoon crops of sugarcane, with a green cane trash blanket. Split applications also had no effect on N uptake by the crop, efficiency of N fertiliser use and residual N levels in soil. Apparent recovery of fertiliser N was mostly in the range of 20-35% and not increased by split applications. These results were obtained from experiments conducted on duplex soils and one on a gradational soil, near Mackay. Sulfate of ammonia was surface banded on cane rows at rates up to 300 kg N/ha in three experiments, while ^{15}N labelled sulfate of ammonia was buried in cane rows at 160 kg N/ha in the fourth experiment.

INTRODUCTION

Australian canegrowers usually fertilise their ratoon crops of sugarcane with a nitrogen/phosphorus/potassium mixture in one application at 0-3 months after harvesting. Two applications are usually made to plant crops, which receive a mixture at planting, followed by a sidedressing of N fertiliser at about 2-3 months later at stooling.

Bureau Sugar Experiment Station extension advice is to delay fertilising ratoon crops until a new root system has developed and the crop is 0.5 m high. This can increase yield above that of fertilising immediately after harvesting (Calcino & Burgess 1995) which is probably due to better utilisation of N. Utilisation of N fertiliser by a sugarcane crop is usually low with 20- 40% of the N applied utilised by the crop in the year of application (Chapman et al 1994; Vallis et al 1995). The N reserve in soil, mostly in organic form, is an important source of N for the crop. Keating et al (1993) reported that between 59 and 76% of the N found in cane crops in south Queensland and north New South Wales could be attributed to N mineralised from this source. A proportion of the fertiliser N does replenish this soil organic pool, with typical values of 26% of fertiliser N being found in soil organic matter 12 months after application (Chapman et al 1994; Vallis et al 1995).

Canegrowers are concerned about the low utilisation of N fertilisers by the crop, because of the additional costs of unused fertilisers, and the possibility that fertiliser losses may lead to adverse downstream effects

An objective of this research was to increase the efficiency of utilisation of N fertiliser. Synchronisation of N applications to match crop uptake is one likely strategy to increase the efficiency of N use. This report presents data from experiments conducted to evaluate the following issues: can splitting of N applications reduce the fertiliser applied without affecting yield; can splitting fertiliser applications improve the N uptake by the crop?

METHODS

Treatments

The four different split-N fertiliser trials, each with four replicates of the fertiliser treatments, were conducted on ratoon crops at three sites. Three trials had application rates of 0, 100, 200 and 300 kg N/ha as sulfate of ammonia banded on top of the trash in the cane row. N fertiliser was applied either as single, two (split-2) or three (split-3) applications at 0, 4 and 10, or 4, 10 and 16 weeks after ratooning, respectively. The 300 kg N/ha rate in Expt. 1 and 2 was applied only as a single application, while the split-3 treatments were included for this rate in Expt. 3(a).

The proportion of fertiliser applied at each time was: 0.25, 0.75 for split-2, and 0.25, 0.50,0.25 for Expts. 1 and 2; 0.50, 0.50 for split-2, and 0.33, 0.33, 0.33 in Expt. 3(a). Adjacent to Expt. 3(a) was Expt. 3(b) which had ^{15}N labelled sulfate of ammonia at 160 kg N/ha buried 100mm deep in a band in the cane row. This fertiliser was applied

as single or split-3 applications with the proportion in each split being 0.33 applied at 4, 10 and 16 weeks after ratooning.

Sites

The experiments were conducted at three locations near Mackay (149.21°E, 21.46°S). Soil types were: Expt. 1 - brownish black, sandy loam, topsoil with reddish brown, sandy clay loam, subsoil (Typic Ustochrept); Expt. 2 - greyish yellow, sandy loam, topsoil with yellow, sandy clay, subsoil (Typic Haplustalf); Expts. 3(a) and (b) - brown, sandy clay loam, topsoil with brown mottled medium clay, subsoil (Typic Haplustalf). All sites had trash mulches retained from green-cane harvesting, and this practice had been in place respectively for 2, 1 and 4 years for Expts 1, 2 and 3 prior to the treatments being applied. Total rainfall for the experiments were 840, 3193 and 1293 mm, respectively. All experiments were irrigated as required for commercial cane production, and after each fertiliser application to activate fertiliser if rain was insufficient for this purpose.

Soil mineral N

In Expt. 1 and 2, soil was sampled by coring 50mm diameter by 300 mm depths at 4, 10, 16, 22 and 52 weeks after ratooning. Soil samples were extracted with 2M KCl and analysed for NO_3 plus NO_2 (Best 1976) and NH_4^+ (Rowland 1983) in Expt. 1 and 2. In Expt. 3(b) soil was excavated to 300 mm and cored, 50mm diameter by 1.2 m depth. Samples provided measurements of NO_3- plus NO_2- and NH_4^+ as above, total N (Bremner & Mulvaney 1982) and ^{15}N. by steam distillation of digests (Saffigna & Waring 1977), and isotope ratio (Ross & Martin 1970). Measurements were conducted at 10, 16, 22 and 52 weeks after ratooning.

N in crop

In Expts. 1 and 2, crop yield was measured at 10, 16 and 22 weeks after ratooning by multiplying the average weight of 10 stalks by total number of stalks. At harvest after 52 weeks, yield of cane was measured by weighing mechanically cut billets from an area of 68m². In Expt. 3(b), only cane from a 0.75 m section of the ^{15}N treated areas was used for measuring N uptake at 10, 16, 22 and 52 weeks. All cane was harvested, weighed, ground, subsampled and analysed for moisture, total N and ^{15}N. Biomass was partitioned into stalks, tops and trash at 10, 16, 22 and 52 weeks after ratooning. Total N in the crop was divided by the cane yield at harvest to give a measure of efficiency of N usage. N uptake for the non-fertilised treatments in Expts 1, 2 and 3(a) was subtracted from N uptake of the fertilised treatments to give a measure of N uptake which could be attributed to fertiliser. This was compared with the fertiliser applied to give a measure of apparent fertiliser N recovered by the crop. Sugar yield at harvest was determined as the product of cane yield by CCS measured in juice crushed from 6-stalk samples.

RESULTS

Sugar yield

N fertiliser applications significantly increased sugar yields by 3.4,4.8 and 5.5 t/ha for Expts. 1, 2, and 3(a) respectively. Maximum yields were
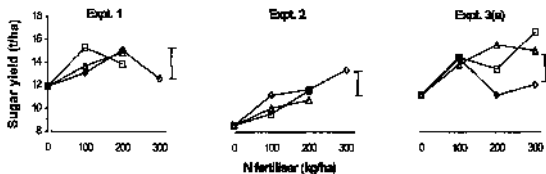
Fig. 1 *Effect on sugar yield of rates of N fertiliser applied as single ◇, two (split-2 □) or three (split-3 △) applications for three experiments.*

by applications of 100 kg N/ha for Exp.1 and 300 kg N/ha for Expts.2 and 3(a) (Fig. 1). There was no significant yield increase from splitting fertiliser applications in Expts. 1 and 2. In Exp.3(a) there was a negative response to single rates in excess of 100 kg N/ha suggesting an apparent benefit from splitting, but this result may be anomalous.

Soil mineral N

Total mineral N in soil increased in response to amounts of N fertiliser applied. Temporal changes in mineral N levels were similar for Exp. 1 and 2, so only data for Exp.2 are presented (Fig. 2a). Single applications had maximum soil mineral N at 10 weeks (6 weeks after application). Values were 14, 74, 65 and 195 kg N/ha for the 0, 100, 200 and 300 kg N fertiliser levels. Mineral N levels then fell until 22 weeks when the levels ranged from 4 to 8 kg N/ha, and remained at this level at harvest, except for the 300 kg N/ha fertiliser application which had 40 kg N/ha

at 22 weeks. Mineral N never exceeded 45 kg N/ha in the split-2 and split-3 treatments. The split-2 treatment had 50 and 150 kg N/ha of fertiliser applied at 4 and 10 weeks but only 25 kg N/ha of mineral N in the soil, which appears unusually low. Crop uptake at this point was 70 kg N/ha, some of which could be attributed to mineralisation. This indicates a substantial loss of fertiliser N or immobilisation by trash. This period between 10 and 16 weeks coincided with a high rainfall event of 138mm followed by consistently wet conditions giving a total rainfall of 274mm. These conditions would favour N fertiliser loss by leaching and denitrification, but the loss processes in this experiment were not measured. By harvest, 52 weeks, mineral N levels in the single and split treatments were low.

For the fertilised treatments, the majority of the mineral N present was as NH_4^+ which ranged from 0.56 to 0.86 of the total mineral N. The
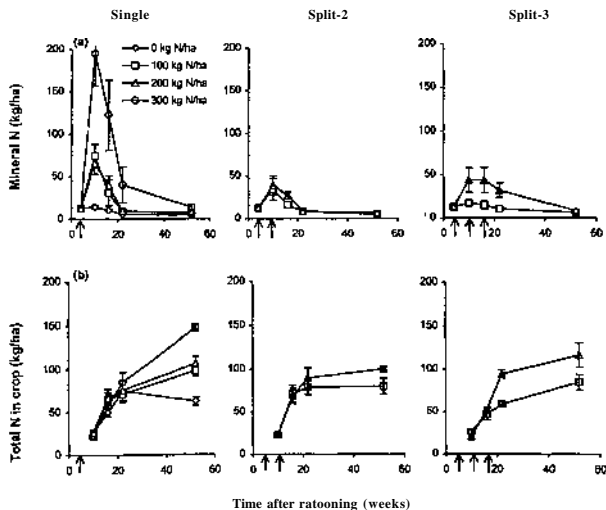
Fig. 2 *Effect of N fertiliser applications (single, split-2, split-3) on (a) soil mineral N (NH_4^+ plus NO_3^-) and (b) N uptake by the crop for Expt. 2. [Error bars indicate standard error of means, and arrows represent time fertiliser applied.]*

highest fertiliser rates tended to have the highest proportion of mineral N present as NH_4^+.

Total N in crop

Nitrogen uptake and efficiency of N fertiliser use
Total N uptake was significantly increased by higher N fertiliser rates of application. N uptake was maintained at a high rate until 22 weeks, and then slowed dramatically, except for the 300 kg N treatment (Fig. 2 a). Total N content at harvest was 62, 98, 107 and 148 kg N/ha respectively for single applications of 0, 100, 200 and 300 kg N/ha of fertiliser (Fig. 2 a). Split applications had no significant impact on total N in the crop. (Figs. 2 b, c)

The ratios of total N uptake: cane yield increased as higher amounts of fertiliser were applied for Expts. 1 and 2 (Table 1). Within any one N application level, the ratio for single, split-2 and split-3 fertiliser treatments was similar, suggesting that the split applications had not influenced the efficiency of N fertiliser use. The data from Expt. 3 (b) (^{15}N experiment) gave comparable results to Expts. 1 and 2, except for the split-3 treatment which had a ratio of 1.67 compared to 1.25 for the single fertiliser application (Table 1).

Apparent recovery of fertiliser N
Another way of evaluating the N uptake by the crop at harvest was to compare it to the fertiliser applied, that is, the apparent recovery of fertiliser N. Fertiliser N taken up by the crop for single applications in Expts. 1 and 2 ranged between 22 and 35%. Splitting fertiliser applications did not improve the apparent recovery of fertiliser N. The ^{15}N data from Expt. 3(b) also supported these data and failed to indicate better recovery of fertiliser N by split applications.

The apparent recovery of N by the crop varied considerably in the initial 6 weeks after each split application of 55 kg N/ha in Expt. 3 (b). The apparent recoveries were 33, 19 and 66%, respectively, for the N applied at 4, 10 and 16 weeks and these apparent recoveries changed little by the 52 weeks sampling. Factors affecting these apparent recoveries of fertiliser were, the small cane at 4 weeks application, the heavy prolonged rainfall after the 10 weeks application, and the well developed crop with full leaf canopy at the 16 weeks application. Soil data from Expt. 3 (b), not presented here, produced no evidence of leaching losses of fertiliser N at 0.9 to 1.2m depth at 10, 16, 22 and 52 weeks. This does not mean that soil N was not leached, but supports the view that denitrification was the main loss process for fertiliser N.

DISCUSSION

Experiments reported here indicate that splitting N fertiliser applications had no beneficial effect given the soil, weather and management conditions of these experiments. Split applications of N are likely to be most beneficial when sub-optimal rates are used. These trials

demonstrated no increase in yield at an application rate of 100 kg N/ha. Mean yields for single, split-2 and split-3 applications were 12.9, 13.1 and 12.6 tonnes sugar/ha, respectively in Expts. 1,2 and 3 (a). Splitting did not increase the total N in the crop compared with single applications; from Expt. 1 and 2, using 100 kg N/ha of fertiliser, the kg total N/tonne of cane was 1.3, 1.29 and 1.26 kg for single, split-2 and split-3 applications. Likewise, recovery of fertiliser N by the crop in the same experiments was 34, 31 and 24%.

The low utilisation of N fertilisers by the crops in these experiments agrees well with similar results from a wide range of past experiments on sugarcane using different sources of N. In these experiments sulfate of ammonia was used so NH_3 volatilisation losses would be negligible on these acidic soils (pH 5.2 to 5.6). Data from the ^{15}N experiment did not indicate leaching, but leaching could not be excluded. Leaching was not expected to high in the duplex of soils Expt. 2, but could be significant for the gradational soil of Expt. 1. Consequently, denitrification was probably the major process leading to poor utilisation of N fertilisers. Vallis et al (1995) demonstrated that there are compensatory losses which lead to low utilisation of N fertiliser, that is, if leaching loss is low then denitrification loss is likely to be high, and vice versa. They showed that soil type had no consistent effect on efficiency of N use, as was the case in this study. Also, efficiencies of use calculated by subtracting uptake of N by the unfertilised crop from uptake by die fertilised crop in these experiments (Table 1) gave similar results to those of Vallis et al (1995).

CONCLUSION

There is little empirical evidence for embracing the concept of split fertiliser applications to gain more efficient use of N fertiliser. Commercially, splitting fertiliser N applications increases labour, machinery and fuel costs because more field operations are necessary. Also, there is little possibility of late fertiliser applications being applied by machinery when cane is too high and soil conditions too wet. Splitting of N applications can be achieved with drip irrigation (R Ridge, personal communication) but this has not improved yield.

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Table 1 *Effect of rate of N fertiliser and split applications on the ratios of total N in crop to cane yield (kg N/t) and the apparent recovery of N fertiliser, viz. crop uptake of N fertiliser ((total N in crop minus N in unfertilised crop) as a % of fertiliser N applied.*

Experiment	Fertiliser applied (kg N/ha)	Total N in crop/cane yield			Crop uptake/fertiliser applied		
		Single	Split-2	Split-3	Single	Split-2	Split-3
		—kg N/t cane—			—%—		
1	0	**1.01**	-				
	100	1.25	1.26	1.17	33	46	31
	200	1.45	1.53	1.46	35	33	31
	300	1.70	–	–	23		
2	0	1.16		.	.	.	.
	100	1.44	1.33	1.36	36	17	22
	200	1.52	1.41	1.73	22	19	27
	300	1.85		.	.	.	.
3(b)	160	1.25	–	1.67	39[1]	–	32[1]

[1] Crop uptake of N fertiliser calculated from ^{15}N data.

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EFFECT OF DEPTH OF UREA APPLICATION ON LOSS OF NITROGEN BY VOLATILISATION FROM ACID SOILS

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ABSTRACT

The effect of depth of soil cover on loss of nitrogen by ammonia volatilisation from urea fertiliser in a humic gley soil and a red earth soil was investigated. Both soils were acid (pH 5.6 • 5.7),but their cation exchange capacities differed greatly (humic gley - 20.3 cmol/kg, red earth - 2.8 cmol/kg). The moisture content of the soil was adjusted to 50% of water holding capacity and nitrogen-15 labelled urea was placed at three depths (25, 50, 75 mm) in potted soil at a rate equivalent to 160 kg N/ha banded urea. The soil was allowed to stand for four weeks in the glasshouse, after which nitrogen-15 recovery was determined. There was no loss of nitrogen from the humic gley regardless of depth of fertiliser application. Urea fertiliser would have to be placed below 75 mm to eliminate volatilisation losses on the red earth soil, although application of urea at 50 mm depth instead of 25 mm reduced the losses by 55%. The losses were presumed to be due to volatilisation of ammonia, because nitrogen-15 recovery from ammonium sulphate was complete.

INTRODUCTION

Urea is the main nitrogenous fertiliser currently used in sugarcane production in Australia, because its cost per unit of nitrogen (N) and transportation expenses are less than for other fertilisers. On-farm experiments with ^{15}N labelled urea in Queensland and New South Wales demonstrated a plant uptake of only 16-29% of the fertiliser N and losses of 47-61% from the plant/ soil system (Keating et al 1993). In these studies, losses of N via volatilisation of ammonia were minimised by placement of urea at depth in the soil. Ammonia volatilisation is a major pathway of N loss from soils with the factors determining its loss from sugarcane fields having been extensively studied. Studies have shown that cation exchange capacity (CEC) (Freney et al 1983, Campbell et al 1984), pH (Freney et al 1983, Campbell et al 1984) and clay content (Campbell et al 1984, Preez et al 1987) all influence the rate of ammonia volatilisation from soil. High CEC, low pH and high clay content all reduce the rate of volatilisation. Gaseous nitrogen can also be lost by nitrite decomposition, and this is enhanced by a high organic matter content in the soil (Nelson 1982).

Results from experiments on depth of urea placement have given variable results, presumably due, at least in part, to variation in soil characteristics. Studies on alkaline soils have found that urea buried at -25 mm showed no significant loss of N (Bouwmeester et al 1985, Rachhpal-Singh et al 1988), while other studies have recorded N losses from urea banded at 12.5 and 25 mm (Campbell 1984). However, large N losses have been found when urea is banded into acid soils.

Approximately 20% of added N was lost via ammonia volatilisation when urea was banded 254 mm below the surface of a sandy soil for 21 days (Overrein & Moe 1967). Haysom et al (1990), found that 60% of the N was lost from surface applied urea after 8 days, with N loss reduced to 20% when the urea was buried at a depth of 25 mm.

In this study, we investigated the effect of depth of soil cover on the loss of urea - N from two different acid soils. Nitrogen-15 labelled urea was placed in bands at three depths in pots of soil in a glasshouse for four weeks. The nitrogen losses were estimated by the difference between the initial and final contents of ^{15}N in the soils.

MATERIALS AND METHODS

Two soils were used; a humic gley (HG) *[Vertic Ochraqualf]* (Stace et al 1968) from Broadwater, New South Wales, and a red earth (RE) *[Rhodic Kandiustuli]* (Stace et al 1968) from Childers, Queensland (Table 1). The soil was collected from the top 20 cm at both locations. Nitrogen-15 labelled urea (10 atom% [15]N excess) was placed at depths of 25, 50 and 75 mm. To validate the ^{15}N recovery techniques, labelled ammonium sulphate (5 atom% [15]N enriched) was placed in separate pots at a depth of 50 mm. All treatments were replicated four times.

Table 1 *The characteristics of soils used in the study*

Measurement	Humic gley soil	Red earth soil
pH (1:5 water)	5.60	5.68
CEC (cmol/kg)	20.3	2.8
Initial bulk density (Mg/m³)	1.10	1.29
WHC (%)[1]	55.4	38.0
Total carbon (%)	250	2.07
Sand (%)	63.3	32.3
Silt (%)	17.4	13.1
Clay(%)	19.3	54.6

[1] Water content after draining for 1 h with zero suction.

The soils were air dried to enable easy mixing before passing through a 1 cm sieve, and then placed in pots (15 cm square and 20 cm deep). Water was added to the soil in the pots to 50% of laboratory water holding capacity (WHC); the pot contents were then emptied and thoroughly mixed. Part of the soil was re-packed to the required depth, the ^{15}N labelled urea added as a 10 cm long band, and the remainder of the soil then replaced. The urea was added at a rate equivalent to 160 kg/ha of N applied as a single band on rows spaced 1.5 m apart for the RE, and a band either side of rows for the HG. The resulting rate on the basis of the area of the soil surface in the pots was 1600 kg N/ha for the RE and 800 kg N/ha for the HG. The pots were placed in a glasshouse for four weeks during which moisture loss and temperature were monitored.

After four weeks the pots were weighed and the soil mixed and sub-sampled for moisture determination (~175g, 105°C), air drying (~200g), and pH determination. The air dried samples were ground through a 2 mm sieve, with a further 5g ground to <250mm. Duplicate samples of ~50 mg each were taken for total N and ^{15}N analysis using an Automated Nitrogen and Carbon Analyser - Mass Spectrometer (ANCA-MS) (Barrie 1991).

RESULT

The maximum soil temperature in the pots during the four week glasshouse trial ranged from 20.5 to 33.0°C, with the soils losing 0.12-0.14g H₂0/g of dry soil. Recovery of N, from ammonium sulphate was 106 ± 10% for the HG and 103 + 6% for the RE. The sources of error in the losses were probably associated with sub-sampling and mixing a moist soil, since the N fertiliser was applied as a concentrated band and therefore not evenly distributed.

There was no significant loss of ^{15}N from any depth in the HG after fertiliser application at a rate equivalent to a split band (Figure 1). The pH of the HG soil at all depths of application did not change significantly

Table 2 *The final pH found at three depths in the two soils after incubation in the glasshouse for 4 weeks (Standard error in brackets)*

Fertiliser	Depth (mm)	Final pH	
		Red earth	Humic gley
Urea	25	7.13(0.05)	5.90(0.19)
	50	7.25 (0.01)	5.74 (0.04)
	75	7.63 (0.06)	5.55 (0.03)
Ammonium Sulphate	50	5.38 (0.12)	4.65 (0.02)

Fig. 1 *The loss of urea from the two soils after placement at three depths and incubation in the glasshouse for 4 weeks.*

from the initial pH of 5.60 to the final pH of 5.73 (Table 2). However there were significant losses of ^{15}N at all depths in the RE following fertiliser application at a rate equivalent to a single band. When urea was placed at 25 mm, the loss of ^{15}N was 31%. The loss reduced to 14% at a depth of 50 mm while the loss at 75 mm was 11% which is only slightly lower than that at 50 mm (Figure 1). The pH of the RE samples increased markedly from 5.68 to -7.34 for all three depths (Table 2). The most probable reason for the difference in N loss between the soils is the large difference in their CEC (Freney et al 1983, Campbell et al 1984) as both soils were initially acidic.

Ammonia volatilisation, nitrite decomposition and denitrification were the possible pathways of N loss in this study. Since the water content of the soils was 50% or less of the laboratory WHC, denitrification would have been negligible (Linn & Doran 1984). Further, if denitrification was occurring, deeper placement would not be expected to reduce the loss in a well mixed soil. Loss of fertiliser N by nitrite decomposition is promoted by soil organic matter (Nelson 1982), and therefore the total carbon content of these two soils would suggest it would be greater in the HG than in the RE (Table 1). However there was no loss of N from the HG which suggests that loss of N by nitrite decomposition was unlikely.

CONCLUSION

The losses of N from urea fertiliser in this study were presumed to be due to ammonia volatilisation because (a) complete recovery of fertiliser N was achieved with ammonium sulphate, which was not subject to ammonia volatilisation in acid soils, (b) loss of N decreased with increasing depth of application, and (c) N recovery was complete for the HG with its high soil organic matter content which is inconsistent with loss by nitrite decomposition.

This study showed that very different management practices could be used to eliminate ammonia volatilisation from different soil types. The application of 160 kg N/ha urea as a split band at 25 mm below the soil surface was adequate for the HG while an equivalent single band application on the RE resulted in large losses of N. The N loss was inversely related to depth of application of urea fertiliser, but a treatment which eliminated N loss on the RE was not found. Urea fertiliser would have to be placed below 75 mm to eliminate volatilisation losses on the RE, although application of urea at 50 mm instead of 25 mm reduced the losses by 55%.

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EVALUATION OF NITROGEN FERTILISER MANAGEMENT STRATEGIES IN SUGARCANE USING APSIM-SWIM

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ABSTRACT

The systems model APSIM-SWIM was used to evaluate nitrogen fertiliser management strategies in sugarcane. After satisfactorily testing crop and soil components of the model against data from a field experiment, two potential management scenarios were analysed. These both examined responses to variation in rate of N fertiliser application, in terms of crop yield and nitrate leaching below the wet zone. The simulations were run for a period of 33 years using historical weather data for Bundaberg, Queensland, to account for the effect of temporal variability in weather. The scenarios differed in irrigation management strategy which resulted in different levels of crop yield and N leaching losses. The results highlight the value of APSIM-SWIM as a tool in research towards improved management of cropping systems.

INTRODUCTION

Agricultural management increasingly needs to take a whole systems approach, in which management decisions are evaluated in terms of impacts on production, profit and the environment. A problem that this approach faces is that some of the systems components are difficult or impossible to measure directly. This has led to the use of systems models that can integrate the experimental observations and predict current and future behaviour of the whole system. Systems models have the additional advantage that they can simulate temporal variation due to variable weather patterns, which is otherwise difficult to capture fully in experimental designs.

The comprehensive modelling capability that has been developed within the APSIM (Agricultural Production systems SIMulator) framework is particularly suited to this approach (Mc Cown et al 1996). This systems model has a modular structure in which crops and major soil processes are dealt with in separate modules. A configuration of modules can thus be chosen that best reflects the system to be simulated. Recently, the soil-water-solute model SWTMv2, which is based on the Richards' and advection-dispersion equations, has been interfaced with APSIM. This combination allows a detailed description of the movement of water and solutes in the soil-plant-atmosphere continuum.

Here we have used the APSIM-SWIM model to simulate a sugarcane system. We tested the model against data from a field experiment. While these data did not allow complete verification of all aspects of model performance, we developed sufficient confidence to make longer-term predictions of N leaching losses for the same soil under different management conditions. These scenarios allowed us to examine the relationship between rate of N fertiliser application and N leaching losses, and the extent to which this relationship was influenced by crop yield, in this case altered via differences in irrigation management.

MATERIALS AND METHODS

Field experiment

The field study near Bundaberg, Queensland, was in a crop of first ratoon CP51-21. The soil was a red-yellow podsolic with a marked textural change at 0.8 m depth. The experiment included three rates of fertiliser N (0, 160, and 320 kg N/ha) banded below the soil surface as urea (Catchpoole & Keating 1995). A bromide tracer was applied to some of the sub-plots. Both soil and cane were sampled seven times during the season. Rainfall during the dry 1992/1993 season was 499 mm. Overhead irrigation varied from 440 to 757 mm. Soil hydraulic properties were obtained in the field (hydraulic conductivity near saturation) and using undisturbed soil cores in the lab (bulk density and water retention curves).

APSIM-SWIM simulations

For the simulations **APSIM** was configured with the soil-water-solute module SWIMv2, a sugar crop module (Keating et al 1996), a surface

residue module (Probert et al 1996) and a soil N module (SOILN, Probert et al 1996). Most of the parameters in these modules were obtained from independent measurements or estimated on the basis of previous experience with similar systems. Where parameters had to be optimised, the same values were used across the various treatments. Important soil parameters that were optimised because of a lack of independent data included the dispersion coefficient for bromide and the runoff and surface sealing parameters. Important crop parameters optimised included the leaf size profiles, which influence overall leaf area development, and, to some extent, crop N demand, as these data were unavailable for the cultivar.

RESULTS AND DISCUSSION

Simulation of experimental data

The water content and bromide tracer data were used as a test of the water and solute transport routines in the model, as bromide does not undergo any transformations and, under the conditions of this experiment, is subject to minimal crop uptake (less than 10 kg Br/ha from a 200 kg Br/ha application). Recovery of the applied (banded) bromide varied (56 - 104%). On the assumption that this was not due to losses, the experimental data presented in Fig. 1b were, therefore, scaled to 100% recovery. The predictions compared well with the
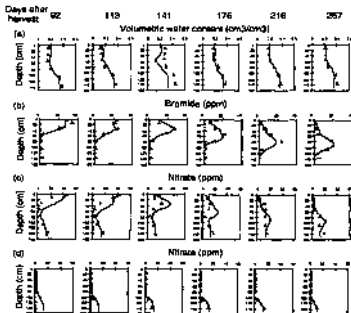
Fig. 1   Observed (A) and predicted (—) (a) water contents, (b) bromide and (c) nitrate profiles of the N320 treatment and (d) nitrate profiles of the N0 treatment at 6 sampling times.

measured data across treatments (see e.g., Fig. 1a,b), indicating that average soil water flux and storage were predicted with sufficient accuracy.

Crop biomass was predicted satisfactorily with the above limited optimisation of crop parameters (e.g. Fig. 2a,b). Observed values of N uptake by the above ground biomass varied from 105 to 217 kg N/ha, depending on the treatment. The combination of crop demand limitations in the fertilised treatments, receiving high N rates, and soil supply limitations in the unfertilised treatments meant that good simulations of crop N were achieved over this treatment range (e.g. Fig. 2c,d).
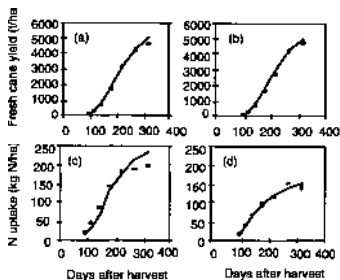
Fig. 2 *Observed (●) and predicted (—) fresh cane yield for the (a) N320 and (b) N0 treatments, and observed (●) and predicted (—) crop N uptake for the (c) N320 and (d) N0 treatments.*

The N-transformations were difficult to parameterise, especially in the high fertiliser treatments. Initial fresh organic N-pools (e.g. decomposing roots) were based on equilibrium values obtained in long term simulation runs for the same site. The zero N fertiliser treatment was simulated well (Fig. 1d). In the high N fertiliser treatments, nitrate in the soil was overpredicted (Fig. 1c). As recovery of the applied fertiliser N in the experimental data was only about 50% one month

after application, we suspect that the sampling technique could have missed some of the (still banded) nitrate on the first few sampling occasions. In addition we have not excluded the possibility that the over-prediction could be due to effects of banded fertiliser application on urea hydrolysis or volatilisation that are not accounted for in the model. Further research is currently underway to address this problem. The decrease in over-prediction with time (initially 95%, decreasing to 16%) is, however, encouraging. This indicates that it is unlikely that the simulations missed any major sources of N loss. While confident, therefore, that the next step of evaluating potential management scenarios is valid, one should still remain cautious and interpret the results in a relative sense.

Evaluation of potential management scenarios

The two scenarios addressed in this study were both based on a sugarcane cropping system with the following characteristics:

- A 14-month plant crop followed by four 13-month ratoon crops and a 6-month fallow.
- A "cool" burning regime at harvest time which removed 70 % of the trash and left the remainder on the soil surface.
- Automatic irrigation in response to soil water status or at set times (e.g. at sowing).
- Planting and harvest windows consistent with local practice.

Irrigation was applied in small amounts of 20 mm per application, except at sowing when 50 mm was applied. The two scenarios differed in the frequency of irrigation. In the first ("wetter") scenario water stress was avoided by applying irrigation as soon as the fraction of plant available water was less than 0.5, while in the second ("drier") scenario the crop could become stressed by allowing this fraction to become 0.25 before irrigation was applied. The small frequent irrigation regimes were designed to minimise confounding differences in crop production level with differences in runoff losses. Both scenarios were run for 9 different N fertiliser rates from zero to 320 kg N/ha. The simulated sugarcane system was not affected by diseases or pests. Yields are, therefore, higher than they would be under most field conditions. By running the simulations with the 1957-1989 Bundaberg weather data the year to year variation in system performance could be assessed and long-term average performance predicted. As the 1957 initialisation influenced the simulations for the first three years, these years were excluded from the analysis.

The results of the "wetter" scenario (Fig. 3) show that in the zero N treatment, the plant crops are less N-stressed than the ratoon crops due to mineralisation of crop residues and soil organic matter during the preceding fallow. For both plant and ratoon crops the N160 and N320 treatments lead to similar yields.
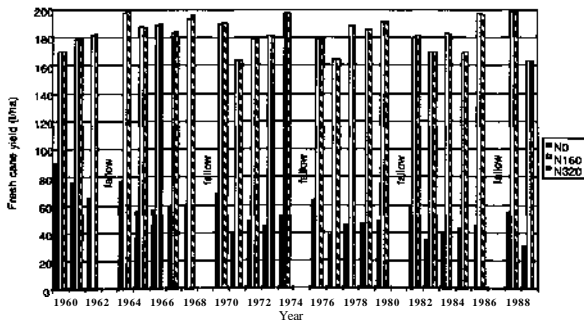
Fig. 3 *Predicted fresh cane yield for individual seasons of the 30-year run of the "wetter" scenario at three different fertiliser rates.*

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This can also be seen in Fig. 4 which shows that when crop biomass reaches a plateau, nitrate leaching starts to increase. In general, fertilising the cane above the threshold value (in our case, 160 kg N/ha) does not increase production, but could have a negative impact on the environment. As expected, the "drier" scenario resulted in significantly lower yields (Fig. 4).

As a consequence, less N was taken up by the crop as compared with the "wetter" treatment, allowing it to accumulate in the soil profile and introducing the risk of enhanced N leaching during subsequent rainfall events. The increase in N leaching in the "drier" scenario starts, therefore, at a lower fertiliser rate. This suggests that N fertiliser rates should be adjusted for a crop that, for reasons other than N supply, is not expected to reach its potential yield.
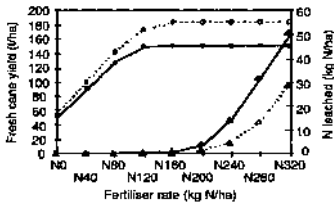
Fig. 4 *Predicted fresh cane yield (circles) and N leached (triangles) averaged over 30 years for "wetter" (—, open symbols) and "drier" (---- closed symbols) scenarios at different fertiliser rates.*

ACKNOWLEDGMENTS

It should be noted that the two scenarios represent two fairly efficient irrigation strategies, where the main impact on N leaching results from differences in crop N uptake. High frequency, inefficient irrigation could lead to increased deep drainage and hence increased risk of N leaching.

CONCLUSION

Research is on-going to ensure that the modules connected into the APSIM crop-soil systems framework adequately simulate the important processes determining crop performance and the soil water and N balance. Whilst acknowledging this model validation process is incomplete, the long-term simulations reported here illustrate that there are opportunities to limit off-farm impacts of fertiliser N if a balance is maintained between N uptake and supply. The use of N fertiliser rates that exceed crop requirements substantially raises the risk of N losses from leaching.

This study shows that comprehensive agricultural systems simulation tools, such as APSIM-SWIM, can be used in a highly effective way in the evaluation of N fertiliser management strategies and more generally in research towards improved management of cropping systems.

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NUTRIENT MANAGEMENT IN SUGARCANE IN HARYANA STATE: KEY TO IMPROVED SUGAR PRODUCTION

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ABSTRACT

In plant crop of sugarcane, sugar yield per unit area increased substantially with the application of both nitrogen (N) and phosphorus (P), an affect mainly due to increase in cane yield. The potassium (K) response on cane yield was marginal but the sugar recovery improved resulting in higher sugar yield. Application of zinc (Zn) increased cane and sugar yields and sugar recovery, up to 10 kg Zn/ha only. Application of 225 kg N/ha, 33 kg P/ha, 104 kg K/ha and 10 kg Zn/ha increased sugar yield by 26.3%, 11.8%, 10.5% and 6.4%, respectively. Application of several iron (Fe) fertilizers was found equally effective in correcting Fe deficiency in sugarcane grown on soils with low available Fe and/or in soils high in CaCO$_3$. In sugarcane monoculture, the balanced nutrition of plant and ratoon crops with NPKZn increased cane yield by 31% to 106%, sugar recovery by 0.2 to 0.4 units and total sugar production by 42% to 105%.

INTRODUCTION

Sugarcane is an important cash crop of Haryana state (India) covering 0.11 Mha and producing 6.4 Mt cane in 1993-94. Though Haryana ranks sixth in area under sugarcane cultivation in the country, its productivity level is low (58.1 t/ha). It is even less than the national average of 67.5 t/ha. This low yield is attributed, besides other factors, to inadequate and improper nutrient addition to the soils which is resulting in depletion of soil fertility. The area, production and productivity for the last 25 years in Haryana have compound growth rates of -0.68% (c.v. 16%), 0.35% (c.v. 19%) and 1.02% (c.v. 14%)/ annum, respectively, illustrating the poor situation in the state (Chaudhary et al 1995). Sugar production in the state has generally remained lower than sugar consumption during the past decade. To meet the sugar requirement of an ever growing population, the state has to produce 11.5 Mt of cane from an area of 0.165 Mha with an average yield of 70 t/ha by the year 2000. This paper deals with various aspects of nutrient management in sugarcane to increase sugar production in Haryana, India.

MATERIALS AND METHODS

Experiments were conducted at C C S . Haryana Agricultural University, Regional Research Station, Uchani, Karnal, at the Sugarcane Sub-Station at Buria (Yamuna Nagar), and on farmers' fields (Table 1). The soils of these areas were sandy loam to clay loam in texture without the problems of alkalinity (pH 7.7-8.7) and salinity (EC 0.03-0.31 dS/m). These were low in organic C (0.24-0.57%), low in P (2.5-4.6 ppm), medium to high in K (50-234 ppm), low to medium in Zn (0.75-1.10 ppm) and low to medium in Fe and with CaCO$_3$ present in A- and B-horizon in some areas. In each of the experiments, sugarcane setts were planted in furrows in the month of March with four replications in randomised block design. All plant crops of sugarcane received 150 kg N/ha and 22 kg P/ha and ratoon crops received 225 kg N/ha and 22 kg P/ha as basal dose except otherwise mentioned. Nitrogen, P, K and Zn were applied through urea, single super phosphate, muriate of potash and zinc sulphate, respectively. In plant crop, full doses of P, K, Zn and N were applied in furrows at planting. The remaining N was top-dressed in two equal splits in the months of May and June. This crop was harvested in February-March. In ratoon crops N and full doses of P, K and Zn were applied along the rows during March followed by ploughing. The remaining N was top-dressed in two equal splits in the months of May and June. Ratoon crop was harvested in December. The pol readings of clarified juice were taken on a Bausch and Lamb polariscope and sugar content calculated as described by Meade & Chen (1977).

The optimum dose of nutrient application in sugarcane was calculated from the quadratic equation (Y=a+bx+cx^2; the values of a, b and c were calculated by the least square method. In the fitted equation, the derivate -b/2c gives the rate of nutrient for obtaining maximum yield.

The most profitable rate of nutrient (Nut$_{opt}$) was calculated by:

$$Nut_{opt} = 1/-2c \ (q/p-b),$$

where b and c are quadratic equation constants; p and q are the price of sugar and nutrient/kg, respectively. Since sugar production is the ultimate aim, optimum rate of nutrient application in sugarcane was calculated on the basis of sugar yield instead of cane yield. The benefit of fertilizer application was calculated by subtracting the cost of fertilizer and its application from the additional income due to its application.

RESULTS AND DISCUSSION

The experimental results are presented as increase in yield over the control treatment in each of the different fertilizer trials.

Nitrogen

The increasing rates of applied N increased cane and sugar yields, though the increases were not directly proportional to the rates of applied N (Table 1). The increase in sugar production was 750, 1240 and 1400 kg/ha at 75, 150 and 225 kg N/ha. Based on sugar yield, the optimum rate of N application for plant crop was found to be 225 kg N/ha. Sugar yield per kg of applied N decreased from 9.9 kg at N$_{75}$ to 6.2 kg at N$_{225}$. Sugar recovery decreased slightly up to 150 kg N/ha. However, it decreased significantly at 225 kg N/ha (Table 2). The decrease in sugar recovery with added N could be due to the presence of unused N which enhances continued vegetative growth as harvest approaches, induces higher moisture levels within the cane, and higher reducing sugars and lower sucrose at harvest (Humbert 1968).

Phosphorus

The increasing rates of applied P increased cane yield, though not in direct proportion to the rates of applied P. Increasing rates of applied P increased sugar yield up to 33 kg P/ha and there was a decrease in sugar yield at 44 kg P/ha. The sugarcane varieties varied in sugar yield responses due to P application: variety Co 7314 showed the highest yield followed by CoH 3 and CoS 767 (data not shown). Therefore, the optimum rate of P application for plant crop also varied: 33 kg P/ha for Co 7314,28 kg P/ha for CoS 767 and 30 kg P/ha for CoH 3. Average sugar yield per kg of applied P varied from 11.6 to 25.8 kg (Table 2). Increase in sugar yield was mainly due to increase in cane yield,

Sugar recovery decreased significantly at 33 kg P/ha (Table 2). These results endorse earlier observations of Kadian et al (1981) and Misra et al (1964) but are not in conformity with those of Samuels et al (1952) and Samuels & Landrau (1956). This variation in results may be attributed to the much higher increase in cane yield (5-20%) due to P application in the present study as compared to 5-6% increase obtained by them. The decrease in sugar recovery could be attributed to the diversion of metabolites towards cane growth resulting in increase in cane yield and less conversion into sucrose.

Table 1 *Description of the fertilizer experiments and soil of experimental areas*

	N-Expt.	P-Expt.	K-Expt.	Zn-Expt.	Fe-Expt.	NPKZn-Expt.
Location	Buria,Y.Nagar	3farmers'fields	Buria.Y.Nagar	Uchani, Karnal	Taharpur, Darnal	Uchani, Karnal
Crop	Plant	Plant	Plant	Plant	Plant	Plant,3 ratoons
Variety	Co 7717	Co 7314 CoS 767 CoH3	Co S767	Co 7717	CoJ64	Co 767 CoS 767
Texture	S.L.	S.L.	S.L.	Loam	C.L.	C.L.
pH	7.75	8.1-8.5	8.6	7.7	8.7	8.6
EC (d Sm^{-1})	0.05	0.03-0.15	0.15	0.05	0.20	0.31
$CaCO_3$ (%)	Traces	Traces	Traces	Traces	1.0	Traces
Organic C (%)	0.57	0.33-0.56	0.45	0.54	0.24	0.41
Available P (ppm)	3.5	2.5-4.5	3.5	3.5	4.6	4.0
Available K (ppm)	52	125-175	60	50	100	234
DTPA-Zn (ppm)				1.1	0.75	0.78
DTPA-Fe (ppm)					4.3	

S.L.: sandy loam, C.L.: clay loam

Table 2. *Effect of fertilizers on the average increase compared to the unfertilized control treatment of cane and sugar production, yield per kg applied fertilizer, sugar recovery (%) and the benefit/cost ratio in rupees in the plant crop of sugarcane in Haryana*

Treatments	Yield increase (kg/ha)		Yield/kg nutrient (kg)		Sugar recovery (%)	Benefit/cost[1] ratio	
	Cane	Sugar	Cane	Sugar		Cane	Sugar
Nitrogen[2]							
N_0	.	.		.	13.58		
N_{75}	5900	750	79	9.9	13.50	4.6	10.1
N_{150}	9700	1240	65	8.2	13.42	4.2	9.4
N_{225}	11500	1400	51	6.2	13.22	3.2	7.2
LSDp=0.05	3200	318	-	-	0.31		
Phosphorus[3]							
P_0	.	.		.	1-1.87		
P_{11}	2456	176	223	15.9	11.62	2.2	2.7
P_{22}	7360	568	334	25.8	11.28	4.0	5.1
P_{33}	9180	641	278	19.4	11.04	3.2	3.6
P_{44}	10203	510	231	11.6	10.72	2.5	1.7
LSDp=0.05	637	231	-	-	0.48		
Potassium[4]							
K_0	.	.		.	11.30	-	
K_{415}	600	790	14	19.0	12.15	0.1	22.5
K_{622}	400	830	6	13.3	12.22	0.0	15.8
K_{830}	1000	910	12	10.9	12.24	0.0	13.0
K_{1037}	1300	1010	13	9.7	12.30	0.0	11.5
LSDp=0.05	NS	479	-	-	0.75		
Zinc[5]							
Zn_0	.	.		.	1316		
Zn_5	1100	167	220	33.4	13.19	1.8	5 8
Zn_{10}	2500	433	250	43.3	13.34	2.4	8.3
Zn_{15}	2400	375	160	25.0	13.27	1.2	4.5
LSDp=0.05	684	346	-	-	NS	-	

[1]Current prices per kg for N, P, Zn, Zn, cane and sugar are Rs 7.21,42.90,7.81,45.70,0.66 and 10.4 respectively and labour charges per hour are Rs. 6.6
[1] Sugarcane variety Co 7717, average of two years
[3] Average of three varieties viz. Co 7314, CoS 767 and CoH 3, three locations and two years
[4] Sugarcane variety CoS 767, average of two years
[5] Sugarcane variety Co 7717, average of two years

Potassium

Application of K did not bring significant increase in cane yield of plant crop; cane yield increased by only 1300 kg/ha with the application of 104 kg K/ha. However, increasing rates of K significantly increased sugar yield largely through an increase in the sugar recovery (Table 2). The relationship between sugar yield, sugar recovery and rates of K application followed Mitscherlich response. Application of 104 kg K/ha increased sugar recovery by 1.0%. Sugar yield response per kg of applied fertilizer ranged from 9.7 kg at 104 kg K/ha to 19.0 at 41.5 K/ha (Table 2). Samuels & Landrau (1956) observed that K deficiency significantly reduced sugar in juice and cane, pol %, brix and purity.

Zinc

Application of Zn brought about a progressive increase in cane and sugar yields with the increasing rates up to 10 kg Zn/ha (Table 2). Higher rate of Zn application appeared to result in a slight decrease in cane and sugar

yields. The highest yield response over control was 250 kg cane and 43.3 kg sugar for each kg of applied Zn at 10 kg/ha (Table 2). The optimum rate of Zn application has been calculated to be 12.5 kg Zn/ha.

Iron

Application of four different Fe fertilizers after the prominent development of Fe-chlorosis symptoms (first week of July) in sugarcane varieties CoJ 64 and CoS 767 grown on a known Fe-deficient soil significantly increased the sucrose contents in both the varieties. However, the sugar contents were still lower than when the same cultivars were grown on an Fe-sufficient soil (Fig. 1). Different sugarcane cultivars responded differently in sucrose contents in relation to the form of Fe applied. Soil-applied FeSO$_4$ (normal form) gave highest sucrose contents for CoJ 64, and soil-applied FeSO$_4$ (super-digested form) in highest in CoS 767. Sharma & Kanwar (1985) reported that in Fe-chlorotic plants, chlorophyll synthesis and accumulation of sugar were adversely affected.
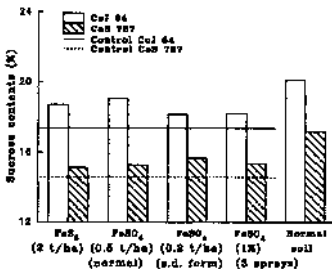
Fig. 1 Effect of different Fe fertilizer application to an Fe-deficient soil on the sucrose contents of two sugarcane varieties. L.S.D.'s (p=0.05 for sugarcane varieties CoJ 64 and CoS 767 were 0.65 and 0.50, respectively.

Benefit/cost ratio

Application of N progressively increased the net profit on the basis of sugar yield from Rs 7101 at 75 kg N/ha to Rs 12779 at 225 kg N/ha. However, the cost/benefit ratios decreased with increasing rates of applied N indicating decreased N responses. Nevertheless, the cost/benefit ratio was more than 1:7 indicating good economic returns even at 225 kg N/ha (Table 1). Application of P increased the net profit up to 33 kg P/ha at Rs 5224 and return per rupee investment on fertilizer P only up to 22 kg P/ha (1:5.1) and decreased at 33 kg P/ha. Application of 41.5 kg K/ha gave a net profit of Rs 7865 which increased to Rs 9666 with the application of 104 kg K/ha. The return per rupee investment on K fertilizer was highest among N, P or Zn (Table 1). Although, the benefit/cost ratio decreased with the increasing rates of applied K, still it was 1:11.5 at 104 kg K/ha. Application of Zn increased the net profit (Rs 4020) and benefit/cost ratio (1:8.3) only upto 10 kg Zn/ha and decreased at 15 kg Zn/ha (Table 1). The net profit and benefit/cost ratio on the basis of cane yield followed similar trends but the magnitude of profit obtained and return per rupee investment were low as compared to sugar yield (Table 1). This indicates the immense importance of fertilizers in increasing sugar production.

Balanced nutrition

In sugarcane monoculture cropping system, application of N alone substantially increased the sugar yield of plant and ratoon crops of
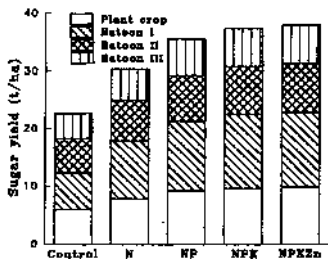
Fig. 2 Effect of different fertilizer combinations on sugar yield of plant and first, second and third ratoon crops of sugarcane variety CoS 767 in Haryana. L.S.D.'s (p=0.05) were 0.69, 1.32, 0.60 and 0.79 for plant, first, second and third ratoon crop, respectively.

sugarcane (Fig. 2). This was further improved by using P along with N. The increase in sugar yield due to addition of K and Zn over NP and NPK, respectively, was marginal (Fig. 2). The balanced fertilization with NPKZn in plant crop increased sugar yield by 63%, in first ratoon crop by 105%, in second ratoon crop by 42% and in third ratoon crop by 47% (Fig. 2). Similarly, the balanced fertilization of plant and ratoon crops with NPKZn increased cane yield by 31% to 106% and sugar recovery by 0.2% to 0.4% (data not shown).

CONCLUSIONS

Maximum cane and sugar production in sugarcane monoculture required annual application of a balanced NPKZn fertilizer at 225 kg N, 33 kg P, 104 kg K and 12.5 kg Zn/ha to sustain high yield, maximum profit and to maintain soil fertility.

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PERFORMANCE OF DIFFERENT GREEN MANURING CROPS IN SUPPLEMENTING N AND INCREASING YIELD OF A SUBSEQUENT CANE CROP

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ABSTRACT

Green manuring with Crotolaria juncea, Sesbania rostrata, Sesbania aculeata, and Indigofera tinctoria were evaluated as to their performance in supplementing N to increase cane yield. Crotolaria juncea was the most suitable green manure crop in respect of its fast growth, biomass production and its contribution to soil N. It contributed 52 kg N/ha, followed by Indigofera tinctoria (47 kgN/ ha), Sesbania aculeata (45 kg N/ha) and Sesbania rostrata (42 kg N/ha). Green manuring and inorganic fertiliser N increased cane yield 7- 25% and 21-48% respectively. Organic C, total N, available P and S and exchangeable K status of the soil were slightly increased by green manuring. Green manuring which supplemented N also improved the efficiency of inorganic N use for cane yield.

INTRODUCTION

Legumes are known to benefit the succeeding crop by way of symbiotic nitrogen fixation and mobilization of lesser available forms of plant nutrients, improving soil nitrate and decreasing leaching losses of nutrients. Green manuring has been advocated to improve yields and to partially substitute the N requirement of the crop (Singh 1984), increase soil N, concentrate P, maintain and renew organic matter and improve the physical and chemical condition of the soil (Jiao 1983). Legumes, which are used as green manures, have high amounts of nutrients and low C to N ratios (range 10-20). 1000 kg of fresh matter contains about 5 kg N, 0.44 kg P and 3.3 kg K (Lizhi 1988).

Many legume species have been studied and tried as green manure crops in different countries. *Crotalaria juncea* and *Sesbania aculeate* were the most acceptable to Indian farmers (Meelu & Morris 1986). Recently, *Sesbania rostrata* has been reported as a potential green manuring crop (Rinaudo et al 1983). Among the species that appear to have significant potential as green manures in Philippine rice production systems, *Indigofera tinctoria* is unique in that it is actually used by farmers on a considerable scale (Dennis et al 1990). Keeping in view the above considerations, the present study was designed to evaluate green manuring crops for their ability to produce a high biomass and contribute N to increase the efficiency of N use and yield of a following sugarcane crop.

MATERIALS AND METHODS

The field experiment was a split-plot design conducted during the 1992-93 and 1993-94 crop season at North Regional Station farm, Thakurgaon, Bangladesh on a sandy loam soil (Eutric Cambisols), having pH 4.9, organic C 1.2% and total N 0.075%. Green manure crops were raised during the summer season (May-July) and ploughed into soil after 60 d, following recording of their fresh biomass weights. Two-budded sugarcane setts of variety Isd 24, raised in a soil bed, were transplanted in November each year and harvested in December in the following year. All plots of sugarcane were fertilized with 125 kg P_2O_5J ha, 222 kg K_2O/ha and 33 kg S/ha as triple super phosphate, muriate of potash and gypsum, respectively. N as urea was applied at different rates according to treatment (see later) in three splits; first after establishment of setts, second at tiller initiation stage, and third at tiller completion stage. The control in main plots did not receive any green manure but N doses as treatment in sub-plots. Again, control in sub-plots received no N but green manures or no green manure. Plots were irrigated three times. Curaterr-5G @ 2 kg active ingredient per hectare was applied in March and April to control white grub and top shoot borer. Necessary intercultural operations and mechanical control of insect pests were done as per normal practice.

Treatments

Four green manure crops *Crotalaria juncea, Sesbania rostrata, Sesbania aculeata* and *Indigofera tinctoria*, in addition to a fallow (control) treatment were established as main plots. Five levels of N equivalent to

0, 50, 100, 150 and 200 kg/ha was applied (as described earlier) to sub-plots with 4 replicates.

Statistical test DNMR, was done for different parameters of green manures and sugarcane (Duncan 1955)

Evaluation of treatments

The height of the green manure crops was measured at 10 d interval and samples of their herbage were collected at plough-down and dried in a forced draught oven at 60° C for 24 h. The samples were then weighed for dry matter (DM) determination, ground (<1mm) and used for determination of N content and their potential N contribution to

On the sugarcane crops, tiller counts were taken in April, and number of millable cane stalks, yield, stalk height and thickness, and field brix were recorded for each plot at harvest. The experiment was harvested at a time leaving border lines of the plots to prevent border-effect. Millable cane stalks were counted and yield was recorded of the harvested area in each plot. Ten cane stalks were selected randomly from each plot to measure stalk height and thickness. Stalk thickness was measured from the top, middle, and bottom portion of the cane. The brix (%) was recorded by hand refractometer. Nitrogen use efficiency was calculated as the cane yield per kg N added.

Soil samples (0-200 mm) were collected from each main plot before transplanting of 60 d old sugarcane settlings (90 d after green manures ploughed in), and later analysed for nutrient status. Soil pH was measured in soil: water suspension at 1: 2.5 by glass electrode, total N for plant and soil was determined by Kjeldhal method (Jackson 1973) and organic C by Walkly and Black wet oxidation method (Black 1965). Available P (NaHCO, extractable) by standard ammonium molybdate method of Olsen (Black 1965), exchangeable K (NH_4OAc extractable) by flame photometer (Black 1965) and available S (NH_4OAc extractable) by turbidimetric method of Bardsley and Lancaster (Black 1965).

RESULT

Biomass and N yield of green manures

Significant potential differences among green manures 1) in respect to biomass production and N content were observed (Table 1). *C. juncea* produced significantly more fresh biomass and dry matter, and added higher N to the soil than the other green manuring crops. It was also superior to the other crops for its fast early growth as shown by its rapid increase in plant height (data not shown). The two species of *Sesbania* produced similar quantities of fresh biomass, but significantly more than did *I. tinctoria* which produced the lowest (Table 1). The latter species has been found to have slow growth rate in other studies (Dennis et al 1990).

Dry matter yield was identical for *S. rostrata, S. aculeata* and *I. tinctoria* in first year and for *S. rostrata* and *I. tinctoria* in second year (Table 1). Though N % was the lowest in *C. juncea* it contributed the highest

amount of N because of its higher dry matter yield. /. *tinctoria* contained the highest % N (2.1) among all the green manuring crops. As a result, it contributed substantial quantities of N to the soil even though its dry matter yield was low. N content was higher in *S. rostrata* than *S. aculeata* but N contribution to the soil was higher for *S. aculeata* due to its higher dry matter yield.

brix was similarly unaffected by green manure treatments. The increase in cane yield (t/ha) by green manuring with *C. juncea, S. rostrata, S. aculeata* and/, *tinctoria were* 12.2 (25%), 3.2 (7%), 6.4 (13%), and 7.1 (15%) in 1992-93, and 9.5 (19%), 4.4 (9%), 5.6 (11%) and 4.8 (9%) in 1993-94, over control, respectively.

Table 1 *Biomass, N-content and N-contribution to soil by different green manure crops.* [Figures with same letter do not differ significantly at 5% level as per DNMR test (see methods)]

Crop year	Treatment	Fresh biomass (t/ha)	Dry matter (t/ha)	N-in DM (%)	N-contribution (kg/ha)
1992-93	*Crotolaria juncea*	17.2a	3 9a	1.4c	4.6
	Sesbania rostrata	13.1b	2.3b	1.9b	1.4
	Sesbania aculeata	13.3b	2.5b	1.8b	5.0
	Indigofera tinctoria	10.1c	2 4b	2.1b	0.4
1993-94	*Crotolaria juncea*	15.3a	3.1a	1.6a	9.6
	Sesbania rostrata	10.3b	2.1c	2.0a	2.0
	Sesbania aculeata	11.6b	2.5b	1.8a	5.0
	Indigofera tinctoria	7.8c	2.1c	2.1a	4.1

Effect of green manure on the sugarcane crop

Green manuring had significant effects on the succeeding sugarcane crops. Total tiller number, number of millable cane stalks, yield, stalk height, thickness and brix were increased by green manuring (Table 2). Cane yield was significantly increased in both years but number of tillers and millable cane stalks, and brix, were increased significantly in the first year only. Increase of stalk height and thickness were not significant in any year. In both years, *C. juncea* produced a significantly higher cane yield than the other green manures which produced statistically identical cane yields. All green manures produced statistically identical number of tiller and millable cane stalks, except *S. rostrata* which produced a lower number in year 1. Maximum stalk height and thickness were unaffected by green manure treatment. Field

Green manuring alone produced cane at 51-60 t/ha. that is about 3.2-12.2 t/ha more than the control, from an addition of organic material containing the equivalent of inorganic N at 50-63 t/ha. Prolonged supply of N due to mineralization of green manure might have led to such increase in yield (Nagarajah 1988; Panda et al 1991). The higher the N contributed by the green manure crop then the higher the cane yield obtained, viz. *C.. juncea* contributed the highest N (54.6kg/ha in 1992-93 and 49.6 kg/ha in 1993-94) and yielded the highest cane (60.3 t/ha in 1992-93 and 60.2 t/ha in 1993-94). Inorganic N fertiliser at 150-200 kg N/ha produced the highest cane yield (61.1 t/ha in 1992-93 and 62.1 t/ha in 1993-94) (Table 2).

Effect of inorganic N

N application as urea significantly increased number of tillers and millable cane stalks, and cane yield but had no significant effect on stalk thickness (Table 2). Stalk height and field brix were increased significantly with N application in first year only. Tiller production progressively increased with increase of N application but cane yield was increased only up to 150 kg N/ha. Number of millable cane stalks were increased up to 150kg N/ha in first year but the increase was inconsistent beyond 100 kg N/ha in second year (Table 2).

Interaction effect of N and green manuring

Interaction of the inorganic N and green manuring was significant in cane yield for 1993-94 only (Table 3). The increase in cane yield following N fertiliser were greater for crops following *C. juncea* than for those after the other green manures or with no green manure. Chatterjee et al. (1979) also reported that inorganic N source applied in combination with organic sources is better utilized than inorganic source alone.

Table 2 *Effect of green manuring and fertiliser N on the yield and yield parameters of a sugarcane crop.* [Figures with same letter do not differ significantly at 5% level as per DNMR test.]

Crop year	Treatment	No. tillers (x10³/ha)	No.millable cane stalks (x10³/ha)	Cane yield (t/ha)	Stalk height (m)	Stalk thickness (cm)	Brix (%)
1992-93	**Green manures**						
	C. juncea	160.5a	107.0a	60.3a	2.2a	1.83a	20.3a
	S. rostrata	154.6b	88.4b	51.3bc	2.1a	1.86a	20.3a
	S. aculeata	173.5ab	95.0ab	54.6b	2.0a	1.84a	20.5a
	I. tinctoria	199.6a	95.86b	55.2b	2.1a	1.87a	20.5a
	Control	158.4b	93.6b	49.1c	2.0a	1.83a	19.7b
	N (kg/ha)						
	0	110.0d	83.4d	41.2d	1.9c	1.79a	19.6c
	50	167.4c	80.7c	49.7c	2.0bc	1.81a	20.7a
	100	187.9b	103.8b	56.8b	2.1ab	1.88a	20.2b
	150	206.3a	114.9a	61.1a	2.2a	1.88a	20.4ab
	200	215.3a	113.3a	60.4ab	2.1a	1.87a	20.4ab
1993-94	**Green manures**						
	C. juncea	192.1a	78.0a	60.2a	2.4a	1.99a	18.5a
	S. rostrata	179.7a	77.0a	55.1b	2.2a	1.99a	18.4a
	S. aculeata	184.2a	77.9a	56.3b	2.3a	1.95a	18.2a
	I. tinctoria	177.5a	77.2a	55.5b	2.3a	2.05a	18.7a
	Control	176.8a	76.5a	50.7c	2.3a	1.99a	18.1a
	N(kg/ha)						
	0	153.0c	64.9c	42.7d	2.2a	2.02a	18.3a
	50	172.7b	73.6b	53.8c	2.3a	1.98a	18.6a
	100	190.8a	81.9a	58.1b	2.4a	2.01a	18.4a
	150	195.5a	81.6a	62.1a	2.4a	2.02a	18.2a
	200	198.0a	64.5a	61.1ab	2.4a	2.01a	18.1a

Table 3 *Interaction of fertiliser N and green manuring on cane yield (t/ha) during 1993-94.*
[Figures with same letter do not differ significantly at 5% level as per DNMR test.]

Fertiliser N	*C. juncea*	*S. rostrata*	*S. aculeata*	*1. tinctoria*	Control
	42.7c	45.1b	43.7c	40.0b	42.0c
	52.8b	55.0a	56.7b	58.3a	46.4c
	70.2a	57.1a	56.3b	58.9a	48.0bc
	67.6a	58.0a	65.7a	57.6a	61.4a
	68.0a	60.2a	59.4ab	62.6a	55.5ab

Nitrogen use efficiency

Efficiency of N use was highly increased when fertiliser N was applied following green manuring as may be seen by comparison with the kg cane/kg N applied for the control treatment (Table 4). The highest efficiency of N was obtained after green manuring with *C. juncea* and the lowest when applied without green manuring. Mosdy, the efficiency was greater at lower level of N fertiliser (50-100 kg N/ha) when applied after green manure or as the control plots. This result agree with Panda et al (1994).

Effect of green manuring on soil nutrient status

The soil under investigation was medium in organic matter and highly deficient in total N. There was some indication (Table 5) of slight increase in pH, organic C, total N, P, K and S over the control. The soil under *C. juncea* had a lower pH than under the other green manures. These trends were in general agreement with those observed by Swarup (1991).

CONCLUSION

Green manuring significantly increased cane yield even when N fertiliser was also applied. Green manure was the most suitable green manure crop because of its fast growth, higher biomass and dry matter yield, and higher contribution of N to the soil. Green manuring not only supplemented N but also increased the efficiency of inorganic N use. Green manuring perhaps also slightly increased organic C, total N, available P and S and exchangeable K status of the soil.

The results thus showed that green manuring is extremely important for maximizing and sustaining productivity of sugarcane and maintaining

fertility status of the soils. Therefore, it is suggested that green manuring should be done in sugarcane soils of Bangladesh or elsewhere as an interim crop in the production system, to prevent soils from nutrient depletion, and maintaining productivity.

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Table 4 *Influence of green manure and N fertiliser application rate on N-use efficiency calculated as kg cane per kg N fertiliser applied*

Crop year	Fertiliser N (kg/ha)	*C. juncea*	*S. rostrata*	*S. aculeata*	*1. tinctoria*	Control
1992-93	50	359	260	262	267	117
	100	225	215	194	201	157
	150	191	119	175	209	105
	200	150	98	113	134	89
1993-94	50	215	260	293	325	88
	100	282	151	143	169	60
	150	171	107	158	104	130
	200	130	91	87	103	68

Table 5 *Soil nutrient status sampled 90 days after green manure ploughing- n of the crops.*

Crop year	Treatment	PH	Organic-C (%)	Total N (%)	Avail. P (ppm)	Exch. K (me/100g)	Avail. S (ppm)
1992-93	*C. juncea*	4.94	**1.26**	0.09	38	**0.13**	46
	S. rostrata	5.04	**1.25**	0.08	35	**0.14**	36
	S. aculeata	5.00	**1.36**	0.11	36	**0.13**	45
	1. tinctoria	5.02	**1.30**	0.09	36	**0.12**	49
	Control	4.96	**1.25**	0.08	35	**0.11**	42
1993-94	*C. juncea*	4.79	**1.23**	0.10	36	**0.16**	44
	S. rostrata	4.87	**1.30**	0.09	33	**0.13**	43
	S. aculeata	4.83	**1.34**	0.11	34	**0.13**	43
	1. tinctoria	4.86	**1.31**	0.10	33	**0.13**	43
	Control	4.80	**1.22**	0.09	32	**0.13**	41

4.3 Irrigation and soil physical problems

IMPROVING THE EFFICIENCY AND PROFITABILITY OF FURROW IRRIGATION FOR SUGARCANE PRODUCTION

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ABSTRACT

Trials were conducted on commercial sugarcane properties to investigate the efficiency and financial benefits associated with alternative furrow irrigation management practices. Modifications to the furrow shape to produce a narrow "v" shape with surface compaction were shown to reduce water use throughout the season by 45%. This represented a saving of $218/ha/yr to the grower and a potential saving of $1.74M annually to the Burdekin sugar industry. Where furrow lengths of 300 m were used instead of 600 m, the volume of irrigation water applied was reduced by 42%. These shorter furrows were found to produce a net return of either $132 or $210/ha/yr after the capital and production costs were assessed, depending on the nature of the water delivery system installed.

INTRODUCTION

Previous research investigating the efficiency of furrow irrigation practices has shown that the efficiency of current practices in the Australian sugar industry are highly variable. Raine (1995) found that seasonal water application efficiencies (defined as the proportion of the applied water available for use by the crop) for furrow irrigation of sugarcane ranged between 31 and 62% on farms where tailwater was not recycled, while efficiencies for individual irrigations ranged between 14 and 90%. Low water application efficiencies were generally found in areas of highly permeable soils with the majority of water loss attributed to deep drainage. In these areas, significant improvements in the irrigation efficiency are possible using a variety of management practices including shorter furrow lengths and changes in furrow shape to introduce surface compaction (Raine 1995). However, growers are generally reluctant to adopt these alternative irrigation strategies unless the operational and financial benefits are demonstrated under commercial conditions.

This paper reports the results from trials conducted on commercial cane farms to improve the efficiency of furrow irrigation by encouraging the adoption of shorter furrow lengths and narrow furrow shapes with surface compaction. It also presents the results of preliminary cost-benefit analyses conducted to identify the on-farm commercial implications of these strategies.

MATERIALS AND METHODS

All trials and analyses were conducted on commercial, furrow-irrigated farms in the Burdekin River Delta area during 1994-95 using typical management practices and operational costs. Each site was located on highly permeable alluvial soils that are typical of 20% (c.8000 ha) of the Delta area.

Irrigation efficiency

The effect of furrow shape and surface compaction on the application efficiency of the first irrigation after hill-up was initially investigated at sites in the Colevale and Maidavale areas. Paired trial sites were established at each location with treatments consisting of a normal "u" shape furrow produced without surface compaction using hill-up boards, and a modified "v" shape furrow with surface compaction produced by tilting the hill-up boards forward approximately 10-15%. The irrigation performance of eight individual furrows in each treatment was measured. To investigate the effect of this treatment on the rate of irrigation water advance along the furrow and application efficiency for the whole season, all irrigations conducted between hill-up and harvesting were monitored at a third site located in Home Hill. The equipment and methodologies employed to monitor irrigation performance at each site were the same as used

by Raine (1995). Cane production at the Home Hill site was also calculated from the mill bin weights at harvest.

The effect of furrow length on irrigation efficiency was determined using the surface irrigation model SIRMOD (Walker 1993). Model input parameters were derived using data obtained from actual irrigations conducted in the Burdekin Delta area. Furrow lengths of 300 m and 600 m were chosen to evaluate the water application efficiency and conduct a cost-benefit analysis because they represented typical alternatives confronting growers in the area.

Cost-benefit analyses

Cost-benefit analyses were conducted to evaluate each of the alternative irrigation strategies outlined above. However, only the costs and benefits that were directly attributable to the change in irrigation management were used for these analyses. The prices and costs used were typical of those encountered in the Burdekin during 1995. In this area, the growers pay for water based on a flat levy on the tonnage of cane produced. Hence, the actual cost of the water (expressed per ML) is a function of (a) the levy, (b) the amount of cane produced (irrespective of actual water usage) and (c) the fixed pumping and maintenance costs associated with applying the water. In general, the actual cost of the water is between $3 and 3.50/t cane. Thus, assuming an average water cost for the Delta growers of $3.25/t cane (including electricity/fuel, pump maintenance, depreciation and levies), an average production of 1241 cane/ha and a water use of 25 ML/year, the average cost of the water in this area is $16.12/ML. For these analyses, the gross return for cane production to the grower was assumed to be $35/

For the comparison between the furrow shape treatments, only the water cost was included as there was typically no additional time, equipment or resource implications associated with implementing this treatment. However, for the analysis of change in furrow length, the costs associated with the purchase and installation of additional water supply works were included, along with the production losses associated with the additional headland area. This analysis was conducted for a 12 ha block with 600 m furrows which could be split into two, 6 ha blocks with 300 m furrows. Two water delivery installation options were considered, a permanent underground pipe system with an assumed working life of 15 years, and a temporary above ground layflat flume system with an assumed working life of 5 years. All capital expenses are based on the retail cost of purchasing and installing the required works. Based on field observations for these soils, production losses associated with inadequate watering of the long furrows were also included in the analysis. It was assumed that there was no decrease in production and commercial cane sugar (CCS) for furrows up to 500 m in length. However, production was assumed to decrease linearly with furrow length after 500 m by 0.03 kg cane/m and CCS by 0.03 units/m. No allowance

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has been made in the analysis for any additional labour, tillage or harvesting associated with shorter furrows.

RESULTS

Furrow shape and surface compaction

The effect of furrow shape on the volumes of water applied in the first post hill-up irrigation at the Colevale and Maidavale sites are shown in Table 1. For both soils, the modified "v" shape furrow required the application of less than 40% of the volume applied to the normal 'u' shaped furrows. This effect appeared to be similar for subsequent irrigations throughout the season as the average volume of water applied for each of 15 irrigations monitored at the Home Hill site, were 1.99 ML/ha and 1.09 ML/ha for the normal and modified furrow shapes, respectively. No difference in production was observed at this site with the "u" and "v" shaped furrows producing cane yields of 161 and 1591/ha, respectively. As there were no additional capital or operating requirements to produce the "v" shaped furrows, and there was no difference in production, the only direct financial effect is associated with the reduced water usage. Based on water use figures at the Home Hill site, using "v" rather than "u" shaped furrows produced a financial benefit of $218/ha/yr. This represented a saving of $10,900 per annum for a 50 ha farm or $1.74M per annum for the local industry assuming that similar benefits could be obtained on one-fifth (c. 8000 ha) of the area farmed in the Burdekin Delta.

Table 1 *The effect of changing furrow shape on water usage in the post hill-up irrigation at the Colevale and Maidavale sites.*

Furrow shape		Irrigation water applied (ML/ha)		
		Colevale		Maidavale
		Average	Range	Average	Range
Norma	"u"	8.3	8.1-8.4	7.3	4.6-12.9
Modified	"V"	2.9	2.8-3.4	2.9	2.8-6.5

A substantial time saving (up to 40%) was also obtained by changing to the "v" shaped furrows from the "u" shaped furrows. At the Home Hill site, the average period of irrigation for a 470 m furrow was reduced from 12 to 8 h per irrigation set. This enabled the farm to be irrigated quicker and produced a number of other benefits including a reduced requirement for additional and/or larger pumping and supply systems, and the introduction of irrigation scheduling which was previously limited by the time required to irrigate.

Furrow length

Decreasing the furrow length from 600 m to 300 m would decrease the volume of irrigation water required to be applied from 1.78 to 1.03 ML/ha/irrigation. Table 2 shows the production costs and benefits associated with changing from the 600 m to 300 m furrow length. The costs associated with the two options for redistributing the water are also indicated. For both options, a positive economic return was found with an annual benefit of $210/ha and $132/ha for the temporary and permanent installations, respectively. This indicates that the financial return for implementing this change over an average 50 ha farm would be either $6600 or $10,500 per year depending on the water delivery system installed.

Table 2 *The annual costs and benefits associated with converting a 12 ha sugar cane block with 600 m furrow lengths into two, 6 ha blocks with 300 m furrow lengths.*

Item	Benefits/Cost ($)
Benefits	
Water saving	2080
Production gains	2052
Total	4132
Costs (Option 1 - Permanent installation)	
Pipeline ($20250 depreciated at 6.7% p.a.) 1350	
Risers ($3000 depreciated at 6.7% p.a.)	200
Fluming and cups ($610 depreciated at 20% p.a.)	122
Headland production (0.2 ha)	868
Total	2540
Costs (Option 2 - Temporary installation)	
Supply fluming ($2100 depreciated at 20% p.a.)	420
Fittings ($1000 depreciated at 20% p.a.)	200
Fluming and cups($610 depreciated at 20% p.a.)	122
Headland production (0.2 ha)	868
Total	1610

Note that in this example, almost half of the projected benefit is due to an increase in production associated with higher water distribution uniformities on the shorter furrows. Where there is no production benefit because the initial furrow length achieves high uniformities, the benefit associated with adopting shorter furrows is limited to the water saving. However, these water savings also decrease rapidly as the initial furrow length decreases, with the direct financial benefit greatly influenced by the cost of the water. It should also be noted that the actual return in each case may be smaller than that indicated as there is no allowance in these calculations for any additional labour, tillage or harvesting costs which may occur with shorter furrows.

CONCLUSION

Substantial improvements in the efficiency of furrow irrigation for sugar cane production can be obtained on high infiltration soils in the Burdekin Delta through the adoption of alternative irrigation management strategies. While the introduction of changes in furrow shape and furrow length may be substantial, further work is required to fully cost these irrigation strategies under a range of farm conditions and to include the effects on farm labour, tillage and harvesting requirements.

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THE EVAPORATION MINIPAN: A SIMPLE IRRIGATION SCHEDULING TOOL FOR THE CANEGROWER

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ABSTRACT

Participatory action learning processes have been an integral factor in the adoption of evaporation minipans as irrigation scheduling tools by Burdekin district canegrowers. Earlier attempts by Bureau of Sugar Experiment Stations (BSES) staff to facilitate irrigation scheduling have been uniformly unsuccessful. With evaporation minipans growers were encouraged to calibrate the minipan for their own soils, through a simple procedure of stalk measurement following an irrigation.

Improved irrigation scheduling with evaporation minipans has been shown to increase cane yield by 10-30%.

INTRODUCTION

The Burdekin district of North Queensland is the largest sugarcane irrigation area in Australia, with 60,000 ha currently producing 7.4 Mt tonnes of cane and production likely to reach 10 Mt tonnes by the year 2000.

Given the seasonally erratic nature of the district's rainfall, it is hardly surprising that Burdekin canegrowers rely almost exclusively on irrigation. In the newly expanded lands of the Burdekin River Irrigation Area (BRIA) growers apply on average 10 MLwater/ha/crop at water use efficiencies (WUE) of 8-121 cane/ML of irrigation water. Whereas on the freely drained soils of the traditionally farmed delta, most growers apply at least 20 ML/ha/crop predominantly from unmetered bores. WUE for the delta are in the order of 3-8 t cane/ML. Poor irrigation scheduling associated with low irrigation application efficiency has been largely responsible for these WUE being below the 12.2 t cane/ML, shown by Kingston (1994) to be achievable in field trials.

Historically, Burdekin cane growers have shown a reluctance to grasp the concept of irrigation scheduling. Crops have been irrigated from grower experience and often when visual signs of moisture stress are evident on sandy portions of blocks. There have been several attempts by BSES to encourage cane farmers to adopt less subjective irrigation scheduling practices, primarily involving predicted crop water use from daily evaporation figures. In one campaign in the early 1990s, growers were instructed to use a water balance chart system. Unfortunately, growers found these charts difficult to use. Furthermore, the use of imprecise estimates of plant available soil water capacity led to predictive inaccuracies and a lack of confidence in the system.

The introduction of the evaporation minipan came in 1991 when a **(BRIA)** cane farmer, Mr Mark Lewis, requested BSES assistance in setting up his own evaporation recording system. Using a section of a 200L salt drum to record evaporative losses and using calculated soil storage capacities, an appropriate minipan deficit figure was deduced. However, although a small number of minipans were being used by BRIA fanners on cracking clay soils the adoption rate between 1991 and 1994 was quite limited.

The initial use of minipans was in the BRIA, where the soils had been intensively mapped and reasonable estimates of soil moisture characteristics were available. This was not the case for the 40,000 ha of the Burdekin delta. Several attempts at selecting appropriate minipan deficit figures for delta soils were grossly inaccurate and so it was apparent that for the minipan concept to be more widely adopted, minipans must be calibrated for soil types.

Robinson (1963) showed the response of stalk growth to soil moisture levels, and Desornay and Davidson (1959) showed a correlation of 80% between stalk growth and final yield. Given this earlier work, stalk length measurement and crop growth rate were used to calibrate minipans for diverse soil types and cane varieties. This procedure not only provided growers with a fairly accurate reflection of the readily available soil moisture of a particular soil, but they became increasingly aware of the response of their crop to soil moisture level and the rapid decline in crop growth rate as soil moisture decreased.

MATERIALS AND METHODS

Growers were shown how to calibrate their individual minipans by marking out 25 stalks in a crop with full canopy cover, and measuring the height of each stalk to the top visible dewlap. The minipan was filled to simulate the soil being filled to field capacity (as it should be under furrow irrigation) immediately after an irrigation. Stalk height was recorded to determine the daily growth rate during the post-irrigation period. This allowed determination of when the growth rate had fallen to 50% of the observed maximum rate. At that time, the depth of water evaporated from the minipan was noted and became the minipan deficit figure for that block. Once the calibration procedure had been conducted by a key grower, small grower meetings (6-15 growers) were held. These gatherings were informal and organised by the key grower who invited neighbours along to discuss the results. After the meetings, growers wishing to conduct their own calibrations were supplied with minipans and measuring sticks by BSES. At these meetings, other irrigation topics were discussed including water use efficiency, soil moisture holding capacity and ways to improve irrigation application efficiencies.

RESULTS AND DISCUSSION

Minipan calibration

Over the 1994/95 summer, more than 70 Burdekin canegrowers collected their own crop growth rates, in order to calibrate evaporation minipans for their soils. Stalk elongation rates reached a peak 3-8 d after cessation of the irrigation (Fig. 1) and only maintained the maximum level for 2-3 d, depending on climatic conditions and soil type. Maximum stalk elongation rate of up to 48mm/d were recorded for Q127, 44 mm/d for Q96, and 38 mm/d for Q1 17.
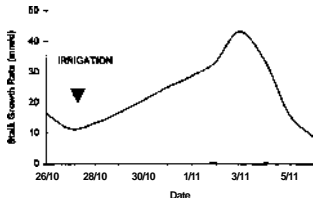
Fig. 1 *Typical stalk elongation growth rates after irrigation of an early plant crop ofQ96 on a sandy loam*

The calibration procedure was found to reinforce the concept of readily available soil moisture with significant differences found between the minipan deficit figures for each of the soil types. In the Burdekin Delta, minipan deficit figures varied from 50 mm for the sands to 120 mm on the clay loams. The cracking clay soils of the BRIA had a minipan deficit of 110-120 mm, whilst the deficit for the sodic duplex soils ranged from 60 to 110 mm. The measurement of crop growth rate not only highlighted the responsiveness of the crop to variations in soil moisture but also to growth reduction caused by overcast conditions.

Yield response to minipan scheduling

The introduction of evaporation minipans increased Burdekin cane yield for the 1994 and 1995 harvests. Representative responses to better irrigation scheduling using minipans can be seen in Table 1, which compares cane yields in the similarly dry production years (200mm effective rainfall) of 1993/94 and 1994/95. Usually cane crops produce lower yields with increasing ratoon age, but this was not the case with the irrigation scheduling comparisons harvested in 1994. Scheduled crops harvested in 1994 produced more cane than their unscheduled earlier ratoon crops.

Table 1 *Yield response (t/ha) to irrigation scheduling using evaporation minipans (R=ratoon)*

Soil	Variety	Unscheduled 1993 (t/ha)	Scheduled 1994 (t/ha)	Production Increase (%)
Alluvial	Q117	124 (1R)	136 (2R)	10
Duplex	Q117	107 (1R)	120 (2R)	12
Duplex	Q96	88 (2R)	110 (3R)	25

At one of the minipan calibration sites in the BRIA during the 1994/95 season there was difficulty obtaining sufficient irrigation supply to irrigate on the minipan schedule. As a demonstration area, a section of a block of late plant cane was irrigated at a higher minipan deficit figure (150 mm) than indicated by the calibration procedure (120 mm). The results show a substantially lower cane and sugar yield for the section of the block irrigated on the basis of the 150mm deficit (Table 2).

Benefits of the calibration procedure

The participatory action learning process used in the calibration of the minipans was instrumental in the ready adoption of evaporation minipans as an irrigation scheduling tool. By the end of November 1995, more than 180 minipans had been distributed to Burdekin growers, whilst a further 30 were being used by canegrowers in the Mareeba and Proserpine districts.

Table 2 *Effect of minipan deficit on cane and sugar yields*

Minipan deficit (mm)	Cane yield (t/ha)	CCS (t/ha)	Sugar yield (%)
120	132.9	15.6	20.73
150	104.2	15.6	16.26

Through the involvement of growers in the collection of the crop growth rate data, growers became aware of the responsiveness of stalk growth (as elongation) to soil moisture levels and the difficulty of visually identifying the initial reduction in crop growth rate as the soil moisture level was depleted. More than 80% of growers involved in the calibration procedure became advocates of the evaporation minipan system, adding further credibility to the concept which helped to extend the system to a broad section of the Burdekin canegrowing community.

Potential productivity gains through better irrigation scheduling across the entire Burdekin district (60,000 ha) were conservatively estimated at a 10% yield increase on one third of the area cropped to sugarcane. With an average yield of around 124 t/ha this represents an increase of 12 t/ha over 20,000 ha or 240,000 t cane per year. The current value (to both millers and growers) of this additional production is in excess of $12 million annually.

CONCLUSION

Evaporation minipans have gained a significant foothold in the Burdekin cane growing culture. They are seen as a simple yet effective way of irrigation scheduling. The participatory process used to calibrate the minipans and the group activities disseminating the data no doubt helped reinforce the concept within the cane farming community.

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SUSTAINED INTENSIVE CROPPING USING DRIP IRRIGATION- A CASE STUDY

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ABSTRACT

This paper examines the potential benefits of using drip irrigation for sugarcane, some concerns with its implementation, and management considerations to achieve the system's potential. Drip irrigation has the potential to place water together with nutrients and/or pesticides directly into the zone of maximum root density. This can optimise water use efficiency. Increased yields of approximately 30 % for the same quantity of water are reported. Further development work is required to determine the ideal depth and horizontal placement of the tape, spacing of emitters, and the scheduling of water use for the range of soils used for sugar production. On-farm management of drip irrigation requires a high level of expertise and extension agencies can assist with adoption by providing technical information, training and monitoring of systems. This paper examines the potential of drip irrigation by use of a case study where the system is in commercial use.

INTRODUCTION

The recent drought in Queensland has focused attention on the value of irrigation to sugarcane production. In the 1970s, a major irrigation scheme was implemented in the Bundaberg district to provide supplementary irrigation to the district which is one of the driest sugar producing areas in the state.

The Bundaberg Irrigation Area irrigates 57800 ha of crops (QDPI Annual Report 1993-94). The area of sugarcane irrigated in 1993-94 was approximately 47240 ha. The nominal water allocation for irrigation is 238497 ML or 4.13 ML/ha(413 mm).

Kingston (1994) showed that the long term average annual water requirement for optimum yield of sugarcane at Bundaberg is 1450 mm. He calculated the average effective rainfall for the period 1980 - 90 to be 786 mm, leaving a deficit of 664 mm to be met by irrigation. Therefore, the irrigation required for optimum yield, assuming an irrigation distribution efficiency of 70 % was 949 mm over this period.

This large discrepancy between irrigation requirement (949 mm) and water available (413 mm) has focused attention on methods of improving irrigation efficiency such as drip irrigation. Hewson et al (1995) state that efficiency of water application with drip irrigation approximates 90% compared to around 70% for water winches and 60% for furrow irrigation. This improvement in irrigation efficiency has prompted the development of drip irrigation at Bundaberg.

With available irrigation water and suitable agricultural land now close to maximum utilisation, there is a need for the sugar industry to look to vertical expansion rather than lateral expansion while not causing environmental problems. This is particularly important as the sugar industry seeks to develop a clean, green image. Recent studies (Keating et al 1995), have identified the potential for nutrient and pesticide contamination of groundwaters. This is of particular concern in the Bundaberg area where the groundwater system, consisting of multi-strata aquifers, also supplies much of the domestic water for the Bundaberg area.

This paper examines the use of drip irrigation to optimise the u scarce water resource while at the same time, minimising mover excess irrigation through to the ground water. A case study on one property which has implemented the use of drip irrigation is outlined with its concerns and benefits. Measurements of water distribution using a drip system are presented and the ramifications of the use of this technique for the sugar industry are discussed.

CASE STUDY

Farmer-initiated trials with sub-surface drip irrigation began on the Churchward farm in 1992. The Red Podzolic (Red Dermosol) soils have a loamy sand surface horizon to 0.15-0.35 m. The drip system was examined as a potential alternative to overhead spray irrigation through water winches which needed to be used weekly on the farm's sandy soils to supply crop water requirements, and were expensive to power. The trials also sought to understand the elements of yield decline/ soil pathogens which inhibit cane growth where monoculture has been practiced over many years.

Churchward & Curd (1995) found that drip irrigation installed at 300mm depth increased cane yield by 25-30 t/ha compared with water winches. There were additional financial gains due to lower pumping costs and electricity costs per megalitre for drip (about one third those for water winches). These on-farm observations prompted the further development of drip irrigation on the Churchward farm. Once the system was operating, the importance of irrigation scheduling became clear.

The EnviroSCAN (Sentek Pty. Ltd.) soil moisture measuring equipment was installed on the Churchward farm to improve the soil moisture monitoring process and assist irrigation scheduling decisions. In conjunction with this equipment, the Department of Primary Industries installed gypsum blocks to investigate the soil moisture distribution pattern around the emitter. While it is recognised that gypsum blocks are not reliable at the wetter end of the moisture range, they will indicate a wetting front.

EnviroSCAN sensors

The EnviroSCAN soil water monitoring system provides a continuous measure of the volumetric moisture (mm) down the profile. Sensors were installed at 5 depths (100,200,400,600 and 1000 mm) recording at 30 minute intervals. A graphical output of volumetric moisture over time can be produced at the various depths (Fig. 1). From this data, irrigation refill points can be determined and strategic irrigation scheduling can be applied for optimum plant growth.
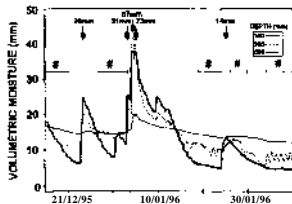
Fig. 1 *Change in volumetric soil moisture (mm) over time for three depths using EnviroSCAN monitoring equipment. Rainfall (˜) and irrigation(#) events are indicated.*

Following the installation of an EnviroSCAN, increased volumetric moisture was detected at the 1000mm sensor following irrigation (data not shown). This indicated that the schedule supplying 18mm over 12 hours every third day was causing deep drainage losses due to the low water holding capacity of the wetted area. To avoid these losses, the schedule was reduced to 3 hours every day supplying 4.5 mm (eg. Fig. 1 at 600mm depth)

Gypsum blocks

Gypsum blocks   were installed at 5 depths (100, 300, 600, 1000 and 1200 mm). Three lateral spacings were chosen: on row, 200 and 400 mm from, but parallel to, the row and 3 distances from the emitter (0,200 and 300 mm) with this layout replicated once (Fig. 2). Blocks were not positioned at all locations, but were strategically placed to monitor the wetting front. Installation occurred immediately after the August 1995 harvest to allow for soil consolidation. Soil'water potential was recorded at 30 minute intervals.
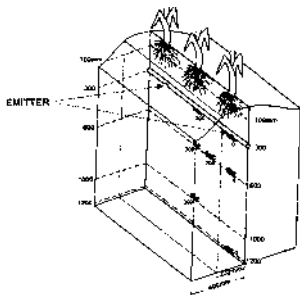
Fig. 2 *Gypsum block layout for 5 depths, 3 lateral spacings parallel to row and 3 distances from emitter. (200mm and 400mm positions not shown)*

Soil moisture potential monitoring over time for three depths, on the row showed the rate of wetting and drying occurring down the profile (Fig. 3) . Despite the poor sensitivity of gypsum blocks at potentials near field capacity, some distinct moisture patterns emerged. At the 100 mm depth, there was a decrease in soil moisture potential under daily irrigation whilst the 300 and 600 mm depths remained at approximately field capacity. Rainfall on the 24/01/96 refilled the soil profile at 100 mm to field capacity and   reduction   e potential recommenced from the 29/01/96.

The loss of moisture at the 100 mm depth indicated that this was the active root zone supplying the required amounts of water and nutrients for plant growth. The occasional small change in moisture potential at 300 mm would indicate that little water was used at this depth while virtually no change was recorded at 600 mm, indicating minimal use from this layer. Gypsum blocks located at 1000 mm and 1200 mm (data not shown) remained at potentials less than -100 kPa for regular irrigation of 4.5 mm for 3 hours. However wetting up of the profile to these depths (< -50 kPa) did occur after several large rainfall events. This would suggest that deep drainage was not occurring with irrigation.

The sub-surface drip system may be used to apply fertilisers such as nitrogen and potash. On farm experience suggests application rates of nitrogen may be reduced from 160 to 130 kg N/ha to obtain the same yields.

Tape maintenance

The way drip irrigation is operated and maintained is critical to its success (Hewson et al 1995). Batchelor & Soopramanien (1993) stated
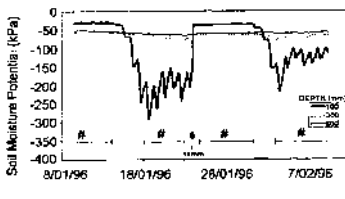
Fig 3 *Change in soil moisture potential (kPa) over time for three depths as measured by gypsum blocks. Rainfall (¯) and irrigation(#) events are indicated.*

that the drip irrigation systems in Mauritius tended to fail catastrophically when not maintained correctly. Steps should be taken to avoid roots intruding into the emitters and to prevent dirt and algal build up in the tubes. Regular flushing (once/week) of dripper lines by opening the flushing valve, together with preventative chlorine and acid treatments are essential to keep the system operating efficiently.

The keys to successful drip irrigation maintenance are well-trained and motivated system operators and access by the system operators to manufacturer's instruction manuals. Most training in the Bundaberg area has been by the tape manufacturers and resellers. BSES have also set up User Groups to facilitate the transfer of information and experiences between users and has produced the Reference Manual on Drip Irrigation for Sugarcane (Hewson et al 1995) to complement manufacturer's current manuals such as Netafim's Installation and Maintenance Manual and T-Tape's General Maintenance and Injection Procedures.

CONCLUSIONS

The experience on the sandy, infertile soils of the Churchward farm in the Bundaberg area has demonstrated that sub-surface drip irrigation is a viable alternative and has the potential to produce more cane from the restricted water resource. By placing the irrigation water in the active root zone, higher water use efficiency can be achieved and environmentally detrimental deep drainage reduced. The application of this technology requires a higher level of management and the regular use of monitoring systems. Further studies into the water application rates and tape placement for the range of soils occurring in this district are required.

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REDUCING SOIL PHYSICAL CONSTRAINTS UNDER SUGARCANE

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ABSTRACT

There is incompatibility between crop row spacing and equipment track widths in the current management system for growing sugarcane. This work compares growing sugarcane in 1.5m rows with 1.8m dual rows as a strategy to reduce soil physical constraints to sugarcane productivity. Trials were established at Tully and Ingham to cover a range of environmental conditions. Undisturbed soil cores were collected to measure bulk density and saturated hydraulic conductivity. Soil cone resistance was measured in the field to assess the effect of traffic.

Bulk density tended to be higher in the near-row and row position under 1.5m rows compared with 1.8m dual rows. Saturated hydraulic conductivity and cone resistance reflected the density results. The 1.8m dual rows yielded higher than the 1.5m rows, with one exception where Q138 yielded higher in 1.5m rows. It is suggested that keeping infield traffic further away from the crop row results in less compacted soil near the crop growth area. This in time may translate into reduced yield decline over the crop

INTRODUCTION

Productivity loss through soil structural degradation and soil compaction is of increasing agricultural concern. This can occur through excessive cultivation (Adem et al 1984) and high axle load traffic at inappropriate soil water contents (Voorhees et al 1986). Controlled traffic has been instigated to reduce structural decline (Tisdall & Adem 1988) and to restrict the spread of soil compaction (Taylor 1986). In some instances there was little or no yield response due to controlled traffic (Williford 1985; Braunack et al 1995). However, in other instances significant yield increases have resulted from the adoption of controlled traffic (Perdok & Lamers 1985; Hadas et al 1990).

This paper presents data from a project investigating the effect of matching crop row spacing to equipment track widths on soil properties and ratoon yield of sugarcane.

MATERIALS AND METHODS

Field trials have been established in north Queensland on BSES Sugar Experiment Station Tully and on a cooperator's property at Ingham. The soil types are classified as Uf 6.34 and Ug 3.2 (Northcote 1979) for Tully and Ingham, respectively. Some physical and chemical properties are given in Table 1. The Tully soil is non shrink-swell whereas that at Ingham has a slight self-mulching tendency (it cracks when dry).

Trial details

Trials were planted at Tully and Ingham in 1993 and 1992, respectively. Treatments consisted of planting single rows 1.5m apart (current commercial practice) compared with dual rows (0.3m apart) at 1.8m spacing. Both treatments were fertilised at the same rates based on area. Plots at Tully consisted of 7 rows by 17m long with four replicates, while those at Ingham were 4 rows by 30m long with five replicates. Trials were harvested with conventional equipment common in each area (wheel track width 1.8m). Each interrow was trafficked twice by the harvester and at least once by a haulout, with some being trafficked twice.The soil at the time of harvest was below the plastic limit at both sites (Table 1). The experimental layout was a randomised block design with results analysed by ANOVA. Two cane varieties were grown at each site, namely Q117 and Q138 at Tully and Q115 and Q124 at Ingham.

Soil measurements

Undisturbed soil cores (75mm diameter, 50mm high) were taken to a depth of 300mm from the row position one week before and usually one week after harvest in 1994 and 1995 at Tully, and 1993 and 1994 at Ingham, to determine bulk density (BD) and saturated hydraulic conductivity (Ks). Soil cone resistance as a measure of soil strength was measured before and after harvest.

Table 1 *Selected soil propeties at each experimental site in north Queensland*

Site	Depth (mm)	Clay <2m (%)	Silt 2-20m (%)	Sand 20-2000m (%)	pH (1:5)	Ca	Mg (cmole/kg)	K	PL (w/w)
Tully	0-100	45.5	24.5	30.8	5.60	2.6	0.8	0.20	32
Ingham	0-250	44.8	25.5	27.3	4.65	4.2	3.2	0.46	32

PL= Plastic Limit

Crop measurements

Crop response was determined from stalk counts and height measurements at regular intervals through the season. Crop yield was assessed by weighing the two central rows in each plot at harvest.

RESULTS

Soil properties

These data pertain to the row position only and for the first ratoon crop. There was no significant difference in bulk density between the 1.5m and 1.8m systems at both Tully and Ingham, although BD tended to be higher at the 1.5m spacing. There were, however, significant differences with depth, with the 0-100mm layer having lower BD compared to the 150-300mm depth (Table 2).

Table 2 *Changes in soil bulk density (Mg/m²) with depth under single (1.5m) and dual (1.8m) rows at Tully and Ingham experimental sites in north Queensland.*

Site	Depth (mm)	Management	
		1.5m	1.8m
Tully	0-50	0.95	0.90
	50-100	1.07	1.02
	100-150	1.05	1.04
	150-200	**1.09**	**1.10**
	200-250	**1.14**	**1.13**
	250-300	**1.11**	**1.07**
Ingham	0-50	0.83	0.90
	50-100	1.06	1.01
	100-150	**1.10**	**1.01**
	150-200	1.05	1.01
	200-250	**1.06**	**1.03**
	250-300	1.05	1.10

Tully LSD (P<0.05) = 0.09; Ingham LSD (P<0.05) = 0.08

Saturated hydraulic conductivity was higher at the shallow than the deeper soil depths, and tended to be higher under the 1.8m than 1.5m system at both sites (Table 3). This is in agreement with the BD measurements. There was, however, no significant difference between the 1.5m and 1.8m systems.

Table 3 *Changes in saturated hydraulic conductivity (mm/s) under single (1.5m) and dual (1.8m) rows at Tully and Ingham experimental sites in north Queensland.*

Site	Depth (mm)	Management	
		1.5m	1.8m
Tully	0–50	0.67	0.67
	50–100	0.28	0.57
	100–150	0.32	0.37
	150–200	0.22	0.25
	200–250	0.17	0.37
	250–300	0.32	0.57
Ingham	0–50	0.82	1.27
	50–100	0.43	0.60
	100–150	0.33	0.74
	150–200	0.43	0.67
	200–250	0.52	0.48
	250–300	0.32	0.40

Tully LSD (P<0.05) = 0.61; Ingham LSD (P<0.05) = 0.25

Soil cone resistance was variable with no distinct trend between the single and dual rows being evident (Fig. 1). Cone resistance for 1.5m rows tended to be slightly greater at Tully, and lower at Ingham, than for 1.8m rows. A zone of high resistance occurred in the surface soil layer under 1.5m rows at both sites. This zone extended to 150mm depth at Tully, but only to 80mm at Ingham (Fig. 1). Generally cone resistance increased with depth for both sites.
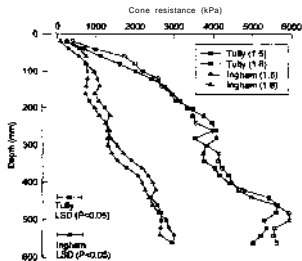
Fig. 1 *Soil cone resistance under single and dual rows.*

Crop response

Since crop response in stalk number and height was similar at both sites, only data for Tully is presented. There were no significant differences between 1.5m and 1.8m rows, however, early population counts show that for the plant and first ratoon crop the 1.8m rows contained a slightly higher stalk population than the 1.5m rows. However, the reverse was the case for the late season counts (Fig. 2). Stalk heights at maturity were slightly higher with the 1.5m rows than with the 1.8m rows, but there was some variation during early growth (Fig. 3). There were, however, significant differences between varieties. Cane yield response to management varied between sites with the 1.8m rows yielding less than the 1.5m rows at Tully, but the reverse occurred
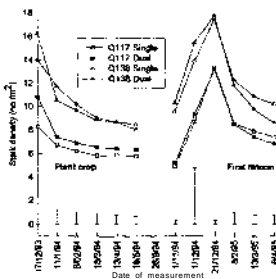
Fig. 2 *Trends in stalk density with time for plant and first ratoon crop of two cultivars (Q117, Q138) grown in single (1.5m) and dual (1.8m) rows at Tully. Vertical bars are LSD(P<0.05) between treatments at each time of measurement.*
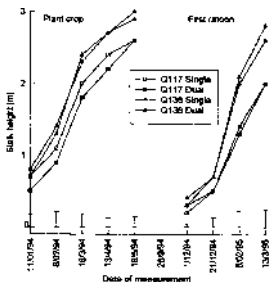
Fig. 3 *Stalk heights for plant and first ratoon crop of two cultivars (Q117, Q138) grown in single (1.5m) and dual (1.8m) rows at Tully. Vertical bars are LSD (P<0.05) between treatments at each time of*

Table 4 *Cane yield (tonnes/ha) for Q117 & Q138 at Tully and Q115 & Q124 at Ingham grown in single (1.5m) and dual (1.8m) rows.*

Site	Crop	Variety	Management		LSD
			1.5m	1.8m	(P<0.05)
Tully	Plant	Q117	89.7	97.7	
		Q138	111.6	102.4	12.9
	1st Ratoon	Q117	94.1	92.3	
		Q138	129.4	101.1	17.5
Ingham	Plant	Q115	87.1	91.6	
		Q124	109.4	113.9	13.3
	1st Ratoon	Q115	81.6	84.7	
		Q124	97.9	101.6	6.2

at Ingham (Table 4). Although there was no difference between 1.5m and 1.8m rows there was a significant difference between varieties (Table 4).

DISCUSSION

There is some indication that BD increased more under the row with 1.5m rows than it did with 1.8m rows. This was presumably due to the proximity of traffic to the row in the narrow system compared with the wider system possibly resulting in lateral movement of soil closer to the stool. The Ks values also reflect this result. Soil cone resistance measurements suggest that a zone of high strength may be developed at a shallower depth with 1.5m rows compared with 1.8m rows. The increased BD and cone resistance would restrict root growth and reduce root exploitation of the profile. The reduced Ks may result in greater runoff and less water movement through the profile. Depending on seasonal conditions this may result in water stress and yield loss. It is speculated that in time the root growth zone would be reduced to a greater extent under 1.5m rows than under the 1.8m rows. Crop growth showed variable response to the changes in soil physical properties, a similar result was observed by Williford (1985). There is a need for long term data to enable valid assessment of the benefits or otherwise of matching crop row spacing and equipment track widths. This is needed because sugarcane is grown in the same rows for up to five years and experiences the same traffic intensity each year. Seasonal conditions can vary greatly thus the need for long term data. Previous studies have concentrated on soil properties in the seedbed, whereas in this study we are attempting to study the effect of harvesting traffic and the benefit of increasing the distance of traffic from the stool area and subsequent crop response.

There is a greater chance for direct damage to the stool by harvesting traffic under 1.5m rows than under 1.8m rows This is due to the incompatibility between current row spacing and equipment track widths. However, the problem of elevator length needs to be resolved when harvesting 1.8m rows as the current elevators are too short to enable even filling of haulout bins. However, visibility may be improved at the wider spacing, thus allowing more accurate trafficking during harvest. By restricting soil compaction, soil degradation will be reduced and more favourable root growth zones may develop with time, especially in conjunction with reduced tillage.

CONCLUSION

Soil physical properties in the row were less favourable for plant growth under 1.5m rows compared with the 1.8m rows due to the proximity of traffic to the stool.

However, crop response was variable to these changes in soil properties.

ACKNOWLEDGMENTS

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4.4 Disease and pest management

THE YELLOW SUGARCANE APHID: A POTENTIAL THREAT TO THE AUSTRALIAN SUGAR INDUSTRY

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ABSTRACT

Yellow sugarcane aphid is a potential pest of sugarcane, sorghum, cereals and pastures in northern and eastern Australia. It feeds on a wide variety of grasses and can have numerous generations in a year. The most likely source of introduction is Hawaii. Insecticides and parasite introductions are potential methods of control, but host-plant resistance probably offers the best long-term option for management. Preemptive and postintroduction responses for the Australian sugar industry are outlined.

INTRODUCTION

In the last 2 years at least seven insect pests of horticulture, cotton, turf and pastures have been introduced into Australia. The response to each has been to first determine the extent and potential distribution of the introduction, the crops at risk and the likely economic losses, the biology and ecology of the pest, and control options. This information is essential to the development of rational response and management options, but the time taken to amass such data (often from dispersed sources) has lead to delays in implementation of the appropriate response. Such delays allow the introduction to establish and spread.

The Australian sugar industry faces a number of potential pests from Indonesia, Papua New Guinea and Hawaii. There are more than 72 potential sugarcane pests in Papua New Guinea alone, some of which are innocuous in that country, but may become pests in a new environment. The establishment of the Ord River sugar industry has increased the potential for the introduction of new pests; the Ord area is in the same weather pattern as central Indonesia.

The introduction of a new exotic pest would place an additional burden on the Australian sugar industry by adding costs and decreasing productivity. For example, if sugarcane borer had been kept out of Australia, the industry would not now be facing losses of $1.8M per year. Delays in dealing with introductions allow the pest time to establish and spread. These delays can be minimised if contingency plans have been developed BEFORE the pest is introduced. A similar approach has been taken by the Australian wheat industry in response to the threat posed by Russian wheat aphid (Hughes & Maywald 1990).

Here we outline one approach to a contingency plan using the yellow sugarcane aphid (YSCA), *Sipha flava* (Forbes), as an example. The components of the contingency plan are:
(i) Determine which species are most likely to be introduced and cause economic damage (in this case YSCA).
(ii) Collect available literature on YSCA and use data on biology and distribution with the CLIMEX climate-matching program to determine likely establishment and spread within Australia,
(iii) Determine the damage caused and the likely economic impact,
(iv) Determine whether other agricultural industries would be affected,
(v) Determine available control measures and provide recommendations for preemptive and postintroduction responses.

The Australian government, as a signatory to the International Agreement on the Application of Sanitary and Phytosanitary Measures, has an obligation to provide pest risk data to support cases for excluding the importation of plant material and plant products. This agreement places an increased emphasis on being able to assess the likely threat of the introduction of exotic pests into Australia.

DESCRIPTION AND BIOLOGY OF YSCA

The wingless adult is small, oval, and yellow with numerous long bristle-like hairs on the body; hair are often paired. The dorsum is covered with transverse intersegmental markings. The winged adult has a yellow

abdomen with a variable dorsal pattern of dark markings (Blackman & Eastop 1985). This combination of colour, hairs and dorsal markings distinguishes it from Australian aphids.

Over much of its range YSCA reproduces asexually with sexual reproduction only occurring in regions with winters that are sufficiently cool. Both sexual and asexual reproduction occurs on grasses. Development is temperature dependent with a generation being completed in as little as 6 days and with as many as 16 generations occurring over an 8 month period (Gaud et al 1967).

HOST RANGE

YSCA feeds on the undersides of leaves of a range of tropical and temperate grasses including species of *Andropogon, Avena, Axonopus, Chloris, Digitaria, Echinochloa, Eragostris, Eriochloa, Festuca, Holcus, Hordeum, Leptochloa, Lolium, Panicum, Pennisetum, Paspalum, Polytrias, Saccharum, Setaria, Sorghum, Trichachne, Triticum* and *Zea* (Gaud et al 1967). Crop hosts often infested include sugarcane, sorghum and millet. In the USA, wheat and other cereals are affected every 10 years or so.

CURRENT DISTRIBUTION

Until 1988, YSCA was confined to the continental USA (Texas, Oklahoma, Louisiana, Florida, Georgia, Kansas, Arkansas), the Caribbean (Jamaica, Dominican Republic, Puerto Rico, Cuba, Barbados) and Central and South America (Mexico, Venezuela, Colombia, Trinidad & Tobago, Guyana, Argentina). In late 1988, YSCA was detected for the first time in Hawaii.

Fig. 1 *The predicted range of YSCA in Australia.*

PREDICTED DISTRIBUTION IN AUSTRALIA

Despite YSCA being considered a serious pest of several grass crops and pastures, there has been very little work on its biology. Consequently, there is no specific biological information which is useful

for the usual way in which the CSIRO climate matching program CLIMEX (Sutherst & Maywald 1985) is used. However, by choosing the climates of locations within the known distribution of YSCA in the USA and the Caribbean a useful prediction of its likely distribution within Australia can be made. For this prediction we have used Tulsa (Oklahoma), Brownsville (Texas), Thomasville (Louisiana) and Santo Domingo (Puerto Rico). Figure 1 illustrates the predicted range within Australia where the climate match is 50% or greater to at least one of the three locations used. CLIMEX predicts that the eastern coast of Australia is at greatest risk from YSCA, including areas with major sugarcane production and tropical pastures.

PEST STATUS

Sugarcane

YSCA causes damage through both the transmission of sugarcane mosaic virus and feeding. In sugarcane, affected plants become stunted and feeding causes leaves to turn yellow then red. Iron-coloured spots often occur as feeding continues. Leaves eventually turn brown and die. Newly planted cane and recently ratooned cane are generally worst affected (Gaud et al 1967). In Puerto Rico, YSCA is mainly a pest during the warmer months although dry spells and drought greatly increase the level of damage. Populations often have a spring and autumn peak with the spring infestation causing more damage (Gaud et al 1967; Miskimen 1970; Oakes & Sierra-Bracero 1972). On sugarcane in Hawaii, YSCA is considered a chronic pest. Damage is most severe in hot, dry conditions when cane is under stress. These conditions occur in Aug/Sept and are thought to suppress natural enemy activity. Feeding damage can lead to severe reductions in plant growth, with total crop loss a possibility. Aponte et al (1989) suggest an economic injury level (EIL) of 4 aphids/plant by 20 days after emergence. YSCA may also become a problem after rain when waterlogging results in nitrogen shortage (Metcalfe 1965).

Sorghum

YSCA is an occasional pest of sorghum in the USA. When it occurs YSCA can be very damaging causing significant reductions in yield as well as delaying maturity and so exposing the flowering panicles to higher densities of sorghum midge than would otherwise occur. Feeding damage causes leaf yellowing and death. Plants are usually infested at the seedling stage and only for a short time, but at this stage infestations of 1 per plant can cause total plant losses. Infestations of as few as 5-10 aphids per plant can kill plants <450 mm tall (Webster 1990). When seedlings are infested panicle yield can be reduced by 2.5% per aphid (Breen & Teetes 1986). Older plants are more tolerant of infestations. The cause of the damage is uncertain, but a toxic saliva is suspected (Breen & Teetes 1986). The EIL is dependent on the age of the crop. For plants 35 mm tall the EIL is 27% of plants infested with at least 1 aphid, 43% for plants 45 mm tall and 100% for plants that are 55 mm tall.

Pastures

In Hawaii, YSCA causes severe damage to tropical pastures. In the USA, Puerto Rico and the Virgin Islands, for tropical pasture grasses such as pangola grass, losses of up to 75% plant protein content readily occur (Oakes & Sierra-Bracero 1972).

Cereals

In the USA, YSCA is an infrequent pest of cereal crops where it causes economic losses in small grains every 10 to 15 years (Webster et al 1994). YSCA is especially damaging as infested plants fail to recover even after spraying has removed the aphids.

MANAGEMENT

In sugarcane, YSCA appears to be most of a problem in young cane or during dry periods when it can retard growth. Periods of heavy rain will reduce numbers (Miskimen 1970), similar to that seen in Australia with grey sugarcane aphid (*Melanaphis sacchari* (Zehntner)), but excessive rain will cause short-term nitrogen deficiency and increase the severity and probability of damage (Metcalfe 1965).

Insecticides

A wide range of general aphicides have been used for control of YSCA; demeton-S-methyl, diazinon, dimethoate, malathion, methyl-parathion,

mevinphos, fenitrothion, phorate and temephos (Metcalfe 1965; Gaud et al 1967; Denmark 1988; Aponte et al 1989). Of these only diazinon is registered for use in Australian sugarcane (for locust control; Allsopp et al 1993); rates used for locusts are likely to be effective against YSCA. Other insecticides would need maximum residue levels (MRLs) to be set before they could be used in Australia.

More recent insecticide screening in Hawaii has shown that bifenthrin at 112 g ai per hectare suppressed YSCA for at least 5 weeks after treatment, that imidacloprid at 67 g ai per hectare suppressed YSCA for 4 weeks and that acephate at 280 g ai per hectare suppressed YSCA for 3 weeks (Ota 1995). Chlorpyrifos was ineffective in these experiments. None of these are registered for use on Australian sugarcane, although MRLs are being developed for bifenthrin and imidacloprid as part of field testing these compounds for the control of other sugarcane insects.

Natural enemies

A wide range of predators has been implicated in control of YSCA; coccinellids, lampyrids, chrysopids, syrphids, anthocorids, reduviids, spiders, birds and lizards (Hayward 1944; Guagliumi 1962; Metcalfe 1965; Gaud et al 1967; Osada Kawasoe 1969; Miskimen 1970). These are all generalist aphid predators, responding to population increases in any aphid species. Given their generalist nature, they would not be likely candidates for introduction to Australia. However, Australia already has a similar general-predator fauna and mese species probably will attack YSCA; many already occur in infestations of grey sugarcane aphid.

The aphidiid *Lysiphlebus testaceipes* (Cress.), the eulophid *Pachyneuron siphonophorae* (Ashmead) and the encyrtid *Homalotylus flamineus* Dalm. have been recorded parasitising YSCA in Cuba and Puerto Rico (Guagliumi 1962; Starii 1967), although Gaud et al (1967) casts doubt on the records of the first two with YSCA. Suitable natural enemies were not found in a search in South America by Hawaiian entomologists (Ota 1995), but natural enemies of a closely related aphid exist in Pakistan and may prove useful.

The fungus *Acrostalagmus aphidium* Pruess appears to play an important role in control of YSCA, although its effectiveness is limited by climatic factors (Metcalfe 1965; Gaud et al 1967; Miskimen 1970).

Host-plant resistance

Differences in susceptibility of sugarcane cultivars offers a potential method for managing YSCA (Metcalfe 1965; White 1990), with antibiosis, antixenosis and tolerance being noted in several cultivars. However, all sugarcane cultivars tested by Aponte et al (1989) were susceptible and we know of no recent ratings of sugarcane cultivars and of no ratings of Australian cultivars. Antibiosis has been seen in some lines of sorghum (Webster 1990) and tolerant lines of wheat have been bred in the USA (Merkle et al. 1991). Leaf surface pubescence has also been found to be an effective resistance mechanism in wheat (Webster et al 1994) and there is considerable variation in pubescence in Australian sugarcane cultivars. Cultivars of pangola grass also differ in their susceptibility to this aphid (Oakes & Sierra-Bracero 1972).

Cultural controls

Recommended cultural controls rest on variation in planting dates, on not burning cane before harvest, and on promoting good cane growth. In Venezuela, summer plantings (September) are more prone to YSCA attack than are rainy-season plantings (April-June) (Aponte et al 1989). This correlates with the reduction in YSCA numbers after heavy rains seen by Miskimen (1970). Burning cane at harvest may have a short-term effect in reducing YSCA numbers, but it also reduces predator numbers and could lead to aphid resurgences on the young ratoon crops (Metcalfe 1965).

AUSTRALIAN RESPONSES TO YSCA

The Australian sugar industry needs to be aware of the potential for the introduction of YSCA, especially from Hawaii, and the damage that it may cause once established. Two groups of responses are appropriate:

those implementable before the introduction of the aphid, and those implementable after any introduction. However, we believe that host-plant resistance offers the best long-term option for minimising the impact of YSCA. In addition, there needs to be an awareness of the potential impact of this species on industries based around tropical pastures and on coarse-grain production in Queensland.

Preemptive responses

(i) Obtain data on efficiency and residue levels of bifenthrin and imidacloprid from Hawaii, combine with MRL data from screening tests in Australia, and apply to the National Registration Authority for registration of these materials for use against YSCA.

(ii) Monitor the progress of parasite introductions in Hawaii and consider the introduction of effective agents before the introduction of YSCA.

(iii) Publicise the potential for the introduction of YSCA, the identification of YSCA, the possible effects on the industry of such an introduction, and the immediate responses to an introduction.

(iv) Obtain data from Louisiana, Florida, Hawaii and other recipients on the relative susceptibility of Australian cultivars.

Postintroduction responses

(i) Determine the geographic range of YSCA within Australia.

(ii) Attempt to contain the spread by application of insecticides and quarantine of affected areas,

(iii) Determine cultivar susceptibility,

(iv) Determine the degree of control afforded by indigenous natural enemies.

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EFFECT OF PLANTING AND HARVESTING DATE ON GREYBACK CANEGRUB DAMAGE TO SUGARCANE GROWN IN THE BURDEKIN RIVER AREA

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ABSTRACT

In the 1993/94 and 1994/95 cane growing seasons, harvest date and planting date had a significant effect on greyback canegrub attack. Cane planted early was more likely to be attacked than cane planted later in the same season. Likewise cane harvested early was more likely to be attacked than cane harvested later. It is hypothesised that this is a result of differences in cane height at the time of beetle oviposition.

INTRODUCTION

Greyback canegrub, *Dermolepida albohirtum* (Waterhouse), is the principal pest of sugarcane in the Burdekin River sugar-growing area in northern Queensland. Greyback canegrub is univoltine with beetle emergence and oviposition occurring between October and January. Damage becomes apparent between February and July and is the result of larval feeding on the roots and underground portion of the cane plant (stool) (Allsopp et al 1993). Badly damaged cane is usually ploughed out after harvest and replanted. Damage is currently estimated to cost the sugar industry $5M annually (Anon 1995).

In the Burdekin River sugar-growing area, all cane is irrigated and is grown in a crop cycle usually lasting 3-4 years. The cane is harvested annually and allowed to regrow (ratoon). The insecticide suSCon Blue (140g/kg controlled release chlorpyrifos) is the only chemical currently registered to control greyback canegrubs and has failed to control grubs on approximately 100 farms in the Burdekin (Robertson et al 1995). This is possibly the result of microbial degradation of chlorpyrifos. In the absence of effective chemical control it has become necessary to develop cultural control techniques to minimise economic loss. To facilitate this, a better understanding of greyback canegrub biology is required.

Greyback canegrub attack does not appear to be random with some blocks being severely damaged whilst those adjoining them remain undamaged (AL Ward, unpublished data). An understanding of spatial distribution may allow damage to be concentrated into blocks of cane where economic loss is minimised (e.g. old ratoons) and, in the long term, allow insecticides to be used only when economic gain is likely. An hypothesis to partially explain the spatial distribution of beetle oviposition is that the tallest cane at the time of beetle flight is preferred for oviposition over shorter cane. This was observed and documented by Illingworth (1918). Our study was conducted to determine whether planting time and harvest date have an effect on the location of greyback canegrub damage in the Burdekin River cane-growing area.

MATERIALS AND METHODS

The Burdekin area was broken into three adjoining regions (replicates) represented by the areas serviced by the Inkerman, Ayr and Invicta Cane Protection and Productivity Boards (CPPB). The Inkerman CPPB services the area south of the Burdekin River, Ayr CPPB the area bordered by the Burdekin River to the south and Barratta Creek to the north and the Invicta CPPB the area to the north of Barratta Creek including Clare and Dalbeg.

All records of greyback canegrub damage in the 1993/94 and 1994/95 cane-growing seasons were examined from data collated by the Inkerman, Ayr and Invicta CPPBs. In each year the data were compiled by visits to all farmers in the Burdekin area and by an aerial survey conducted in June using a light plane. Blocks were regarded as damaged if visible injury was evident before harvest or if stools were removed at the time of harvest by the harvester. All blocks on farms with grub infestation were grouped into either plant or ratoon blocks using farm maps obtained from the CPPBs. Plant blocks were considered as either

early or late plant. Early planting occurred between March and the end of the first week in June. Late planting took place from the third week in June through to mid September. Ratoon blocks were grouped according to the week in which they were harvested (Table 1) using information obtained from CSR Cane Supply and Transport Division. In both 1993 and 1994 week zero commenced on the 14 June. As cane is harvested in a series of 5-6 rounds, it was assumed that on each farm there would be blocks fitting into all categories. All plant and first ratoon blocks to which suSCon Blue was applied were excluded from the analysis to avoid any confounding effects the insecticide may cause.

The proportion of ratoon and plant cane blocks damaged were analysed separately using two-way ANOVAs with interactions, the two factors being region and time of planting or harvesting. Year was used as a blocking factor in both analyses. In the ratoon data a regression analysis was then carried out on each data set. Before analysis all data were transformed using an arcsin transformation.

RESULTS

We included 394 blocks on 60 farms in the 1993 analysis and 574 blocks on 72 farms in 1994 (Table 1). These farms covered 2141 ha and 2605 ha in 1993 and 1994, respectively. The ratio of blocks damaged to area damaged did not differ significantly, suggesting that there was no bias in block size in the damaged blocks (P=0.33).

Table 1 *Total number of blocks in the data set for each planting and harvest category in 1993 and 1994 for cane areas Inkerman (Ink), Ayr (Ayr) and Invicta (Inv).*

Treatment	1993			1994		
	Ink(22)[1]	Ayr (16)	Inv (22)	Ink (26)	Ayr (20)	Inv (26)
Plant crop:						
Early plant	9	9	11	32	20	18
Late Plant	8	3	12	24	22	16
Ratoon:						
Harvest Week						
(period)[2]						
0 – 4 (1)	15	13	12	34	9	9
5 – 8 (2)	25	21	22	43	25	23
9 –12 (3)	23	16	26	52	23	13
13 –16 (4)	30	16	15	33	29	25
17 –20 (5)	17	19	26	35	21	13
21 –24 (6)	17	17	12	25	8	22
Total	144	114	136	278	157	139

[1] Figure in brackets indicates the number of farms in the analysis.
[2] Ratoon harvest periods for 1993 and 1994 commenced on 14 June.

In plant cane, early planted blocks had a significantly (P=0.02) greater chance of being attacked by greyback canegrubs than late planted blocks (Fig.1). Locality did not significantly effect damage (P=0.53) and there

was no interaction between locality and planting date (P=0.82). In ratoons there was a significant (P<0.001) effect of harvest date on the proportion of blocks damaged (Fig. 1). Multiple comparisons based on least significant differences showed less damage occurred as the harvest progressed (Table 2). The average proportion of blocks damaged in each of the three cane areas did differ significantly (P=0.002). However, there was no interaction (P=0.11) between cane area and harvest date. Regression analysis of each location over the two study years showed significant negative relationships (R^2 0.63 - 0.93) occurred in all but one of the analyses, and that just missed out at 5% (Table 3).
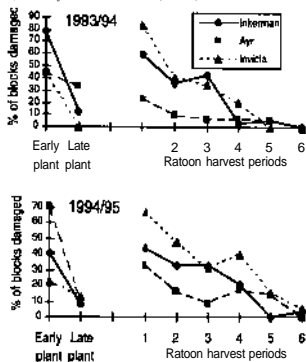
Fig. 1 *Percentage of blocks of sugarcane, grouped according to harvest and planting date, damaged by greyback canegrubs on farms sustaining grub damage in the Burdekin in the 1993/94 and 1994/95 cane growing seasons.*

Table 2 *Average proportion of ratoon cane blocks damaged in each harvesting period.*

Harvest period[1]	N	Means[2]
1	6	0.89 d
2	6	0.65 c
3	6	0.56 cb
4	6	0.40 b
5	6	0.12 a
6	6	0.01 a

[1] See Table 1 for explanation of harvest date
[2] Means followed by the same letter are not significantly different using LSD (P>0.05) following arcsin transformation.

Table 3 *Regression equation (y = a + bx) for the effect of harvesting time on proportion of blocks damaged.*

Location / Year	a	b	R^2	P
Inkerman 1993	0.95	-0.16	0.87	0.0063
Ayr 1993	0.52	-0.08	0.85	0.0089
Invicta 1993	1.09	-0.19	0.93	0.0018
Inkerman 1994	0.83	-0.11	0.74	0.0276
Ayr 1994	0.63	-0.08	0.63	0.0589
Invicta 1994	0.95	-0.12	0.90	0.0043

DISCUSSION

Our study shows that the planting time (early versus late) or harvesting time of a cane block does affect the likelihood of a block of cane sustaining greyback grub damage. This is supported by results taken from three distinct cane areas over two years. An explanation for the effect is the differing height of adjoining cane blocks at the time of beetle oviposition. As all cane in the Burdekin is fully irrigated, the height of cane in each harvest period can be expected to be a continuum. Early harvested cane will be much taller than later harvested cane. The relationship for late planted versus early planted cane was also consistent with the cane height hypothesis with all areas showing significantly more damage in early planted blocks over the two years.

Our results suggest that cane planting and harvest date can be used as a cultural strategy to manage greyback canegrubs in the Burdekin River cane-growing area. In grub-prone areas where chemical control is not available, all cane should be planted late to minimise the risk of damage and early ploughout of the plant crop. In ratoon cane, old ratoons that are to be ploughed out in the coming season should be cut first to focus grub damage on blocks in which economic loss is minimised. On farms where suSCon Blue is effective, early plant cane should be protected with suSCon and cut in the first round of the following season to maximise the chance of it being attacked and the grubs killed with insecticide. Late plant blocks should not have suSCon applied to them and should be cut in the later rounds of the harvesting period. This would provide the benefit of reduced chemical application costs and also limit the amount of ground treated with suSCon. Reducing the area treated may reduce the buildup of the microbial populations responsible for the premature degradation of suSCon in these blocks.

CONCLUSION

Sugarcane planted early or ratooned after being harvested early is more likely to be attacked by greyback canegrubs than sugarcane planted late or harvested late. Our results imply that careful planning of planting and harvesting can minimize damage. On farms where suSCon is effective, the effective life of suSCon can be possibly increased; on farms where suSCon is not effective, economic losses can be minimised.

ACKNOWLEDGMENTS

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NEMATODE PESTS: THEIR ROLE IN YIELD DECLINE OF SUGARCANE AND OPPORTUNITIES FOR IMPROVED MANAGEMENT PRACTICES

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ABSTRACT

Plant parasitic nematodes are one of a number of soil physical, chemical and biological constraints that limit the productivity of sugarcane in Australia. Lesion nematode (Pratylenchus zeae) is ubiquitous and other recognised pests (eg. root-knot nematode (Meloidogyne spp.), stubby root nematode (Paratrichodorus 5pp.), stunt nematode (Tylenchorhynchus spp.), spiral nematode (Helicotylenchus spp.) and dagger nematode (Xiphinema spp.)) are widespread. This paper reports on research designed to assess the economic importance of nematodes on sugarcane and explores opportunities for achieving sustainable nematode control.

INTRODUCTION

Intensive monoculture of soils used for sugarcane in Queensland has resulted in a decline in the physical, chemical and biological fertility of the soil resource. This decline in soil productive capacity, termed 'Yield Decline', is believed to be a major contributor to the productivity plateau that has been apparent in the sugar industry for the last 25 years. A multi-disciplinary research program is now underway to identify possible causal factors and plant-parasitic nematodes are one of the many biological constraints under investigation.

Sugarcane has the most complex nematode fauna of any crop grown in Australia, with more than 30 pest species having been recorded (McLeod et al 1994). Nevertheless, the significance of nematodes in the Queensland sugarcane industry is uncertain and nematodes are recognized as important pests only in the sandy soils of the Bundaberg region (Bull 1981). The work reported here aims to assess the significance of nematodes in other soils and regions. It also aims to determine which species are the key pests on sugarcane and to develop an understanding of their distribution, population dynamics and damage thresholds.

MATERIALS AND METHODS

Nematodes were identified and counted in root and soil samples from fields that were representative of the Australian sugar industry. All fields had grown sugarcane for many years and were sampled when plant or ratoon crops were 6-12 months old. A total of 242 fields from southern districts (Queensland border to Bundaberg) and 160 fields from northern districts (Tully to Gordonvale) were sampled. Soils were also bioassayed for root-knot nematodes (*Meloidogyne* spp.) using tomato seedlings and species were identified using a PCR-based assay of mt DNA (Stanton et al 1996). Soil texture in each field was characterised by particle size analysis and data on cropping history and variety were collected.

Crop loss assessment

Crop losses caused by nematodes will be estimated from nematicide experiments in soils that are representative of large areas of the sugarcane industry. Six experiments were established in 1995 and a further ten are planned for 1996 and 1997. Each experiment consists of a paired comparison of nematicide-treated and untreated plots. The nematicide treatment consists of alternate applications of aldicarb and fenamiphos, the nematicides being applied once every 8-10 weeks in an effort to achieve a high degree of nematode control. Above- and below-ground growth responses to the nematicides will be measured and nematode populations will be monitored throughout the experiments.

Population dynamics and damage thresholds

Data on nematode population dynamics were obtained by collecting roots and soil at regular intervals during a crop cycle in a sugarcane field from northern Queensland. Damage thresholds for *Pratylenchus zeae* and *Meloidogyne javanica* are being determined by inoculating a range of nematode densities onto sugarcane in the glasshouse and in field microplots. Crops wim different levels of susceptibility to each species are also being used to establish a range of population densities in the field so that relationships between yield and nematode density can be determined.

RESULTS

Survey

Lesion nematode (*P. zeae*) was found in all Queensland sugarcane fields, with about 25% of fields showing population densities of more than 5 nematodes/g soil. Root-knot nematode (*Meloidogyne* spp.) was present in about 60% of samples and *M. javanica* was the most common species. Both *Pratylenchus* and *Meloidogyne* occurred in significant densities in all varieties and their distribution was not related to clay content or to region. A wide range of other plant parasitic nematodes were recorded, including stubby-root (*Paratrichodorus minor* and *P. lobatus*), spiral (*Helicotylenchus dihystera*), dagger (*Xiphinema radicicola, X. longotum, X. americanum*), needle (*Paralongidorus* 5pp.), stunt (*Tylenchorhynchus annulatus*), ring (*Nothocrinonema* sp.), *Macroposthonia* sp.), sheath (*Hemicycliophora labiata*), and burrowing (*Radopholus* sp.).

Crop loss assessment

The first six experimental sites encompassed sandy loam and clay loam soils that were typical of situations where growers did not consider nematodes to be a problem. *Pratylenchus*, *Meloidogyne* and a number of other plant-parasitic species were present at each site. Initial data from these experiments showed that nematode populations in nematicide-treated plots were reduced by 30-60% within two months of planting and by 80-95% after five months. At this time, a 25-30% increase in plant height was evident at three of the six sites.

Population dynamics and damage thresholds

Populations of *P. zeae* on sugarcane in northern Queensland fluctuated in a similar pattern from year to year. A decline in population occurred during an 8 month fallow prior to establishing the 'plant crop (Fig. 1). Nematode numbers then increased rapidly during the 'monsoon' season when conditions were optimal for root growth, and declined when soil dried prior to harvest.

When potted sugarcane seedlings were inoculated with *P. zeae* and grown in a glasshouse for 8 weeks, stalk growth was reduced progressively ($R^2 = 0.81$, $P < 0.05$) as inoculum density increased (Fig. 2).

DISCUSSION

Nematodes known to be pathogenic to sugarcane (eg. *Pratylenchus zeae*, *Meloidogyne* spp. and *Paratrichodorus minor*) were widely distributed on sugarcane in both northern and southern regions of Queensland.

Fig. 1 *Population dynamics of* Pratylenchus zeae *under a sugarcane crop in north Queensland. Harvest date is indicated (I).*

Fig. 2 *Effect of inoculation with* Pratylenchus zeae *on the gro sugarcane in the glasshouse.*

Contrary to industry opinion, nematode populations in heavy soils were sometimes as high as they were in sands. Numbers of *P. zeae* were often higher than the population levels found to reduce growth of sugarcane in the glasshouse, suggesting that nematode pests may be more damaging than is currently recognised by the sugar industry. Experiments in progress should quantify losses caused by nematodes and provide information on the population dynamics and damage thresholds of *P. zeae* and *M. javanica*.

Nematode control in the sugar industry currently relies on organophosphate and carbamate nematicides that are subject to concerns about possible health and environmental impacts (eg. mammalian toxicity, groundwater contamination). Opportunities for reducing reliance on chemicals should therefore be explored. Varietal resistance may be a feasible option now that molecular approaches can facilitate transfer of existing sources of nematode resistance to unrelated crop species or can be used to engineer new sources of resistance (Williamson et al 1992; Burrows & Jones 1993). It may also be possible to improve the usefulness of crop rotation by deploying nematode resistance genes into commercially acceptable rotation crops or by introducing crops such as brassicas, which generate chemicals that are toxic to nematodes. Practices which increase soil organic matter (eg. green manure crops, green cane trash blanketing, addition of organic amendments) may also have potential as they are likely to increase the suppressiveness of soils to nematodes (Stirling 1991).

A likely outcome of future research is an integrated approach to nematode control in sugarcane which may involve:

(i)   use of data from nematode surveys and yield loss experiments to more rigorously define the extent of nematode problems at a regional level and identify situations where economic losses are likely.

(ii)   development of monitoring and diagnostic services which aid decision making at the farm level and ensure that control measures are applied when needed and that nematicides are used in a discriminating manner.

(iii)   identification of genes for resistance and tolerance to root-knot and lesion nematodes and their introduction into sugarcane cultivars.

(iv)   introduction of short-term rotation crops which reduce population densities of sugarcane-specific nematode pests.

(v) adoption of management practices which conserve organic matter and increase microbiological activity in soil, possibly enhancing naturally occurring mechanisms of biological control.

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EFFECT OF HEAT, FUNGICIDE AND FUMIGATION TREATMENTS ON THE MICROBIOLOGY OF SUGARCANE SOILS AFFECTED BY YIELD DECLINE

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ABSTRACT

Yield decline affected soil was pasteurised for 1 h at 45-70°C. Pachymetra root rot was eliminated at 55°C. Sugarcane shoot growth continued to increase when soil was treated at temperatures significantly above those required to eliminate known soil pathogens. This suggests the involvement of unknown pathogens and/or nutritional effects in the growth response. Populations of fungi, bacteria, actinomycetes and fluorescent pseudomonads responded differently to pasteurisation treatments. The fungicide mancozeb and the soilfumigant methyl bromide greatly affected soil microbial populations, reducing populations of total fungi and fluorescent pseudomonads, and led to an increase in populations of total bacteria.

INTRODUCTION

The build up of a suite of organisms unfavourable to root growth under sugarcane monoculture appears to be an important basis for sugarcane yield decline, a condition limiting sugarcane yields throughout Queensland (Magarey 1996). Major root pathogens identified include *Pachymetra chaunorhiza* Croft & Dick (Croft & Magarey 1984; Dick et al 1989), *Pythium arrhenomanes* Drechsler (Croft & Magarey 1984; Magarey 1986), and various nematode species (Chandler 1980; Magarey 1996). Recently, Magarey et al (1995) have shown that a group of organisms viz. dematiaceous (dark sterile) fungi, may act as minor pathogens debilitating sugarcane root growth without inducing specific and major root symptoms.

This paper examines the effect of soil fumigation, soil pasteurisation and fungicides on the general soil microflora and sugarcane root pathogens. Groups of organisms assayed include; general fungi, actinomycetes, fluorescent pseudomonads, and bacteria. Experiments were conducted between 1989 and 1994 at Tully, Queensland. Unless otherwise stated assays were conducted on sugarcane rhizosphere soil.

MATERIALS AND METHODS

Pot Experimental Techniques

Soil from sugarcane fields known to be affected by yield decline (YD) (Magarey 1994) was collected to a depth of 200mm. The soil was classified as a Tully series soil (Murtha 1994) and was sieved (0.5 aperture), mixed thoroughly by hand, and weighed (1.4 kg dry weight equivalent) into 150mm diameter terracotta pots. Pregerminated plants 100-150mm high were transplanted into the terracotta pots, one plant/pot, fertilised with 0.343g K_2HPO_4, 0.153g NH_4NO_3, and 0.335g urea at the time of transplanting, and transferred into air-conditioned benches where soil temperature was maintained at 25-30°C (Reghenzani 1984). A randomised complete block experimental design with four replicates was employed. Plants were sub-irrigated using terracotta saucers and an automatic drip irrigation system. After 4-6 weeks plants were harvested; the soil and root mass was removed undisturbed from the pots and 1g of roots plus closely adhering rhizosphere soil collected for general biological assay. Shoot and root dry weight were recorded.

Experiment 1: YD-soil from the Herbert River district was pasteurised for 60 mins. at either 45, 50, 55, 60, 65, or 70°C. Treated soils were assayed for total fungi, bacteria, actinomycetes, fluorescent pseudomonads and root pathogens as described below.

Experiment 2: Soil from the Tully district (Tomasin) was collected and treated with the biocides mancozeb (Dithane®), metalaxyl (Ridomil®) plus fenamiphos (Nemacur®), or fumigated with methyl bromide-chloropicrin and assayed for total fungi, bacteria, actinomycetes and fluorescent pseudomonads.

Biological assays; sample preparation

Each 1g root sample was placed in a 250 mL glass bottle containing 100 mL sterile 0.1 M $MgSO_4$ solution. Bottles were placed on an orbital shaker operating at 60 cycles/minute for 1 hr. A dilution series was established (10^{-3} - 10^{-7}) for each sample using further quantities of sterile 0.1 M $MgSO_4$. For each assay, 0.5 mL of soil suspension was spread evenly over each of two petri dishes and allowed to dry in a laminar flow cabinet. Plates were incubated at 28°C for 2-3 days before colony counts were conducted. Martin's medium (Martin 1950) was used to estimate total fungal populations of rhizosphere soils using dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Starch medium (SCNA) (Williams & Davies 1965) was used to estimate total actinomycete populations using dilutions of 10^{-4}, 10^{-5}, and 10^{-6}. A low power microscope was used to aid the identification of actinomycete (vs. bacterial) colonies. King's medium B (King et al 1954) was used to estimate total bacterial populations using dilutions of 10^{-5}, 10^{-6} and 10^{-7}. The medium of Sands & Rovira (1970) was used to estimate fluorescent Pseudomonad populations using dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Fluorescent pseudomonad colonies were distinguished by a distinct green or green-blue fluorescence under ultraviolet light.

Soil pathogens

Soils were assayed for *P. chaunorhiza* by assessing the percentage of rotted, primary shoot roots (Croft & Magarey 1984). *P. arrhenomanes* was assayed using a sorghum bait bioassay (Croft 1988), and parasitic nematodes by counting after extraction from soil or roots using the Whitehead tray technique (Whitehead & Hemming 1965).

RESULTS

The effect of pasteurisation treatments on various groups of soil organisms is illustrated in Table 1 (over). *Pachymetra chaunorhiza* and *Pythium* spp. were eliminated between 50-55°C, and 55-60°C respectively. Total fungi declined above 55°C while fluorescent Pseudomonads were greatly diminished at 50°C, and completely eliminated > 65°C. At 45°C, both total bacteria and fluorescent Pseudomonads increased (10x) with temperature. Total actinomycete populations fluctuated in response to temperature and were most abundant at 60-65°C.

Shoot and root growth continued to increase in response to soil treatment above those required to eliminate Pachymetra and Pythium root rots. At 45°C, where populations of total bacteria and fluorescent Pseudomonads were much higher than at any other soil temperature treatment, shoot growth appeared to be reduced.

The biocides in experiment 2 had a variable effect both on plant harvest parameters and also on groups of soil microorganisms (Table 2).

Table 1 *Expt. 1: Population density (number/g soil) of organisms in the rhizosphere of sugarcane roots growing in a "yield decline " soil pasteurised for 60 min at different temperatures.*

Attribute		Control	45°C	50°C	55°C	60°C	65°C	70°C
Total fungi	(x10^5)	4.6	4.8	4.6	0.4	3.6	0.4	1.4
Total bacteria	(x10^8)	1.1	11.1	3.9	4.0	2.8	1.9	1.1
Fl. pseudomonads	(x10^7)	1.7	19.3	0.6	0.2	0.1	0	0
Total acti nomycetes	(x 10^7)	2.4	2.8	5.8	4.6	12.2	10.8	2.0
Pythium(% baits colonised)		73	43	37	3	0	0	0
Pachymetra root rot								
(% rotted roots)		84	68	52	0	0	0	0
Shoot weight (g)	*LSD (5%) = 0.96*	4.10	3.82	4.72	4.86	6.70	7.12	6.84
Root weight (g)	*LSD (5%) = 1.32*	2.56	2.72	3.62	5.02	6.16	6.14	7.30

Metalaxyl plus fenamiphos had little effect on fungi, bacteria, or actinomycetes but reduced fluorescent pseudomonad populations. Methyl bromide fumigation led to a four-fold increase in fungi, and a nine-fold increase in bacteria while reducing slightly fluorescent pseudomonad and actinomycetm populations. Mancozeb reduced all groups of organisms except bacteria, which were increased seven-fold. Both mancozeb and methyl bromide appeared to reduce the biodiversity of isolated organisms, though no measure of this parameter was made. Only mancozeb and methyl bromide improved shoot and root growth.

Table 2 *Expt. 2: Population density (number/g soil) of organisms in the sugarcane rhizosphere in soils treatedwith the biocides mancozeb <MZ), metalaxyl plus fenamiphos (M+F), and with the soil fumigant methyl bromide (MB).*

Attribute		Control	MB	MZ	M+F
Total fungi	(x10^6)	17	60	1.5	14
Total bacteria	(x10^8)	6.3	55.5	44.0	5.5
Fl. pseudomonads	(x10^5)	5.2	2.0	1.0	1.2
Total actinomycetes	(x10^8)	2.3	1.7	0.1	2.4
Shoot weight (g)	*LSD (5%) = 1.33*	4.57	6.1	6.05	4.47
Root weight (g)	*LSD (5%) = 0.67*	2.25	3.44	3.35	2.56

DISCUSSION

This paper reports for the first time the relationship between pasteurisation temperature and *Pachymetra chaunorhiza* viability. Soil pasteurisation at 55°C for 1 h was sufficient to eliminate Pachymetra root rot. Pasteurisation at 60°C, 1 h was required to eliminate all Pythium spp.

Heat treatment of soil at temperatures above 55°C led to better sugarcane growth in this experiment. Other experiments (RC Magarey and JI Bull, unpublished data) suggest that soil pasteurisation at 100°C would improve growth further. Biocide research (Magarey & Bull 1994) suggests that besides the known sugarcane root pathogens, other organisms affect sugarcane growth. Results reported from experiment 1 are consistent with this hypothesis. It is possible that altered soil nutrient availability may also affect sugarcane growth at these higher temperature treatments.

Treatment of soil at 45°C disrupted the populations of soil microorganisms with an explosion of the bacterial populations (increasing ten-fold). A small decrease in sugarcane shoot growth was also noted in this treatment. In other experiments with biocides it has been noted that disruptions to natural biocontrol mechanisms can result in more severe root disease (e.g. Pythium root rot, nematodes), and reduced growth. This emphasises the importance of maintaining a

stable biological community in canegrowing soils. Management strategies that favour biological control processes (and hence, reduced pathogen populations) need to be indentified and extended to the sugar industry.

The soil biology of sugarcane soils remains an important area of research for the Australian sugar industry. Little is understood about the complex interaction of organisms (pathogens, saprophytes, growth promoting organisms) in canegrowing soils nor the interaction of soil biology with soil physical and chemical properties. This understanding will be important for the maintenance or enhancement of sugarcane productivity within Australia.

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INTEGRATED PEST MANAGEMENT OF RATOON STUNTING DISEASE OF SUGARCANE IN AUSTRALIA

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ABSTRACT

Ratoon stunting disease (RSD) has been controlled in Australia by integrated pest management (IPM)for 40 yr. IPM has to meet the challenges of changing farming systems and to incorporate new technologies. Disease-free seed is the foundation of the IPM program. Since the mid 1970s there has been an increase in the use of approved seed plots to supply disease-free seed to farmers. New machinery such as mechanical chopper harvesters are difficult to disinfect and this lead to a major weakness in the IPM program. A new serological diagnostic procedure has increased the accuracy and efficiency of diagnosis of RSD and, in 1995, 40,000 samples were processed. Control of volunteer plants which may carry disease has become difficult as farmers have adopted shorter fallow periods. On-going research and extension is required to maintain an effective IPM program for RSD.

In recent years there has been a growing interest in integrated pest management (IPM) for control of insect pests and diseases. IPM has been used for control of ratoon stunting disease (RSD) of sugarcane in Australia for 40 yr (Steindl 1961). The changing requirements for continued success of the IPM program for RSD may provide some directions and warnings for IPM programs for other pests and diseases.

RSD is the most economically important disease of sugarcane in Australia and in most overseas countries (Gillaspie & Teakle 1989). In Australia RSD causes losses of between \$10-20M per year inspite of control measures estimated to cost \$2.4M. RSD is caused by the bacterium, *Clavibacter xyli* subsp *xyli* Davis et al., which infects the xylem of sugarcane causing stunting of growth. RSD is spread by cutting implements contaminated with juice infected with the bacterium, by planting infected cuttings and through carryover of diseased volunteer plants (Steindl 1961). Losses range from 7% to 60% depending on the moisture status of the crop during the season, susceptibility of the cultivar, and presence of other diseases (Gillaspie & Teakle 1989).

IPM of RSD in Australia involves provision of disease-free planting material, disinfection of cutting implements, inspection of plant sources for disease, control of volunteer plants and extension. This paper will briefly outline how changes in industry practices and advances in technology have required constant evolution of the IPM of RSD.

DISEASE-FREE PLANTING MATERIAL

Provision of disease-free planting material is the basis of control of systemic diseases in many vegetatively propagated crops. In all sugarcane districts of Australia local boards known as Cane Protection and Productivity Boards (CPPB) employ staff who oversee supply of disease-free planting material, carry out on-farm plant source inspections and conduct surveys to establish disease status of crops in the district.

Hot water treatment of cane for individual farmers to provide RSD-free planting material was introduced during the 1950s in Queensland (Steindl 1961). The disadvantages of this system are that the eradication may be incomplete if heavily infected cane is treated, treatment requires extensive commitment of CPPB staff time and farmers are often disappointed with the germination of heat-treated cane. In all districts of Queensland there has been a reduction in the hot water treatment conducted for individual growers and the establishment of approved seed plots by CPPBs (Fig.1). Large quantities of approved seed were sold during the early 1980s to control Fiji and leaf scald diseases. Some CPPBs currently provide the approved seed cut, loaded on to vehicles and, if required, delivered to the farm. Farmers pay for the approved seed through their levy to the CPPB and by direct payments per tonne of approved seed. The danger of this system is that if disease enters a plot and is not detected it is spread to many growers. This danger has lead to strict guidelines for operation of the seed plots and for frequent quality assurance checks before plots are approved for use.
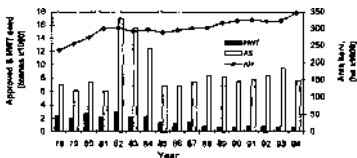
Fig.1 *Trends in use hot water treated (HWT) and approved seed (AS) in the Queensland sugar industry in comparison to the area harvested (AH) for milling.*

Future challenges for the managers of approved seed plots will be to continue to provide farmers with a product suitable for their demands. Farmers who use billet planters are already demanding access to approved seed as billets. This requires careful organisation to prevent the risk of disease entering approved seed plots on contaminated harvesters. Approved seed is currently provided by semi-government organisations (CPPBs) and the true cost of the approved seed includes the price payed by the farmer plus a proportion of the CPPB levy which is jointly payed by the farmer and the milling company. Opening the provision of approved seed to competition between private companies and the semi-government organisations may improve the marketing and delivery of approved seed.

DISINFECTION

The disinfectant, benzalkonium chloride, has been used in Australia since the 1950s to disinfect cutting implements (Steindl 1961). However, the mechanisation of sugarcane production has changed dramatically the whole system of cane farming and new machinery and techniques continue to be developed. Recommendations for disinfecting cane knives were modified to suit mechanical cutter harvesters when they were introduced into the industry. The recommendation was to disinfect the knives on the basecutter of harvesters. Subsequently, research has shown that this is not adequate because diseased juice can be blown or drip down from contaminated chopper boxes and extractor fans (Taylor et al 1988). To disinfect basecutters, chopper boxes and extractor fans is a very time consuming operation and the majority of harvester operators will not disinfect their machines often enough or thoroughly enough to prevent RSD spread. This has resulted in a weakness in the whole IPM program for RSD. Attempts to find more practical methods of disinfecting harvesters have so far been unsuccessful. To overcome this weakness growers are being advised to place even greater emphasis on ensuring that approved seed is used for all plantings and that extreme care is taken to ensure the approved seed is not reinfected. By reducing the sources of RSD it is hoped that economic losses can be kept to a minimum.

New machinery, such as billet planters, trash strippers and stool splitting fertiliser boxes, and changing farming practices, such as the use of contract planters, may require modification of the IPM program for RSD in the next 10 yr.

INSPECTION OF PLANT SOURCES

New technology has improved the efficiency and accuracy of RSD diagnosis. RSD was originally diagnosed by slicing the stalk of cane and looking for red-orange dots in the vascular traces of the nodal region (Steindl 1961), but this is not reliable with all cultivars. Phase-contrast microscopic examination of xylem extracts for the characteristic bacterium has been used for diagnosis extensively in Australia (Amiet 1985) but the technique is time consuming and the number of samples which can be processed is restricted. Serological techniques based on an enzyme-linked immunoassay have recently been developed (Croft et al 1994) and in 1995 40,000 samples were processed in two laboratories in Queensland (Table 1). Disease levels in Queensland were low, but New South Wales had districts with a significant RSD problem. The assay was also extensively used by BSES for research into cultivar resistance to RSD (Table 1).

Table 1 *Summary of samples diagnosed in 1995 for RSD by the evaporative-binding enzyme-linked immunosorbent assay.*

District/ organisation	No. samples	% positive RSD
Northern Qld.	1253	1.4
Central Qld.	17674	0.5
Southern Qld.	1675	1.0
NSW	7809	16.8
BSES-routine	9533	1.2[2]
BSES-RSD research[1]	1823	49.0
CSR-breeding	475	0
Total	40242	6.1

[1] Includes positive controls and samples from fields surrounding BSES experiments.
[2] BSES research into cultivar resistance to RSD and epidemiology of RSD.

Current research is aimed at developing a polymerase chain reaction (PCR) DNA probe based assay. *Clavibacter xyli* subsp *xyli* specific primers have been developed and the format for practical assays are being investigated (M.Fegan, personal communication). Present indications are that the PCR assay will increase the sensitivity of the diagnosis of the disease by 1,000 to 100,000 times compared to serological and microscopic techniques.

VOLUNTEER CONTROL

Volunteer plants which survive from one crop cycle to the next can, if infected with RSD, introduce disease into the new crop. The practice of ploughing out an old ratoon crop and replanting it within 1 - 10 weeks has increased greatly in the last 20 yr, with some northern districts having over 90% of planting conducted in this way. Control of volunteers in this system, with virtually no fallow period, is nearly impossible, especially when soil moisture is high preventing drying out of the stubble of the previous crop. Farmers are currently advised to fallow fields known to contain RSD infected crops for 6 months and also to ensure that areas to be planted with approved seed have been fallowed for at least 6 months and all volunteers destroyed.

EXTENSION

On-going extension programs are essential for successful IPM of RSD. Because RSD does not cause obvious visual symptoms, farmers need to be continually reminded of the benefits of RSD control. Since farming systems are continually changing, IPM for RSD is not static and new advice and new extension techniques are required to ensure that the program continues to be successful. This extension is currently undertaken by BSES extension officers and staff of CPPBs. Innovative use of marketing techniques to increase adoption of approved seed will be an important activity for extension of RSD control in the future.

DISCUSSION

The IPM program for RSD in Australia has been improved in recent years by the development of serological techniques which have increased the efficiency and accuracy of RSD diagnosis. There has been a slight increase in the use of approved seed in recent years but the use of approved seed is inadequate in some districts. Disinfection of mechanical harvesters and billet planting machinery is not widely adopted which is resulting in spread of RSD between fields. The increase in the practice of ploughing out old ratoon crops and replanting them after only a few weeks is increasing the carryover of RSD in volunteer plants. Currently, extension programs are emphasising the need for increased use of approved seed to reduce the losses from RSD.

All industries are facing increasing change and to be successful all components of an industry must adapt to this change. IPM usually involves a range of treatments which require farmers to learn skills in a number of areas and to regularly complete all parts of the program. As well, the program is not static and requires on-going extension and research to meet new challenges. Experience of the last 40 yr with IPM for RSD has shown that without legislative controls it is very difficult to obtain and maintain full acceptance of the IPM program. Legislation is used in the Burdekin district to ensure all farmers use approved seed sources but the IPM program for RSD control is voluntary in all other districts. In Queensland acceptance of the IPM program for RSD control is excellent relative to most overseas sugarcane industries, with 87% of growers using approved or inspected plant sources each year. However, the future continued success of the program will require innovative solutions to meet changing farming practices, particularly extension methods to improved the adoption of approved seed. Involving private enterprises in the provision of approved seed may stimulate new ideas for the marketing and delivery of approved seed. In general, farmers would prefer a simpler form of disease control but economically IPM will probably remain the most viable option.

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5. Resource sustainability and environment

5.1 Soil properties

SOIL MANAGEMENT RESEARCH FOR SUSTAINABLE CANE PRODUCTION IN THE 21ST CENTURY

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ABSTRACT

Poor cane growth with frequent need for crop re-establishment are a feature of the group of grey duplex soils that are common to much of the Australian and South African sugar industries. Soil factors limiting climatic potential include poor water intake due to surface crusting, soil loss through erosion, low available moisture capacity, organic matter loss, acidification and waterlogging during wet seasons. Many current ratoon cane management practices such as interrow ripping, fertilizer timing and placement, burning of crop residues at harvest, harvesting under wet conditions and using heavy infield transport, are incompatible with sound long term management of these soils. Soil management strategies in the 21st century will need to incorporate ecological principles based on crop residue retention, nutrient recycling, minimum tillage, ridge tillage, cover crops and intercropping.

INTRODUCTION

In many cane producing areas in Australia and South Africa, productivity has in recent years remained fairly constant despite the release of better yielding varieties. The plateauing of yield may be due to one or more of the following factors: (i) increased incidence of droughts; (ii) pest and disease problems; (iii) stool damage due to infield loading transport equipment; (iv) the effects of monocropping on degradation of soil properties; (v) management practices not matched with soil type. This paper examines some of the recent research initiatives in the South African and Australian sugar industries concerning the last two aspects.

Soil degradation and yield decline

In South Africa, in the 1970's, Al toxicity and P fixation (Meyer et al 1971) were identified as factors limiting cane growth in the midlands area of KwaZulu-Natal. In 1972 the "Upper Tongaat co-ordinated project" was established to investigate yield decline on 16 000 ha of caneland. The results of laboratory, glasshouse and field trials showed that the problem could not be linked to any single cause. Apart from P, Zn and Mg deficiency, damage due to soil micro-organisms such as nematodes and fungi were listed as possible factors limiting growth (Thompson 1985). Yield responses of up to 50 % were obtained from fumigation with methyl bromide compared with 15 to 30 % when P and Zn were applied. Other studies on soil degradation processes have included erosion (Platford 1982), compaction (Swinford & Boevey 1984), surface crusting (Dewey & Meyer 1989), salinisation (Johnston 1978), irrigation water quality (Culverwell & Swinford 1985), waterlogging (Van Antwerpen et al 1991), and acidification (Schroeder et al 1994). More recently, a survey of 24 paired sites comprising "virgin" and "cultivated" land, has revealed that increasing salinity and sodicity levels contributed to degradation in the irrigated cane areas and acidification in the rainfed cane areas (Van Antwerpen & Meyer 1996). In Swaziland yield decline on irrigated duplex soils was linked to a deterioration in both physical and chemical properties of soils (Henry & Ellis 1995).

In Australia, a study in the 1980s of 19 paired old and new sugarcane sites in the Herbert river valley, revealed that compaction, losses of organic matter and acidification of soils were factors most likely to be associated with the decline in cane productivity in Northern Queensland (Wood 1985). Although yields have subquently improved following the introduction of green cane trash management (Wood 1986), yield decline in the Australian industry is still a major problem, costing A$2OO-300 million annually. (Garside 1995). More recently, a survey of paired old and new land sites at Tully, Herbert and the Burdekin, by researchers of the Yield Decline Joint group, have indicated increased soil acidity, compaction, lower intake rates, lower zinc and copper levels on old land relative to new land just brought into cultivation from forest (Anon 1996). Soil-borne fungi and toxins were secondary effects associated with the deterioration of soil properties following long term sugarcane monoculture. Yield responses of up to 20 % have been obtained on old cane growing land in Queensland following fumigation with methyl bromide.

Soil crusting, erosion and compaction

Duplex soils in both countries crust to varying degrees under both dryland and irrigation. Physical disaggregation of soil particles through the impact of raindrops, causes surface compaction which limits water penetration into the soil. Soil crusting is the precursor to soil erosion and soil erodibility ratings have been determined for different soil types by Platford (1982). Rainfall simulator trials have shown that strong crusts do not form under a surface mulch such as trash. Average results from 5 trials over a 5 year period showed that trash saved 89% of the soil that was lost from the burnt plots. Ameliorants such as phosphogysum, molasses meal, polyvinyl alcohol and various polymers were less effective and far more costly than a trash blanket in reducing runoff and increasing rainfall use efficiency (Meyer & Dewey 1988).

In South Africa, Maud (1960) showed that soils are most susceptible to compaction when their moisture content is near field capacity. Swinford & Boevey (1984) found that compaction in the row reduced yield more than compaction of the interrow. Soil ripping was only slightly beneficial. They concluded that yield decline from infield traffic is as much due to physical damage to stools as to a breakdown in soil structure, particularly under critical soil moisture conditions.

Soil surface crusting leading to reduced water infiltration, increased run-off and erosion, have also been measured in the Australian sugar industry. Prove et al (1986), compared cultivated and virgin soils to determine the effect of compaction on bulk density. For subsoils, the virgin area was lower in bulk density compared with the cultivated areas for both the soil types studied.

Salinity/sodicity and drainage

The effects of soil salinity and sodicity in the irrigated regions of both industries have been extensively studied (Von der Meden 1960; Johnston 1978; Kingston 1985; McGuire 1991). A primary cause of soil salinisation in these regions are high water tables which allow capillary rise of saline ground water into the rooting depth of the crop.

Poor quality irrigation water may be another source of salts. A serious decline in yield on an estate in northern KwaZulu Natal was linked to increasing soil salinity (Culverwell & Swinford 1985). In Australia, it was noted that some light-textured soils irrigated with good quality water in the Burdekin area, dispersed forming a slurry which prevented adequate water penetration (McGuire 1991). Gypsum is now used more widely on these soils to reduce soil dispersion and increase water intake.

A common thread in yield decline studies is poor internal drainage which not only contributes to salt build up but can exacerbate the effects of compaction, oxygen availability and denitrification under a trash blanket. Under protracted periods of waterlogging the potential for the formation of phytotoxic organic acids and hydrocarbon gases such as ethylene increases. The disasterous yields in 1991 from Mossman to Sarina in Australia may be linked to the release of phytoxic levels of ethylene.

Soil acidification

Adverse effects of high levels of exchangeable Al on the growth of sugarcane are well documented for sugarcane in South Africa (Moberly & Meyer 1975). Recently, an assessment of soil fertility trends has shown that sandy soils on the south and lower south coast of KwaZulu-Natal, have progressively become more acidic (Meyer et al 1989). Increased acidification of soils on various estates in KwaZulu-Natal was also recently predicted through the use of a model (Schroeder et al 1994).

Many soils in the Herbert canegrowing areas in Queensland are also highly acidic (<pH 5.0 in water). Much of the exchangeable Al is likely to be in monomeric form and highly toxic, especially when Al is > than 50% of the exchangeable cations. In South Africa, even the most Al-tolerant varieties respond to lime under these conditions.

PREVENTIVE SOIL MANAGEMENT STRATEGIES

Although the consequences of various soil degradation processes are generally well known, it is only recently that research has shifted from reclamation to conservation management to prevent problems arising in the first place. To this end, a knowledge of soil type is extremely important. Sugarcane management should differ according to soil type and this includes crop establishment, varieties, fertilizer management, trash management, harvesting and irrigation scheduling (Moberly & Meyer 1984). Examples of selected practices follow.

Crop establishment

In South Africa, research has shown that minimum tillage (strip tillage). in which glyphosate is used to kill the old crop, results in minimal soil erosion and improved cane yield when compared with conventional methods of land preparation. Other measured benefits included increased cane yield, soil organic matter, reduced soil bulk density, reduced soil and water loss (Iggo & Moberly 1976). Minimum tillage has been widely adopted in the South African sugar industry and is recommended according to slope and erodibility hazard of soils as shown in Table 1. In Australia, minimum tillage using glyphosate is still of minor importance and is confined to sandier soils in the areas north of Ingham in Queensland.

Table 1 *Recommendations foi South Africa.*

Soil group	Soil taxonomy	Erosion hazard	Minimum tillage priority
Grey sands to loams	Inceptisols	High	High
Red loamy sands to clays	Oxisols	Moderate	Clay < 15%
Black clays	Mollisols Vertisols	Low	Not recommended
Brown humic loams	Oxisols Latosols	Low	Only where slope >15%

In recent years there has been renewed interest in green manuring as a fallow crop in order to improve soil physical and chemical conditions and decrease the incidence of pests and diseases specific to sugarcane. Green manure crops such as cowpeas and dolichos bean are widely used as a fallow crop in Australia, except in areas of heavy textured poorly drained soils. In the South African sugar industry, favourable results were obtained from early research into rejuvenating old cane land with velvet beans, sunnhemp, cowpeas, lupins, rape, buckwheat and mungbeans (Pearson 1958). Green manuring is mainly recommended on soils prone to erosion which includes mainly grey sandy soils on slopes in excess of 5% where there is insufficient clay and organic matter to keep the soil together. Bottomland soils with low air filled porosities would also benefit from green manuring.The priority in recommending green manuring according to soil type is similar to the guidelines for minimum tillage shown in Table 1.

In Swaziland, green manuring and crop rotation have been tested at Mhlume Estate on a commercial scale (Hill 1988).The mean yields of 13 fallowed and green manured 40 ha blocks of land compared with the mean yields of 13 non fallowed blocks of land, improved by 45% in the plant crop with residual effects of 25% measured in the 1st and 2nd ratoon crops. Follow up trial work by Nixon (1992) confirmed large responses to bare fallowing (11-29%) and green manuring (10-54%) in the plant crop with small but non significant residual responses measured in the subsequent ratoon crops. Yield increases were related more to prolific rooting brought about by mainly improved soil physical properties, particularly the air-filled porosity at 10kPa suction(AFP) which increased on average from 11.9% (control) to 16.1 % (fallowed). Infiltration rate and resistance to penetration were also significantly improved. Soil organic matter levels were adversely affected by bare fallowing but increased slightly with green manuring. Nitrogen availability was improved at low or zero N fertilizer inputs from green manuring. The benefits of this practice in controlling diseases such as RSD still needs to be quantified by plant pathologists.

Trash management

In Australia, extensive research has been carried out into the evaluation of the effect of crop residues following green cane harvesting (Ridge et al 1979; Wood 1986; Dick & Hurney 1986). More recently, it has been shown that green cane systems have much higher soil microbial biomass and earthworm populations than burnt cane systems thereby providing a mechanism for an increased supply of nutrients for early crop growth (Sutton et al 1994). Most growers in north Queensland have adopted this practice.

In South Africa, trashing is strongly recommended on the more erodible entisol and alfisol soil groups. Thompson (1966) reported average yield responses of 10 tons cane per hectare to trash retention in trials conducted under rainfed conditions on a cross section of soils. He also noted significant increases in soil organic matter and cation exchange capacity, particularly in the top few centimetres of soil. Under irrigation the response to trash retention was found to be much lower. Trash conservation is a very effective means of reducing soil and water losses from sugarcane fields. This is particularly important in KwaZulu-Natal, where slopes are often steep and many of the soil types are highly erodible.

Harvesting programme

In South Africa, cane is mainly manually harvested and mechanically hauled, and the harvesting season is usually April to January. If it extends later into the wet summer months, there is increased risk of infield traffic causing soil compaction, smearing, capping and physical damage to stools. Fields with free-draining soils which are unlikely to compact severely should, where possible, be held back in reserve for harvesting in wet periods. A suggested programme for cane fields according to soil group, and which is equally applicable to both South African and Australian situations, is shown in Table 2.

DISCUSSION

With increasing demands on the soil environment, the key to sustainability in the 21st century will be the extent to which cane producers adopt preventive management strategies using ecological principles from natural ecosystems. According to Hornick & Parr (1987), a sustainable system is any system in which the benefits from soil conservation practices are equal to or greater than the negative effects of the soil degradation processes (see Fig. 1). The concept is equally valid for low-input and high-input systems. Management by soils (MBS), that is, matching management practices to specific soil conditions, should be given a high priority in future research.

Better understanding is needed of how different soils behave under wetting and drying, particularly in relation to crusting, compaction, the supply of nutrients from organic matter pools and the release of

Table 2 *Recommended harvest programme based on soil groups in South Africa and Australia.*

Soil group	Compaction hazard	Soil taxonomy	Suggested harvest season
Valley bottom	High	Inceptisols Entisols	Winter
Grey sandy soils	Moderate	Alfisols Aridsols	Winter/spring
Clays and clay loams	Moderate	Alfisols Mollisols	Spring/summer
Brown humic loams	Low	Oxisols	Summer
Recent sands well drained alluviums	Low/medium	Entisols	Spring/summer

regulatory hyrocarbons such as ethylene. New microprocessor technology allows use of digitized maps in the cabins of chemical spreaders, enabling changes in fertilizer and herbicide applications at predetermined amounts as the machine passes over different soiltypes in the field. When management practices are applied on a soil-by-soil basis, the result will be improved efficiency through the better control of chemicals.

The second research area is the concept of differentially managing zones within the field (Larson & Robert 1990), for example, to manage the row area differently from the interrow. The row area should provide a good soil structure, rooting depth, nutrient and moisture availability and the interrow should be managed to create a surface to maximise intake rate of water, erosion control and be firm for wheel traffic. Tramline and ridge systems for the control of infield traffic have proved very successful in other crop industries for managing soil compaction. The use of controlled traffic zones is currently under investigation in Queensland. One of the systems that looks very promising is planting soya beans as a green manure crop into ridges, destroying the soya beans with glyphosphate six weeks prior to establishing sugarcane, followed by planting the cane setts into the old soybean stubble in the ridge using zero tillage.

The merits of ridge and vertical mulching tillage for improving the quality of duplex soils in the South African Sugar Industry are also currently under investigation in the South African sugar industry (Meyer et al 1992 ; van Antwerpen et al 1991). Apart from increased yields, ridging resulted in improved surface drainage, less compaction damage to the cane row, better aeration and healthier root development and generally improved moisture conservation. Further work is needed in testing the efficacy of combination treatments of vertical mulching on the row at crop establishment followed by ridging up in the ratoon crop. Other areas that warrant further research include the testing of ridging and vertical mulching in combination with green manuring.

CONCLUSIONS

Soil management systems for sustained sugarcane production in the 21st century will have to be proactive and accommodate ecological principles to an increasing extent.Strategies based on MBS as well as emphasising crop residue retention, nutrient recycling, row/interrow management based on minimum tillage, intercropping, and ridge tillage will help to develop productive, profitable and sustainable production systems. Environmental quality issues, particularly air, ground and surface water quality will make it imperative to base soil management practices on an understanding of the ecosystem concept. The growing interest in Europe in 'biosugar production', using organic fanning methods could well gather momentum and in future favour countries producing sugarcane using ecologically based management practices and using enviromental audits based on the international ISO 14000 environmental guidelines.

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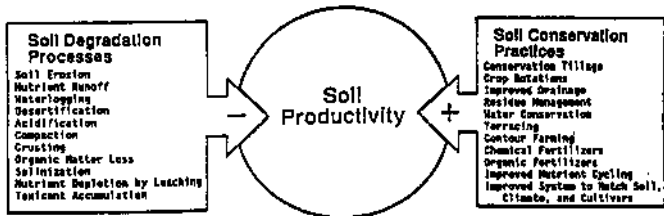Maud RR (1960) The compaction of sugarbelt soils at various moisture

Fig. 1 *Relationship between soil degradation processes, conservation practices and soil productivity*

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SUSTAINABILITY OF CROP AND SOIL PRODUCTIVITY UNDER SUGARCANE-BASED CROPPING SYSTEM

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ABSTRACT

Field studies were conducted during (1992-93 to (1994-95 at Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, India to study the effect of organic manures (FYM, green manure sunhemp, pressmud cake and cane trash) along with 100% and 50% recommended N in sugarcane based crop rotation (sugarcane plant- ratoon - sesamum - paddy) with an objective to monitor the changes in soil fertility for sustained crop production. Integrated use of organic manures and 100% chemical fertilisers not only gave higher yields of crops grown in the rotation but also improved the soil fertility status. When the recommended nitrogen was reduced to 50%, the integrated use of organic manures and inorganic fertilisers gave sustainable crop yields. Among the organic manures, FYM was better in enriching the soil available N and P with lesser depletion of soil available K when compared to green manure and cane trash + press mud cake. The integrated effects of organic manures and inorganic fertilisers were more pronounced on the subsequent crops (sesamum and paddy) than the initial crops (sugarcane plant and ratoon) in the rotation. Enrichment of soil reserves with respect to available P and depletion of available soil K due to higher crop removal (in spite of K addition to all crops in rotation) was found, indicating the need for K application to the crops in rotation for maintaining soil reserves.

INTRODUCTION

Until recently, the use of inorganic fertilisers dominated the nutritional scenario for improved crop production. Now it is well recognised that excessive use of chemical fertilisers and pesticides is leading to irreparable damage to the basic resources like soil, water and yield (Subba Rao 1995). Efforts in regulating their use and lessening the dependence on these chemicals through use of organic wastes for sustainable crop and soil productivity are yielding rich dividends. Mono cropping was proved to be harmful in terms of soil fertility depletion, harbouring pests and diseases (Chatterji & Maiti (1984). Generally a sugarcane plant - ratoon - sesamum - paddy cropping system is followed in North Coastal Andhra Pradesh. Hence field studies were conducted during (1992-93 to (1994-95 to study the effect of organic manures (FYM, green manure sunhemp, pressmud cake and cane trash) alongwith 100% and 50% nitrogen in sugarcane based cropping system with an objective to monitor the changes in soil fertility for sustainable crop production.

MATERIALS AND METHODS

Field experiments were conducted at Regional Agriculural Research Station, Anakapalle in a sugarcane plant - ratoon-sesamum-paddy cropping system in the (1992-93 and (1994-95 seasons. The following treatments were applied :

T$_1$	=	NPK + micronutrients based on the recommended doses for all the crops.
T$_2$	=	T$_1$ + 20 t/ha of farm yard manure (FYM) (FYM to sugarcane plant crop only)
T$_3$	=	20 t/ha FYM (to plant crop) + 50% N + full P and K to each crop.
T$_4$	=	T$_1$ + green manuring with green manure crop once in crop cycle.
T$_5$	=	T + Sulphitation pressmud + trash at 10 t/ha each to sugarcane plant crop only.
T$_6$	=	Green manure as in T$_4$ +50% N +full P and K to each crop.
T$_7$	=	Trash + pressmud cake at 10 t/ha each (to plant crop) + 50% N + full P and K to each crop.
T$_8$	=	T + integrated pest management to each crop.
T$_9$	=	50% N + full P and K to each crop.
T$_{10}$	=	Control (No NPK , micronutrients or manure)

The recommended rates (per hectare) of NPK and micronutrients were 112 kg N + 75 kg P$_2$O$_5$ +120 kg K$_2$O + 50 kg Z n S O$_4$ for plant crop; 224 kg N + 75 kg P$_2$O$_5$ + 120 kg K$_2$O + 50 kg Z n S O$_4$ for ratoon crop; 40 kg N + 60 kg P$_2$Q$_5$ + 30 kg K$_2$O for sesamum; and 80 kg N

+ 40 kg P$_2$O$_5$ + 30 kg K$_2$O for paddy crop. P, K and Zn were applied basally, whereas N was applied in two splits at 45 and 90 days after planting for the sugarcane plant crop; at ratoon initiation and 45 days later for the ratoon crop ; a basal dressing and three weeks later for sesamum. For paddy, N was applied at transplanting, tillering and panicle initiation. The test varieties for sugarcane, sesamum and paddy were Co 72(19, YLM 17 and AKP 70-73. The plant crop was planted in March '92 and ratooned in March' 93. The sesamum crop was sown in January ' 94 and paddy was transplanted in August '94. All the treatments were replicated four times in a randomised block design. Uptake of N, P and K was determined using standard procedures Piper (1967). The experimental soil was a dark brown clay loam, moderately drained, very deep, non-calcareous, neutral in reaction (pH 7.2), normal in conductivity (EC 0.12 dS /m), low in available N(224 kg N/ha), medium in available P$_2$0$_5$ (40 kg /ha) and high in available K$_2$O (346 kg/ha). Standard procedures were followed for determination of soil characteristics (Jackson (1967). The farm yard manure (FYM) had 1.02% N, 0.64% P$_2$0$_5$ and 1.20 K$_2$0. The green manure crop, sunhemp contained 2.3% N, 0.51% P$_2$0$_5$ and 1.79% K.O. The N, P$_2$0, and K$_2$0 contents of cane trash were 0.27%, 0.55% and 0.52% respectively. The available N, P,05 and K$_2$0 contents of pressmud cake were 0.24%, 0.72% and 0.(19%, respectively.

RESULTS AND DISCUSSION

Yield of sugarcane and grain yield of sesamum and paddy are given in Table 1.

Highest yields of sugarcane plant and ratoon crops and sesamum were recorded when the crops received recommended doses of NPK and 10 t/ha each of trash and pressmud cake (applied to the plant crop and gave a residual effect on the ratoon and sesamum crop). However, the grain yield of paddy was highest when the crop received recommended doses of NPK and the residual effect of 20 t/ha FYM (i.e T$_2$). This yield was similar to T$_5$ where residual effect of cane trash + pressmud cake was felt alongwith application of recommended doses of NPK. The yield in T$_4$ treatment was similar to T$_1$ treatment. Thus, all the crops in the crop rotation gave highest yields with an integrated use of organic manures with chemical fertilisers. Singh et al (1993) observed that integrated use of fertiliser N with green manuring in a sugarcane plant crop and its residual effect in ratoon increased N-use efficiency.

When the average yield of these crops at different levels of applied nitrogen and in combination with inorganic and organic manures was considered (Table 2); the yield of all crops in the rotation reduced as applied N reduced from 100% to 0%N. The crop yield gap obtained

Table 1 *Effect of organic manures and inorganic fertilisers on the yield of sugarcane plant crop, ratoon crop, sesamum and paddy.*

Treatment	Cane Yield (t/ha)		Grain Yield (kg/ha)	
	Plant crop	Ratoon crop	Sesamum	Paddy
T_1 = NPK + Zn (100% recommended doses)	92.3	95.1	337	3662
T_2 = T_1 + integrated pest management	93.7	94.3	326	3405
T_3 = T_1 + 20t/ha FYM*	89.9	95.6	336	4328
T_4 = T_1 + greenmanuring*	89.5	89.5	330	3707
T_5 = T_1 + pressmud cake @ 10t/ha + canetrash @ 10t/ha*	96.8	96.0	352	4146
T_9 = 50% N + full P & K	90.7	76.9	281	2966
T_3 = T_9 + 20t/ha FYM*	91.4	89.1	292	3480
T_6 = T_9 + greenmanuring*	92.6	81.1	295	3450
T_7 = T_9 + Pressmudcake @ 10t/ha + canetrash @ 10t/ha*	86.0	89.2	300	3329
T_{10} = Control (no fertilisers or manures)	77.3	56.2	250	2088
LSD P< 0.05	-	16.5	10.6	687

*Organic manures before planting of sugarcane plant crop only and fertilisers were applied to each crop.

Table 2 *Sustainability of crop productivity under sugarcane plant-ratoon- sesamum - paddy cropping system as indicated by average yields for different treatments.*

Level of fertiliser	Cane yield (t/ha)		Grain yield (kg/ha)	
	Plant crop	Ratoon crop	Sesamum	Paddy
100% N	92.4	94.1	336.1	4141
50% N	90.2	84.1	291.8	3556
0 N	72.2	56.2	250.0	2253
100% N + full P & K as inorganic fertilisers	92.3	95.1	331.2	3800
Integrated use of 100% NPK + organic manures	92.1	95.5	339.3	4368
50% N + full P & K as inorganic fertilisers	90.7	76.9	280.3	3190
Integrated use of 50% N + full P and K + organic manures	90.0	86.4	295.5	3678

due to application of 100% recommended NPK and combination of this treatment with organic manures tended to increase as the crop rotation advance from sugarcane plant crop to paddy. For example, whilst the cane yield from application of chemical fertiliser and the integrated use of fertilisers and manures (with applied N reduced to 50%) was almost the same for the sugarcane plant crop, the integrated use of 50% N + full P and K + organic manures increased ratoon cane yield by 12.3%, sesamum grain yield 5.4% and paddy grain yield 15.3% over that of 50% N + full P and K alone. Further, the crop yields from integrated use of 50% N + full P and K + organic manures were nearly equal to those from 100% NPK alone. This indicates that sustainable crop yields can be obtained in a crop rotation from integrated use of chemical fertilisers and organic manures even when the level of applied nitrogen is reduced by 50%. Jadhav et al (1993) reported organic sources like cane trash, pressmud cake, spentwash and their compost, FYM and green manuring reduce the need for chemical fertilisers. Improved sugar yield due to integrated use of 180 kg N/ha and 10 t/ha FYM was reported by Palchamy et al (1994).

The balance sheet of soil available nutrients after completion of the crop rotation are given in Table 3. Maximum removal of N (- 287 kg/ha), P_2O_5 (- 49 kg/ha) and K_2O (- 388 kg/ha) from soil reserves occurred in control plots (T_{10}). When FYM was used on the sugarcane plant crop either with 100% (T_2) or 50% (T_3) N; the N addition to soil was recorded. On the other hand when green manure, pressmud cake + cane trash alongwith 100% N was used, nitrogen was added to soil reserve. Mishra & Mahatim Singh (1991) attributed this to fixing of N by the green manure besides adding N straight to the soil reserve. This indicates the value of integrated use of manures and fertilisers for maintaining soil N fertility. However, when 50% N is added through fertiliser along with green manure or cane trash + pressmud cake, considerable depletion of soil N from soil reserves was observed (-104 and - 74 kg/ha respectively) indicating that the N added was much less than the crop requirement. Among the organic manures, FYM was better in enriching the soil available N (+ 173 kg/ha and + 85 kg/ha at 100% and 50% N respectively) and P (+ 296 kg/ha and + 310 kg /ha at 100% and 50% N respectively) with lesser depletion of soil available K_2O (-131 kg/ha and - 39 kg/ha at 100% and 50% N respectively). Rabindra et al (1985) attributed the superiority of FYM to more favourable effect on soil aggregation resulting in better physical condition of the soil and maintenance of organic matter.

The P_2O_5 applied either through fertilisers or manures was apparently higher than crop removal (Table 3) and hence this nutrient was added to soil reserves.

Despite regular addition of K_2O to all crops in the rotation, the applied K_2O is lower than crop uptake (Table 3). This indicates a need for higher K_2O addition. Sundara & Subramanian (1989) reported that the removal of K was much higher than the amount of K applied whereas lesser N and P were removed than the amount applied in sugarcane based crop rotation. Bansal (1992) reported the removal of K from soil reserves in the absence of K application to the crops in the multiple crop rotation.

CONCLUSIONS

1. Sustainable crop yields can be obtained in a crop rotation from integrated use of chemical fertiliser and organic manures even when the level of applied nitrogen is reduced by 50%. Further, the yields of crops obtained as a result of integrated use of 50% N and organic manures are comparable with the yields of those obtained due to application of 100% recommended dose of inorganic fertilisers alone. Thus there is a scope for saving of inorganic nitrogen to the crops grown in the rotation.

2. The integrated effects of organic manures and inorganic fertilisers were more pronounced in the subsequent crops (sesamum and paddy) than the initial crops (sugarcane plant and ratoon) in the rotation, indicating the long term effects of organic manures in the crop sequence.

3. Integrated use of 100% inorganic fertilisers and organic manures led to nitrogen addition to the soil reserve. The P removal by the crops is less than the P addition and hence P was added to soil reserve. The K removal was much higher than the K addition leading to depletion of soil reserves.

ACKNOWLEDGEMENT

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Table 3 *Balance sheet of available N, P₂O₅ and K₂O after completion of a sugarcane plant - ratoon-sesamum-paddy crop rotation cycle*

Treatment	Nutrient status (kg/ha)			Before crop cycle	Soiltest (kg/ha)		Change to soil reserve[1]
	Applied	Uptake	Balance		Aftercrop cycle	Balance	
Nitrogen (N)							
T₁	456	457	−1	224	220	−4	−5
T₂	660	457	+203	224	194	−30	+173
T₃	432	363	+69	224	240	+16	+85
T₄	514	464	+50	224	228	+4	+54
T₅	507	492	+15	224	236	+12	+27
T₆	286	382	−96	224	216	−8	−104
T₇	279	341	−84	224	214	−10	−94
T₈	456	492	−36	224	231	+7	−29
T₉	228	340	−112	224	226	+2	−110
T₁₀	0	293	−293	224	230	+6	−287
Phosphorus (P₂O₅)							
T₁	250	76	+174	40	45	+5	+179
T₂	378	85	+293	40	43	+3	+296
T₃	378	66	+312	40	38	−2	+310
T₄	263	85	+178	40	42	+2	+180
T₅	377	92	+285	40	41	+1	+196
T₆	263	68	+195	40	41	+1	+196
T₇	377	65	+312	40	42	+2	+314
T₈	250	76	+174	40	45	+5	+179
T₉	250	58	+192	40	42	+2	+194
T₁₀	0	55	−55	40	46	+6	−49
Potassium (K₂O)							
T₁	300	695	−395	346	392	+46	−349
T₂	540	710	−117	346	385	+39	−131
T₃	540	586	−46	346	339	+7	−39
T₄	345	711	−366	346	351	+5	−361
T₅	371	754	−383	346	412	+66	−317
T₆	345	522	−177	346	386	+40	−137
T₇	371	542	−171	346	398	+52	−119
T₈	300	674	−374	346	353	+12	−362
T₉	300	468	−168	346	373	+27	−141
T₁₀	0	420	−420	346	378	+32	−388

1   + added to soil reserve; - removal (ram soil reserve)

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EVALUATION OF SOIL DEGRADATION UNDER SUGARCANE CULTIVATION IN NORTHERN KWAZULU-NATAL

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ABSTRACT

The aim of this study was to quantify the physical and chemical condition of soils under sugarcane production in northern Kwazulu-Natal, because commercial sugarcane yields have failed to break through the "productivity plateau" during the past 15 years. Soil samples from 29 virgin and adjoining cultivated fields were examined with 15 and 14 originating from dryland and irrigated areas respectively. The most prominent soil chemical property to contribute to soil degradation under sugarcane cultivation was increased acidity in dryland areas and increased salinity and sodicity levels in irrigated areas. Other important differences found between paired sites and relative to the virgin area included reduced organic matter, cation exchange capacity, total N and extractable bases and increased soil bulk density. In general dryland areas were acidifying and irrigated areas sodicifying. Remedial measures should include increasing soil organic matter through minimum tillage, trashing and fallowing.

INTRODUCTION

The failure of commercial sugarcane yields to break through the "productivity plateau" during the past 15 years was the main reason for initiating a survey in which the differences in soil properties between virgin and adjoining cultivated soils were examined. Similar studies were conducted in other areas of southern Africa (du Toit & du Preez 1995; Henry & Ellis 1995) but to date not in the South African sugar industry. Studies conducted elsewhere with sugarcane include degradation surveys conducted in the Herbert valley Queensland, Australia (Wood 1985) and in Swaziland, Southern Africa (Henry & Ellis 1995) and trials to improve soil fertility following incorporation of sugarcane trash in India (Jadhav 1995). Haynes et al (1991) reported that intensive cropping systems based on mineral fertilizers, intensive soil tillage and removal of crop residues often lead to decreased levels of soil organic matter. The amount of organic matter present in the soil has been shown to affect soil bulk density (Ekwue & Stone 1995), plant available water capacity (Hudson 1994), porosity, CEC, exchangeable acidity (Wood 1985; Schj0nning et al 1994), the availability of N, P and S (Naidu & Rengasamy 1993) and yield (Jadhav 1995). Du Toit & du Preez (1995) have shown that soil organic matter loss following the introduction of cultivation of selected dryland soils, reached equilibrium in 5-10 years in the warmer, drier ecotypes and 40-60 years in the cooler, wetter ecotypes of southern Africa. The cultivation period of fields used in this survey ranged from 2 to more than 30 years, with the mean at about 25 years.

METHODS AND MATERIALS

Soil samples were collected from 29 paired sites in the northern areas of Kwazulu-Natal. The sites were representative of alfisol, oxisol and vertisol soils and were located between latitude 28°23' and 30°45' south and longitude 31°20' and 32°03' east. Paired sites consisted of uncultivated (virgin) and adjoining cultivated sites under sugarcane no more than 30m apart. Virgin sites included natural bush, and road reserves with natural grassland. At each site, soil samples were taken in triplicate at depths of 0-150, 150-300 and 300-450mm for chemical examination. Duplicate undisturbed soil core samples were taken at depths of 0-20 and 200-220mm for analysis of soil physical properties. In cultivated sites, soil samples for chemical and physical examination were collected in the interrow at crop ages ranging from one to 12 months. The fertilizers used were inorganic blends with a nitrogen to phosphorus to potassium (N:P:K) ratio of either 5:1:5 or 1:0:1. Di-ammonium phosphate, mono-ammonium phosphate and urea were used occasionally. The amount of N, P and K applied per hectare ranged between 120 to 160kg, 20 to 40kg and 100 to 160kg respectively. Fifteen of the 29 paired sites comprised cane grown under dryland conditions and 14 sites were irrigated. On 55% of the dryland sites cane is burned before harvest and of this 91% of the cases the tops are spread after harvest. The remaining 9% tops are raked and burned after harvest. All the irrigation growers are burning their cane before harvest with only 54% spreading the tops after harvest.

Physical properties that were measured included bulk density (core method), soil water retention (pressurised closed system), soil texture (hydrometer method) and electrical conductivity of the saturation extract. Total pore space (TPS) was calculated from bulk density assuming a constant particle density of $2.65 Mg/m^3$ and plant available soil water capacity (PAWC) was calculated from the retention data. Chemical analysis included pH (1:2.5, soil:water), P (Truog), Zn, exchangeable K, Ca, Mg, Na, S (1N ammonium acetate), Al (0.2N ammonium chloride) and total N (Meyer et al 1989), titratable acidity (Thomas 1982), cation exchange capacity (sum of titratable acidity and NH_4OAc extractable K, Ca, Mg and Na), acid saturation (titratable acidity / cation exchange capacity x 100) and organic matter (Walkley & Black 1934). The extract from water saturated soil samples was used to determine electrical conductivity (EC_e) and soluble Na, Ca and Mg concentrations. The significance of differences in analysis between cultivated and virgin sites was determined by means of the students' t-

RESULTS

Statistical differences of selected soil physical properties between paired sites from dryland and irrigated areas are summarised in Table 1. Mean differences in clay content between paired sites were <3% and not significant. Dryland and irrigated areas contained about equal amounts of clay but the former contained about 5% more silt. Soil bulk density was higher for cultivated compared to virgin sites and higher in the irrigated compared to the dryland areas. Mean soil water content at saturation and at matric potentials of -10kPa and -1500kPa were all slightly higher in the virgin areas compared to the cultivated sites and of the same order between dryland and irrigated areas.

The main chemical changes are summarised in Tables 2 and 3. pH values from the virgin sites in the dryland area was about 1.02 pH units more acidic compared to the irrigated area. Soil pH was also lower on the cultivated sites relative to the virgin sites of the dryland area but higher in the irrigated area. Further assessment of the results showed significant increases in titratable acidity, extractable Al, and acid saturation and significant declines in exchangeable Mg, Na and CEC of the intermediate subsoil layer (150 to 300mm). Overall these results suggest that cultivated dryland sites are acidifying relative to virgin sites.

Comparison of the chemical status of paired sites from the irrigated area (see Table 3) showed a buildup of salinity (EC_e), water and ammonium extractable Na in the cultivated sites and a decline of extractable bases, titratable acidity and CEC. Statistical significant differences between paired sites in the irrigated area were obtained for TA, CEC, SAR, EC, NH_4OAc extractable K, Ca and Na and water extractable Na. Although, the topsoil values for Na were below the threshold value of 2 cmol/kg, the subsoil (450mm depth) values in the irrigated area approached this threshold value. It is evident from these results that the cultivated sites in the irrigated area have been sodicified.

Table 1 *Soil physical properties of paired sites from a dryland and irrigatea area.*

Soil property	Depth (mm)	Dryland			Irrigated		
		Mean virgin	Mean cultivated	Mean difference	Mean virgin	Mean cultivated	Mean difference
Clay %	150	19.2	22.3	-3.1	20.6	19.7	0.9
	300	20.7	21.1	-0.4	23.1	23	0.1
	450	21.8	21.0	0.8	25.5	25.3	0.2
Silt %	150	14.8	13.6	1.2	9.2	8.9	0.3
	300	14.8	13.9	0.9	8.3	8.8	-0.5
	450	12.7	12.5	0.2	6.9	8.5	-1.6
Bulk density (Mg/m^3)	20	1.208	1.340	-0.132*	1.396	1.444	-0.048
	220	1.416	1.455	-0.039	1.503	1.602	-0.099
SWC at saturation (v/v %)	20	44.6	40.8	3.8	50.1	47.2	2.9
	220	41.4	41.8	-0.4	47.3	42.2	5.1
SWC at-10kPa (v/v %)	20	24.9	24.7	0.2	29.1	25.8	3.3
	220	25.1	25.4	-0.3	29.5	25.7	3.8
SWC at-1500kPa (v/v %)	20	17.8	15.1	2.7	16.6	13.9	2.7
	220	17.2	17.5	-0.3	18	15.9	2.1
Total pore space (v/v %)	20	54.5	49.4	5.1*	47.3	45.5	1.8
	220	46.6	45.1	1.5	43.3	39.6	3.7
PAWC 0-10kPa (mm/m)	20	197	161	36	211	212	-1
	220	163	164	-1	178	164	14
PAWC -10 to-1500kPa (mm/m)	20	71	96	-25*	125	119	6
	220	79	79	0	115	98	17

SWC = Soil water content   PAWC = Profile available water capacity   * Significant at P=0.05

Table 2 *Soil chemical differences associated with organic matter content changes between paired sites in a dryland and irrigated area.*

Soil property	Depth (mm)	Dryland			Irrigated		
		Mean virgin	Mean cultivated	Mean difference	Mean virgin	Mean cultivated	Mean difference
P (mg/kg)	150	21.83	32.69	-10.87	33.92	23.50	10.42
	300	24.52	29.04	-4.52	16.26	12.49	3.77
	450	17.57	20.84	-3.25	10.20	12.72	-2.52
S (mg/kg)	150	24.87	17.27	7.60	22.86	19.57	3.29
	300	18.92	17.00	1.92	22.07	24.29	-2.21
	450	18.54	19.85	-1.31	33.38	53.31	-19.92
Zn (mg/kg)	150	2.10	2.15	-0.04	1.46	1.30	0.15
	300	1.70	1.65	0.06	1.09	0.87	0.22
	450	1.36	1.05	0.31	0.67	0.77	-0.10
(Ca+Mg)/K ratio	150	21.21	20.36	0.85	15.93	20.98	-5.05
	300	33.21	28.85	4.36	27.98	33.54	-5.56
	450	40.35	42.46	-2.11	40.71	38.56	2.15
Organic matter (%)	150	3.87	3.31	0.56*	2.40	1.88	0.52*
	300	3.33	3.19	0.14	2.08	1.69	0.38*
	450	3.16	3.04	0.12	1.46	1.39	0.08
Total N (cmol/kg)	150	13.85	9.42	4.43*	7.57	5.39	2.18*
	300	7.07	9.28	-2.21	5.44	4.93	0.52
	450	9.35	8.25	1.11	4.78	4.14	0.64
C:N ratio	150	15.32	17.11	-1.79	13.69	13.54	0.15
	300	16.34	16.97	-0.63	16.28	14.92	1.36
	450	17.00	17.10	-0.10	12.50	12.69	-0.19

* Significant at P=0.05   ** Significant at P=0.01

Table 2 summarises the effect of cultivation in dryland and irrigated areas on organic matter (OM), total N, as well as nutrients and nutrient ratios important in the successful production of sugarcane not listed in Table 3. The P status of the dryland area was on average higher in the cultivated sites compared to the virgin sites but the converse applied for the irrigated area. The differences in P between paired sites was greatest in the First soil layer (0 to 150mm) of both dryland and irrigated areas and decreased with increasing depth. A similar trend was noticed for OM. The remaining nutrients (S, Zn and total N) and OM all showed lower levels for the cultivated sites relative to the virgin sites in both the dryland and irrigated areas. The C:N ratio was slightly higher in the cultivated sites compared to the virgin sites in the dryland area but the converse was found for the irrigated area. The (Ca+Mg)/K ratio shows little change between paired sites of both the dryland and irrigated areas. Statistical significant differences between paired sites were obtained for only OM and total N.

Table 3 *Soil chemical differences associated with pH changes between paired sites in a dryland and irrigated area.*

Soil property	Depth (mm)	Dryland			Irrigated		
		Mean virgin	Mean cultivated	Mean difference	Mean virgin	Mean cultivated	Mean difference
pH (water)	150	5.56	5.37	0.19	6.68	6.80	-0.13
	300	5.64	5.35	0.29	6.55	6.81	-0.27
	450	5.71	5.43	0.28	6.75	6.80	-0.05
Al (mg/kg)	150	6.93	9.13	-2.20*	2.55	2.36	0.18
	300	9.00	12.31	-3.31	2.09	2.45	-0.36
	450	9.38	11.77	-2.38	2.50	2.70	-0.20
K[1] (cmol/kg)	150	0.53	0.46	0.08	0.89	0.58	0.31*
	300	0.37	0.30	0.07	0.58	0.38	0.21
	450	0.27	0.21	0.06*	0.41	0.31	0.10
Ca[1] (cmol/kg)	150	6.89	6.14	0.75	8.32	6.97	1.35*
	300	7.21	6.23	0.98	9.17	7.27	1.90
	450	6.88	6.39	0.50	10.08	7.51	2.57**
Mg[1] (cmol/kg)	150	4.13	3.27	0.86*	4.07	3.86	0.20
	300	4.22	3.12	1.09**	4.34	4.24	0.10
	450	4.18	3.40	0.78	5.12	4.19	0.93
Na[1] (cmol/kg)	150	0.54	0.58	-0.03	0.64	1.03	-0.39*
	300	0.64	0.42	0.22**	0.83	1.54	-0.71*
	450	0.65	0.46	0.19*	1.44	2.08	-0.64
Titratable acidity (cmol/kg)	150	0.32	0.50	-0.18*	0.14	0.11	0.03*
	300	0.33	0.53	-0.20*	0.14	0.16	-0.01
	450	0.36	0.48	-0.12	0.18	0.15	0.02
CEC (cmol/kg)	150	12.41	10.94	1.47	14.06	12.57	1.49*
	300	12.76	10.61	2.16*	15.06	13.58	1.48
	450	12.34	10.94	1.40	17.22	14.25	2.97
Acid saturation (%)	150	5.82	10.42	-4.60*	1.67	2.48	-0.81
	300	7.52	13.43	-5.91	1.86	4.17	-2.32
	450	8.35	14.27	-5.93	2.02	4.30	-2.28
Ca[2] (mmol/dm³)	150	0.50	0.60	-0.10	0.74	0.74	0.00
	300	0.52	0.70	-0.18	0.62	0.58	0.04
	450	0.46	0.56	-0.10	0.73	0.60	0.14
Mg[2] (mmol/dm³)	150	0.57	0.59	-0.02	0.57	0.78	-0.21
	300	0.51	0.63	-0.12	0.49	0.57	-0.08
	450	0.44	0.50	-0.06	0.73	0.55	0.18
Na[2] (mmol/dm³)	150	2.70	2.23	0.47	3.00	5.51	-2.51
	300	2.67	2.11	0.56	3.08	5.80	-2.72**
	450	2.46	2.00	0.46	5.34	7.84	-2.50
Sodium adsorption ratio	150	1.97	1.57	0.41	2.51	4.28	-1.77*
	300	1.86	1.43	0.43	2.85	5.29	-2.44*
	450	1.92	1.47	0.45	3.72	6.11	-2.39*
EC_e (mS/m)	150	59.91	60.64	-0.73	63.77	96.92	-33.15
	300	56.56	63.22	-6.67	57.54	88.69	-31.15**
	450	49.67	52.56	-2.89	86.42	107.42	-21.00

* Significant at P=0.05   ** Significant at P=0.01   [1] = extract with ammonium acetate   [2] = extract with water

CEC = cation exchange capacity   ECe = electrical conductivity of the saturated paste extract

DISCUSSION

The overall impression from this work is that the soil chemical properties were more affected by sugarcane cultivation than the physical properties. The small insignificant textural differences between the virgin and cultivated sites indicates that excessive soil variability can be ruled out when interpreting the results.

The higher bulk density of the cultivated sites might be due to structural break down (not measured) as a result of the reduced organic matter content (Schj0nning et al 1994), chemical dispersion of clays through enhanced Na levels (Johnston 1981) and compaction through infield haulage systems (Swinford & Boevey 1984). The difference between paired sites of both the dryland and irrigated areas is relatively small compared to results from a similar study conducted in the Herbert Valley, Australia (Wood 1985). The South African system of mainly manual cutting and stacking and mechanised haulout with harvesting restricts

soil compaction when compared to the complete mechanised harvesting systems used in Australia. The values reported in Table 1 are well below a mean critical bulk density value of 1.72Mg/m³ (calculated from Maud 1960) for the South African sugarcane industry. The higher bulk densities obtained for the irrigated area compared to the dryland area might be due to a higher moisture content in the soil at harvest (Archer & Smith 1972), chemical dispersion through enhanced Na levels (Johnston 1981) and the use of haulout vehicles weighing up to 20 ton when loaded (Swinford & Boevey 1984).

Archer and Smith (1972) have shown that the capacity of soils to store plant available water (PAWC) will initially improve with increasing soil bulk density (BD) to a maximum but thereafter progressively decline. This trend was also evident from the results presented inTable 1 where PAWC (between -10 and -1500kPa) increased with an increasing BD to reach an optimum PAWC level between BD values 1.400 and 1.500Mg/m³.

Continuous monocropping of sugarcane, burning of crop residues and a relatively (to the rest of southern Africa) high rainfall of 900 to 1200mm were some of the major factors contributing to reduced soil organic matter content and CEC of the cultivated sites in the dryland area. This inturn explains the increased acidification, Al, EC and water extractable bases and reduced NH_4OAc extractable bases (Aldrich & Turrel 1950- Wood 1985; Schroeder et al 1994; Henry & Ellis 1995). Aldrich and Turrel'(1950) suggested that the increased water soluble bases and reduced NH OAc extractable bases may largely be due to the replacement of these cations on the exchange complex by hydrogen. At present the prospect of growers breaking the continuous monocropping cycle in sugarcane production is fairly mode as the average number of ratoons for the South African industry is about eight and fallowing is not normally practised.

The most pronounced results for the irrigated area from this survey was the relatively large differences between paired sites in terms of increased salinity and sodicity (Table 2). It was also evident that aIfisols with a duplex character are more prone to sodicity and oxisols to salinity (results not shown). Alfisols are affected by the underlying parent material which is a rich source of Na (Beater & Frankel 1965). Oxisols are normally the better drained soils and are well suited for irrigation. The quality of irrigation water used will thus determine the rate of degradation on these soils. Oxisols occur predominantly in the northern parts of the South African sugar industry where rivers with the poorest water quality are located (Meyer & Van Antwerpen 1995).

Organic matter is a rich source of mineral P, S and N in soil (Naidu & Rengasamy 1993) and it can be expected that soil management practices leading to a decline of OM in soil will reduce the availability of these nutrients to plants. Possible reasons for the reduced P content of cultivated soils in the irrigated area may be due to lower OM, higher crop removal by cane growers and possible precipitation of P as phosphates for Ca and Mg in irrigation water on soils with a high P fixing capacity. P recommendations will probably need to be reassessed for irrigated cane.

The availability of Zn is mainly soil pH controlled (Naidu & Rengasamy 1993) with optimum availability between pH values 5.0 and 5.7 (Schroeder et al 1994). The mean pH of the dryland area was within this range but that of the irrigated area ranged between 6.5 and 6.8 for the virgin and cultivated sites respectively. The pH effect probably accounts for the unchanged Zn status between paired sites in the dryland area and the reduced Zn levels for the cultivated sites in the irrigated area.

The low OM content and C:N ratio found for the irrigated area is in many respects similar to that reported for sodic soils in Australia (Naidu & Rengasamy 1993). The reduced C:N ratio from the cultivated sites in the irrigated area suggest that the loss of OM is larger than that of total N which may be due to the practice of burning nearly all plant residues of cane produced under irrigation. The fact that more plant residues are retained in dryland cane fields compared to the irrigated area could be the reason why the C:N ratios were higher for the cultivated sites which means than losses of total N were greater than OM. The increased (Ca+Mg)/K ratio in the topsoil of the irrigated area on the cultivated sites indicates leaching of K relative to Ca and Mg, suggesting that the current K fertilisation recommendation for the irrigated area is not adequate. Similar results were reported for an irrigation estate in Swaziland by Henry & Ellis (1995).

This survey was not designed to look at the effect that cultivation period over time had on soil degradation. However, a trend was revealed from the samples collected in the dryland area at site number 42 on a coastal loamy sand (results not shown). Two cultivated fields, two and >20 years in production respectively, were sampled within 20 metre of the virgin site. The soil degradation pattern for the old cultivation site followed the mean trend reported in Tables 1, 2 and 3 for the dryland area. Soil degradation for the two year old field had already started to show small differences relative to the virgin site in terms of titratable acidity, acid saturation, CEC, P, S, Al and NH_4OAc extractable K, Ca and Mg. These were however not significant.

CONCLUSION

As in many other parts of the world soil degradation, mainly in the form of increased acidification in the dryland area and salinity/sodicity build up in the irrigated area, are factors that have been positively identified in the South African sugar industry. As these factors have been linked to yield decline steps must be taken to prevent or reduce the rate of soil degradation. The common thread Unking the results in all three tables is the decline in

organic matter levels. Production of sugarcane in South Africa is a monoculture practice with a mean of eight crops before fields are re-established with sugarcane. The cycle of monocropping can only be broken through crop rotation (Capriel et al 1992), green manuring, minimum tillage, greencane harvesting and spreading of crop residues in order to stabilise and even increase soil OM quantities. Growers in the irrigated area should also reduce sodium build up through drainage and the use of $Ca(S0_4)_2$. There may also be merit in the use of ammonium sulphate as the main N carrier in order to acidify the soils to a pH values between 5.5 and 6.0. Growers in the dryland areas will also need to conserve the OM content of their soils and make more use of $CaCO$, to reduce acidification of their soils.

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SUGARCANE GROWTH AND YIELD COMPARISONS FOR PAIRED OLD AND NEW LAND SITES

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Sugar Yield Decline Joint Venture
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ABSTRACT

Growth characteristics, cane and sugar yield were measured for sugarcane crops grown on paired old and new land sugarcane sites at Tully (2 sites) and on a continual sugarcane versus a sugarcane/pumpkin rotation site in the Burdekin River Irrigation Area (BRIA), north Queensland. At one of the Tully sites cane yield was higher on new land whereas there was no difference at the other. There was no difference in cane yield between the continual sugarcane and the sugarcane/pumpkin rotation at the BRIA site. CCS was higher on old land at both Tully sites but there was no difference between the crop systems in the BRIA. Growth, as measured by stalk number per unit area, was always better on new (Tully) or rotation land (BRIA) regardless of sampling time. Whether early growth differences were reflected in ultimate cane and sugar yield appeared dependent on crops not lodging, thus avoiding stalk death, and/or the adverse effects of old land on growth being compensated for by a high input production system. It is concluded that the potential for yield decline exists at each of these sites. However, its expression is dependent on the particular growing conditions.

INTRODUCTION

The Australian sugar industry has been on a productivity (sugar yield/ha) plateau for the past 25 years (SRDC 1995) The precise reasons for the productivity plateau are unknown but are believed to be due to a combination of factors that are related to climate, soil, management and industry development. The phenomenon known as yield decline, defined as the loss of productive capacity of sugarcane-growing soils under long term monoculture, is probably a component of the productivity plateau.

Identifying and overcoming the causes of yield decline is the charter of a major Joint Venture involving the Bureau of Sugar Experiment Station, CSIRO Division of Soils, the Queensland Department of Primary Industries, and the Sugar Research and Development Corporation (Garside 1995) The Joint Venture is taking a farming systems approach to the problem and relating crop growth and yield to soil chemical, physical and biological properties in paired old and new land sites, rotation experiments with other species, and rundown experiments (when new land is planted and continues to grow sugarcane)

In this paper, data are presented on differences in growth, cane and sugar yield between two paired old and new land sites for sugarcane and between a continual sugarcane versus sugarcane/pumpkin rotation site.

MATERIALS AND METHODS

Site selection

Two paired sites (Harney and Costanzo) were selected near Tully to compare sugarcane crop growth between old and new land. Old land at both sites had been under sugarcane monoculture for more than thirty years, whilst the new land was first planted to sugarcane in 1992. The crops measured in these studies were first ratoons. New land had previously been unimproved pasture at Harney and the site of a building at Costanzo.

Tully is located on the wet tropical coast of north Queensland where the mean annual rainfall is 4074 mm (Clewett et al 1994) and sugarcane is grown without irrigation. The Costanzo site was located 20 km north of Tully where the soil was a yellow Dermosol of the Mossman series (Cannon et al 1992) The Harney site was located on the banks of the Tully River 10 km southwest of Tully where the dominant soils are a combination of yellow Dermosol/Hydrosol of the Mossman (Dystropept - Soil Survey Staff 1994) and Hewitt (Typic Tropaquept - Soil Survey Staff 1994) series (Cannon et al 1992) Detailed site descriptions are provided by Bramley et al (1996)

At another site, in the Burdekin River Irrigation Area (BRIA)(Pegararo), sugarcane crop growth was compared between a continual sugarcane versus sugarcane/pumpkin rotation land. Until two years prior to the experiment, all of the land had been under sugarcane for at least 30 years. Then, pumpkins had been grown on part of the site. The entire site was then replanted to sugarcane at the commencement of the experiment. Thus, measurements were made on a plant crop at this

The BRIA is in the dry tropics where the mean annual rainfall is approximately 1000 mm (Clewett et al 1994) and all sugarcane production is under irrigation. The soil at this site is a Brown Chromosol (Udic Haplustalf - Soil Survey Staff 1994) of soil profile class 6Dya (Thompson et al 1990)

All sites were managed by the respective farmers using conventional methods, and within sites agronomic practices were the same on the old and new land and on the two die rotation lands.

Crop growth measurements

At each site three replicates, each 10 m x 1 row were selected in each of the old and new land crops. At the Pegararo site the continual sugarcane and the rotation area were used. These measurement (and subsequent harvest positions) were permanently marked. Stalk counts (stalks/10m) were carried out at monthly intervals commencing soon after planting or ratooning depending on the crop class.

At crop harvest, each 10 m of row was hand harvested, millable stalks were counted, the cabbage (green leaf and immature top of the stalk) was removed, stalks weighed, and a 6-stalk sample was used for CCS (Commercial Cane Sugar) determination (Anon 1970) A further sub-sample was fibrated using a cutter grinder and weighed before drying in a forced air oven at 80°C for 48 h and re-weighed. These data were used to calculate dry stalk biomass.

RESULTS AND DISCUSSION

New land improved sugarcane yield at Harney, had no effect at Costanzo, and there was no difference between continual sugarcane and the pumpkin rotation at Pegararo (Table I) At Harney, CCS was higher on old land than new land. Whereas there was no effect on CCS at either Costanzo or Pegararo. The combined effect of cane yield and CCS resulted in higher sugar yields on new land at Harney. At all sites millable dry stalk biomass reflected sugarcane yield, with stalk moisture being of the order of 70% in all treatments.

At both Harney and Costanzo, stalk numbers were initially much higher on new land than old land and this difference, though subsequently smaller, was maintained through to final harvest. Maximum stalk numbers were recorded two to one month earlier on new land (Fig.1) Similar, though less pronounced trends, were apparent for the rotation and continual sugarcane areas at Pegararo (Fig.1) Stalk numbers

declined at all sites later in the growing period, with the rate of decline being initially much higher on new land. However, after about mid-January the rate of decline slowed considerably and the difference in stalk number between new and old land remained relatively constant until harvest at around 20 - 30/10m row.

At Costanzo, individual stalk fresh weights were higher on old land than on new land, as they were on continuous sugarcane land compared to sugarcane/pumpkin land at Pegararo (Table 1) Hence at these sites, although there were fewer stalks on old land and continuous cane, these stalks weighed more than those grown on the new land and the sugarcane/pumpkin land, respectively. With the result that, as seen above, cane yields were the same in all treatments at these sites. By contrast, at Harney, both the fresh weight and number of stalks per 10m row were greater on new land, as was cane yield.

Table 1 *Growth responses of sugarcane crops grown at three paired sites in north Queensland. Sites at Harney and Costanzo compare old and new sugarcane land, and at Pegararo compared continuous sugarcane with sugarcane/pumpkin rotation.*
[Numbers are the Means of 3 replicates with standard deviations in parentheses.]

Site	Old	New	Pumpkin
Harney			
Cane yield (t/ha)	76(9)	98(9)	
CCS (%)	15.8(0.7)	13.9(0.1)	
Sugar yield (t/ha)	12.1 (2.0)	13.7(1.0)	
Stalk moisture (%)	69(1)	67(1)	
Individual stalk fresh weight (kg)	1.1 (0.2)	1.1(0.2)	
Stalk dry biomass (t/ha)	23.8 (3.4)	31.8(2.2)	
Costanzo			
Cane yield (t/ha)	104 (9)	98(5)	
CCS (%)	15.0(0.1)	14 .5 (0.4)	
Sugar yield (t/ha)	15.6(1.2)	14.3(1.1)	
Stalk moisture (%)	71(1)	70(1)	
Individual stalk fresh weight (kg)	1.2(0.2)	0.8(0.1)	
Stalk dry biomass (t/ha)	30.4 (3.2)	29.0(1.5)	
Pegararo			
Cane yield (t/ha)	179(17)		176(8)
CCS (%)	16.8(0.3)		16.7(0.4)
Sugar yield (t/ha)	30.0 (2.3)		29.4(0.9)
Stalk moisture (%)	68(1)		68(1)
Individual stalk fresh weight (kg)	2.4 (0.3)		2.0 (0.0)
Stalk dry biomass (t/ha)	57.4 (5.8)		55.7(1.0)

Superficial interpretation of the yield data at Costanzo suggests that there has been no loss in productive capacity of the soil after more than 30 years of sugarcane production. Our observations during growth suggest that this was not the case. Crop growth was so vigorous on the new land at this site that severe lodging occurred in February, whereas there was no lodging on the old land. Once the lodged crop rows intermingled it was impossible to continue stalk counts and accurately define the harvest area. Severe loss of mature stalks through decay and numerous new shoots were evident in the new land area by harvest (September) due to the crop having been lodged in a moist, humid environment for some 6 months prior to harvest. Due to the problem of lodging in the new land, we harvested border areas of the new land plots where lodging was not as severe. However, being a border area, there is little doubt that the yield measured did not accurately reflect the potential yield within the new land plots, had they not lodged. We believe that, had stalk death been avoided, the new land crop at this site would have substantially outyielded the old land crop, which did not lodge. As noted in other studies (Muchow et al 1995), the present experience indicates that lodging, and subsequent stalk death, is a serious impediment to maximizing yield.

At the Harney site, where lodging did not occur, the new land produced 22% more cane and 11% more sugar than the old land. The cane and sugar yield, and pattern of stalk development on old and new land here reflect that reported by Muchow et al (1994) for unfumigated and fumigated soil conditions. Numerous other field fumigation studies where only sugarcane yield has been measured suggest similar responses (approx. 20% increase in cane yield) to fumigation (Magarey & Croft 1995) The implication is that soil-related factors, which may be controlled by fumigation, are limiting sugarcane yields under long term monoculture.

Breaking the monoculture for two years with a rotation crop (pumpkins) had no effect on sugarcane yield in this study, even though there were substantially more stalk numbers throughout growth following the pumpkins (Fig. 1) However, as seen in Table 1, the individual stalks from the continual sugarcane land were heavier than those from the sugarcane/pumpkin rotation. This site, in the BRIA, had high inputs of radiation, water and nutrition resulting in relatively high yields (175 t/ha) Muchow et al (1994) only measured a 5% increase in sugarcane yield with fumigation at high yield levels (e.g. 200 t/ha) in the BRIA, whereas substantially greater responses to fumigation have been recorded in less favourable environments (Magarey & Croft 1995) The implication is that the adverse effects of long term sugarcane monoculture may be substantially overcome under favorable growing conditions such as occur and/or can be applied in the BRIA. However, the corollary is that if soil health is improved there may not be the need for such high inputs of water and nutrients to maximize yields.
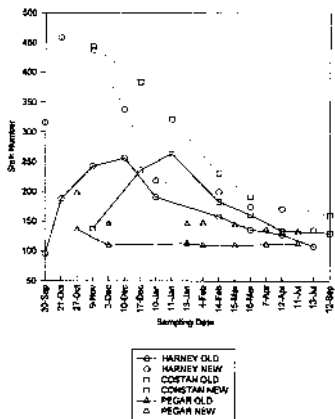

Fig. 1 *Change in stalk number/10 m of row with time on old and new sugarcane land at Harney and Costanzo, and on continual sugarcane and sugarcane/ pumpkin rotation at Pegararo.*

Two important implications emerge from these studies. Firstly, the potential for reduced yields under long term monoculture is likely to be universal, because the current data and numerous other observations suggest that one of the basic yield components, stalk number, is substantially reduced. However, whether or not early treatment effects are realized in differences in final yield appears to be greatly affected by the environmental and cultural conditions under which crops are

grown; conditions that vary between sites and seasons. Secondly, the numerous stalks produced in early growth that subsequently die, presumably through assimilate shortages and/or shading effects later in growth, appears to indicate a substantial waste of resources. An important question needs to be answered: are assimilates from the dying stalks retranslocated to the surviving stalks?

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EFFECT OF LONG TERM CANE PRODUCTION ON SOIL PROPERTIES OF A GLEYED PODZOLIC SOIL NEAR BUNDABERG, QUEENSLAND

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ABSTRACT

Cane production is often associated with intensive soil cultivation and monoculture cropping, resulting in soil compaction and depleted levels of organic matter, with ensuing agronomic problems of soil crusting and poor water infiltration. A comparison was made of two adjacent sites; one used predominantly for cane production since 1985 (cultivated), the other a tract of undisturbed native vegetation (uncultivated). The soil at each site, a sodic dermosolic redoxic hydrosol (gleyed Podzolic), was sampled for a range of physical and chemical analyses. The cultivated site demonstrated reduced soil density and increased total organic carbon levels in the 0.15-0.45m layer of soil. However, despite these favourable changes, there was no improvement in soil stability as the cultivated soil slaked in the top 0.4m. There was a marked decline in microbial biomass carbon in the cultivated site, compared to the uncultivated site; a noted inverse relation with the organic carbon levels. Subsoil compaction (0.65m to 1.1m) was greater in the cultivated site. The potential benefits of controlled traffic with minimal cultivation on soils such as this site are discussed. Restricting wheel compaction solely to the inter-row will ensure long-term benefits of cultivation in the plant row, as demonstrated in this study.

INTRODUCTION

Cane production is often associated with intensive soil cultivation, particularly land preparation before planting and in some areas between ratoons. Cane machinery; especially harvesters, exert high ground pressure on soils that are commonly moist or wet, with subsequent soil compression and deformation. In addition, repeated cultivation and a monoculture can result in depleted levels of soil organic matter with deleterious effects on soil stability, water infiltration and chemical status.

Studies in different cropping systems have related length of time under cropping to changes in soil physical properties. Cotton production for 15 years on a sodic grey clay, Warren, N.S.W. led to increased subsoil compaction, increased soil surface sodicity, increased dispersibility and decreased organic matter, to 0.3m (McKenzie et al 1991). Continuous cropping of krasnozems and euchrozems around Kingaroy, Queensland has reduced water infiltration by one-third, almost doubled bulk density and decreased organic carbon in the surface soil up to five-fold, compared with virgin soils (Bridge & Bell 1994).

This project aims to assess the impact of 10 years of irrigated cane production on selected physical and chemical properties of a gleyed podzolic soil. Soil samples and measurements were taken from the cane Field and also from an immediately adjacent uncultivated treeline.

MATERIALS AND METHODS

The site was on Loeskow farm, 12km southeast of Bundaberg, Queensland and consisted of a cultivated, irrigated cane field and an adjacent area of uncultivated native vegetation. The soil is a sodic dermosolic redoxic hydrosol (Isbell 1993); and may be termed a gleyed podzolic (Stace et al1968).

The cultivated site was cleared and planted to sugarcane in 1985. Cane was grown to 1991, then peanuts were grown followed by tomatoes and zucchinis. A second cane crop was planted in 1993. Prior to this planting the soil was "square ploughed" to a depth of 0.4m. The block is furrow irrigated.

The uncultivated site immediately adjoins the cultivated field and falls within the same soil mapping unit. The vegetation consists of *Melaleuca* spp. and *Themeda triandra* (kangaroo grass).

Soil sampling was done in February 1995 when the crop was in 2nd ratoon and approximately 1.2m tall. 0.1m diameter steel cores were hydraulically driven to 1.1m in the uncultivated site and the row and inter-row of the cultivated site. For the latter, the five cores were taken just 0.1m off-center of the row (to avoid intense rooting of the cane) and in the centre of the inter-space. All cores were cut into lengths of 0.05m for the top 0.2m and into 0.1m lengths to 0.5m, then 0.6-0.7m, 0.8-0.9m and 1-1.1m. Determined at each depth were: bulk density, aggregate stability (Loveday & Pyle 1973), organic carbon (Heanes 1984) and microbial biomass (Vance et al 1987).

RESULTS AND DISCUSSION

Bulk density

There are three soil depth zones of interest (Fig. 1). The 0-0.15m layer in the inter-row is up to 17% denser than the row, and the row up to 10% denser than the uncultivated site. From 0.15-0.45m the above trend reverses somewhat in that the uncultivated site is denser (up to 4%) than the row. The difference is at a maximum at 0.35m where the row is significantly (P<0.01) less dense than the uncultivated site or inter-row at the same depth. From 0.45-1.05m, the trend shows little change with the row and inter-row being on average 6% denser than the uncultivated site. In this zone, the row and inter-row are not significantly different.

The square ploughing, two years previous, probably accounts for the lower bulk density of the row in the 0.15-0.45m layer, and demonstrates that this naturally hardsetting soil responds to loosening if there is no subsequent re-compaction. The soil in the inter-row was also loosened by the ploughing but subsequent, repeated wheelings have apparently packed the soil to high densities. The increase in density below 0.45m in the cultivated soil apparently reflects long-term densification, below ploughing depth.

Aggregate stability

At the first observation time (2h) of the aggregate stability test all aggregates from the cultivated site in the top 0.4m slaked, i.e. they broke down to microaggregates (Table 1). In contrast the original aggregates from the uncultivated site did not slake within this time. Soil to 0.2m depth from the uncultivated site and 0.4m depth from the cultivated site showed only slight dispersion, i.e. breakdown into primary particles of sand, silt and clay at the 20h observation. They were thus remoulded at field capacity for the second part of the dispersion test when all samples (both uncultivated and cultivated sites) dispersed almost immediately upon immersion.

Surface soil aggregate stability has decreased markedly as a result of cultivation, as evident by the rapid slaking of the cultivated soil. On remoulding, the soils of both sites dispersed rapidly, again demonstrating the instability of this soil as a result of wet cultivation.

Table 1 *Dispersion indices for different soil depths of the uncultivated and cultivated sites. (Standard error in parentheses).*

| Depth (m)	Dispersion indices	
	Uncultivated	Cultivated (row)
0.025	3.0 (0)	3.0(0)
0.075	5.5 (0)	5.5 (0)
0.125	5.5 (0)	5.5 (0)
0.175	5.5 (0)	5.5 (0)
0.25	8.7(1.9)	5.5 (0)
0.35	11.3(1.9)	5.5 (0)
0.45	14.8(1.3)	8.6(1.6)
0.65	16.0 (0)	11.0(1.1)
0.85	16.0(0)	14.1 (2.2)
1.05	16.0(0)	16.0 (0)
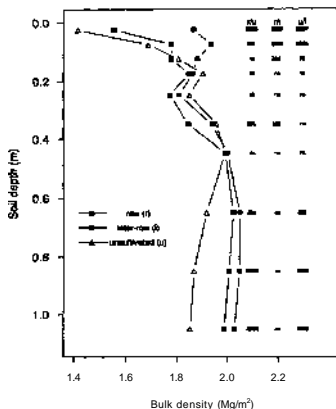
Fig. 1 *Mean bulk densities at different soil depths for the uncultivated and cultivated (row and inter-row) sites at Loeskow's, February 1995. The significance of the difference between the means of any 2 treatments for one depth is given as a probability level, * (P < 0.05); ** (P < 0.01); *** (P< 0.001); ns = nonsignificant*

Organic carbon

There are three distinct zones in the organic carbon profiles of the two sites. The greatest organic carbon content was in the top 0.05m of the uncultivated site with a mean of just over 1.5% (Fig.2). Beneath this depth, the trend was reversed with the cultivated site having up to almost double the amount of organic carbon compared with the uncultivated site. From 0.45-0.65m there was a small but significant difference between the two sites, but below that there was no difference. The increased organic carbon in the 0.05-0.45m layer of the cultivated site was strongly evident in the dark colour of the top 0.5m of the cultivated (cane) soil.

The increase in organic matter in the cultivated soil contrasts with many other studies on the effects of cultivation that indicate a decline in organic carbon content with time (e.g. Dalai & Mayer 1986). Of interest here is that the 33% increase in organic carbon content in the upper
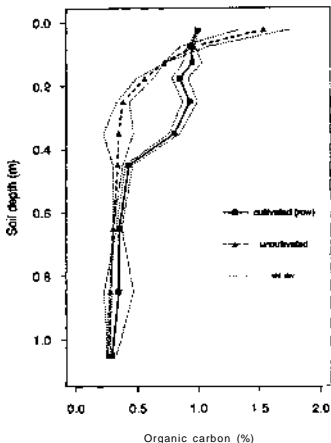
Fig. 2 *Mean organic carbon contents at different soil depths for the uncultivated and cultivated (row) sites.*

0.45m of soil was not reflected in improved aggregate stability. One explanation is that a general improvement in organic carbon is less relevant than an increase in the proportion of fine (gel-like) organic matter. The increased organic carbon may be charcoal, from cane burning. Charcoal is non-labile, so would not improve soil structure.

Microbial biomass

Preliminary evaluation of this type of data indicated that large reductions in microbial biomass occurred as a result of cultivations (Table 2). Replication of this analysis is currently underway. The decrease in microbial biomass in the cultivated soil may be related to the use of soil pesticides and as a consequence there is greater organic carbon content in the cultivated site. Increased quantities of plant material are being added to the soil, but not broken-down.

Table 2 *Microbial biomass carbon for the cultivated (row) and uncultivated sites.*

Site	Depth (m)	Microbial Biomas (mg/kg)	Decline (%)
Uncultivated	0-0.05	342	
Cultivated	0-0.05	232	32
Uncultivated	0.15-0.2	168	
Cultivated	0.15-0.2	29	83

CONCLUSION

Cane production has resulted in favourable changes in several soil physical and chemical properties of the soil at this study site. The importance of this result is emphasised because this soil is considered marginal for cropping. The favourable physical changes include a reduction in bulk density, an increase in organic matter content, and an increased range of soil pore sizes. However, these improvements

have not been reflected in improved aggregate stability or increased microbial biomass.

The results indicate that this soil responds to cultivation if no subsequent compaction occurs. Hence, controlled traffic with minimal cultivation of rows would provide benefits for both plant growth and machinery efficiency on this soil. Soil loosening, so important in this naturally dense soil, would last through ratoons, and even through plant cane crops if the row/inter-row differentiation could be maintained, i.e. the next crop is planted in the same rows as the previous one.

The loosened soil (under the cane row) would remain loose if all wheels were concentrated solely in the "roadway" between the rows. In this way, the plants would benefit from lower soil density in the row and the cane traffic would benefit from increased density (less rolling resistance) in the inter-row.

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USE OF FAME (FATTY ACID METHYL ESTER) ANALYSIS TO QUANTIFY CHANGES IN SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH SUGARCANE YIELD DECLINE

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ABSTRACT

Fatty acid methyl esters (FAMEs) were extracted from soils from three locations in Queensland where plant symptoms (eg. diseased roots) of sugarcane yield decline were present, and from adjacent soils free of this syndrome. The yield decline soils had higher concentrations of several fatty acids including 16:0 ISO, 16:1 w8c and 18:1 w9c and lower concentrations of 21:0 ISO, C18 N alcohol and several unnamed fatty acids. Although the FAME profiles differed for soils from each location, the differences in fatty acid composition between soils with and without yield decline were consistent across all soils and suggest a change in the abundance of eukaryotes (fungi). This is consistent with the documented increase in levels of root pathogenic fungi in yield decline soils.

INTRODUCTION

Yield decline of sugarcane is defined as the diminishing ability of caneland to produce sugar per harvested hectare (Magarey 1994) and has been observed throughout most of the cane-growing areas of Queensland. Production losses due to yield decline and other associated pests, weeds and diseases were estimated to be \$111M, or approximately 20% of the total costs of sugar production in 1995 (SRDC, Research and Development Plan 1995-2000).

Fumigation experiments in soils throughout Queensland suggest that yield decline is associated with deleterious soil organisms (Magarey 1994). It is hypothesised that cane management practices, such as growth of cane as a monoculture, have disturbed the ecological balance between functional groups of the soil biota (eg. heterotrophic bacteria and fungi, actinomycetes, fungivorous protozoa, fungivorous nematodes, microarthropods) and promoted the development of deleterious organisms such as the fungal root pathogens *Pachymetra chaunorhiza*, *Pythium* spp. and *Rhizoctonia* spp.(Magarey 1994).

In order to modify existing cane management practices or develop new ones that will maintain soil health and sustain cane yields, it is important to understand how management practices induce shifts in the ecological balance of soil organisms and how these shifts are linked to the development of yield decline. Management strategies that ultimately lead to reduced pathogen populations may then be identified and exploited by the sugar industry.

We report the use of technology, based on the extraction and analysis of fatty acid methyl esters (FAMEs) from the cells of soil organisms, to study the composition of microbial communities present in sugarcane soils. FAME anaylsis has been developed by Microbial ID, Inc., (MIDI), Newark, DE, USA as a rapid identification tool for soil microorganisms. It has been applied successfully to analysis of soil microbial community structure (Cavigelli et al 1995). Identification of specific "signature" fatty acids in soil FAME profiles can also be used to detect the presence of specific functional groups of soil microorganisms in the soil (Cavigelli et al 1995). We have applied FAME analysis to soils where sugarcane yield decline is present (in land that has been under sugarcane monoculture for many years, designated "old land") or absent, (in land that has been recently assigned to sugarcane, designated "new land"). We show that this technology has considerable promise in (a) demonstrating differences in microbial communities between old land and new land, and (b) identifying the nature of these differences.

MATERIALS AND METHODS

Trial sites and treatments
Replicated air-dried soil samples (0-100 mm soil depth) were obtained from three sugarcane properties in north Queensland where yield decline is present. At two of these properties, Fortini (near Ingham) and Kalamia (near Ayr), soil from old land (where cane had been grown continuously for 20 years or more) was compared with soil from new land (where

sugarcane had not been grown previously). The new land site was a few metres from the old land site on both of these properties. At a third property (Pegararo, near Ayr), old sugarcane land that had been cropped to pumpkins for the past two years was compared with adjacent soil that remained under continuous cane. The growth of sugarcane (stalk counts) in the new land soil at Fortini and Kalamia was significantly greater than that in the old cane land, and at Pegararo the growth of cane in the soil under pumpkins for two years was greater than that in the soil under continuous cane (RC Magarey and RO Nable, *personal communication).*

FAME analysis
Lipids were extracted from 6 g of soil with methanolic NaOH, methylated with methanolic HCl and the fatty acid methyl esters removed with an organic solvent and chromatographed (Cavigelli et al 1995). The chromatograms were analysed using software developed by Microbial ID, Inc., (MIDI). Newark, DE, USA. The software identifies the fatty acid peaks and computes their area.

FAME profiles were compared using principal component analysis. The peak area for each fatty acid in a FAME profile was transferred to a spread sheet and values from replicate profiles averaged. The peak areas for the fatty acids from the new land soils were then subtracted from those of the old land soils. These differences were summed across the soils and the 12 peaks showing the greatest increase and the 8 peaks showing the greatest decrease were selected.

RESULTS

PCA analysis of FAME profiles of Fortini, Kalamia and Pegararo soils
Principal component analyses of the FAME profiles obtained from Fortini and Kalamia and for the ± pumpkins soils from Pegararo are shown in Figs 1 and 2.

Although a minimum of three replicated samples were available for each soil, the FAME profiles obtained for replicates within soils were grouped together. On the basis of this grouping, the old land and new land profiles for the Fortini and Kalamia soils can be regarded as different from each other (Fig. 1). This would suggest that the composition of the microbial communities present in these soils was different. A similar, but less clear separation of the old FAME profiles was observed for the Pegararo ± pumpkin soils (Fig. 2). Here one of the 3 replicated soil samples appeared to be different from the other 2 in each soil type, but the grouping of the 3 replicates, as shown in Fig. 2 still suggests that the ± pumpkin soils are different.

Fatty acid analysis of the FAME profiles

A comparative analysis of the fatty acid composition of the FAME profiles for the different soils is shown in Fig. 3. There were several major differences in the fatty acid composition between the Fortini and Kalamia old and new land soils and the Pegararo ± pumpkin soils. Of particular note was the increased levels of peak # 9 (18:1 w9c) and the
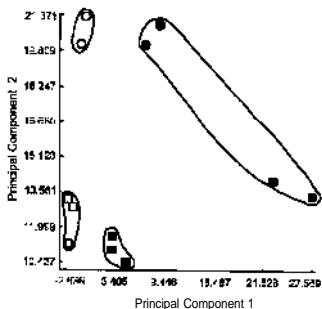
Fig. 1 *Principal component analysis of FAME profiles for old (open symbols) and new (solid symbols) land at Fortini (○, ●) and Kalamia (□, ■).*

Fig. 2 *Principal component analysis of FAME profiles for Pegararo soils after two years of pumpkins (●) or continuous cane (○).*

decreased levels of peaks # 13 (summed feature of 19:1 w7c / 19:1 w9t), #15 (21:0 ISO) and #19 (C18 N Alcohol) in the old land and - pumpkin soils. The fatty acid 18:1 w9c is characteristic of eukaryotes (eg. fungi) (Cavigelli et al 1995). This would suggest that the abundance of eukaryotes was higher in the old land and - pumpkin soils. However, further experiments are required to confirm this.

CONCLUSION

In this limited study, we have demonstrated that FAME analysis of soils is a useful technique for detecting differences in the composition of microbial communities in soils that are associated with sugarcane yield decline. We have found several common differences between the fatty acid composition of these communities present in soils with and without yield decline. These differences may reflect an increased abundance of eukaryotes (eg. fungi) in the yield decline soils. This would be consistent with the documented increase in levels of root pathogenic fungi in yield decline soils (Magarey 1994). Further comparisons between soils with yield decline and soils where the decline-causing
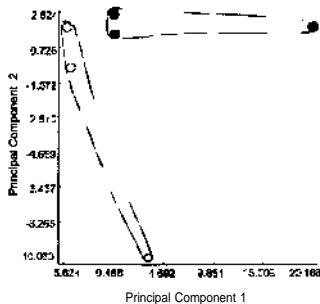
pathogens are absent or reduced (eg. following crop rotations) are necessary to confirm and extend these findings.

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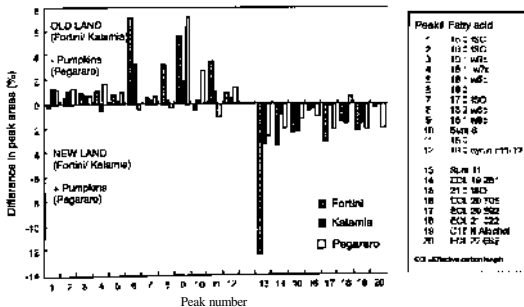
Fie 3 *Difference in peak areas (%) for 20 individual fatty acids following subtraction of peak areas of microorganism fatty acids in soil samples from new land or land after two years rotation of pumpkins from peak areas of old land or cane land without pumpkin rotation.*

ADVANCES IN QUANTIFYING SOIL ACIDITY AND ACIDD7ICATION RATES IN THE SOUTH AFRICAN SUGAR INDUSTRY

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ABSTRACT

The adverse effects of soil acidity on crop production are well documented but little attention has been paid to acidification of sugar producing soils in South Africa. The current criteria used for determining lime requirement needed reconsideration in light of the above and the possible detoxification of aluminium by sulphur in humic soils. Soil acidification rates were determined and compared with those predicted by a mechanistic soil profile acidification model. Aluminium saturation was evaluated as an index for determining lime requirement. Soil acidification was found to be associated with a range of soils in sugarcane areas, and was reasonably well correlated with pH decline predicted by the model. Aluminium saturation in conjunction with the Al:S ratio was found to be a more reliable index for determining lime requirement than exchangeable aluminium per se. Varietal differences in terms of tolerance to acidity were apparent.

INTRODUCTION

The detrimental effects of toxic levels of exchangeable aluminium on crop production (Meyer & Wood 1976; Noble et al 1986) and the acidification of soils due to continuous cropping, are recognised as problematic in many South African soils (Scotney & Dijkhuis 1990). Specifically, much attention has been given to amelioration practices of the acid soils of kwaZulu-Natal (Moberly & Meyer 1975; Sumner et al 1986; Farina & Channon 1988) after it was recognised that Al toxicity, rather than acidity *per se*, was the primary limiting factor in acid soils (Kamprath 1970). Little attention was, however, paid to the acidification of the sugar industry soils because the soil nutrient information retrieval system (NTRS) linked to the fertiliser advisory service (FAS) indicated little build up of acidity in the various regions of the sugar belt (Meyer et al 1992). The demise of soil pH as the basis for determining lime requirement resulted in the adoption of acid saturation in the local maize industry (Farina et al 1980), and in the use of an exchangeable Al index (EAI) based on a 0.01 M NH_4Cl extraction, in die sugar belt of kwaZulu-Natal for this purpose (Moberly & Meyer 1975). This apparent difference caused considerable confusion amongst growers involved in mixed agriculture. In addition, the fact mat soil Al *per se* in the form of die EAI) may have been overestimating the amount of lime required for sugarcane in some circumstances, prompted re-evaluation of the current criteria for determining lime requirement for sugar producing soils. Impetus was added to this approach after it became apparent that some of the more recently released South African Sugar Association Experiment Station (SASEX) varieties were responding differently to lime application than the standard NCo376 variety.

The objectives of mis paper are to review the latest research aimed at providing the South African sugarcane grower with the best possible advice for lime application. This involved assessment of acidification of the sugar belt soils and evaluation of a soil Al saturation index in conjunction with the Al:S ratio for determining lime requirement.

PROCEDURE

Rates of acidification were investigated by calculating changes in soil pH, exchangeable base cation (K, Ca and Mg) concentrations and increases in EAI values in a range of sugar belt soils (Schroeder et al 1994). The Soil Profile Acidification Model (SPAM) developed by scientists at the NSW Agriculture Research Institute, Australia (Robinson et al 1995) was used to predict acidification rates of various soils in the industry.

Data from a large number of field and pot trials conducted in the South African sugar industry over 25 years were used to evaluate the Al:S ratio (Schroeder et al 1993) and/or an Al saturation index (ASI) (Schroeder et al 1995) for modifying lime requirement. ASI values were calculated by expressing the EAI values (cmol/kg) as a percentage of

the sum of the extractable cations (K, Ca, Mg and EAI as cmol/kg). All data are based on analytical techniques routinely used in the FAS laboratory. Yield responses to amelioration were expressed as percentage increase in dry matter production over the yield obtained in the control treatment. To assess the apparent varietal differences in response to acidity, ASI data sets pertaining to individual sugarcane varieties were examined separately. The ASI values were compared with acid saturation

RESULTS

The following two examples illustrate the occurrence of soil acidification in the industry;

i)   Analysis of >200 soil samples taken over 12 years from an estate on the south coast of kwaZulu-Natal, indicated a general decline in pH and base cation concentration, and an increase in EAI values with continuous cropping (Fig. 1).

ii) The actual rates of acidification of soils on a Zululand estate were well correlated (r=0.65) with those predicted by the SPAM model (Table 1) when it was assumed that the 12 month old rainfed crop had adequate fertilisation.

Table 1   *Measured annual pH decline and predicted pH decline from the SPAM model for individual fields on a Zululand sugarcane estate over a six year period.*

Field	Actual	Predicted
6	0.11	0.13
16	0.18	0.15
30	0.03	0.11
32	0.08	0.16
36	0.22	0.00
44	0.03	0.13
59	0.06	0.16
62	0.14	0.13
66	0.03	0.15
74	0.19	0.10
80	0.10	0.11
86	0.04	0.12
Mean	0.10	0.125

In terms of yield data from various field and pot trials, significant responses to applied lime and/or gypsum did not always occur, despite EAI values above the recognised threshold value of 3.5 cmol/kg clay. The use of the Al:S ratio as an index for predicting yield responses to amelioration was substantially better than using EAI alone, as the correlation coefficient (r) improved from of 0.48 to 0.70. It was found

that the incidence of significant and non-significant yield responses could generally be separated by an Al:S ratio of 2 (Fig. 2). This largely explained the lack of yield responses reported when cane was grown on humic soils due to the mineralisation of substantial amounts of S from the organic matter (Schroeder et al 1993).

The composite trial results also showed that, when data relating to variety N12 and soils with Al:S values <2 were excluded, yield response as a function of the Al saturation index (ASI) gave a correlation of r=0.802. Significant and non-significant responses could generally be separated by an ASI value of 20% (Schroeder et al 1995). Variety N12 appeared to be negatively affected by liming to an ASI value of less than 40% (Fig. 3). ASI values were well correlated with the traditionally quoted acid saturation figures (r= 0.93).
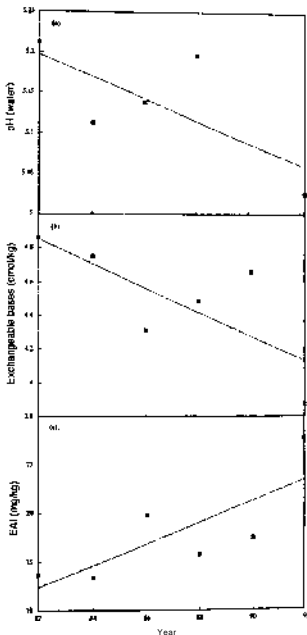

Fig. 1   Acidification of soils over 12 years on a sugarcane estate in kwaZulu-Natal in terms of mean (a) pH, (b) exchangeable base and (c) Al index values.

DISCUSSION AND CONCLUSIONS

Despite the fact that widescale build up of acidity was not previously indicated in various regions of the South African sugar belt, acidification of soils is in fact occurring. The global application of NIRS had probably
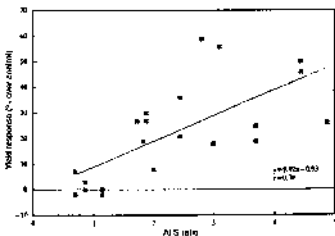

Fig. 2   Regression of relative cane yield response to applied lime and/ or gypsum vs Al:S ratio from field and pot trials.
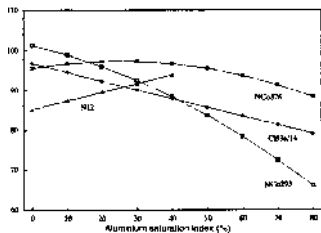

Fig. 3 Tolerance of four varieties of sugarcane to soil acidity measured by Al saturation index.

masked the actual changes on individual estates. The reasonably good correlation obtained between actual pH decline and that predicted by SPAM, indicated that increases in acidification due to crop removal of bases and leaching effects are real, and in line with similar process noted elsewhere. The use of a model such as SPAM for estimating acidification rates of soils in conjunction with soil test values will lead to improved lime recommendations.

The improved correlation between relative yield response and ASI compared with EAI *per se.* appears to justify the use of Al saturation as the norm for determining lime requirement in the South African sugar industry. As the lack of response to lime on the humic soils is attributed to the detoxification of Al by SO_4^{2+}, it is proposed that the Al:S ratio be used in conjunction with the proposed ASI for determining lime requirement of the humic and sulphur rich soils of the sugar belt. Based on the ASI data available for variety N12, it is considered important to evaluate other new varieties in terms of their tolerance to acidity and/ or reaction to lime application.

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5.2 Environmental impact

DEVELOPMENT OF A FLEXIBLE DECISION SUPPORT ENVIRONMENT FOR USE IN EVALUATING OFF-SITE IMPACTS OF CANE PRODUCTION

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ABSTRACT

Sugar production does not occur in isolation but in landscapes characterised by complex land and natural resource use including alternative primary industries, residential and recreational use and conservation. Managing the impacts of cane production in this landscape is a significant challenge. The development and use of decision support systems that take advantage of, and integrate, advances in geographical information systems (GIS), modelling and knowledge-based systems may facilitate this process. With evolving but far from complete understanding of the environmental impacts of production, a complex and changing legislative and political environment and rapid advances in technology, the design and implementation of operationally relevant decision support systems is demanding. Three proof of concept decision support tools (a tool to assess land use suitability, a land use allocation tool and a tool to explore the hydrological impacts of land use) and their integration into a higher level tool are described. These illustrate both the types of decision support that are appropriate for managing sugar production and expansion in a broader environment, and approaches to the flexible delivery of such support.

INTRODUCTION

The sugar industry faces a significant challenge in natural resource management and planning to minimise detrimental off-site environmental impacts. Given the complexity of the natural and social systems in which sugar production occurs, this process is demanding and often only partly achievable. A decision support system (DSS) may play a powerful role in facilitating such deliberations (thereby fostering a more integrated and strategic approach to planning) by providing means of making more effective use of current data and understanding.

In practice, the development of a decision support system for natural resource planning in sugar-producing areas is exacting. Many planning issues are one-off or infrequent (assessing the potential environmental impact of large scale expansion proposals for example). Decisions are made at a range of scales (spatial and temporal) and in the light of incomplete data and understanding. As a result, decision -making often relies heavily on intuition and experience and approaches to decision making can frequently change as a result of improving information and understanding (Walker et al 1995). Furthermore, the process of DSS development can have a profound impact on the users' understanding of the issues addressed. Hence, decision support systems for environmental management may become redundant before they are completed.

These challenges have been addressed in the development of a prototype DSS focussing on the impact of land use and land use change in the Herbert Catchment of north Queensland. In order to provide a flexible system, a 'tool-kit' based on the use of a 'task language' is being developed (Walker & Johnson 1995). The task language is designed to allow resource managers with limited computing skills to develop new tools to address novel requirements using a range of resources.

This paper describes a suite of four tools implemented within the prototype decision support environment as a proof of concept exercise. Extensive interviewing of potential users of a DSS for natural resource management within the Herbert River catchment (principally officers within state government agencies) was undertaken as part of a 'needs analysis' process in system development. Each interviewee described the range of natural resource planning or management tasks in which he or she was involved. Those tasks that might be facilitated by decision support were then analysed in sufficient detail to provide a basis for specifying a potential decision support tool to be used in addressing that task (Walker & Johnson 1995). For the purposes of 'proof of concept', three of the 38 tasks identified in these interviews were then implemented as decision support tools within the prototype decision support environment. The tasks selected and resulting tools are outlined in this paper. The flexibility of the toolkit approach is then illustrated by considering the combination of these tools into a fourth tool.

THE TASKS ADDRESSED

Land suitability

The Queensland Department of Primary Industry (QDPI) Land Suitability criteria provide means of evaluating comparative land use suitability for a range of crops in the humid tropics on the basis of a range of biophysical data (Anon 1990). Deriving a land use suitability for a location is a key criterion used in land use planning and extension. Land Suitability classification is a comparatively data intensive operation and time consuming where undertaken manually. However, both data access and classification are highly amenable to automation.

Land use allocation

Translating land use policy into land use allocation can be demanding. For example, consider development of a proposed reserve allocation strategy for conservation purposes within the Herbert catchment (which has high conservation as well as production value) derived from data about the catchment and a set of policy guidelines for reserve allocation.

Hydrological impacts of land use change

Offsite impacts of cane production in terms of water quantity and quality have emerged as key issues in the expansion of the sugar industry in tropical floodplains. Impacts may occur within cane areas (where altering drainage in one area may, for example, impact on flooding in another) and may impact on other natural resources (mangroves, for example). In order to help to identify constraints on expansion, policy makers and planners need to be able to explore the likely impacts of land use change to cane on hydrological flows and water quality.

THE TOOLS DEVELOPED

Tools designed to help the user address the above tasks were created in the prototype Herbert Catchment Decision Support System. The tools are written in a 'task language' (Kendon et al 1995) that was developed for use within this system (Walker & Johnson, 1995). The task language is essentially a high level programming language that allows the user to edit existing tools or create new tools by combining or recombining analytical resources and input / output devices. Use of the task language is therefore similar to the creation of macros in, for example, spreadsheet packages.

The implemented tools illustrate the range of functionality and tasks that might be addressed within a flexible toolkit environment. They are developed for the purposes of illustration only - results should not therefore be taken as any statement of priorities or issues in the Herbert catchment.
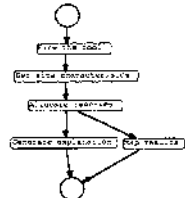
Each of these tools is outlined below and accompanied by a data flow diagram which provides a high level specification for that tool. The
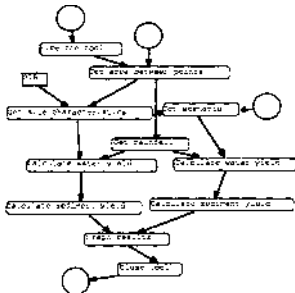
262
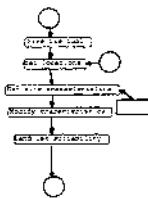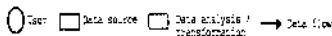
a) Land use suitability   b) Land use allocation

Key:

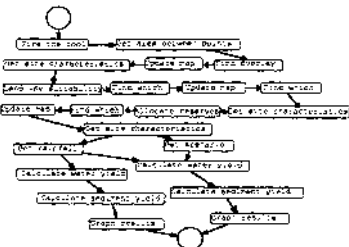c) Land use change and hydrology

d) A combined tool

Fig 1 *The four 'proof of concept' tools represented as data flow*

tool definition (ie. the actual program) for the third tool is also presented in order to illustrate the correspondence between data flow diagrams and the implemented tools.

Land suitability
This tool derives the land suitability for sugarcane for a specified UMA (Unique Mapping Area - a geographical area defined by homogenous soil, landscape and vegetation features, see Basinski 1978) or set of UMAs by applying the QDPI land suitability criteria for the Wet Tropical Coast. UMAs are selected from a map of the catchment. The information required for each UMA in order to calculate land use suitability is retrieved from a data base. The user has the opportunity to review and modify these data (this enables the user to update information, e.g. by using more detailed local knowledge / inspection of the site and to explore scenarios e.g. the impact of modifying the drainage characteristics of a site on land use suitability). The land use suitability is then derived and an explanation of the basis for the Land Use Suitability for any UMA is provided - including, for example, the factors not considered due to missing data. The definition for this tool is presented as a data flow diagram in Fig. 1a.

Land use allocation
In this example, a hypothetical reserve allocation strategy for the Herbert River catchment is derived from a set of rules and data about the catchment stored in a GIS. The procedure is based on a reimplementation of work described by Goldsborough & Robertson (1994), itself based on work on computational reserve allocation described by Cocks &Baird (1989). The process of reserve allocation was chosen because the same approach could be applied to a range of allocation tasks.

In the hypothetical example undertaken, the objective was to provide a reserve allocation strategy for four vegetation communities / habitat types. The proposed reserve allocation strategy was derived from the rules presented in Table 1. These were applied to the 16 000 cadastral units (the land parcels for which separate tenure, ownership or zoning are legally defined) in the lower Herbert Catchment. Each site was therefore considered to be a potential reserve and each overlaid with information on vegetation and current ownership. The definition for this tool is presented as a data flow diagram in Fig. 1b.

Table 1 *Rules applied in deriving a proposed reserve allocation strategy*

A site can legitimately be added to the list of proposed reserves if:
it is already a National Park; or
it has the largest area of the remaining cadastral units and has at least one of the communities at the site; or
it is a remaining cadastral units in which the community least represented in the list of reserves occurs; or
it is a site on which one of the communities occur ; or
it is an existing state forest reserve; or
it is an existing lowland forest reserve.

In the final strategy :
Each community should occur at least 4 times
The number of state forest reserves must exceed 10
The number of lowland forest reserves must exceed 10
All existing reserves appear in the list of selected reserves
The sum of the area of all the reserves should not exceed 15 000 ha.
The total number of reserves should not exceed 50

Hydrological impacts of land use change
This tool illustrates decision support using simulation models. The tool is based on application of a prototype model of water and sediment yield in the Herbert Catchment. The tool derives water and sediment yield between two user specified points in the catchment given current land use and provides the user with a variety of alternate mechanisms for altering model parameters in order to specify alternate land use scenarios. Cumulative discharge and turbidity loads under current
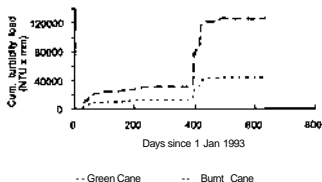
Fig. 2   Exploration of the potential impact of a change from burnt to green cane harvesting for a sub-catchment of the Herbert river. Green cane harvesting is based on a model derived from data for the period shown. For burnt cane, the mean turbidity load is changed from 130 NTU x mm to 200 NTU x mm (estimate based on Prove et al 1995) and the index of average instantaneous turbidity generation to instantaneous discharge is changed from 1.5 to 1.3. The output illustrates the significant increase in cumulative turbidity that would be predicted (on the basis of these assumptions) with a return to burning cane in the Herbert prior to harvest.

conditions and one or more land use scenarios are presented graphically. For example, this tool might be used to explore the potential impacts of changing from burnt to green cane harvesting in a subcatchment of the Herbert on sediment yields (Fig. 2). The definition for this tool is presented as a data flow diagram in Fig. 1c.

IMPLEMENTATION

The code used to implement the land use change and hydrology tool in the task language is presented in Table 2. While understanding or generating this code places some demands on the user it is considerably less complex and more intuitive implementing the tools from scratch in the underlying programming languages (in this instance Prolog, Avenue and C). Future versions of the toolkit will enable the user to edit existing tools or create new tools through a diagram-based interface. Furthermore, a variety of means of helping the user to translate a task into a tool will be incorporated into the toolkit. While operational development of decision support tools in such an environment by state government agencies is by no means a certainty, and remains some way off, we believe that it is reasonable to aim for routine operational application in natural resource management.

Table 2   The land use change and hydrology 'proof of concept' tools as implemented in the task language

```
fire_tool ('g:\p_con_3\working\tooldesc.txt', 'Land use change and
hydrology
1'g:\p_con_3\working\pofc3.apr'),
get_area_between_points (Catchment),
get_site_characteristics(p_con_3,Site_characteristics),
get_rainfall(Catchment,Rainfall),
calculate_water_yield(Site_characterisics,Rainfall,
Current_water_yield),
calculate_sediment_yield (Site_characteristics, Current_water_yield,|
Current_sediment_yield),
graph_results (Current_water_yield,'CUM DISCH COMP'),
graph_results (Current_sediment_yield,'CUM TURB LOAD'),
get_scenarios (Catchment, Scenario),
calculate_water_yield (Scenario, Rainfall, Scenario_water_yield),
calculate_sediment_yield (Scenario, Scenario_water_yield,
Scenario_sediment_yield),
graph_results(Scenario_water_yield,'CUM DISCH COMP'),
graph_results (Scenario_sediment_yield,'CUM TURB LOAD'),
close_tool ('Land use change and hydrology 1').
```

WHY USE A TOOLKIT-BASED APPROACH?

The principle justification for the development and application of the toolkit based approach is that this enables flexible adaptation of existing tools or the creation of new tools to meet new or evolving needs. Although some new functionality had to be added to the task language in order to implement the three tools described above, creating each of these new tools was considerably less time consuming than had each been generated individually from scratch. However, to illustrate the flexibility of the task language approach, consider the integration of the functionality in the three tools described above into a single tool (Fig. 1d). This tool prompts the user to specify a subcatchment from a map. It then calculates land use suitability for all the UMAs within that area; finds those UMAs with a land use suitability for cane of 1, 2 or 3 (i.e. appropriate for cane production); finds those areas within this area that are not currently under cane; and removes from this area of potential expansion for cane any cadastral units that fall into the reserve allocation strategy previously described. Having therefore identified the potential area for expansion, the hydrological model is used to explore the potential impacts of such expansion on water and sediment yield from the sub-catchment.

DISCUSSION AND CONCLUSIONS

Management of the environmental impacts (particularly off-site) of the sugar industry demands integrated consideration of a range of natural resource management issues. These issues often require considerable data and complex analysis. While usually incomplete, such data and appropriate analytical tools may often exist but not be accessible to planners - who typically have decreasing time available to tackle increasingly complex tasks. The customisable decision support environment described here is designed to provide planners and resource managers with powerful but flexible means of combining and synthesising data and understanding that is currently dispersed and difficult to access and integrate. The tools described in this paper were developed as a 'proof of concept' to illustrate the range and flexibility of a task language approach. As the functionality available in the task language is expanded and the associated data resources are rationalised and enhanced through trial application on real problems the toolkit should enable natural resource managers to explore and increasing range of issues with increasing ease. The intention is that development and initial use and, subsequently, operational use of this DSS will evolve as the issues, analytical tools and data faced by and available to planners evolve.

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IMPACT OF SUGARCANE CULTIVATION ON WATER QUALITY IN MAURITIUS

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ABSTRACT

With approximately 90% of the 90000 ha of existing arable land under sugarcane, cultivation of the crop represents, in public opinion, the greatest uncontrolled threat to surface and ground waters in Mauritius. Yet monitoring of nitrate and herbicide residues soluble in drinking water sources have shown the concentrations of nitrate and herbicide residues to be far below the maximum level permissible in the 1991 Environment Protection Act of Mauritius (e. g. 50 mg nitrate/L and 3 mg atrazine/L). The data obtained however do not provide a complete assurance that sugarcane cultivation does not degrade water quality or the wider environment. Data on emission of nitrous oxide to the atmosphere and on contamination of water courses by agrochemicals carried by sediment during erosion need to be obtained and examined before such assurance can be given.

INTRODUCTION

Since sugarcane occupies 90% of the 90000 ha of existing arable land area, agriculture in Mauritius is therefore dominated by sugarcane cultivation. On average. 620000 t sugar is produced annually contributing about 30% of the gross export earnings of Mauritius. This level of sugar production requires annual inputs of 10000 t nitrogen (N), 4000 t phosphorus (P_2O_5), 12000 t potassium (K_2O) and 1100 t herbicidal products.

Despite the substantial economic and accompanying social benefits accruing from the use of agricultural chemicals in sugar production, the present public mood about their use remains one of fear and mistrust. The public concern is primarily centered around health hazards posed by the agricultural chemicals. That concern has spurred the Government of Mauritius to introduce in 1991 an Environment Protection Act setting water quality standards and permissible limits of pollutants in drinking water and effluents. The act also decreed that pollution must be controlled at its source, and its prevention is the direct responsibility of the activity or sector causing it.

In the above context, to determine whether our current agricultural practices involving sugarcane are discharging unacceptable quantities of nitrate and pesticide residues into receiving water bodies in Mauritius, nitrate levels in tap water and herbicide residues dissolved in ground and surface waters in the country have been monitored. This paper presents some of the results from these monitoring studies.

MATERIALS AND METHODS

Nitrate levels in drinking water at 25 locations in Mauritius were determined colorimetrically every month from 1991 to 1994 using a 5% resorcinol solution (Velghe & Claeys 1985). In a separate study, water of 10 groundwater basins (2 sampling points/basin) and of 6 rivers (3 sampling points/river) were sampled at fortnightly intervals during 1995. The concentration of soluble residues of 6 herbicides (atrazine, hexazinone, 2,4-D, diuron, ioxynil and linuron) frequently used in sugarcane fields were determined in the water samples by high performance liquid chromatography (Soniassy et al 1994).

RESULTS AND DISCUSSION

Nitrate levels

Surface reservoirs and aquifers each supply 50% of the drinking water in Mauritius. Typical fluctuations of NO_3 concentration during the 4-year monitoring period are illustrated for one surface reservoir (Fig 1A) and for one aquifer (Fig 1B). At each site monitored, the level of NO_3 never exceeded the maximum limit of 50mg N0/L recommended by the World Health Organization or permitted in the 1991 Environment Protection Act of Mauritius. In fact, more than 700% of the 700 water samples analysed contained less than 15mg NO_3/L and concentration higher than 25 mg NO_3/L was found in less than 10% of the water samples (Fig 2).
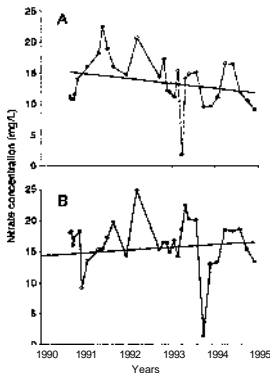

Fig. 1   *Examples of nitrate concentration in drinking water and their evolution with time from 1990 to 1994 in Mauritius - (A) for water at Beau Bassin from a surface reservoir and (B)for water at Fond du Sac from an aquifer.*

The most interesting observation however was, that as shown in Fig. 1 (A and B), there was no tendency for NO_3 level to rise with time despite the fact that the sugarcane planting community did not withhold application of fertilizer N in any single year.

Safe levels of NO_3 in drinking waters are to be expected. Indeed when ^{15}N-labelled fertilizer N at the currently used rate of 120 kgN/ha was applied to sugarcane in field lysimeter studies, not more than 5% of the fertilizer N moved below the 1m soil depth (Ng Kee Kwong & Deville 1984). Rapid biological immobilization of the fertilizer N close to the soil surface had efficiently removed the NO_3 from solution leaving little or none available for leaching (Ng Kee Kwong et al 1986).

Herbicide residues

Only 3 herbicides, namely atrazine, hexazinone and diuron, could be found dissolved in ground and river waters (Fig 3). Even then the frequency of their noted presence in ground and river waters did not exceed 50%. In addition, the maximum permissible limit of 3 mg atrazine/L, 14 mg diuron/L and 270 mg hexazinone/L was never exceeded.
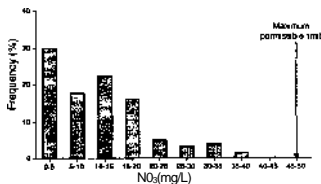
Fig. 2 *Frequency of different nitrate levels in drinking water in Mauritius in samples taken between 1991 and 1994 (n = 700).*
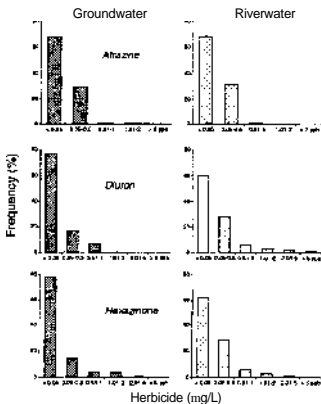

Fig. 3 *Frequency of different atrazine, diuron and hexazinone concentrations in river waters (n = 488) and groundwaters (n = 306) of Mauritius during 1995. Maximum permissible limits are 3 mg atrazine/L, 14 mg diuron/L and 270 mg hexazinone/L.*

The absence or very low concentrations of soluble herbicide residues in ground and river waters was also to be expected on the basis of studies already done on the degradation and leaching of herbicides in Mauritius. Indeed, herbicides are rapidly degraded in soils (Umrit & Ng Kee Kwong 1995). For instance 65 to 80% of 2, 4-D (applied at 2.0 kg a.iVha) was found to have been degraded in less than 1 week while more than 50% ioxynil (applied at 0.20 kg a.i./ha) was decomposed in not more than 2 weeks. As result of their rapid degradation in soils, less than 5% of any of the herbicides studied moved deeper than 1 m below the soil surface.

The public fear and mistrust of agricultural chemicals used in sugarcane cultivation in Mauritius cannot therefore be supported on the basis of nitrate levels and concentrations of herbicide residues solubilized in ground and surface waters. This, however, should not be construed to mean that sugarcane cultivation does not impair water quality in Mauritius. More research is needed to convince the general public that agricultural chemicals really do not pose a risk to their health. In this context, agrochemicals are discharged not only in a soluble state but also in particulate forms attached to sediment in run-off during erosion (see e.g. Smith et al 1993). With the undulating or hummocky topography often encountered in Mauritius and the fact that 70% of the yearly rainfall (which may exceed 4000 mm in the superhumid areas) occurs as high intensity downpours between January and April, significant amounts of fertilizers and herbicides could have been moved in particulate forms. Whether the leakage of agrochemicals in particulate forms to surface waters is sufficiently extensive to pose eutrophication problems, rendering the water unfit for recreation or fishery purposes needs to be elucidated.

Furthermore, in the context of the wider environment, 40% of fertilizer N applied to sugarcane in Mauritius could not be accounted for (Ng Kee Kwong & Deville 1987). As reviewed by Katyal (1993), NO_3 and carbon substrate availabilities together with low oxygen diffusion potential and temperatures above 10°C are the prime conditions inducing denitrification to occur at significant rates. In Mauritius the soils are high in organic matter (30 to 120 g/kg soil) and are highly aggregated (Parish & Feillafe 1965). In addition, on the account of the high rainfall, the soils are often moist resulting in low oxygen diffusion potential. These factors together with the fact that temperatures are always above 10°C provide good *a priori* evidence that much of the fertilizer N unaccounted for is lost to the atmosphere by denitrification as nitrous oxide and nitrogen gas.

ACKNOWLEDGEMENTS

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SUGAR FACTORY WASTEWATER MANAGEMENT STRATEGIES FOR ENVIRONMENTAL PROTECTION IN MAURITIUS

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ABSTRACT

Sugar factory wastewaters consist mainly of non-toxic substances namely, dissolved sugar, fine bagasse particles, smut, furnace ash and oil/grease. Seventy-five mg/L COD appears to be an achievable target during week day crushing, if pollution reduction at source is practised to reduce the hydraulic, BOD, COD and solids loading. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) refer to the oxygen required to biograde or to chemically oxidize the waste constituents of the effluents. However, during weekend and end-of-crop washing, the effluent volume is low but the COD content is high. Certain factories have special or sedimentation ponds for wastewater which is released gradually into the irrigation network. This practice appears to be a better approach than diluting the objectionable effluent with raw water to the Government effluent discharge norms.

INTRODUCTION

Mauritius has an average annual production of about 620 000 t sugar produced from some 6.5 Mt of cane by seventeen sugar factories having an average crushing capacity of 125 t cane per hour. The volume of wastewater produced could be as high as 20m^3/t cane processed, that is 2500m^{3}per hour for a crushing season of 107 days.

The main sources of water pollution in a sugar factory stem from product spillages, cooling water for mill bearings which may be contaminated with oil/grease, condensate with entrained sugar, effluents with smut and furnace ash, boiler blow down, factory floor washings, washwaters of equipment during weekend and end-of-crop shut down.

The ideal situation would be where there is no discharge from the factory into public water courses, however, due to water rights laws, factories which abstract water from rivers have to return wastewater for users downstream. Of the present seventeen sugar factories, six have achieved zero discharge, eleven make use of their wastewater for irrigation of cane fields, eight discharge part of their effluents into the rivers, while three have partial discharge into the open sea.

One sugar factory (A) which releases all its effluents into a river is selected as case study. Comparison is made with another factory (B) which is also bound to return the water abstracted, and which has adopted a pollution prevention programme to meet the Government effluent discharge guideline. Water consumption and effluent volume at the two factories were measured with the objective of limiting the total volume of effluent to a strict minimum and of limiting the cost of eventual waste treatment. Effluent qualities at both factories were also assessed with respect to the proposed effluent standard. In-plant effluent streams were characterised at factory A, to identify the sources of pollution and the means of minimising them are discussed.

MATERIALS AND METHODS

Flow rates of raw water input and effluent output at the two factories were measured using a Swoffer model 2100 series current velocity meter.

To assess effluent quality, the effluents at the two factories were sampled during three one-week periods at the beginning, mid and end of the milling season. Hourly grab samples were composited over 12-hour period, preserved and analyzed for chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total suspended solids (TSS). Temperature and pH on each fresh sample were also recorded. COD was analysed by a semi-micro digestion method followed by colorimetry, BOD was determined by the dilution method, and TSS by the filtration method through Whatman GF/C filter (Wong Sak Hoi 1992, STASM 1991).

For in-plant characterisation of wastewater streams in factory A, the sampling points are illustrated in the water circuit diagram (Fig 1 ,over).

All the streams were then combined and diluted with raw water (stream 10) before discharge into the river. The parameters measured were pH, temperature, TSS and COD.

RESULTS AND DISCUSSION

Comparison of effluent flow rate and quality at factories A and B
The flow rates of water input and wastewater output taken at the beginning, mid and end of the crushing season at factories A and B are shown in Table 1. Factory A had the highest island water intake of 17 m^3/t cane, the lowest figure being 1 m^3/t cane while that for factory B varied from 2-10 m^3/t cane depending on whether water re-circulation was practised through the use of a spray pond/cooling tower, or a once-through system was adopted. From Table 1 it can be seen that the effluent ouput at Factory A exceeded the water input because the final wastewater was diluted with river water prior to discharge.

Table 1 *Flow rates (m^3/h)of input raw water and output wastewater at factories A and B, with water intake rates of 17 and 10 m^3/t cane respectively.*

	Factory	
	A	B
Input raw water	1930	1098
	1845	923
	1966	901
Mean	1880	974
Output wastewater	3020	1099
	-	834
	2860	805
Mean	2950	979

A comparison of certain parameters for wastewater quality at the two factories is given in Table 2. Temperature and pH did not pose any problem and were well within the proposed norms of 40°C and 5-9 pH. However, for COD, BOD and TSS, it is evident that although factory A had the highest island effluent produced per tonne cane (26m^3), the mean COD, BOD and TSS of its final effluent still exceeded the Government proposed uniform effluent standard of 30,20 and 30 mg/L respectively. Factory B with a lower effluent per tonne cane (10m^3) had even higher COD contents of 71 mg/L. Effluent treatment should be envisaged for compliance with the effluent standard.

From Table 2, COD and BOD levels in effluents at Factory A throughout the milling season appeared reasonably low, except for the peaks at 158 mg/L COD , 104 mg/L BOD and 1495 mg/L TSS. For factory B, COD fluctuated between 27 and 119 with a mean value of 71 mg/L. Recent daily factory data showed that a lower COD with peak at 75 mg/L was achievable by further separating the more polluted

Fig 1 *Factory A: Water Circuit Diagram*

stream, e.g. the excess condensate, for surface irrigation during weekday crushing.

Wastewater management strategy at factory B

Factory B has adopted a pollution prevention approach for the last three years, and the most important measures taken are :

i)   spilled products are swept up and returned back to process where possible, limiting the use of water hoses to a strict minimum

ii)   an oil trap has been installed at each mill in the milling train to reduce oil contamination in the effluent

iii)   wastewater with high COD loading due to juice spillage during weekday processing andwash water during weekend shut

down, are channelled into the cane interrows with no noticeable detrimental effect on cane growth

iv)   end-of-crop washings are also used for irrigation.

Since it is more efficient to use river water for cooling purposes as its temperature is lower than the re-circulated spray pond-cooled water, a once-through system is adopted by factory B for its barometric condenser cooling water. It is only during periods of drought that the condenser water is re-circulated through the use of a spray pond. For easier monitoring, all wastewater canals in factory B are made to converge to one effluent stream. Daily COD monitoring during the last three milling seasons has shown that, with such a wastewater management strategy, the COD in effluent being discharged to the river peaked at 75 mg/L .

Sources of pollution in factory A and its wastewater management policy

The analytical results of in-plant characterisation of effluents at factory A are shown in Table 3 - The pH values of all streams were within the Government effluent discharge norms, the temperature of all streams except 5, 7, 8 and 9 were within limits, and TSS contents in all streams were negligible, contrary to the results obtained in Table 2 which indicated that the subsider shown in Figure 1 was overloaded. Wastewaters in the milling department (streams 1,2 and 4) were low in COD but the presence of oil could be minimized by the use of oil/grease trap.

Wastewaters from the crystalliser (stream 3) may contain dissolved sugar due to massecuite leakage.

Excess condensate (stream 8) is the main source of pollution, as measured by COD levels (Table 3). This pollution could be reduced in several ways: part of it could be used for hot imbibition of cane, the remainder could be impounded in a stabilisation pond to reduce COD

Table 2 *Wastewater quality at factories A * and B, with effluent rates of 26 and 10 m^3/t cane respectively*

		Temp. (oC)	pH	COD (mg/L)	BOD (mg/L)	TSS (mg/L)
Factor/A	Mean	34.5	6.5	44	31	226
	Min	26.5	6.2	0	0	0
	Max	38.1	6.9	158	104	1495
Factory B	Mean	33.9	6.9	71	34	89
	Min	28.9	6.7	27	0	0
	Max	38.7	7.6	119	62	962
Effluent Standard		40	5-9	30	20	30

* After dilution with clean river water before release

Table 3 *In-plant characterisation of potential pollution sources in various streams in factory A; measured levels of TSS were negligible in all streams*

Streams	pH	Temperature $(^{\circ}C)$	COD (mg/L)
1. Mill bearings	6.9	25	3
2. Mill turbines	6.9	25	8
3. Cooling crystallisers	6.9	26	44
4. Turbo alternator	7.0	26	12
5. Evaporation condenser	7.0	42	28
6. Pan condensers	7.0	39	60
7. Condensate to boiler	6.8	85	11
8. Overflow condensates	7.8	55	865
9. Overflow subsider	8.3	56	121
10. Combined effluent	7.1	36	83
Mauritian Standards	5-9	40	30

to lower level, and later released for irrigation. The condensate from the second effect evaporators could be used as boiler feed water if it is ascertained as being free from sucrose.

Barometric tail pipe condensed water (streams 5 and 6) was the next significant source of pollution. Sucrose arrestors in the pans as well as polybaffles in the pan condenser must be sized appropriately and maintenance carried out to ensure that they are working properly. This is important to minimize the loss of sucrose in the condensed water, but it also has the effect of reducing the COD loading.

It is most likely that the effluent discharge guideline in Mauritius will be of uniform effluent standard. Because factory A is situated in a region where water is plentiful and it is not economical to pump the wastewater uphill for irrigation, it seems logical to dilute the effluent with clean raw water to meet the discharge guideline. The ratio of dilution water/original undiluted effluent flow rate was as high as 3.6 times. However, the fact that there was a surge of COD, BOD and TSS in the combined wastewater (Table 2), suggests that there is no guarantee that compliance with the effluent standard will not fail.

An effluent standard of 30 mg/L COD, is not justifiable when the wastewater is non-toxic, since the cost involved in treating the wastewater down to 30 mg/L COD is much too expensive. Also, a time

scale should be provided to enable the right technical and economical measures to be taken to reach the desired goal in pollution control.

With proper planning, zero effluent (Hsieh et al 1995) can be achieved by : (i) re-circulating the overflow from the fly ash subsiders in the system, and (ii) installing a spray pond to cool down the condensed water for re-circulation.

CONCLUSION

The uniform effluent standard approach to control pollution from point sources currently in force in Mauritius does not take into consideration the variations in the assimilative capacity of the receiving waters (Van der Merwe & Grobler 1990) and the impacts of effluent discharged on water quality of receiving waters. It encourages the dilution of the effluent to within the acceptable limits, which is wasteful of good quality water, a scarce resource in Mauritius.

A pollution prevention approach based on limiting the input of pollutant into the water environment by practising waste reduction, recycling, recovery and re-use, appears to be a better wastewater management strategy.

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NITROGEN ISOTOPE RATIOS AS INDICATOR OF THE SOURCE OF NITRATE CONTAMINATION IN GROUNDWATER

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ABSTRACT

Monitoring of the nitrate concentration in domestic and investigation bores in the Bundaberg district has indicated that some contamination of the groundwater is occurring. This study was conducted to determine whether it was possible to distinguish between organic and inorganic sources of groundwater contamination by using $\delta^{15}N$ values. Groundwater samples were collected from domestic and investigation bores and nitrate concentrations determined. ^{15}N enrichment was then determined on dried distillates using natural abundance stable isotope ratios and $\delta^{15}N$ values calculated. Localised sources of contamination were suggested, with 11.6% of S^4N values ranging from +8 to +16 "/ which was indicative of nitrate contamination, possibly from non-sewered settlements. Contamination from inorganic sources was also identified, with $S^{15}N$ values ranging from -3 to +2 $^0/_{00}$ the majority of which were associated with known areas of high recharge. We conclude that $\delta^{15}N$ values may be used as a semiquantitative tool in differentiating between organic and inorganic sources of nitrate contamination of groundwater.

INTRODUCTION

Nonpoint nitrate contamination of groundwater by agricultural practices is of growing global concern (Bogardi & Kuzelka 1991). In the Bundaberg district in south-eastern Queensland, where landuse is dominated by sugarcane (with horticulture forming the balance), an unconfined aquifer underlies the agricultural area and supplies a source of potable water to over 80% of the general community. The combination of a growing urban and rural-residential population and extensive fertilisation of sugarcane and horticultural crops, raise the potential for nitrate contamination of the groundwater through leaching. Identification of sources of nitrate in groundwater can be resolved semiquantitatively by monitoring the changes in the natural abundance ratio of the two stable isotopes of N, N-14 and N-15. Kreitler & Jones (1975) established three N-isotope ($\delta^{15}N$) ranges for nitrate from different sources:- for nitrate from unfertilised, cultivated fields, values ranged from +2 to +8 $^0/_{00}$ (2) for nitrate from an inorganic source, values ranged from -8 to +6 $^0/_{00}$ with 90% between -3 and +2 $^0/_{00}$ and (3) for nitrate from an organic source, values ranged from +10 to +20 7 . These ranges were confirmed in a study by Gormly & Spalding (1979) when investigating the sources of nitrate contamination in the groundwater in the Central Platte Region of Nebraska. Exner & Spalding (1994) used the technique to differentiate between nonpoint sources of nitrate contamination at two sites in the Nebraska Panhandle. At site one, they found values between +5.8 and +8.8 $^0/_{00}$ which was indicative of a mixed source of nitrate contamination. At the second site, values between +4 and +5 $^0/_{00}$ were recorded which was indicative of leachates from an inorganic fertiliser source.

In the study reported here, $\delta^{15}N$ values were used as a semi-quantitative tool to distinguish between inorganic and organic sources of nitrate in the groundwater beneath the Bundaberg district.

MATERIALS AND METHODS

Groundwater samples were collected from selected domestic and investigation bores in the Bundaberg district using a Grundfos MP1 submersible monitor pump. Two to four times the volume of the bore water volume were pumped to waste to rid the bore of standing water before a representative sample was taken for analysis. Samples were immediately placed in ice until storage was possible at 2°C. The samples, again packed in ice, were transported to the laboratory where nitrate concentration was determined by colorimetric analysis (Henzell et al 1968). Groundwater samples to be used for $\delta^{15}N$ analysis had the NH_4^+-N removed first by steam distilling with MgO and collection of NH_3 in boric acid. Devarda's alloy was then used to reduce NO_3^--N (and NO_2^--N if present) to NH_4^* which was collected by steam distillation (Bremner, 1965). Distillates were evaporated to dryness and NH_4^+ converted to N_2 gas using the method of Ross & Martin (1970) for isotope ratio analysis. The ^{15}N enrichment was calculated from the 29:28

signal ratio measured on a Micromass 602E mass spectrometer. The data was then expressed as $\delta^{15}N$ where

$$\delta^{15}N\ (7 J = [(^{15}N/^{14}N)_{Mbuple} - (^{15}N/^{14}N)_{amospht:OT}] / [(^{15}N/^{14}N)_{amospht.}J * 1000$$

The standard to which $\delta^{15}N$ is usually referred is atmospheric N_2.

While 425 samples were initially collected from bores distributed over the entire land surface overlying the aquifer during October and November 1993, $\delta^{15}N$ analyses were undertaken only on samples with nitrate concentrations >25 mg NO_3^-/L. These represented 69 samples or 16% of the total bores sampled.

RESULTS AND DISCUSSION

The $\delta^{15}N$ values of the NO_3^--N found in the water samples ranged from -3.9 to +14.0 $^0/_{00}$ (Fig. 1). Approximately 52% of these samples had $\delta^{15}N$ values <+2 $^0/_{00}$ and experience elsewhere (Exner & Spalding 1994) strongly suggests that this nitrate is of inorganic origin, most probably nitrogenous fertiliser. $\delta^{15}N$ values for N fertilisers have been variously reported as -87 ^1 to +6.2 $^0/_{00}$ (Kreitler & Jones 1975; Kreitler et al 1978). A small number of samples examined (12%) had $\delta^{15}N$ values >+87 $^0/_{00}$ which suggests that this nitrate is of organic origin (Spalding et al 1993). The remainder of the samples , 36%, exhibited $\delta^{15}N$ values between +2 and +8 $^0/_{00}$ and was of indeterminate origin. These waters could represent N mineralised from cultivated fields (Kreider & Jones 1975) or may represent some mixing of organic (say from septic systems) and inorganic (from fertilisers) sources. There was little clear relationship between nitrate concentration in water samples and $\delta^{15}N$ values (Fig. 1).
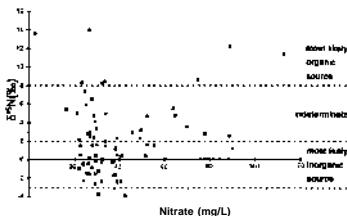

Fig. 1 *Relationship between $\delta^{15}N$ values and nitrate concentration as found in the domestic and investigation bores in the Bundaberg region. Ranges of $S^{15}N$ values are also indicated as a means of identifying the origin of the possible sources of nitrate contamination.*

Examination of the spatial distribution of the bores from which these water samples were derived, in relation to the $\delta^{15}N$ values obtained, is somewhat instructive (Fig. 2). Firstly, those samples that recorded $\delta^{15}N$ values $>+8^o/_{oo}$, indicating a strong signal from an organic source, appear to be most closely associated with some small urban settlements such as Moore Park and Burnett Heads and the rural-residential areas to the east of Bundaberg city. Septic waste disposal systems are widespread in these areas. The second noteworthy point is the large clustering of bores with elevated nitrate of low $\delta^{15}N$ signal, indicative of a fertiliser N source, in the Oakwood-Gooburrum area to the north-east of Bundaberg city. This region is characterised by intensive sugarcane and horticultural production and freely draining soils that result in enhanced rates and quantities of groundwater recharge (Fig. 2).

The $\delta^{15}N$ values calculated for the NO_3^- found in the groundwater beneath the Bundaberg district are in agreement with values found in studies on groundwater in Nebraska, Washington and New York (Exner & Spalding 1994; Spalding et al 1982; Kreitler et al 1978). Exner & Spalding (1994) suggested that, in highly aerated and rapidly drained soils, volatilisation and denitrification are assumed to be limited, with the $\delta^{15}N$ values of the nitrate closely reflecting those of the nitrogen source. In the Bundaberg region, >50% of the $\delta^{15}N$ values were recorded from bores in well-drained, aerated soils which also happened to be in areas of high recharge. These areas were associated with the growing of highly fertilised crops which suggested that the nitrate contamination could be occurring through leaching of the inorganic source. In other areas of the Bundaberg region, where urban regionalisation was occurring, heavier isotopic ratios were recorded and these were indicative of nitrate contamination occurring from human or animal wastes. This suggests that, in these areas of high $\delta^{15}N$ values, either denitrification or ammonia volatilisation of the N present had occurred (Kreitler et al 1978). However, unknown factors such as the time delay for nitrate to move through the soil and into the aquifer and the extent of denitrification in the aquifer further complicate a reasonable explanation of the sources of nitrate that are found in the groundwater (Keating et al 1995). In conclusion, the $\delta^{15}N$ values were found to be a useful tool for identifying possible sources of nitrate contamination in the groundwater underlying the Bundaberg district.

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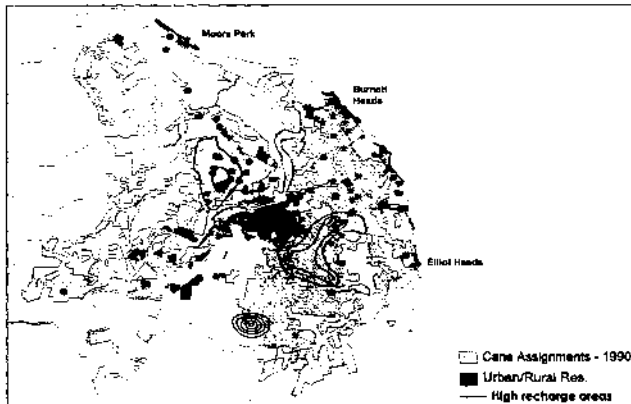
Fig. 2 *Land use and location of bores in the Bundaberg district which exhibited signs of nitrate contamination from an organic, inorganic or possible unfertilised source. (* - most likely organic source eg. septic; * - indeterminate; • - most likely inorganic source eg. fertilisers)*

TRACE GAS EMISSIONS FROM A TRASH BLANKETED SUGARCANE FIELD IN TROPICAL AUSTRALIA

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ABSTRACT

The effect of trash on the production or sequestration of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from sugarcane fields in northern Australia was measured because of a need to quantify earlier estimates. Plastic (PVC) cylinders (250 mm diam.) were pushed into the soil to a depth of 200 mm in rows of sugarcane. ^{15}N-labelled potassium nitrate (KNO_3) or urea (both 99 atom % ^{15}N excess) were broadcast onto the soil surface at a rate of 160 kg N/ha. Two soil moistures, equivalent to 50 mm and 25 mm of rainfall, were used. Production of gases was measured by placing covers on the microplots and sampling the headspace after 0 and 60 minutes. This procedure was repeated every 6 hfor 10 days. CO_2 evolution increased on the addition of trash to the soil surface from between 175 to 290 kg CO_2-C /ha. Soil respiration decreased in the presence of N fertiliser with urea having a more significant effect than KNO_3. Net uptake of CH_4 occurred in all microplots, the addition of trash increasing CH_4 oxidation at the higher soil moisture whereas the presence of N fertiliser increased CH_4 oxidation at both soil moistures. N_2O production was greatest from microplots fertilised with KNO_3, the presence of trash enhancing N_2O evolution. In conclusion, the presence of trash on the soil surface of this sugarcane field influenced the production of CO_2 and Nfl and the uptake of CH_4

INTRODUCTION

Annual production of CO_2 from Australian agricultural cropping systems in 1990 was estimated to be 13.6 Mt CO_2 (Russell 1991). Estimated CH_4 production from the burning of crop stubbles, sugarcane and grazing lands in Australia in 1990 was 495 kt/year (Galbally et al 1992) whereas N_2O production from Australian agriculture was estimated at 155 kt/year (Pulsford 1991). The atmospheric concentration of these three gases is increasing at an annual rate of 0.5%, 0.9% and 0.25% respectively (Houghton et al 1990) and are estimated to be collectively responsible for between 72% and 83% of global warming. Consumption of CH_4 can occur through oxidation by soil bacteria, while soil organic matter increases from improved pastures, reduction in burning of crop stubbles and the storage of CO_2 in live vegetation and dead litter, provide the largest sink for CO_2. The main sink for N_2O is photolytic reduction by UV radiation in the stratosphere.

The production or uptake of all three gases is affected by changes in agricultural management practices. Such a change has occurred in the sugar industry where 40 % of the crop is now harvested green, resulting in the return of 15 - 20 tonnes of organic matter/ha to the soil surface. An understanding of the effect that this change may be having on the production or sequestration of CO_2, CH_4 and N_2O is necessary if modified management procedures are to be introduced to curtail gaseous contamination.

The objective of this study was to measure the emission of CO_2, CH_4 and N_2O from a sugarcane field where the grower uses green cane trash blanketing and to estimate their contribution to atmospheric contamination.

MATERIALS AND METHODS

The experimental site was established on a green cane, trash blanketed sugarcane farm in the Herbert River Valley, 21 km west of Ingham (18.7°S, 146.2°E) in northern Queensland. The soil type was a bleached grey clay, 0.11% N, 1.86% C, containing 41% clay, 27% silt and 32% sand. The climate of the area is tropical with a mean annual rainfall of 2275 mm. Microplots were formed by pushing PVC cylinders (250 mm diam. by 250 mm long) to a depth of 200 mm into the soil beside the rows of cane. The experimental design was a factorial of three nitrogen fertiliser treatments (nil, potassium nitrate and urea), plus and minus trash, and two soil water contents with three replications, making 36 microplots. On the 14 September, 1994, trash was removed from the soil surface of 12 microplots, urea (99 atom % ^{15}N excess) broadcast on the soil surface at a rate of 160 kg N/ha and washed into the soil with 200 mL water. Trash was then replaced on the soil surface of 6 microplots. On 2 October, 1994, potassium nitrate (99 atom % ^{15}N

excess) was broadcast on the soil surface of a further 12 microplots at a rate of 160 kg N/ha, following removal of trash. Trash was then replaced on the soil surface of 6 of the microplots. Water was then applied to all 36 microplots, with 18 receiving water equivalent to 50 mm of rainfall and 18 receiving water equivalent to 25 mm of rainfall. Following water application, covers were placed on the microplots for 1h every 6h and gas samples taken after 0 and 60 minutes. This gas sampling procedure was repeated for 10 days. The gas samples were analysed for N_2O, CH_4 and CO_2 by gas chromatography (Mosier et al 1991, Weier et al 1991) and ^{15}N-N_2O by Europa Trace Gas Analyser. After 4 days, all microplots received additional water at their respective application rates. No natural rainfall occurred at the experimental site during the study period.

RESULTS

Carbon dioxide

The presence of trash on the soil surface of the microplots resulted in increased rates of soil respiration when compared to the no trash treatments (Table 1). CO_2 evolution ranged from 175 to 290 kg CO_2-C/ha and was greatest from the control plots. The presence of N fertiliser (particularly urea) had a depressing effect on the CO_2 evolution rate. Except for the control plots, there was no significant effect of soil moisture on CO_2 evolution from the microplots.

Table 1 *Total cumulative values (kg CO_2 - C/ha) obtained for the control and N fertiliser treatments after 9.5 days following application of the water and trash treatments [Values are the means of three replications with standard errors in parenthesis].*

Treatment	Control	Urea	$^{K}N O_3$
(+) Trash - 50mm	290 (50)	204 (37)	253 (3)
(-) Trash - 50mm	182 (2)	111 (27)	144 (12)
(+) Trash - 25mm	252 (80)	175 (18)	230 (22)
(-) Trash - 25mm	83 (8)	103 (8)	156 (42)

Methane

Net consumption of atmospheric methane occurred in all microplots regardless of the treatments imposed (Table 2). The addition of trash resulted in greater oxidation rates at the higher soil moistures in all three N treatments with values ranging from 6.9 to 19.1 kg CH_4-C/ha. Methane oxidation rates were also greater in the presence of KNO_3, whereas no significant difference in uptake rates could be found between urea and the control.

Table 2 *Net consumption of methane (kg CH₄- C/ha) over 9.5 days obtained for the control and N fertiliser microplots following application of the water and trash treatments [Values are the means of three replications with standard errors in parenthesis].*

Treatment	Control	Urea	KNO₃
(+) Trash - 50mm	6.9(1.5)	7.1(2.3)	19.1(1.8)
(-) Trash - 50mm	4.0(1.2)	6.8(1.4)	12.9(4.3)
(+) Trash - 25mm	2.7(0.8)	4.3(3.7)	9.0(1.1)
(-) Trash - 25mm	6.1(2.5)	7.8(1.1)	8.7(2.3)

Nitrous oxide

The production of N_2O-N was higher from 4 of the 6 microplots when trash was present on the soil surface (Table 3). N_2O evolution was greatest from microplots fertilised with KNO_3 with values ranging from 145 to 241 g N_2O-N/ha over 4.25 days. For the + trash microplots fertilised with urea, N_2O emissions ranged from 128 to 181 gN_2O-N/ha.

Table 3 *Total cumulative values (g N_2O - N/ha) obtained for the control and N fertiliser microplots after 4.25 days following application of the water and trash treatments. Values are the means of three replications with standard errors in parenthesis.*

Treatment	Control	Urea	KNO
(+) Trash - 50mm	140 (16)	181 (12)	192(14)
(-) Trash - 50mm	120 (3)	125 (28)	241 (94)
(+) Trash - 25mm	125(12)	128 (24)	207 (28)
(-) Trash - 25mm	83 (1)	138 (7)	145(15)

DISCUSSION

The increase observed in soil respiration beneath the trash blanketed microplots is consistent with the increased soil moisture and soil microbial biomass found beneath trash blanketed systems (Wood 1991). He found that soil moisture maintained a consistent level of 15% under trash while soil microbial biomass was twice that of the burnt system. A reduction in CO_2 production in the presence of fertiliser N was also found and may be due to a decrease in microbiological activity caused by the combined effects of osmotic tension and excess salt levels (Johnson & Guenzi 1963). However, there was a more pronounced effect of urea than KNO_3 on CO_2 production and may suggest a further effect of increased pH on microbial activity even though the urea was broadcast on the soil surface and not applied in bands (Wetselaar et al 1972).

The oxidation of CH_4 in sugarcane soils is in agreement with results obtained for other cropping systems (Bronson & Mosier 1993) although CH_4 uptake was much lower than found here, averaging 186 g CH_4-C/ ha. The occurrence of greater oxidation rates at the higher soil moistures is indicative of soils that have been intermittently flooded. These soils inherently have a greater capacity for CH_4 uptake due to the presence of increased methantrophic bacteria (Nesbit & Breitenbeck 1992). The greater CH_4 oxidation observed in the KNO_3 treated plots suggests that NO_3-N was not involved in the inhibition of CH_4 oxidation which is in contrast to the findings of Mosier et al (1991) who found that both N fertilisation and cultivation decreased CH_4 uptake. Nesbit & Breitenbeck (1992) also found that CH_4 oxidation was only inhibited in the presence of high concentrations of NO_3-N whereas Whittenbury et al (1970) reported that NO_3-N was used as a N source by the methanotrophic bacteria.

The increased production of N_2O from the KNO_3 treatment in the presence of appropriate soil water was consistent with what was expected in the presence of two of the three main factors that influence the denitrification process (Weier et al 1993). The third factor, available

C, may also be present through leaching of soluble C from the trash blanket on application of the water treatments. Percolation of soluble organic C into the soil profile after rainfall has been found to provide the denitrifying bacteria with the substrate necessary for the reduction of NO_3-N to occur (Weier et al 1991). The lower N_2O emissions from the urea treated microplots was due to the length of time required for urea to be transformed into NO_3-N (Chapman et al 1991) as >80% of the N was still in the NH_4-N form at the end of the sampling period.

In conclusion, the presence of a trash blanket on the soil surface in the sugarcane field enhanced the release of CO_2 and N_2O to the atmosphere and increased the uptake of atmospheric CH_4.

ACKNOWLEDGEMENTS

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POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZO-FURANS IN TOPSOILS FROM NORTHERN QUEENSLAND, WITH A HISTORY OF DIFFERENT TRASH MANAGEMENT PRACTICES

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ABSTRACT

Two topsoils from northern Queensland sugarcane fields with a history of different trash management practices were sampled and analysed for polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF). High levels of octachlorodibenzodioxin were found in both samples. The concentrations of all PCDD and PCDF in the soil from the plot which was "raked and burned" were about double the concentrations in the plot where trash blanketing has been practised since the early 1980s.

INTRODUCTION

Polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) include some of the most toxic xenobiotics known and are also very persistent. Combustion of chlorinated compounds such as chlorinated hydrocarbons used as pesticides is a likely pathway for the formation of PCDD/PCDF. To investigate concentration levels as an indication for a potential source of PCDD/PCDF, soil from two plots with different trash management practices on a sugarcane farm in northern Queensland were sampled.

MATERIAL AND METHODS

Site

The soils analysed in this study came from a sugarcane farm in the Herbert Valley (35 km NW of Ingham), Queensland. The soils are classified as red earth (well drained Haplustalfs). The climate is described as tropical, with a high humidity and an annual rainfall > 2000 mm (wet season in summer).

Treatment and soil sampling

Soils from two different trash management treatments were sampled viz: 1) Green cane harvest, trash raked and burnt, normal cultivation, fertiliser banded beneath the soil surface (RB); and 2) green cane harvest, trash blanket, zero cultivation, fertiliser broadcast or dropped as a band on top of the interspace (TB).

Prior to establishment of the trash management trial, the cane was burnt and harvested in the conventional manner at both sites. The study was an addition to a large trash management trial which was established in 1982 to determine annual cane yields and sugar content as well as a range of physical and chemical soil properties as a function of different trash management practices. Full details of the trial have been published by Wood (1986). Replicate soil samples were taken from the surface 25 mm of each treatment, bulked together and mixed thoroughly. Residual cane trash was removed manually before subsamples were oven dried at 40°C for 24 h. Sampling methods have been described in detail elsewhere (Sutton et al 1994).

Sample analysis

Samples were transported in sealed containers by courier to the dioxin laboratory in Bayreuth, Germany. Before extraction, a mixture of 12 $^{13}C_{12}$-labelled 2,3,7,8-substituted PCDD/PCDF congeners representing the 10 Cl_4- Cl_8 homologue groups was added to the extraction solvent (toluene). All samples including a laboratory blank were Soxhlet extracted for 20 h. The extracts were first cleaned up on a combined Na_2SO_4, H_2SO_4/silica gel, silica gel and NaOH/silica gel column that was eluted with hexane. The purified extract was then fractionated on a basic alumina column. The PCDD/PCDF fraction was reduced almost to dryness, a labelled recovery standard added, the sample again reduced almost to dryness and then taken up in a small amount of toluene. The samples were analysed using a HP5890 II gas chromatograph coupled to a VG-Autospec Ultima mass spectrometer operating in EI mode at

34 eV with a resolution of 10,000. The soil samples were analysed three times on two different columns (DB-5-MS and RTX-2330) to (i) quantify the homologues, (ii) quantify the 2,3,7,8-substituted congeners, and (iii) confirm the results on a mass fragment trace.

RESULTS AND DISCUSSION

The recovery of internal standards (> 70%) as well as the results of the laboratory blank (0.04 - 1.7 pg/sample) were satisfactory. The results of this study are summarised in Table 1. When the quantity of a congener in the sample did not exceed the maximum blank level by a factor of three the quantity is represented in the table as '< detection limit'.

Table 1 *PCDD/PCDF concentrations in soils (pg/g soil dry weight) from sugarcane fields in north Queensland with either green trash blanket (TB) or trash raked and burnt(RB) management. (< indicates that concentration was less than detection limit given)*

Homologue group	TB	RB
Σ Tetrachlorodibenzodioxins (TCDD)	2.9	3.2
Σ Pentachlorodibenzodioxins (PeCDD)	1.2	4.4
Σ Hexachlorodibenzodioxins (HxCDD)	9.0	24
Σ Heptachlorodibenzodioxins (HpCDD)	110	210
Octachlorodibenzodioxin (OCDD)	5200	9000
Σ Tetrachlorodibenzofuran (TCDF)	3.9	8.6
Σ Pentachlorodibenzofuran (PeCDF)	0.97	1.9
Σ Hexachlorodibenzofuran (HxCDF)	0.59	0.71
Σ Heptachlorodibenzofuran (HpCDF)	0.20	0.31
Octachlorodibenzofuran (OCDF)	2.0	3.3
2,3,7,8 substituted congeners		
2,3,7,8-TCDD	< 0.04	0.13
1,2,3,7,8-PeCDD	0.09	0.39
1,2,3,4,7,8-HxCDD	0.52	1.0
1,2,3,6,7,8-HxCDD	0.46	1.1
1,2,3,7,8,9-HxCDD	1.2	2.2
1,2,3,4,6,7,8-HpCDD	51	91
2,3,7,8-TCDF	< 0.03	0.80
1,2,3,7,8-PeCDF/1,2,3,4,8 PeCDF[1]	0.08	0.14
2,3,4,7,8-PeCDF	0.03	0.05
1,2,3,4,7,8-HxCDF/1,2,3,4,7,9 HxCDF[1]	< 0.02	0.04
1,2,3,6,7,8-HxCDF	< 0.03	0.05
1,2,3,7,8,9-HxCDF	< 0.23	< 0.26
2,3,4,6,7,8-HxCDF	< 0.04	< 0.03
1,2,3,4,6,7,8-HpCDF	0.08	0.13
1,2,3,4,7,8,9-HpCDF	< 0.02	< 0.02
Σ Polychlorinated dibenzodioxins (PCDD)	5300	9200
Σ Polychlorinated dibenzofurans (PCDF)	7.7	15
Toxic Equivalents (International) (I-TEq)	6.1	11

[1] Sum of two congeners which could not be separated

A number of individual PCDDs and PCDFs could be identified in both soil samples. The PCDD/PCDF concentrations in soils from plot RB ("rake and burn") were consistently higher for all homologue groups as well as for the individual congeners. The contamination level, expressed as international toxic equivalents (I-TEq) - a widely used method to express the total toxic potential of all PCDD/PCDF in a sample was found to be 6.1 pg I-TEq/g dry soil on plot TB and 11 pg I-TEq/g dry soil on plot RB. It is interesting to note that, for example, German authorities have restricted most agricultural food crop production to soils which do not exceed 5 pg I-TEq/g.

However, the concentrations found should not be of great concern with regards to consumption of sugar which comes from sugarcane grown in such a soil since PCDD/PCDF soil - shoot transfer has shown to be insignificant for most plant species (e.g. Muller et al 1993). Further, very hydrophobic substances such as PCDD/PCDF are unlikely to accumulate in relatively polar phases such as sugar.

A large fraction of the total toxicity is due to very high concentrations of one congener - octachlorodibenzodioxin (OCDD). This congener is so dominant that it accounts for 98 % of the total PCDD/PCDF soil burden. Compared, for example, with soils from agricultural areas in Germany, the OCDD levels in these two soil samples from Queensland are 50 to 100 fold greater. Heptachlorodibenzodioxin (HpCDD) is also higher but to a much lesser extent, while all other homologues can be considered as typical for "clean" background soils. PCDD/PCDF homologue patterns which are dominated by OCDD to such an extent are rare in soils but have been reported from Japan (Nakamura et al 1995). Sediments from the Mississippi River (Rappe et al 1995) and sewage sludge samples (Horstmann et al 1992) have also been shown to be dominated by OCDD.

These relatively high OCDD levels plus the fact that all homologues and congeners are increased under RB compared to TB suggest that trash management practices such as burning of sugarcane enhance the formation and release of PCDD/PCDF and especially OCDD.

CONCLUSION

Based on this preliminary study the authors can only speculate about the formation of these compounds. Combustion of organochlorine compounds during burning could lead to the formation and emission of PCDD/PCDF into the environment. The combustion of pesticides, possibly residuals, might play a role, but other formation processes can not be excluded. Whatever the cause, the authors recommend that this is further investigated, since the formation of these pollutants should be minimised.

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NITROGEN BALANCES FOR SUGARCANE PLANT AND FIRST RATOON CROPS IN THE WET TROPICS

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ABSTRACT

Because of increased interest in potential off-farm effects of agricultural practices, nutrient balances were investigated in a field-based project for sugarcane in the Johnstone River catchment. Data for nitrogen (N) from plant and ratoon crops indicate that leaching and gaseous losses are major N loss pathways. Nitrogen flux pathways can be influenced beneficially by management techniques including mounding of the row, sub-surface placement of narrow fertiliser bands, reduced fertiliser rates, trash retention, and timing fertiliser application to coincide with conditions optimum for uptake by the plant.

INTRODUCTION

The Queensland sugar industry occupies a coastal region in close proximity to many rivers, the Great Barrier Reef (GBR) and World Heritage listed rainforest. Both of the latter regions, which are of great ecological and economic value, may suffer long term damage due to off-site effects of some agricultural practices (Yellowlees 1991). A few studies have suggested links between land use practices and increased nutrient concentrations in streams (Bramley et al 1994) and in the GBR lagoon (Walker &0'Donnell 1981; Bell 1991). As reported by Keating et al (1993), past research in which the pathways of nutrient movement in sugarcane crops have been measured directly, have concentrated on one aspect of the balance. Apart from a desk study (Vallis & Keating 1994), no attempt has been made to construct a complete N balance for sugarcane. Such a balance is necessary to understand processes which determine N availability and in formulating strategies to improve fertiliser use efficiency. Accordingly, a project "Nutrient Balances and Transport from Agricultural Lands", was commenced on sugarcane in July 1992, with the encouragement of the north Queensland Johnstone River Catchment Coordinating Committee. In addition to complete balances for nitrogen (N), the size and sensitivity of transport pathways to management are reported in this paper for a plant and first ratoon crop of sugarcane.

MATERIALS AND METHODS

Detailed materials and methods were outlined by McShane et al (1993). The site in south Johnstone mill area, was located in a field at 145° 59'E, 17° 34.5'S. which had a 9% slope on a Ferrosol (Isbell 1993). Rainfall in the region is characterised by monsoonal falls during a distinct wet season, with an annual average in excess of 3000 mm. Nutrients applied in kg/ha were: 825 Ca, 75 Mg, 170 N, 31 P, 136 K, and 3 S to the plant crop, with 160 N and 95 K applied to the first ratoon crop. Calcium (Ca) and magnesium (Mg) were applied before final seed-bed preparation and planting to the sugarcane cultivar Q138.

Two plots, 70 m x 10 rows (15 m), were instrumented to monitor and sample leachate, surface runoff (dissolved and particulate components) and bedload as defined by Houghton & Charman (1986). Samples of rainfall received during the plant crop were analysed and input of N from this source was calculated based on an average N concentration of 0.252 mg/L. Actual values in rainfall will be obtained from analysis of resin column traps. Nutrient concentration in the crop was measured by sampling and analysing tops, trash and cane from three 60 stalk sub-plots (approximately 2m x 3 rows), for each treatment. Total plot yields and sub-plot nutrient concentrations were used to calculate nutrient removed in millable cane. The harvest residue components of trash blanket, stubble and roots were also sampled. Soil samples taken to 1 m (100 mm increments) from both the row and inter-row at establishment and after each harvest were analysed for inorganic (mineral) and easily mineralisable N. Net change in soil N for each crop period was regarded as an input to or output from the balance if

the value decreased or increased respectively. Ammonia volatilisation loss during the first ratoon was measured using a micro-meteorological technique and results have been reported by Freney et al (1994). Other gaseous losses, including denitrification, were calculated by difference. Experiments using ^{15}N labelled urea were undertaken during the first ratoon crop to validate the above approach.

Due to the cost of conducting this trial, only two unreplicated treatments could be evaluated each year. However, three sampling points were located within each plot for collection of leachate, estimation of crop uptake, and determination of harvest residue left in the field. The two management practices evaluated in the plant crop were: flat soil profile with cultivation for weed control, and mounded rows with herbicides treatment for weed control. Nitrogen was applied to the plant crop, initially with P and K at planting, and secondly with K during cultivation to fill in the drill. The crop was harvested green and residues were retained on the soil surface as a trash blanket. For the first ratoon crop, N was applied as urea with K in a 300 mm band on the surface of the row of the flat profile plot, without cultivation, and in a 30 mm wide sub-surface band, behind a disc coulter which split the mounded row of the other plot.

RESULTS

Fertiliser was the major N input for the plant crop (Table 1). Additional N came from net mineralised N, rainfall and unaccounted sources. Plant crop uptake (harvest residue plus millable cane) accounted for approximately three quarters of the total output balance. The major loss pathway for the plant crop was through leachate, with runoff and bedload erosion loss constituting small output components. There was a small increase in the soil net mineral pool during the plant crop, thus contributing to the output component of the balance.

In the ratoon crop, as for the plant crop, fertiliser was the major N input (Table 2). The combined inputs of mineralised N, rainfall N, release from the crop residue pool, recycling from >600 mm, and mineral N, constituted less than a quarter of the inputs. Although removal of N in harvested product was the largest single output component in the first ratoon crop (Table 2), other losses to volatilisation, current fertiliser N denitrification, leaching, unaccounted output and runoff, constituted approximately three quarters of the output balance. Leaching, denitrification and, depending on treatment, volatilisation, were major loss pathways in the first ratoon crop.

DISCUSSION

Management practices designed to minimise N loss and maximise N use efficiency by the crop must reduce leaching, volatilisation and denitrification losses, while maximising crop N uptake and retention of N in pools less subject to loss. Mounding reduced water infiltration through the row and reduced early leaching losses (data not presented here). Although mounding had little effect on total N leached during the plant crop, a trend towards

Table 1 *Nitrogen balances for sugarcane plant crops grown under two different management practices (kg N/ha).*

	Flat profile Cultivated	Mounded profile Min-till
INPUTS		
Fertiliser	170	170
Net mineralised	54	44
Rainfall	6	6
Unaccounted[1]	19	30
Total input	**249**	**250**
OUTPUTS		
Millable cane	79	96
Harvest residue[2]	115	91
Leached	54	56
Net mineral	1	3
Runoff[3]	<1	3
Bedload	0	1
Total output	**250**	**250**

[1] Calculated by difference between the sums of measured inputs and measured outputs.
[2] Includes tops, trash and cane left in the field after harvest, and underground stubble and roots.
[3] Includes particulate and dissolved total N.

Table 2 *Nitrogen balances for sugarcane first ratoon crops grown under two different management practices (kg N/ha).*

	Flat profile surface urea	Mounded profile split-row urea
INPUTS		
Fertiliser	160	160
Net mineralised	19	20
Rainfall	10	10
Harvest residue[1]	7	11
Net mineral	4	0
Recycled[2]	3	4
Total input	**203**	**205**
OUTPUT		
Millable cane	63	73
Volatilisation	60	10
Leached	18	30
Denitrification[3]	6	33
Runoff	4	6
Unaccounted[5]	54	53
Total output	**203**	**205**

[1] Release of N during the first ratoon crop due to a reduction in harvest residue (defined in Table 1).
[2] Assuming that 15% of leached N is absorbed by roots at >600 mm depth.
[3] From current fertiliser, estimated by ^{15}N mass balance.
[4] Includes particulate and dissolved total N.
[5] Calculated by difference as for Table 1, possibly denitrification of non-fertiliser N.

greater N in the millable cane harvested from the mounded plot, suggests improved N uptake efficiency associated with mounding. There was substantial surplus unaccounted N in plant crop balances, which suggested an unmeasured N source. It is suggested that recycling of N from below 600 mm, the depth at which leaching was measured, may be a source of the additional N. Evidence of net positive charge in Ferrosols at depth by Gillman and Abel (1987), suggests these soils may have layers capable of retaining and acting as a source of N.

Substantially less N was leached from the first ratoon crop, than from the plant crop, despite receiving more rainfall (3845 mm by the first ratoon and 2369 mm by the plant). A possible explanation is that nitrate interception was more likely by the established root system of the ratoon crop. Such uptake would be improved if fertiliser application was timed to match crop uptake. There were slightly greater runoff losses from the mounded plot in both plant and ratoon crops due to increased slopes on the edges of mounds and the less permeable, uncultivated interspace in the mounded plant crop. There was no soil erosion loss from both first ratoon plots due to protection of the soil surface by the trash blanket. The most notable feature of the first ratoon balance was that sub-surface placement of urea reduced N volatilisation loss by 50 kg/ha.

There was effectively additional N in the mounded profile, split-row urea plot amounting to 35 kg/ha when compared to the other plot. This additional N (in kg/ha), came mainly from reduced volatilisation losses (50), and slight increases in availability from mineralised (1) and recycled (1) sources, discounted by reduced input from above ground harvest residues from the previous crop (13) and less release from mineral sources (4). Changes in N output pathways (in kg/ha) which resulted from the increase in N input were, increases in losses to denitrification (27), leaching (12) and runoff (4). While there was an increase in output to millable cane (10) for the mounded first ratoon plot, uptake by other crop components was less (17).

Discounting the increased losses of N (43), by the reduced total crop uptake (7) and slightly less unaccounted loss (1), results in a net increase in N loss of 35 kg/ha from the mounded first ratoon plot when compared to the other plot, and this accounts for the increased N supply outlined above. It can be seen that management options which reduce N volatilisation, although increasing N output in millable cane, can in effect increase losses to other pathways. It is suggested that the management options in place in an attempt to reduce denitrification and leaching, were only partially effective without an accompanying reduction in fertiliser input.

Nitrogen volatilisation losses can be reduced by either sub-surface fertiliser placement, or by the use of ammonium forms which do not volatilise as rapidly as urea under acid soil conditions (Denmead et al 1990). A reduced rate of fertiliser N application may, however, be necessary to control losses through other pathways. There were substantially greater gaseous loss from the ratoon crop (volatilisation + unaccounted + denitrification), than for the plant crop where gaseous losses were less than the unaccounted N source. Chapman et al (1992), suggested that denitrification of crop residues may occur, and this may account for denitrification losses measured here in the first ratoon crop. The trend towards greater N in the millable cane harvested from the mounded, split-row urea placement plot in the ratoon crop suggests improved N uptake efficiency with this management practice.

The magnitude of balance components published for volatilisation (Freney et al 1994), plant uptake (Chapman et al 1981) and leaching (Baver 1963) are similar to values reported in this paper. The magnitude of denitrification reported here is in line with the opinion of Chapman et al (1991), who considered denitrification the major loss process in their trial. Average potential N loss to leaching plus denitrification for a plant and four ratoons of a trash retained system has been estimated from a desktop balance study at 109 kg/ha/yr (Vallis & Keating 1994). For a similar crop cycle, average leaching loss was calculated from our data at 30 kg/ha/yr. Assuming first ratoon unaccounted loss as denitrification from non-fertiliser N, average annual loss of N to denitrification would total 73 kg/ha. Thus average loss to leaching and denitrification sums to 103 kg/ha/yr, which is remarkably similar to the Vallis and Keating estimate.

CONCLUSIONS

Major loss pathways for N in sugarcane have been identified from a nitrogen balance study as leachate loss for the plant crop and leachate, denitrification and volatilisation loss for the first ratoon crop. It has been shown that pathways of loss are sensitive to management. Use of mounding, sub-surface fertiliser placement and placement of fertiliser in narrow bands in the mound, reduce losses which should result in fewer adverse off-farm effects. Timing of fertiliser application to coincide with uptake by the crop and reduced fertiliser rates are suggested as additional management options which may improve fertiliser use efficiency.

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6. Research, development and extension

BUILDING A STRATEGIC INFORMATION SYSTEM FOR THE SUGAR INDUSTRY

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ABSTRACT

The sugar industry has major impacts on the communities in which it is based the land on which it is situated, and its surrounding environment. There is abundant information describing aspects of the industry collected by groups within or associated with the industry. But for the whole industry, these information collections are not available for all locations, are difficult to acquire or are in incompatible formats (where they are available in digital formats). There are also information gaps. This project (commenced in June 1995) builds an industry wide information system for the whole sugar industry.

This paper gives the reasoning behind the development of the system, discusses the issues which must be resolved in its production and shows how it is being made available to assist in decision making, communication and understanding of issues in the sugar industry. Data are held in a geographic information system and relational database; examples demonstrate the ability of such a system to integrate dissimilar data from a variety of sources and provide powerful analyses of sugar industry issues. The system provides flexibility in the way in which information can be made available to people in the sugar industry; various means of access to the information are discussed.

INTRODUCTION

The sugar industry has major impacts on nearby communities and the surrounding environment. These impacts are as various as economic benefits to coastal communities; major changes in wildlife habitat and the visual landscape for tourists. While some impacts of the industry were realised decades ago, many continue through the dynamics of the agronomic and edaphic system; through expansion and contraction of areas affected; through changes in farm management, milling and waste disposal, and as a result of increased competition for land in the coastal areas. The perception of the importance of these impacts changes over time as objective measurements are obtained or understanding gained or with the growth of the environmental ethic in society as a whole.

To reduce negative impacts and to obtain the greatest returns from change in the industry, change must be and is planned. The effectiveness of this planning depends in part on the ability to relate and integrate dissimilar sets of information either technically (in terms of establishing common frames of reference) or conceptually (in terms of understanding the inter-relationships inherent in the information). This establishes a context for decisions and can make the data clearly understood and shared by all parties to decisions. Its effectiveness depends to a large extent on the availability and quality of information about the current state and the dynamics of change.

Conceptual analytical tools have increased in sophistication and availability with the emphasis in such areas as resource and information economics, urban and regional planning and socio-economic analysis. Information technology tools have increased in power and decreased in price; the numbers and breadth of people able to and choosing to access digital data in its various forms has increased; and there is increasingly a network which allows exchange of data as well as remote access to data sources. This has greatly increased the ability to deal with the data already available but it is increasingly the case that the products and outcomes achieved from these tools and approaches is dependent on data - its presence, scale and quality (Jhoty et al 1995).

There is abundant information describing aspects of the sugar industry; but much of this information is not available for all locations or is difficult to acquire. Use of this information requires it to be in digital format. Much of it is not digital and the information in digital format may not be compatible or appropriately stored. There are also significant information gaps.

This paper describes a project which addresses key aspects of this issue by collecting information sets likely to be important for broad-scale decision-making in the sugar lands. The information set will be available to all decision-makers in the Queensland sugar industry in a form which they can use (including a variety of electronic methods as well as paper

reports). The project will also establish a process within the Department of Primary Industries to review and update data on a regular basis after the project finishes.

METHODOLOGY AND DESIGN

Design and development of the system involves five non-sequential

Stage 1: Accumulation of information which impinges on land use decisions. Information sets include: land suitability and soils; current cane assignments; current land use; local government zoning & planning; industry expansion proposals; environmentally sensitive areas; cultural places of significance; valuable alternative uses; irrigation; industry infrastructure; other infrastructure (roads, railway etc); land tenure and topographic features.

These information sets fall into three classes. Class 1 includes digital information which can be captured or accessed readily, by identifying the custodians of these information sets and arranging the capture of or access to the data. Class 2 includes information which is not in digital form or requires a significant investment to convert to a form which is readily accessed. Class 3 is information (for example, current land use) that still remains to be collected.

Stage 2: Design of the information system and its delivery to interested parties in the industry. The core of the system will be a hybrid of information held at the Resource Management Institute at Indooroopilly in Brisbane and maintained links to other data custodians. Information at Indooroopilly will be held in a combination of ARCINFO (a GIS software package) and INGRES (a relational database management system) applications. External data systems will vary and it will be necessary to establish exchange or access mechanisms (Fig. 1).

Stage 3: Construction of an index/directory to the system; both paper-based and electronic index (in both Microsoft Access and dBase formats)

Stage 4: Development of access mechanisms to the system.

Stage 5: Communication of the system and its capabilities to industry groups. This started through several meetings with peak industry bodies designed to discuss:

i)   the information system being developed to determine interest in the project;

ii)   what information each group currently utilises which would be of relevance to the project; and

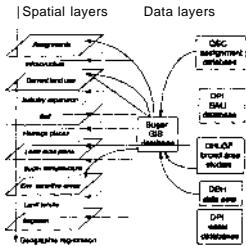iii)   the types of information they would like to have access to within the information system.

Fig 1A *diagrammatic representation of the proposed information system for the sugar industry. The figure illustrates the connections which must be established to construct the system and maintain it over time. (QSC - Queensland Sugar Corporation; DPI - Department of Primary Industries; SAU - Soil and Land Information database of DPI; DHLGP - Department of Housing, Local Government and Planning; DEH - Department of Environment and Heritage)*

The subsequent communication strategy was built on acquiring partners in the process of building the system, to represent both custodians and users of the developed system. This will be supplemented by small and large group meetings and seminars to acquaint a large audience with the system and its potential uses.

An example of the processes necessary to establish the system is the cane assignments which regulate sugar production in Queensland. While copies of cane assignment data are held at various locations throughout the sugar industry, the official version is held by the Queensland Sugar Corporation (QSC) in a relational database application (Microsoft Access software) in Brisbane. The data are not spatial and consist of

statements linking the owner of an assignment to one or more cadastral references (for example: portion of lot 1243 on plan 6789). With some data transformation, it is possible to attach the assignment data to the Digital Cadastral Data Base (DCDB) which is the statewide spatial dataset of land parcels and thus create a spatial coverage of assignments. Assignment data change frequently and the project will establish mechanisms to update as the official data changes.

The completed system will have the ability to integrate and view information spatially (Fig. 2); a facility which will enhance planning and decision-making in the sugar industry.

ISSUES OF AN INTEGRATED INFORMATION SYSTEM

Decision support system or data-centred approach

The development of information systems for an industry such as the sugar industry can concentrate in either of two broad directions. It can focus on the access tools and develop "front ends" which are tailored to the capabilities and needs of major players in the industry or it can concentrate on developing robust mechanisms for collecting and making available the data which feed such systems. The former is more likely to obtain immediate community acceptance but is confronted by the twin issues of rapid change in information technology (both hardware and software) which limits the life of any software system and difficulties in data maintenance. The latter approach puts more emphasis on data acquisition which increases the probable value of the database system but runs the risk of inadequately meeting the immediate needs of users.

This project is taking the latter approach for the following reasons:

i) The system is not complete in itself; it complements systems and approaches being developed throughout the industry.

ii) Many potential clients of the system have or are investing heavily in their own spatial data systems. Their systems are capable of absorbing new layers of data but are unlikely to easily use a sophisticated interpretative system. A decision support system will then be an additional tool requiring financial and skills investments above the current enhanced levels.

iii) Software packages are now available which simply display and retrieve data at a level which previously would have required a major programming effort. They are increasing in sophistication and decreasing in price. It is feasible for the project to use two of
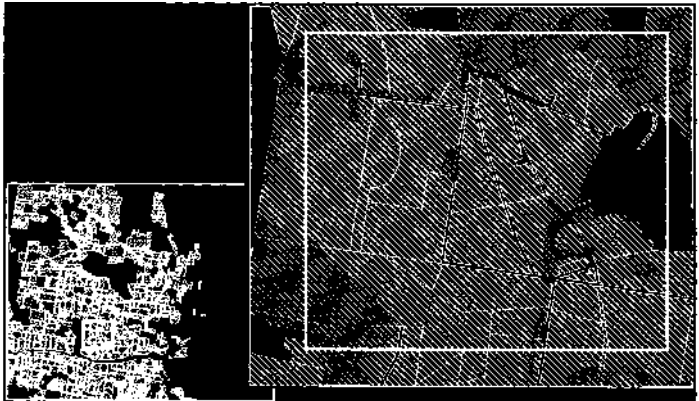

Fig. 2 *An example of the ability to integrate and view information spatially in a geographic information system. Assigned land (striped) is superimposed on land actually growing cane (dark grey)*

the more popular versions of this software as a means of broad access to the information system.

iv)   The channels to information are broadening. Information services through cable or satellite television and various components of the global Internet are examples. The return to investment in any one form is likely to decrease with time.

Data storage and management
The proposed system consists of an array of data layers which have a number of custodians. The system can be implemented by accessing data where it is currently stored, thus avoiding having two or more copies of the same dataset. While that is desirable, its effective implementation depends on adequate information communication infrastructure which is not uniformly in place. Consequently, the system in its initial stages, will be stored on computer systems at the Resource Management Institute's Indooroopilly complex and will involve protocols for the maintenance and updating of data which are copies of master sets held elsewhere.

Use of information in the system
Considerable onus is placed on the user of the information to know the quality and applicability of the data. This includes the purpose for which the data were collected and stored, as well as the accuracy, error levels and limits of application (Perritt 1995). The project will produce a complete index to the system which includes each of these aspects. Nevertheless, the heterogeneous nature of the system requires caution and expertise in its use. Notable sources of error could be:

i)   Scale variations - Information ranging from farm or block scale (better than 1:10,000) to regional data sets which are less accurate than 1:100,000.

ii)   Artefacts in overlay analysis - A major utility of a system based on GIS technology is the ability to overlay and integrate dissimilar datasets based only on their common geographic position. There are at least two major sources of error in overlay analysis. Firstly, creation of spurious polygons or areas of land resulting from inexact locations of boundaries (Smith & Campbell 1989). Secondly, the intersection of one polygon by another or by a line or point assumes a homogeneous distribution of information content across the polygon which is rarely the case, particularly in natural resource data (MacDougall 1975).

iii)   Geographic registration - Spatial data must be accurately located in space. The methods of achieving this involve significant compromises and result in error in geographic positioning.

Issues of copyright, confidentiality and security
Some of the data layers will include confidential or sensitive information (particularly if current and historic production data are added to the system). It will be necessary to establish the degree of confidentiality (which groups are to be denied access, for example), whether it is possible and useful to grant wider access to the data in an aggregated form, and the degree and form of security to be implemented. The dimensions of this issue will be resolved during close consultation with major stakeholders in the system. In addition, wider access to the system as a whole will depend on approval from the holders of copyright which applies to each layer in the system.

ACCESS TO THE DATA SYSTEM

This system is being designed at a time of major change in the way in which public information is made available to the community. In the United States, there has been a considerable investment in the National Information Infrastructure - *a system of telecommunications pathways and connections that transmits and receives voice, video, and data[1]* - which will enable enhanced access to information through the global internet (Bauman Foundation 1995). There are similar though less ambitious programs in Australia (Baker 1994) and substantial initiatives in the interfacing of and access to diverse government databases. The Queensland Land Information System (QLIS), for example, is developing a flight path to simple one-stop access to information (Eden & Baker 1994) and has identified a set of Foundation Information which underpin many land use decisions in the state (Webbnet Land Resources

Services 1995). Nevertheless, broad access systems are as yet poorly developed in Queensland and northern New South Wales.

The closest to a form of broad access is the global internet and, in particular, the World Wide Web which can make information available to users in a range of forms. This could include access to the data, interpreted or aggregated forms of the data and/or information from or about the system in hypermedia. Hypermedia is an information environment which can increase the utility of information by allowing 'browsing' of the information; users choose the degree of specialisation of information appropriate to their needs (Carrascal et al 1995). A similar set of data and information in hypermedia can be packaged on a CD-ROM. This project will use both avenues as a form of access.

Additionally, many groups in the sugar industry have the potential to use the information produced by the system directly in a GIS as a complement to their existing spatial data. Most use either MAPINFO or ARCVIEW-ARCINFO software and data will be produced and disseminated in these forms. Groups, who have no access to the system electronically, will have the ability to frame questions of the system based on an understanding of the data and the potential for integration. For these purposes, the Department of Primary Industries will act as a bureau service to major stakeholders in the system.

The success of the system depends on the breadth of access as well as an understanding of the scope of the system and its capabilities.

CONCLUSION

The strategic information system is being designed to meet the needs of those who require a spatial overview of the sugar industry and its environment. Consequently it will be useful to those responsible for planning in the various sectors of the industry such as industry groups and authorities; various government agencies; mills, regional bodies, agribusiness, local authorities and others for whom a planning approach is crucial. In order to ensure its usefulness to these groups, it is being developed with their cooperation and guidance.

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CRC FOR SUSTAINABLE SUGAR PRODUCTION: A FRAMEWORK FOR SCIENCE, INDUSTRY AND UNIVERSITY COLLABORATION

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ABSTRACT

The Cooperative Research Centre (CRC) for Sustainable Sugar Production was established in 1995 to foster an internationally competitive and environmentally sustainable Australian sugar industry. The CRC is a joint venture involving 13 parties from the growing and milling sectors of the sugar industry and its major research and development (R & D) and training providers. The CRC undertakes R&D, training and extension designed to provide a scientific basis for sustainable sugar production in three program areas: Protecting the Environment, Sustaining Soil & Water Resources, and Enhancing Productivity, with the focus on crop, soil and water management practices. The CRC provides a framework whereby industry, R&D agencies and universities can jointly identify priority issues and plan, resource and implement activities to address them in a coordinated way. It is a bold attempt to enhance the integration of research, extension and education relating to sugar production and provide a holistic, multidisciplinary approach to the complex sustainability issues facing the industry. Like most significant initiatives, the venture is not without its challenges.

INTRODUCTION

The Australian sugar industry has a long history of investment in research and development (R & D). From the turn of the century until the 1980s, R & D for the industry was largely funded from levies from growers and millers, and most of the industry's R & D was carried out by two providers totally dedicated to sugar R & D : the Bureau of Sugar Experiment Stations (BSES) and the Sugar Research Institute (SRI). The BSES is a Queensland Government statutory authority with a primary focus on R & D including extension for the cane production sector. The SRI is the R & D arm of the Australian raw sugar milling industry and focuses on improving raw sugar processing and equipment design. A small amount of R & D has also been undertaken by technical groups attached to the larger sugar milling companies. Historically, sugar industry R & D has been production-oriented, with a strong applied, problem-solving focus.

During the 1980s, the Queensland and New South Wales governments substantially increased their funding for sugar industry R & D, and the Sugar Research Council, later to become the Sugar Research and Development Corporation (SRDC), was formed enabling the industry to obtain matching Australian Government funding. The SRDC is an Australian Government statutory authority whose role is to allocate, on a competitive basis, funds derived from industry levies and matching government support, for R & D and training providers to undertake work on priority issues. Several trends were associated with these changes. The overall investment in sugar industry R & D increased by more than half, and there was significant diversification in R & D providers (most notably through the involvement of public-funded agencies such as the Queensland Departments of Primary Industries and Natural Resources, the CSIRO, and the Universities). A wider range of issues was addressed, with greater emphasis on longer-term strategic research, but with some compartmentalisation between agencies.

The Cooperative Research Centre (CRC) for Sustainable Sugar Production was one of ten new centres selected for establishment in 1995 as part of the Australian Government's program to foster better linkages between science, industry and the universities. The CRC is a joint venture between the growing and milling sectors of the Australian sugar industry and its major R & D, and education providers. Each of the joint venture parties contributes cash or in-kind resources (professional staff, infrastructure support), which are augmented with considerable new cash funding from the Australian Government. The government funding is to facilitate the necessary cooperative arrangements linking the industry, R & D and university joint venture parties, and enable significant new R & D initiatives to be implemented.

RATIONALE FOR THE CRC

The aim of the CRC Program is to foster better integration of public- and industry-funded R & D and training in the universities, and in turn, better linkage of these functions with the industries that they serve (Department of Industry, Science and Technology 1995). A further pre-requisite for CRC Program support is a demonstrated need for the commitment of additional public funding to address significant industry and community problems. The major problem facing the Australian sugar industry, and the target of researchers in the new CRC, is how to manage several emerging issues to ensure both the sustainability and the future international competitiveness of the industry.

The location of the industry adjacent to expanding urban and tourist developments has heightened environmental awareness and raised concerns about possible adverse effects of sugar production. These fears are exacerbated by the industry's proximity to environmentally sensitive areas such as the Great Barrier Reef, coastal wetlands and tropical rainforests. As with most rural industries, the sugar industry's 'terms of trade' continue to decline. The industry faces greater international market pressure as competitors rapidly adopt improved technology. Meanwhile, despite advances in production technology, there has been limited improvement in average crop productivity in Australia over the past two decades. In recent years, there has been a major expansion of the Australian industry, and further expansion is expected into the next century. The expansion has been associated with a progressive decline in government regulation of industry operations, a trend unlikely to be reversed when the current Sugar Industry Review Working Party reports at the end of 1996.

SUSTAINABILITY FOCUS

The Standing Committee on Agriculture (1991) defined three requirements for agricultural practices to be considered *sustainable*: they must be profitable, they must maintain the industry's natural resource base, and they must not damage the off-farm environment. The CRC focuses on R & D to satisfy these three requirements, with a view to complementing existing R & D activities and providing leadership in developing the capacity to integrate and generalise research experience. The practical outputs of the CRC's activities are scientific information, technologies and agronomic practices that enhance the productivity and profitability of sugar production, sustain the soil and water resources on which sugarcane depends, and minimise adverse effects of crop, soil and water management practices on the off-farm

On- and off-farm issues being directly addressed by the CRC include most aspects of crop, soil and water management, such as the timing of key operations and the implications for industry profitability; optimising

the mix of varieties; yield estimates and forecasting; tillage practices; conservation of crop residues; soil water management and irrigation; resource use planning; fertiliser management and nutrient supply; management of soils with adverse physical and chemical properties; liming and other soil amelioration practices; waste/effluent disposal on caneland; surface drainage; and the effects of these various practices on the quality of surface and groundwaters, and on the movement of soil, nutrients, chemicals and water through the soil profile, and across the land surface and in adjacent catchments.

The CRC does not directly address all of the on-farm issues conceivably affecting the sustainability of sugar production. Issues beyond the immediate scope of the CRC include biodiversity, habitat protection, biotechnology, the breeding of new cane varieties, protecting the crop from various pests and diseases, and agricultural engineering associated with planting, harvest and transport of cane. These issues may, however, be addressed from time to time through collaborative linkages to agencies with a direct interest in these issues. Sustainability issues relating primarily to other industry sectors such as milling, transport, storage, refining and marketing are also beyond the immediate scope of the CRC.

KEY ELEMENTS OF THE CRC

Parties and resources
Approximately 27% of the total resources of the CRC are directly or indirectly sourced from the sugar industry. Industry parties to the CRC include the five major milling companies (Table 1) which collectively represent 90% of the industry's crushing capacity. These companies have committed in-kind resources totalling $6.4 million (1994/95 value) over the seven year life of the CRC. A further $4.2 million has been committed in cash and in the form of R & D project support by the SRDC. The Australian Canegrowers Council Ltd, representing the nation's 6900 growers, is also a party to the CRC. Three R & D agencies, and three universities (Table 1) contribute a further $17 million of in-kind resources, the BSES component of which is also partly industry funded. $14,256 million has been committed by the Australian Government through the CRC Program. In addition to covering the networking costs of the new Centre, the Commonwealth funds support additional professional staff and a postgraduate training program.

Professional staff and students
The equivalent of 24 full-time professional staff (FTSE) are seconded to the CRC through the part-time involvement of *c*. 70 professional staff (an average level of involvement of 35%). An additional 13.5 FTSE scientists and upwards of 10 postgraduate students are being recruited using the Commonwealth funds to fill skill gaps. The new staff and students are not directly employed by the CRC, but are recruited by the parties using funds provided through the CRC, and seconded back to the CRC to work on CRC-funded activities. Collectively, the new and seconded staff represent a unique professional resource for the industry. Almost all of the disciplines (e.g. agricultural and soils es, biological and environmental sciences, chemistry and other

physical sciences, economics and social sciences) needed to address the complex sustainability issues relating to sugar production are represented in the CRC. There is also a complementary mix of research, extension, and academic skills that span the spectrum from short-term applied to longer-term strategic perspectives.

Research programs
Seconded staff from the various parties work in collaborative multidisciplinary teams within three research programs that reflect the key elements of sustainability defined above: *Protecting the Environment; Sustaining Soil & Water Resources;* and *Enhancing Productivity*. Within the research programs, activities are coordinated within subprograms focusing on specific priority issues and objectives (Table 2). Much of the work, particularly that requiring a 'holistic' multidisciplinary approach, is new or is work that would not have been initiated in the foreseeable future had the CRC not been formed.

Protecting the Environment focuses on establishing the nature and extent of environmental issues and problems, both real and perceived; on developing a framework designed to assist the industry and local authorities to minimise any adverse downstream effects of production; and on how to manage canefields for disposal of solid wastes and effluents.

Sustaining Soil and Water Resources focuses on using soil and water resource inventories to extrapolate experience across regions and assist industry planning; on developing conservation tillage strategies such as green cane trash blanketing; and on reducing the effects of soil constraints caused by acidity, salinity, compaction and waterlogging.

Enhancing Productivity focuses on ways to increase productivity through optimising irrigation and fertiliser management practices; better yield forecasting and crop scheduling; and better matching of the crop to the environment.

Cross-programs
In addition to research, subprogram teams participate in cross-program activities in three areas: *Education, Technology Transfer* and *Systems Analysis and Modelling*. The cross-program structure is designed to ensure that these activities are effectively integrated with the research. Within each cross-program, a small leadership group is responsible for coordinating participation in education, technology transfer and systems integration activities.

Education links subprogram teams to the three universities (Table 2) participating in the CRC. Opportunity is provided for postgraduate students enrolled in the universities to gain direct industry experience by working under the supervision of CRC scientists on industry problems as part of their thesis research requirements. The aim is to enhance professional and technical expertise within the industry, by increasing the number of postgraduate training opportunities, and broadening the range of specialist expertise available for student supervision and teaching.

Table 1 *Parties to the Cooperative Research Centre for Sustainable Sugar Production*

Industry organisations	R & D agencies	Universities
• Bundaberg Sugar Ltd	• Bureau of Sugar Experiment Stations	• James Cook University (Centre Host)
• CSR Ltd	• CSIRO (Division of Soils and Tropical Crops & Pastures)	• Central Queensland University
• Mackay Sugar Cooperative Association Ltd	• Queensland Department of Natural Resources	• The University of Queensland
• NSW Sugar Milling Cooperative Ltd		
• Sugar North Ltd		
• CANEGROWERS		
• Sugar Research and Development Corporation		

Table 2 *CRC for Sustainable Sugar Production: Key research objectives*

Program 1: Protecting the Environment

• Assessment of current environmental scenarios and budgets for inputs to and losses from sugarcane production systems at farm and catchment scales

• Development and promotion of production practices consistent with environmental protection and integrated catchment management principles

• Guidelines for dealing with unacceptable drainwaters from canelands, and use of canelands as 'environmental kidneys' for solid wastes and sewage effluents

Program 2: Sustaining Soil & Water Resources

• Use of computer-based inventories of soil, water and climatic resources to aid industry planning and extrapolation of research results

• Development and promotion of soil conservation practices based on conservation of crop residues

• Technologies to minimise problems associated with adverse soil chemical and physical properties (salinity, sodicity, waterlogging and nutrient imbalances)

Program 3: Enhancing Productivity

• Better management of water and nutrient supply to match sugarcane needs and avoid losses to the environment

• Tools to enable better crop scheduling and yield forecasting

• Strategies for better matching of genetic potential of sugarcane varieties to environmental production constraints

Systems Analysis and Modelling aims at integrating information from the different aspects of the cane-production system being addressed in the different research subprograms. The strategy is to encourage a multidisciplinary focus to more effectively integrate outputs from component research and develop a 'holistic' or systems approach to industry problems. This cross-program also focuses on ways to help generalise results from field experiments across canegrowing regions and from year to year.

Technology Transfer aims to effectively interface the CRC with industry and community stakeholders, to ensure that the research undertaken is sharply focussed on industry and community concerns and that the benefits from the CRC's activities will be rapidly realised. To achieve this, the CRC is strongly linked with the existing industry extension service, operated by the BSES and the Crop Protection and Productivity Boards associated with the milling companies. Research staff are encouraged to incorporate technology transfer and communication objectives, in consultation with extension staff, into their projects at the inception stage. Consistent with this philosophy, research staff are expected to assume some responsibility in the technology transfer process.

Management

The strategic directions of the CRC are driven by industry and community stakeholders in sustainable sugar production, within the context of two agreements which broadly outline its nature, structure and function. A Board comprising representatives of the joint venture parties, and supported by a consultative committee representative of grower, miller, agribusiness, environmental and community interests, is responsible for policy formulation and strategic oversight of the Centre. At operational level, activities are planned and implemented under the guidance of a director, and program and subprogram leaders drawn from the parties. In effect, the CRC provides a framework whereby representatives from industry, R & D providers and the universities jointly define priority sustainability issues, and then plan, resource and implement appropriate research, extension and education activities to address them.

The CRC functions as a cooperative research network, rather than as a 'physical centre' as such. Staff are located at 18 laboratories and experimental stations dispersed from Mossman in north Queensland to Harwood in northern New South Wales. The operation of the network is supported though a small secretariat headquartered at James Cook University in Townsville, which as well as being a party within the CRC, serves as Centre Agent and Centre Host. In these latter capacities, the University acts as 'banker' and provides access to administrative services and accommodation for the secretariat.

CHALLENGES

The aim of the CRC is more effective integration of R & D, and enhanced collaboration between the sugar industry and the research and training agencies, so that the benefits from R & D are more rapidly generated and future needs are met for trained scientists in the sustainability area. The scientific issues associated with sustainability are complex and practical solutions to industry problems, in a rapidly changing regulatory and commercial environment. will in most cases require an integrated approach that also considers economic and social factors. As such, they require a multidisciplinary approach combining the unfettered efforts of the industry's brightest and its most practical minds.

To be successful, the CRC must generate the synergy potentially available from the integration of its staff into effective multidisciplinary and multifunctional teams. There are considerable challenges in coordinating activities that encompass diverse disciplines and functions and are regionally-dispersed. All involved in the CRC share a strong commitment to benefit the Australian sugar industry. Nonetheless, the initiative brings together personnel from organisations with differing functions and 'cultures'. Consequently, those involved, from the individual scientist to the Board member, bring forward a range of differing perspectives on issues from strategic priorities to operational procedures. Individuals partially seconded to the CRC can face the problem of 'divided' loyalties where collective decisions do not coincide with those preferred by their host party. Effective collaboration also requires considerable initial investment of time and effort, while the benefits take time to emerge.

The dispersed location of the staff poses specific logistical difficulties for communication. Considerable emphasis has therefore been placed on developing the physical capacity for enhanced communication between remotely-located staff. The Queensland Department of Small Business, Industry and Tourism has committed significant funds for an 'advanced communications capability' for the CRC. Key elements include a computer network with shared software and databases, linking to scientists at all major centres through the Internet or through telecom modem, and a videoconferencing facility with multi-site capability and interactive software sharing capacity.

The challenge for all involved in the CRC is to contribute to an environment that embraces diversity, enhances the opportunities for synergy, and at the same time meets requirements for accountability to the joint venture parties and to the CRC Program. It will inevitably take some time, perhaps of the order of 18 months, for the CRC to begin to operate at near full capacity. Even then, the need will persist for acceptance of progressive, adaptive change if the CRC is to maintain effective linkages with industry and community stakeholders and ensure its activities retain sharp focus on stakeholder priorities.

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CONTRIBUTION OF AGRICULTURAL ECONOMICS TO SUGARCANE RESEARCH AND DEVELOPMENT IN MAURITIUS

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ABSTRACT

Agricultural research in Mauritius dates back more than 100 years. Since 1953, the Mauritius Sugar Industry Research Institute (MSIRI) has been responsible for all research work pertaining to sugarcane. The emphasis has been on sugar processing and sugarcane agronomy. During the 1980s, a consensus emerged that agricultural economics was a key factor in sugarcane Research and Development. The importance, achievements, and future orientation of the discipline to sugarcane research in Mauritius are discussed.

INTRODUCTION

In Mauritius, sugarcane is grown by miller planters and large and small "owner" planters on 79500 ha, about 43% of the island's total area. Miller planters own c. 40000 ha and produce nearly 62% of annual sugar production. Farm size ranges between 750 and 5200 ha. Large planters (>40 ha) harvest c. 12000 ha, and small planters (farms <10 ha) cultivate about 25000 ha subdivided into 65000 plots. The major constraints to productivity for small planters are rockiness and lack of water. The size of their plots, together with the high degree of fragmentation, prevents an efficient form of management being undertaken. Statistics related to area harvested and productivity for millers and owner planters are given in Table 1.

Table 1 *Yields and area harvested by millers and planters (1990-1994)*

Year	Miller planters		Planters[1]	
	Area harvested (M ha)	Yield (t/ha)	Area harvested (M ha)	Yield (t/ha)
1990	0.039	85.7	0.036	59.6
1991	0.036	87.9	0.038	61.8
1992	0.036	87.8	0.037	67.6
1993	0.037	76.8	0.036	62.8
1994	0.035	74.2	0.036	59.0

[1] Large and small planters

Under normal climatic conditions, annual sugar production of 0.62 Mt may be expected. A record production of 0.72 Mt was achieved in 1973 (Anon 1974). About 75% of the sugar is sold to the European Union under the Sugar Protocol of the Lome Convention and the balance sold locally. In the late 1970's, sugar production was the predominant contributor to the Mauritian economy. With rapid expansion of tourism and manufacturing, its relative contribution to gross domestic product is now 6% (MEPD 1994). Sugar production is being carried out under increasingly difficult conditions. Cost of production is being severely affected by expansive wage policies, while agricultural labour is becoming increasingly scarce. Miller planters and large planters can partly solve the above problem through mechanization but this is not the case with small planters. Another source of concern is the reduction in area cultivated. Julien et al (1995) estimate that from 1980 to date, some 6000 ha of cane lands have been converted to other uses.

The sugar industry is strongly backed by research. Organized research started more than 100 years ago, and added impetus was given to research on breeding, physiology, nutrition, plant protection, as well as processing of sugarcane, with the creation of the Mauritius Sugar Industry Research Institute (**MSIRI**) in 1953. This organization also carries out research on foodcrops grown in association with, or in rotation with, sugarcane. However, the need to focus on agricultural economics as a means to advance the industry has only recently been recognised.

This paper discusses the relevance of agricultural economics to sugarcane research in Mauritius under the themes of :

(i)   Importance of agricultural economics;
(ii) Achievements; and
(iii) The present and future orientation of agricultural economics research.

IMPORTANCE OF AGRICULTURAL ECONOMICS

Technology generation and innovation processes have always been geared to all categories of sugarcane producers. The contribution of the small planters to the growth of the industry has not been equal to expectations as they have lagged behind in the adoption of new techniques and technologies when compared with the large planter group. This is because the resource base, objectives, priorities, and problems of the small planters do not allow them to benefit to the maximum from research findings and recommendations.

Large and miller planters are the advanced sector of the industry and perform cultural operations using the latest technology available. However, it has been observed that it was becoming increasingly difficult for them to take advantage of some research products as the information disseminated was purely of a technical nature without being supplemented by economic considerations. They operate as private companies with a strong sense of profit maximization as their objective and as such they need to be guided on the relative financial and economic merits of alternative technologies proposed by researchers.

Furthermore, in the early 1980s, the country was undergoing substantial economic transformation and resources were being transferred away from agriculture to other sectors. There was a need for a revision of the research strategy and the adoption of alternative approaches which could strengthen the research-client linkages and which were likely to contribute to the development process. Consequently, Agricultural Economics was introduced as a research discipline at MSIRI in 1985, when an economist was employed for the first time. Presently, resources allocated to this discipline represent 0.8% of MSIRI recurrent budget.

ACHIEVEMENTS

The role of the agricultural economist was initially construed to assess the financial and economic attractiveness of technology generated or adapted through agricultural research. Such appraisals were to be undertaken at the end of the research cycle, prior to dissemination of results. The economists' duty statement was subsequently enlarged to include socio-economic research and economics of resource use. Achievements in these two areas are outlined below.

Socio-economic studies

The main objectives of socio-economic research carried out at MSIRI have been to identify planter priorities and objectives as well as the set of constraints within which the small planter attempts to achieve his/her implicit goals (Berthelot 1991). It is expected that this will enable more accurate definition of small planters' needs in terms of research and extension and will facilitate the process of technology generation, transfer, and adoption. Over the past ten years, 3 major projects which required the collaboration of other institutions have been completed.

The Souvenir Farming Systems Research Project

This project involved setting up a drip irrigation pilot scheme on small planters' fields. It also entailed describing the traditional farming system in the project area as well as the monitoring of changes induced by the new technology. It was observed that average cane yield improved by 75% for irrigated plots, that planters were overfertilizing their fields, and that chemical weeding was not adopted. The study confirmed the potential value of using socio-economic findings to modify information to suit the client's needs, and in identifying issues requiring the attention of agricultural scientists. For instance, the effect of non-adherence to fertilizer recommendations requires further study. The research also highlighted issues of wider relevance viz ; the need for increased extension input, planter participation in research planning, and improved coordination between institutional servicing planters, etc. These findings are pertinent to attempts at regrouping small planters into LAMUs (Land Area Management Units) which are blocks of adjoining plots owned by small planters and managed collectively.

Labour and transport problems of small-scale planters

A survey was carried out in 1989 in four factory areas to assess the extent of labour shortages and transport problems among small planters. Survey respondents opined that solutions to problems could be envisaged if collective actions were undertaken at their level. The need for mechanization of field operations was also expressed.

Socio-economic profile of small planters

A survey conducted in the early 1990s in four regions of the island aimed to develop a clear understanding of the characteristics and constraints of the small planter. The study showed that small planters operated within an environment characterised by socio-economic and technical constraints and that they had a limited resource base. The findings led to an improvement in institutional services for small growers.

Main findings from the three socio-economic studies are summarised in Table 2.

Resource economics

Emphasis in resource economics research has been mainly on cropping systems, irrigation technologies, and labour use.

Cropping systems

The financial merits and demerits, as well as the managerial difficulties associated with the traditional 12-month cane crop compared to 24-month (Hawaiian style) crop were assessed. Results showed that the longer crop cycle reduced profitability.

Another project aimed at evaluating cropping systems, based on sugarcane and food crops, in two localities of the island. Mixtures of cane and either maize, groundnut or bean were compared to sole cane planted in winter and summer. Financial analysis carried out over a two-year period showed that these food crops, grown in rotation or in interrows of sugarcane (Table 3) were unlikely to bring additional benefits to miller planters.

Comparative studies of drip and overhead irrigation systems have shown that although the former brings higher returns (due mainly to higher yields, low and stable operational expenses, and efficiency in water use), it requires a higher capital investment and highly trained personnel for efficient management. Financial and economic analysis have indicated that investment in drip irrigation is profitable in the sub-humid zone only (Table 4). Between 1988 and 1993, the area under drip irrigation on sugar estate land increased slowly from 225 to 569 ha (Anon 1989; Anon 1994). Also, the number of small-scale drip irrigation schemes for small planters financed by government has constantly increased since 1988.

Labour use

Agricultural labour is becoming increasingly scarce and the alternative is to partly or fully mechanize some field operations. Attempts have been made in the past to identify and quantify periods of shortage and surplus through the development of labour profiles and requirement

Table 2 *Main findings from socio-economic studies*

Souvenir Farming Systems Research (1987-1992)

Plot size range (ha)	0.1 -1.4
% of planters overfertilizing fields	
- before introduction of irrigation (n=23)	56
- after introduction of irrigation (n=104)	59
Yields (t/ha)	
non-irrigated (n=23)	**65**
irrigated (n=104)	**114**
Gross margins[1] (US$ x 1000/ha)	
non-irrigated (n=23)	3.6
irrigated (n=104)	8.6

Labour Shortage and Transport Problems of Small Planters (1989)

	R Belle n=136	St Felix n=147	Solitude n=126	FUEL n=137
		% planters		
Hiring labour for harvest	72	56	78	68
With labour problems	78	76	68	84
Hiring lorry for transport of cane	97	83	69	92
With transport problems	64	52	64	99

A Socio-economic Study of Small Sugarcane Planters in Mauritius (1990)

	R Belle n=200	St Felix n=200	Solitude n=200	FUEL n=200
		% planters		
>40 years of age	78	75	77	76
No level of schooling	31	24	29	20
Factors limiting yields				
Unfavourable climatic cond.	34	59	72	53
Poor weed control	25	20	2	4
High cost/shortage of labour	72	72	42	65

[1] Prices in 1996 US$

Table 3 *Gross Margins' (US$ x 1000/ha) for selected cropping systems based on sugarcane and foodcrops*

	Constance	Mon Tresor Mon Desert
SSPC+ 1R	3.6	4.4
Maize[2] + LSPC	2.1	
Maize[2] + LSPC + Groundnut[3]& maize[3]	2.4	
SSPC + maize[3] + 1R		4.4
SSPC + bean[3] + 1R + maize[3]		3.5

[1] Prices in 1996 US$ [2] Full stand [3]intercropped

LSPC: Long Season Plant Cane (summer planting)
SSPC: Short Season Plant Cane (winter planting)
1R: First Ratoon

Table 4 *Return to drip and overhead irrigation in 3 agro-climatic zones*

	Drip irrigation	Overhead irrigation
Investment costs[1] (US$/ha)	4184	2150
Annual running costs[1] (US$/ha)	284	100-200
Financial rates of return (%)		
Humid (up to 299 mm)[2]	1	**0**
Intermediate (300-600 mm)[2]	8	6
Sub-humid (over 600 mm)[2]	14	11
Minimum acceptable rate of return : 10%		

[1] Prices in 1988 US$ [2] Annual moisture deficit

charts. These have helped to identify the potential for mechanization and devise labour planning strategies.

Case Studies

During the past five years, there has been a growing demand from sugar estates for individual case studies. These have ranged from water allocation problems to construction of farm plans, and the studies have helped them in their long term development strategies.

PRESENT AND FUTURE ORIENTATION OF AGRICULTURAL ECONOMICS RESEARCH

The Mauritian sugar industry is a dynamic one. Technological change, new market opportunities, existing operational constraints, as well as macroeconomic issues will dictate the pace at which it develops. Hence, agricultural economics will have to contribute more towards improving its efficiency. This section deals with projects which are either part of on-going research or which will be implemented in the near future. Comments on the usefulness of econometrics in applied research are made and the involvement of the agricultural economist in the research cycle is briefly discussed.

Socio-economic studies

Socio-economic studies will focus on the rehabilitation of abandoned cane lands and the economics of regrouping small planters into large co-operative units with more effective management and operational

Rehabilitation of abandoned cane lands

An island wide survey is being undertaken to determine major reasons for abandonment of cane land and to quantify the extent to which land can be recuperated for future cane cultivation. Survey findings will allow the authorities concerned to elaborate strategies to accelerate the rehabilitation of abandoned plots.

The economics of small planter regrouping

Given the small size and fragmented nature of their plots, it is becoming more and more uneconomical for individual planters to continue cane cultivation. One way to overcome this sizable constraint is to encourage planters to regroup themselves into larger units (LAMUs). Since 1989, Government, through the Farmers Service Corporation, has provided integrated services to assist the creation of LAMUs. Services offered to planters include general extension advice, provision of machines for land preparation, procurement of cane setts, coordination of field operations and liaison with institutions servicing planters. Some 65 LAMUs covering about 537 ha of land have been set up so far. Investigations are being carried out to determine the cost-effectiveness of this approach as well as the optimal size of LAMUs. It is expected that the findings of these studies will serve as a guideline to decision-makers in formulating future development strategies and will also provide organizational and management guidelines for planters who would like to create their own groupings. The project will reach completion in about 4 years time.

Resource and production economics

Areas of key interest which fall under the production economics heading are crop cycle studies, mechanization, irrigation and cost of production analysis.

Crop cycle studies

These studies become pertinent in the light of changing technology and resource availability for sugar production. The determination of the optimal cane cycle under varying agronomic, ecological, and managerial conditions is in progress. The practice of the economically viable number of ratoons is a key determinant for higher levels of profitability and productivity. Research is also carried out on the economics of summer and winter plantings.

Mechanization

Mechanization of field operations is costly and is undertaken with the objective of supplementing labour during times of shortage rather than displacing labour already available. Studies will emphasize choice of machinery and equipment together with cost estimation of selected field operations like planting, harvesting, loading, etc. The impact of mechanization on labour demand and organization will also be assessed. The main immediate gains are likely to occur through mechanization of harvest.

Irrigation is a major means to increase productivity, but requires costly investments. Financial and economic viability studies of drip irrigation have already been completed and attention will now be given to other technologies like centre pivot and dragline systems. As water is a scarce resource, and different irrigation systems with varying levels of efficiency of water use are available in Mauritius, the economics of extensive and intensive irrigation systems will also be compared.

Cost of production analysis

Research carried out by the various technical disciplines under the present research programme of MSIRI is geared towards reducing the cost of production at field level. This is vital to the long term efficiency and competitiveness of the industry. Cost of production profiles once established for all three categories of producers and monitored periodically can provide a powerful tool for analysis of trends. They also enable the effectiveness of cost reducing technologies and techniques to be measured when applied to real production conditions. Furthermore, they provide a basis for targeting research interventions. This project will be given a high priority on the research agenda as there is an urgent need to investigate the causes of the low production per hectare achieved over the past five years. Most agricultural scientists claim that adverse climatic conditions are responsible for poor crop performance. In addition to these constraints, the economist is advocating a need to verify whether in the face of ever increasing costs, canegrowers are adopting a low input strategy which has had a detrimental effect on yield.

Econometrics

Agricultural policies, and broad macro-economic policies can affect the various groups of cane producers differently. Econometric techniques provide a framework for analysis and forecasting response of the agricultural sector as a whole, or of individual producer groups, to changes in policies. In our local context, there are several cases which require investigations.

One example that is particularly pertinent to the Mauritian situation is the effect of diversification policies on sugar production. Efforts have been made in the past to promote the production of foodcrops which are grown mainly on sugar estates with various incentives like input subsidies, guaranteed prices, etc. Except for potato, these policy measures have not achieved the desired objectives. In order to intensify food crop production further, a new set of policy instruments has been introduced. For example, a rebate on an already-existing export tax on sugar is obtainable if some cane fields are put permanently under foodcrops and fruits, or if larger areas of rotational land or areas under plantcane, are rented out to vegetable growers. The impact of these measures on overall sugar production needs to be analysed.

The research cycle

A typical research project consists of the following stages : identification, selection, experimentation, analysis, recommendation and dissemination. In projects initiated by other research disciplines, the agricultural economist used to intervene at the end of the cycle prior to the dissemination process. There is now full recognition that in many projects, the economist's participation is required from the initial stages. Two main reasons justify the new approach. First, funds allocated to research are becoming more and more scarce, and there is urgent need to select and prioritise projects. The economist is able to assist research administrators in these tasks. Secondly, the economic evaluation of the impact of research *(ex-post* evaluation) is an important guide for future research orientation. The latter exercise is more effective if agricultural economists are involved in all stages of the research cycle.

CONCLUSION

In relation to the size and importance of the sugar industry, the contribution of agricultural economics to sugarcane research and development has been relatively modest to date. However, at the research level, the economist's capability in addressing the needs of producers, which are not covered by purely technical solutions, is gaining recognition. The major objective of the sugar industry at large is to maintain its long term viability and competitiveness. This will have to be achieved through productivity improvement at field level, more efficient resource use, resorting to economically viable technologies,

and by developing the ability of the different producer groups to respond to innovations. Agricultural economics as a research discipline will be instrumental in helping the industry to meet these challenges.

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QUALITY ASSURANCE AND IMPROVEMENT IN RESEARCH, DEVELOPMENT AND EXTENSION (R, D AND E)

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ABSTRACT

Quality assurance is difficult to implement in R, D & E in agriculture for various reasons. This poster paper: (1) Outlines and defines the intent of quality assurance (and of the related concepts of total quality management, world best practice, and bench-marking) rather than the way that quality assurance has been implemented in production line manufacturing industry; (2) Outlines the need to accept that quality assurance is a dynamic, evolving system, rather than a static prescriptive set of procedures and rules; and (3) Highlights the perhaps unique, but at least different features or characteristics of R D and E in agriculture that will have an impact on how stakeholders respond operationally to the difficulties of implementing quality assurance.

INTRODUCTION

Quality assurance (QA) and The International Organization for Standards (ISO) 9000 series of quality standards are becoming of vital interest and concern to scientists and R & D bodies. Most people have heard of these new procedures and of the associated statutory requirements, however, few have any idea of what they really mean. The more cynical may well have a vague idea or suspicion that it is yet another management fad from overseas. Like many of these fads in the past they make lots of money for consultants fluent in their jargon and expert in their implementation, but add little to what the better R & D providers and managers are currently doing and have been doing for many years.

THE INTENT OF QUALITY ASSURANCE

The terms QA (and the related concepts of total quality management, world best practice and bench-marking, among others) have largely been corrupted by abuse both in technical terms and in every day usage. A basic definition of QA needs to be made in the context of a philosophy, a system to achieve the philosophy, a documentation of what is supposed to be done and an accreditation of what is actually being done. Thus, total quality management is a philosophy aimed at making the best use of available resources. Quality control is a system used to achieve the best use of available resources. QA is the documentation of how quality control is undertaken. Quality audit is the accreditation, usually by third parties, that the quality control as documented by QA is in place and operating.

Here we can see one of the reasons why people are confused and frustrated, that is, the term quality assurance is technically only the documentation. However, it is frequently used to mean all aspects of QA even though the documentation aspect of QA is the only aspect of QA with which people have contact or experience. Furthermore it is frequently imposed with little perceived need or benefits. Thus, in this paper I will use the term QA in a general sense to include all aspects of QA, in the same way as it may well be used by many of the readers at Sugar 2000.

A definition of world best practice and of bench-marking is that they are two of many ways of determining the quality of your organization. They compare what your organization is doing with what other similar organizations are doing, and determine what is the best of what any organization is doing.

The International Organization for Standards (ISO) 9000 series of quality standards identifies the key elements of a quality system for manufactured products. The scope of these standards is: selection of documents; design, development, production, installation, and servicing; production and installation; final inspection and test; quality management and systems elements.

For clarification and discussion on what is or what is not meant by any of these or other related concepts please refer to the extensive literature that is widely available.

One of the merits of QA is that it has been extensively documented, and if QA is implemented "by the book", all of its aspects are consistent with each other. Outcomes are then largely independent of the individual person actually doing the work, which is important in production line manufacturing. This is in stark contrast to the inconsistencies, changes of mind, unknowns and success being largely dependent on a given individual doing the work, that are a common occurrence in the way many organizations operate. QA has been successful in many production line manufacturing organizations in reducing material waste, and the size of inventories and time delays. It has also been successful in maintaining quality when there is a clearly agreed measure of quality and when this measure is easy to quantify. An example would be quality measured as time in fulfilling a customer's request for the purchase of a defined production line item or spare part. A second example would be the number of defects that had to be repaired after the delivery of a

However, when there is no clearly agreed measure of quality, or even if there is one, that is difficult to quantify, then QA has only had limited success. Furthermore having a philosophy, a system to achieve the philosophy, a documentation of what is supposed to be done, and an accreditation of what is actually being done, is not unique to QA.

Now let us turn to our area of interest. Historically, the aims of the better R & D providers and managers have included, as with QA, the best use of available resources. The system to achieve this has been that the practices and processes that underpin R D and E in agriculture, have always included in-built and on-going internal and external review. In earlier times, they have been variously called peer review both of proposals for funding and of papers, editorial committees of papers, applications or need for external funding, or the need for projects to be funded by "soft money". At times, these practices and processes have been implicit and low key; at other times explicit and high profile. The outcome has been an improvement in R & D that has been slow and incremental, with occasional "revolutions". The control and magnitude of the review process has usually been in the hands of senior R & D staff. These practices and processes have only recently become known generally as quality assurance.

Undoubtedly QA will make a contribution to the on-going improvement in R & D. However, to maximize its contribution, we need to focus more on the intent of QA and less on the way that it has been implemented in production line manufacturing industry. This focus is needed for two reasons. First, because successful implementation of QA is usually highly industry and workplace specific. Secondly, because of the perhaps even unique, but at least different, features or characteristics of R D and E in agriculture that will have an impact on how stakeholders respond operationally to QA.

Four aspects of the intent of QA that may help understand some of the difficulties of implementing QA to the on-going improvement of R D and E in agriculture are:

(i)   QA may be driven for transformation and innovation or QA may be driven for accountability;

(ii) QA may be achieved by reduction in areas of most waste, over the total organization, or by reduction of waste over only part of the organization;

(iii) QA may be achieved by staff having an increased ability to express their skills more as generic skills and less as context specific skills;

(iv) QA may be achieved by an agreed management process to deliver agreed outcomes and/or QA may be achieved by a visionary leadership that identifies new outcomes and facilitates improvements in the agreed management process and in the agreed outcome. For details and references see Monypenny (1996).

QUALITY ASSURANCE AS A DYNAMIC AND EVOLVING SYSTEM

To varying degrees, the problems encountered with attempting to implement QA into the on-going improvement of R D and E in agriculture are in part caused by the complexity both of the issues and of the decision support required. For example, see Eden et al (1983). Furthermore, the problems with QA cannot be solved in a "once and for all manner", because as one is solved another will acquire priority. QA is very much a dynamic and evolving system. Systems thinking has a long experience with complex issues like QA. There are many systems methodologies that could be used. The main contribution of all of these methodologies is to provide a framework and a terminology that are largely generic rather than largely context specific to QA. The need or reason to be generic is to increase the clarity of the perception of the relative importance of the many aspects of the issues related to QA.

For this work, I have chosen Systems Engineering Methodology (Sage 1977, 1983) because of its attention to institutional or organizational aspects of systems. For details and references see Monypenny (1995). A central purpose of the Systems Engineering Methodology is to assist clients in the organization of knowledge for the formulation, analysis, and interpretation of issues and problems that are of large scale and scope. It is most appropriate for use when one or more of the following applies: (i) There are many considerations and interrelations; (ii) There are far-reaching and controversial value judgements; (iii) There are multi-disciplinary and interdisciplinary considerations; (iv) Future events are difficult to predict; and (v) Structural and institutional considerations play an important role. Each of these apply to QA and improvement in R, D and E in agriculture.

CHARACTERISTICS OF R, D AND E IN AGRICULTURE

R & D in agriculture has a number of features or characteristics (as distinct from production line manufacturing). These characteristics make the efficient use of resources difficult, let alone the implementation of QA. These characteristics also have implications for most aspects of how staff and R & D organizations respond to issues like QA. These characteristics are:

Many stakeholders
The number of stakeholders or interested parties is usually high. In alphabetical order some are: Community representatives on the board of the research institution; Elected politicians with responsibility for research and funding; Funding authorities; Individual researchers; Industry representatives on the boards of funding authorities; Research Directors.

Range of expectations
Not only are there usually a large number of stakeholders but these stakeholders usually have a large range of expectations. In alphabetical order some are: Express community concerns; Find answers to current problems; Just do their job; Make available solutions to existing problems; Make contributions to knowledge; Make money; Stay within their budget.

Difference in planning time horizons
The stakeholders also usually have different lengths of planning time horizons. At the short end, this is likely to be a financial or calendar year. At the long end, it could be the next generation of industry or policy decision makers.

Difference in weighting given to cash and to environmental aspects
Stakeholders make recommendations or take decisions based on their perceived utility function. The weightings they give to each aspect of their utility function, and thus to their decision making, will vary between stakeholders. Particularly in the case of agriculture, the weighting given to cash or money aspects and to environmental aspects of a decision may vary significantly between stakeholders.

Difference in weighting given to private and social costs and returns
Stakeholders will also vary in terms of the weightings they give, in their utility function, to the importance and distribution of private and social costs and to private and social benefits.

Mixture of private and public sector funding
Priorities given to funding often varies between private sector decision makers and public sector funding authorities.

Difference in policy objectives
Above all these characteristics in R, D and E in agriculture, there are often significant differences between stakeholders in terms of their policy objectives or direction. There are often also significant differences even in what is or should be considered as viable alternatives. For example, the difference between moving towards free trade or towards increased protection; or the difference between mining or not mining uranium.

IMPROVEMENT

Most of these features or characteristics are not common, and rarely seen together, in production line manufacturing industry, where QA has been successful. Thus to achieve QA aims in R, D and E in agriculture, the implementation needs to be different. It is not appropriate to just take ISO Series 9000 and apply it to R, D and E in agriculture, if for no other reason than Series 9000 was designed for implementation in large scale production line manufacturing industry. Operationally this means that QA by or for R & D managers, R & D funders, scientists, agricultural extensionists, agricultural consultants, canegrowers, and the management of human resources, of patents and of property rights, needs to be different. If QA is to be developed and put in place in R & D. it needs to be done because of the perceived need and benefits that will accrue, rather than because QA has been successful in production line manufacturing.

Given the above characteristics and the considerable differences that they represent. QA and improvement in R, D and E in agriculture is more likely to come from:

(i) Leadership that identifies and clearly communicates what is to be achieved;

(ii) Management that delivers agreed outcomes using agreed processes;

(iii) Doing better, what the better providers and managers of R, D and E are currently doing and have been doing for many years;

(iv) Minimizing intermittent distractions through:
    distorted communication between stakeholders;
    turmoil (political, technological, personal, moral, etc);
    fads and fashions (productivity, returns to R and D, QA, and
    one of the next ones, quality platform);
    spurious panacea hunting.

In closing, the message from this poster paper is: Quality R, D and E has never been, and never will be easy but in spite of distractions as history shows, when the collective will is there, quality R, D and E is achieved.

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7. By-products

SOCIAL, ECONOMIC AND ENVIRONMENTAL ISSUES ASSOCIATED WITH THE BIODUNDER BY-PRODUCT FROM THE SARINA ETHANOL DISTILLERY

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ABSTRACT

Since August 1989, CSR Distilleries Operations has conducted a continuous Biostil fermentation process which uses molasses as a feed stock to make ethanol. This process replaced an old batch plant which produced a by-product called dunder, which posed a serious environmental problem. Dunder was used as sugarcane fertiliser, but because of its dilute nature, it was uneconomic to transport it any distance, so only a portion of the dunder produced could be utilised. Thus, dunder in excess of that used on canefarms was sprayed onto an extensive land disposal site, which sometimes caused unmanageable environmental problems of run-off into marine wetlands, smells, and fly breeding.

The new Biostil produced a by-product, marketed as Biodunder, which was more concentrated than the old dunder, and consequently could be fully utilised as sugarcane fertiliser. A distribution program was developed which is a commercial and environmental success, in the Mackay district. It is also a social success, for it has generated employment as twenty-five trucks, with drivers and maintenance crews were required to spread 200ML of Biodunder onto 45000 ha of caneland in 1994.

INTRODUCTION

Before 1989, the CSR Distillery at Sarina made ethanol at a cost to the environment. Only some of the by-product could be recycled onto nearby cane farms because its nutrient worth was exceeded by the transport cost. Distribution was only economic on nearby cane farms where it's value as a fertiliser was well established. (Bieske 1979; Usher & Willington 1979; Webb & Chapman 1987).

A land disposal system was developed on 800 ha of land to evaporate surplus dunder. This imposed high nutrient levels, mainly potash, and developed barren soil areas. Also, between sprayings, cultivation of plots was necessary to break the soil crust and to increase the surface area for evaporation. During high rainfall events, discharges of high biological oxygen demand (B.O.D.) material from these areas into the adjacent estuary systems depleted oxygen levels causing marine life to suffocate. Fly breeding and pungent odours developed at the same time, much to the discomfort of the local population.

The original Sarina Distillery was built in 1927 to process molasses into ethanol. Full production of ethanol from this plant occurred in 1978, CSR having purchased the Sarina Distillery a few years earlier. Consumption of molasses was 190000 tonnes per year with a resultant output of 600ML. The 1978 fertiliser value of this quantity of dunder was $4.2M. Unfortunately for the local environment, no effort was made to recycle dunder even though its fertiliser value was known in the early 1960s.

Biostil provided technology to produce a more concentrated by-product. CSR built a new plant in 1989, for it was realised that Biodunder could be economically distributed onto all farms in the Mackay region, thus reducing environmental issues. This paper discusses various aspects which assisted the successful introduction of Biodunder as a nutrient recycling programme onto sugarcane farms.

BIOSTIL TECHNOLOGY

CSR had to solve the problems of dunder disposal if ethanol production was to continue. Biostil technology was developed in Sweden and evaluated in trials at Sarina Distillery. This technology uses semi-continuous fermentation which can increase the concentration of inorganics in the by-product by up to four times of that from the batch process. This provided the opportunity to reduce substantially road transport and field distribution costs. If also eliminates the need for a land disposal system, and reduced the amount of product to be stored during periods when on-farm distribution is not possible. CSR, therefore, invested $25M in a new plant in 1989, and the concentrated by-product was marketed as Biodunder.

PRODUCT QUALITY CONTROL

Biodunder is stored in earth dams at Oonooie, 5km south of Sarina. Contents of dams are subject to precipitation and evaporation, so that nutrient concentrations are stratified with depth. The main fertiliser value of Biodunder is potassium (K) and Chapman (1995) showed that concentration is correlated with spindle Brix.

(K% = 0.105 Brix - 0.23, $r^2 = 0.998$)

Biodunder is marketed with a 3% K content. By testing the concentration in the dam with a Brix spindle, the depth at which Biodunder is pumped from storage can be adjusted to maintain quality. Discharge of Biodunder from the Distillery varies from 2 - 4% K, and the product in storage can range from 0 - 4% depending on rainfall and evaporation. Brix of Biodunder is monitored daily from June to December, and the specific gravity is 1.15 for 3% K w/v concentration.

Bureau of Sugar Experiment Stations (BSES) staff monitored the nutrient content of Biodunder being supplied to canegrowers for four years, from 1990-1993, by collecting ten samples per year. K content exceeded 3%K w/v by 10-28%.

BSES recommendations for K applications up to 120 kg/ha are based on soil analyses. The maximum K recommendation is provided by $4m^3$/ha of Biodunder. At this application rate other nutrients provided in kg/ha are nitrogen 36, phosphorus, calcium 38, magnesium 23, sulfur 14, plus traces of minor elements. This provides an input of nutrients to offset those removed from the field as sugarcane is harvested.

When Biodunder is used, canegrowers have to provide other fertilisers to meet their crops' full nutritional requirements.

DISPENSING BIODUNDER

The preferred transport and field distribution equipment is a tandem truck with a trailer, giving a total load of 23m³. The tandem is used for field distribution, and is refilled on-farm from the trailer. B-doubles and semi-trailers are also used, but they do not have the field manoeuvrability of tandems. Delivery pipes from transport tanks are

at constant head to ensure uniform discharge rates. Normally, Biodunder is applied to a width of seven rows of cane, each 1.5m wide. Solenoid valves on each outlet per row allow fewer rows, or rows on angled headlands to be fertilised accurately without spillage onto headlands.

Application rates vary according to customer requirements. Metering is controlled by changing nozzle size of outlets at each row and by varying ground speed of application. Tracks are fitted with 0-20 km/hr speedometers to monitor ground speed.

Biodunder application is accurate and rapid because of the ease of application of liquid compared to solid fertiliser and provides a cost effective fertiliser for canegrowers.

INDUSTRY ACCEPTANCE

The appointment of a BSES extension officer at Sarina and the subsequent establishment of field demonstration trials in 1977 was the start of a slow and steady acceptance of dunder (Fig 1).
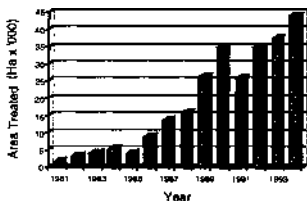
Fig. 1 *Area of sugarcane treated with dunder [(1981-mid 1989) and Biodunder (mid 1989 - 1994)]*

The area treated with the batch plant dunder in 1989 was 28000ha, but only amounted to approximately 50% of the total output as it was uneconomic to transport it further afield. About 45000ha were treated with concentrated Biodunder utilising the total distillery output in 1994. Most Biodunder produced is used as a sugarcane fertiliser with a small amount used on cotton, pastures and horticulture. It is also used as a molasses substitute for stock food and as a binder in the coal industry.

ECONOMIC ISSUES

Mackay district canegrowers have readily accepted Biodunder as a K fertiliser source because of cost savings. Biodunder can be applied to canefields at 55 to 95% of the cost of purchasing equal amounts of K as muriate of potash. Not only is Biodunder cheaper, it is applied at no additional cost and it also provides other plant nutrients.

An opinion survey by Hamilton & Crossman (1993) showed that 68% of customers agreed, and 28% strongly agreed that Biodunder provided the best value fertiliser for sugarcane. Sixty-five percent of customers definitely will, and 35% probably will, continue to purchase Biodunder. The same survey also revealed that after price, the next important aspect that CSR should address was to develop a value-added Biodunder which would supply the total nutrient supply for the crop (Fig. 2). This would capitalise on the existing cost of the application and transport of Biodunder which does not supply sugarcane's complete nutritional requirements.

A second survey by Blockley & Majewski (1995) confirmed that canegrowers would readily use Biodunder enriched with nitrogen and sulfur. The convenience of using enriched Biodunder has wide

appeal because of its rapid and precise application as well as is cost advantage. This was despite the possible loss of some urea-N by ammonia volatilisation and its low P content. Chapman (1995) suggested that as P fertiliser is relatively stable in the soil, a single heavy application of P fertiliser for the whole crop cycle before planting could be used and this advice is being followed by some canegrowers. "Liquid One Shot" was the preferred name selected for enriched Biodunder in a survey of canegrowers and truck contractors.

1. Timeliness of supply
2. Australian ownership
3. Quality Control
4. Location of supply
5. Nutrient enrichment
6. Price Competitiveness
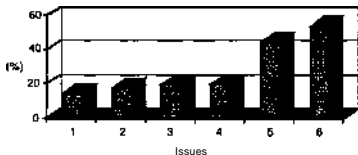
Fig. 2 Percentage of Biodunder customers who considered the issues 1-6 important from a survey in the Mackay District [after Hamilton & Crossman 1993].

EVALUATION OF "LIQUID ONE SHOT"

The decision to proceed with marketing "Liquid One Shot" was based on the results of the above surveys and applied research by BSES/CSR. Ammonia volatilisation losses were shown to be as high as 40% of the N broadcast onto cane trash as urea following green cane harvesting by Denmead et al (1990). Conditions which favoured loss were heavy dews and light falls of rain which were not sufficient to dissolve urea and wash it into the soil. When urea was dissolved in Biodunder and applied to cane trash, there was an initial delay in ammonia volatilisation for four to five days, followed by a loss from Biodunder/urea slightly less than from solid urea, namely, 22 and 19 kgN/ha respectively (Chapman et al 1994). Volatilisation losses when Biodunder/urea was applied to soil was approximately half of that when applied to green cane trash. The placement of Biodunder/urea can also affect its efficiency of use by the cane crop, and Wood & Chapman (1990) showed that nutrient uptake was quick by placement on the cane row rather than in the inter-row.

ENVIRONMENTAL AND SOCIAL ISSUES

Clayton & Pearson (1992) working for the Australian Centre for Tropical Freshwater Research evaluated the impact of Biodunder use on freshwater streams in the Sarina area. They showed no impact on aquatic fauna in streams which drained Biodunder-treated caneland. Fly breeding and odours associated with the old dunder dispersal area are no longer an issue as all Biodunder production is now utilised.

When the old batch plant was closed, voluntary retrenchment did occur. Despite this, there was a net gain in local employment as a much enlarged work force was required to recycle the Biodunder onto a much larger area. As all K for sugarcane is imported into Queensland the area treated in 1994 resulted in reduced imports equivalent to 12000 tonnes of potassium chloride.

Fish kills associated with run off from the dispersal area have also ceased in the marine wetlands of Llewellyn Bay. This previously occurred after heavy rainfall as runout could not be contained in dams constructed in the disposal areas.

THE FUTURE

With the completion of the batch blending facility for "Liquid One Shot", this will enable the trial mixing of P and other additions according to customer demand. The precision application should allow canegrowers to match their nutrient inputs more closely with their crops' needs, as determined by soil nutrient analysis. A further development may include the use of high flotation in-field application trailers to enable fertiliser application to occur under field conditions which may be too wet to permit the use of trucks.

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SUGAR ANALYSIS OF INDIAN CANE MOLASSES BY GLC METHOD

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ABSTRACT

A Gas-liquid chromatographic method for the simultaneous determination of fructose, glucose and sucrose in Indian cane molasses is described. The multi level calibration is employed to achieve accuracy and precision of the data. The GLC analytical data are in close agreement with those obtained by chemical method. The proposed GLC method is found to be quite precise, rapid and sensitive as compared with the conventional chemical methods of analysis.

INTRODUCTION

Molasses, a by-product of sugar industry is a vital raw material for the production of alcohol which itself is a basic feedstock for a wide range of chemicals and liquors. The composition of molasses has a direct bearing on its price as well as on the quality and yield of products derived from it. Hence there is a growing realisation among molasses users of need for an accurate, reliable and fast method of molasses quality analysis.

Though the determination of sugars in various sugar containing products ranging from sugarcane juice to molasses have been reported (Sweeley et al 1963; Sawardekar et al 1965; Vidauretta & Fournier 1970; Wong Suk Hoi 1982; Schaffler & Day-Lewis 1983; Schweer 1983); the gas-liquid chromatography method has not been applied in any Indian Sugar Industry products. ICUMSA (International Commission For Methods of Sugar Analysis) has officially adopted GLC method for the estimation of sucrose, glucose and fructose in cane juice and cane molasses (Schaffler 1986). Initially this work was undertaken to develop the GLC method for the determination of sugars in cane juice (Yewale 1995; Yewale & Shivade 1995:1996) and then further was extended to molasses.

Such an approach not only evaluates the quality of molasses in terms of suitability to other by-products but also reflects upon the glucose to fructose ratio in the molasses samples. Changes in the G/F ratios quantify the microbial contamination at milling stations due to poor sanitation leading to sucrose inversion.

MATERIALS AND METHODS

Reagents and Materials

Chemically pure sugars (glucose, fructose and sucrose) dried at 105°C for 3 hours were used. The derivatisation reagents hydroxylamine hydrochloride, dimethyl aminoethanol, hexamethyl disilazane (HMDS) and trifluoro acetic acid (TFA) were used without further purification. Pyridine was used as a derivatisation solvent.

The molasses samples for the 1994-95 season were collected from various sugar factories.

Calibration standards

Aqueous solutions (50 g) containing sugars of interest, viz. sucrose, glucose and fructose, were prepared. The concentrations of these sugars were selected as per their respective range, generally found in final molasses (30% sucrose, 20% reducing sugars). These solutions were prepared in duplicate and chromatographed three times to verify the response factors of individual sugars in the mixture. These calibration standards were also analysed before and after the molasses samples to minimize the instrumental errors.

Synthetic mixtures

A standard synthetic mixture of sugars was prepared by dissolving 0.75 g sucrose, 0.3 g fructose and 0.2 g glucose in 50 g of distilled water. This mixture was used as a reference standard for the quantitation of sugars in molasses. The composition of this sugar mixture was done as per the range of these sugars found in Indian molasses.

Molasses solution

Molasses 2.5 g was diluted to 50 mL with distilled water. It was assumed that on the average basis Indian molasses consists of 50% sugars and 50% non-sugars. The molasses solutions were prepared freshly every day and then subjected to derivatisation.

Oximation Reagent

This reagent was freshly prepared by dissolving 2.5 g hydroxylamine hydrochloride in 100 mL. pyridine followed by careful addition of dimethyl ethanol (at the rate of 55 mL/mL) to the above mixture.

Oximation and Sillylation

In a 5 mL screw - capped hypovial 500 mL of freshly prepared oximation reagent was gradually added to 5uL of the sugar solution. The vial was sealed with teflon screw cap and heated at 80°C. After cooling to room temperature hexamethyl disilazane (HMDS, 450mL) was added and mixed with agitation. Addition of trifluoro acetic acid (50mL) then followed with continuous stirring. The mixture in the sealed vial was reheated at 80°C for 10 minutes and then cooled to room temperature at which time precipitation occurred. The supernant liquid (1mL) was subjected to GLC ANALYSIS.

Gas Chromatography

A Perkin-Elmer Sigma 2000 model equipped with hydrogen air flame ionisation detection system and LCI-100 as integrator system was used. The other details of the established parameters for this analysis are reported in Table 1. A stainless steel column packed with 5% ov-17 phase on chromosorb W (6 ft x 1/8") was conditioned at 300°C (Stationary phase of maximum temperature 350°C) and used for this analysis at high temperature. The glass column packed with 10% UCW-982 (maximum temp. 250°C) on chromosorb W (HP) (3.3 ft. X 1/4") as suggested in 1990 by ICUMSA tentatively adopted GLC method was also taken for this analysis.

Table 1 GLC conditions for molasses analysis.

Column used	- 5%ov-17 on ChromosorbW, 100-120 mesh (max. Temp. 350°C)
Column dimensions	- S.S. 6' x 1/8'
Temperatures	
Oven	- Programming from 210°C, 225°C to 250°C with 5°C/min. rate and hold up time is 5 minutes for each segment.
Injection port	- 275°C
Detection Port	- 275°C
Sample size	- 1 mL
Carrier gas, N₂	- 20 mL/min., 85 psi
Hydrogen gas	- 40mL/min., 40 psi
Compressed air	- 440 mL/min., 40 psi
Analysis time	- 20 minutes

The calibration standards, synthetic mixture of the sugars and molasses were derivatised in the similar manner as discussed above. The calibration standards were chromatographed before and after the molasses samples to obtain response factors for individual sugars in the mixture. The quantitation of individual sugar (fructose, glucose and sucrose) was obtained by comparing the peak areas from samples with the peak areas of known amounts of the sugars in the synthetic

RESULTS AND DISCUSSION

The stationary phase 10% UCW 982 with the chromosorb W (HP) solid support, as proposed by ICUMSA subject 8 in 1990 was compared with the 5% ov-17 stationary phase (max. temp. 350°C) proposed in this paper. This column was selected for such analyses due to its high resolution efficiency and the steady base line at higher temperatures (>250°C). Multilevel calibration provided both accuracy and precision for the individual sugars. The drift in retention time of individual sugars was avoided by chromatographing the standards before and after the molasses samples. The precision of GLC analyses of individual sugars is well illustrated by coefficient of variation values (Table 2), relating to the sugar analyses as shown in Table 3.

Table 2 *Statistical analysis of GLC results; standard deviation (S.D.) and coefficient of variation (C.V.) are shown.*

	S.D.	C.V.%	S.D.	C.V.%	S.D.	C.V.%
Molasses						
	0.32	3.06	0.06	1.32	0.18	0.60
2	0.04	0.40	0.10	2.63	0.22	0.69
3	0.01	0.07	0.03	0.56	0.17	0.48
4	0.04	0.69	0.03	1.04	0.01	0.02
5	0.08	0.90	0.05	0.81	0.34	0.09

Temperature programming allowed monosaccharides (fructose and glucose) and sucrose to resolve nicely (Fig. 1a,b). Other than glucose and fructose no monosaccharides were found in significant concentrations in molasses. The ratio of glucose to fructose varied from 0.4 to 0.7 (Table 3) which reflects overall sugar processing. The fresh molasses samples were simultaneously analysed by known chemical method of analysis (Lane-Eynon method) for reducing sugars (F+G) and sucrose. Sugars in molasses as determined by GLC method agree well with those values obtained by Lane-Eynon method (Table 3). The sucrose content in molasses as observed with GLC was generally higher (±0.5 - 1.0%) in 4 out of 5 samples (Table 3) than by Lane-Eynon method. The reducing sugars obtained by chemical method were higher than those obtained by GLC method, in 3 out of 5 samples (Table 3), perhaps due to presence of other reducing substances in molasses.

The sugar contents in the molasses were not, however, compared in this work with the traditional method, viz. polarimetry, since the technique is incompatible to isolate the individual component of sugars, reducing sugars and other optically active components if present in the molasses samples.

Calibration, linearity and sensitivity

The instrument was calibrated everyday by analysing calibration standards of various concentrations of sugars. The response was found to be linear over the range of concentration of sugars generally found in molasses, viz. sucrose 25-75 mg/g, and glucose and fructose 2.5-7.5mg/g (data not shown). The multilevel calibration technique employed for such analyses minimizes

Table 3 *Analysis of sugars in molasses, comparison of proposed GLC and conventional Chemical methods.*

	GLC					Lane and Eynon			
Molasses samples	Fructose %	Glucose %	Reducing sugars %	Sucrose %	SGLC	Reducing sugars %	Sucrose %	SL&E %	G/F
1	10.65	4.76	15.41	30.11	45.52	16.86	29.51	46.37	0.45
2	9.20	3.85	13.05	32.39	45.44	12.98	31.75	44.73	0.42
3	6.89	4.55	11.44	34.74	46.18	12.09	33.71	45.80	0.66
4	6.30	3.57	9.87	35.40	45.27	09.80	35.97	45.77	0.57
5	8.43	5.98	14.41	34.38	48.79	15.68	33.87	49.55	0.71
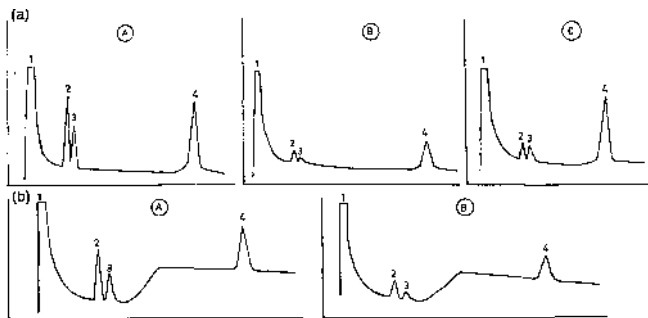
Fig.1 *Gas-liquid chromatograms using columns (a) 5% ov-17 stainless steel and (b) 10% UCW-982 glass showing (A) Calibration mixture, (B) molasses 1 and (c) molasses 2*
[peaks identified:- 1. pyridine, 2. fructose, 3. glucose, 4. sucrose]

the instrumental errors and the errors sometimes encountered with the use of internal standard technique. The sensitivity of the FID detection system and the proposed stationary phase to resolve monosaccharides from dissaccharides was found to be quite good.

Precision Test

To assess the precision of the proposed method five molasses samples from different sugar factories were analysed three times each by the GLC method for fructose, glucose and sucrose and by the Lane-Eynon method for reducing sugars and sucrose. The analytical data and subsequent statistical analysis are represented in Tables 2 and 3.

CONCLUSION

The GLC method for the analysis of sugars in cane molasses is described. The multilevel calibration technique is employed to minimize the instrumental errors. The derivatisation gave the stable derivative in the aqueous system. The proposed method is rapid, accurate, precise and informative as compared to traditional chemical and polarimetric methods of analysis. The sucrose content and G/F ratio can focus on the exact composition of molasses and sugar processing. Therefore GLC may be employed as a process-monitoring analytical tool in Indian Sugar Industries. The use of 5% OV-17 stainless steel column is suggested over 10% UCW-982 glass column as recommended by ICUMSA (1986), taking into account the resolution efficiency of the column, the avoidance of base line drift, and its accuracy in quantitation of sucrose at higher temperature.

ACKNOWLEDGMENT

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OXYGEN TRANSFER STUDIES ON THE PRODUCTION OF BIOGLYCEROL : A PROMISING BY-PRODUCT OF SUGAR INDUSTRY

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ABSTRACT

Bioglycerol production from renewable and cost effective raw materials such as sugarcane juice or molasses is assuming increasing importance with better understanding of factors affecting metabolic activities of osmophilic yeasts. The study reports the effect of aeration and agitation on time course profiles of sugar utilization and cell mass and product formation by an osmophilic yeast, Hansenula Anomala NCIM - 3341 . Optimum product yield has been obtained at an airflow rate of 0.5 vvm and agitation rate of 600 rpm. The volumetric mass transfer coefficient ($K_L a$) and the maximum oxygen transfer rate (OTR) has been estimated to be 132/hr and 6.6 mmol/L/hr respectively.

INTRODUCTION

Fermentative glycerol production from sugarcane molasses by yeast "steered" with sulphite or alkali (Neuberg 1918) has been known for some time. During the First World War, a great amount of glycerol was produced in Germany (Connstein & Ludecke 1915) and in England (Eoft el al 1919). The main draw backs of these processes were poor yield (25% based on sugar) and very low concentration of glycerol (3-4 %) in fermented broth.

Nickerson and Carrol (1945) first reported that an osmophilic yeast then classified as *Zygosaccharomyces acidifanciences* produced glycerol in yields up to 22% of the sugar fermented in the absence of steering agents. It was demonstrated by Onishi (1960) and Spencer (1957) that many osmophilic yeasts can produce glycerol and related polyols under aerobic conditions and in the absence of steering agents. The yield of these polyols varied from 2 - 60 % of sugar utilized depending on the yeast strain employed and fermentation conditions (Spencer 1978).

India imports a substantial quantity of glycerol and the present price of this polyol is c.S 2100/tonne. Although glycerol can be produced from propylene, it is not possible for country like India to rely on imported petrochemicals or crude. Glycerol is produced in the country mainly as a by-product from manufacture of soaps and from fats and oils. In view of escalating crude prices and shortage of fat and oils on one side and availability of cheap raw materials such as molasses ($ 10- 15/tonne) or even sugarcane juice on the other side, it is imperative for a country like India to establish alternative route based on fermentation for glycerol production.

The paper reports the effect of Oxygen transfer on the kinetics of glycerol synthesis by *H. anomala*.

MATERIALS AND METHODS

Culture

Various strains of osmophilic yeasts were screened for glycerol production (Patil & Borawake 1989) and *H. anomala* NCIM-3341 was selected for further studies on the basis of higher yield, fast fermentation rate and the presence of invertase activity. The culture was maintained on MGYP- agar slants.

Inoculum preparation

The inoculum was prepared on a rotary shaker at 30°C in a medium having composition (w/v glucose 10%, yeast extract 0.5%, urea 0.1% and pH 5.5-6.0). The liquid culture volume corresponding to 0.16 % (w/v) dry cell weight was centrifuged under aseptic conditions. The residual cell mass was resuspended in the fermentation medium and used to inoculate the fermenter.

Fermenter studies

The fermentation medium composition was (w/v) glucose 30%, yeast extract 0.25%, urea 0.1%, casein hydrolysate 0.1%. $MgSO_4.7H_2O$ -

0.025%. The fermentations were carried out in a 7.5 L capacity "Chemap" laboratory fermenter and fermentation medium volume was 2.5 L. The pH of the medium after sterilization was adjusted to 6.0 with 0.25 N NaOH and the temperature maintained at 30°C. Just before inoculation the medium was aerated to bring the 0_2 saturation to 100% level. At regular intervals, samples were withdrawn aseptically for estimation of cell mass, residual sugar and polyol concentration. The effect of aeration and agitation rate were studied at 0, 0.18, 0.36, 0.54, 0.72 vvm and 500, 600 & 700 rpm respectively.

Analytical methods

Total polyols were estimated by a colorimetric method (Lambert & Neish 1950). Individual polyols were separated by TLC on silica gel with ethanol:acetone:chloroform (1:1:2) solvent system and detected with Molisch's reagent. Reducing sugars were determined with alkaline copper and arsenomolybdate reagent method (Somogyi 1945; Nelson 1944). Yeast growth was estimated after centrifugation and drying to a constant weight.

RESULTS AND DISCUSSION

The time/concentration profiles for product and substrate were found to be linear, whilst the time/cell mass concentration relation fitted to a second degree polynomial. Results are expressed in terms of specific growth rate (m), viz. rate of growth/unit cell mass; specific rate of product formation (m_p), viz. rate of product formation/ unit cell mass; and the overall yield ($Y_{p/s}$) based upon the sugar consumed. These quantities are calculated from the equations fitted for the time course of the respective concentrations.

Effect of aeration

The effect of aeration on m,m_p and $Y_{p/s}$ is shown in Fig.1 a,b and c. m. and m_p were maximum at an air flow rate of 0.36 vvm. $Y_{p/s}$ increased steeply with air flow up to 0.2 vvm, with a further slow increase from 0.2 to 0.6 vvm and a subsequent decline.

Three phases of cell growth were identified : the first involved rapid growth under 0_2 limiting conditions with little polyol formation. At lower aeration rates, near anaerobic conditions prevailed due to high 0_2 demand for growth. This phase ended after c. 18 h. The second phase was characterised by increase in the dissolved 0_2 concentration and maximum product formation with total utilization, of sugar. Generally, polyol yield increased with increased 0_2 saturation. The third phase began when all sugars were consumed with increased cell growth, presumably, due to the switch over of metabolism of the yeast from sugar to glycerol as the substrate. The fermentation process should be terminated at the beginning of this phase.

Effect of agitation

Agitation rate had little influence on m (Fig. 2a) and m_p (Fig. 2b) except in the early phase of fermentation, whilst yield was maximum at 600 rpm in the early period (10-30h), although this effect was nullified in the later phase (Fig. 2c).
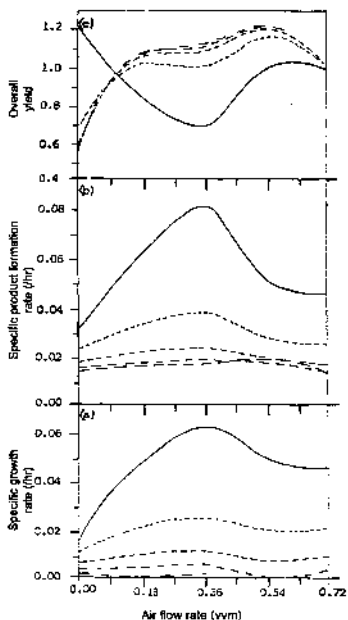
Fig. 1 *Effect of air flow rate on (a) specific growth rate, (b) specific product formation rate, and (c) overall yield at fermentation times of 10 (——), 30 (———), 60 (—·—·), 90 (·····), 120 (——) hours.*
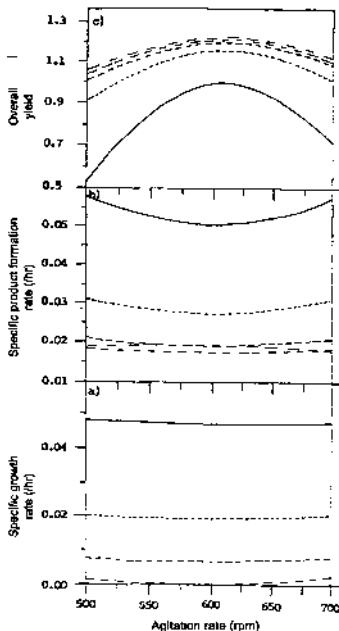
Fig. 2 *Effect of Agitation rate on (a) specific growth rate, (b) specific product formation rate, and (c) overall yield at fermentation times of 10 (——), 30 (———), 60 (—·—·), 90 (·····), 120 (——) hours.*

Estimation of K_L a and OTR

K_La and OTR were determined by the dynamic method of gassing out (Bandopadhyay & Humphrey 1967; Taguchi & Humphrey 1966) on the basis of the following equations:

$$N_A = \frac{dC_L}{dt} = K_L a (C^* - C_L) \quad (I)$$

$$C_A = C^* - \frac{1}{K_L a} (r X - \frac{dC_L}{dt}) \quad (II)$$

Where, N_A = Volumetric oxygen transfer rate (mMol/L/h)
C_L = Conc. of dissolved O_2 in fermentation broth (mMol/L)
t = Time(h)
K_L = Mass transfer coefficient (cm/h)
a = Gas liquid specific surface area (cm')
C^* = Equilibrium dissolved O_2 conc.(mM/L)
r = Specific O_2 uptake rate per unit weight of cell (mM/g/h)
X = Dry weight of cells per unit volume (g/L)

The procedure involves stopping of air supply to the fermentation broth, which results in a linear decline in the dissolved O_2 concentration due the respiration of the cells as shown in Fig. 3. This also corresponds to a conditions where K_La becomes zero. The slope of the line between "air off" and "air on" points yields an explicit value of the volumetric oxygen demand rate of the organism, rX. The air is turned on after a specified interval of time and the dissolved O_2 concentration rises accordingly. Thus, from equation (II), a plot of C_L versus $(dC_L/dt) + rX$, yields a straight line, the slope of which is equal to $-1/K_L$a and the intercept on Y-axis corresponds to C^*

Measurement for K_La were made at 90 h during the fermentation process. The "air off period was kept at 1 minute. The fermentation conditions during this period were:sugar concentration. 8 g/L, dissolved O_2 concentration, at 100% O_2 saturation 15 mg/L, air flow rate 0.54 w.m, stirrer speed 600 rpm, cell mass concentration 28 g/L. Volume and pH of broth were 3.3 L and 6.0 respectively.

The slope of line from Fig. 3 gives a value of 0.0084 mg/L/sec. which is equal to the volumetric oxygen demand rate, rX. The specific oxygen
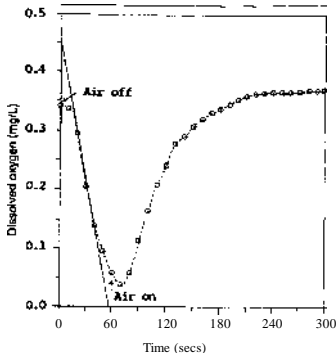
Fig. 3 *Estimation of volumetric oxygen demand rate.*

uptake rate of 0.256 g/g. h determined in the study compares well with maximum oxygen uptake rate of 0.256 g/g.h reported in the literature (Mavituna & Sinclair 1988) for *S cerevisiae*. The value of K_La obtained as per equation (II) in this study is 132/ hr as compared to the values in the range of 180 to 240 / h for *S cerevisiae* reported in the literature. The K_La obtained by the dynamic method gives maximum oxygen uptake rate of 6.6 mM/L/h.

CONCLUSION

Our investigations show that optimum aeration rate for maximum product yield was 0.54 vvm beyond which the yield declined presumably because of a "flooding effect" involving a drastic decrease in the gas liquid interfacial area available for O_2 transfer. Agitation rate had little effect on (m and m_p). Optimum product yield was obtained at 600 rpm. Total polyol concentration at the end of fermentation (103 h) was 105.4 g/L. Based on TLC analysis, the polyol mixture consisted of glycerol and arabitol in 4:1 ratio.

K_La, which is a measure of aeration capacity of the fermenter and OTR are essential features for the scale-up any fermentation process and the values obtained are comparable with those reported in the literature.

For improving the economic viability of the process, we are targeting a yield of minimum 50% and polyol concentration of 15%. Effect of the following factors need to be further investigated.

i)   Effect of initial sugar concentration and alkaline conditions on polyol yield.
ii)   Use of alternative raw materials such as molasses, sugarcane juice or starch hydrolysate.
iii)   Fed-batch/continuous fermentation process for extending polyol formation phase.
iv)   Potential commercial use of arabitol.
v)   Use of advanced separation techniques such as reverse osmosis for separation of polyols.

Preliminary calculations based on the above assumption indicate that 1 tonne of Indian molasses ($10-15/tonne) can yield 0.25 tonne of polyols (glycerol @ $ 2100/tonne).

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