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BIODETERIORATION OF HARVESTED SUGARCANE IN JAMAICA

by

RICHARD HENRY TILBURY

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UNIVERSITY OF ASTON IN BIRMINGHAM

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S U M M A R Y

The microbiological, physical and chemical changes which occur in stored, harvested sugarcane were studied in Jamaica and the United Kingdom. The degree of deterioration was proportional to time of storage, and was revealed by a statistically significant reduction in sucrose content. Other symptoms included a fall in pH, and increases in reducing sugars, dextran, viscosity, and microbial count. Cut cane was universally infected with _Leuconostoc mesenteroides_, which reached a maximum count of 10^7 to 10^8 organisms per ml. juice within 3 to 4 days of harvest. Counts of other microorganisms were generally insignificant, except for occasional lactobacilli. A new dextran-forming species was named _Lactobacillus confusus_. Microorganisms isolated from deteriorated cane were screened for their ability to cause deterioration of a sterile, synthetic cane juice. _L. mesenteroides_ strains were the most deteriogenic, but attempts to reproduce the symptoms of 'sour' cane by inoculation of this organism into cut cane were only partially successful. _L. mesenteroides_ was present in the soil and the epiphytic flora of the stalk. The principal vector of infection appeared to be the cutters' machete, especially in wet weather. Cane harvested by a chopper machine deteriorated more rapidly than hand-cut whole-stalks. Economic losses due to deterioration of harvested cane were estimated to be 9.2% of the initial recoverable sugar for the 1969 crop at Frome Estate, Jamaica. Dextran content was a useful indicator of cane biodeterioration. The dextran content of mill juices was correlated with rainfall, and significant correlations were obtained between dextran content and viscosity of mill syrups and the amount of sugar lost in final molasses; it also caused the formation of elongated crystals. Attempts to control sour cane by chemical and physical methods were unsuccessful, and it was concluded that the only solution is to mill cane within 24 hours of harvest. A novel method for removal of dextran from mill juices by enzymic treatment with dextranase was developed and patented.

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CHAPTER I INTRODUCTION

Post-harvest deterioration of the sugarcane (Saccharum officinarum) has been observed in most major cane sugar producing areas. It occurs during the storage interval between harvest and milling of the cane. Juice extracted from deteriorated stalks exhibits a reduction in sucrose content, frequently accompanied by increases in 'gum' content and acidity. The latter causes a characteristic sour odour which explains the colloquial name of 'sour cane'. The significance of this deterioration lies in its harmful economic effects; firstly, it reduces the amount of sucrose in cane prior to milling, and secondly, the 'gums' interfere with the manufacture of sugar in the factory, leading to a reduction in throughput and recovery of sugar.

Most of the research to date has been undertaken in Louisiana, Queensland, and Natal. In Louisiana, sour cane is often experienced when cane is damaged by a freeze prior to harvest, followed by a period of warmer weather. Here post-harvest deterioration is accelerated by infection with the lactic acid bacterium Leuconostoc mesenteroides (Cienkowski) van Tieghem, which forms the polysaccharide dextran from sucrose and gives rise to the so-called 'viscous fermentation' (Owen,1949). It is thought that infection is facilitated by freeze damage which causes splits in the rind of the cane stalks. In Queensland the problem has been accentuated by the recent introduction of mechanical harvesters which chop the cane stalks into short billets; this chopped-up cane deteriorates much more rapidly than manually-cut, whole-stalk cane. It was clearly shown in a series of papers by Egan (1964;1965a;1965c;1966;1967b;1968b) that this deterioration is due to infection by L.mesenteroides, facilitated by mechanical damage caused by the harvester. Research in Natal has concentrated on the chemical changes which occur in harvested cane, with special reference to polysaccharides (Bruijn, 1966a,b;1970). Microbiological aspects were not investigated, but it appears from the nature of the polysaccharide formed that L.mesenteroides is not a major cause of post-harvest deterioration in Natal. Similar results were

obtained by Nicholson & Lilienthal (1959) with regard to whole-stalk cane in Queensland.

Many other papers have been published on this topic, but in general an empirical approach was adopted, in which observations were made on the physical and chemical changes that occur during storage of cane after harvest, without attempts to elucidate the cause.

Sugar factory staff on Tate & Lyle estates in the West Indies (British Honduras, Jamaica and Trinidad) reported that 'sour' cane is a frequent occurence, especially after elongated delays between harvesting and milling, which tend to occur in the rainy season, or as a result of mechanical breakdowns, strikes, arson, etc. At such times the loss of recoverable sugar and reduction of the factory capacity was estimated to be severe, although economic assessments of the losses were not made. These observations, together with the likelihood of introduction of mechanical harvesting in the region, and a lack of published research on post-harvest deterioration of cane in the West Indies, stressed the need for a research investigation there. The work reported in this thesis attempts to meet this need.

It was concluded from the literature that two main types of deterioration occur in harvested sugarcane. In healthy cane, physical and chemical changes in juice composition are caused by enzymes naturally present within the stalk. Some of these changes are the result of the continued respiration and metabolism of the cells. Other reactions are probably stimulated by physical shock due to harvest and subsequent handling. They may also be affected by pre-harvest burning of the cane (Rizk & Normand,1969), climatic conditions before and after harvest, variety of cane, maturity of cane, soil type, etc. These autolytic changes take place during the gradual death of the plant cells. This type of deterioration will subsequently be referred to as 'autolysis' or 'staling' of cane, and is considered to be an aspect of plant physiology.

The other type of deterioration appears to be caused by infection of harvested cane with specific microorganisms, and is considered here to be a problem in biodeterioration. Hueck (1965) defined biodeterioration of materials as "any undesirable change in the properties of a material caused

by the vital activities of organisms"; a material was defined as "the substance or matter of which any thing is made or to be made (by humans)". However, it can be argued that the cells of freshly-harvested sugarcane are still alive, in which case the problem may be one of plant pathology. This view was held by Egan (1965c) who regarded the phenomenon as a disease, named 'sour storage rot', and the causative organism was described as a pathogen. The resolution of this question is irrelevant at this stage, because the fundamental principles of plant pathology and biodeterioration are almost identical (Hueck,1965); it is discussed more fully in Chapter VI. Biodeterioration of harvested sugarcane is subsequently referred to as 'sour cane'.

The emphasis of research in this thesis was on sour cane rather than stale cane, because the symptoms of the phenomenon in the West Indies appeared to indicate microbiological deterioration.

Research was initiated in October 1966. A major constraint was that initial research had to be executed at the Research Laboratories of Tate & Lyle Ltd., in England, where sugarcane was grown in a greenhouse under artifical conditions and was only available in very limited supplies. Therefore much of the work utilised sugarcane air-freighted from the West Indies. This limited the scope of the research because large quantities of cane could not be sent, and it was impossible to guarantee the freshness of the cane on arrival, despite efforts to preserve it by refrigeration. The experiments were planned with these facts in mind. Efforts were made to minimise the variability of parameters such a geographical location, climate and local practice, by the restriction of research to Frome Estate in Jamaica, where the problem of sour cane was reported to be severe. However, a few samples of cane juice from British Honduras and Mauritius were also examined. Later research was executed on location at Frome Estate during the periods February - August 1969 and February - April 1970.

Hueck (1965) advocated a systematic approach to biodeterioration, based on a comparison with pathology. This method was considered useful and is adopted here as a logical approach to post-harvest biodeterioration of sugar-

cane in the West Indies.

Descriptions of the sugar cane plant, its cultivation, harvest and the process of sugar manufacture in Jamaica are given in the Appendix, together with definitions of special terms in the sugar industry.

Symptoms

The deterioration occurs in juice contained in the parenchyma of the cane stalk. The composition of cane juice (Table I,1.) enables it to support growth of a wide range of microorganisms. This growth causes changes in juice composition which give rise to the symptoms of sour cane.

	Constituent		%	% of soluble solids
Cane	Water		73-76	
	Solids		24-27	
		Fibre (dry)	11-16	
		Soluble solids	10-16	
Juice Solids	Sugars		75-92	
		Sucrose		70-88
		Glucose)reducing sugars		2- 4
		Fructose) or 'invert'		2- 4
	Salts		3.0-7.5	
		of organic acids		1.0-3.0
		of inorganic acids		1.5-4.5
	Free organic acids		0.5-2.5	
		carboxylic acids		0.1-0.5
		amino acids		0.5-2.0
	Other organic non-sugars			
		protein		0.5-0.6
		starch		0.001-0.150
		gums		0.3-0.6
		wax,fats & phosphatides		0.05-0.15
		unidentified		3.0-5.0
juice pH 5.3-5.6				

Table I,1. Composition of sugar cane and juice solids (Meade, 1963)

The most important symptom is a loss of sucrose, which is due partly to its inversion to reducing sugars, and partly to its conversion to polysaccharides. Inversion (sucrose hydrolysis) is catalysed by acids and invertase enzymes, which are present in normal cane and some microorganisms.

In the sugar industry the sucrose content of juice is usually expressed by its pol and purity; a decrease in pol and purity is usually accompanied by an increase in reducing sugars.

The production of organic acids by microorganisms increases the juice acidity, which may be measured by a rise in titratable acidity and a drop in pH (Fort & Lauritzen,1938a). Lactic and acetic acids are the principal acids formed, which give rise to a 'sour' smell (Friloux,Cashen & Cangemi,1965).

Another important symptom is a rise in 'gum' content, which leads to increased juice viscosities and interference with the manufacturing process (Foster,1969c). It is manifested by difficulties in juice clarification, poor circulation in vacuum pans, reduced recovery of sugar, production of elongated crystals, and poor filtrability of raw sugar. The composition of the 'gums' or polysaccharides responsible for these effects has not been fully investigated. In early work, mainly based on examination of extracted juice in the mill, these gums were often found to be dextrans produced by _Leuconostoc mesenteroides_ and other lactic acid bacteria, or levans produced by _Bacillus_ species (Perquin,1940). However, recent studies showed that the polysaccharide isolated from stored, whole stalks is an α-glucoside differing from _L.mesenteroides_ dextran and not associated with growth of this organism. (Nicholson & Lilienthal,1959; Bruijn,1966b;1970). This polysaccharide was named 'sarkaran' by Bruijn (1970); it is probably a natural product of the cane. The gum formed in deteriorated frozen cane (Irvine & Friloux,1965) or chopped-up cane (Egan,1965c;1966) is almost certainly _L.mesenteroides_ dextran.

Since there was little information on post-harvest deterioration of cane in Jamaica, it was considered of prime importance to obtain basic data on the physical, chemical and microbiological changes that occur during storage of harvested cane under Jamaican conditions. It was hoped this data would indicate what type of deterioration occurs; what factors affect deterioration; what economic losses are incurred, and what, if any, microorganisms are involved. Therefore, storage experiments on freshly-harvested Jamaican cane were initiated. During 1966-1968, these experiments utilised cane air-freighted from Jamaica, but in 1969 extensive trials were performed

at Frome Estate. This work is reported in Chapter III. In addition, attempts were made to assess the effects of sour cane on the factory process.

Diagnosis

It is desirable to be able to diagnose sour or stale cane before it enters the mill, so that suitable corrective measures can be taken. In extreme cases, cane of poor processing quality should be rejected because of its harmful effects in the factory process. Therefore many attempts have been made to devise a quality control test to diagnose and estimate deterioration.

A suitable test should be rapid, simple, accurate and sensitive, and should accurately indicate either the degree of deterioration of the cane or its processing quality. The known symptoms of deteriorated cane have enabled several parameters to be considered for such a test.

Sucrose and reducing sugar content are of little use unless their original values in the corresponding fresh cane are known, but this is seldom the case. Furthermore, the variability of these parameters in fresh cane is too great to permit comparisons with average 'fresh' analyses.

Early workers in Louisiana favoured the use of titratable acidity or 'excess acidity' for freeze-damaged cane (Fort & Lauritzen, 1938a), but recently Irvine & Friloux (1965) found that gum content was a more sensitive parameter. This has been confirmed by Egan (1966) in Queensland and Bruijn (1966a) in Natal. However, the techniques used for determination of gums were not sufficiently rapid for quality control purposes.

Most recently Keniry, Lee & Davis (1967a,b) showed that 'dextran' content was a better index of processing quality than either gum or lactic acid content for chopped cane in Queensland. The technique was based on turbidimetric measurement of the 'haze' formed by addition of 50% ethanol to starch-free, protein-free, cane juice. The advantages of this method were its rapidity, simplicity and accuracy, and it was claimed that 'dextran' was not present in freshly-harvested cane. Evidence to the contrary was subsequently published by Foster (1969a), but since the method of Keniry et al (1967a) is not specific for dextran, Foster's results may be explained by

the presence of cane polysaccharides different from L.mesenteroides dextran. The choice of a standard dextran to calibrate the haze analysis technique is difficult because the dextrans produced by L.mesenteroides vary considerably in physical properties between strains (Jeanes et al,1954). Despite these disadvantages it was felt that determination of 'dextran' by haze analysis warranted extensive study in Jamaica.

It was also considered that the viscosity of cane juice may be a suitable indicator of ezcessive polysaccharide formation. It can be determined rapidly and easily, and calculation of specific viscosity eliminates the effect of the inherent viscosity of the fresh juice.

Other parameters, such as volatile acids, alcohols, non-volatile organic acids, amino acids (Bruijn,1966a) and pH (Egan,1966) were reported to be unsuitable.

Diagnosis of microbiological deterioration should ideally include microbiological tests, but most available methods were unsuitable for quality control purposes because they require a long incubation time. Direct microscopic examination does not differentiate between live and dead cells or between biodeteriogens and harmless organisms. However, rapid methods such as the methylene blue reduction test are widely used to assess the bacteriological quality of milk and justified their evaluation for estimation of sour cane.

Etiology

The criteria for establishment of an organism as the causative agent of a disease were first defined in the three classical postulates of Koch (1882), and applied to biodeterioration by Hueck (1965). These principles are adopted here.

Although little work has been published on the microbiology of harvested cane, the microflora of extracted cane juice in the mill was studied by several authors. Since a high proportion of organisms present in mill juice are derived from cane, some qualified conclusions may be drawn from this work in regard to the etiology of sour cane.

Early work was reviewed by Thaysen & Galloway (1930), Perquin (1940), Hucker & Pederson (1942) and Owen (1949). The organism most often associated with gum and slime formation in mills was Leuconostoc mesenteroides, but in some cases the causative organisms were lactobacilli or Bacillus species. Ln Louisiana, McCalip & Hall (1938) isolated L.mesenteroides in almost pure culture from frost-damaged cane, whilst later studies by McCleskey, Faville & Barnett (1947) showed that this organism was the principal cause of gum formation in cane juice. However, Mayeux (1960) and Duncan & Colmer (1964) suggested that coliform bacteria (Aerobacter species) may also be important. In Australia the work of Egan (1964;1965a;bc;1966;1967a,b;1968a,b) and Egan & Rehbein (1963) conclusively demonstrated that L.mesenteroides is the principal cause of the rapid deterioration of mechanically harvested, chopped cane, although in whole-stalk cane Nicholson & Lilienthal (1959) showed that this organism was not associated with the formation of a new, non-dextran polysaccharide.

It was concluded from the circumstantial evidence in the literature that L.mesenteroides might be a principal biodeteriogen of whole-stalk cane, but research was needed to test this hypothesis in accordance with Koch's postulates. The role of other potential deteriogens such as lactobacilli, yeasts and Bacillus species also required investigation.

Research on the etiology of sour cane in Jamaica had three main objectives. Firstly, the effect of storage time on quantitative and qualitative changes in the microbial flora of harvested cane was examined by means of various selective media (Chapter III). Secondly, pure cultures of predominant types of organism were selected from these media and identified to specific level (Chapter IV). It was hoped these results would enable valid conclusions to be made with respect to Koch's first and second postulates. Finally, it was necessary to distinguish between deteriogenic and non-deteriogenic organisms and satisfy the conditions in Koch's third postulate. Since most of this work had to be executed in the U.K., where cane was in short supply, an 'in vitro' screening test was devised in which the 'deteriogenic power' of selected isolates was tested by their inoculation

into a 'synthetic cane juice'. A few isolates were subsequently inoculated into cane stalks (Chapter V).

Biodeteriogensis

According to Hueck (1965), the development of biodeterioration phenomena may be divided into the phases of infection, incubation and manifestation.

Infection requires suitable environmental factors and the presence of a suitable infection mechanism. Both these items are governed by factors which vary according to local practice and geographical location. Assuming that healthy, standing cane does not contain microorganisms inside the stalk, and that potentially deteriogenic organisms are in contact with the exterior of the plant, it is likely that initial infection occurs through mechanical damage to the stalk. In warm, tropical climates the principal entry mechanism is probably through the cut ends at harvest. The only study of this is the work of Egan (1964;1965s;1965c) on the infection of mechanically-harvested chopped cane with L.mesenteroides in Queensland. This organism was not detected inside standing cane but it was found in small numbers inside the cut ends within 10 minutes of harvest. Chopped cane was infected more heavily and more frequently than whole-stalk cane; this was explained by the greater degree of mechanical damage and increased number of potential points of infection in the former. In colder climates, such as Louisiana and Argentina, it appears that entry of L.mesenteroides into stalks is facilitated by splits in the stalk caused by frost damage (McCalip & Hall,1938; Cross, 1966). In Jamaica temperatures do not fall below 66°F, so this entry mechanism is not applicable, but the infection of freshly-cut ends with L.mesenteroides was examined for a few whole-stalks and mechanically-harvested billets (Chapter VI).

In many countries, including Jamaica, cane is burnt prior to harvest to remove unwanted foilage. It is widely held that burnt cane deteriorates more rapidly than green cane, partly because in some varieties burning causes splits in the stalk which provide potential entry mechanisms for organisms (Stephenson & Doolan,1947; Owen,1949; Balch,Broeg & Lautitzen,1950), but in

Queensland, Waddell (1952,1954) obtained the opposite result. It is likely that the effects of burning are greatly influenced by rainfall; burning also influences the autolytic changes in harvested cane (Rizk & Normand,1969).

Since the practice of burning cane in Jamaica is well-established and likely to remain so for economic and social reasons, only a few comparisons were made between the deterioration of green and burnt harvested cane (Chapter III). However, attempts were made to assess the 'sterilising' effect of burning on the content of L.mesenteroides in the epiphytic flora of standing cane.

Damage of cane by insects or disease may also facilitate infection (McCaig & Fort,1936; Mayeux,1960). In Jamaica the cane borer insect (Diatrea saccharalis) is prevalent, so a comparison was made between the L.mesenteroides count of juice from healthy and borer-damaged cane (Chapter VI).

The mechanism by which L.mesenteroides penetrates the stalk after infection and colonises the parenchyma, with resultant changes in juice composition, is not known. Egan (1965a) found that penetration and growth of this organism in chopped cane was very rapid, and postulated that its initial spread throughout the plant occured by passive transport in the vascular bundles. In whole stalks, the rate of penetration and growth of the organism was slower (Nicholson & Lilienthal,1959). There is no evidence whether L.mesenteroides is a plant pathogen and kills living cells or whether it is merely saprophytic. Time did not permit an investigation of these mechanisms here, but the rate of penetration and growth of L.mesenteroides in cane was determined (Chapter VI).

The length of the incubation period, before symptoms of sour cane become evident, depends on the degree of initial infection (or mechanical damage) and environmental conditions. As expected, the degree of deterioration of harvested cane is directly proportional to its time of storage. Most workers agree that whole stalks should be milled within 24 to 48 hours of harvest to prevent significant sucrose loss (Scott,1926; Haldane,1933; Beata Neves,1935; Subbaiya,1938; Rosenfeld,1941; Wold,1946; Stephenson & Doolan,1947). With

chopped-up cane, however, significant losses occur within 14 to 24 hours, depending on climatic conditions (Keniry et al,1967b; Kirby,1968; Vickers,1968).

The effect of climatic conditions on deterioration of harvested cane is difficult to assess because of the inability to control this parameter in large-scale experiments. It is probable that the rate of deterioration is proportional to storage temperature (Deerr,1921; Lauritzen & Balch,1935), but the effect of rainfall and humidity is uncertain. Autolytic changes in cane are probably enhanced by the death of plant cells due to loss of turgor in hot, dry weather, and retarded when the cane remains moist. However, moist climatic conditions probably increase the degree of infection and accelerate the growth rate of microorganisms. Some observations on these factors are reported in Chapters III, VI and VII.

The rate of deterioration of harvested cane in Jamaica wasinvestigated in a series of storage experiments over a five month period in 1969 (Chapter III). At this time all cane in Jamaica was manually cut, but in 1970 the first mechanical chopper-harvester was introduced for trials at Frome Estate. Advantage was taken of this to compare the deterioration rates of manually-cut, whole-stalk cane and mechanically-harvested, chopped-up cane.

It is known that there are varietal differences in the rate of post-harvest deterioration of sugarcane. Haldane (1933) postulated that these differences are due to variation in resistance to damage or moisture loss, but nothing is known of the resistance of varieties to infection with L.mesenteroides. This was not investigated in this thesis, since most of the storage experiments utilised variety B4362 which comprised 73% of the cane reaped in Jamaica in 1969. Similarly, although the age and maturity of cane is thought to influence its rate of deterioration after harvest, the experiments were standardised by using mature cane approximately 12-15 months old.

Nothing is known of the effect of strain, inoculum size and physiological state of the organism on infection of harvested cane and its subsequent deterioration. These parameters were not studied here.

Ecology

Some evidence shows that L.mesenteroides is present in the epiphytic flora of cane and in the soil of canefields (Mayeux,1960; Mayeux & Colmer, 1961; Egan,1965a); it is also found frequently in raw sugar factories (Owen, 1949). No studies have been reported of vectors of transmission of this organism from its primary sources of infection to the cut ends of harvested cane, although Egan (1965a) isolated it from the juice-soaked mud of a chopper-harvester.

Since L.mesenteroides has not been detected inside standing cane stalks, it is postulated that a principal means of infection of harvested whole-stalk cane is the transmission of this organism from the soil and exterior of the stalk to the cut ends by means of the cutting instrument, or machete. Attempts were made to verify this hypothesis by examination of soil, the exterior of the stalk, and machetes for L.mesenteroides (Chapter VI). It is considered that direct contact between the cut ends of cane and the ground may also be a significant method of infection. In addition, some attempts were made to assess the role of insects and airborne dust as vectors of L.mesenteroides. The possible enrichment of soil with this organism by application of factory filter-cake mud as a fertiliser was examined.

The reported occurrence of sour cane in many different parts of the world indicates the wide geographical distribution of deteriogens such as L.mesenteroides.

Control

The simplest method of control of sour cane is to mill the cane before economic losses become significant, but this is often impossible due to unforeseen and uncontrollable circumstances. Research on deterioration of mechanically-harvested chopped-up cane in Queensland showed that an improvement in the logistics of cane cutting and transport was the only practical solution to the problem (Allen,1967; Egan,1968b;1969; Foster,1969b). Therefore attempts were made to assess the efficiency of harvest and transport systems in Jamaica by means of time studies (Chapter VII).

An alternative solution may be the prevention of infection or inhibition of growth of the deteriogen after infection by application of bacteriostatic or bactericidal chemicals. These approaches were tried by Egan (1965b,1968a), who dipped or soaked the cut ends of freshly-harvested cane in various bactericides, and sprayed the chopper-harvester blades and cut cane. This proved ineffective, due to failure of the chemicals to penetrate the stalk. Fumigation of cut cane billets in bins with formaldehyde achieved some success but was found to be uneconomic; this approach could not be considered for whole-stalk cane in Jamaica.

In the present research, many bactericides were screened in the laboratory for their ability to inhibit L.mesenteroides and to penetrate cane stalks (Chapter VIII).

Inhibition of Leuconostoc growth by modification of the physical environment does not appear practical. However, the possible application of molasses to reduce water activity in the cut ends of the stalk was investigated (Chapter VIII).

Prophylaxis of some cane diseases by the breeding of resistant varieties is widely practised (Martin, Abbot & Hughes,1961), but this approach was considered to be beyond the scope of the present investigation. Since it is not known whether L.mesenteroides is a plant pathogen or a saprophyte, it is uncertain whether this method would be applicable to sour cane.

One practical approach to help minimise sour cane may be the application of a penalty system to cane growers, based on the results of a quality control test at the factory which estimates the degree of deterioration of incoming cane. The difficulty here is to find an acceptable quality control test; attempts to solve this problem are discussed in Chapters III and VIII.

It was apparent that the chances of finding a satisfactory method for prevention of sour cane were slight. However, the harmful economic effects of sour cane include not only loss of sucrose prior to milling, but interference by polysaccharides such as dextran with subsequent factory performance. A novel method of removing harmful dextran from cane juice in the mill prior to processing, by treatment with the enzyme, dextranase, was

investigated (Chapter VIII).

Economic losses

Post-harvest deterioration of cane results in two main sources of economic loss. Firstly, both the weight of cane and its sucrose content are reduced prior to milling, which causes a drop in the yield of tons of sugar per acre. Since the grower is paid on the basis of recoverable sugar in the actual tonnage of cane delivered at the factory, this represents a financial loss to the grower. Secondly, deteriorated cane adversely affects factory performance in many ways, notably, poor juice clarification and the requirement of extra lime to neutralise acidity; increased viscosities of process materials, which retard boiling and crystallisation and hence reduce the effective capacity of the factory; production of elongated crystals which are not acceptable in many sugar refineries, and which cause difficulties in purging; a reduction in exhaustability of molasses and recovery of sugar, with an accompanying increase in the amount of sucrose in molasses, and the production of raw sugar of poor filtrability which does not handle well in the refinery.

Despite a wealth of papers from many countries in which the rate of loss of sucrose in harvested cane is measured on a experimental scale, no assessments of actual economic losses appear to have been made. Such an assessment was considered to be of prime importance in the present study, because this information is essential to the rational development of control measures. Economic losses of sucrose in the field were assessed for the 1969 crop at Frome Estate. Mean rates of loss of recoverable sugar in stored, cut cane were determined for the principal variety over a 5-month period. The distribution of age of cane post-harvest at the mill was determined by time studies for the same period, and this data was used to estimate the actual loss of recoverable sugar in cane (Chapter VII).

In recent years the increased incidence of sour cane due to mechanical harvesting in Queensland has stimulated research on the effects of dextran on processing (Sutherland,1960a,b; Keniry et al,1967a; Sutherland,1968; Sutherland & Paton,1969; Leonard & Richards,1969). Again, although some of

these effects were studied quantitatively, no attempts were made to estimate the economic loss incurred.

In practice, the economic effects of sour cane on the factory process are extremely difficult to assess, owing to the continuous nature of the process. However, some attempts were made to estimate these losses at Frome in 1969 and part of 1970. Daily determinations of the dextran content of mill juice were made, and for limited periods the crystal elongation of c-massecuites was examined (Chapter VII).

CHAPTER II STANDARD METHODS AND MATERIALS

A. MICROBIOLOGICAL METHODS

1. Enumeration of microorganisms in cane juice

Viable counts of organisms were determined by the pour plate technique. Tenfold serial dilutions of neat cane juice were made in sterile $\frac{1}{4}$-strength Ringer solution (Oxoid). 1 ml. aliquots of three selected dilutions were pipetted into petri dishes and mixed with molten medium at 45-50°C. Plates were incubated for the selected time and temperature, and the colonies counted. One plate at each dilution was used for routine work, but plates were duplicated when greater accuracy was required. Selective media were used to isolate and enumerate selected groups of microorganisms.

2. Total spoilage organisms

A medium was sought which would permit the growth of all microorganisms capable of growing in normal, fresh cane juice. Alford & McCleskey (1942) compared four media for isolation of bacteria from cane juice. A medium containing sucrose 10%, trytone 1%, and yeast extract 0.1% (sucrose tryptone agar) gave consistently higher counts than either nutrient agar or raw juice tryptone agar (1% tryptone in sterile cane juice). Further work by Faville (1947) and McCleskey, Faville & Barnett (1947) utilised raw sugar agar (tryptone 1%, yeast extract 0.5%, raw sugar 10%, pH 6.7) for isolation of *Leuconostoc mesenteroides* from cane juice.

The following medium was selected for isolation of total spoilage organisms in cane juice. Its composition was intended to be a simple, nutritionally adequate, reproduceable imitation of fresh, natural cane juice. It was named sucrose tryptone agar.

Sucrose Tryptone Agar (STA)

Sucrose	100g.
Glucose	10g.
'Difco' Tryptone	10g.
'Difco' Yeast Extract	5g.
'Oxoid' Ionagar No.2	20g.
Distilled water	1L
pH 5.5	

It was sterilised in the autoclave at 15 p.s.i. for 10 minutes. Colonies were counted after aerobic incubation at 30°C for 2-3 days. The medium was clear and permitted the easy enumeration of colonies. Organisms which produced slime from sucrose were recognised by their mucoid colonial appearance. It supported the growth of lactic acid bacteria, *Bacillus* spp., Gram negative rod-shaped bacteria (including coliforms), yeasts, moulds and actinomycetes.

3. Lactic acid bacteria

The closely related genera *Leuconostoc* and *Lactobacillus* were the most common organisms in cane juice. These organisms have similar growth requirements and grow in the same selective media.

Selective media for lactobacilli were reviewed by Sharpe (1960), who recommended that of Rogosa, Mitchell & Wiseman (1951) for isolation of lactobacilli from human, animal and dairy sources.

a) U.K. Experiments

'Oxoid' Rogosa Agar (Modified, pH 5.4), or 'Difco' Rogosa SL broth plus agar (1.5%) was used. Initially the plates were incubated for 5 days at 30°C in an atmosphere of 88% N_2 / 12% CO_2, but later it was found that aerobic incubation for 3 days at 30°C was satisfactory. Yeasts were inhibited by incorporation of Mycostatin 50 units/ml. (E.R.Squibb & Sons Ltd.,Moreton, Cheshire) in the medium. This inhibited growth of a wide range of yeast species without effect on the lactic acid bacteria. Counts of *Leuconostoc* species on this medium were similar to those obtained on STA.

b) Jamaica Experiments

Rogosa agar was not considered suitable for research work in Jamaica because of its high cost, complexity, and non-availability. Therefore a more simple, economic and easily prepared alternative was sought. Two chemicals were considered for incorporation in STA to make it selective for lactic acid bacteria. Thallous acetate 0.1% was recommended by Sharpe (1955) for selection of lactobacilli, whilst sodium azide 0.005-0.3% was used by Mayeux & Colmer (1961) for selection of *Leuconostoc*. A preliminary experiment

showed that 0.005% sodium azide in STA inhibited growth of Bacillus species and Gram-negative rods, but did not inhibit several yeast species. Increases in azide concentration up to 0.03% progressively inhibited both yeasts and lactic acid bacteria.

A comparison was made between three media for their ability to support growth of 87 isolates of lactic acid bacteria (Leuconostoc mesenteroides and Lactobacillus species), 5 yeast species, 7 Bacillus species and 18 isolates of Gram-negative aciduric rods. The media were :-

A. STA + Seitz filtered sodium azide (BDH Ltd) 0.005% concn.

B. STA + Seitz filtered thallous acetate (BDH Ltd) 0.1% final concn.

C. 'Difco' Rogosa SL broth + agar, 1.5%

Medium B permitted more rapid growth of lactic acid bacteria than 'A' or 'C'. Media A and B inhibited growth of all non-lactic organisms, but 'C' allowed growth of 3 yeast strains and 2 Gram-negative rods. All organisms grew well on plain STA which was used as a control. It was concluded that Medium B was the most satisfactory selective medium for lactic acid bacteria, since it permitted good growth of nearly all these organisms after two days incubation, whilst all other organisms were inhibited.

Therefore the medium chosen for selection of lactic acid bacteria from cane juice in Jamaica was STA plus thallous acetate 0.1%, denoted STATA. The thallous acetate was autoclaved in the medium without loss of effect. Plates were counted after 2-3 days aerobic incubation at 30°C.

c) Differentiation between Leuconostoc mesenteroides and Lactobacillus species.

Mundt & Hammer (1966) reported that 7% ethanol inhibits growth of Leuconostoc mesenteroides but permits growth of Lactobacillus species. 39 strains of L.mesenteroides and 33 Lactobacillus species isolated from cane juice were screened for their ability to grow in 'Difco' Rogosa SL broth containing 7% ethanol. Only 3 strains of L.mesenteroides grew compared with 24 isolates of lactobacilli. This technique was used for rapid differentiation of colonies of Leuconostoc and Lactobacillus, which are of similar appearance on Rogosa agar.

4. Catalase-positive, Gram-negative, aciduric bacteria

The selective medium adopted for isolation of these organisms was recommended by Carr (1966) for acetic acid bacteria :-

Glucose Yeast Extract Agar (GYE)

Glucose	20g.
'Difco' Yeast Extract	10g.
'Oxoid' Ionagar No.2	30g.
Distilled water	1L
pH 4.8	

It was sterilised in the autoclave at 10 p.s.i. for 10 minutes.

Non-aciduric organisms were inhibited by the low pH, whilst lactic acid bacteria were inhibited by Actinomycin, 2PPM, (Calbiochem, Los Angeles, California) (Beech & Carr, 1960), and yeasts were inhibited by Mycostatin, 50 units/ml. The antibiotics were added to the molten, sterile medium before use. The plates were incubated aerobically for 2-3 days at 30°C. In practice it was found that all organisms isolated on this medium were catalase-positive, Gram-negative, aciduric, rod-shaped bacteria.

5. Yeasts and Moulds

a) Mesophilic yeasts

These were isolated on Wort Agar (Oxoid) containing 500 PPM Aureomycin (Lederle Laboratories Division, Cyanamid of Gt.Britain, London) to inhibit bacteria (Beech & Carr,1955). Moulds were inhibited by the evaporation of 0.1 ml. saturated alcoholic diphenyl on the lid of the petri dish prior to incubation (Ingram,1958). The plates were incubated aerobically for 3 days at 30°C.

b) Osmophilic yeasts

A modification of the osmophilic agar medium of Scarr (1959) was used :-

Difco Wort Agar, 50.6g. was dissolved in 1 L of a syrup which contained sucrose 35%, and glucose 5%, in distilled water. The pH was 5.0-5.5. The medium was sterilised in the autoclave at 10 p.s.i. for 15 minutes. Plates were incubated aerobically for 3-5 days at 30°C.

c) Moulds

Wort agar (Oxoid) containing 500 PPM Aureomycin was used. Plates were examined after 2, 3 and 5 days aerobic incubation at 30°C.

6. Bacillus species

The sample dilutions were heated for 20 minutes at 80°C to kill vegetative cells. 1 ml. aliquots were then plated on nutrient agar (Oxoid) and the plates were incubated aerobically for 5 days at 30°C. This method gives a count of bacterial spores of the genus *Bacillus*.

7. Coliform bacteria

Maconkey Agar No.3 (Oxoid) was used. Yeasts were inhibited by the addition of 50 units/ml. Mycostatin. Plates were counted after aerobic incubation for 24 hours at 37°C.

8. Growth and maintenance media

After isolation on selective media, organisms chosen for further study were grown and maintained on media shown in Table II,1.

Group of Micro-organism	Growth Media	Maintenance Medium
genus *Leuconostoc*	Yeast extract glucose citrate (YGC) broth or agar (Garvie,1960)	Yeast extract glucose skim milk (Garvie, 1960)
genus *Lactobacillus*	MRS broth or agar (de Man, Rogosa & Sharpe, 1960)	Yeast extract glucose skim milk (Garvie, 1960)
Catalase-positive, Gram-negative bacteria	GYE broth or agar pH 7.0 (Carr,1966)	GYE agar slopes, pH 7.0 (Carr,1966)
Mesophilic yeasts	Malt extract broth and Sabouraud Dextrose agar (Oxoid)	Wort agar slopes (Oxoid)
Osmophilic yeasts	Osmophilic agar (modified after Scarr, 1959)	Osmophilic MYGP broth (Brewing Industry Research Foundation, Nutfield, Surrey)
Coliform bacteria	Maconkey Agar No.3 (Oxoid)	Nutrient agar slopes (oxoid)
genus *Bacillus*	Nutrient broth or agar (Oxoid)	Nutrient agar slopes (Oxoid)

Table II,1. Growth and maintenance media for microorganisms isolated from cane juice.

Maintenance cultures were stored at 4°C.

Some strains of leuconostocs and lactobacilli were preserved by freeze-drying, using a modification of the method of Rose (1970).

9. Microscopic examination

Smears of pure cultures were stained by Gram (Hucker modification).

10. Catalase test

Plates were flooded with hydrogen peroxide 10 volumes %. Evolution of gas bubbles denoted the presence of catalase.

11. Preservation of cane juice samples

All samples of cane juice were examined within one hour of extraction, where possible. When this was impossible, juice for microbiological examination was stored at 4°C in a refrigerator and analysed within 24 hours. Control tests showed that the microbial flora of chilled juice remained unchanged for this period, but after 2 days storage the count increased slightly. Freezing of juice was unsatisfactory as it reduced the count by 90%.

Juice for chemical analysis was preserved for periods up to 24 hours by the addition of 0.1 ml. saturated alcoholic mercuric chloride per 100 ml. juice. Juice was frozen when the delay prior to analysis exceeded one day.

B. PHYSICAL AND CHEMICAL METHODS

General methods of sugar analysis were selected from the recommendations of the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) (de Whalley,1964), and the Manual of Methods of the Jamaica Association of Sugar Technologists (JAST) (Innes,1965).

Definitions of terms are given in the Appendix.

1. Brix

Refractive Brix was determined in an Abbé refractometer.

2. Polarisation

Direct polarisation was determined in a polarimeter by the official ICUMSA method (de Whalley,1964).

3. pH

Electrometric determinations of pH were made by a pH meter.

4. Reducing sugars (R.S.)

a) United Kingdom

The R.S. content of cane juice was determined by the Luff-Schoorl volumetric technique (Runeckles,1961).

b) Jamaica

The R.S. content of cane juice was determined by the official ICUMSA Lane and Eynon volumetric technique (de Whalley,1964).

c) Screening of microorganisms for deteriogenic power

Many microorganisms were screened for their ability to cause deterioration of a synthetic cane juice (Chapter V), including the formation of reducing sugars from sucrose. A method for determination of R.S. was sought which would enable rapid analysis of large numbers of samples. The required range of measurement of R.S. was 0 - 0.1% with an accuracy of ± 0.005% R.S. The methods of Luff-Schoorl and Lane and Eynon were too time-consuming for this purpose. The selected method was a modification of the EDTA titrimetric technique of Knight & Allen (1960) :-

Synthetic cane juice was diluted 1:100 with distilled water to give R.S. contents between 0 and 0.1%; the original R.S. content of the uninoculated juice was 1.8%, and the sucrose content 16.5%. 1 ml. aliquots were pipetted into 6 x $\frac{3}{4}$ inch test tubes, diluted with 5 ml. distilled water and mixed with 2 ml. copper reagent. The tubes were immersed in a boiling water bath for 5 minutes, cooled, and the contents titrated against 5mM EDTA using murexide indicator. Standard solutions were prepared with Analar glucose and invert-free sucrose to give R.S. contents of 0 to 0.1% at a constant total sugar concentration of 0.18%. There was a linear relationship between % R.S. and EDTA titre over this range, and the method was reproducable.

5. Sucrose

The official ICUMSA Clerget method was generally used (de Whalley,1964). This is a double polarisation technique, before and after enzymic inversion of sucrose with invertase.

Some determinations were made by the ICUMSA chemical method (de Whalley, 1964), in which R.S. are determined by the Lane & Eynon technique before and after enzymic inversion.

The sucrose and R.S. content of cane juice from experiments on stored, harvested cane at the Research Centre in 1968 were determined on a Technicon Auto-Analyser, by a technique using sodium borohydride and Prussian blue (Runeckles & Dann, 1968).

6. Gum

'Gums' in cane juice are defined as soluble, alcohol-precipitable polysaccharides of high molecular weight. This includes pectins, hemicelluloses, dextrins, dextrans and levans, but excludes starch which is in the insoluble, granular form in unheated cane juice.

Two methods of determination were used in initial experiments :-

a) The Louisiana Method (Roberts & Friloux, 1965)

Starch was first removed from the juice by centrifugation. 10 ml. supernatant was treated with 30 ml. ethanol and the precipitated gum was removed by centrifugation, washed in 80% ethanol, and re-dissolved in distilled water. The total sugar content of the solution was determined colorimetrically (490 nm) by the phenol-sulphuric acid method, with glucose as standard. Results were expressed as gum % Brix.

b) The Natal, or Sugar Milling Research Institute (S.M.R.I.) Method (Bruijn, 1966a)

Starch was first removed from the juice by filtration through Whatman No.40 filter paper. 15 ml. juice was treated with 100 ml. acidified ethanol (150 ml. 95% ethanol, 15 ml. conc. HCl, 15 ml. water) and the solution allowed to stand overnight. The precipitate was filtered through a Gooch crucible, dried, weighed, and the weight corrected for included ash after ignition. Results were expressed as gum % Brix.

This method was rapid and gave good agreement with pure dextran standards.

7. Dextran

Dextrans are defined as homologous polymers of D-glucopyranose with predominantly α-(1-6) linkages. The high molecular weight of native dextran renders them insoluble in 50% ethanol. This is the basis of the 'haze analysis' technique for estimation of dextran content of cane juice, used by Keniry, Lee & Davis (1967a,b), based on earlier work by Nicholson & Horsley (1959). However, the method is not specific for dextran, since it determines all the polysaccharide material precipitated from a starch-free and protein-free solution by 50% ethanol. Despite this disadvantage, the method was adopted because it is rapid, accurate, and suitable for routine determinations of 'dextran' in the cane factory.

a) Raw cane juice

50 ml. juice was treated with 10 ml. trichloracetic acid, 10% w/v, to precipitate protein. 2 g. diatomaceous filter aid was added and the mixture was filtered under vacuum through Whatman No.5 filter paper to remove starch and protein. The filtrate was divided into two 10 ml. aliquots. One aliquot, the 'blank', was mixed with 10 ml. distilled water; to the other was added 10 ml. ethanol. After 15 minutes, the optical density of the 'haze' in the test solution was determined colorimetrically at 720 mμ against the corresponding water 'blank'.

The dextran concentration was determined from a standard curve prepared with a pure dextran at concentrations from 0 to 0.4% w/v in a 20° brix sucrose solution. Initially the standard dextran was prepared from a growing culture of Leuconostoc mesenteroides strain L20, (isolated from sour cane), by the method of Jeanes (1965). Later, a commercially available, high molecular weight dextran was used (food grade dextran), MW 5-40 million, catalogue no.1527d; Koch-Light Laboratories Ltd., Colnbrook, Slough, England). The standard curve obtained with these two dextrans was almost identical.

Dextran content was expressed as dextran % brix.

A later paper by Keniry, Lee & Mahoney (1969) recommended several improvements to their earlier method, including an extension of the incubation time to 20 minutes. This was adopted in work during 1970.

b) Process materials

The method of Keniry et al (1969) was used for syrups, massecuites and molasses.

The process material was diluted with water to 40° brix. 50 ml. was treated with a mixture of ion-exchange resins, consisting of equal parts by weight of Amberlite IR-120 (H) and Amberlite IR-45 (OH), (B.D.H. Ltd.), at the following rates :- syrup, 1g; massecuite, 5g; molasses, 10g. 0.5ml. fungal α-amylase (Novo II, Globe Products Ltd., Accrington, England) solution was added. The enzyme was prepared as a 0.4 % w/v stock solution in acetate buffer, pH 5.5 and stored at 4°C. The mixture was incubated in a water bath at 40°C for one hour with occasional shaking, to remove soluble starch and inorganic salts. It was then decanted through a 100-mesh gauze and made up to 100 ml. with distilled water, including washings. 10 ml. trichloracetic acid, 10 % w/v, was added, and the solution was filtered under vacuum through Whatman No.5 filter paper with filter aid. The dextran content of the filtrate was determined as in (a).

8. Viscosity

The viscosity of cane juice and synthetic cane juice was determined by the capillary tube method in a Ostwald viscometer, according to the technique described by Findlay (1960).

Simple Ostwald-type viscometers were used, with a viscosity range of 2 to 20 centipoises (cP) (Baird & Tatlock Ltd., Chadwell Heath, Essex, England). They were calibrated with distilled water and checked against an NPL calibrated viscometer using a standard 20% w/v sucrose solution.

The viscometers were immersed in a water bath maintained at 25°C $\pm$ 0.1°C (U.K. experiments) or 30°C $\pm$ 0.1°C (Jamaica experiments). All cane juices were filtered free of particulate matter prior to test. Juices were equilibrated in the water bath for 15 minutes, then the time of outflow, 't' seconds, was measured by a stopwatch to the nearest 0.1 second; the mean of three determinations was calculated.

The absolute viscosity η of juice was calculated from the equation :-

$$\eta = k \rho t$$

where η = absolute viscosity in cP

k = viscometer constant

ρ = density of juice at 25°C, determined from standard tables of density of sucrose solutions at various values of brix (Table 22, Meade, 1963).

and t = mean time of outflow in seconds

The specific viscosity η spec. of cane juice was calculated from the equation :-

$$\eta_{spec.} = \frac{(\eta - \eta_o)}{\eta_o}$$

where η = absolute viscosity of juice in cP

η_o = absolute viscosity of a pure sucrose solution at the same brix and temperature as the test solution, taken from standard tables.

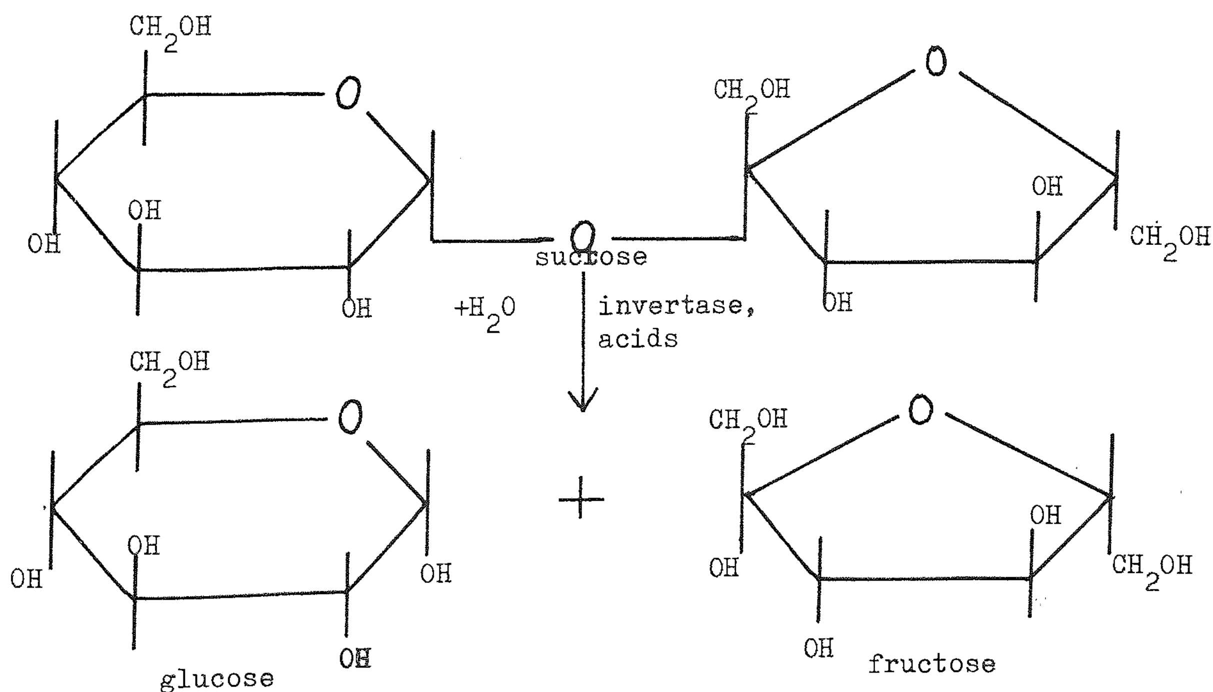
CHAPTER III THE MICROBIOLOGICAL, PHYSICAL AND CHEMICAL CHANGES WHICH OCCUR DURING STORAGE OF HARVESTED SUGARCANE.

A. INTRODUCTION

1. Symptoms of deteriorated cane

The literature contains many publications from most cane-growing countries on the physical and chemical changes which occur in harvested sugarcane. Some general conclusions may be drawn from this work which appear to be universally valid despite the wide range of variation in parameters such as variety of cane, climatic conditions, harvest practice, etc.

The most important fact is that the amount of recoverable sugar in harvested cane (='yield') decreases with time of storage. This is due to a combination of two main factors. Firstly, sucrose is lost by its conversion to other compounds, principally reducing sugars and polysaccharides. These changes are mainly due to invertases and other enzymes found naturally within the stalk; and also to enzymes produced by invading microorganisms. Invertases catalyse the hydrolysis of sucrose to glucose and fructose, which may be utilised as energy sources in respiration and growth of the plant cells. This reaction ('inversion') is also catalysed by acids and proceeds at a rate directly proportional to the acidity.



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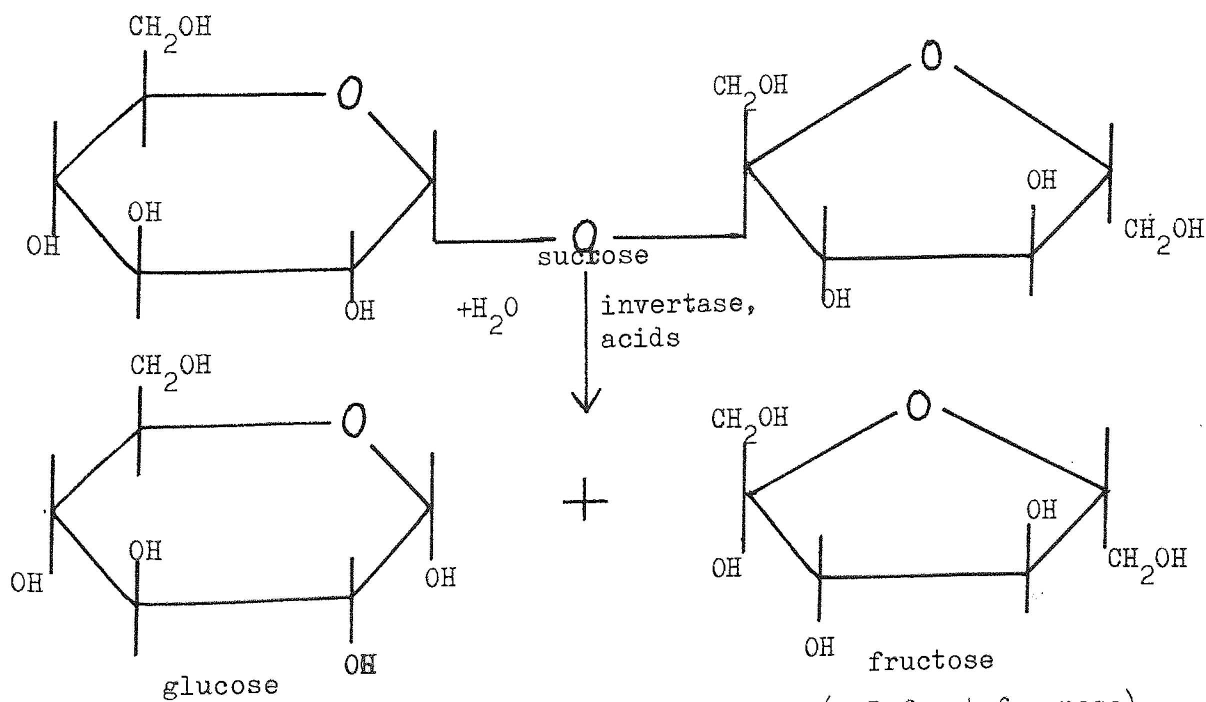
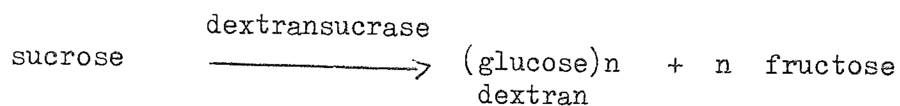
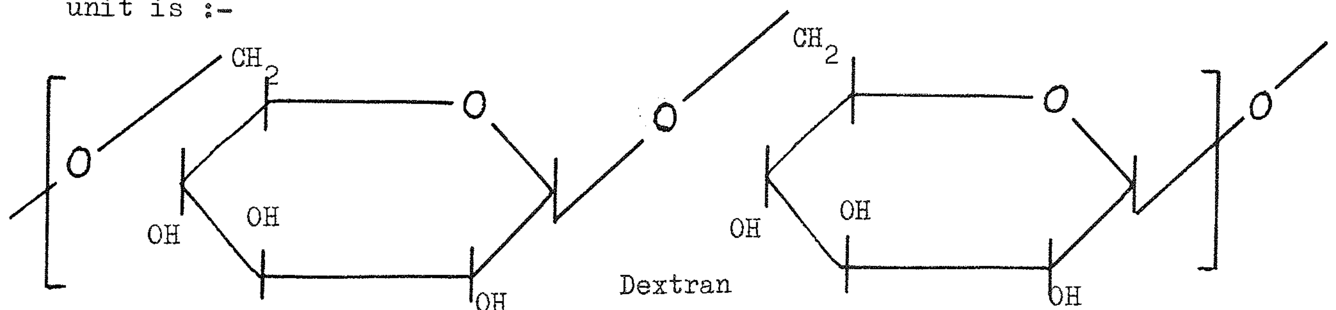


At least two invertases are present in sugarcane, one being most active under acid conditions, the other under neutral conditions; their activities vary relative to each other, and absolutely, in different parts of cane stalks at different stages in maturity of the plant (Rizk & Normand, 1969b).

The effect on sucrose of microbial growth in harvested cane is dependent on the species of organism and its associated metabolism. Many organisms, notably some species of yeast, produce extracellular invertase. Lactic acid bacteria typically convert sugars to lactic and acetic acids, mannitol, and in some species, carbon dioxide. Leuconostoc mesenteroides produces in addition the enzyme dextransucrase which catalyses the formation of fructose and the polysaccharide, dextran, from sucrose (Anderson, 1963) :-

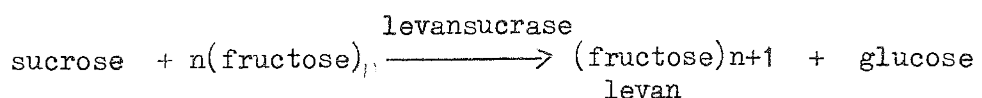


Dextran is a predominantly α-1,6 linked polyglucan in which the repeating unit is :-

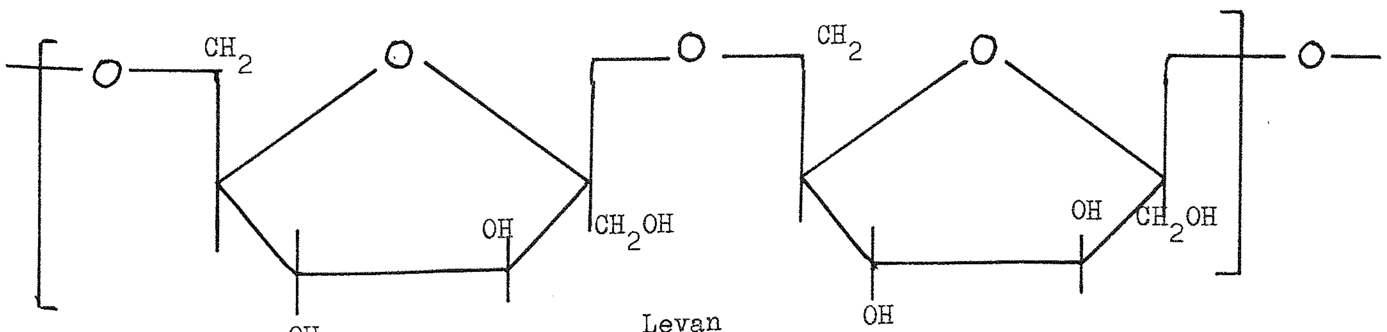


Dextran

Various species of Bacillus, e.g. B. subtilis, produce the extracellular enzyme levansucrase, which catalyses the formation of glucose and the polysaccharide, levan, from sucrose (Anderson, 1963) :-



Levan is a β-2,6 linked polyfructosan, in which the repeating unit is :-



Levan

There is also evidence for a polysaccharide in harvested, whole-stalk cane, whose formation appears to be due to metabolic changes in the plant rather than the growth of microorganisms. This polysaccharide is a glucan containing 25-30% α-1,6 and 70-75% α-1,4 glucosidic linkages (Nicholson & Lilienthal,1959; Bruijn,1966b,1970); it was named 'sarkaran' by Bruijn (1970).

The results of the changes undergone by sucrose in stored, cut cane are manifested by the following symptoms :- a reduction in true sucrose, pol and purity of both cane and expressed juice; an increase in reducing sugars; an increase in soluble polysaccharides ('gums'), especially dextran; an increase in acidity, and reduction in pH of the juice.

The second factor which reduces the yield is a loss in weight of the cane due to evaporation of moisture. The effects of this moisture loss are reflected in increased Brix of expressed juice and increased fibre % cane. The latter causes a reduction in the percentage extraction of juice in the mill, because the proportion of juice bound by the fibre is increased.

No previous studies on the microbiology of stored, harvested, whole-stalk cane appear to have been published. Therefore it is not possible to deduce the significance of microorganisms in the production of symptoms described above. However, the widespread and well-documented occurrence of harmful microbial activity in mill juice, which is principally ascribed to *Leuconostoc mesenteroides*, combined with the frequent incidence of sour cane and its harmful effects on the manufacturing process, suggest that under certain conditions microorganisms cause biodeterioration ofharvested cane. This evidence is fully discussed in subsequent chapters.

2. Factors which influence the rate of deterioration

Quantitative comparisons are made difficult because of the wide differences that exist in varieties of cane, climatic conditions and harvest practice between the various cane-growing areas. Although much of the published work is empirical in nature, some factors which influence the rate of post-harvest deterioration of cane have been investigated :-

a) Variety of cane Research in many countries has clearly shown that there is a marked varietal difference in susceptibility to deterioration after harvest. The earliest reports were those of Hall (1914) in Louisiana and Cross & Belile (1915) in Argentina. A wealth of confirmatory evidence was subsequently published by Lauritzen and his co-workers in Louisiana (Lauritzen & Balch, 1934,1935,1948; Lauritzen, Balch & Fort, 1939; Lauritzen, Balch, Davidson & Arceneaux, 1949). These results may be due to differences in susceptibility of varieties to moisture loss (Haldane, 1933) which in turn may depend on the thickness of the waxy layer on the stalk (Lauritzen, 1942). Where cane is harvested in cold weather, e.g. Louisiana, the result may be explained by differences in varietal resistance to freezing (Lauritzen et al, 1949). Another contributory factor may be the variation in amount and distribution of cane invertases in the stalks (Rizk & Normand, 1969b). In the Caribbean area, varietal differences in post-harvest deterioration have been reported in Puerto Rico (Boneta-Garcia & Lugo-Lopez, 1962; Samuels & Cayere, 1967); Cuba (Rodriguez, 1968); and Trinidad (Anon, 1956). In the latter publication variety B4362 showed the greatest rate of deterioration amongst six varieties tested; this variety is at present predominant in Jamaica. Similar work has recently been published in India (Gupta, Juneja, & Narain, 1967; Gupta, Juneja & Shuckla, 1968) and Mexico (Perdomo & Ramos, 1969).

b) Climatic conditions It is hard to assess the effect of climatic conditions on rate of deterioration of cut cane, partly because of the difficulty in controlling temperature, rainfall and humidity in large-scale experiments, and partly due to the complexity of interaction between these parameters. However, it is likely that climatic factors are the most important of all parameters which influence deterioration rate.

Several early studies showed that cut cane stored in the shade deteriorated more slowly than cane stored in the sun (Cross & Belile, 1915; Barnes, 1916; Lee, 1923; Sanyal, 1925; Rosenfeld, 1935; Boneta-Garcia & Lugo-Lopez, 1962). This was ascribed to a direct effect of temperature on

invertase activity, but it now seems more probable that the indirect effect of temperature on moisture content is more important. Cross & Belile (1914) and Cross & Harris (1916) in the Argentine, and Lauritzen & Balch (1934,1935) in Louisiana, demonstrated that piles of cut cane deteriorated more slowly when kept moist by regular sprinkling with water than if not so treated. Further work by Lauritzen & Balch (1948) showed that the rate of deterioration of cane stored under controlled environmental conditions was almost independent of temperature provided that the relative humidity (R.H.) was high; at constant temperature the rate of loss of moisture and rate of inversion were inversely proportional to the R.H. The beneficial effect of high moisture retention was thought to be due to the maintenance of turgor in the cane cells.

Lauritzen & Balch (1935) also reported that cane deteriorates more alowly in large piles than in small piles, due to the lower temperature and higher R.H. that exist in the centre of a large pile. In Hawaii, however, Wold (1946) observed the opposite effects.

The effect of rainfall is controversial. Lauritzen & Balch (1935) concluded that wet weather tended to minimise inversion in stored whole-stalk cane, presumably due to its beneficial effect on R.H. and moisture content. More recent work in Australia showed that post-harvest deterioration was greater in warm, wet weather than in dry weather, both for whole-stalks (Egan, 1964) and chopped-up cane (Keniry, Lee & Davis, 1967b). Here, however, this effect was associated with increased rate of infection of the cane with <u>Leuconostoc mesenteroides</u>. In Caribbean conditions it is widely held that the incidence of sour cane increases in the rainy season. In Puerto Rico, Samuels & Cayere (1967) reported that harvested whole-stalk cane deteriorated more rapidly in wet weather than in dry conditions. It seems probable that in the presence of <u>L.mesenteroides</u>, rainfall may be an important vector of infection of cut cane by this organism.

A special climatic factor is important in those areas which lie at the extreme northern and southern limits of sugar cane cultivation. Here the [illegible] temperature may drop to freezing point during the harvest season,

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resulting in frost-damage to the cane. This damaged cane exhibits severe symptoms of deterioration, which appear to be enhanced by infection with *L.mesenteroides*; the organism enters the stalk through splits in the rind (McCalip & Hall, 1938). Research on this phenomenon in Louisiana was reviewed by Owen (1949) and in Argentina by Cross (1966). Fortunately, in the Caribbean area the ambient temperature always remains above freezing point, so that this phenomenon is not relevant.

c) Pre-harvest burning It is generally acknowledged that burnt cane deteriorates more rapidly than green cane after harvest (Stephenson & Doolan, 1947; Owen, 1949; Balch, Broeg & Lauritzen, 1950; Turner & Rojas, 1962; Foster, 1969a). However, in Jamaica there was found to be no difference (Anon, 1958), whilst in Australia green cane deteriorated more rapidly than burnt (Waddell, 1952,1954). The reasons for these differences are not clear, but it seems likely that burning induces changes in the invertase systems of the cane plant (Rizk & Normand, 1969a,b,c). Polysaccharide formation occurs more rapidly in harvested burnt cane than in green cane, and the qualitative nature of the polysaccharide may also differ (Leonard & Richards, 1969; Foster, 1969a). Burning may also facilitate infection of the cane by microorganisms through splits in the rind.

d) Method of harvest The introduction of mechanical harvesters which chop the cane into short billets has greatly accentuated the problem of sour cane in Queensland, and resulted in a wealth of publications (Vallance & Young, 1959; Egan & Rehbein, 1963; Egan, 1964, 1965a,b,c, 1966, 1967a,b, 1968a,b; Keniry, Lee & Davis, 1967a,b,; Kirby, 1968; Vickers, 1968; Foster, 1969a). It was clearly shown that the rate of post-harvest deterioration of chopped cane greatly exceeds that of whole-stalk cane. This is mainly due to increased incidence of infection with *L.mesenteroides*, since chopped cane possesses many more available points of infection than the equivalent length of whole stalk. Significant deterioration of chopped cane can be observed within 12 hours of harvest in unfavourable climatic conditions, compared with more than 30 hours for whole stalks. The degree of mechanical damage to the cut ends of the bill

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found to influence the rate of deterioration of cane (Egan & Rehbein, 1963); similar results with whole-stalks were reported in Hawaii (Wold, 1946).

e) Maturity of cane There is limited evidence which suggests that cane which is either overmature or immature deteriorates more rapidly than mature cane after harvest (Dymond, 1924; Lauritzen & Balch, 1935; Anon, 1967; Egan, 1969), although Turner & Rojas (1962) did not observe any significant differences between cane aged 13 and 14½ months at harvest.

f) Miscellaneous When large areas of standing cane are accidently or maliciously burned, it is often impossible to harvest and mill all the cane within the normal time period. A decision must then be made either to leave the cane standing and harvest it gradually over a period of days or to cut all the cane rapidly and mill it gradually. Limited experimental evidence suggests that the latter practice results in more favourable recoveries of sugar than the former (Calma, 1941; Stephenson & Doolan, 1947; Anon, 1967; Samuels & Cayere, 1967). One report, however, revealed the opposite result (Young, 1962).

Other factors which could influence the rate of deterioration of harvested cane but which have not been investigated include soil type; ratoon crop, and presence or absence of potentially deteriogenic microorganisms in the cane area.

3. Plan of investigation

It was apparent from the literature that post-harvest deterioration is greatly influenced by factors such as climate, variety and harvest practice, which vary widely between different cane growing areas. Therefore the present investigation was confined to one area, Jamaica, in order to limit the variability of these factors.

During the first part of the investigation samples of cane and cane juice were air-freighted from Jamaica to England under refrigeration. This cane was stored in a controlled environment and the changes in its microbiological, physical and chemical composition were determined at intervals. Although the experimental conditions could not simulate those existing in the field, it was hoped that useful qualitative information could be obtained.

Fifty storage trials on harvested, burnt, whole-stalk cane were conducted at Frome between March and July, 1969. The experimental conditions closely simulated field practice, and climatic conditions were recorded. The majority of experiments utilised variety B4362, which constituted 73% of the crop, but some trials with B51129 (12% of the crop) were included for comparison. Measurements were made of the changes in physical, chemical and microbiological properties of cane during storage periods up to 10 days; special emphasis was placed on aspects which had not previously been studied, such as dextran content and Leuconostoc count. The objectives were :-

a) To estimate the rate of deterioration and loss of recoverable sugar.

b) To examine the significance of microorganisms in deterioration.

c) To select a suitable parameter for detection and measurement of deterioration and/or processing quality.

d) To study the effects of climatic conditions, maturity and variety of cane on deterioration.

In 1970 advantage was taken of the presence at Frome of a mechanical chopper-harvester to oompare the deterioration rates of mechanically-harvested, chopped-up cane and manually-cut, whole stalks.

B. EXPERIMENTAL

1. Experiments on material air-freighted to U.K., 1966-68.

Details of ten batches of material air-freighted to the Research Centre are summarised in Table III,1. The examination of these samples is reported below :-

a) Experiment 'a' Samples of mixed and crusher juice were taken by Dr. M.P.Scarr from several factories in Jamaica and Trinidad in June, 1966, chilled, and sent to the U.K. On receipt the juices were examined microbiologically by plating on Shapton medium (Oxoid CM269) containing 25% sucrose, pH 5.8, and tomato juice agar (Oxoid CM114). Quantitative data was not obtained, but the predominant organisms were Lactobacillus and Bacillus species. Selected colonies were isolated, purified and identified by methods described in Chapter II.

16 isolates were identified as follows :- Lactobacillus plantarum, 4 isolates; L.casei var. alactosus, 4 isolates; Lactobacillus new species, (Group II), 2 isolates; Bacillus subtilis, 5 isolates; B.megaterium, 1 isolate. Leuconostoc mesenteroides was not detected, but since there was a long delay between sampling and examination of the juice it is possible that this organism had died out.

b) Experiment 'b' A sample of chilled, fresh, mixed juice from a factory in Mauritius was examined within two days of despatch.

Viable plate counts of microorganisms were determined by methods described in Chapter II for total spoilage organisms (STA); lactic acid bacteria (Rogosa agar); Bacillus spp., and yeasts. The count of total spoilage organisms and lactic acid bacteria was identical at 4.0×10^7 /ml. The lactic acid bacteria were predominantly dextran-formers; 22 colonies were selected, purified and identified as Leuconostoc mesenteroides, 17 isolates; Lactobacillus plantarum, 1 isolate; Lactobacillus new species (Group II), 4 isolates. The count of Bacillus sp. spores was 2.0×10^3 /ml.; 11 colonies were selected, purified and identified as Bacillus pumilus, 8 isolates;

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Experiment Code No.	Source of material	Date despatched	Date received	Transit time in days	Details of material
a	Various T & L Estates in Jamaica and Trinidad	June 1966	June 1966	not known	Cane juice from mills, sampled by Dr.M.P.Scarr. Deep-frozen on receipt
b	Mauritius	29.8.66.	30.8.66	1	500ml. mixed juice from mill, sampled on 29.8.66. and chilled in transit.
c	Frome Estate, Jamaica	Nov. 1966	16.11.66	not known	Daily samples of crusher & mixed juice taken by Dr.M.P. Scarr between 23.6. & 8.7.66.. Deep-frozen until despatch in November.
d	Frome Estate, Jamaica	23.3.67.	28.3.67.	5	Crusher juice extracted from green & burnt cane after storage for 1,3 & 7 days. Chilled before & during transit. Green & burnt cane stalks harvested 23.3.67. and chilled.
e	Frome Estate, Jamaica	18.4.67.	19.4.67.	1	Crusher juice extracted from green & burnt cane after storage for 1,3,7 & 15 days. Chilled before & during transit. Green & burnt cane stalks harvested 18.4.67. and chilled.
f	Tower Hill Estate, British Honduras	23.5.67.	27.5.67.	4	Crusher juice extracted from burnt cane after storage for 1,3 & 7 days. Chilled before & during transit.
g	Mauritius	23.10.67.	24.10.67.	1	Crusher juice from mill, sampled 23.10.67. & chilled.
h	Frome Estate, Jamaica	10.5.68.	15.5.68.	5	Burnt cane stalks harvested 10.5.68. and chilled.
i	Frome Est. Jamaica	7.7.68.	11.7.68.	4.	Burnt cane stalks, harvested 7.7.68. and chilled.
j	Frome Est. Jamaica	2.7.68.	17.7.68.	15	Burnt cane stalks, harvested 2.7.68. and chilled.

Table III.1. Samples of cane juice and cane stalks air-freighted to the Research Centre, 1966-68.

1.0×10^3ml; it was noted that some pellicle-forming yeasts were present.

The result of a physical and chemical examination of the juice, by methods given in Chapter II, was :- refractive brix, 15.0°; pH 5.25; sucrose, 10.74% w/w; reducing sugars, 0.89% w/v; gum (Natal method), 0.83% w/v.

It was concluded that the juice was beginning to deteriorate, since its Leuconostoc content and gum content were high.

c) Experiment 'c' Daily samples of crusher and mixed juice were taken by Dr. M.P.Scarr at Frome Estate, Jamaica, in June/July 1966 and deep-frozen. They were sent to the U.K. in November and were received in a thawed state on 16th November 1966. Since the juices had deteriorated in transit, only limited tests were carried out. Microbiological examination revealed that the total spoilage organism count was 1.0×10^4 to 1.8×10^5 ml; the Bacillus spore count was 3.7×10^4 ml. and the yeast count was 3.0×10^4ml. in pooled samples. Few dextran-forming colonies were observed on STA medium. The brix and pH of pooled samples were 16.8° and 4.85 respectively.

It was concluded that freezing of the juice had killed most of the non-sporulating bacteria, whilst Bacillus spores and yeasts had survived and caused deterioration of the juice in transit. Therefore freezing was not recommended as a method of preservation for subsequent samples.

d) Experiment 'd' At Frome Estate, samples of green and burnt mature cane (variety B4362) were cut from adjacent fields on 13th March 1967. Each sample was mixed and stored in a pile in the open in the factory yard. Sub-samples were crushed in a small 3-roller mill after storage periods of 1,3 and 7 days. The extracted juice was analysed at the factory for brix, pol, purity and reducing sugars. Each juice sample was placed in a sterile container and refrigerated at 4°C until air-freighted to the U.K. on 23rd March 1967.

On the 23rd March, similar stalks of green and burnt cane were harvested, chopped into 18" billets, wrapped in polythene bags, packed in ice, and air-freighted to the U.K.

The juice and cane samples were received at the Research Centre on 28th March, after a 5-day delay in transit. The juice samples were examined

immediately. The cane stalks were incubated at 28-30°C in polythene bags, and five billets each of green and burnt cane were removed after storage periods of 1,3,7 and 15 days. The surface of the billets was 'sterilised' by swabbing and flaming with methylated spirit, and each batch was crushed aseptically in a flame-sterilised, 2-roller, hand mill. The juice was collected aseptically and analysed immediately.

All juices were examined by microbiological, physical and chemical methods described in Chapter II. Microbiological results are summarised in TableIII,2, and physical/chemical analyses are summarised in Table III,3.

The juice samples obtained from cane stored at Frome were visibly fermenting on receipt and had obviously deteriorated during storage and the 5-day transit period. This eliminated all differences between treatments and reduced the value of quantitative analyses. However, it was apparent that the high total spoilage organism count consisted mainly of lactic acid bacteria, although yeasts constituted between 1/10th and 1/100th of the total flora. Other groups of micro-organism were not significant. Several isolates of lactic acid bacteria were identified as _Leuconostoc mesenteroides_, 4 isolates, and _Lactobacillus plantarum_, 3 isolates.

The microbiological results for cane stored in the U.K. indicated that it had already deteriorated in transit prior to the initiation of the storage experiment; hence the real post-harvest storage periods were 6,8 and 12 days. There were no significant differences between green and burnt cane. The reduction in count of total spoilage organisms and lactic acid bacteria during storage suggests that they may have attained maximum counts in transit. 6 isolates of lactic acid bacteria (LAB) were identified as _Leuconostoc mesenteroides_. Yeasts and catalase positive, Gram-negative, aciduric rods (AAB) constituted a significantly high proportion of the flora, whereas other groups contributed only a small proportion.

A comparison of the U.K. results with those obtained at Frome, for cane stored at Frome, confirmed that the juices had deteriorated in transit. This was revealed by the low pH, higher reducing sugar content, and high gum content

Juice Sample Source	Storage Time (days)	Total Spoilage Organisms	Lactic Acid Bacteria	Aciduric, Gram-negative Rods	Mesophilic Yeasts	Osmophilic Yeasts	Moulds	*Bacillus* spores	Coliform Bacteria
		Count/ml. of Juice							
Green stalks stored in U.K.	1	1.0×10^{7}	1.0×10^{7}	1.4×10^{3}	$>2\times10^{6}$	$>10^{6}$	1.1×10^{4}	1.7×10^{3}	NT
	3	2.1×10^{6}	$<10^{3}$	3.5×10^{5}	9.5×10^{5}	5.0×10^{5}	2.7×10^{4}	1.3×10^{3}	1.7×10^{2}
	7	1.4×10^{6}	$<10^{3}$	$>10^{6}$	2.3×10^{6}	1.6×10^{6}	1.1×10^{5}	1.5×10^{3}	2.9×10^{3}
Burnt stalks stored in U.K.	1	1.6×10^{7}	1.0×10^{7}	3.8×10^{5}	7.5×10^{5}	2.0×10^{5}	3.2×10^{4}	5.1×10^{2}	NT
	3	7.1×10^{6}	8.3×10^{6}	1.0×10^{5}	2.0×10^{5}	1.0×10^{5}	$<10^{3}$	2.0×10^{3}	$<10^{1}$
	7	1.0×10^{6}	2.3×10^{4}	8.5×10^{5}	4.8×10^{5}	3.1×10^{5}	2.0×10^{3}	1.5×10^{3}	$<10^{1}$
Juice from green stalks stored at Frome	1	2.9×10^{8}	3.7×10^{8}	4.2×10^{3}	1.1×10^{7}	1.0×10^{7}	$<10^{1}$	4.4×10^{2}	6.0×10^{3}
	3	1.4×10^{8}	1.6×10^{8}	3.6×10^{3}	8.5×10^{5}	3.6×10^{5}	$<10^{1}$	4.1×10^{2}	6.7×10^{2}
	7	5.5×10^{8}	5.1×10^{8}	1.0×10^{4}	1.2×10^{6}	1.5×10^{6}	$<10^{1}$	5.7×10^{2}	1.4×10^{4}
Juice from burnt stalks stored at Frome	1	1.7×10^{8}	8.0×10^{7}	2.5×10^{2}	5.9×10^{6}	3.3×10^{6}	2.0×10^{1}	9.0×10^{1}	4.0×10^{3}
	3	6.5×10^{8}	3.8×10^{8}	4.2×10^{4}	6.4×10^{6}	1.1×10^{6}	8.0×10^{1}	3.3×10^{3}	1.6×10^{4}
	7	1.4×10^{8}	1.3×10^{8}	2.0×10^{3}	1.2×10^{7}	8.2×10^{6}	1.1×10^{2}	3.0×10^{3}	2.0×10^{3}

Table III.2. Microbiological examination of cane juices, Experiment 'd'.

NT = Not Tested

Juice Sample Source	Storage Time (Days)	pH (U.K.)	°Brix		°Pol	Purity	R.S.% w/v		% Gum w/v U.K.*		Viscosity (U.K.) cp 23.5°C
			U.K.	Frome	Frome	Frome	U.K.	Frome	(i)	(ii)	
Green stalks stored in U.K.	1	5.25	19.2	-	-	-	1.46	-	0.34	0.24	1.89
	3	4.53	22.1	-	-	-	10.35	-	0.25	0.09	2.01
	7	4.18	22.2	-	-	-	16.70	-	0.29	0.12	2.11
Burnt stalks stored in U.K.	1	4.90	22.0	-	-	-	1.13	-	0.23	0.12	2.14
	3	4.70	23.9	-	-	-	4.60	-	0.28	0.17	2.25
	7	4.20	26.8	-	-	-	12.70	-	0.67	0.48	2.89
Green stalks stored at Frome	1	3.50	19.2	20.1	18.36	91.34	3.4	0.88	1.00	1.04	3.59
	3	3.30	20.5	21.6	18.71	86.62	5.2	1.90	1.61	1.86	5.91
	7	3.50	22.0	22.4	16.00	71.43	3.7	3.25	0.48	0.41	2.45
Burnt stalks stored at Frome	1	3.40	19.6	20.6	19.07	92.57	3.6	0.41	2.59	3.05	13.52
	3	3.55	20.2	21.2	19.05	89.86	2.3	0.50	0.39	0.45	2.48
	7	3.40	19.5	21.8	18.60	85.32	2.3	1.17	0.75	0.49	3.00

Table III,3. Physical and chemical examination of cane juices, Experiment 'd'.

* (i) Bruijn (1966)

(ii) Roberts & Friloux (1965)

and viscosity of the U.K. analyses. However, the Frome results indicate that the green cane deteriorated more rapidly than burnt cane during storage, in terms of its pol, purity and R.S. content. Significant deterioration was observed within three days of harvest. Similar results were obtained with cane stored in the U.K., for pH and R.S., but the gum content and viscosity of burnt cane exceeded that of green cane.

e) Experiment 'e' The samples were similar to those of Experiment 'd'. They were despatched on 18th April 1967 and received two days later; the cane was fairly fresh and the ice-packing was still semi-solid, but the juice samples were visibly fermenting.

The stalks were analysed after storage periods of 1,3,8 and 15 days. Microbiological results are shown in Table III,4, and Fig.III,1; physical and chemical analyses are summarised in Table III,5, and Fig.III,2.

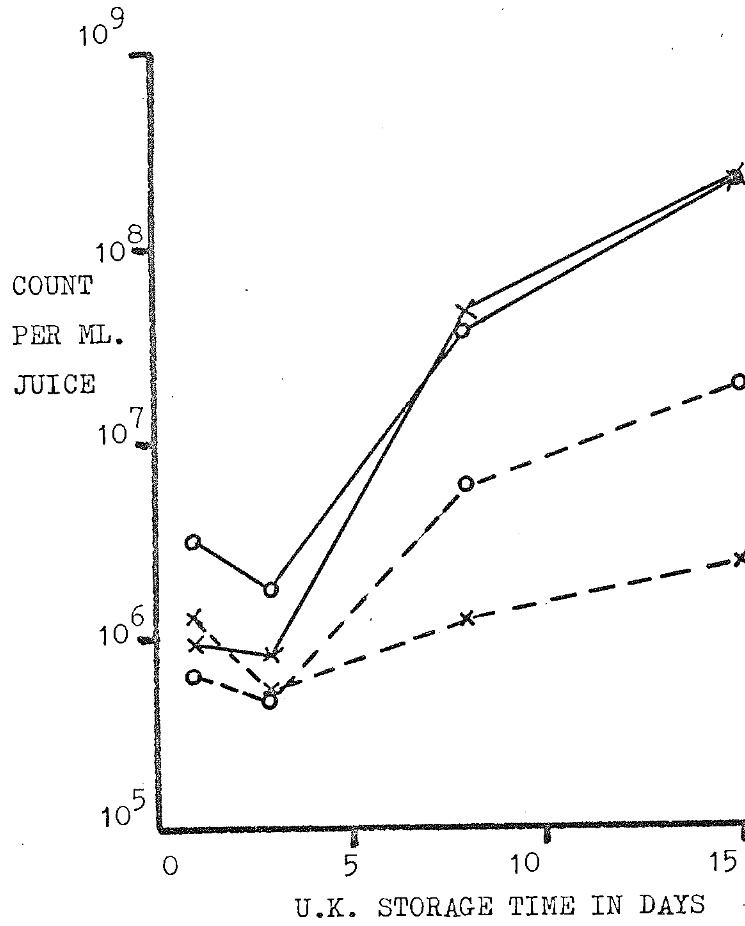
The microbial flora of juice from cane stored at Frome was similar to that of the equivalent samples in Experiment 'd'. 13 isolates of predominant LAB were identified as Leuconostoc mesenteroides, 7 isolates; L.paramesenteroides, 1 isolate; L.dextranicum, 1 isolate; Lactobacillus confusus (new species), 3 isolates; Lact.plantarum, 1 isolate.

The cane stored in the U.K. was two days old post-harvest at the beginning of the experiment; therefore the real storage periods were 3,5,11 and 17 days. This was revealed in the relatively high initial counts of spoilage organisms (10^6 - 10^7 per ml.). Burnt cane showed a higher count of spoilage organisms and LAB than green cane up to 8 days storage time, after which die-off occurred in burnt cane only. The predominant organisms were again LAB; 12 isolates were identified as follows :- Leuconostoc mesenteroides, 3 isolates; Lactobacillus confusus (new species), 1 isolate; Lact.plantarum, 2 isolates; Lact.casei var. casei, 3 isolates; Lact.casei var. alactosus, 3 isolates. The lactobacilli appeared to be more numerous than the leuconostocs as storage time increased. AAB were the next most numerous organisms, but attained only 1/10th of the LAB count. Yeast counts were high but increased only slowly during storage; other organisms were insignificant.

Juice Sample Source	Storage Time (days)	Count/ml. of Juice							
		Total Spoilage Organisms	Lactic Acid Bacteria	Aciduric Gram-negative Rods	Meso-philic Yeasts	Osmo-philic Yeasts	Moulds	*Bacillus* spores	Coliform Bacteria
Green stalks stored in U.K.	1	$3.2x10^6$	$9.0x10^5$	$6.4x10^5$	$1.3x10^6$	$1.0x10^6$	$3.0x10^3$	$2.9x10^2$	$1.0x10^1$
	3	$1.8x10^6$	$8.0x10^5$	$4.6x10^5$	$5.0x10^5$	$1.9x10^5$	$3.5x10^2$	$1.9x10^2$	NT
	8	$3.6x10^7$	$4.5x10^7$	$6.0x10^6$	$1.2x10^6$	$2.9x10^5$	NT	$1.0x10^1$	$< 10^1$
	15	$2.1x10^8$	$2.0x10^8$	$1.9x10^7$	$2.2x10^6$	$1.9x10^6$	$5.0x10^3$	$3.6x10^3$	$< 10^1$
Burnt stalks stored in U.K.	1	$1.4x10^7$	$9.1x10^6$	$1.0x10^6$	$1.0x10^6$	$4.0x10^5$	$1.0x10^3$	$2.3x10^2$	$< 10^1$
	3	$1.5x10^7$	$1.3x10^7$	$5.0x10^5$	$2.8x10^6$	$5.0x10^5$	$1.0x10^3$	$1.0x10^1$	NT
	8	$5.8x10^7$	$6.6x10^7$	$6.0x10^6$	$2.1x10^6$	$5.0x10^5$	NT	$5.0x10^1$	$< 10^1$
	15	$2.2x10^7$	$3.7x10^7$	$1.0x10^6$	$7.0x10^5$	$6.0x10^5$	$2.0x10^3$	$4.0x10^1$	$< 10^1$
Juice from green stalks stored at Frome	1	$3.7x10^8$	$6.3x10^8$	$7.0x10^4$	$1.0x10^6$	$1.1x10^6$	$1.2x10^3$	$2.5x10^3$	$2.0x10^1$
	3	NT	$8.0x10^8$	$9.4x10^4$	$8.0x10^5$	$7.0x10^5$	$4.0x10^3$	$1.1x10^3$	$< 10^1$
	7	$3.3x10^8$	$3.9x10^8$	$1.2x10^5$	$2.5x10^6$	$1.0x10^6$	$3.0x10^2$	$5.7x10^2$	$2.0x10^1$
Juice from burnt stalks stored at Frome	1	$2.8x10^8$	$3.5x10^8$	$5.9x10^4$	$9.0x10^5$	$5.0x10^5$	$1.0x10^1$	$2.1x10^3$	$<10^1$
	3	$6.0x10^8$	$5.5x10^8$	$2.2x10^5$	$1.0x10^6$	$4.0x10^5$	$4.0x10^2$	$2.6x10^3$	$3.0x10^1$
	7	$2.6x10^8$	$1.9x10^8$	$5.0x10^4$	$1.5x10^6$	$3.0x10^5$	$2.0x10^3$	$5.0x10^3$	$2.0x10^1$

Table III.4. Microbiological examination of cane juices, Experiment 'e'.

NT = Not Tested



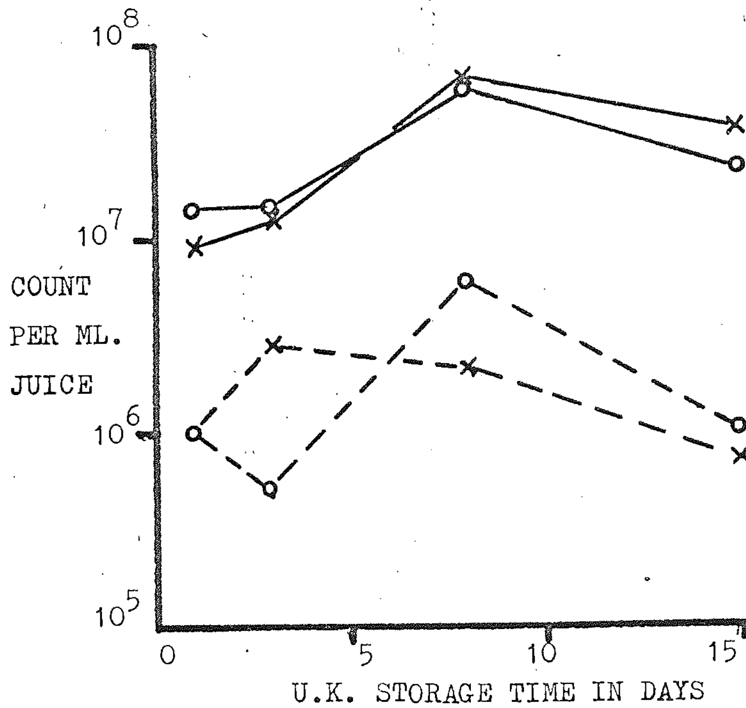


Fig.III,1. The effect of storage time on the microbial flora of juice from harvested cane, Experiment 'e'.

Key
o———o Total spoilage organisms
x———x Lactic acid bacteria
o- - - - -o Catalase positive, Gram-negative, aciduric rods
x- - - - -x Mesophilic yeasts

Juice Sample Source	Storage Time (days)	pH		^{o}Brix		^{o}Pol		Purity		R.S.% w/v		Gum% w/v (U.K.)*		Viscosity cp 11.5^{o}C (U.K.)
		U.K.	Frome	U.K.	Frome	U.K.	Frome	U.K.	Frome	U.K.	Frome	(i)	(ii)	
Green stalks stored in U.K.	1	4.90	-	23.8	-	20.13	-	84.57	-	2.10	-	0.37	0.05	3.23
	3	4.50	-	23.6	-	19.01	-	80.56	-	2.56	-	0.35	0.07	3.20
	8	3.92	-	25.0	-	18.83	-	75.34	-	4.36	-	0.33	0.15	3.30
	15	3.40	-	22.0	-	10.23	-	46.49	-	8.90	-	0.55	0.43	3.13
Burnt stalks stored in U.K.	1	4.85	-	23.5	-	21.04	-	89.52	-	0.75	-	0.42	0.06	3.21
	3	4.25	-	23.8	-	20.18	-	84.78	-	1.52	-	0.31	0.08	3.25
	8	3.72	-	24.1	-	15.11	-	62.70	-	5.60	-	0.49	0.15	3.16
	15	3.10	-	22.2	-	3.40	-	15.31	-	12.90	-	0.85	0.54	3.30
Green stalks stored at Frome	1	3.40	5.40	23.4	23.2	-	21.69	-	93.49	6.30	0.68	2.88	3.125	7.44
	3	3.40	5.40	23.4	23.0	-	21.40	-	93.04	5.80	0.64	3.16	3.00	8.39
	7	3.25	5.20	23.0	23.0	-	19.14	-	83.22	5.50	2.08	1.62	1.68	6.11
Burnt stalks stored at Frome	1	3.35	5.40	22.6	22.5	-	20.88	-	92.80	3.80	0.64	2.46	2.80	24.95
	3	3.44	5.40	22.8	22.6	-	20.97	-	92.79	4.10	0.70	2.92	3.13	27.20
	7	3.30	5.00	20.0	20.5	-	17.99	-	87.76	3.10	0.91	1.62	1.65	15.45

Table III.5. Physical and chemical examination of cane juices, Experiment 'e'.

* (i) Bruijn (1966)

(ii) Roberts & Friloux (1965)

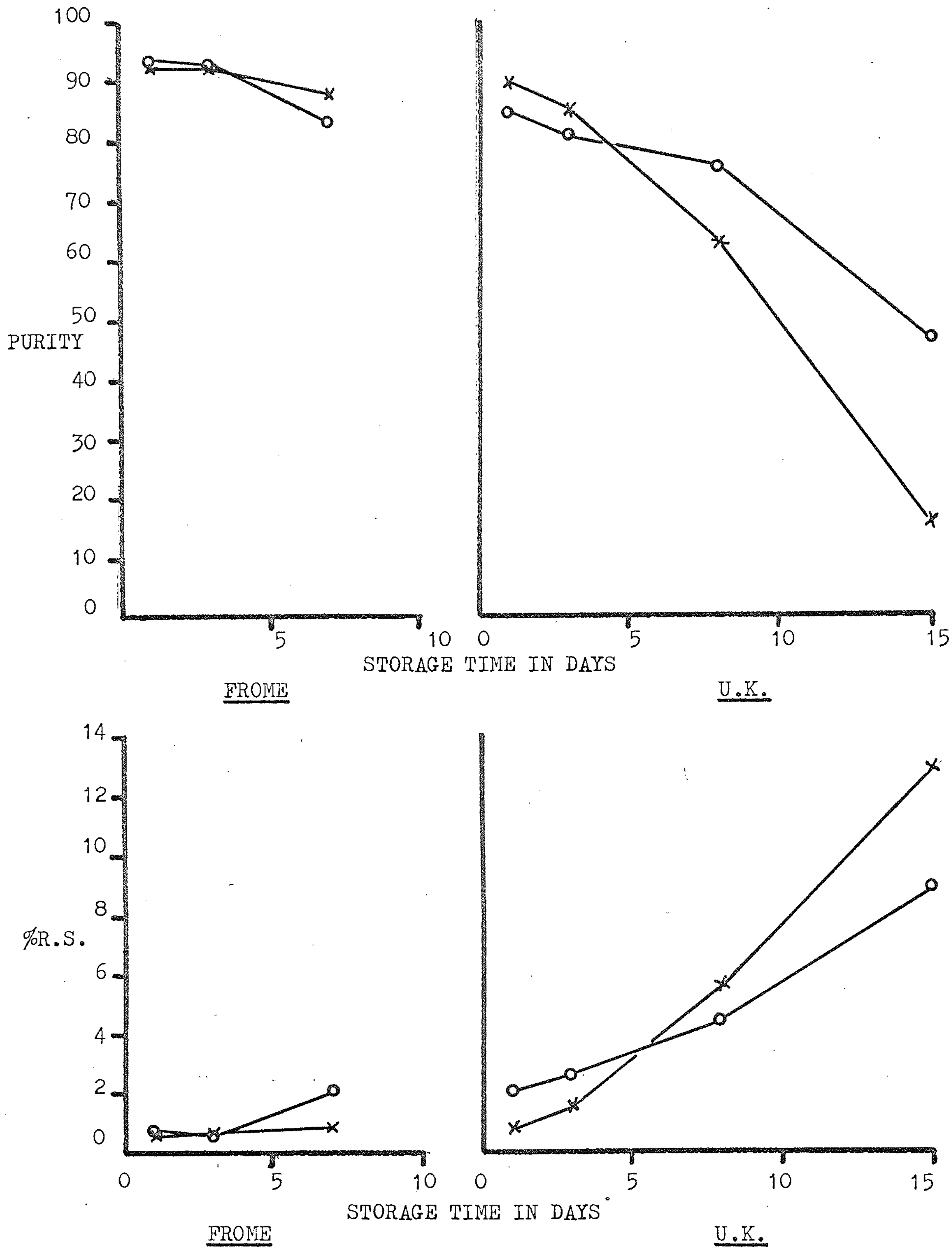


Fig.III,2. The effect of storage time on the purity and R.S. content of juice from harvested cane, Experiment 'e'

Physical/chemical analyses of juice from cane stored at Frome were similar to the equivalent samples in Experiment 'd', and again indicated severe deterioration in storage and transit. The results obtained at Frome again showed that the green cane had deteriorated more rapidly than burnt cane (Fig.III.2). However, the cane stored in the U.K. revealed the opposite effect. The gum content increased only slightly during storage, and did not cause any significant increase in viscosity; this may be explained by the predominance of lactobacilli over dextran-forming leuconostocs. The much greater rate of deterioration observed with cane stored in the U.K. with that stored at Frome may have been due to the use of short billets in the former case; however, it was impossible to air-freight whole stalks under refrigerati

f) Experiment 'f'. Samples of crusher juice were taken from burnt cane stored for 1,3 and 7 days at Tower Hill factory, B.Honduras. The juices were refrigerated until despatch on 22nd May 1967, but they were not received in the U.K. until 27th May. Two of the three thermos flasks were broken in transit and all the samples were badly deteriorated and very viscous on receip

The following microbial counts were obtained :-

	Count/ml. juice
total spoilage organisms	8.7×10^{8} - 2.5×10^{10} ;
LAB	8.0×10^{8} - 3.5×10^{10} ;
Yeasts	7.6×10^{4} - 2.0×10^{5} ;
All other microorganisms	$< 2 \times 10^{3}$

The predominant organisms were LAB, which showed very high counts. There was no significant difference between samples. Four isolates of LAB taken from 10^{-7} dilutions of Rogosa agar plates were identified as :- Leuconostoc mesenteroides, 2 isolates; Lactobacillus confusus (new species), 1 isolate; Lact.casei var. alactosus, 1 isolate.

Physical/chemical analyses of the samples are summarised in Table III,6.

The high gum content and viscosity and low pH were probably due to growth of Leuconostoc mesenteroides.

No. of days storage	°Brix		% Pol	Purity	pH	% R.S.	% gum*	Viscosity in cp at 25.0°C
	U.K.	BH	(BH)	(BH)	(U.K.)	(U.K.)	(U.K.)	(U.K.)
1**	18.8	18.6	16.02	86.04	4.05	6.72	3.96	***
3	18.8	19.8	16.62	83.94	3.80	1.56	0.35	2.24
7**	23.8	24.0	18.28	76.17	3.85	10.96	5.33	***

Table III.6. Physical and chemical analyses of cane juices. Experiment 'f'.

* Bruijn (1966)
** Broken thermos flasks - badly deteriorated samples
*** Viscosity too high for determination in Ostwald viscometer

g) Experiment 'g' A sample of crusher juice was taken from a factory in Mauritius on 23rd October 1967, chilled, and despatched the same day. It was received in the U.K. on 24th October, refrigerated, and analysed on 26th October.

The following microbial counts were obtained :-

		Count/ml. juice
Total spoilage organisms	-	1.1×10^{10}
LAB	-	1.0×10^{10}
Yeasts	-	1.2×10^{6}
AAB	-	2.3×10^{5}
All other microorganisms	-	$< 10^{4}$

LAB were again predominant and present in very high numbers; 7 isolates were identified as :- Leuconostoc mesenteroides, 3 isolates; Lactobacillus plantarum, 4 isolates.

The physical/chemical analysis is given below :-

pH	3.85
^{o}Brix	13.90
Total sugars	13.44 % w/v
Gum	0.81 % w/v (Bruijn,1966)
Viscosity	2.46 cp at 25°C

Again the high gum content and viscosity and low pH were indicative of microbial spoilage by LAB, especially Leuconostoc mesenteroides.

h & i) Experiments 'h' and 'i' Two batches of freshly harvested, burnt cane stalks, (variety B4362), designated samples 'h' and 'i', were received from Frome by air freight on 15th May and 11th June, 1968, respectively. Each batch consisted of 30 stalks, 15" in length; each stalk was double-wrapped in polythene, and the container was kept chilled with bags of dry ice. The time between harvesting and examination was 6 days, during which 5 and 4 days respectively were occupied in transit, the balance in refrigeration. Both samples were moderately fresh on arrival, and most individual stalks were fairly dry, although the ice had melted.

Storage experiments on the cane were carried out by methods similar to those previously described in Experiment 'd', with the following exceptions. The stalks were stored in sealed glass tanks over constant R.H. solutions at 24° to 28°C. Sample 'h' was stored over saturated KCl (ca 85% R.H.), whilst sample 'i' was stored over saturated $(NH_4)_2SO_4$ (ca 80% R.H.). At each interval, 3 (sample 'h') or 4 (sample 'i') stalks were removed for crushing, but the juices were analysed separately instead of being pooled as in previous experiments. In addition to previous analyses, the dextran content was estimated by a method based on that of Keniry et al (1967a, 1967b). The results are summarised in Tables III,7 and III,8.

Analysis of the microbiological results reveals that the initial total counts were already fairly high at 10^{7} to 10^{9} organisms per ml. These increased rapidly on storage, reaching 10^{11} to 10^{12} per ml. in two days. Actual counts were impossible to obtain beyond this stage, but they appeared to be about 10^{13} after 5 to 7 days. The predominant organisms in almost every

Storage Time in Days	Stalk No.	pH		purity		%w/v R.S.		%gum w/v		%Dextran w/v		Specific Viscosity		Microbiological Examination counts per ml. juice: Total Spoilage Organisms	LAB	AAB	Yeasts
			mean		mean		mean		mean		mean		mean				
	1	4.55		86.7		1.73		0.91		.078		.094		$3.2x10^{7}$	$4.1x10^{7}$	$5.1x10^{4}$	$2x10^{4}$
0	2	5.12	4.86	78.5	84.7	0.96	1.19	0.80	0.76	-	.066	.049	.058	$4x10^{7}$	$2.8x10^{7}$	$3.5x10^{6}$	$7x10^{2}$
	3	4.91		89.0		0.87		0.56		.054		.031		$1.8x10^{7}$	$7.1x10^{6}$	$8x10^{4}$	$4x10^{3}$
	4	4.90		93.9		1.50		0.54		.019		.026		$3x10^{7}$	$2.4x10^{7}$	$3.9x10^{5}$	$1.5x10^{5}$
1	5	4.95	4.42	97.3	88.9	0.91	1.24	1.15	1.03	.100	.100	.056	.279	$8.3x10^{8}$	$8.3x10^{8}$	$2.5x10^{6}$	$5.4x10^{3}$
	6	3.41		75.5		1.32		1.41		.180		.754		$5x10^{10}$	$5x10^{10}$	$2x10^{5}$	$6.6x10^{3}$
	7	4.50		86.9		3.32		0.94		.056		.050		$3.3x10^{11}$	$4.6x10^{11}$	$5x10^{6}$	$1.5x10^{4}$
2	8	4.05	4.10	84.5	81.4	1.60	2.80	0.97	1.18	.015	.194	.158	.198	$5x10^{12}$	$1x10^{13}$	$2x10^{7}$	$3.1x10^{5}$
	9	3.75		72.9		3.49		1.64		.510		.385		$2x10^{12}$	$> 10^{13}$	$1.5x10^{7}$	$2.1x10^{5}$
	10	4.90		82.0		1.78		1.21		.016		.097		ca 10^{12}	ca10^{12}	$2.1x10^{7}$	$8.5x10^{6}$
5	11	4.49	4.67	83.2	84.2	3.66	2.56	0.64	0.92	.004	.007	.049	.101	$6x10^{7}$	$3x10^{7}$	$3.4x10^{6}$	$4x10^{3}$
	12	4.63		87.3		2.23		0.91		.000		.156		ca$3x10^{12}$	ca$1.5x10^{12}$	$8.7x10^{4}$	$1.5x10^{4}$
	13	3.75		64.6		4.93		0.65		.222		.223		ca$6x10^{13}$	ca$5x10^{13}$	$3.3x10^{7}$	$9x10^{6}$
7	14	4.27	4.01	53.2	58.1	7.62	5.51	0.68	0.71	.012	.086	.108	.155	ca$1.5x10^{13}$	ca$1.5x10^{13}$	$2.2x10^{6}$	$3.1x10^{6}$
	15	4.02		56.6		3.99		0.80		.023		.134		ca$1x10^{13}$	ca$7x10^{12}$	$1.6x10^{7}$	$8x10^{6}$
	16	3.40		38.3		9.41		1.06		.254		.246		NT	NT	$1x10^{8}$	$9.4x10^{7}$
12	17	3.50	3.50	45.5	42.0	5.73	7.10	0.82	1.03	.026	.278	.131	.258	NT	NT	$9.4x10^{6}$	$7x10^{6}$
	18	3.50		44.2		6.15		1.21		.554		.398		NT	NT	$2.7x10^{7}$	$1.7x10^{7}$

Table III,7. Analyses of cane juices, Experiment 'h'.

NT = Not Tested

Storage Time in Days	Stalk No.	Physical and Chemical Examination: pH	mean	purity	mean	%w/v R.S.	mean	% gum w/v	mean	%Dextran w/v	mean	Specific Viscosity	mean	Microbiological Examination counts per ml. juice: Total Spoilage Organisms	LAB	AAB	Yeasts
	19	4.80		80.2		2.64		0.28		.003		.025		$2.6x10^{7}$	$1.8x10^{7}$	$8.2x10^{6}$	$3.9x10^{4}$
0	20	5.45	5.10	96.5	94.1	1.58	1.74	0.65	0.47	.036	.011	.017	.075	$1.2x10^{9}$	$5.2x10^{6}$	$1.1x10^{7}$	$ca3x10^{6}$
	21	5.10		100!		2.07		0.58		.003		.257		$1.5x10^{9}$	$6.5x10^{8}$	$2.3x10^{7}$	$2.2x10^{5}$
	22	5.03		99.8		0.65		0.36		0		0		$3.2x10^{9}$	$3x10^{9}$	$1.2x10^{7}$	$1.1x10^{4}$
	23	4.80		NT		NT		0.66		.186		.163		$2.4x10^{9}$	$1.3x10^{9}$	$ca\ 10^{8}$	2.5x105
1	24	4.88	4.67	NT	NT	NT	NT	0.73	0.78	.120	.173	.061	.173	$1x10^{11}$	$2.9x10^{10}$	$2.2x10^{6}$	$1.1x10^{4}$
	25	4.50		NT		NT		0.81		.044		.203		$4.1x10^{10}$	$3.3x10^{10}$	$9.8x10^{6}$	$1.3x10^{5}$
	26	4.50		NT		NT		0.91		.342		.326		$> 10^{12}$	$ca5x10^{12}$	$1.3x10^{7}$	$1.5x10^{6}$
	27	4.65		95.5		0.51		0.87		.134		.124		$3.8x10^{8}$	$3.3x10^{9}$	$8.3x10^{6}$	$9.6x10^{6}$
2	28	4.70	4.46	100!	92.9	0.49	0.73	0.45	0.84	.058	.250	.076	.268	$1.3x10^{11}$	$4.3x10^{11}$	$1.1x10^{8}$	$1.1x10^{6}$
	29	4.50		76.1		1.43		0.69		.130		.089		$2.1x10^{10}$	$3.1x10^{11}$	$3.4x10^{6}$	$1x10^{6}$
	30	4.00		100!		0.49		1.36		.678		.782		$ca3x10^{13}$	$ca8x10^{13}$	$8.1x10^{6}$	$1.4x10^{7}$
	31	3.65		73.9		4.81		0.38		.042		.126		$3.1x10^{12}$	$ca3x10^{13}$	$1.1x10^{9}$	$2.6x10^{5}$
3	32	3.90	4.33	94.9	85.5	1.41	1.71	0.92	0.60	.466	.136	.407	.158	$3.2x10^{14}$	10^{16}!	$7x10^{5}$	$5.3x10^{4}$
	33	4.87		93.2		0.13		0.57		.018		.054		$ca7x10^{10}$	$5.8x10^{10}$	$1x10^{7}$	$2.1x10^{6}$
	34	4.90		79.9		0.48		0.63		.019		.043		$3x10^{7}$	$6x10^{7}$	$3.3x10^{6}$	$3.4x10^{6}$
	35	3.88		73.9		0.87		0.72		.041		.181		$ca2x10^{15}$	$2.6x10^{12}$	$2.2x10^{10}$	$7.2x10^{7}$
7	36	5.00	4.42	82.8	76.6	0.51	0.85	0.63	0.73	0	.022	.064	.165	$5x10^{7}$	$1x10^{7}$	$7.5x10^{6}$	$2.7x10^{4}$
	37	4.37		73.0		1.18		0.85		.026		.249		$2.1x10^{11}$	$3.4x10^{11}$	$3x10^{7}$	$1.1x10^{7}$

Table III.8. Analyses of cane juices, Experiment 'i'.

NT = Not Tested

case were LAB, although AAB were almost as numerous initially. The latter, however, multiplied only slowly and within two days were insignificant compared with the former. The yeast population also increased slowly but did not attain numbers higher than 10^8/ml., even after 12 days. The LAB appeared to be predominantly Leuconostoc mesenteroides, forming raised, clear, slimy colonies on sucrose agar, although other types (probably belonging to the genus Lactobacillus) also appeared. The principal AAB were probably Aerobacter aerogenes initially, but after storage, other types appeared, including yellow, pigmented mucoid organisms.

The physical and chemical analyses showed very wide variations between individual stalks on any one day, and since the number of replicates was necessarily few, mean values are probably not truly representative. However, some general trends are apparent. Deterioration during storage was shown by reduction in pH and purity, and increases in reducing sugars (R.S.), gum, dextran and viscosity. The rise in R.S. content was steady in sample 'h', but did not occur in sample 'i', nor did the mean purity decrease very much in sample 'i'. Gum and dextran contents, and viscosity appeared to reach maximum values by day 2 and then decline. A possible explanation is that the enzyme dextransucrase produced by Leuconostoc mesenteroides is inactivated at pH values much below 4.8 (Jeanes, 1965), and also the molecular weight and viscosity of dextran decreases. Sample 'i' had not deteriorated as much as sample 'h' after 7 days storage, and the bacterial counts were generally lower this may be due to the lower relative humidity during storage. The results reveal that significant sucrose loss can occur in stalks stored for two days prior to milling (e.g. the purity drop was 3.3% in sample 'h' and 1.2% in sample 'i').

j) Experiment 'j' A batch of freshly harvested, burnt cane stalks was despatched by air freight from Frome, Jamaica, on 2nd July 1968. The batch was similar to previous samples 'h' and 'i', except that a BOAC strike delayed its arrival until 17th July and 15 days elapsed between harvesting and examination; the refrigeration history of the sample was unknown.

Storage Time in Days	Stalk No.	Physical and chemical examination						Microbiological examination counts per ml. juice			
		pH	Purity	R.S. %w/v	Gum %w/v	Dextran %w/v	Specific Viscosity	Total	LAB	AAB	Yeasts
0	38	4.04	31.0	4.08	0.22	0.043	0.124	2.4×10^{11}	2.0×10^{10}	4.6×10^{7}	6.7×10^{7}
	39	4.00	60.4	3.08	1.34	0.960	1.210	8.4×10^{10}	1.3×10^{9}	$< 10^{3}$	4.5×10^{5}
	40	4.38	70.7	2.98	0.77	0.600	0.432	5.8×10^{10}	8.0×10^{8}	1.1×10^{6}	2.0×10^{4}
	41	4.50	61.1	2.46	0.53	0.094	0.106	7.2×10^{10}	5.2×10^{10}	2.6×10^{6}	2.6×10^{5}
	42	3.95	56.1	3.70	0.96	0.804	0.847	8.0×10^{10}	3.5×10^{11}	$< 10^{3}$	2.0×10^{5}
	43	4.02	39.1	2.29	0.57	0.144	0.435	1.2×10^{10}	1.1×10^{10}	$< 10^{3}$	6.1×10^{5}
1	44	5.13	98.8!	0.77	0.61	0.048	0.152	6.2×10^{8}	8.6×10^{8}	8.8×10^{5}	1.1×10^{5}
	45	3.73	47.4	4.34	1.32	0.888	1.300	8.0×10^{7}	5.0×10^{7}	$< 10^{3}$	1.2×10^{6}
	46	4.60	96.5!	1.16	0.30	0.054	0.215	3.0×10^{9}	2.7×10^{9}	1.2×10^{7}	4.7×10^{5}
	47	4.25	40.2	5.70	3.08	1.320	7.500	1.1×10^{9}	1.3×10^{9}	4.3×10^{7}	3.4×10^{6}
	48	4.80	100!	0.60	0.55	0.048	0.209	1.5×10^{9}	1.3×10^{9}	9.4×10^{6}	8.6×10^{5}
	49	3.96	80.4	1.83	0.26	0.084	0.171	2.7×10^{9}	3.0×10^{9}	2.4×10^{7}	1.1×10^{6}
5	50	3.70	77.6	1.70	0.56	0.038	0.096	8.8×10^{8}	2.2×10^{8}	2.3×10^{9}	5.3×10^{7}
	51	4.00	99.3!	1.18	0.58	0.038	0.130	1.9×10^{8}	8.0×10^{7}	2.2×10^{7}	8.2×10^{5}
	52	3.45	52.1	2.76	0.43	0.101	0.169	2.2×10^{8}	8.0×10^{7}	2.0×10^{5}	4.1×10^{6}
	53	3.02	54.0	4.11	1.17	0.636	0.965	2.5×10^{9}	1.8×10^{9}	2.6×10^{9}	2.6×10^{7}
	54	3.50	69.8	2.43	0.67	0.156	0.394	1.8×10^{10}	2.5×10^{9}	1.6×10^{9}	4.4×10^{6}
	55	3.67	84.3	1.14	0.39	0.036	0.159	2.4×10^{9}	1.5×10^{9}	2.5×10^{9}	2.6×10^{6}

Table III.9. Analyses of cane juices, Experiment 'j'.

The cane had deteriorated in transit so that storage experiments were of limited value. The experiments were similar to those for samples 'h' and 'i', except that six stalks were examined at intervals of 0, 1 and 5 days, and only the principal groups of microorganisms were counted. The cane was stored over saturated KNO_3, providing 91% R.H. at 30°C.

The results are summarised in Table III,9.

Nearly all samples possessed an initial high count of LAB, and had marked symptoms of deterioration (i.e. low pH, low purity, high R.S., gum and dextran content, and high viscosity). After one day's further storage the counts of LAB dropped slightly, whilst those of the AAB and yeasts rose. The AAB were mainly large, mucoid, yellow or cream colonies. There were some anomalous results; i.e. stalks 44, 46 and 48 were not badly deteriorated, whereas stalks 45 and 47 were. At 5 days the LAB count had decreased further, whilst yeasts and AAB had increased. The AAB were small, orange-brown colonies. Juice analyses showed very low pH values (3.02 to 4.00), but purities were inconsistent and the gum content was not excessive except in stalks 53 and 54.

It is probable that most of the deterioration had occurred in transit. In view of this, no attempts were made to correlate symptoms with storage time, or physical and chemical analyses with microbiological observations.

21 isolates of LAB were identified as :- Leuconostoc mesenteroides, 13 isolates; L.dextranicum, 6 isolates; Lactobacillus confusus (new species), 2 isolates.

k) Relationship between gum content, dextran content and viscosity of cane juice One of the most harmful effects of sour cane is an increase in the content of gums, especially dextran, in the extracted juice; this increases the viscosity of the juice and leads to processing difficulties in the factory (Chapter VII). Therefore it is desirable to be able to estimate the effect of gums and dextran on the viscosity of cane juice. Furthermore, these three parameters warranted consideration for use as a method of assessment of the degree of deterioration of cane (Chapter VIII).

A study was made of the relationships between dextran content, gum content and viscosity, utilising the data obtained from juice analyses of 37 stalks of stored, harvested cane in Experiments 'h' and 'i'. Statistical analysis was used to calculate regression and correlation coefficients for the data. Linear relationships were found between gum content and $\log_{10}$ specific viscosity (Fig.III,3), dextran content and $\log_{10}$ specific viscosity (Fig.III,4), and gum content and dextran content (Fig.III,5). The correlation coefficients are shown in Table III,10.

In another experiment, the relationships between gum content, dextran content and absolute viscosity were studied using pure cultures of known gum-forming organisms isolated from sour cane. 43 isolates of Leuconostoc mesenteroides and Lactobacillus species were inoculated into sterile synthetic cane juice medium (SCJ). The cultures were incubated for 3-5 days at 30°C and then centrifuged to remove bacterial cells. The supernatants were analysed for gum content, dextran content and viscosity by methods described in Chapter II. The correlation coefficients are given in Table III,10.

Parameters	Correlation coefficient,'r'	
	cane stalks 1-37	SCJ medium
~~Green~~ Gum content and $\log_{10}$ specific viscosity*	+ 0.446	+ 0.535
Dextran content & $\log_{10}$ specific viscosity	+ 0.416	+ 0.658
Dextran content and gum content	+ 0.405	+ 0.429

Table III,10. Comparison of the correlation coefficient between gum content, dextran content and viscosity in natural and synthetic cane juice.

There was a significant correlation between all three pairs of parameters, but the correlation coefficient 'r' was low, and the standard error of the means was high. This indicates that it is not possible to estimate accurately the viscosity of cane juice from either its gum or dextran content, and vice-versa. Similar arguments are applicable to the relationship between gum and dextran content. The low values of 'r' may be explained by the wide

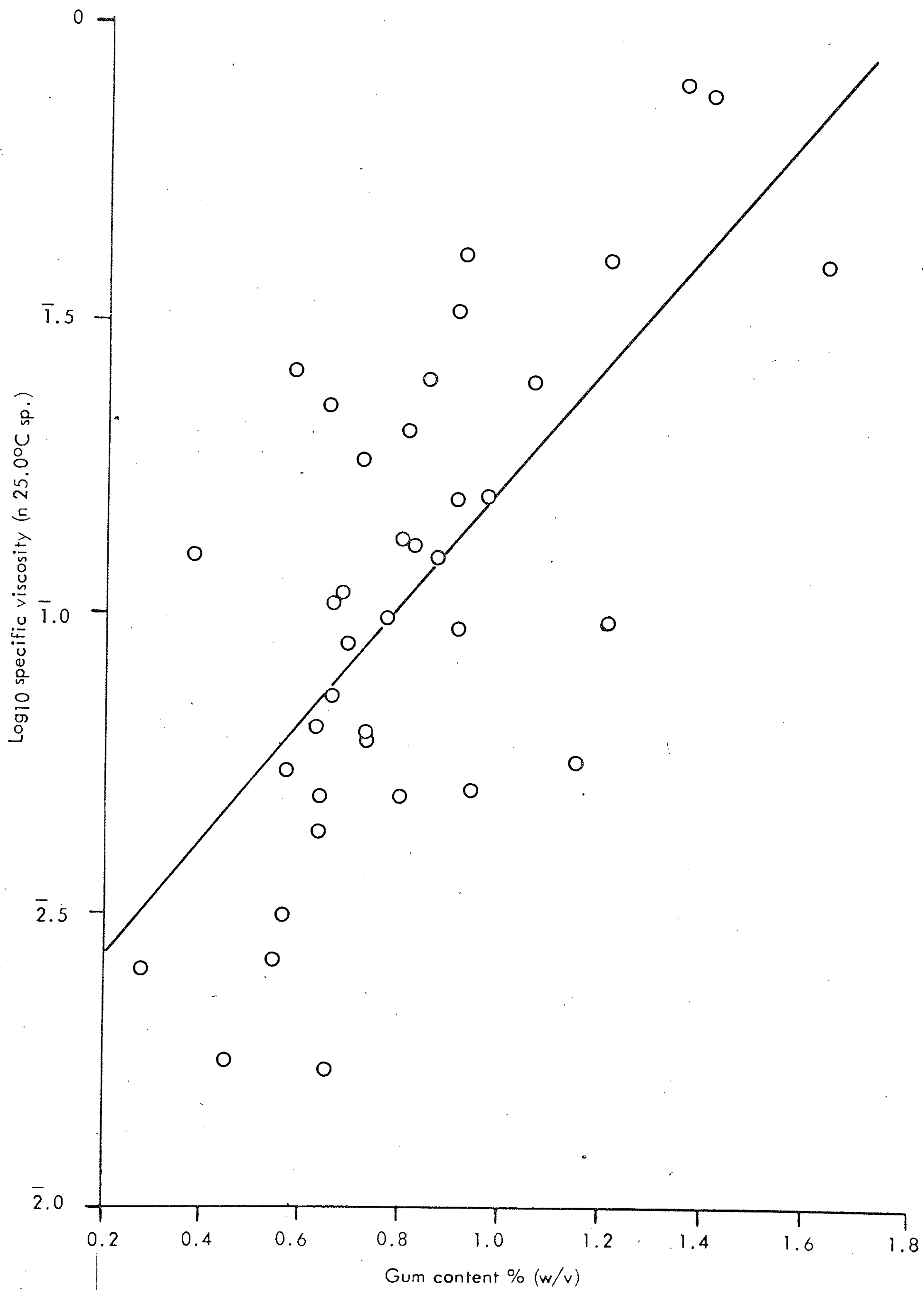


Fig.III,3. The relationship between gum content and specific visocosity of cane juice from Experiments 'h' and 'i'.

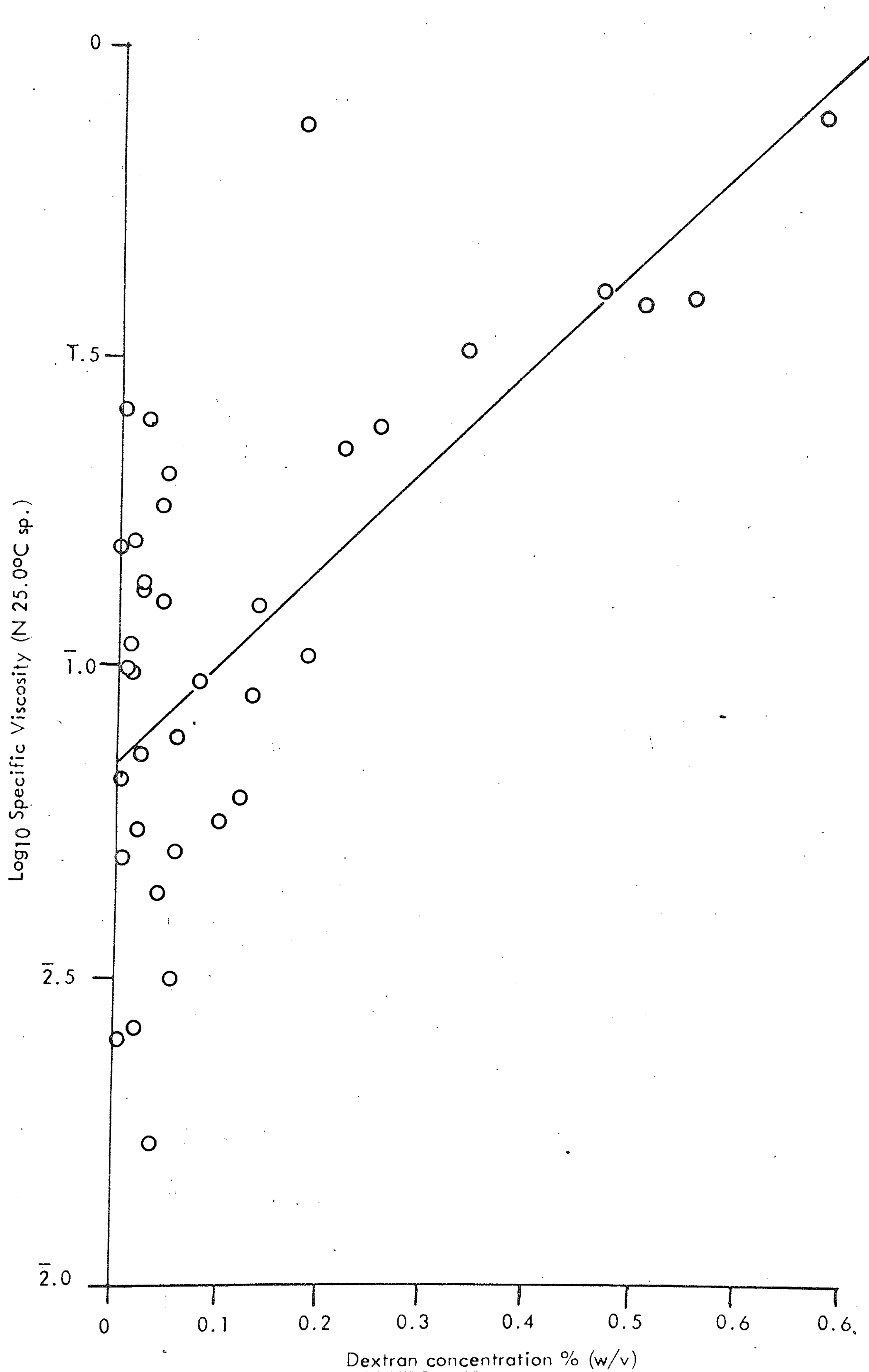


Fig.III,4. The relationship between dextran content and specific viscosity of cane juice from Experiments

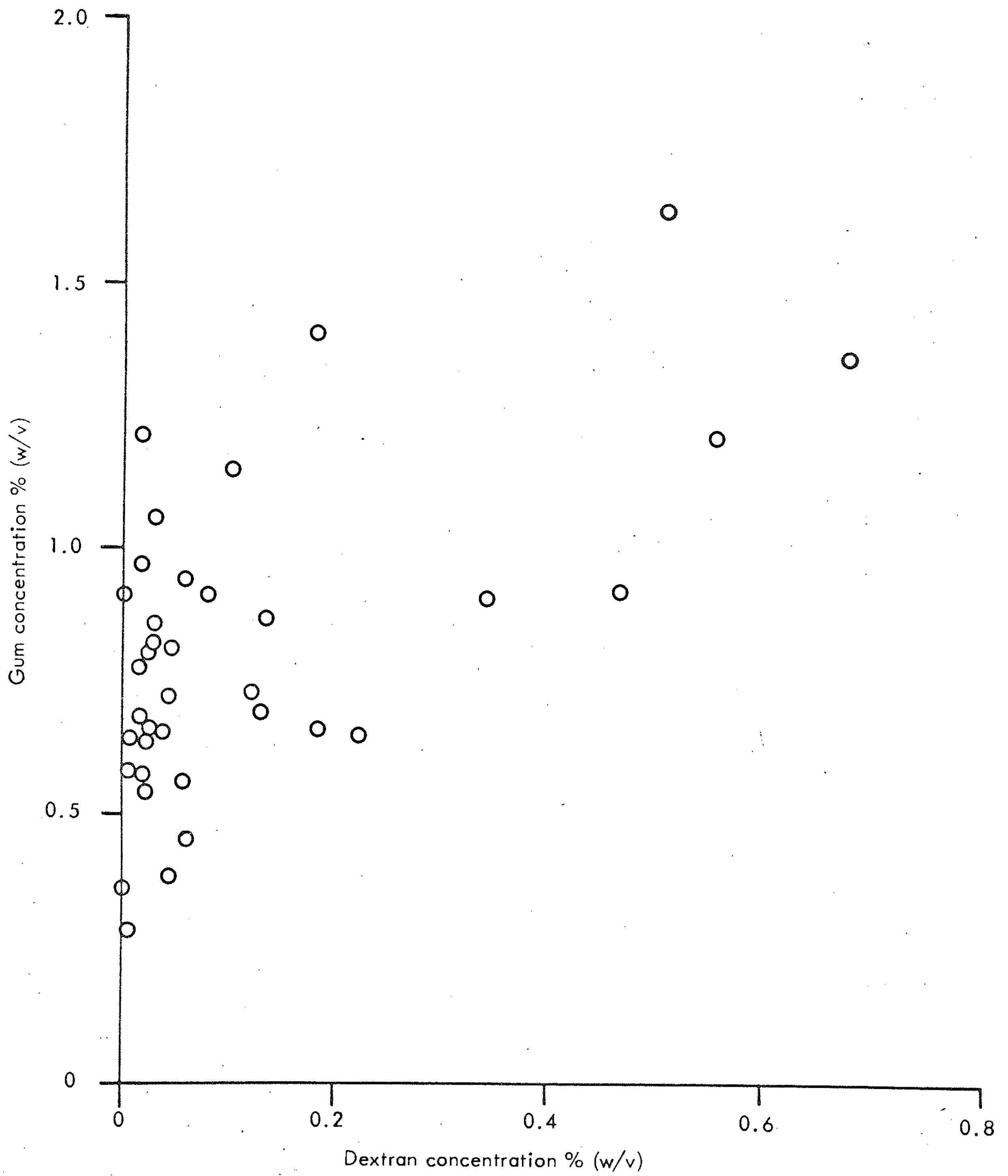


Fig.III,5. The relationship between dextran and gum content of cane juice from Experiments 'h' and 'i'.

variation reported in properties of dextrans formed by different strains of *Leuconostoc mesenteroides* (Jeanes et al, 1954; Jeanes, 1965). Despite these objections, all three parameters appeared to justify further study for their potential application in estimation of cane deterioration, in the apparent absence of more suitable alternatives (Chapter VIII).

2. Storage experiments on burnt, manually-harvested, whole-stalk cane, Frome, 1969.

The experiments reported in section 1., which utilised cane air-freighted from Jamaica to the U.K., provided qualitative indications of the changes that occur in harvested cane during storage. However, due to the imposition of unavoidable constraints, these results were obtained under '*in vitro*' conditions which differed significantly from those existing in the field in Jamaica. Therefore a series of replicated trials on harvested cane were undertaken at Frome during the 1969 crop, in order to obtain statistically reliable data on the microbiological, physical and chemical changes which occur during storage under '*in vivo*' conditions.

Several factors were standardised throughout the trials in order to minimise sources of uncontrolled variation. All experiments utilised manually-harvested, whole-stalk, mature cane from fields which were reaped according to the Estate's own programme for factory supply. All the cane was burnt one day prior to harvest, since this is the normal practice in Jamaica. The fields were selected at random from each of the Estate's ten farms throughout the period studied, in order to obtain a representative cross-section of cane grown under a range of conditions of soil type and climate.

57 replicated trials were initiated over the period March - July, 1969, of which 45 trials were successfully completed. 39 experiments utilised variety B4362, and 6 experiments utilised variety B51129, which constituted 73% and 12% of the harvested crop respectively. Three experiments were initiated per week, commencing on a Monday, Tuesday and Wednesday respectively.

The trials were classified into 4 groups, A,B,C and D, based on climatic data and degree of maturity in the period studied, (Table III,11).

Experimental Period	Date: week ending (Sat.)	No. of Wet Days Total Weekly *	Rainfall (inches) Total Weekly	Rainfall (inches) Total Weekly Mean	Temperature °F Mean ** Maximum	Temperature °F Mean ** Minimum	Age of cane at harvest (months) Mean	Experiment Nos. B4362	Experiment Nos. B1129
A	April 5	4	2.65	2.11	89.9	69.1	13.2	7	-
	April 12	6	5.21					10;12	11
	April 19	3	2.37					13;14	15
	April 26	0	0					18	16
	May 3	4	1.41					19;21	-
	May 10	2	1.00					22;24	23
B	May 17	5	4.16	5.82	90.3	71.5	14.1	25;26;27	-
	May 24	5	5.24					28;29;30	-
	May 31	7	8.07					32;33	31
C	June 7	0	0.18	1.08	90.6	73.4	14.8	36	-
	June 14	3	1.35					37;38;39	-
	June 21	2	2.48					40;41;42	-
	June 28	1	0.29					43;44;45	-
D	July 5	4	1.37	2.28	92.2	72.2	15.1	46;48	47
	July 12	3	0.67					49;50;51	-
	July 19	4	3.01					52;53;54	-
	July 26	5	4.68					55;56;57	-
	Aug. 2	3	1.69						
								-	-

Table III.11. Climatic data and distribution of 45 experiments on the storage of harvested cane, Frome, 1969.

* Days which received more than 0.1" rain   ** Recorded in the factory area

For various reasons the 1969 crop extended beyond its planned finishing date, and extended into the rainy season. This resulted in progressive overmaturity and decline in cane quality between April and July. Period A (April and early May) had light to moderate rainfall and slightly lower temperatures than the other periods; Period B (mid- to late-May) contained the high rainfall associated with the normal May/June rainy season; Period C (June) had generally light rainfall and higher ambient temperatures, whilst period D (July) had moderate rainfall, high ambient temperatures and overmature cane.

The experimental procedure used in each trial is described below :-

200 healthy stalks were cut from the chosen field between 8-9 am. In order to obtain a representative sample, eight sampling locations were selected these were distributed at equidistant intervals along the two opposite ends of the field. 25 stalks were cut from each location, at a depth of 20-30 feet in along the row. The cane was immediately transported to the factory where it was mixed thoroughly and re-distributed into eight 25-stalk bundles. These were tied, labelled, weighed to the nearest $\frac{1}{4}$lb., and laid on level grassy ground near the factory, without protection from the elements.

One bundle was crushed and analysed immediately (time lapse between cutting and crushing was $1\frac{1}{2}$ to 2 hours); the remainder were stored, and one bundle was removed for crushing at daily intervals ($\pm$ one hour) up to ten days storage time, except for days 8 and 9. Each bundle was re-weighed before crushing. The cane was crushed in an electrically-driven, 3-roller, small mill at constant pressure. In experiments 1-35 (Periods A & B) the cane was passed through the mill until the weight of extracted juice was 50% $\pm$ 1% of the weight of cane at crushing; in subsequent experiments, (Periods C & D), the cane was passed through the mill a standard three times, and the weight of extracted juice recorded; the latter procedure parallels more closely the actual conditions existing in the factory. Between each sample the mill was 'sterilised' by physical cleaning and treatment with chloride of lime solution, followed by a rinse with boiler condensate water. Juice samples were collected in clean containers and mixed thoroughly before sub-samples were taken for analysis.

Where possible, extracted juices were analysed immediately; otherwise, they were preserved by methods described in Chapter II. The following physical, chemical and microbiological analyses were determined for each juice

Brix, pol, pH and reducing sugars (R.S.) were determined by standard methods described in Chapter II; the apparent refractive purity was calculated True sucrose content could not be determined because of lack of facilities. The dextran content and absolute viscosity of the samples was determined by methods described in Chapter II; specific viscosity was calculated from the absolute viscosity. Gum content was not determined, because dextran was thought to be a more sensitive indicator of deterioration. Microbiological examination was confined to total spoilage organisms, lactic acid bacteria, and yeasts, by methods given in Chapter II. Selected microorganisms were chosen for purification and subsequent identification. The choice of these three groups of organism was based on earlier observations, reported in section 1., that LAB and yeasts were the predominant microorganisms in juice from stored cane. However, the use of STA permitted growth of any microorganism, including AAB, capable of growth in normal cane juice.

An example of the complete data for one typical replicate experiment (no.30) is presented in Table III,12. This trial was conducted during the high rainfall Period B, and heavy rainfall occurred on the day of harvest and each subsequent day of the experiment. The cane shows clear evidence of bio-deterioration, since it was heavily infected with Leuconostoc mesenteroides. This organism multiplied rapidly, reaching a maximum count of 10^8 per ml. after three days storage time; its growth was accompanied by rapid increases in dextran content, viscosity, and R.S., and rapid decreases in pol, purity and pH. Only slight weight loss occurred, due to the high rainfall and ambient relative humidity.

In contrast, the results of another experiment (no.50), are shown in Table III,13. This trial was conducted in Period D (July), when the weather was hot and mainly dry; no rainfall occurred on the day of harvest. The cane had a low initial microbial count, which increased slowly at first but

Parameter	Storage Time in Days							
	0	1	2	3	5	6	7	10
Original wt.lb.	74.0	71.0	74.5	73.5	66.5	68.5	74.5	68.5
Wt. at grinding	74.0	70.5	73.0	72.25	64.5	66.5	71.0	67.25
% wt. loss	nil	0.7	2.0	1.7	3.0	2.9	4.7	1.8
Wt. juice	38.0	36.0	36.25	36.25	32.0	34.5	35.25	34.25
Juice % cane	51.3	51.0	49.6	50.1	49.6	51.8	49.6	51.0
Phys/Chem.Analysis								
Ref. brix	20.6	20.2	20.0	19.2	20.4	20.2	21.6	19.4
% pol	19.0	19.1	18.3	17.5	17.4	16.3	16.4	14.4
App. purity	92.3	94.6	91.4	91.4	85.3	80.6	76.1	74.1
R.S. % w/w	0.496	0.616	0.683	0.526	1.05	1.25	1.17	1.27
pH	5.0	5.0	4.7	4.8	4.6	4.6	4.2	3.9
Dextran % Bx	0.049	0.025	0.177	0.484	0.821	1.250	0.900	1.055
Specific Viscosity	0	0.005	0.004	0.100	0.112	0.193	0.174	0.238
Microbiological Analysis								
Total spoilage count/ml.	9.9×10^5	4.1×10^6	3.1×10^7	8.8×10^7	7.5×10^7	1.8×10^7	3.9×10^7	2.4×10^7
Leuconostoc count/ml.	9.1×10^5	2.8×10^6	2.0×10^7	1.1×10^8	6.5×10^7	2.3×10^7	2.3×10^7	1.7×10^7
Yeast count/ml.	2.3×10^3	6.0×10^4	1.5×10^4	2.1×10^4	NT	NT	NT	7.6×10^4
Climatic conditions during storage								
Daily rainfall (inches)	1.71	0.42	0.85	1.78	0.32(4) 1.45(5)	0.65	1.73	0.67(8) 1.10(9) 2.15(10)
Temp. °F Maximum	90	88	89	89	91(4) 90(5)	90	90	86
Minimum	70	74	74	73	71(4) 73(5)	72	74	74

Table III.12. The effect of storage time on harvested cane, Experiment no.30.

Date reaped: 21.5.69; Date burnt: 20.5.69; Time reaped: 9 am; Variety: B4362; Farm: Shrewsbury (No.4); Field: Pear Tree; Age of cane: $13\frac{3}{4}$ months; Ratoon: 5th.

NT = Not Tested

Paramter	Storage Time in Days							
	0	1	2	3	4	6	7	10
Original wt. lb.	84.5	80.0	92.5	85.75	83.0	90.5	94.0	85.25
Weight at grinding	84.5	79.0	90.5	82.25	78.5	82.0	84.0	73.5
% Weight loss	nil	1.3	2.2	4.1	5.4	9.4	10.6	13.8
Weight juice	39.5	37.5	46.5	39.5	32.0	33.5	36.0	32.0
Juice % cane	46.7	47.5	51.4	48.0	40.8	40.9	42.9	43.5
Phys./Chem. Analysis								
Ref. brix	17.2	17.8	17.2	17.6	17.6	18.4	18.4	17.8
% pol	15.2	14.6	15.1	15.3	14.7	13.4	14.0	13.8
App. purity	89.7	82.0	87.7	87.1	83.7	72.7	76.1	77.3
R.S. % w/w	0.630	0.670	1.130	1.120	0.975	1.020	1.120	3.250
pH	5.5	4.5	4.3	5.0	4.6	4.6	4.4	4.5
Dextran % Brix	0.078	0.224	0.256	0.188	0.247	0.282	0.137	0.320
Specific viscosity	0.042	0.034	0.059	0.036	0.064	0.112	0.048	0.038
Microbiological Analysis								
Total spoilage count/ml.	$1.8x10^5$	$1.5x10^6$	$5.2x10^6$	$3.6c10^6$	$7.2x10^6$	$3.3x10^6$	$3.0x10^6$	$4.4x10^6$
Leuconostoc count /ml.	$1.7x10^5$	$7.0x10^5$	$5.3x10^6$	$3.7x10^6$	$2.3x10^6$	$1.7x10^6$	$1.9x10^6$	$9.0x10^5$
Yeast count/ml.	$9.2x10^4$	$2.8x10^4$	$3.0x10^5$	$2.0x10^4$	$1.9x10^5$	$8.0x10^5$	$3.8x10^5$	$9.3x10^5$
Climatic conditions during storage								
Daily rainfall (inches)	0	0.11	0.16	0	0	0(5)0(6)	0.20	1.48(8) 0.43(9)0(10)
Temp. ^oF Maximum	91	94	93	93	NT	92(5)95(6)	93	91
Minimum	72	72	74	72	NT	72(5)71(6)	71	73

Table III.13. The effect of storage time on harvested cane, Experiment no.50.

Date reaped: 8.7.69.; Date burnt: 7.7.69.; Time reaped: Noon; Variety: B4362; Farm: Barham (No.2); Field: Grange No.3, No.70; Age of cane: $16\frac{3}{4}$ months; Ratoon: 7th. NT = Not Tested

levelled out at a count of less than 10^7 per ml. juice. The predominant organism was Leuconostoc mesenteroides, but yeasts constituted approximately 1/10th of the total spoilage flora; no significant counts of other organisms were obtained. No significant increase occurred in dextran content and viscosity, and the decreases in pol, purity and pH were significantly less than in Experiment 30. The cane lost weight rapidly due to evaporation in the hot, dry climatic conditions.

It was concluded that the changes observed in Experiment 50 were mainly caused by normal staling, whereas in Experiment 30 the cane became sour due to infection by L.mesenteroides and exhibited more rapid deterioration. It is likely that the infection of cane in Experiment 30 was facilitated by moist climatic conditions, especially on the day of harvest, whereas in Experiment 50, dry conditions prevailed.

The overrall results are summarised graphically in Figs.III,6 to III,17. The effect of storage time on each parameter is presented in the form of six graphs; graphs A, B, C and D represent the mean data for all experiments in the corresponding seasonal period, variety B4362 only; graph E shows the mean results for all experiments with variety B4362, over the whole season; graph F is the mean of all experiments with variety B51129. Each point represents the mean of 10 replicate trials in Period A; 8 replicates in Period B; 10 replicates in Period C; 11 replicates in Period D; 39 replicates in 'E' and 6 replicates in 'F'. At storage times of 4, 5 and 6 days, however, the points are generally based on fewer replicates than those stated above, and are therefore of less significance.

The data for weight loss, pol, purity and extraction were analysed statistically in a computer. The regression equation, correlation coefficient, significance and 95% confidence limits were calculated.

The data for each parameter are discussed in the following sections. It should be noted that within each parameter the six graphs have a common scale and intercept on the x and y axes.

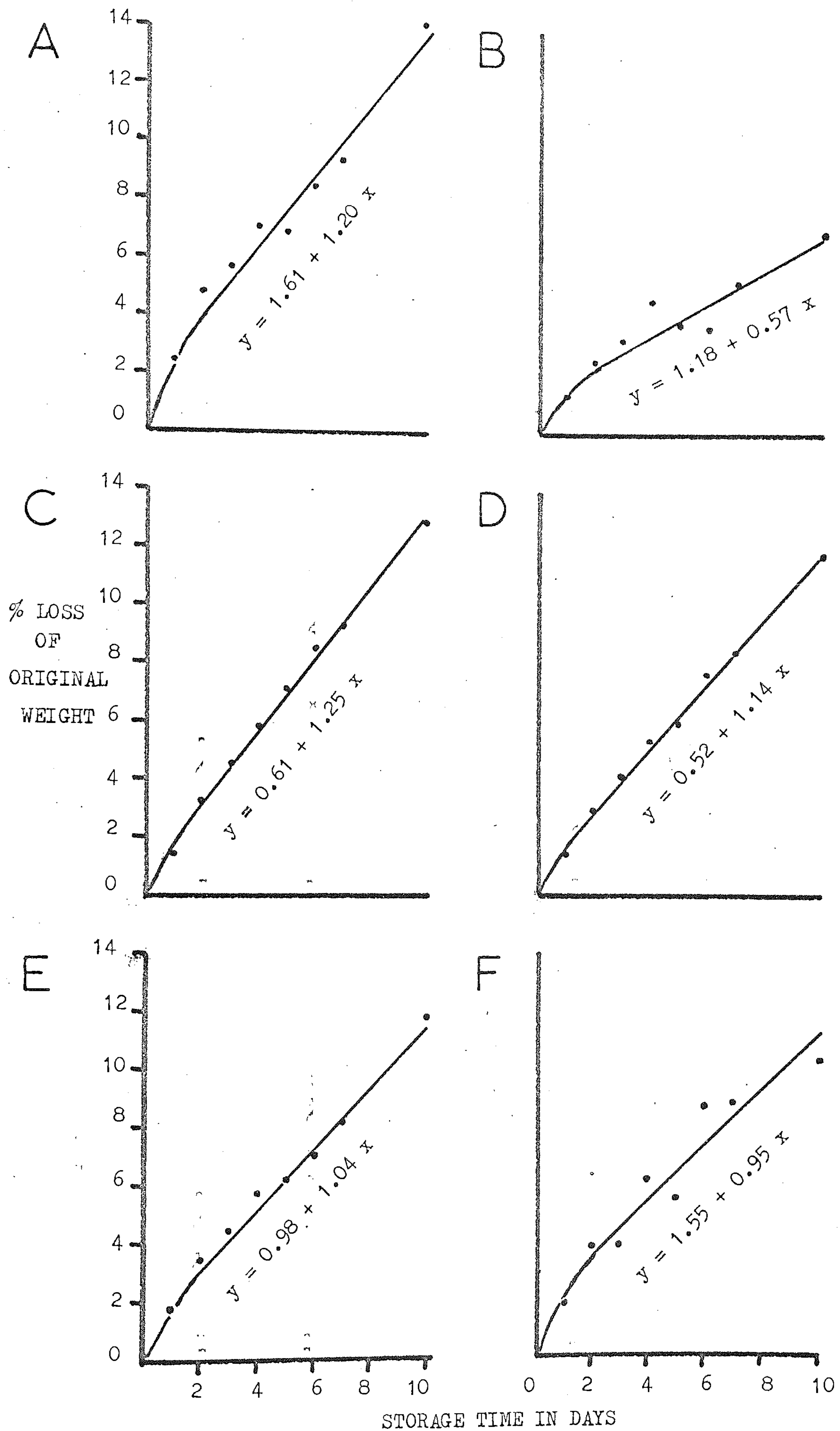


Fig.III,6. The effect of time of storage on the % weight loss in harvested cane.

a) % Loss of original weight of cane The results are summarised in Fig.III, 6, and the statistical analysis is summarised in Table III,14.

Experiment Group	Correlation Coefficient 'r'	Significance of 'r'- p	Mean Value of 'y'	Stand. deviation of 'y'	Variance ratio F	Significance of F - p	Mean daily % loss of original weight
A	.807	.001	7.45	4.49	113.6	.001	1.20
B	.692	.001	4.03	2.38	42.18	.001	0.57
C	.932	.001	6.61	3.87	448.4	.001	1.25
D	.931	.001	5.92	3.56	479.6	.001	1.14
E	.841	.001	6.00	3.58	271.0	.001	1.04
F	.720	.001	6.24	3.91	38.68	.001	0.95

Table III,14. Statistical analysis of the effect of time of storage (x) on loss of weight of harvested cane (y).

In all cases there was a highly significant correlation between weight loss and time of storage, but the standard deviation of the means was high; this indicates a wide between-replicate variation. The overrall mean rate of weight loss was similar for both varieties (E & F). Amongst variety B4362 the mean rate of weight loss was significantly lower in period B than in periods A, C & D. This may be explained by the wet climatic conditions that prevailed in period B, since the rate of loss of moisture from cane is inversely proportional to the ambient relative humidity.

b) Refractive Brix The results are summarised in Fig. III,7.

During periods A and B the brix was not significantly affected by time of storage, whereas in periods C and D the brix increased slightly in proportion to storage time. These differences are probably explained by the difference in crushing technique between these periods. Variety B51129 (F) showed a greater rate of brix increase than variety B4362 (E).

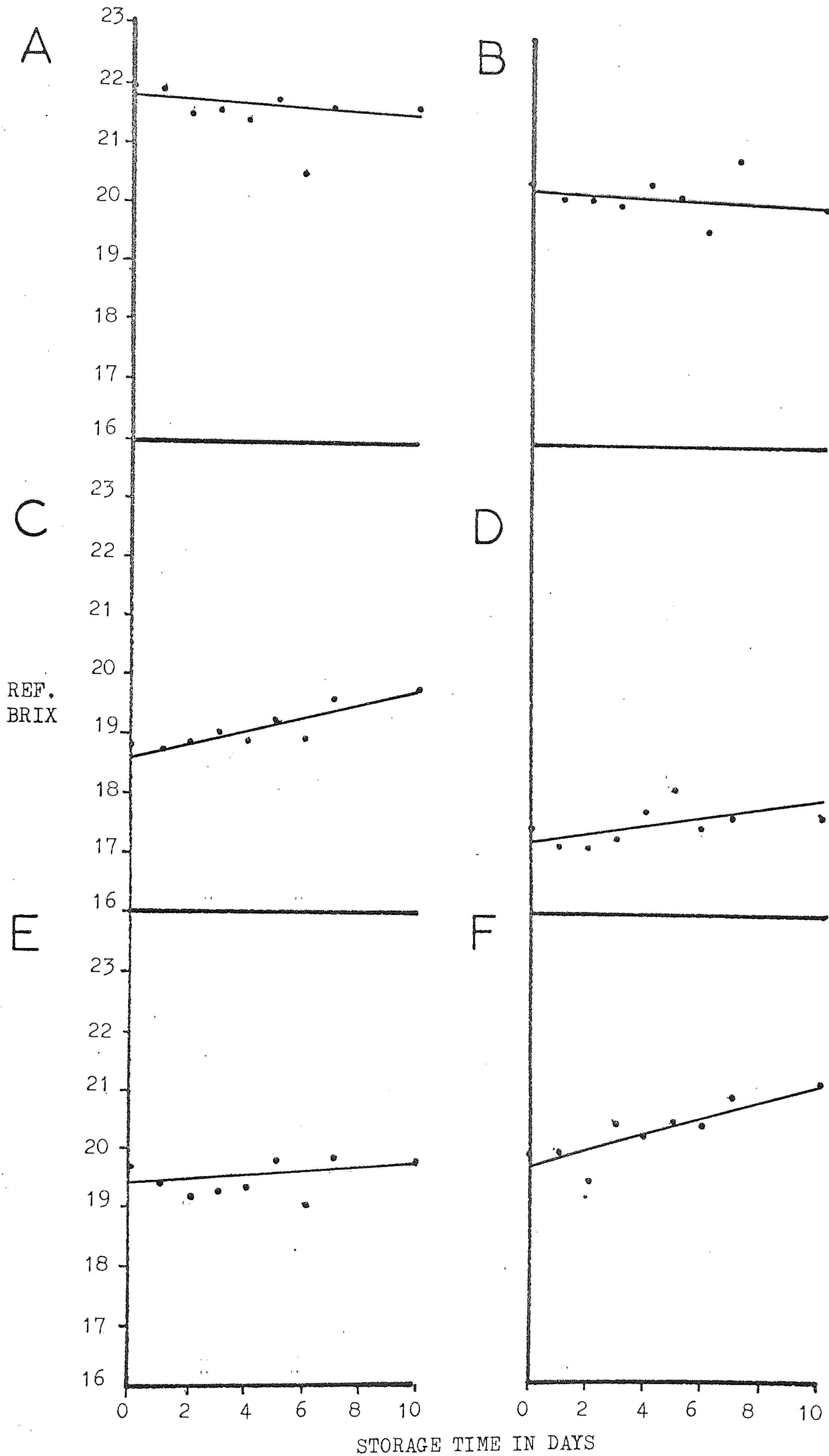


Fig.III,7. The effect of time of storage on the refractive brix of juice expressed from harvested cane.

c) % Extraction The results are shown in Fig.III,8, and statistical analyses are summarised in Table III,15.

Experiment Group	Correlation Coefficient 'r'	Significance of 'r'–p	Mean Value of 'y'	Stand. Deviation of 'y'	Variance ratio F	Significance of F – p	Mean daily loss in % extraction
A	– .249	.05	50.28	2.11	4.613	.05	0.16
B	–9.61	N.S.	49.83	1.65	0.5504	N.S.	0.05
C	– .529	.001	46.40	2.67	28.78	.001	0.44
D	– .263	.02	44.66	2.79	6.171	.05	0.24
E *	– .396	.001	45.53	2.73	17.46	.001	0.34
F	– .095	N.S.	50.58	3.17	0.3829	N.S.	0.09

Table III,15. Statistical analysis of the effect of time of storage (x) on % extraction of juice from harvested cane (y).

* E here = mean of periods C & D only

N.S. = not significant (p => 0.1)

In periods A and B, when the cane was crushed to give a weight of juice 50%, ± 1%, of the weight of cane at grinding, time of storage did not significantly affect the extracted juice % cane, and the objective was attained. However, in periods C and D, when cane was crushed by a standard three passes through the mill, the extracted juice % cane was significantly reduced with an increase in storage time. This is due to moisture loss by evaporation, which increases the insoluble fibre % cane and hence the proportion of non-extractable juice bound by the fibre. This effect reduces the yield of tons sugar per ton cane.

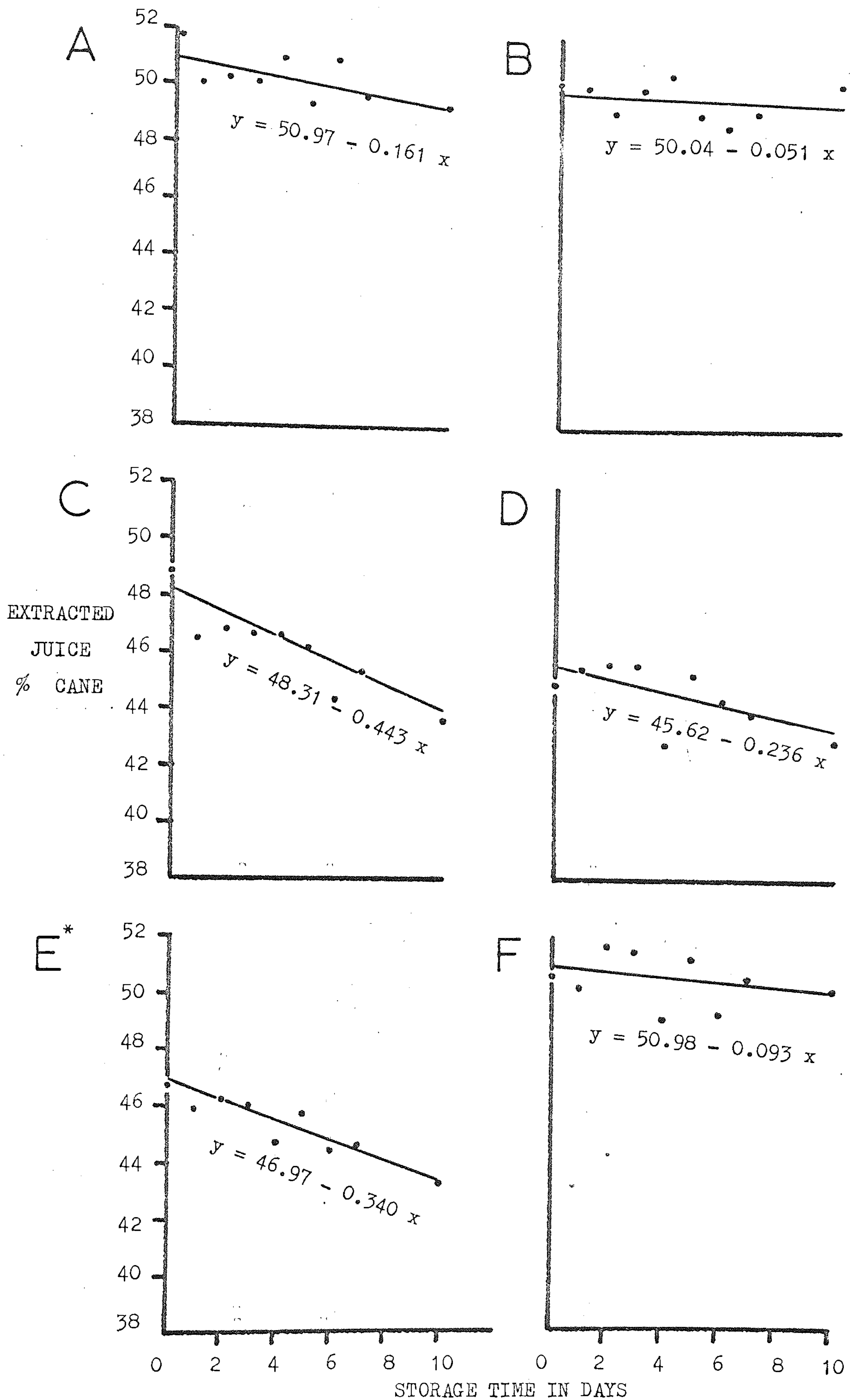


Fig.III,8. The effect of time of storage on the expressed juice % cane of harvested cane.

d) % Pol in juice The results are summarised in Fig.III,9, and statistical analyses are summarised in Table III,16.

Experiment Group	Correlation Coefficient 'r'	Significance of 'r' -p	Mean Value of 'y'	Stand. Deviation of 'y'	Variance ratio F	Significance of F - p	Mean daily loss in % pol in juice
A	- .675	.001	18.30	1.40	59.53	.001	0.29
B	- .773	.001	17.03	1.53	87.82	.001	0.38
C	- .732	.001	15.77	1.35	89.99	.001	0.32
D	- .680	.001	14.01	1.87	73.25	.001	0.40
E	- .715	.001	16.28	1.54	77.65	.001	0.35
F	- .284	.05	17.15	2.06	3.671	.1	0.18

Table III,16. Statistical analysis of the effect of time of storage (x) on % pol in juice of harvested cane (y).

There was a highly significant negative correlation between storage time and % pol in juice in all experiments with variety B4362 (A,B,C,D,E), but this relationship was only just significant for variety B51129 (F). The mean daily loss of % pol in juice was significantly greater in period B than in the periods before (A) and after (C). It is postulated that this was due to the increased rate of infection and growth of L.mesenteroides in the cane during this wet period. However, in July (period D) the rate of loss in pol reached a maximum; this may be due to overmaturity of the cane and greater activity of natural cane invertases. Variety B51129 (F) lost its pol at a rate only half that of B4362 (E), which suggests that it is less susceptible to post-harvest deterioration than the latter.

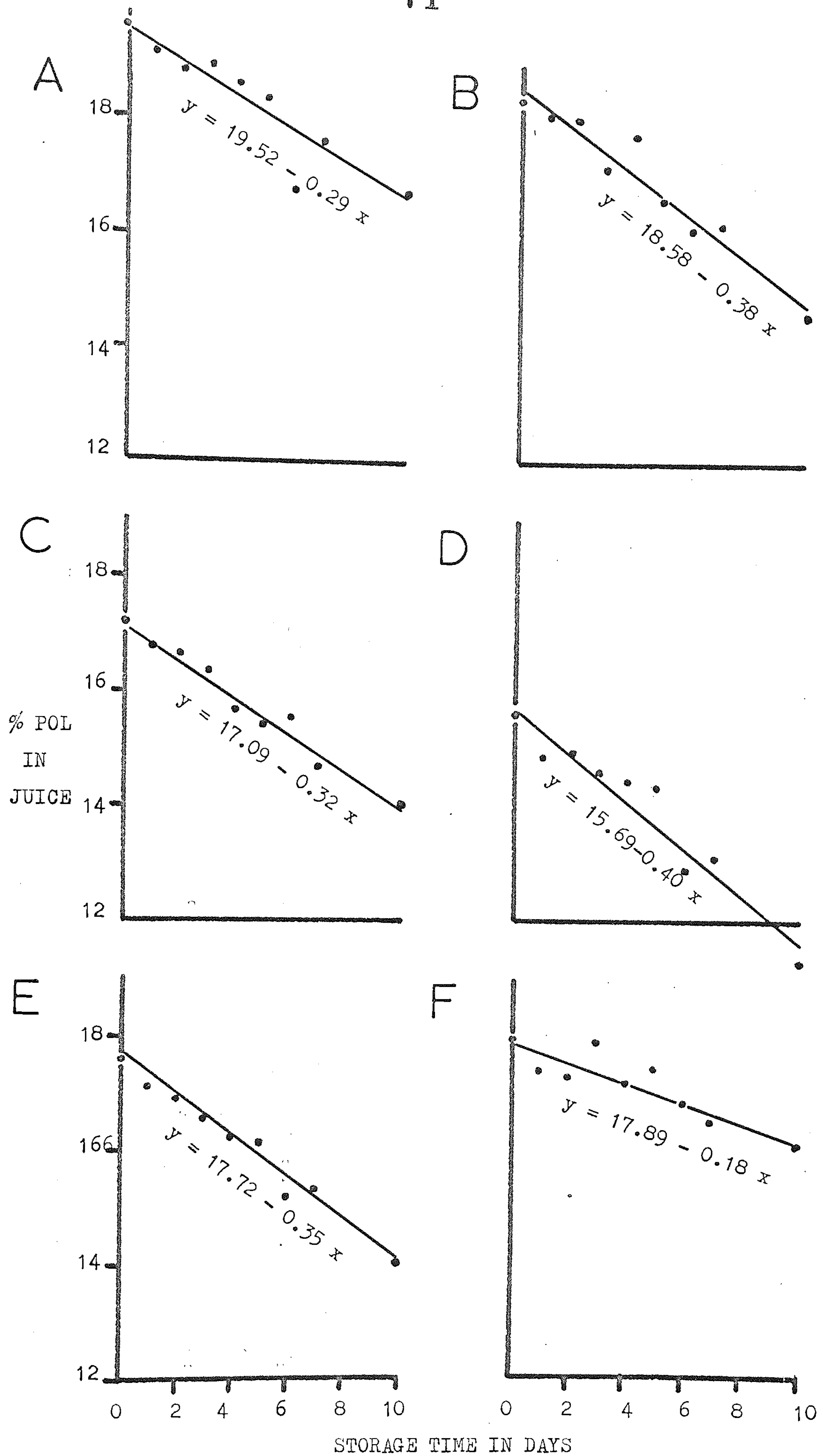


Fig.III,9. The effect of time of storage on the % pol in juice of harvested cane.

e) Apparent purity The results are summarised in Fig.III,10, and statistical analyses are summarised in Table III,17.

Experiment Group	Correlation Coefficient 'r'	Significance of 'r'-p	Mean Value of 'y'	Stand. Deviation of 'y'	Variance ratio F	Significance of F - p	Mean daily loss in apparent purity
A	- .665	.001	84.51	5.97	56.44	,001	1.22
B	- .845	.001	83.61	6.64	144.4	.001	1.78
C	- .869	.001	82.50	7.65	239.9	.001	2.12
D	- .846	.001	80.06	9.61	213.8	.001	2.58
E	- .806	.001	82.67	7.47	163.6	.001	1.93
F	- .793	.001	84.45	6.24	69.45	.001	1.53

Table III,17. Statistical analysis of the effect of storage time (x) on the apparent purity of juice from harvested cane (y).

In all experiments there was a highly significant negative correlation between storage time and apparent purity. With variety B4362 the rate of drop in purity increased throughout the season, which may be caused by progressive overmaturity of the cane. Unlike % pol, the rate of purity drop was not greater in period B than in periods A and C; therefore it cannot be concluded that the enhanced infection of cane by L.mesenteroides during period B had a significant effect on this parameter. Variety B4362 (E) appeared to drop in purity at a significantly greater rate than variety B51129 (f).

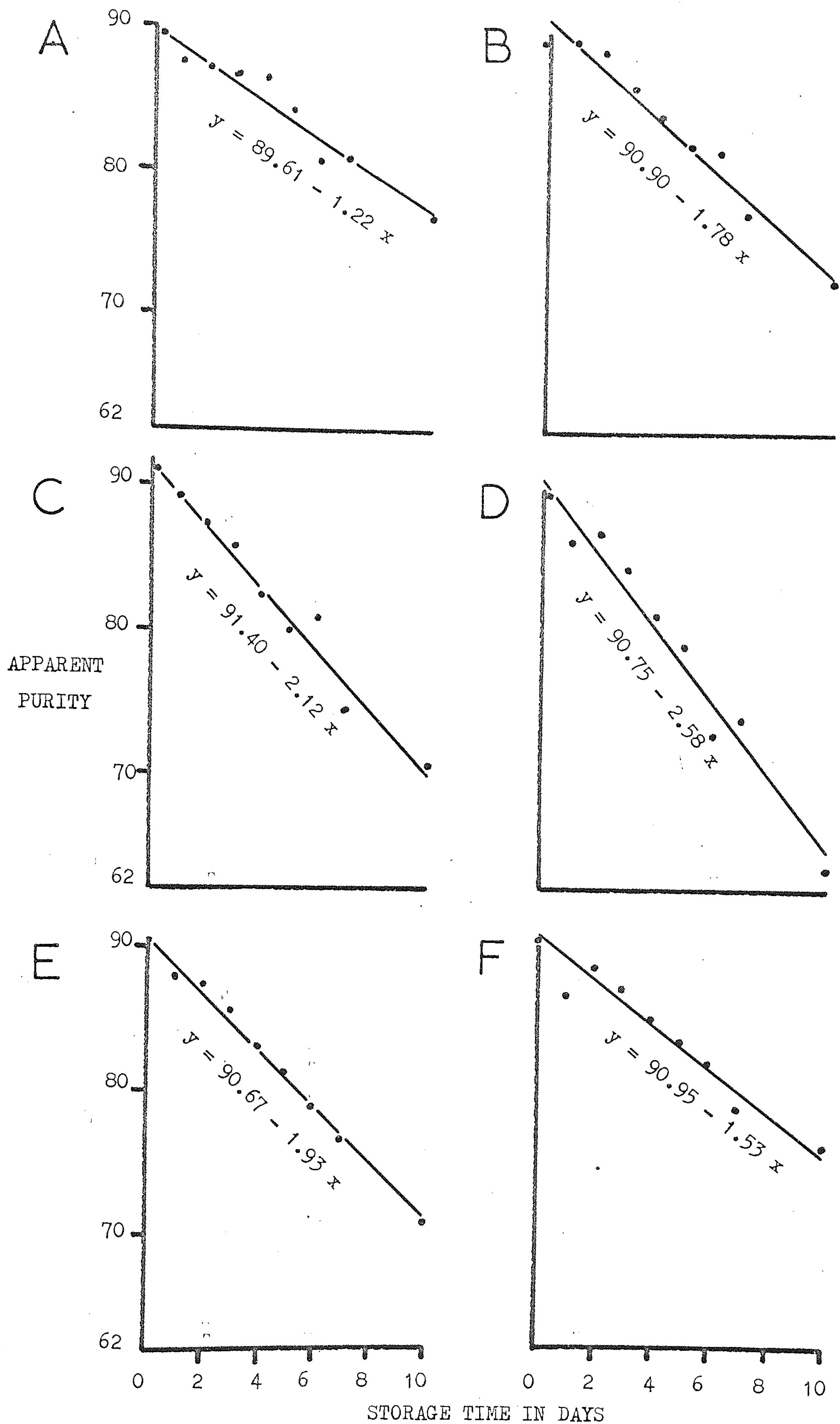


Fig.III.10. The effect of time of storage on the apparent purity of juice from harvested cane.

f) <u>Reducing sugar content (% R.S.)</u> The results are shown in Fig.III,11.

In all periods the R.S. content was directly proportional to storage time. However, in periods A, B and D the relationship appeared to be non-linear, although the shape of the curves was dissimilar. The mean results for variety B4362 (E) showed a smooth linear regression.

The mean daily increase in % R.S. was calculated for each graph by dividing the difference between final R.S. and original R.S. by ten :-

Experiment Group	Mean daily increase in % R.S.
A	0.017
B	0.069
C	0.080
D	0.096
E	0.067
F	0.021

The rate of increase in R.S. shows a steady rise throughout the season for variety B4362 (A,B,C,D), which again may be caused by progressive over-maturity of cane. Period B shows a higher rate of increase in R.S. than expected from the overmaturity effect, especially between storage times of 3 to 6 days. The shape of the curve is similar to the logarithmic growth curve of bacteria, and may be partly explained by the rapid growth of *L.mesenteroides* in this cane, accompanied by R.S. formation. Variety B51129 (F) showed significantly less R.S. formation than variety B4362 (E).

The overrall mean rate of increase in % R.S. for B4362 was 0.067% per day, compared with a mean drop in % pol of 0.35% per day; therefore only one-fifth of the sucrose lost was converted to R.S. as an end product.

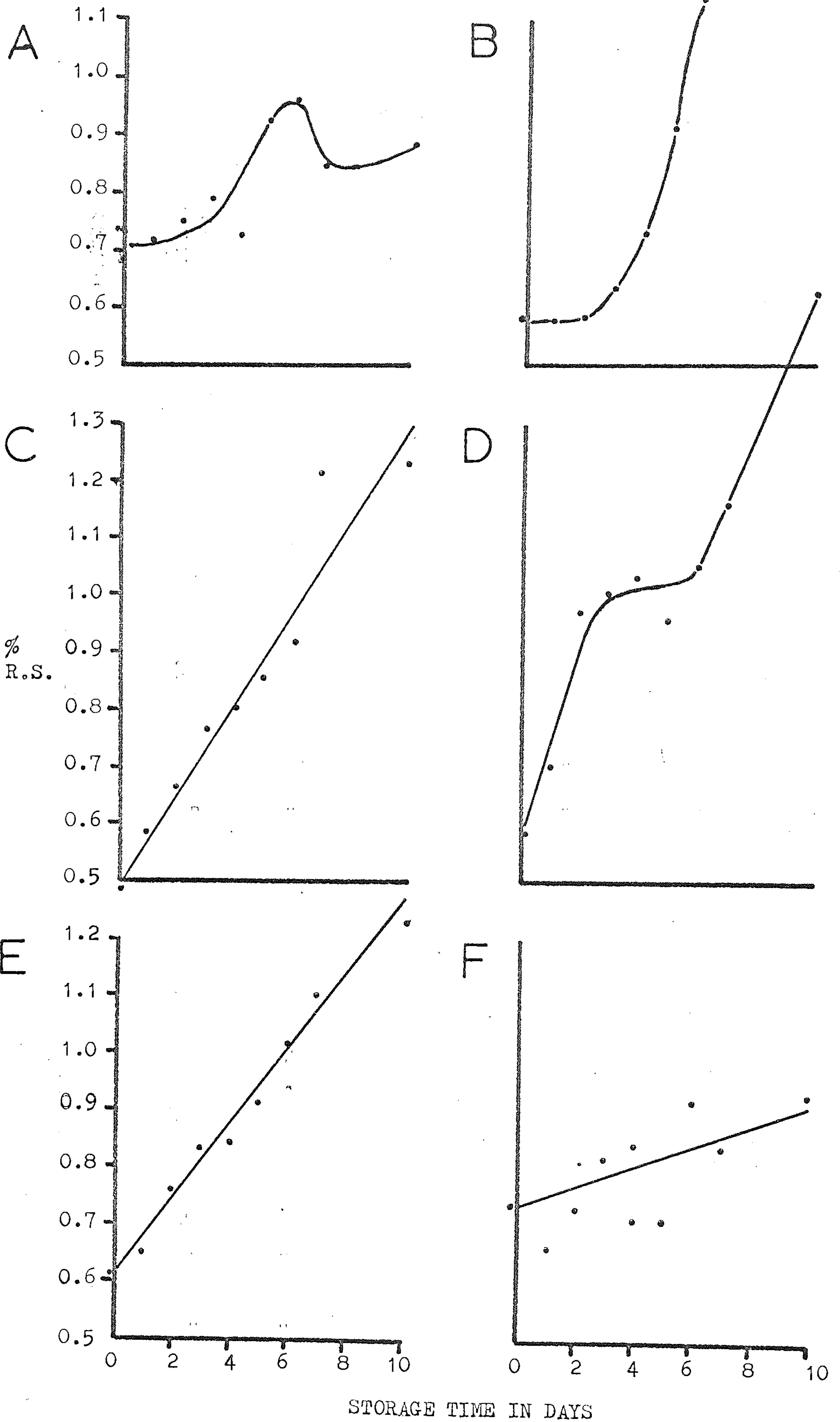


Fig.III.11. The effect of time of storage on the reducing sugar (R.S.) content of harvested cane.

g) pH The results are shown in Fig.III,12.

In all experiments the pH was inversely proportional to storage time. The relationship was almost linear, but the initial rate of fall in pH was generally faster than the final rate. Individual experiments showed a wide variation in pH at a given storage time. The mean daily drop in pH was calculated to be :-

Experiment Group	Mean daily drop in pH (units)
A	.049
B	.100
C	.072
D	.065
E	.072
F	.070

Overmaturity did not appear to cause a significant increase in the rate of pH drop, and the mean rates for both varieties was similar. However, the rate of fall in pH was significantly higher in period B than in any other period for variety B4362, which may be due to more rapid growth of *L.mesenteroides*.

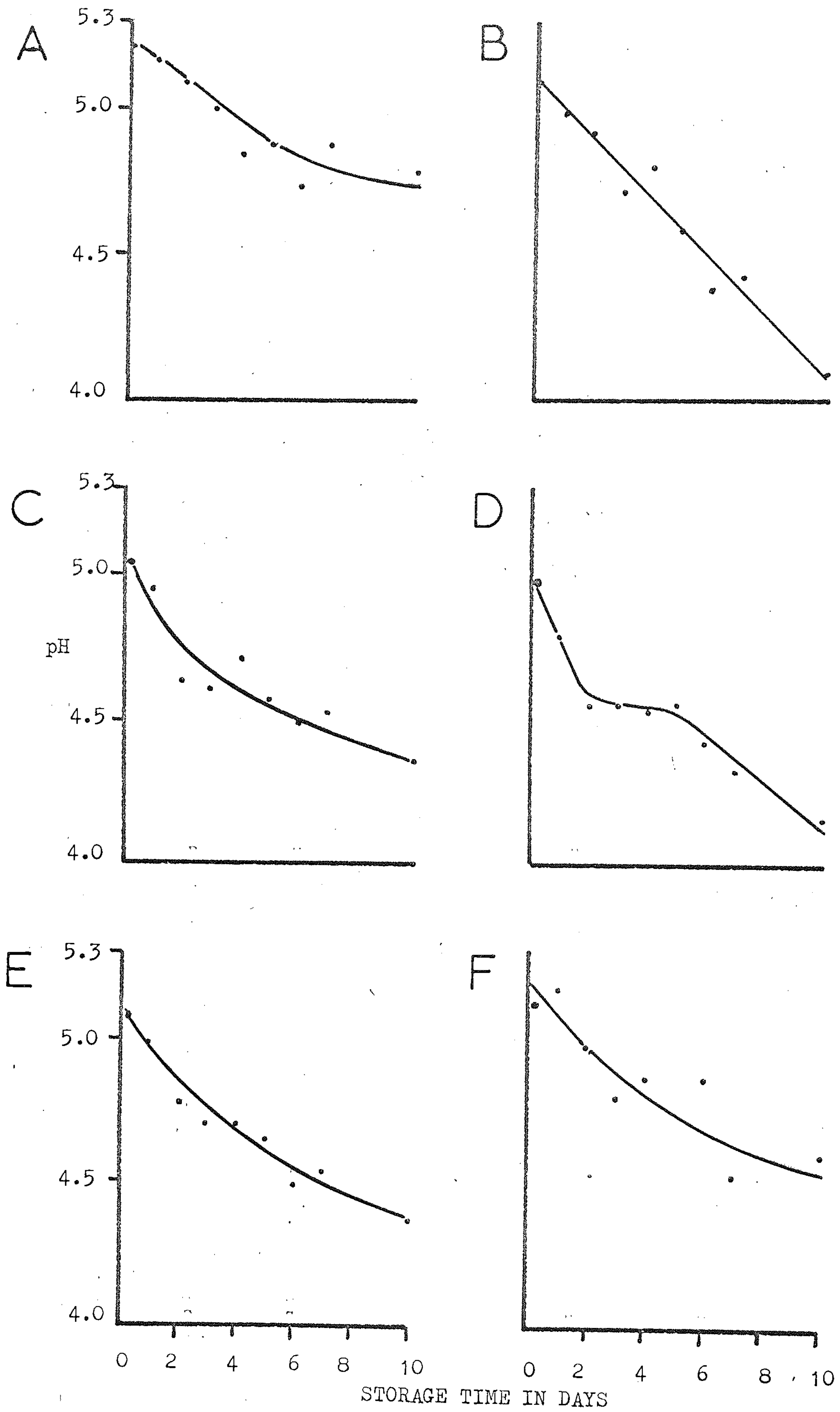


Fig.III.12. The effect of time of storage on the pH of juice from harvested cane.

h) Dextran content and specific viscosity The results are shown in Figs. III,13 and III,14, respectively. They may suitably be discussed together.

In general, both dextran content and specific viscosity were directly proportional to time of storage. Although the general relationship appeared to be linear, both parameters displayed peaks and troughs which are probably statistically significant and could not be represented by a linear regression equation; such an equation would probably contain at least five terms and would be difficult to derive. The curves for B4362 (all season), E, exhibited two peaks for dextran content at days 4 and 6, and two troughs at days 5 and 7, whereas specific viscosity showed peaks and troughs which were displaced by a factor of one day prior to those for dextran.

These results may be explained in terms of dextran production by L.mesenteroides. It is shown in Fig.III,16, that L.mesenteroides attains its maximum count in infected cane 3 to 4 days after harvest, and then slowly dies out. It is also known that pure cultures of this organism in a liquid sucrose medium produce dextran which causes an initial viscosity increase, followed by a decrease (Jeanes, 1965). This was explained by changes in molecular weight and degree of cross-linking of the dextrans, which influence its viscosity and water-solubility. This phenomenon could satisfactorily explain the peaks and troughs in dextran content for harvested cane between 4 and 7 days storage. However, it is difficult to explain the lack of correlation between peaks and troughs of dextran and viscosity, without further investigation.

The continued increase in both dextran content and specific viscosity beyond day 7, when L.mesenteroides counts are declining, could have several explanations. Firstly, it may be caused by the continued activity of the enzyme dextransucrase, which is produced extracellularly by L.mesenteroides. Alternatively, it may be due to formation of similar polysaccharides by certain lactobacilli (Chapter IV) which grow more slowly than Leuconostoc. Finally, it may be due to formation of a non-microbial polysaccharide such as sarkaran, which would be detected by the 'haze analysis' technique. Resolution of this problem requires further research.

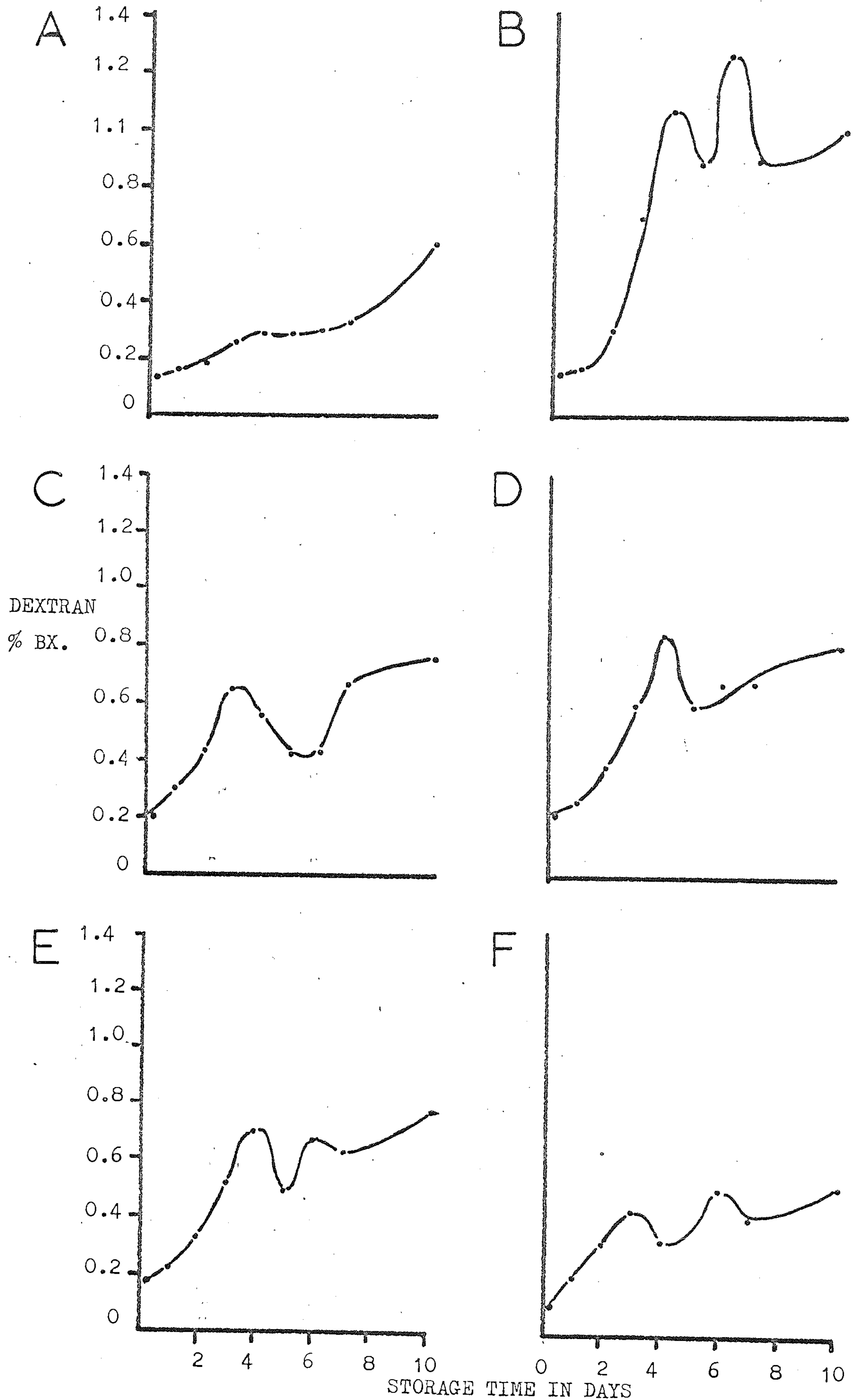


Fig.III,13. The effect of storage time on the dextran content of juice from harvested cane.

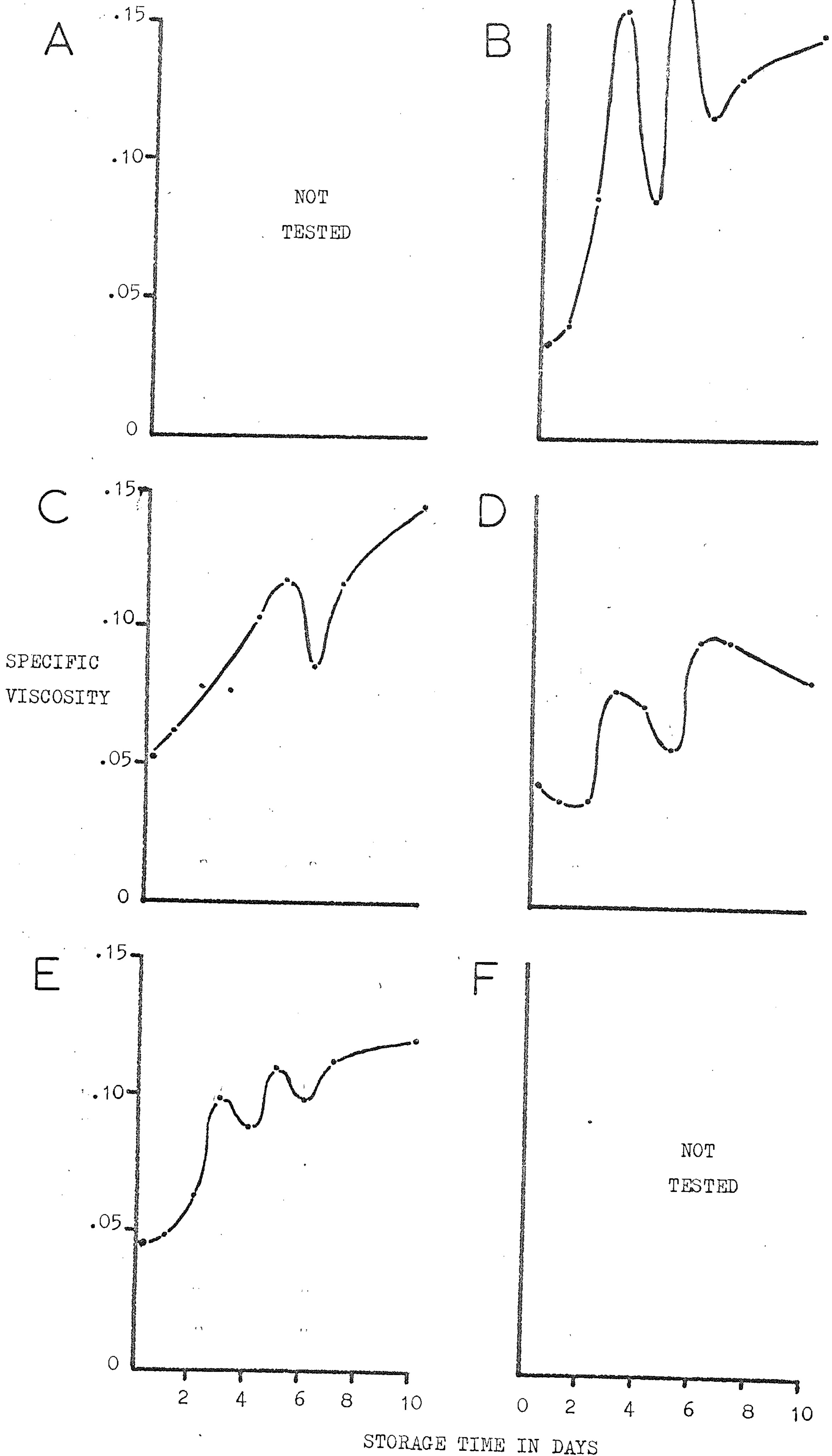


Fig.III,14. The effect of storage time on the specific viscosity of juice from

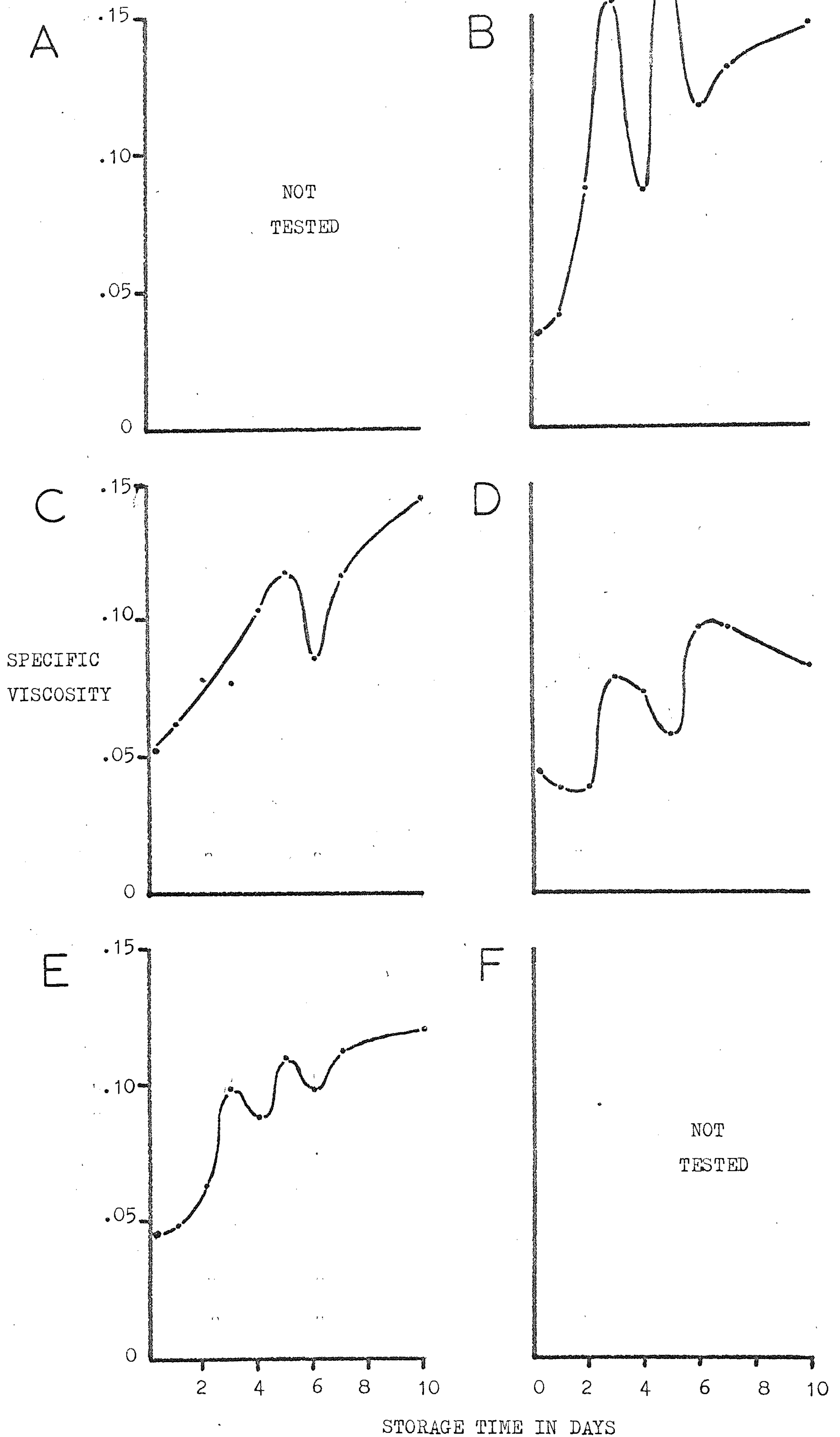


Fig.III,14. The effect of storage time on the specific viscosity of juice from harvested cane.

The rate of increase of dextran and viscosity was significantly greater in period B than in other periods. This is almost certainly due to the greater degree of infection of this cane with L.mesenteroides. Period A showed the least dextran formation, and variety B4362 was slightly more susceptible to dextran formation than B51129.

It is of interest that many samples of freshly-harvested cane contained 'dextran' when analysed by the haze analysis technique. The maximum figure observed was 0.51% Brix, in Experiment 56. Mean figures for initial dextran % Brix are shown below :-

Experiment Group	Mean initial dextran % Bx.
A	0.13
B	0.16
C	0.20
D	0.22
E	0.18
F	0.09

Within variety B4362 there is a small, but probably significant, increase in initial 'dextran' content throughout the season; this may be an effect of over-maturity on formation of 'sarkaran'. This observation confirms similar reports by Foster (1969c) for burnt, chopped cane in Queensland, and contravenes the findings of Keniry et al (1967b), who did not detect 'dextran' in fresh cane.

It may be concluded that dextran content and viscosity are suitable indicators of processing quality of harvested cane, since the harmful effects of dextran are probably directly proportional to the dextran content and viscosity of the juice. However, they are less satisfactory as indicators of the age of cane post-harvest, owing to the observed non-linear relationship of these parameters to time of storage, and the presence of 'dextran' to varying degrees in fresh cane. Dextran content is more suitable than viscosity for use as a routine quality control test, because its determination is more rapid and accurate, and its rate of increase is greater.

i) Microorganisms Results on the counts per ml. juice of total spoilage organisms, lactic acid bacteria and yeasts are summarised in Figs.III,15,16 and 17 respectively.

In all periods studied the growth curves of total spoilage organisms and lactic acid bacteria are almost identical which indicates that the latter group were the predominant spoilage organisms. Significant numbers of other groups of microorganisms were generally detected only in fresh cane; these were usually yeasts and AAB. Yeasts multiplied slowly during storage but rarely attained counts higher than 10^6 per ml; initial yeast counts were usually between 10^3 and 10^4 per ml. juice. The AAB did not appear to multiply in harvested cane except in a few isolated experiments.

The lactic acid bacteria consisted mainly of *Leuconostoc mesenteroides*, but *Lactobacillus* species often became more prevalent towards the end of the 10-day storage period. Initial counts of *L.mesenteroides* were usually between 5.0×10^4 and 5.0×10^5 per ml. They multiplied rapidly in harvested cane and exhibited the characteristic exponential growth curve. Maximum counts were obtained within 3 to 4 days of harvest, after which a slight decline in numbers occurred.

L.mesenteroides was detected in juice from freshly-harvested cane in every replicate experiment, but the rate and extent of subsequent multiplication varied widely between experiments, The highest mean maximum counts were observed in period B, whereas the mean maximum counts recorded in periods A, C and D were similar. It is probable that the high rainfall and moist ambient conditions of period B facilitated infection and multiplication of *L.mesenteroides* in harvested cane. However, the mean initial LAB count in period B did not differ significantly from the subsequent periods C and D, although it was higher than in period A.

Overmaturity of the cane did not appear to affect its degree of infection with *L.mesenteroides*, neither was there any significant difference between the varieties B4362 and B51129.

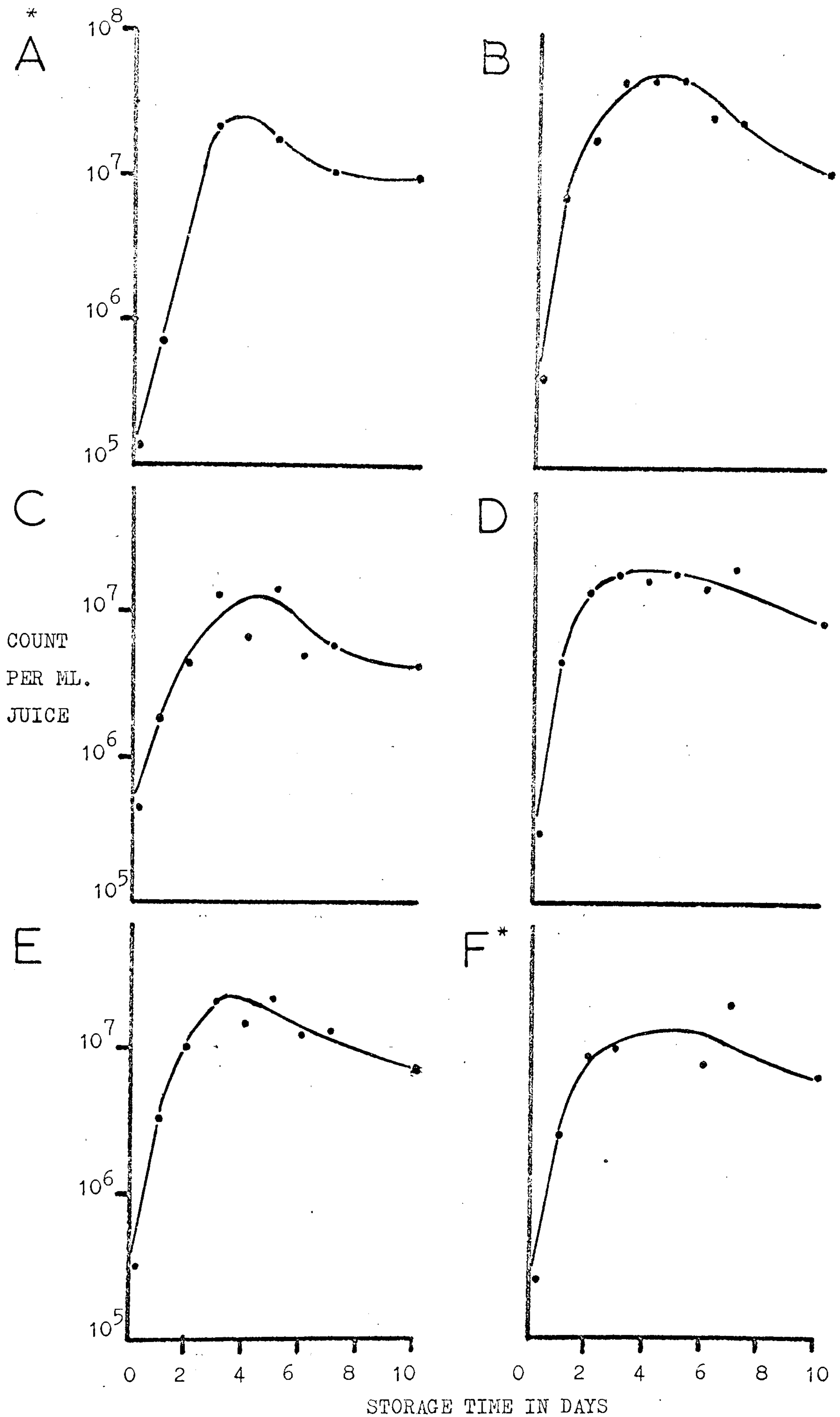


Fig.III.15. The effect of storage time on the count of total spoilage microorganisms in juice from harvested cane.

* Each point represents mean of 3 replicates only

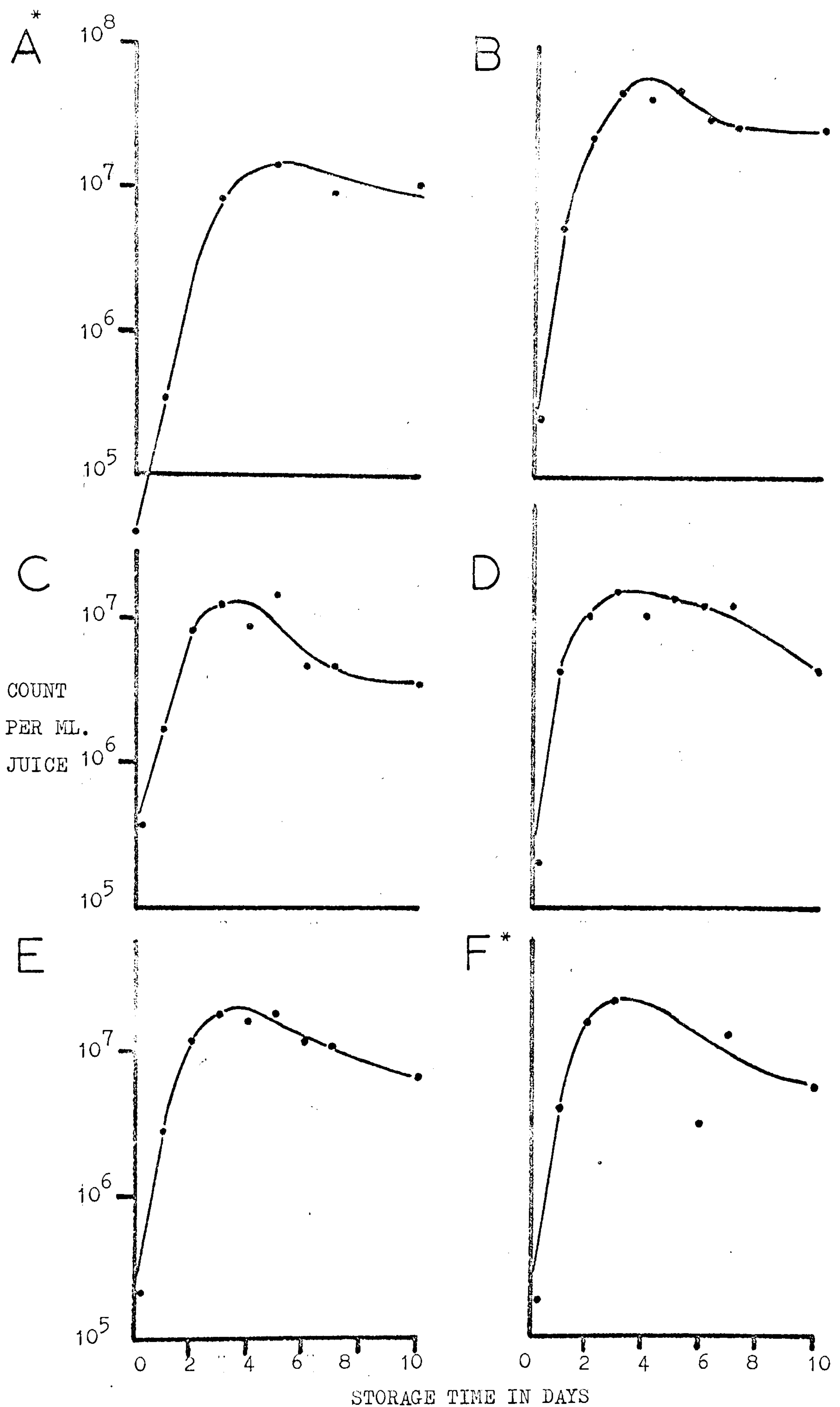


Fig.III.16. The effect of storage time on the count of lactic acid bacteria in juice from harvested cane.

* Each point represents mean of 3 replicates only

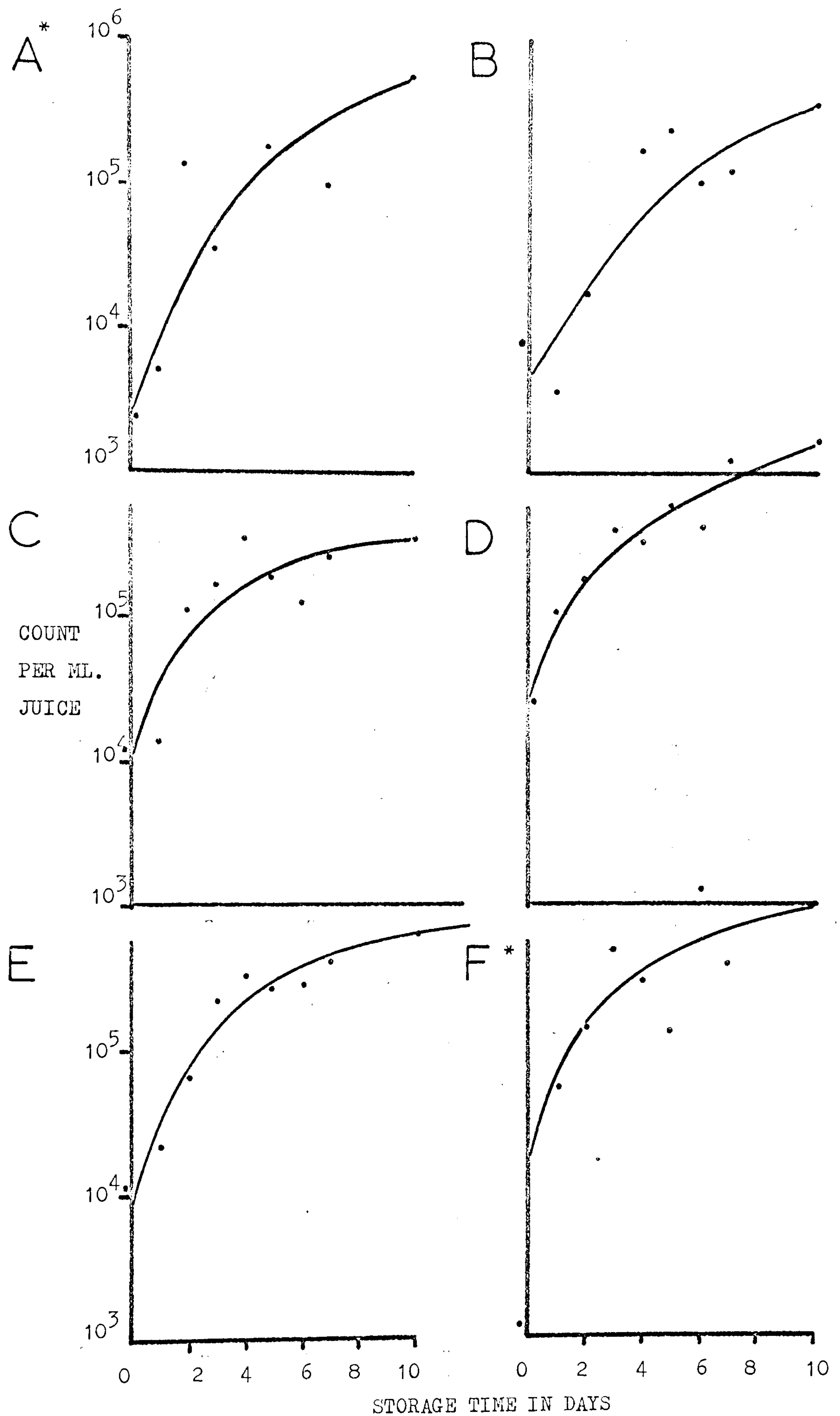


Fig.III.17. The effect of storage time on the count of yeasts in juice from harvested cane.

* Each point represents mean of 3 replicates only

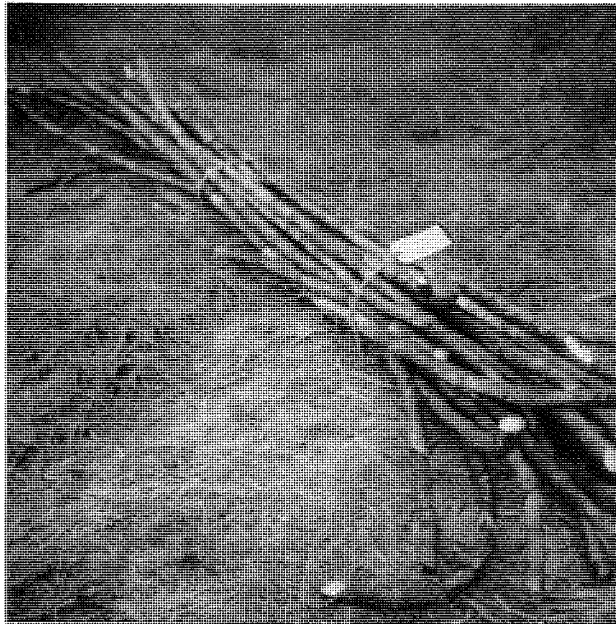
Many isolates of LAB were selected for identification. 16 isolates were Leuconostoc mesenteroides, 8 were identified as L.dextranicum, and several isolates of homofermentative and heterofermentative lactobacilli were obtained.

It was concluded that under Jamaican conditions, harvested cane is almost always infected with L.mesenteroides. In most cases this organism multiplies rapidly inside the cut stalks, but its degree of infection and growth is enhanced by wet climatic conditions at harvest and during subsequent storage. When Leuconostoc counts exceed 10^7 per ml. juice, it is highly probable that this organism causes significant losses of sucrose and formation of potentially harmful dextran in the stalks. However, since the infection is ubiquitous, it is difficult to distinguish between the effects of Leuconostoc growth and normal staling of cane, except where wet conditions obviously enhance microbial growth.

In many experiments, especially in wet weather, harvested cane developed growth of the fungus Monilia sitophila at the nodes and cut ends. This organism became visible after 2 - 3 days storage, and was recognised by its characteristic red-pink colour (Fig.III,18.). Although it was of very common occurrence on cane stored in the factory yard, it was not considered to be of significance in causing sour cane, because it appeared to grow saprophytically on juice exuded from the stalks. However, its presence is indicative of the age of cane after harvest. This organism was a troublesome contaminant in the microbiology laboratory, where it can rapidly spread over the surface of agar petri-dish cultures and sporulates profusely at the junction of the lid and base. It was successfully inhibited by use of diphenyl on the lid, (Chapter II).

3. Comparison of post-harvest deterioration of mechanically-harvested, chopped-up cane with hand-cut, whole-stalk cane.

Two adjacent fields of mature cane of the same age and variety (HJ5741) were selected. One was burnt in the usual manner, the other was left green. 72 whole stalks of burnt cane were cut by hand from a short length of a row



(a)

(b)

FIG. III,18.

Storage experiments on harvested, burnt, whole-stalk cane. Variety B4362, commenced 25th June 1969.

(a) Appearance of a bundle of cane immediately after harvest.

(b) Appearance of same bundle after 3 days storage in the open, showing growth of *Monilia sitophila* at the nodes.

adjacent to the green field. 144 whole stalks were hand cut from the green field near this point. In the burnt field, the billets of mechanically-harvested cane were collected from the row next to that previously sampled, over a similar distance. All the cane was immediately transported to the factory. The burnt, whole stalks were mixed and divided into six bundles of 12 stalks, whilst the green cane was mixed and divided into 12 bundles of 12 stalks each. 6 of these green bundles were chopped by hand into billets approximately 11" long. The burnt, chopped cane was divided into 6 piles, each of similar weight to a bundle of whole cane.

The bundles and piles were labelled and weighed to within $\frac{1}{4}$lb, laid on grass, then stored in the open. One sample of each treatment was analysed immediately and after storage times of 12, 24, 36, 48, 72 and 96 hours.

Each sample was weighed, then crushed in a 2-roller electric mill. The juice was collected and weighed, and analysed for the following :- Leuconostoc count; dextran content; Brix; pol; sucrose; reducing sugars. Chemical analyses were kindly carried out by the S.M.A. Research Dept., Mandeville, on frozen juices. Two trials were performed; Experiment 1 commenced on 3rd March, and Experiment 2 on 23rd March, 1970.

The results of Leuconostoc counts are shown in Fig.III,19 and dextran content in Fig.III,20. Chemical analyses obtained in Experiment 2 are given in Table III,18.

Both the Leuconostoc count and dextran content of mechanically-harvested cane were much greater than that of hand-cut cane at any given storage time. The initial Leuconostoc count of the mechanically chopped billets was high, but hand chopped billets of green cane were not significantly more contaminated than whole-stalk cane. This shows that the rough treatment of cane in the harvesting machine is probably responsible for its heavy infection. There was no significant difference between burnt and green whole stalks. The rate of loss of weight due to drying in whole-stalk cane was approximately half that of chopped cane. Weather conditions during the experiment were hot and dry.

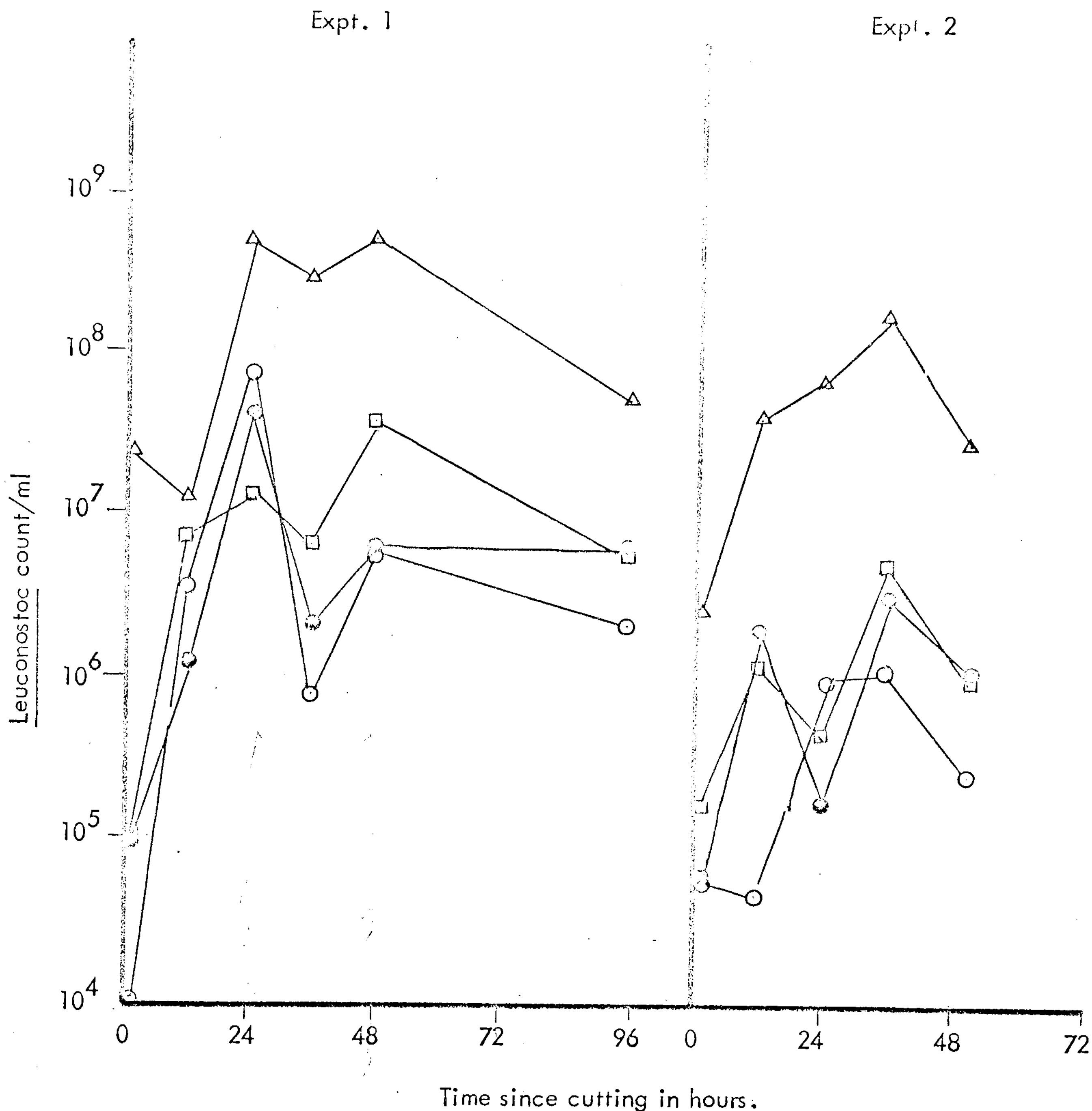


Fig.III,19. The effect of storage time on the Leuconostoc count of juice from hand-cut and mechanically-harvested green and burnt cane.

Key

- o Green, whole-stalk, manually-cut
- □ Green, chopped (by hand)
- ● Burnt, whole-stalk, manually-cut
- △ Burnt, chopped, mechanically harvested

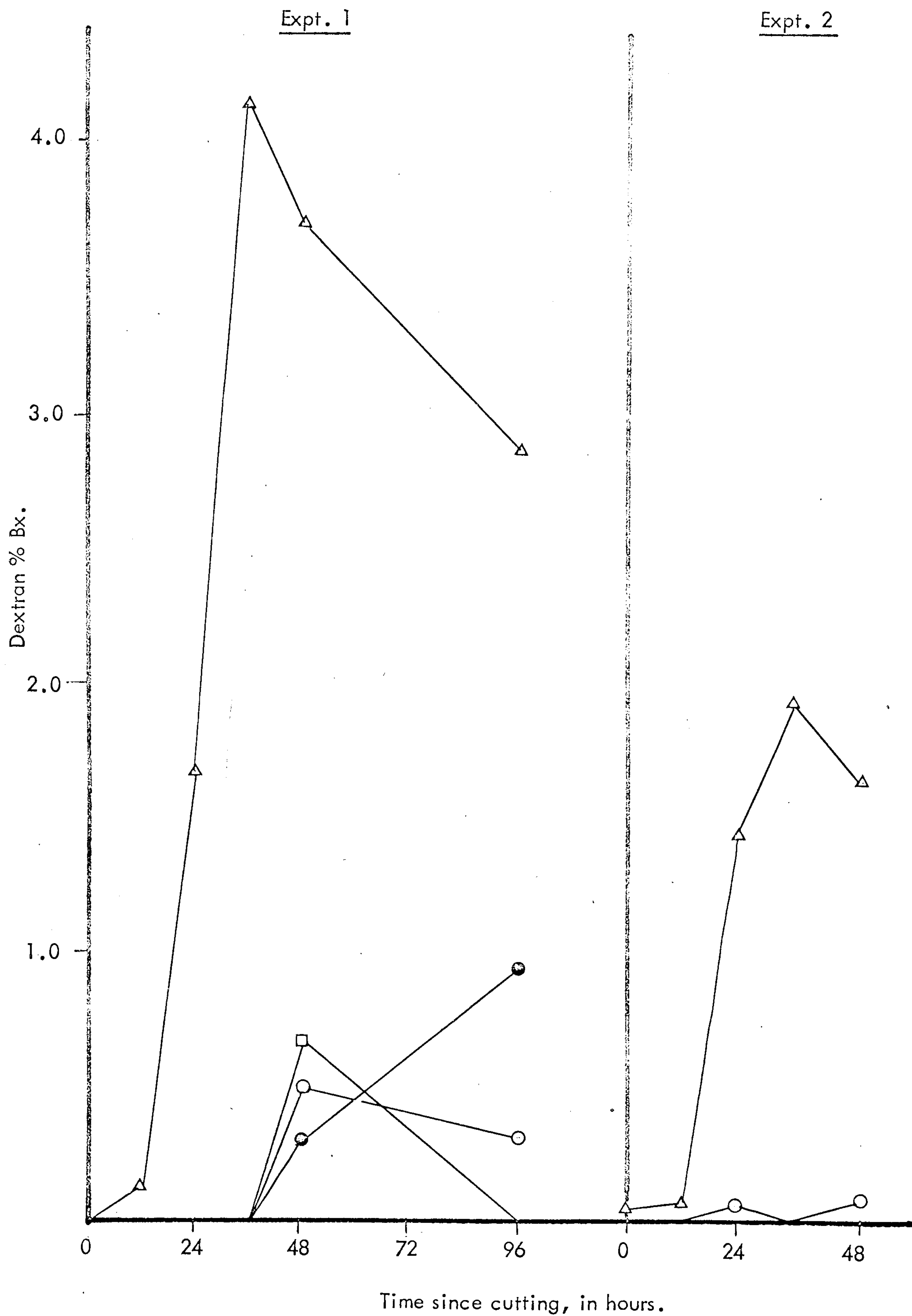


Fig.III,20. The effect of storage time on the dextran content of juice from hand-cut & mechanically harvested green & burnt cane.

Key

○ Green, whole, hand-cut

◐ Burnt, whole hand-cut

□ Green, chopped (by hand)

△ Burnt, chopped, mechanically harvested

Storage time in hours	Treatment	Analysis				
		R.S.%w/w	Sucrose % w/w	°Brix	% Pol	% Weight Loss
0	Green, whole	0.44	13.36	21.28	18.12	-
	" chopped	0.52	11.58	19.68	16.07	-
	Burnt, whole	0.44	13.83	20.48	18.30	-
	" chopped	0.90	12.37	19.76	16.79	-
12	Green, whole	0.44	13.94	20.48	18.40	1.2
	" chopped	0.48	13.95	20.28	18.35	2.2
	Burnt, whole	0.56	12.23	19.88	16.70	0.9
	" chopped	0.92	12.55	18.96	16.72	1.2
24	Green, whole	0.64	13.63	20.28	18.06	1.0
	" chopped	0.48	13.98	20.68	18.49	2.3
	Burnt, whole	0.44	14.27	20.68	18.75	1.4
	" chopped	1.51	12.69	18.96	16.84	1.6
36	Green, whole	0.64	13.59	21.08	18.26	3.6
	" chopped	0.74	13.81	20.88	18.40	6.1
	Burnt, whole	0.62	14.07	21.48	18.81	3.6
	" chopped	1.41	12.46	19.16	16.69	8.5
51	Green, whole	0.74	13.92	20.68	18.44	2.6
	" chopped	0.81	12.55	20.28	17.10	7.7
	Burnt, whole	0.66	13.97	21.68	18.78	4.2
	" chopped	1.51	11.67	19.16	15.99	6.3
80	Green, whole	NT	11.58	23.28	17.13	6.2
	" chopped	NT	11.06	23.68	16.79	11.7
	Burnt, whole	NT	8.36	22.88	14.16	7.3
	" chopped	NT	NT	20.48	NT	14.5

Table III,18. Changes in juice composition and weight of cane during storage of green and burnt cane harvested by machine and chopped-up, and harvested manually.

(Chemical analyses determined by S.M.A. Research Dept.)

NT = Not Tested

C. CONCLUSIONS

1. Qualitative and quantitative data were obtained on the microbiological, physical and chemical changes which occur during storage of harvested cane. Initial experiments utilised cane air-freighted from Jamaica to the U.K., but this was often delayed in transit and suffered deterioration prior to test. In every case the predominant spoilage microorganisms were lactic acid bacteria, especially Leuconostoc mesenteroides.

2. 45 replicated experiments on the storage of burnt, manually-cut, whole-stalk cane were executed at Frome in 1969. The results were analysed statistically, and the effect of storage time on the following parameters was studied :- weight loss in cane; juice extraction % cane; brix; pol; purity; reducing sugars; pH; dextran; viscosity; viable count of spoilage organisms, lactic acid bacteria and yeasts.

Variety B4362 deteriorated more rapidly after harvest than B51129. With variety B4362, the rate of deterioration increased significantly during periods of high rainfall. This was coincident with an increase in the maximum count of L.mesenteroides in the cane. Overmaturity of the cane appeared to enhance its rate of deterioration. Nearly all the cane was infected with L.mesenteroides, which was the predominant spoilage organism in every case. Its growth was accompanied by significant increases in the dextran content and viscosity of cane juice. Dextran content was more suitable for use as an indicator of cane processing quality than juice viscosity.

3. Two experiments compared the rate of post-harvest deterioration of manually-cut, whole-stalk cane and mechanically-harvested, chopped cane. The latter deteriorated most rapidly, and exhibited high Leuconostoc counts and dextran contents within 15-20 hours of harvest.

4. It was concluded that whole-stalk, manually-cut cane should be milled within 24 hours of harvest to minimise economic losses, especially in hot, wet weather.

CHAPTER IV IDENTIFICATION OF MICROORGANISMS ISOLATED FROM STORED HARVESTED SUGARCANE

A. INTRODUCTION

The microbiology of harvested, whole-stalk cane during storage has not been studied previously, but some investigations on the microflora of extracted cane juice in the factory mill have been reported. Several of these include microbial identification to species level. It is assumed here that many of these microorganisms are derived from the crushed cane.

Early work on the microbiology of mill juices and the cane plant was reviewed by Thaysen & Galloway (1930), Hucker & Pederson (1942), and Owen (1949), who lists the known microorganisms isolated from cane juice. Amongst the bacteria, *Leuconostoc mesenteroides* is most frequently reported, but other gum-forming bacteria were also identified. Levan formers were probably members of the genus *Bacillus*, whilst rod-shaped dextran-formers were probably lactobacilli. The occurrence of the latter group in cane and beet factories was reviewed by Perquin (1940), who described *Betabacterium vermiforme* as the causative organism of an outbreak of slime in a beet factory.

Several papers were published in Louisiana on the identification of organisms in cane juice. *L. mesenteroides* was isolated from frost-damaged cane by McCalip & Hall (1938), whilst Alford & McClesky (1942) identified the principal slime-forming organisms in juice as *L. mesenteroides* and *L. dextranicum*. Later work by Faville (1947) and McClesky, Faville & Barnett (1947) showed that these '*L. dextranicum*'isolates were in fact *L. mesenteroides* strains which fermented pentoses slowly. More than 700 isolates of *L. mesenteroides* were studied and divided into 4 types, A, B, D, & F, based on colonial morphology. Mayeux (1960) and Duncan & Colmer (1964) studied coliform bacteria on the cane plant and in mill juice and found that the predominant species were *Aerobacter aerogenes* and *A. cloacae*.

In Cuban mill juice the predominant genera were *Bacillus*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, *Escherichia* and *Leuconostoc*. (Pederson & Hucker (1946).

The causative organism of the deterioration of mechanically harvested chopped-up cane in Queensland was identified as *L. mesenteroides* (Egan, 1965c); colony types A & D predominated.

The significance of polysaccharide-producing bacteria of the genus *Bacillus* in sugar refining was discussed by Skole, Newman & Barnwell (1968), who identified members of this group in Louisiana raw sugars as *B. subtilis* and *B. cereus*, but they did not examine cane juice.

Slime-forming bacteria in cane juice were identified as *Acetobacter xylinum* in an early paper by Browne (1906).

Very little work has been published on yeasts and moulds present in cane juice. El-Tabey Shehata (1960) identified many yeast isolates from fresh Brazilian cane juice; the eight predominant species were *Saccharomyces carlsbergensis var. alcoholiphila* (n.var); *S. cerevisiae*; *Pichia membranefaciens*; *P. fermentans*; *Torulopsis stellata*; *Candida krusei*; *C. guilliermondii*, and *C. intermedia var. ethanophila*. Kopeloff & Kopeloff (1919) found that the most common mould spores in Louisiana cane juice were *Aspergillus sydowi*, *A. flavus*, *A. niger*, *A. repens*, *Penicillium divaricatum*, and members of the genera *Citromyces*, *Cladosporium*, *Syncephalastrum* and *Trichoderma*.

In a study of osmophilic yeasts on the cane plant, Scarr (1954) identified 35 isolates as *Torulopsis spp*. (30 strains); *Saccharomyces bisporus* (3 strains); *S. elegans* (2 strains); and *S. rouxii* (1 strain). The xerophitic mould *Aspergillus glaucus* was also obtained.

It is apparent from the literature that extracted cane juice contains a wide range of microorganisms, many of which may be implicated in the souring of harvested cane. Quantitative and qualitative changes in the microbial flora of stored, harvested cane were reported in the previous chapter, whilst the present chapter describes the identification of microorganisms isolated in these experiments.

Selective media were used to isolate various groups of microorganisms thought to be of potential significance in sour cane, i.e. lactic acid bacteria (*Leuconostoc* and *Lactobacillus* species); aerobic spore-forming

rods (*Bacillus* species); coliforms; acetic acid bacteria and other aciduric Gram-negative bacteria; mesophilic and osmophilic yeasts and moulds.

More than 500 isolates were selected from a range of sources and media and maintained in a stock culture collection. The colonial morphology, Gram stain reaction, microscopic appearance and catalase reaction of all the isolates were recorded, and representative strains were chosen for identification tests at both the generic and specific levels using methods recommended by current leading authorities on each group.

B. EXPERIMENTAL

1. Lactic acid bacteria

Sharpe, Fryer & Smith (1966) defined lactic acid bacteria as Gram-positive, non-sporulating cocci or rods dividing in one plane only (with the exception of the pediococci); catalase negative (some strains may possess a 'pseudocatalase' detectable on low sugar-containing media); usually non-motile, obligate fermenters, producing mainly lactic acid and sometimes also volatile acids and CO_2. They are subdivided into four genera as follows:-

Streptococcus - homofermentative cocci in pairs or chains

Leuconostoc - heterofermentative cocci in pairs or chains

Pediococcus - homofermentative cocci dividing in two planes to give tetrads which may appear as clusters

Lactobacillus - homo- or hetero-fermentative rods

The strains examined here were isolated on STA medium (Chapter II) or on media selective for lactic acid bacteria, i.e. Rogosa agar or STA + 0.1% thallous acetate, designated STATA (Chapter II). They were identified to generic level by the tests recommended by Sharpe et al (1966), i.e. microscopic appearance of Gram-stained films; catalase test; fermentative use of carbohydrate (Hugh & Liefson (1953)); homo- or hetero-fermentative, by production of gas from glucose (Gibson & Abd-el-Malek (1945)). Most of the isolates belonged to the genus *Leuconostoc*; the remainder were *Lactobacillus* species.

a) Genus Leuconostoc van Tieghem, emend. Hucker & Pederson

Heterofermentative streptococci were first grouped in the genus Betacoccus by Orla-Jensen (1919) but Hucker & Pederson (1930) placed them in the genus Leuconostoc (van Tieghem (1878) and classified three species, L. mesenteroides, L. dextranicum and L. citrovorum. Later work by Garvie (1960) divided the genus into six groups; three were L. cremoris, L. lactis and L. dextranicum , whilst three were L. mesenteroides. Whittenbury (1966) studied leuconostocs isolated from grass and silage and found some strains which differed significantly from the four named species. Most recently, Garvie (1967a, b) described two new species of Leuconostoc, L. oenos from wine, and L. paramesenteroides, previously regarded as a non-dextran-producing variant of L. mesenteroides. Within the species L. mesenteroides isolated from cane juice, McCleskey et al (1947) distinguished 4 types A, B, D and F, based on differences in colonial morphology on 10% sucrose agar. These types also differed in certain fermentation reactions, in amount of dextran, gas and acid produced, and in the temperature and pH requirements for growth. Serological and bacteriophagic studies showed that type A constituted a distinct and homogeneous group, whereas types B, D and F were heterogeneous (Leiva-Quiros & McCleskey (1947)). Garvie (1960) did not find such clear-cut differences in the strains she examined. The growth factor and amino acid requirements (Garvie, (1967b)), and the lactic dehydrogenases (Garvie (1969)) of the genus were also studied.

In the present study the identification methods of Garvie (1960) were used. These were based on the following physiological and biochemical tests:- growth at 37° and 45°C; heat resistance (55°C for 15 mins); action on litmus milk; dextran synthesis from sucrose; diacetyl production; carbohydrate fermentation - production of acid from arabinose, xylose, salicin, sucrose, melibiose, lactose and trehalose; hydrolysis of aesculin and arginine. All the strains examined were catalase negative, heterofermentative, Gram-positive, oval cocci occurring in pairs and short chains. Isolates which hydrolysed arginine and/or grew at 45°C were considered to be heterofermentative lactobacilli. Lactic acid configuration was not

determined, neither were serological nor nutritional tests attempted. The classification scheme of Garvie (1967a) was used.

87 isolates belonged to the genus Leuconostoc. 85 formed dextran from sucrose and fermented a pentose sugar, and therefore were placed in either Group V (L. dextranicum) or Group VI (L. mesenteroides). The species were separated by their ability to ferment arabinose. 63 isolates fermented arabinose and were classified as L. mesenteroides (Cienkowski) van Tieghem. 22 isolates did not ferment arabinose but fermented xylose and were placed in L. dextranicum (Beijerinck) Hucker & Pederson. One isolate formed dextran but fermented neither pentose; it was identified as L. dextranicum (Group IV). The single non-dextran-forming isolate fermented both pentoses and was classified as L. paramesenteroides Garvie (nov.spec.) (Garvie Group III).

The characteristics of 85 isolates are compared with the standard descriptions of Garvie (1967a) in Table IV,1.

The pattern of physiological properties of cane isolates identified as L. mesenteroides is similar to that of Garvie's Group VI, except that a few strains did not ferment trehalose. However, isolates classified as L. dextranicum differed significantly from Garvie's Group V in two respects. 73% of cane strains fermented salicin compared with only 19% of Gp.V. strains, and 95% of cane strains hydrolysed aesculin, compared with 50% in Gp.V. In both cases the percentage of positive strains approximates more closely to Gp.VI than to Gp.V. Therefore these isolates appear to be intermediate between Groups V and VI, but since they failed to ferment arabinose they should be classified as L. dextranicum, Group V.

The origins of the Leuconostoc isolates are shown in Table IV,2.

Test	L.mesenteroides % strains positive		L.dextranicum % strains positive	
	Garvie Gp. VI	Tilbury	Garvie Gp. V	Tilbury
No. of strains examined	31	63	16	22
Acid from:- arabinose	100	100	0	0
xylose	77	60	100	100
salicin	77	89	19	73
melibiose	81	76	94	86
lactose	52	46	62	64
trehalose	100	87	100	86
Hydrolysis of aesculin	94	97	50	95
Resistance to heat 55°C,15 mins.	77	84	75	53

Table IV,1. Physiological and biochemical characteristics of 85 strains of Leuconostoc isolated from cane juice compared with L. mesenteroides (Gp.VI) and L. dextranicum (Gp.V) (Garvie, 1967a).

All isolates possessed the following characteristics:- gas produced from glucose; no growth at 45°C; arginine not hydrolysed; dextran and acid formed from sucrose; growth at 37°C; diacetyl not produced.

Experiment Code No.	Origin	Culture collection code number of isolates: L. mesenteroides	L. dextranicum
b	Mauritius,1966	G6,G7,G8,G9,G10,G11,G12, G12a,G13,G14,L5s,L61,L16, L17,L18,L19,L20.	-
d	Frome,1967, I.	1b,16b.	1a,4a,4b,9a,14b,14c, 17b.
e	Frome,1967, II.	62a,65a,68b,69a,70a,71a, 71b,72a,73b,73c.	66b,70b.
f	B.Honduras,1967	122a,122c.	-
g	Mauritius,1967	131,132,133.	-
j	Frome,1968,III.	152,153,155,158,164,166, 169,170,172,173,174,201, 202.	156,159,161,162, 163,200.
k	Frome,1969	J3,J5,J9,J12,J15,J18,J19, J33,J53,J58,J61,J62,J91.	J2,J6,J8,J28,J44,J46, J67.
l	Frome,1970	J93,J98,J99.	J97.

Table IV,2. Origin of 86 isolates of Leuconostoc from cane juice.

L. mesenteroides was isolated from cane juices from Jamaica, British Honduras and Mauritius over several years. The isolates were obtained from fresh cane juice and from cane stored for various periods up to 10 days after harvest; the majority were selected from 10^{-7} dilutions of cane juice. This indicates the wide distribution of the organism and its predominance in stored, harvested cane.

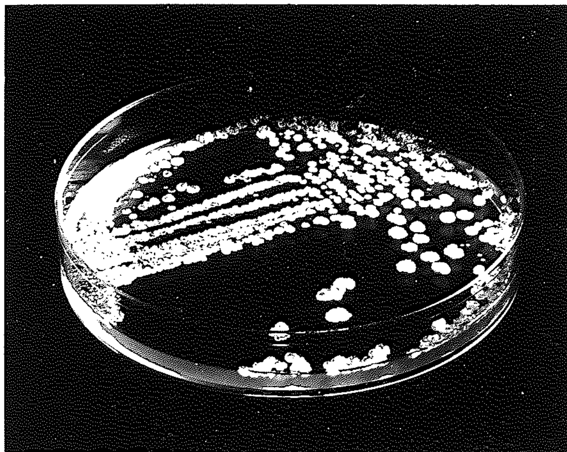
The colonial morphology of dextran-forming strains on STA included the 4 types A,B,D and F observed by McCleskey et al (1947), but types B and D predominated. The appearance of these types is shown in Figure IV,1. Type A colonies (3 isolates) were smooth, semi-transparent, round, entire, and convex with a low elevation; the slime was watery. Type B colonies (30 isolates) were rugose, opaque, cartilaginous, raised, often surrounded by clear, watery slime. Type D colonies (18 isolates) were smooth, transparent, round, entire, large (8-12 mm diameter), convex, with a high elevation (4-8 mm), and frequently dripped onto the lid of the inverted petri-dish. Type F colonies (12 isolates) were similar to 'D' but smaller and opaque. The colony types were randomly distributed between samples, and there did not appear to be any correlation between colonial morphology and physiological characteristics.

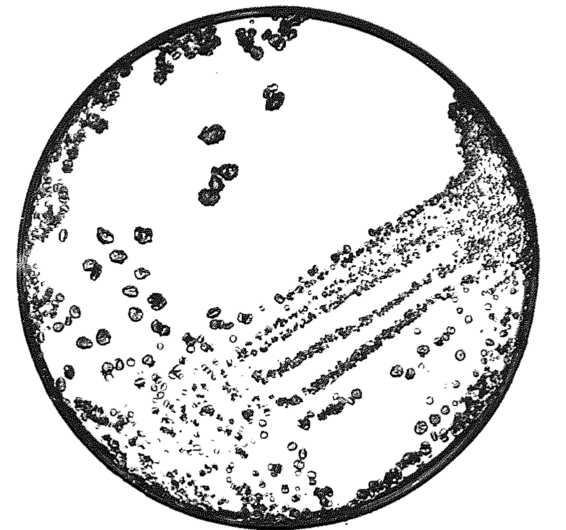
b) Genus Lactobacillus Beijerinck

The most recent authoritative review on the taxonomy of lactobacilli is that of Sharpe (1962), whilst the identification of lactobacilli is described by Sharpe et al (1966). In both these publications the genus Lactobacillus is conveniently divided into the three sub-genera Thermobacterium, Streptobacterium and Betabacterium, first suggested by Orla-Jensen (1919), although these are not recognised in Bergey's Manual (Breed, Murray & Smith 1957). Thermobacterium contains homofermentative, thermophilic species, whilst Streptobacterium includes homofermentative species which grow at low temperatures and Betabacterium comprises heterofermentative species. The 67 lactobacilli strains isolated from cane were classified in the sub-genera Streptobacterium (30 isolates) and Betabacterium (37 isolates).

The methods of identification to specific level were those of Sharpe et al

(a)





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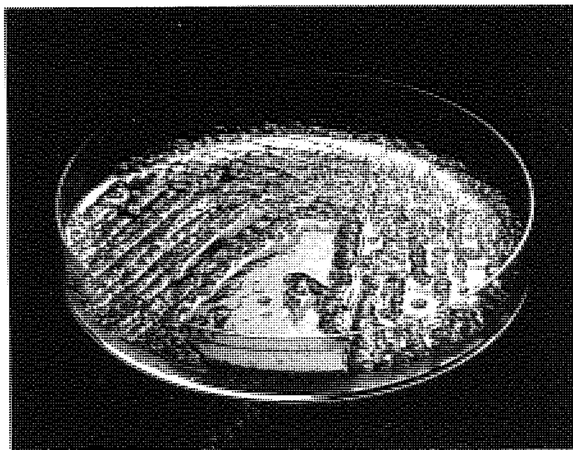
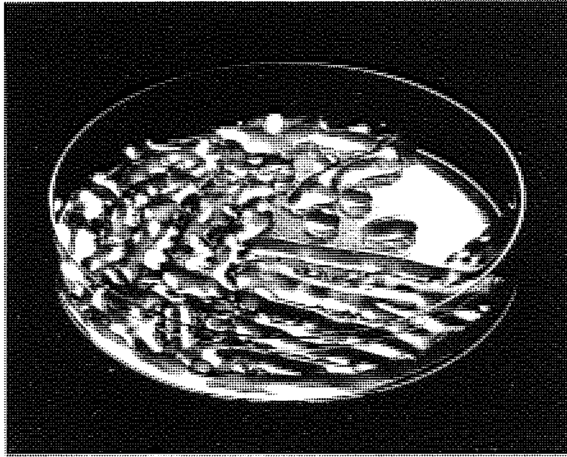


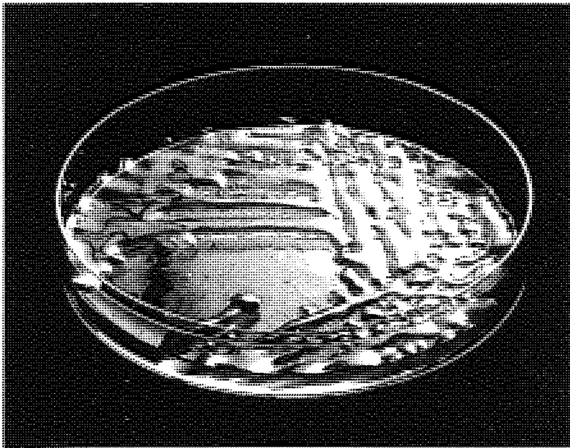
(c)

FIG. IV.1. Colonial morphology of *Leuconostoc mesenteroides* cultures on sucrose tryptone agar, 72 hours at 30°C (2/3 actual size)

Key (a) Type A. Strain no. 62a.
(b) Type B. no watery slime. Strain no. 68b.
(c) Type B. Watery slime present. Strain no. 69a.



(d)

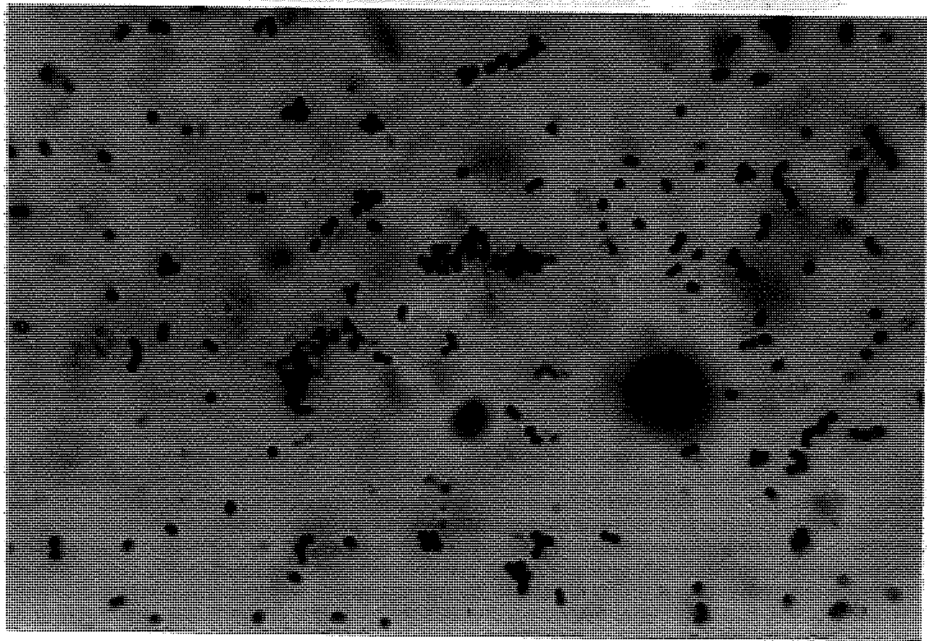


(e)

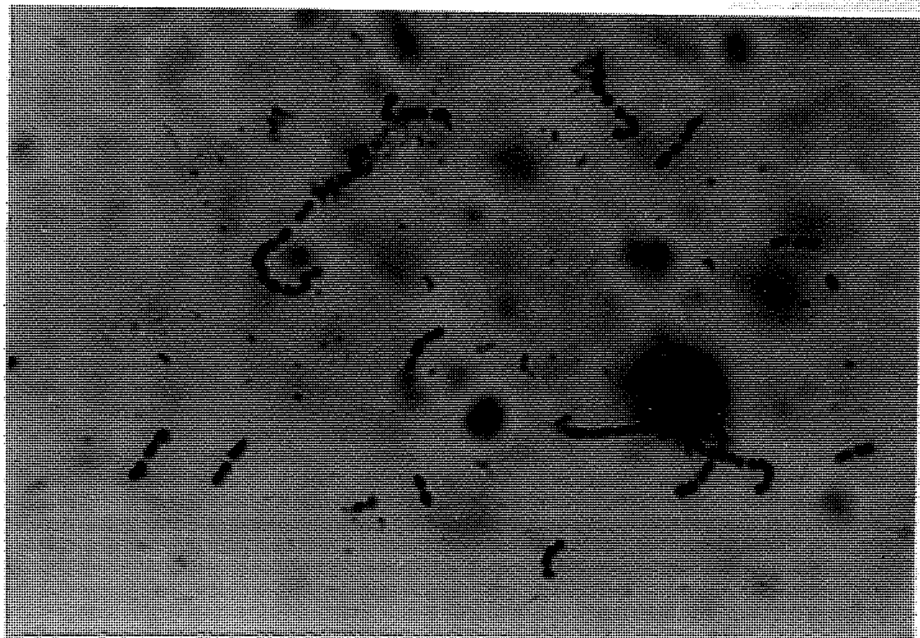
FIG. IV.1.

Colonial morphology of *Leuconostoc mesenteroides*
Cultures on sucrose tryptone agar, 72 hours at 30°C
(2/3 actual size)

Key (d) Type D. Strain no. L20.
(e) Type F. Strain no. 70b.



(f)



(g)

FIG. IV,1.

Microscopic appearance of *Leuconostoc mesenteroides*

Gram-stained smears, magnification x 1650

Key			
	(f)	Strain L20.	Culture grown on YGC agar 48 hours at 30°C
	(g)	Strain 14b.	Culture grown in YGC broth 24 hours at 30°C

(1966). The following physiological and biochemical characteristics were determined:- gas production from glucose; growth at 37° and 45°C; hydrolysis of arginine; production of dextran from sucrose; % acid in milk; growth in 0.4% Teepol; aesculin hydrolysis; fermentation of arabinose, cellobiose, melezitose, melibiose, maltose, galactose, lactose, mannitol, salicin, and sucrose. Lactic acid configuration was not determined, neither were serological nor nutritional tests attempted.

(i) Streptobacterium Orla-Jensen. The identification of 30 isolates of Streptobacterium is given in Table IV,3, in comparison with standard descriptions of the species (Sharpe (1962)).

17 strains closely resembled L. plantarum (Orla-Jensen) Holland except in fermentation of xylose; growth at 45°C and % acid formed in milk. The two latter differences appear to be significant, but in view of the similarity in the more important fermentation reactions, and ability to grow in 0.4% Teepol, this group is considered synonymous with L. plantarum.

The 5 strains identified as L. casei v. casei (Orla-Jensen) Holland also differed from the standard description in xylose fermentation, growth at 45°C and % acid formed in milk, and, in addition, fermentation of arabinose and mannitol. However, the ability to ferment lactose but not rhamnose, and failure to grow in 0.4% Teepol, clearly distinguishes this group from the other species of Streptobacterium.

The 8 isolates placed in L. casei v. alactosus (Orla-Jensen) Holland agree closely with the standard description in most respects. The group is distinguished from L. casei v. casei and L. casei v. rhamnosus by its inability to ferment lactose and rhamnose respectively.

An interesting property of most strains of L. casei v. casei and v. alactosus was the ability to form dextran from sucrose. This property was noted in isolates of streptobacteria obtained from 'frogspawn' in a beet-sugar factory by Perquin (1940), who proposed that they belonged to a new species, named Streptobacterium dextranicum. This species differs from Sharpe's description of L. casei v. alactosus only in its absence of galactose fermentation, and from cane juice isolates in its absence of arabinose fermentation.

Test	*L.plantarum*		*L.casei v.casei*		*L.casei v.alactosus*	
	Sharpe	Tilbury	Sharpe	Tilbury	Sharpe	Tilbury
No. of strains examined	NS	17	NS	5	NS	8
Fermentation of:						
arabinose	±	±(14)	–	+(5)	–	±(5)
lactose	+	+(17)	+	+(5)	–	–(0)
melibiose	+	+(16)	–	–(0)	–	–(0)
raffinose	+	+(13/13)	–	–(0)	–	∓(2)
rhamnose	±	±(9/13)	–	–(0)	–	∓(2)
sucrose	+	+(16)	+	+(5)	+	+(8)
xylose	±	∓(7)	–	±(3)	–	–(1)
amygdalin	+	+(12/13)	+	+(5)	+	+(8)
cellobiose	+	+(13/13)	+	+(5)	+	+(8)
galactose	+	+(13/13)	+	+(5)	+	+(8)
maltose	+	±(7/13)	+	+(4)	+	+(8)
mannitol	+	+(12/13)	+	∓(1)	+	±(4)
salicin	+	+(17)	+	+(5)	+	+(8)
sorbitol	+	+(12/13)	+	+(5)	+	+(8)
Growth at 15°C	+	+(12/13)	+	+(4)	+	+(8)
Growth at 45°C	±	∓(2)	±	–(0)	–	∓(3)
% acid in milk	0.3-1.2	5.7-11.0	1.2-1.5	3.3-10.1	0	0.7-2.7
Growth in 0.4%Teepol	+	+(13/13)	–	–(0)	–	–(0)
Hydrolysis of aesculin	+	+(17)	+	+(5)	+	+(8)
Hydrolysis of arginine	–	–(0)	–	–(0)	–	–(0)
Dextran from sucrose	NS	∓(1)	NS	+(5)	NS	±(4)

None produced gas from glucose.

Table IV,3. Physiological and biochemical characteristics of 30 homofermentative *Lactobacillus* strains (*Streptobacterium*) isolated from cane juice, compared with standard descriptions of species (Sharpe, 1962).

Key

- \+ all strains positive
- ± majority of strains positive
- ∓ majority of strains negative
- – all strains negative
- NS not stated

Figures in brackets denote number of strains giving a positive result.

The creation of a new species to include the dextran-forming strains of L. casei does not seem justified.

(ii) Betabacterium Orla-Jensen. 37 isolates of heterofermentative lactic acid bacteria either hydrolysed arginine or grew at 45°C (or both) and therefore were classified in the sub-genus Betabacterium. Full identification tests were determined for 10 isolates, whilst more limited tests were applied to 18 strains and 9 isolates were only tested at generic level.

None of the isolates resembled any of the 5 species of Streptobacterium described by Sharpe et al (1966) i.e. L. fermenti, L. buchneri, L. brevis, L. cellobiosus, and L. viridescens.

34 of the strains formed copious amounts of dextran from sucrose; 27 of these were classified into 6 distinct groups on the basis of arginine hydrolysis, growth at 45°C, and fermentation of arabinose, melibiose, trehalose, xylose, lactose and salicin. The scheme is shown in Table IV,4.

Group No.	No. of isolates	Arginine hydrolysis	Growth at 45°	Fermentation of:-					
				arabinose	melibiose	trehalose	xylose	lactose	salicin
I	6	+	−	+	± (3)	∓ (2)	+	± (3)	+
II	6	+	+	−	−	− (1)	+	± (3)	+
III	7	+	−	−	− (1)	∓ (2)	+	∓ (2)	∓ (2)
IV	3	+	−	+	+	−	−	−	−
V	3	−	+	+	∓ (1)	∓ (1)	± (2)	∓ (1)	∓ (1)
VI	2	−	+	−	−	−	+	−	± (1)

All strains produced dextran and fermented sucrose, and hydrolysed aesculin.

Table IV,4. Characteristics of 6 groups of Betabacterium which differed significantly from named species described by Sharpe et al (1966).

Key
+ all isolates positive
± majority of isolates positive
∓ majority of isolates negative
− all isolates negative.

Figures in brackets denote number of isolates giving a positive result

Test		Group I (6 isolates)	Group II (6 isolates)
Isolate numbers:		65b; 68a; 69b; 123a; 167; 168	L15; G3; G5; L51; J2; JE1
Fermentation of:	arabinose	+	–
	cellobiose	+ (4/4)	+
	melezitose	– (0/4)	–
	melibiose	± (3)	–
	raffinose	∓ (1/4)	–
	trehalose	∓ (2)	– (1)
	xylose	+	+
	amygdalin	+ (4/4),	+
	rhamnose	+ (4/4)	–
	sorbitol	∓ (1/4)	–
	maltose	– (0/4)	+
	galactose	± (3/4)	+
	lactose	± (3)	± (3)
	mannitol	– (0/4)	–
	salicin	+	+
	sucrose	+	+
Growth at 15°C		+ (4/4)	+
Growth at 45°C		–	+
Hydrolysis of aesculin		+	+
Growth in 0.4% Teepol		– (0/4)	–
Hydrolysis of arginine		+	+
% acid formed in milk		2.0 – 2.4 (4)	0.7 – 3.0
Production of dextran from sucrose		+	+
Gas from glucose		+	+

Table IV.5. Physiological and biochemical characteristics of two groups of dextran-forming, heterofermentative lactobacilli (Betabacterium) isolated from cane juice.

Key:
- \+ all strains positive
- ± majority of strains positive
- ∓ majority of strains negative
- – all strains negative

Figures in brackets denote number of strains giving a positive result.

Seven of the isolates which were identified only to generic level grew at 45°C, hydrolysed arginine and formed dextran from sucrose; therefore these probably belong to Group II.

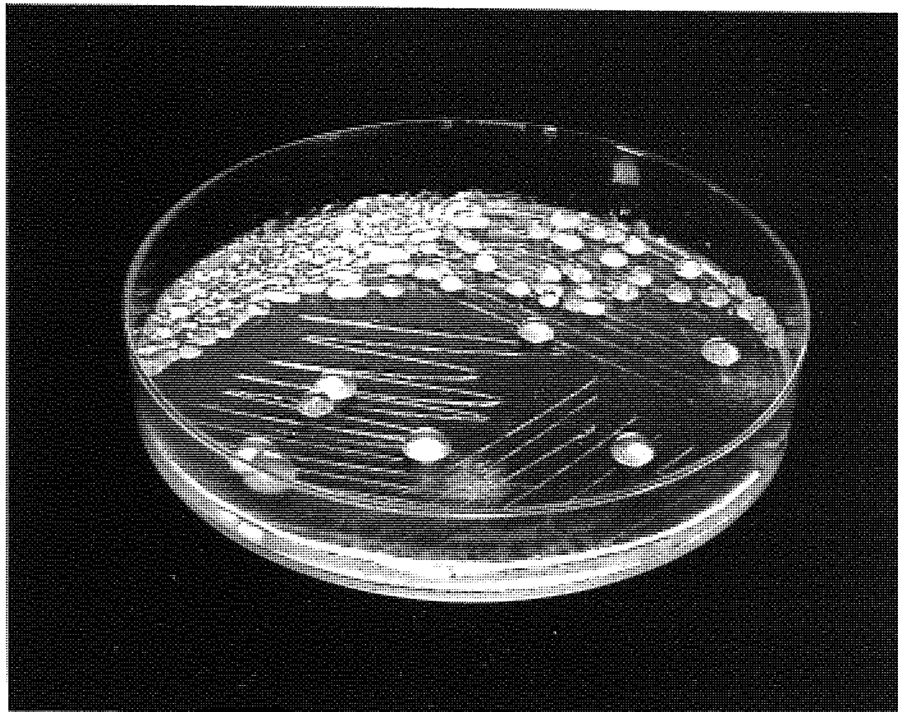
Three isolates which did not produce dextran from sucrose were not identified.

The complete biochemical characteristics of Groups I and II are shown in Table IV,5. The macroscopic and microscopic appearance of a typical isolate ~~are~~ is shown in Fig. IV,2.

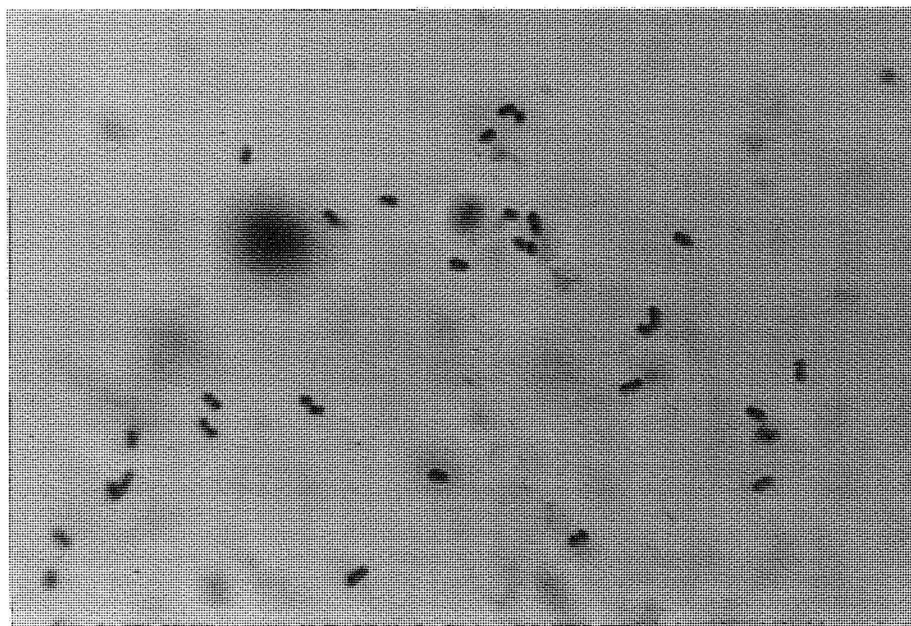
Groups I and II are distinguished from each other by fermentation of arabinose, maltose and rhamnose, and growth at 45°C. They clearly differ from any of the named species of Sharpe et al (1966) in the pattern of growth at 45°C, arginine hydrolysis, aesculin hydrolysis, growth in 0.4% Teepol, and fermentation of amygdalin. Therefore it is suggested that these Groups constitute two new species of betabacteria. A comparison of these groups with Betabacterium vermiforme (Ward) Mayer described by Perquin (1940) shows certain similarities, but the fermentation patterns differ significantly.

Strains 65b, 69b and 123a were sent to Dr. Sharpe for identification. She confirmed that these isolates differ significantly from previously named species of betabacteria, but are similar to several dextran-forming strains recently isolated from different sources, (culture numbers 930 and 889).

The qualitative nature of the extracellular polysaccharides of these five strains was compared in order to seek further evidence on the homogeneity of the proposed new species. Pure samples of gum were obtained from the organisms grown in a sucrose medium, by the method of Jeanes (1965). The gums were hydrolysed to their constituent simple sugars by mild acid hydrolysis. 10mg. gum was boiled with 45% formic acid in a sealed tube overnight; the hydrolysates were evaporated under vacuum, washed three times in distilled water, and finally dried in a vacuum oven at 40°C. Gas-liquid chromatography was performed on the trimethylsilyl derivatives, by the method of Sweeley, Bentley, Makita & Wells (1963).



(a)



(b)

FIG. IV.2.

Macroscopic and microscopic appearance of Lactobacillus confusus.

Key (a) Colonial morphology. Strain 65b grown on sucrose tryptone agar 3 days at 30°C (actual size)

(b) Microscopic appearance of Gram-stained film, magnification x 1650. Strain 69b grown in YGC broth for 24 hours at 30°C

Two peaks were observed in the chromatograms of all the hydrolysates; these were identified as α and β glucose. No other sugars were detected at the operating temperature of 190°C or at higher temperatures. It was concluded that glucose is the sole constituent simple sugar of the gums, which are probably the homo-glucan polysaccharide, dextran. Therefore the five isolates appear to be homogeneous with respect to extracellular polysaccharide formation. The proposed new species is tentatively named *Lactobacillus confusus*, and is described by Sharpe, Garvie & Tilbury (in preparation).

Further work is necessary to establish the taxonomic position of Groups II - VI, but it appears that they may also comprise one or several new species of *Lactobacillus*.

2. Genus *Bacillus* Cohn.

The genus *Bacillus* comprises Gram-positive rod-shaped bacteria which sporulate aerobically and produce catalase.

17 isolates were selected from plates of raw sugar agar, pH 5.5, after inoculation with 10^{-2} dilutions of cane juice heated for 20 minutes at 80°C to kill vegetative organisms. The juices were obtained from various West Indian sources (Expt. 'a') or from Mauritius (Expt. 'b', Chapter III).

The identification methods were those of Smith & Gordon in the 7th Edition of Bergey's Manual (Breed et al (1957)), which are a revision of the monograph of Smith, Gordon & Clark (1952). At the time of test the more recent methods of Wolf & Barker (1968) were not published.

The isolates were examined by the following tests:- microscopic appearance of cells grown on glucose agar, especially sporangium swelling, shape and location of spores, presence of vacuoles and diameter of cells, indole production; acetylmethylcarbinol production (Voges-Proskauer test); formation of acid and gas from glucose and mannitol; motility; growth in 7% NaCl; comparative growth on nutrient agar, glucose agar and soya agar; growth in glucose broth anaerobically; reduction of nitrates to nitrite, and formation of gas from nitrates anaerobically; pH of glucose broth cultures; starch hydrolysis and production of crystalline dextrin from starch.

Test	B.pumilus		B.subtilis		B.megaterium	
	Bergey	Tilbury	Bergey	Tilbury	Bergey	Tilbury
No. of strains examined	NS	8	NS	7	NS	2
Sporangium swollen	–	–	–	–	–	–
Spore shape oval to cylindrical	+	+	+	+	+	+
Spore location	central	central	central	central	central	central
Vacuoles in glucose agar cultures	–	–	–	–	+	+
Cell diameter (microns)	<0.9	0.5–0.9	<0.9	0.5–0.7	>0.9	1.5
Growth in 7% NaCl	+	+(7)	+	+	NS	–
Starch hydrolysis	–	–	+	+	+	+
Crystalline dextrins from starch	–	–	–	–	–	–
Anaerobic growth in glucose broth	–	–	–	∓(2)	–	∓(1)
Nitrites produced from nitrates	–	–	+	+	∓	–
Gas formation from nitrates	–	–	–	–	–	–
Acid from:- glucose	+	+(7)	+	+(6)	+	+
mannitol	+	+(6)	+	±(4)	+	±(1)
Motility	±	+	±	+	±	±(1)
Indole production	NS	–	NS	–	NS	–
Voges-Proskauer reaction	+	+	+	+	–	–
Growth on:- nutrient agar	+	+	+	+	+	+
glucose agar	+	+	++	++(5)	++	++
soya agar	++	+	++	+	++	+
pH of glucose broth cultures	>5.2	5.3–5.5	>5.2	5.5–5.7	NS	5.3
Isolate numbers:	B1;B2;B5;B8;B9; B11;B12;B13		B7;F1d;F5;MA; MB;MC;L13		B3; TSFa	

Table IV,6. Characteristics of 17 isolates of the genus Bacillus obtained from cane juice, in comparison with standard descriptions of species in Bergey's Manual (7th Edition).

Key:
+ all isolates positive
± majority of isolates positive
∓ majority of isolates negative
– all isolates negative
NS: not stated

Figures in brackets denote numbers of isolates giving a positive result.

Eight isolates were identified as _B. pumilus_ Gottheil; seven were _B. subtilis_ Cohn, and two were _B. megaterium_ de Bary. The results are compared with standard descriptions of the species, taken from Bergey's Manual, in Table IV,6.

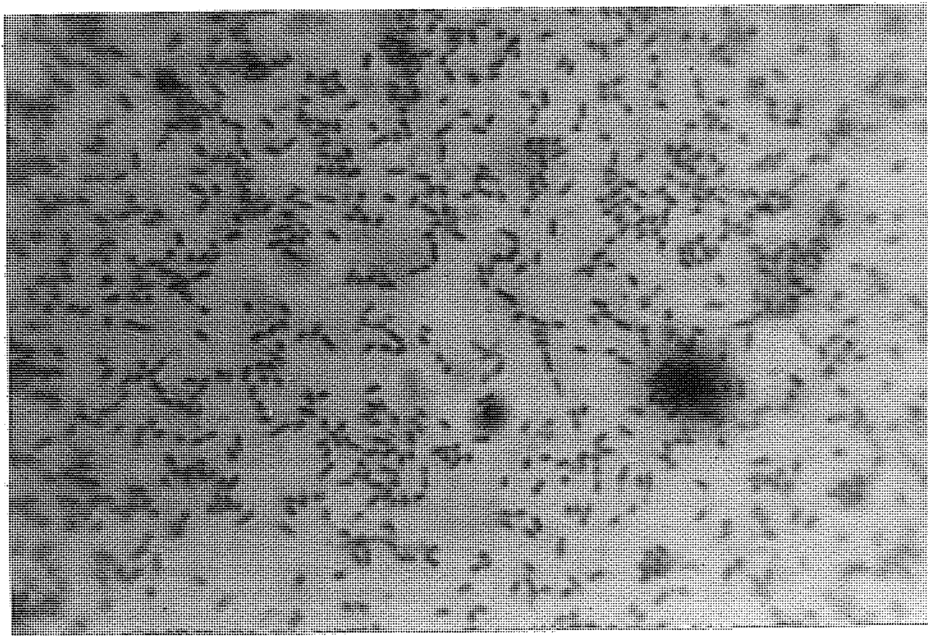
There was close agreement between the characteristics of the cane juice isolates and the standard species description. The exception was one isolate, B5, which was classed as _B. pumilus_ despite its inability to grow in 7% NaCl. This might be identified as _B. coagulans_, but it did not hydrolyse starch and the pH of a glucose broth culture was more than 5.0.

3. Gram-negative Bacteria

Many isolates from harvested cane were obtained on GYE agar, pH 4.8, containing nystatin to inhibit yeasts and actinomycin to inhibit lactic acid bacteria (Chapter II). All the isolates were catalase positive, Gram-negative, rod-shaped, bacteria. In general the population of these organisms in fresh juice was high, but they increased slowly during storage to reach a count 1/100 to 1/1,000 that of the lactic acid bacteria. Several different types of colony were observed, and most were pigmented. One type included small, round, opaque colonies with a low convex elevation, which ranged in colour from pale buff, pale orange, to dark brown. Another type consisted of yellow, smooth, shiny, flat, spreading colonies. The third type contained very large, opaque, domed, smooth, mucoid colonies, creamy white to pale yellow in colour; submerged colonies usually produced splits in the agar due to gas production, whilst surface colonies frequently 'dripped' onto the lid of inverted petri-dishes. A characteristic 'sweet acid' smell was noted. A typical isolate is shown in Fig. IV,3.

Typical isolates of each type were selected for further study. Screening tests for deteriogenic power showed that most of these isolates were not significantly deteriogenic when inoculated into a sterile, synthetic cane juice (Chapter V), but the mucoid types produced moderate gum formation. Therefore 14 isolates of the mucoid type were selected for identification tests.

(a)



(b)

FIG. IV.3.

Macroscopic and microscopic appearance of Enterobacter sp., strain no. 142.

Key (a) Colonial morphology on sucrose tryptone agar, 3 days at 30°C (actual size)

(b) Microscopic appearance of Gram-stained film, magnification x 1650. YGC broth culture, 24 hours at 30°C.

The scheme of Vanderzant & Nickelson (1969) for identification of Gram-negative bacteria was adopted. The criteria studied were Gram stain; growth on Maconkey agar; presence of oxidase (Kovacs 1956); fermentative or oxidative utilisation of carbohydrates (Hugh & Liefson 1953); motility and pigmentation.

All the isolates were Gram negative rods with rounded ends, in pairs, grew on Maconkey agar, oxidase negative, and fermentative; therefore they were members of the family *Enterobacteriaceae* Rahn.

Identification to genus level was determined by the reactions in Kohn's Two-Tube Medium (Oxoid Ltd.), as described by Carpenter, Lapage and Steel (1966).

All the isolates showed identical reactions:- acid and gas from glucose; acid from mannitol and sucrose/salicin; urease negative (one isolate was positive); H_2S and indole not produced; motile. On this basis they were classed in the genus *Enterobacter* Hormaeche & Edwards (late *Aerobacter* Beijerinck), but the species was not determined. *Aerobacter aerogenes* and *A. cloacae* are widely distributed in nature in plants, soil and water, and were found in large numbers on cane and in cane juice by Mayeux (1960) and Duncan & Colmer (1964).

The identity of yellow pigmented isolates was not determined, but many may belong to the genus *Erwinia* Winslow et al (Hendrie, Mitchell & Shewan, 1968). Wolzogen Kuhr (1923) found that the predominant bacteria of the epiphytic flora of sugarcane were yellow-pigmented, short rods which resembled *Bacterium herbicola a. aureum*, now classified as *Erwinia herbicola*.

4. Yeasts and Moulds

a) Yeasts:

Mesophilic and osmophilic yeasts were isolated from Frome cane (Experiments 'd' and 'e', Chapter III) on wort agar and osmophilic agar respectively. 53 isolates were screened for deteriogenic power by inoculation into a sterile, synthetic cane juice (Chapter V); 21 showed the ability to invert sucrose and were selected for identification.

The methods of identification were those of Lodder & Kreger-Van Rij (1952). The following tests were applied:- cell size, shape and pellicle formation in malt extract broth; pseudomycelium formation in slide cultures on potato dextrose agar; ascospore formation on carrot plugs; fermentation of glucose, galactose, maltose, lactose, sucrose and raffinose; assimilation of glucose, galactose, maltose, lactose, sucrose, and ethanol in Bacto Yeast Nitrogen Base; nitrate assimilation in Bacto Yeast Carbon Base, and aesculin hydrolysis. In addition, some of the tests recommended by Kreger-Van Rij (1964) were included, i.e. growth at 37°C; growth on osmophilic agar, and growth in Bacto Vitamin-Free Yeast Base.

At the time of test the two simplified schemes for identification of yeasts of Beech, Davenport, Goswell and Burnett (1968) had not been published. A new edition of 'The Yeasts' (Lodder 1970) has been published but not yet received.

The characteristics and identification of the yeasts are shown in Table IV,7.

None of the isolates formed ascospores and therefore were classified in the family _Cryptococcaceae_. In the absence of arthrospores and pigment production they were further classified in the sub-family _Cryptococcoideae_. Most of the isolates possessed round to oval cells which reproduced by multilateral budding, and did not form pseudomycelium or capsules; they belonged to the genus _Torulopsis_ Barlese. A few isolates possessed round to oval cells and a well developed pseudomycelium and were classed in the genus _Candida_ Berkhout.

Few of the isolates conformed exactly to the species description of Lodder & Kreger-Van Rij (1952) but the differences in properties were sufficiently slight to enable their classification in known species rather than create new species.

Isolate No.87d differed from the standard description of _Candida pseudotropicalis_ (Castellani) Basgal only in its positive assimilation of nitrate and smaller cell size. Numbers 102 and 103 were identified as _Candida tropicalis_ (Castellani) Berkhout although their cell size was smaller.

Test	C.pseudo-tropicalis	C. tropicalis	T. etchellsii	T. dattila	T. stellata	T. holmii	T.sake	T. versatilis
No. of isolates tested	1	2	1	3	5	5	2	2
Number of isolates	87d	102; 103	85a	95;105; 107	43a;92; 100;104 106	33a;39b 40a;48d 61b	45d; 60a	44b; 96
Ascospore form.ⁿ	−	−	−	−	−	−	−	−
Fermentation of:								
glucose	+	+	+	+	+	+	+	+
galactose	+	+	−	−	−	+	+	+
maltose	−	+/−	+	−	∓(1)	−	+	+
lactose	+	−	−	−	−	−	−	−
sucrose	+	+	−	+	±(4)	+	+	+/−
raffinose	+	−	−	−	−	±(4)	+/−	−
Assimn. of:								
glucose	+	+	+	+	+	+	+	+
galactose	+	+	−	+	−	+	+	+
maltose	−	+	+	+	−	±(4)	+	+
lactose	+	−	−	−	−	−	−	−
sucrose	+	+	−	+	+	+	+	+
ethanol	+	+/−	−	∓(1)	−	+	+	+/−
Pseudomycelium formation	+	+	−	−	−	−	−	−
Pellicle form.ⁿ	−	−	−	−	−	−	−	−
Cell size (μ)	2.0-3.0 x 2.6-5.0	3.7-6.1	2.4-4.2 x 2.4-4.4	2.0-6.1	2.8-5.4	2.0-5.4	3.0-5.0	2.4-5.9
Cell shape	oval	round	short/ oval	round	round	round	round	round
Nitrate assimn.	+	+/−	−	±(2)	±(4)	±(4)	+	+
Aesculin hydrolysis	+	−	−	∓(1)	−	∓(1)	+	−
Growth at 37°C	+	+	−	±(2)	∓(1)	∓(1)	+/−	+
Growth on osmo.agar	+	+	+	+	+	+	+	+
Growth on vit. free medium	−	−	−	−	+	±(4)	−	+/−

Table IV,7. Characteristics and identification of yeasts isolated from Frome cane.

Key:

+ all isolates positive

± majority of isolates positive

∓ majority of isolates negative

− all isolates negative

Figures in brackets denote number of strains giving a positive result.

Strain 85a was identical with Torulopsis etchellsii (Etchells et Bell) Lodder et Kreger-Van Rij except for its negative assimilation of galactose and nitrate.

The three strains identified as T. dattila (Kluyver) Lodder possessed smaller cells, assimilated nitrate (2/3) and did not ferment raffinose 1/3.

Five strains were classified as T. stellata (Kroemer et Krumbholtz) Lodder although they assimilated nitrate (4/5) and did not ferment raffinose 1/3. A variety of this species, T. stellata v. cambresieri, had been isolated from a sugar mill, and El-Tabey Shehata (1960) had previously found this species in Brazilian cane juice.

The five isolates placed in T. holmii (Jorgensen) Lodder differed from the standard description only in positive nitrate assimilation (4/5 strains).

Similarly, isolates Nos. 45d and 60a differed from T. sake (Saito et Ota) Lodder et Kreger Van Rij in their positive nitrate assimilation.

Finally, nos. 96 and 44b were identical with T. versatilis (Etchells et Bell) Lodder et Kreger Van Rij except for their negative fermentation of lactose and raffinose, but this fermentation was only weak in the standard description.

All the isolates were able to grow on osmophilic agar, but in general the strains within a species were isolated on both osmophilic and wort agar. Two species, T. etchellsii and T. versatilis, had previously been isolated by the author from British Guiana raw sugar (Tilbury 1967).

Pellicle forming yeasts were frequently observed in cane juice but were not identified as they did not exhibit deteriogenic power.

b) Moulds:

The identification of moulds from stored cane was not studied. However, the pink fungus Monilia sitophila (Montagne) Saccardo (perfect stage Neurospora sitophila) was widely distributed in large numbers on stored, harvested cane, where it generally became visible within 3-4 days of harvest, especially in wet weather. (Chapter III). This was a troublesome contaminant of laboratory petri-dishes and in order to prevent its overgrowth of plates it was necessary to inhibit it by the evaporation of diphenyl on the petri-dish lid prior to

incubation (Ingram, 1958).

C. CONCLUSIONS

1. 206 microorganisms were isolated in pure culture from juice extracted from stored, harvested cane. Selective media were used to isolate the different types of microorganisms thought to influence the biodeterioration of cut cane, i.e. lactic acid bacteria; Bacillus species; Gram-negative, catalase-positive, aciduric, rod-shaped bacteria, and yeasts. The isolates were identified by methods recommended by current leading authorities for each group.

2. The 154 isolates of lactic acid bacteria comprised 87 cultures in the genus Leuconostoc and 67 in the genus Lactobacillus.

3. Leuconostoc isolates were identified as 63 strains of L. mesenteroides, 23 strains of L. dextranicum and 1 strain of L. paramesenteroides. Only the latter isolate did not form dextran from sucrose. The L. mesenteroides strains were similar to the Group VI of Garvie (1967a), but L. dextranicum strains differed significantly from Garvie Groups IV and V in their higher proportion which fermented salicin and hydrolysed aesculin. The four types of colonial morphology described by McCleskey et al (1947) were observed, but these types were not correlated with differences in biochemical properties. The organisms were widely distributed and predominated in juice from stored, harvested cane.

4. Lactobacillus isolates were separated into the sub-genera Streptobacterium (30 isolates) and Betabacterium (37 isolates) on the basis of gas production from glucose.

5. The homofermentative streptobacteria were identified as L. plantarum (17 strains) L. casei v. casei (5 strains) and L. casei v. alactosus (8 strains). The characteristics of these strains were in general agreement with the standard species description of Sharpe (1962). Many strains of L. casei v. alactosus and v. casei formed dextran from sucrose.

6. The heterofermentative betabacteria differed significantly from the descriptions of the six species named by Sharpe (1962) and Sharpe et al (1966). All formed dextran from sucrose and hydrolysed aesculin. They were divided

into six groups on the basis of arginine hydrolysis, growth at 45°C and fermentation of six sugars. Detailed studies were made of Groups I and II, which each contained six strains. Group I strains appeared to be a new species of *Lactobacillus*, tentatively named *L. confusus*, discussed in a paper by Sharpe, Garvie & Tilbury (in preparation). The characteristics of this species include hydrolysis of arginine, failure to grow at 45°C and in the presence of 0.4% Teepol, and fermentation of arabinose, xylose, cellobiose, amygdalin, rhamnose, salicin and sucrose.

7. 17 isolates of the genus *Bacillus* were identified as *B. pumilus* (8 strains); *B. subtilis* (7 strains) and *B. megaterium* (2 strains).

8. 14 isolates of Gram-negative, catalase-positive, aciduric bacteria formed large, opaque mucoid colonies on sucrose agar and produced significant amounts of gum in a synthetic cane juice. They were classed in the genus *Enterobacter* (Carpenter et al 1966) (late *Aerobacter*), but the species was not determined. It is suggested that isolates of yellow-pigmented bacteria belong to the genus *Erwinia*.

9. 21 isolates of yeast which were able to invert sucrose in a synthetic cane juice were identified as follows:- *Candida pseudotropicalis* (1 strain); *C. tropicalis* (2 strains); *Torulopsis etchellsii* (1 strain); *T. dattila* (3 strains); *T. stellata* (5 strains); *T. holmii* (5 strains); *T. sake* (2 strains) and *T. versatilis* (2 strains). The characteristics of the isolates were in general agreement with the species' descriptions of Lodder and Kreger-Van Rij (1952).

10. The mould *Monilia sitophila* (perfect form *Neurospora sitophila*) frequently occurred on the stalks of harvested cane during storage.

CHAPTER V THE ETIOLOGY OF SOUR CANE

A. INTRODUCTION

Etiology is the study of the cause of a phenomenon. The phenomenon considered here is biodeterioration of sugar cane caused by microorganisms ('sour cane'), as distinct from autolytic changes which occur in non-infected cane ('stale cane').

The fundamental principles for establishment of an organism as the causative agent of a disease were defined in the classical work of Koch (1882). Hueck (1965) applied Koch's postulates to biodeterioration, and adapted them as follows:-

1. A biodeteriogen must be present in or near materials showing phenomena of biodeterioration, in such numbers and such a distribution that their presence may explain the phenomena in question.

2. It must be possible to isolate the biodeteriogen from the material showing phenomena of biodeterioration and grow it as a pure culture.

3. It must be possible, with the pure cultures thus obtained, to reproduce under controlled conditions, phenomena of biodeterioration comparable to the original phenomena. The microorganism must be observed in, and recovered from, the experimentally infected material.

These criteria are adopted in this study.

Despite many publications on the post-harvest deterioration of sugarcane, few include microbiological aspects. Most of these investigations studied extracted cane juice in the factory mill, where the principal emphasis was on the effect of bacteria in the mill on inversion of sucrose between crushing and clarification, and the formation of 'dextran' gums and slimes which clog pipelines and retard sugar manufacture. It can reasonably be assumed that a large proportion of microorganisms present in factory mill juices are derived from the extracted juice, although many originate from the epiphytic flora of the cane plant, soil, trash, and the milling plant itself. The nature of the microflora of mill juices therefore provides some information on the microflora of harvested cane. Furthermore, it can be argued that many

of the changes in extracted juice composition brought about by these organisms in the mill could also occur inside the stalks of infected, cut cane.

Early work on the microbiology of mill juices, both in cane and sugar beet factories, was reviewed by Thaysen & Galloway (1930), Hucker & Pederson (1942), and Owen (1949). In general, the occurrence of dextran slimes and gums was due to the growth of Leuconostoc mesenteroides, but in a few cases levan gums were formed by Bacillus vulgatus and related species. Perquin (1940) reviewed the literature on the formation of gums from sucrose by rod-shaped bacteria, and studied a case of dextran formation in a beet sugar factory which was due to a lactic acid bacterium, identified as Betabacterium vermiforme.

Later work was mainly executed in Louisiana, where deterioration of frozen cane resulted in severe processing difficulties. McCalip & Hall (1938) isolated L. mesenteroides in large numbers and almost pure culture from the stalks of deteriorated, frost-damaged cane; when inoculated into sterile 10% sucrose solutions, pure cultures of this organism reproduced the symptoms of frost-damaged deteriorated cane, i.e. loss of sucrose, drop in pH, and increase in viscosity and dextran content. Millstein, Tobin & McCleskey (1940), Alford & McCleskey (1942), McCleskey, Faville & Barnett (1947) and Faville (1947) studied gum-forming bacteria in cane juice, and found that L. mesenteroides was the principal organism. More recently, however, Mayeux (1960) and Duncan & Colmer (1964) reported the occurrence of large numbers of coliform bacteria (Aerobacter aerogenes and A. cloacae) in mill juice. The latter authors found that pure cultures of A. cloacae produced slime when inoculated into sterilised cane juice, and suggested that coliform bacteria may play a significant part in the deterioration of cane juice. Their results hardly justify this hypothesis because the coliform counts were only a small fraction of the total number of organisms.

The microflora of sugar mills in Cuba was studied by Pederson (1938) and Pederson & Hucker (1946). In freshly-extracted juice the predominant

microorganisms were species of the genera Bacillus, Micrococcus, Flavobacterium, Achromobacter and Escherichia; L. mesenteroides was frequently present, but did not predominate until a later stage in the process.

The most recent work is that of Egan in Queensland, who made an extensive study of the deterioration of mechanically-harvested, chopped-up cane. (Egan & Rehbein 1963; Egan 1964, 1965 a, b & c; 1966; 1967 a,b; 1968 a,b). His work clearly showed that this phenomenon, named 'sour storage rot', was due to infection of cane by L. mesenteroides. It was isolated in almost pure culture and in very high numbers from nearly all samples of chopped-up cane billets examined; the degree of deterioration was correlated with the extent of infection. These results satisfy the conditions of Koch's first and second postulates. Egan & Rehbein (1963) also did experiments which satisfied Koch's third postulate; inoculation of chopped cane with pure cultures of L. mesenteroides reproduced the symptoms of 'sour storage rot' whilst 'sterile' controls did not deteriorate significantly in replicated trials, despite some cross-infection. Comparable studies were not carried out on whole-stalk cane.

An earlier paper by Nicholson & Lilienthal (1959) reported the formation of a new polysaccharide in whole-stalk cut cane in Queensland; the polysaccharide was a glucan with 29% α 1-6 links and 60% α 1-4 linkages. Inoculation of sterilised cane with pure cultures of L. mesenteroides showed that slight growth occurred during subsequent storage, but it was not correlated with this polysaccharide formation. Similarly, there was no correlation between L. mesenteroides count and polysaccharide content in regions of a naturally infected stalk 14 days after harvest; in another experiment, naturally infected cane stored over a 14-day period showed significant increases in L. mesenteroides count, but no formation of the polysaccharide. This evidence suggests that L. mesenteroides was not responsible for the formation of the new polysaccharide; Leuconostoc-type dextran was not detected in the stalks.

The significance of levan-forming organisms belonging to the genus Bacillus in sugar refineries was discussed by Scarr (1949) and Skole, Newman & Barnwell (1968), but the effects of this group in harvested cane deterioration have not previously been studied.

In Jamaica, Smith (1956) determined microbial counts of mill juices in one factory at daily intervals over a three year period. The mean count per ml. of first expressed juice was 3.4×10^6, with a minimum of 1.0×10^6 and maximum of 7.4×10^6. About 30% of the total microbial count was yeasts, 60% 'slime moulds', and 10% was miscellaneous moulds and bacteria. However, it seems probable that the 'slime moulds' were in fact Leuconostoc colonies.

It is concluded from the literature that there is circumstantial evidence which favours Leuconostoc mesenteroides as the principal biodeteriogen in sour cane, but other microorganisms may also be significant. Since the bulk of this evidence was obtained from extracted juice in the mill rather than the cane itself, it does not satisfy the etiological principles of Koch's first and second postulates. No work has been reported which satisfies Koch's third postulate for whole-stalk cane. Therefore research was initiated to elucidate the etiology of sour cane.

Qualitative and quantitative changes in the microbial flora of harvested cane during storage were investigated in a series of experiments in the United Kingdom and Jamaica, reported in Chapters III and IV. The results enable valid conclusions to be drawn with respect to Koch's first and second postulates.

The differentiation between biodeteriogens and non-biodeteriogens amongst the microorganisms isolated from a material is of prime importance in etiology. This problem can only be resolved by tests which satisfy Koch's third postulate. It was not considered feasible to screen large numbers of microorganisms by the inoculation of pure cultures into aseptically-harvested cane, due to the inadequate supply of cane at the Research Centre. Instead, a preliminary screening test was devised which

determined the ability of pure cultures to reproduce the effects of sour cane when inoculated into a sterile synthetic cane juice. A few selected isolates were subsequently tested in cane stalks.

B. EXPERIMENTAL

1. Koch's 1st and 2nd Postulates

The results of experiments in Chapters III and IV show that *Leuconostoc mesenteroides* is the predominant organism in stored cane, and that its growth is associated with loss of sucrose, drop in pH, increase in reducing sugars, formation of gum and dextran, and increase in juice viscosity. These changes occur more rapidly in heavily infected cane than in lightly-infected cane. It is concluded that *L. mesenteroides* satisfies Koch's 1st and 2nd postulates with regard to its establishment as a causal organism of sour cane.

However, it was also observed that on extended storage other micro-organisms may attain a high population and therefore may contribute to deterioration, e.g. lactobacilli, yeasts, and catalase positive, Gram-negative, rod-shaped, aciduric bacteria.

2. Screening of microorganisms isolated from cane juice for deteriogenic power in a synthetic cane juice.

A synthetic medium was formulated in an attempt to obtain a defined, reproduceable, imitation of natural, fresh, cane juice. Its composition was based on analyses of cane juice published by Spencer & Meade (1963) and unpublished data from Frome Estate:-

Synthetic Cane Juice Medium (SCJ)

	g/L
Sucrose	156.0
Dextrose	14.0
KH_2PO_4	1.5
$MgSO_4.7H_2O$	0.5
$CaSO_4$	0.5
Soluble starch	1.0
Tryptone (Bacto)	5.0
Yeast extract (Difco)	5.0
Distilled water to	1 L
pH	5.4

The medium was distributed in 50 ml. amounts in 100 ml. rimless conical flasks fitted with aluminium caps, and sterilised by autoclaving for 10 minutes at 10 p.s.i.

Stock cultures of microorganisms were grown in appropriate liquid media for 24 - 48 hours and 0.1 ml. volumes were incoulated into flasks of SCJ medium. The flasks were incubated for 5 days at $30^{\circ}C$ ($\pm 2^{\circ}$) with occasional hand shaking.

After incubation the cultures were centrifuged for 6 minutes at 3,000 RPM and the supernatants analysed for the principal symptoms exhibited in sour cane juice:-

<u>acid production</u> - pH was determined by pH meter

<u>inversion of sucrose</u> - reducing sugars were determined by a modification of the EDTA titrimetric method of Knight & Allen (1960), described in Chapter II.

<u>'gum' formation</u> - 'gum' was determined by the method of Bruijn (1966a), and viscosity by the Ostwald's viscometer method described in Chapter II.

Sterile controls were included in each test.

The ability of an organism to reproduce the symptoms of sour cane juice was expressed by its 'deteriogenic power'.

The classification scheme adopted is shown below:-

Classification of Deteriogenic power	Acidity	Inversion	Gum *	
	pH	% R.S. + % Gums	% Gum	Viscosity (cP at $25^{\circ}C$)
0 - None	> 5.2	< 2.6	< 0.33	< 1.70
1 - Weak	4.5 - 5.2	2.6 - 5.0	0.33 - 0.50	1.70 - 2.00
2 - Moderate	3.5 - 4.45	5.05 - 7.5	0.51 - 1.00	2.10 - 3.40
3 - Strong	< 3.5	> 7.5	> 1.00	> 3.40

(*Classification of 'gum' is based on the highest results of either % gum or viscosity).

These criteria were based on a comparison with results obtained from 13 sterile control juices:-

	pH	%R.S.	% Gum	Viscosity(cPat25°C)
Mean	5.40	1.80	0.23	1.630
Maximum	5.70	2.25	0.33	1.700
Minimum	5.25	1.10	0.14	1.530

During 1966-1968 the biodeterioration of imported, stored, harvested cane was studied at the Research Centre in a series of experiments coded 'a' to 'j' (Chapter III). More than 300 representative microorganisms were isolated from these samples on various selective media, and maintained in a stock culture collection. They were classified in groups down to genus level, and many were identified to species level (Chapter IV). 215 of these isolates were screened for 'deteriogenic power' in SCJ medium:-

Group of microorganisms	No. of isolates tested	Results in Table
Leuconostoc species	42	V,1.
Lactobacillus species	34	V,2.
Catalase-positive, Gram-negative, aciduric rods	52	V,3.
Bacillus species	22	V,4.
Coliform bacteria	9	V,4.
Actinomycetes	3	V,4.
Yeasts	53	V,5.

The results of these tests are summarised in Table V,6.

Moulds were not tested because they are generally present in small numbers in stored cane, coupled with their relatively slow growth rate and obligately aerobic growth requirement.

The characteristic pattern of 'deteriogenic power' of *Leuconostoc mesenteroides* and *L. dextranicum* was moderate acidity, weak to strong inversion, and moderate to strong gum formation; the viscosity of several isolates was too high to be tested in an Ostwald viscometer. It was concluded that *L. mesenteroides* and *L. dextranicum* can cause severe deterioration of cane juice.

Isolate No.	Sample source	Screening Test Result				Deteriogenic Power		
		pH	%R.S.	% Gum	Viscosity (cP at25°C)	Acidity	Inversion	Gum
G6	b	3.65	2.50	0.380	2.501	2	1	2
G7	b	3.80	4.85	1.220	3.527	2	2	3
G8	b	3.70	2.50	0.700	2.256	2	1	2
G9	b	3.70	3.75	0.700	2.365	2	1	2
G10	b	3.65	3.00	0.760	2.347	2	1	2
G11	b	3.75	2.90	0.474	1.872	2	1	1
G12	b	3.65	2.10	0.360	1.782	2	0	1
G12a	b	3.85	2.30	0.260	1.673	2	1	0
G13	b	3.70	4.70	2.086	839.5	2	2	3
G14	b	3.75	2.70	0.380	1.963	2	1	1
L16	b	3.70	4.20	1.334	6.659	2	2	3
L17	b	3.70	2.90	0.474	1.692	2	1	1
L18	b	3.75	3.40	0.934	2.579	2	1	2
L19	b	3.80	2.55	0.660	2.415	2	1	2
L20	b	3.60	6.00	NT	255.2	2	3	3
16b	d	3.80	7.95	5.540	229.9	2	3	3
62a	e	3.65	1.45	0.453	3.039	2	0	2
65a	e	4.05	NT	1.270	2.389	2	NT	3
68b	e	3.50	1.60	0.440	1.816	2	0	1
69a	e	3.70	NT	0.800	2.110	2	NT	2
70a	e	3.90	1.31	0.600	2.129	2	0	2
71a	e	3.70	1.75	0.527	1.933	2	0	2
71b	e	3.50	2.20	0.433	1.868	2	1	1
72a	e	3.85	>2.00	0.247	2.625	2	1	2
73b	e	3.90	5.35	2.367	30.91	2	3	3
73c	e	3.80	1.70	0.051	2.171	2	0	2
122a	f	3.70	3.60	0.827	2.588	2	1	2
122c	f	3.50	2.70	0.680	2.627	2	1	2
131	g	3.15	3.40	2.470	1.440	3	2	3
133	g	4.10	1.85	0.430	1.990	2	1	1
152	j	4.10	5.20	4.28	TV	2	3	3
153	j	4.10	4.60	4.39	TV	2	3	3
155	j	4.10	3.50	3.81	TV	2	2	3
14c	d	3.70	2.70	0.813	2.085	2	1	2
17b	d	3.90	1.80	0.253	1.646	2	0	0
66b	e	3.60	1.95	0.640	2.300	2	1	2
70b	e	3.80	1.70	0.253	1.673	2	0	0
156	j	3.95	0.90	1.61	3.215	2	0	3
159	j	3.75	3.00	2.82	16.85	2	2	3
200	j	3.80	2.30	1.14	3.023	2	1	3
72b	e	3.80	1.10	0.247	1.856	2	0	1
154	j	4.10	5.40	4.08	TV	2	3	3

Table V.1. Deteriogenic power of microorganisms isolated from cane juice, estimated by a screening test in SCJ medium:- Leuconostoc species

Key to isolates. All isolates are *Leuconostoc mesenteroides*, except nos. 14c, 17b, 66b, 70b, 156, 159 & 200 which are *L. dextranicum*, and 154 which is an unidentified *Leuconostoc* species, and 72b which is *L. paramesenteroides*.

NT : Not Tested

TV : Too viscous to be tested in Ostwald viscometer.

Isolate No.	Identification	Sample source	Screening Test Result				Deteriogenic Power		
			pH	%R,S,	% Gum	Viscosity (cP at25°C)	Acidity	Inversion	Gum
J5a2	L.casei v. alactosus	a	3.55	1.70	0.233	1.623	2	0	0
J5bs		a	3.50	2.00	0.300	1.665	2	0	0
64b		e	3.35	1.85	0.334	1.756	3	0	1
64c		e	3.30	2.00	0.400	1.815	3	0	1
67b		e	3.70	4.85	0.300	2.198	2	2	2
124a		f	3.30	9.95	4.866	3.147	3	3	3
48b	L.casei v.casei	d	3.50	5.85	1.493	2.095	2	2	3
50b		d	3.50	5.75	1.480	2.396	2	2	3
115b		e	3.55	5.50	1.247	2.302	2	2	3
116a		e	3.50	2.50	0.427	1.772	2	1	1
116b		e	3.30	6.00	1.874	2.428	3	3	3
T1(1)	L. plantarum	a	3.45	1.85	0.207	1.624	3	0	0
TI(2)		a	3.95	1.20	0.213	1.622	2	0	0
L6s		b	3.80	10.40	0.287	5.678	2	3	3
7a		d	3.50	2.20	0.200	1.615	2	0	0
12a		d	3.50	1.70	0.220	1.603	2	0	0
12b		d	3.60	2.50	0.280	1.595	2	1	0
54a		d	3.50	1.65	0.227	1.634	2	0	0
63a		e	3.35	1.25	0.380	1.673	3	0	1
63b		e	3.35	1.40	0.180	1.632	3	0	0
73a		e	3.60	0.57	0.787	1.691	2	0	2
74b		e	4.30	1.40	0.267	1.626	2	0	0
138		g	3.50	1.55	0.260	1.600	2	0	0
139		g	3.65	2.00	0.270	1.630	2	0	0
140		g	3.55	1.10	0.270	1.650	2	0	0
141		g	3.45	2.30	0.330	1.640	3	1	1
65b	L. nov.spec. Group I	e	4.15	2.80	1.367	4.583	2	1	3
68a		e	3.80	NT	1.160	2.105	2	NT	3
69b		e	4.15	2.70	1.033	3.147	2	1	3
123a		f	4.15	3.30	1.660	5.145	2	1	3
62b	Unidentified	e	3.70	1.40	0.440	1.715	2	0	1
66a		e	4.10	NT	1.720	4.693	2	NT	3
67a		e	3.70	NT	0.787	2.057	2	NT	2
134		g	3.90	2.95	0.310	1.770	2	1	1

Table V.2. Deteriogenic power of microorganisms isolated from cane juice, estimated by a screening test in SCJ medium:-

Lactobacillus species

NT : Not Tested

Group	Isolate No.	Sample source	Screening Test Result				Deteriogenic Power		
			pH	%R.S.	% Gum	Viscosity (cP at25°C)	Acid-ity	Inver-sion	Gum
I	80	e	5.35	0.54	0.213	1.744	0	0	1
	117a	e	5.20	2.05	0.167	1.517	1	0	0
IIa Misc.	JC43b	a	4.10	0.00	0.220	1.533	2	0	0
	L12	b	4.50	0.45	0.327	2.197	1	0	2
	13b	d	4.75	1.10	0.220	3.650	1	0	3
	27c	d	4.20	1.30	0.280	1.742	2	0	1
	31e	d	4.10	2.50	0.213	1.675	2	1	0
	32b	d	4.75	3.40	0.213	1.530	1	1	0
	74a	e	4.30	0.00	0.340	1.744	2	0	1
	74d	e	4.20	0.90	0.280	1.643	2	0	0
	75b	e	4.30	0.10	0.260	1.691	2	0	0
	76c	e	4.90	0.54	0.240	1.750	1	0	1
	77	e	4.40	0.00	0.247	1.780	2	0	1
	78b	e	4.15	1.20	0.207	1.655	2	0	0
	78c	e	4.05	2.00	0.233	2.140	2	0	2
	79b	e	4.05	0.90	0.293	1.667	2	0	0
	81a	e	4.05	0.53	0.307	1.733	2	0	1
	83b	e	4.40	1.55	0.207	1.583	2	0	0
	84a	e	4.30	0.95	0.173	1.556	2	0	0
	118a	e	4.20	1.40	0.207	1.563	2	0	0
	118b	e	4.05	1.55	0.180	1.515	2	0	0
	136	g	4.20	4.75	0.210	2.510	2	1	2
IIb cream, mucoid	142	h	4.35	0.00	0.597	1.739	2	0	2
	147	i	4.35	1.00	0.548	1.652	2	0	2
	148	i	4.30	0.20	0.630	1.915	2	0	2
	149	i	4.30	0.90	0.476	1.805	2	0	1
	180	j	4.30	1.50	0.646	1.939	2	0	2
	183	j	4.50	1.20	NT	1.774	1	NT	1
	184	j	4.35	0.20	0.477	1.689	2	0	1
	185	j	4.30	0.40	0.586	2.065	2	0	2
IIc yellow mucoid	175	j	4.75	0.40	0.599	2.884	1	0	2
	178	j	4.80	1.00	0.615	2.784	1	0	2
	179	j	4.80	0.00	0.516	3.042	1	0	2
III	J4	a	3.30	2.00	0.233	1.689	3	0	0
	28b	d	3.60	1.80	0.253	1.571	2	0	0
	29a	d	3.75	1.95	0.260	1.659	2	0	0
	30	d	3.35	2.05	0.213	1.693	3	0	0
	31a	d	3.35	1.30	0.287	1.586	3	0	0
	31b	d	3.50	9.85	0.233	2.178	2	3	2
	31c	d	3.30	1.50	0.260	1.553	3	0	0
	31d	d	3.20	6.75	0.253	1.746	3	2	1
	52	d	3.75	1.55	0.207	1.539	2	0	0
	74c	e	3.55	3.70	0.207	2.998	2	1	2
	75c	e	3.75	1.05	0.247	1.638	2	0	0
	78a	e	3.15	4.45	0.253	1.681	3	1	0
	117b	e	3.55	1.25	0.200	1.512	2	0	0
IV	27a	d	2.90	9.65	0.220	2.251	3	3	2
	75a	e	2.70	>2.00	0.307	2.814	3	1	2
	76a	e	2.85	5.40	0.213	3.136	3	2	2
	79a	e	2.80	1.40	0.307	1.850	3	0	1
	82a	e	2.90	>2.00	0.297	2.607	3	1	2
	82d	e	2.95	2.50	0.220	1.588	3	1	0

Table V.3. Deteriogenic power of microorganisms isolated fron cane juice, estimated by a screening test in SCJ medium:- catalase positive, Gram-negative, acid-tolerant rod-shaped bacteria.

Group No.		
I	Final pH in SCJ medium	>5.00
II	"	4.05 - 5.00
III	"	3.00 - 4.00
IV	"	<3.00

NT : Not Tested

Isolate No.	Identification	Sample source	Screening Test Result pH	%R.S.	% Gum	Viscosity (cP at 25°C)	Deteriogenic Power Acidity	Inversion	Gum
	<u>Bacillus species</u>								
TSFa	B.	a	4.90	4.85	0.213	1.670	1	2	0
B3	megaterium	b	4.80	4.45	0.247	1.651	1	1	0
B1	B.pumilus	b	4.70	1.50	0.187	1.582	1	0	0
B5		b	4.75	1.70	0.313	1.560	1	0	0
B8		b	5.25	1.05	0.240	1.547	0	0	0
B12		b	4.70	1.65	0.240	1.604	1	0	0
MB		a	5.80	8.15	0.149	2.545	0	3	2
F5		a	5.70	>10.0	0.153	2.560	0	3	2
B7	B.subtilis	b	4.90	7.05	0.320	2.429	1	2	2
22b		d	4.90	4.85	0.264	1.774	1	2	1
23a		d	4.75	1.40	0.187	1.545	1	0	0
114b		e	5.90	7.90	0.247	1.594	0	3	0
20a		d	5.00	3.55	0.287	1.714	1	1	1
24a		d	3.95	1.65	0.233	1.566	2	0	0
25a		d	5.80	8.70	0.191	2.005	0	3	2
56a	Unidentified	d	6.80	1.80	0.347	1.583	0	0	1
57a		d	4.65	1.40	0.287	1.579	1	0	0
111		e	4.90	4.15	0.220	1.653	1	1	0
112		e	4.75	1.40	0.227	1.632	1	0	0
113		e	4.70	1.10	0.320	1.577	1	0	0
114a		e	5.05	4.25	0.200	1.681	1	1	0
121b		e	5.00	0.75	0.290	1.616	1	0	0
	<u>Coliform bacteria</u>								
1a	Aerobacter aerogenes I	c	4.15	0.80	0.260	1.601	2	0	0
19a		d	4.35	1.35	0.300	1.663	2	0	0
19b		d	3.90	1.55	0.233	1.597	2	0	0
55a	Unidentified	d	4.55	0.00	0.263	1.591	1	0	0
108a		e	4.15	4.15	0.313	1.546	2	1	0
108b		e	4.35	1.70	0.226	1.598	2	0	0
109		e	4.45	1.10	0.350	1.640	2	0	1
110		e	4.15	2.00	0.371	1.990	2	0	1
125		g	4.35	0.65	0.290	1.900	2	0	1
	<u>Actinomycetes</u>								
28a	Unidentified	d	5.50	1.90	NT	NT	0	0	NT
29c		d	5.55	0.00	NT	NT	0	0	NT
32a		d	5.50	1.40	NT	NT	0	0	NT

<u>Table V.4.</u> Deteriogenic power of microorganisms isolated from cane juice, estimated by a screening test in SCJ medium:-

<u>Bacillus</u> species; coliform bacteria and actinomycetes.

NT : Not Tested

Isolate No.	Identification	Sample source	Screening Test Result			Deteriogenic Power	
			pH	%R.S.	Viscosity (cP at25°C)	Acidity	Inversion
87d	Candida pseudo-tropicalis	e	4.05	5.95	NT	2	2
102	C.	e	4.15	9.00	NT	2	3
103	tropicalis	e	3.95	9.65	1.504	2	3
95	Torulopsis	e	4.20	8.65	NT	2	3
105	dattila	e	4.15	4.00	NT	2	1
107		e	4.10	4.75	NT	2	1
85a	T.etchellsii	e	4.25	9.35	NT	2	3
33a	T.holmii	d	4.50	>10.0	NT	1	3
39b		d	4.30	>10.0	NT	2	3
40a		d	4.40	>10.0	NT	2	3
48d		d	4.10	4.60	NT	2	1
61b		d	4.20	>10.0	NT	2	3
45d	T.sake	d	4.25	>10.0	NT	2	3
60a		d	4.25	>10.0	NT	2	3
43a	T.stellata	d	4.40	>10.0	NT	2	3
92		e	4.40	6.50	NT	2	2
100		e	4.00	8.15	NT	2	3
104		e	4.40	>10.0	NT	2	3
106		e	4.25	6.45	NT	2	2
44b	T.	d	4.10	6.60	NT	2	2
96	versatilis	e	4.30	>10.0	NT	2	3
34a		d	5.30	1.40	NT	0	0
36a		d	4.45	0.65	NT	2	0
38a		d	4.30	1.70	NT	2	0
45c		d	5.40	1.70	NT	0	0
47a		d	4.50	0.80	NT	1	0
86b		e	4.30	0.20	NT	2	0
87a		e	5.20	1.85	NT	1	0
88b	Unidentif-	e	4.65	0.00	NT	1	0
89a.	ied,	e	4.85	0.75	NT	1	0
90d	isolated	e	4.55	0.75	NT	1	0
49	from wort	d	3.95	2.80	NT	2	1
35a	agar	d	4.25	0.35	NT	2	0
87c		e	4.60	1.10	NT	1	0
90e		e	4.30	1.05	NT	2	0
91		e	4.10	0.75	NT	2	0
93		e	4.30	0.90	NT	2	0
94		e	4.45	0.75	NT	2	0
119a		e	5.20	2.25	NT	1	0
120b		e	4.35	1.35	NT	2	0
129		g	4.30	0.80	NT	2	0
130		g	4.25	>10.0	NT	2	3
135		g	4.35	0.00	NT	2	0
41a	Unidentif-	d	4.35	0.00	NT	2	0
42a	ied,	d	4.30	0.30	1.457	2	0
58a	isolated	d	4.65	0.00	NT	1	0
59a	from	d	4.60	0.50	1.550	1	0
97	osmophilic	e	4.30	0.00	1.420	2	0
98b	agar	e	4.30	0.80	NT	2	0
99		e	4.25	1.25	NT	2	0
101a		e	4.70	0.00	NT	1	0
101b		e	4.30	1.70	1.413	2	0
128		g	4.40	1.85	NT	2	0

Table V,5. Deteriogenic power of microorganisms isolated from cane juice, estimated by a screening test in SCJ medium:- Yeasts

NT : Not Tested

Organism	No. Isolates	Acidity* 0	Acidity* 1	Acidity* 2	Acidity* 3	Inversion* 0	Inversion* 1	Inversion* 2	Inversion* 3	Gum* 0	Gum* 1	Gum* 2	Gum* 3
Leuconostoc species													
L. mesenteroides	33	0	0	32	1	6	15	5	5	1	7	14	11
L. dextranicum	7	0	0	7	0	3	3	1	0	2	0	2	3
L. paramesenteroides	1	0	0	1	0	1	0	0	0	0	1	0	0
Unidentified	1	0	0	1	0	0	0	0	1	0	0	0	1
Lactobacillus species													
L. casei v. alactosus	6	0	0	3	3	4	0	1	1	2	2	1	1
" v. casei	5	0	0	4	1	0	1	3	1	0	1	0	4
L. plantarum	15	0	0	11	4	12	2	0	1	11	2	1	1
L. nov. spec.	4	0	0	4	0	0	3	0	0	0	0	0	4
Unidentified	4	0	0	4	0	1	1	0	0	0	2	1	1
Gram-negative rods													
Group I	2	1	1	0	0	2	0	0	0	1	1	0	0
IIa	20	0	4	16	0	17	3	0	0	11	5	3	1
IIb) Enterobacter	8	0	1	7	0	7	0	0	0	0	3	5	0
IIc) sp.	3	0	3	0	0	3	0	0	0	0	0	3	0
III	13	0	0	7	6	9	2	1	1	10	1	2	0
IV	6	0	0	0	6	1	3	1	1	1	1	4	0
Bacillus species													
B. megaterium	2	0	2	0	0	0	1	1	0	2	0	0	0
B. pumilus	4	1	3	0	0	4	0	0	0	4	0	0	0
B. subtilis	6	3	3	0	0	1	0	2	3	2	1	3	0
Unidentified	10	2	7	1	0	6	3	0	1	7	2	1	0
Coliform bacteria	9	0	1	8	0	8	1	0	0	6	3	0	0
Actinomycetes	3	3	0	0	0	3	0	0	0	NT	NT	NT	NT
Yeasts													
C. tropicalis/ pseudotropicalis	3	0	0	3	0	0	0	1	2	NT	NT	NT	NT
T. dattila	3	0	0	3	0	0	2	0	1	"	"	"	"
T. etchellsii	1	0	0	1	0	0	0	0	1	"	"	"	"
T. holmii	5	0	1	4	0	0	1	0	4	"	"	"	"
T. sake	2	0	0	2	0	0	0	0	2	"	"	"	"
T. stellata	5	0	0	5	0	0	0	2	3	"	"	"	"
T. versatilis	2	0	0	2	0	0	0	1	1	"	"	"	"
Unidentified, mesophilic	22	2	7	13	0	20	1	0	1	"	"	"	"
Unidentified, osmophilic	10	0	3	7	0	10	0	0	0	"	"	"	"

Table V,6. Deteriogenic power of microorganisms isolated from cane juice, estimated by a screening test in SCJ medium:- Summary of results

* Key to classification of 'deteriogenic power' :

0 - none
1 - weak
2 - moderate
3 - strong

Of the lactobacilli, the most common homofermentative isolates were *Lactobacillus casei* and *L. plantarum*. In general these produced moderate to strong acidity, little or no inversion, and little or no gum formation. However, *L. casei v. casei* isolates produced moderate inversion and strong gum formation. Isolates of a proposed new heterofermentative species of *Lactobacillus** where characterised by strong gum formation. It was concluded that lactobacilli may contribute to significant deterioration of cane juice when they attain high populations in cane stored for long periods.

Many bacteria in stored cane were isolated on glucose yeast-extract agar at pH 4.8. Although heterogeneous they possessed several common properties, e.g. catalase-positive, Gram-negative, rod-shaped, aciduric. Little attempt was made to identify these organisms. They were often present in relatively high numbers in juice from fresh cane, but increased only slowly during storage. They probably originated from the soil and the epiphytic flora of the cane plant. This group was arbitrarily classified into 4 sub-groups on the basis of the final pH observed in SCJ cultures.

~~0 = none : 1 = weak : 2 = moderate : 3 = strong~~

Group II were the most common isolates; these were further sub-divided into Group IIa, miscellaneous; Group IIb, creamy, mucoid colonies; and Group IIc, with yellow mucoid colonies. Groups IIb and IIc were identified as *Enterobacter* species. The characteristic deteriogenic power of Group II isolates was weak to moderate acidity, no inversion, and variable gum formation. The *Enterobacter* species mainly produced moderate gum formation, but in Group IIa the majority showed little or no gum formation. GroupIII produced moderate to strong acidity, little or no inversion, and little or no gum formation. Group IV isolates were characterised by strong acidity, weak to moderate inversion, and moderate gum formation. It was concluded that these organisms are not normally of great significance in sour cane, because loss of sucrose by inversion is slight, and the numbers do not reach very high levels; gum formation, however, might be significant during extended storage of cane.

* Not yet named - see Chapter IV

The *Bacillus* isolates in general did not show significant deteriogenic power, except *B. subtilis* which caused moderate to strong inversion and some gum formation. These organisms, however, were not isolated in high numbers from stored cane and probably are not significant.

Although the coliform bacteria (isolated on Maconkey agar) produced moderate acidity, they did not cause inversion; a few formed weak amounts of gum. These organisms do not appear to be significant in sour cane, contrary to the suggestions of Duncan & Colmer (1964) and despite the high counts sometimes observed in fresh cane juice.

The three isolates of actinomycetes showed very weak growth in SCJ medium, and can be dismissed as insignificant.

Viscosity and gum content were not generally determined for the yeast isolates as it was considered that loss of sucrose by inversion would be their main contribution to spoilage. About 2/5 of the yeast isolates inverted sucrose with the consequent formation of large amounts of reducing sugars. These isolates were identified and found to be predominantly *Torulopsis* species. Nearly all the yeasts produced moderate acidity. It was concluded that many yeasts of the genus *Torulopsis* may cause significant inversion of sucrose in cane after extended storage, when they often reach high numbers.

3. The growth of mixed cultures of deteriogenic microorganisms in SCJ medium:

Evidence from storage experiments (Chapter III) shows that *Leuconostoc mesenteroides* can multiply rapidly inside cut cane, reaching maximum counts of 10^8 - 10^9/ml. juice after 2 - 4 day's storage, and then dies out slowly. Counts of other organisms, such as lactobacilli, Gram-negative aciduric rods, and yeasts increase more slowly but can reach high counts in 5 - 10 days. The preceeding experiment shows that each of these types of organism may produce symptoms of deterioration when grown in synthetic cane juice. A comparison was made between the growth of these organisms in mixed culture in SCJ medium and observations on their relative growth in naturally infected, harvested cane.

Four test organisms were selected from those screened for deteriogenic power; a typical representative of each major deteriogenic group was chosen:-

Deteriogenic group	Isolate No.	Deteriogenic Power		
		Acidity	Inversion	Gum
Leuconostoc species - *L.mesenteroides*	122a	2	1	2
Lactobacillus species - *L.plantarum*	12b	2	1	0
Mesophilic yeast - *Torulopsis holmii*	61b	2	3	NT
Gram-negative,aciduric rod (Group IIa)	83b	2	0	0

Each organism was grown in a suitable liquid medium for 24 hours at 30°C.

Cultures were then diluted in sterile Ringer solution to give haemocytometer counts of ca. 10^5 cells/ml.

0.5 ml. each culture was inoculated into separate 50 ml. sterile SCJ medium, and one flask was inoculated with all four organisms, to give theoretical initial counts of ca. 10^3/ml. The flasks were incubated at 30°C with occasional hand shaking. Viable plate counts were determined initially and after 1, 2 and 5 days incubation. Selective media were used to count each type of organism, by methods described in Chapter II:-

Leuconostoc & *Lactobacillus* - Rogosa agar + Nystatin

83b - GYE agar, pH 4.8, + Nystatin and Actinomycin

T. holmii - wort agar + Aureomycin

Differential counts of *Leuconostoc* and *Lactobacillus* from the mixed culture were obtained by the technique of Mundt & Hammer (1966). 30 colonies on a plate of Rogosa agar were picked off at random and inoculated into Rogosa SL broths with and without 7% ethanol; the broths were incubated for five days at 30°C and examined for growth indicated by turbidity. *L. mesenteroides* 122a did not grow in the presence of 7% ethanol, whilst *Lactobacillus plantarum* 12b did grow.

The results are summarised in Table V,7 and Figure V,1.

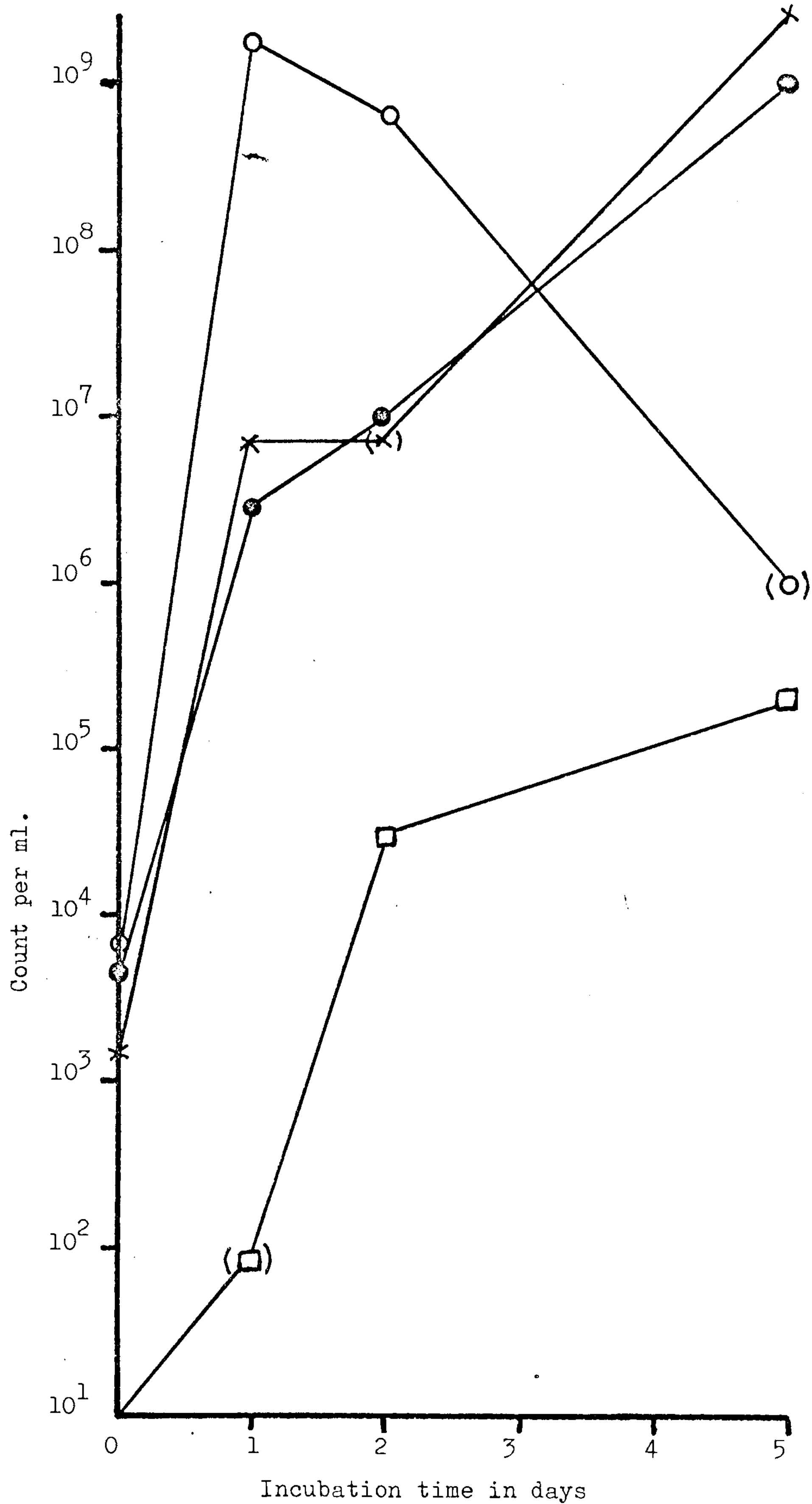


FIG. V.1 Growth of 4 microorganisms in mixed culture in synthetic cane juice

Key

O———O	Leuconostoc mesenteroides	122a
●———●	Torulopsis holmii	61b
×———×	Lactobacillus plantarum	12b
□———□	83b	

Brackets around a point indicate its approximate position.

Treatment	Organism	Count per ml. at stated storage time (days)			
		0	1	2	5
Pure cultures (separate)	L. mesenteroides	6.9×10^3	$> 10^{11}$	6.0×10^8	$< 10^5$
	Lact. plantarum	1.5×10^3	$> 10^{10}$	1.2×10^8	5.2×10^9
	T. holmii	5.6×10^3	5.3×10^7	1.1×10^8	1.5×10^{10}
	83b	$< 10^1$	6.0×10^6	4.4×10^6	1.0×10^9
Mixed culture	L. mesenteroides	6.9×10^3	1.8×10^9	6.7×10^8	$< 10^7$
	Lact. plantarum	1.5×10^3	6.9×10^6	$< 10^7$	2.7×10^9
	T. holmii	4.7×10^3	3.0×10^6	9.8×10^6	1.0×10^9
	83b	$< 10^1$	$< 10^2$	3.0×10^4	2.0×10^5

Table V.7. Growth of four microorganisms in SCJ medium in separate and mixed culture. (Counts are mean of duplicate plates)

In separate culture, L. mesenteroides and Lactobacillus plantarum grew most rapidly and attained maximum counts within one day. L. mesenteroides then died out slowly and after five day's storage only low numbers were viable; in contrast, L. plantarum counts remained high. The final pH was 3.5 - 3.6 in both cultures; L. mesenteroides therefore appeared to be more acid sensitive than L. plantarum. The yeast T. holmii increased slowly in numbers and reached its maximum count at five days. Isolate 83b was inoculated in very low numbers, but grew slowly to reach a maximum count after five days; this count was less than that of the yeast.

In mixed culture all isolates grew more slowly and maximum counts were lower than in separate culture. L. mesenteroides grew most rapidly, reached a maximum count at one day and then slowly died out. The growth of L. plantarum was markedly retarded in comparison with its pure culture; its growth rate was almost identical to that of the yeast, T. holmii. Both organisms attained a similar maximum count after five day's storage. The bacterium 83b did not grow well even after five day's storage, but its low initial inoculum may have hindered its ability to survive competition. The final pH was 3.50.

The results of this pure culture study agree closely with observations on the microbial flora of harvested cane during storage. There appears to be a natural succession of microorganisms in cane juice. Leuconostoc

mesenteroides establishes itself very rapidly and reaches high counts within 1 - 2 days, but as the pH falls it dies out and the more acid-tolerant organisms such as lactobacilli and yeasts become predominant after 4 - 5 day's storage. Significant conclusions could not be made for aciduric, Gram-negative catalase positive rods. The etiological significance of these results is that *L. mesenteroides* is probably the main cause of sour cane in short to medium storage periods, but on extended storage other organisms such as lactobacilli and yeasts may become significant.

4. Koch's third postulate:- The effect of pure cultures of *L.mesenteroides* on aseptically harvested sugarcane

Conclusive proof that *L. mesenteroides* is the causative organism of sour cane requires that Koch's third postulate is satisfied; i.e. inoculation of harvested cane with pure cultures of the organism must reproduce symptoms of sour cane. Several attempts were made to verify this using sugarcane grown in a greenhouse at the Research Centre.

In these experiments, 11-month old cane grown in pots was used, but its Brix and sucrose content were low and only a few stalks were available. The stalks were swabbed and flamed with methylated spirit, then 'harvested' aseptically and cut into 15 inch lengths with flame-sterilised shears. The cut ends were sealed in sterile polythene bags and the cane was handled with sterile tongs.

The test organisms were strains of *L. mesenteroides* isolated from sour cane:- L20 (Expt.1); L16 (Expt.2); 172 (Expt.3). 24 hour MRS broth cultures were diluted in Ringer solution and checked by haemocytometer to give an inoculum of ca. 10^3 cells per ml. (Expt.1), 10^3 and 10^6 cells per ml. (Expt.3), or used undiluted at ca. 10^{10} cells per ml. (Expt.2). The actual inoculum level was checked by plate counts on STA. Test stalks were inoculated by dipping the cut ends in 10 ml. inoculum in a sterile dish; sterile control stalks were dipped in sterile Ringer solution; the contact time was five minutes.

After inoculation the ends were replaced in bags and the stalks were

stored at 30°C and R.H. 91% in a glass tank containing saturated KNO_3 solution. Control and test stalks were removed for analysis after three days (Expt.1), 5 and 7 days (Expt.2) and 3 and 6 days (Expt.3). The stalks were crushed aseptically in a sterilised 2-roller, hand mill; the extracted juice was examined for Leuconostoc count on STA agar, pH, refractive brix, reducing sugars and sucrose content, gum and dextran content, and viscosity by methods described in Chapter II.

The results are summarised in Table V,8.

In Experiment 1, stalk 'D' was successfully infected with Leuconostoc, but stalk 'C' was not. Both the control stalks 'A' and 'B' and stalk 'C' showed high counts of other microorganisms. Analyses of juice from 'D' did not differ significantly from control juices.

Experiment 2 showed that all the 'test' stalks were infected with Leuconostoc whilst the 'sterile' controls were not, although stalk 'H' was heavily infected with other organisms. After 5 day's storage, 'G' showed a higher dextran content and specific viscosity than the control, but gum content and pH did not differ significantly. The Leuconostoc count of 'G' was low, but it may have been due to death of the organisms after reaching an earlier maximum count. At 7 days Leuconostoc counts were higher than at 5 days, but juice analyses did not differ significantly between tests and the control.

Experiment 3 was unsuccessful because juice volumes were too small to permit analysis. However, the test stalks were successfully infected with Leuconostoc,(except stalk 'U') at both inoculum levels. The controls were again free of Leuconostoc but heavily contaminated with other organisms. Leuconostoc counts were higher at 6 days than after 3 days storage, but no significant differences in pH were detected.

These experiments failed to demonstrate that L. mesenteroides is able to cause sour cane, but this may have been due to various undesirable features of the technique. Firstly, the cane was atypical and immature, with a low Brix and sucrose content; juice volumes were too low to permit adequate analysis in some instances. Secondly, despite careful aseptic

Experiment No. and Test Organism	Storage time (days)	Stalk No.	Treatment	*Leuconostoc* count (colonies/ml. juice)	pH	Refractive °brix	Gum (%solids)	Dextran (%solids)	Specific Viscosity (cP at 25°C)
1. L20 (10^3/ml)	3	A	Control	0	5.10	12.5	2.52	.34	.016
		B	Control	0	5.25	11.0	4.53	.47	.016
		C	Test	0	5.00	7.1	6.04	.40	.033
		D	Test	5.5×10^8	5.10	13.5	4.43	.30	.045
2. L16 (10^{10}/ml)	5	E	Control	0	5.10	15.0	3.15	.08	.061
		F	Test	6.3×10^6	5.10	8.2	5.92	.23	.027
		G	Test	2×10^5	5.20	14.0	4.33	1.26	.092
	7	H	Control	0	4.95	11.0	3.92	NT	.033
		I	Test	1.4×10^7	5.10	10.4	3.15	.50	.021
		J	Test	8×10^6	5.10	8.2	4.23	.15	.027
3. 172	3	M	Control	0	5.15	10.4	NT	NT	NT
		N	Control	0	5.20	11.6			
		O	Test 10^3/ml	$> 10^4$	5.10	11.3	NT	NT	NT
		P	Test 10^3/ml	1.2×10^5	5.25	12.6			
		Q	Test 10^6/ml	1.3×10^5	5.20	7.5	NT	NT	NT
		R	Test 10^6/ml	6.0×10^5	5.10	10.0			
	6	S	Control	0	5.25	NT	NT	NT	NT
		T	Control	0	5.20				
		W	Test 10^3/ml	1.2×10^8	5.20	NT	NT	NT	NT
		X	Test 10^3/ml	6.0×10^7	5.25				
		U	Test 10^6/ml	$<10^3$	5.05	NT	NT	NT	NT
		V	Test 10^6/ml	2.0×10^8	5.15				

Table V.8. The effect of *L. mesenteroides* on harvested sugar cane during storage

NT : Not Tested

technique, the 'sterile' controls were often infected heavily with other organisms which may also have caused deterioration. The principal contaminants were usually similar to Enterobacter species. Finally, infection of the test stalks with Leuconostoc was not reproduceable, since several stalks remained 'sterile'. A large inoculum appeared to be necessary to guarantee infection, and final counts of Leuconostoc in the stored cane were often lower than those observed 'in vivo'.

Experiment 3 was repeated at a later date using mature cane. The results are shown in Table V,9.

The initial controls, Y and Z, showed no Leuconostoc count, dextran or specific viscosity, but contamination and cross-infection of the controls with Leuconostoc occurred during storage. After 6 days storage this was clearly revealed in the results for G and H.

Leuconostoc counts appeared to be influenced by the inoculum level, since higher counts were consistently obtained with the larger inoculum (10^6/ml.). All the test stalks were successfully infected, and final counts were slightly higher than those observed under 'in vivo' conditions.

Significant increases in dextran content and viscosity were observed in stalk F after 3 days and in K at 6 days storage. Stalk L showed a high dextran content but no significant change in viscosity.

Gum content and pH did not differ significantly between tests and controls. Inversion was significant in samples I and K.

It was concluded from this experiment that inoculation of harvested cane with L.mesenteroides can cause several of the symptoms of sour cane. However, the results were not reproduceable in duplicate stalks, and no single stalk displayed all the symptoms. Cross-infection of controls reduced the significance of the results. The variable results may be due to the artificial nature of the experiment, the lack of adequate replication, and large differences between the properties of individual stalks. Future work should utilise cane grown naturally in the field with a significant number of replicates at each treatment, but the prevention of infection of the controls remains a difficult problem.

Storage time in days	Treatment Inoculum count/ml	Stalk No.	*Leuconostoc* count/ml juice	pH	Sucrose % w/w	Reducing Sugars % w/w	Specific viscosity	Dextran %Brix	Gums %Brix
0	0	Y	0	5.60	19.27	0.42	0	0	.57
		Z	0	5.60	18.18	1.18	0	0	.33
3	0	A	3×10^3	5.45	17.71	0.71	.072	.002	.57
		B	1.3×10^4	5.40	14.88	1.47	.010	.002	.56
"	10^3	C	2×10^7	5.40	13.67	0.37	.070	.002	.57
		D	7.2×10^8	5.35	NT	NT	0	.002	.53
"	10^6	E	5.8×10^9	5.45	NT	NT	.007	.003	.50
		F	$>10^{10}$	5.50	15.07	1.07	.109	.082	.38
6	0	G	6.5×10^6	5.45	19.48	0.19	.013	.161	.57
		H	1.7×10^7	5.50	21.21	0.62	.045	.215	.63
"	10^3	I	6×10^5	5.20	10.17	3.35	0	0	.27
		J	8×10^5	5.45	18.65	0.98	.032	.079	.64
"	10^6	K	4×10^{13}	5.30	19.52	2.79	.076	.121	.30
		L	4×10^{10}	5.40	20.14	0.38	.018	.321	.58

Table V.9. The effect of *Leuconostoc mesenteroides* No. 172 on harvested sugarcane during storage.

NT : Not Tested

The results do not fully satisfy Koch's third postulate but the evidence of this preliminary experiment suggests that L.mesenteroides could be responsible for increase in the dextran content of sour cane.

C. CONCLUSIONS:

1. The results of storage experiments on harvested cane, reported in Chapters III and IV, satisfy the etiological principles of Koch's first and second postulates for the establishment of Leuconostoc mesenteroides as a principal causative microorganism of sour cane. Other microorganisms such as lactobacilli, yeasts, and aciduric Gram-negative rods may also contribute to deterioration of cut cane stored for long periods.

2. 215 microorganisms of different groups were isolated from deteriorating cane and screened for their ability to produce the symptoms of sour cane when inoculated into a sterile, synthetic cane juice. All isolates of Leuconostoc mesenteroides and L. dextranicum were strongly deteriogenic. Some strains of lactobacilli, especially Lactobacillus casei v. casei and a proposed new heterofermentative species,* caused inversion and gum formation. Most isolates of Gram-negative aciduric rods did not show significant deterioration, but Enterobacter species and a few unidentified isolates were able to cause inversion and moderate gum formation. Amongst the genus Bacillus, only B. subtilis exhibited deteriogenic power; this species showed inversion and gum formation. Since this group was not detected in large numbers in stored cane it was not considered to be significant. Approximately 2/5 of the yeast isolates caused inversion of sucrose and a low pH; most of these belonged to the genus Torulopsis. Coliform bacteria and actinomycetes were not deteriogenic.

3. When a mixed culture containing equal numbers of Leuconostoc mesenteroides, Lactobacillus plantarum, Torulopsis holmii and the Gram-negative, aciduric, catalase positive, rod-shaped isolate 83b were inoculated into sterile synthetic cane juice, a microbial succession was observed. L. mesenteroides grew most rapidly and reached a maximum count within one day's incubation; it then died out slowly as the pH decreased. L. plantarum and T. holmii grew more slowly at approximately equal rates and reached a

* Not yet named

high maximum count after 5 day's incubation. 83b did not compete successfully, but its initial inoculum was very small. The results confirmed observations on the microbial succession in naturally-infected, harvested cane during storage. It was concluded that L. mesenteroides may be the main cause of sour cane in short to medium storage periods, but on extended storage lactobacilli and yeasts may become significant.

4. Inoculation of pure cultures of L. mesenteroides into stalks of aseptically harvested cane did not reproduce all the symptoms of sour cane, but this may have been due to difficulties in the experimental technique which could not be eradicated. However, significant increases in dextran content were associated with Leuconostoc growth in some stalks. It was concluded that L. mesenteroides did not fully satisfy the requirements stated in Koch's third postulate.

5. The overall etiological evidence suggests that Leuconostoc mesenteroides and L. dextranicum are the principle microorganisms responsible for souring of harvested cane. These organisms usually predominate in juice extracted from harvested cane stored for periods up to 5 days, and they can reproduce the symptoms of sour cane when inoculated into sterile, synthetic, cane juice. However, inoculation of L. mesenteroides into aseptically harvested cane did not reproduce all the symptoms of sour cane as stipulated in Koch's third postulate. Therefore it is not conclusively proven that L. mesenteroides is the main cause of sour cane.

CHAPTER VI THE INFECTION PROCESS AND ECOLOGY OF LEUCONOSTOC MESENTEROIDES

A. INTRODUCTION

Experimental evidence suggests that Leuconostoc mesenteroides is the principal deteriogen in souring of cane. In order to devise appropriate control measures, information was sought on the ecology of this organism, and the infection process.

The literature shows that L. mesenteroides is geographically widespread, but its substrates appear to be narrowly defined. It is frequently isolated from natural or man-made foods which contain large amounts of fermentable carbohydrate under conditions of acid pH and reduced oxygen tension, e.g. dairy products, especially milk, cheese and starter cultures (Garvie, 1960); plant materials and vegetables (Mundt & Hammer, 1966); fermenting vegatables and canned tomatoes (Hucker & Pederson, 1930); grass and silage (Whittenbury, 1966), and alcoholic beverages such as cider (Carr and Whiting, 1970). However, its most frequently reported habitat is probably in cane and beet sugar factories, where its ability to form dextran gels or "frogspawn" attracts much attention.

The first microbiological study of slime formation in a sugar (beet) factory was probably that of Cienkowski (1878), who named the causative organism Ascococcus mesenteroides. Van Tieghem (1878) studied the same organism and re-named it Leuconostoc mesenteroides. Since then, there have been numerous reports of the occurrence of this organism in sugar factories, adequately reviewed by Thaysen and Galloway (1930); Hucker and Pederson (1942); Faville (1947) and Owen (1949). However, none of these investigators studied the primary sources of infection of L. mesenteroides.

1. Primary sources of infection

It was suggested by Boekhout (1900) and Velich (1903) that L. mesenteroides enters raw juices from the soil in which, according to Stoklasa and Vitek (1904), they have their natural habitat (cited by Thaysen and Galloway,

1930). Wolzogen Kühr (1923) demonstrated that sugarcane possesses a natural epiphytic flora which consists of various saprophytic bacteria, but L. mesenteroides was not described.

The microbial flora of sugarcane and soil from cane fields in Louisiana was recently studied by Mayeux (1960) and Mayeux & Colmer (1960). L. mesenteroides was detected in small numbers by an enrichment technique on the washings obtained from freshly-harvested stalks. It was not detected on standing cane in normal or diseased leaves, in leaf sheath water, or in cane-borer dust. Mayeux concluded that this organism only multiplies rapidly under the conditions met in the extracted juice in the mill. Samples of soil taken near cut-cane stubble revealed the presence of Leuconostoc-type colonies after enrichment; the highest counts were obtained at a distance of 18 inches. Copious slime formation on stubble in wet weather was found to be due to A. aerogenes rather than the expected L. mesenteroides.

L. mesenteroides was isolated from 1 in 1,000 dilutions of soil of Louisiana cane fields by Mayeux & Colmer (1961), by means of a selective agar medium containing 0.005% sodium azide.

The most recent work is that of Egan (1964; 1965a; 1965c) in Queensland. L. mesenteroides was detected in low numbers on the surface of both green and burnt standing stalks, but it was not found inside healthy cane tissue. It was also isolated from soil in a cane field, and from cane stubble.

In view of the little quantitative information on the occurrence and distribution of L. mesenteroides in the soil and on the cane plant, it was decided to investigate these aspects more fully.

2. Secondary sources and vectors of infection

Since L. mesenteroides occurs in the soil of cane fields and on standing cane stalks, but not inside the healthy cane tissue, it is necessary to consider how the organism is transmitted to the cut cane.

It seems probable that infection only occurs when the cane is damaged in some way that enables organisms to enter the sucrose-containing

parenchyma inside the stalk. In a healthy stalk, this does not arise until harvest when the cut ends are exposed. The most obvious vector of infection is the cutting instrument itself, which is probably contaminated with L. mesenteroides by contact with soil and the exterior of the stalk. The only publication on this subject is that of Egan (1965a), who isolated L. mesenteroides from the juice-soaked mud of a mechanical chopper-harvester, and from cane tissue at the ends of harvested chopped-up cane within ten minutes of harvest. He concluded that infection occurred at the instant of cutting. No studies of hand-cut, whole-stalk cane or the cutter's machetes have been made.

The other obvious means of transmission is by direct contact of the cut cane with soil on the ground..

The role of aerial infection by dust particles and insects has not been investigated, but dust raised by mechanical harvesters in operation may be significant.

Nicholson & Lilienthal (1959) postulated that rainfall acted as a vector of infection, since sour cane is usually observed late in the season, when rainfall occurs. They suggested that the cane stalk is frequently contaminated with L. mesenteroides and, after burning, the cane is splashed with juice from bursting stalks. This infected material is washed down the stalk by rain and lodges in the leaf axil, in contact with the bud. The bud is corky late in the season and it was demonstrated by inoculation experiments that it is permeable to L. mesenteroides.

Owen (1949) suggested that the cane factory itself is a major secondary source of L. mesenteroides infection. The factory contains many places where dilute sugar solutions accumulate and become heavily infected despite regular 'good housekeeping'. Organisms must be released as airborne dust particles as these liquids dry out. The constant circulation of cane transport between factory and field ensures a regular interchange of microbial flora, and the vehicles themselves probably contaminate the cut cane in transit. Furthermore, filter-cake mud from the factory is applied as a fertiliser to newly-planted cane fields, which ensures a regular enrichment of the soil

with organisms adapted to sucrose containing materials.

The mechanisms of transmission of *L. mesenteroides* from primary sources of infection to the cut cane appeared to warrant further investigation.

3. *Colonisation of the host plant tissue*

Little is known about the mechanisms which enable *L. mesenteroides* to rapidly colonise the cane tissue after initial contact with the cut end at harvest. Nicholson & Lilienthal (1959) measured the *L. mesenteroides* count of whole stalk cane within each node and internode at various storage times up to 14 days after harvest. The cane was initially free of *Leuconostoc*, and was infected naturally. The counts increased progressively with time of storage and were inversely proportional to the distance of the site from the cut end. The highest count recorded was 450,000 per g. at the node closest to the cut end after 14 days storage. Egan (1964; 1965a; 1965c), studied the rate of spread and population build-up of *L. mesenteroides* in mechanically-harvested, chopped-up cane billets, of average length 14 inches. The results showed a rapid spread of the organism along the length of the billet, ~~and a rapid spread of the organism along the length of the billet~~, and a rapid increase in population. Counts of 10^8 organisms per g. cane tissue were obtained near the cut ends within 51 hours of harvest.

Egan postulated that at the moment of cutting, the stalk is compressed by the chopper-blades, after which the sudden decompression draws any available liquid into the stalk, together with microorganisms. He suggested that the initial distribution of the organism within the stalk occurred by passive transport in the xylem of the vascular bundles, followed by active extension into the storage tissue (parenchyma). Some justification for this theory was obtained by observations on the movement of water-soluble dyes in the xylem after application of the dye to the freshly-cut ends. Colour in vascular bundles was detected up to 3 inches, or the nearest node, within 5 minutes of cutting.

Whole stalks usually retain some green leaves at the top after a pre-harvest burn, so that at the moment of cutting a transpiration pull exists which may help to transport the organism. These tops, however, are usually

removed at harvest.

The mechanism by which L. mesenteroides colonises the parenchyma, with resultant sucrose loss and other changes, has not been studied.

Egan (1965a) suggested that this process is relatively slow but is accelerated by mechanical damage to the tissues, e.g. bruising due to blunt knives and rough handling. Although this subject is of considerable academic interest, it was not investigated in the present work. Some brief speculations, however, are relevant.

L. mesenteroides is non-motile, so that it cannot actively invade tissue. It probably escapes from the xylem into intracellular spaces of the parenchyma via the pits of the xylem. Here the intracellular moisture contains traces of dissolved sugar and nutrients in a diffusion gradient between the phloem and the parenchyma, which may enable the organism to multiply. The pH and oxygen content of this solution are probably near optimum for growth of the organism. It is not known whether L. mesenteroides acts as a true pathogen or whether it multiplies saprophytically. In the former case, the parenchymal cells may be killed by toxic extracellular metabolites of the organism such as lactic and acetic acid, which can reduce the pH of cane juice to 4.2 - 4.5. If so, leakage of sucrose from the parenchymal cells might occur. The sucrose could then be attacked by the extracellular dextransucrase of the organism, and converted to fructose and dextran. Alternatively, this enzyme may be able to enter the living parenchymal cell and exert its effect without killing the cell. In this case, the organism would be considered saprophytic. Bieleski (1958) reported that cane tissue remains alive in harvested, healthy stalks for at least 800 hours under normal storage conditions.

Until this question is resolved, the correct description of post-harvest biodeterioration of sugarcane associated with L. mesenteroides cannot be stated. Egan (1964) treats the problem as one of plant pathology, in which the organism is described as a pathogen, and the effects it produces are a disease, which he names 'sour storage rot'. Here it is preferred to treat the problem in terms of biodeterioration, in which the

causative organism is the biodeteriogen, and the symptoms are called 'sour cane'.

4. <u>Factors influencing initial infection</u>

a) <u>Organism</u> In common with plant pathogenic bacteria, it is probable that the infectivity of <u>L. mesenteroides</u> is influenced by the strain of organism, inoculum size and physiological state of the organism, but no information is available on this subject.

b) <u>Soil</u> Differences in soil type might be expected to influence its <u>Leuconostoc</u> content. Nothing is known of the ability of the organism to survive in soil for long periods. Although it does not form spores, its dextran capsule may enable it to resist dessication.

c) <u>Cane</u> The variety, age and physiological state of the cane may influence the degree of its natural resistance to infection. It is well known that the rate of deterioration of cane after cutting varies widely with variety, and that immature or overmature cane deteriorates more rapidly than mature cane. However, these differences may be due to variations in the degree of 'autolysis' (non-microbiological deterioration) or to differences in resistance to mechanical damage or moisture loss. It was shown by Browne (1906) that freshly cut cane exerts a slight germicidal action on some bacteria, but the effect disappears at 8 - 24 hours after cutting. He ascribed this effect to natural plant oxidases.

d) <u>Mechanical damage</u> The two most important factors which influence the degree of infection of cane are probably mechanical damage and climatic conditions; these are frequently inter-related. These factors influence both the initial infection and the rate of deterioration; only the former will be discussed here:-

(i) <u>Harvest damage</u> Wold (1946) in Hawaii was the first to observe that badly bruised cane, produced by rough handling and cutting with blunt blades, deteriorated more rapidly than cleanly cut cane; he ascribed this to increased bacterial infection. The amount of harvest damage is small with hand-cut, whole-stalk cane. Each cane, 8 - 10 feet long, has only

two cut ends through which an organism can enter; when cane is mechanically harvested by chopper machines, the average billet length is about 12 inches, so that there are 16 - 20 potential points of infection. In addition, the machines frequently produce jagged, bruised cuts and splits, compared with the generally clean cuts of a hand machete. These facts have become extremely significant in the last decade because the rising cost of labour in many countries has forced the wide-scale replacement of men with machines. Early experiments simulated machines by comparing the deterioration of whole stalk and chopped cane, both hand-cut. Vallance and Young (1959) in Queensland showed that this chopped cane deteriorated twice as rapidly as whole-stalk cane over a four day period, but in Louisiana Coll, Davidson, Stewart & Guilbeau (1962) detected no differences. Severe losses of sugar were first observed in mechanically harvested, chopped-up cane during weekend storage in the 1962 Queensland season. Egan & Rehbein (1963) found that these losses were due to L. mesenteroides infection; the degree of infection was proportional to the degree of mechanical damage. For example, Egan (1964) found that in dry weather nearly every sample of chopped cane was infected by L. mesenteroides, whereas whole stalks were almost sterile. In wet weather however, the latter also became infected. Since then a great deal of research has been carried out on this subject in Queensland, especially by Egan (1964; 1965a; 1965b; 1965c; 1966; 1967a; 1967b; 1968a); this work is summarised in two recent papers (Egan 1968b; 1969). Important papers were also published by Kirby (1968); Vickers (1968); and Foster (1969). In all these studies it was clearly shown that mechanically harvested, chopped-up cane deteriorates much more rapidly than hand-cut, whole-stalk cane. This is mainly due to greater initial infection with L. mesenteroides.

(ii) Burning In many countries, it is normal practice to set fire to cane fields shortly before harvest. This removes most of the green leaves and trash from the cane stalk, which makes the cane easier to cut and improves efficiency by reducing the amount of non-sucrose containing material transported to the factory.

It is generally recognised that burnt cane deteriorates more rapidly than green cane after harvest (Owen 1949), but whether this is due to greater initial infection with microorganisms or to changes in the autolysis of cane is not clear. Waddell (1952; 1954), however, found that in dry conditions green cane deteriorated more rapidly than burnt cane after cutting. He ascribed this to surface sterilisation of the stalk by burning, coupled with the production of a charred, sticky coating which acted as a barrier to infection. In contrast, Foster (1969) postulated that this film of syrup on the stalk increased the susceptibility to infection. These differences may be due to climatic variations, especially rainfall. In many varieties burning causes splits in the stalk which increase the potential points of infection. In view of the fact that during a burn the internal temperature of the stalk rises to between 125 and 190°F (Questel & Bregger, 1959), it is likely that observed differences between the deterioration of green and burnt cane are mainly due to enzymic changes within the cane. For example, many of the cells must be killed, since Bieleski (1958) showed that above 40°C (104°F) the respiration system of cane cells becomes permanently damaged. Similarly, it may be postulated that the oxidases which inhibit bacterial growth (Browne, 1906) are inactivated, and render the cane more susceptible to infection (Owen, 1949). Rizk & Normand,(1969) demonstrated that burning alters the ratio of natural acid and neutral invertases within the stalk.

In Jamaica most cane is burnt prior to harvest. The effect of burning on the L. mesenteroides content of the epiphytic flora of cane was therefore studied.

(iii) Disease Mechanical damage to standing cane is caused by disease, insects and rodent attack. McKaig & Fort (1936) studied the composition of juice from Louisiana cane injured by the cane borer (Diatraea saccharalis) and red rot disease (Colletotrichum falcatum Went). The sucrose content was lower and the nitrogen content higher than in healthy cane, but microbiological tests were not performed. Later, McCalip & Hall

(1938) noted that in frost damaged Louisiana cane, heavy infection by L. mesenteroides in the lower joints was usually associated with damage from borer infestation and splits in the stalk. Most recently, Mayeux (1960) found that leaves diseased by red rot contained significantly greater numbers of bacteria than healthy leaves. Cane borer dust was also shown to be very highly contaminated with bacteria, principally Aerobacter aerogenes. The incidence of L. mesenteroides was not recorded, but it is likely that it is able to enter cane tissue in this manner. Rodent damage to cane is widespread in the West Indies, but its effect on microorganisms has not been studied.

In view of the high incidence of cane borer in Jamaica, the effect of this insect damage on L. mesenteroides content of cane was investigated.

(iv) Freezing Semi-tropical countries which harvest cane in cold weather frequently experience sour cane following a freeze. The cane cells are damaged or killed by a freeze, and splits occur in the rind which permit entry of microorganisms; these organisms then multiply rapidly if the weather becomes warmer. This 'viscous fermentation' has often caused serious economic losses in Louisiana. The organism responsible has been identified as L. mesenteroides, which appears to compete more successfully than other microorganisms at the low prevailing temperatures (Browne 1906; Walton & Fort 1931; Fort & Lauritzen 1938b; McCalip & Hall 1938; Lauritzen et al 1949; Owen 1949; Coleman 1952; Irvine & Davidson 1963; Friloux et al 1965). This phenomenon also occurs in Argentina (Cross, 1966). Owen (1949) thought that increased susceptibility of freeze-damaged cane to microbiological deterioration was due to reduced inhibitory action of the plant oxidases at low temperature, coupled with an increase in soluble nitrogen compounds. L. mesenteroides has also been isolated from frost-damaged sugar beets (Schneider et al 1968). Fortunately temperatures in the West Indies never reach freezing point.

e) Climate The effect of climatic conditions on degree of infection of cane is very difficult to assess because it cannot be controlled in large

scale '*in vivo*' experiments. However, it seems that warm, wet weather favours microbiological deterioration (Gupta et al 1967; Samuels & Cayere 1967; Egan 1969; Foster 1969b). This is probably due to the greater contamination of both cutting instruments and cut cane stalks with infected, sticky, moist soil in wet conditions. Water itself acts as a vector of infection, and by local dilution of cane juice, enhances the penetration and growth rate of microorganisms in the cut ends. Increase in temperature also accelerates the growth of *L. mesenteroides* up to a temperature of 30°C. However, in hot, dry weather many organisms die due to dessication, and the cut ends of cane dry out rapidly.

After harvest, cane is either stored in small piles on the ground, or loaded directly into carts, trucks or bins. In Jamaica, the weight of cane in a transport cart is about 8 tons. It is probable that climatic conditions during storage are generally favourable to infection and growth of *L. mesenteroides*. Only the surface layers of a storage pile are exposed to the atmosphere; the depths of the pile are protected and the cane has its own micro-environment, in which the temperature and humidity are probably high. Furthermore, the cane is in intimate contact with soil, trash and other contaminated surfaces. Several of these factors were studied by Wold (1946).

B. EXPERIMENTAL

All experiments were carried out at Frome Estate, Jamaica, during March - August 1969 and February - April 1970, unless otherwise stated.

All *Leuconostoc* counts were determined by the pour plate technique on STATA medium described in Chapter II. Typical mucoid, catalase-negative colonies were assumed to be *Leuconostoc mesenteroides*, although a small proportion may have been *L. dextranicum*.. Occasional microscopic examinations were made to check that these organisms were Gram-positive, oval cocci, as distinct from rod-shaped lactobacilli.

1. Primary sources of infection of *L. mesenteroides*

a) Soil in canefields

Soil samples were taken in cane fields at different distances from the cane stalks and at several depths. The fields were selected from various farms, in order to provide a range of soil types and climates.

Surface samples were taken by scooping soil into sterile jars; depth samples were taken by means of an augur, and soil from the desired depth was transferred to a sterile jar. The soils were examined the same day for the presence of *L. mesenteroides*. 10 g. soil were aseptically added to 90 ml. sterile $\frac{1}{4}$ strength Ringer solution (= 10^{-1} dilution), and thoroughly shaken for two minutes. The *Leuconostoc* content was then determined. The results are summarised in Table VI,1.

A total of 30 samples from 11 fields was examined. Of these, eleven contained *Leuconostoc* in detectable numbers, with counts ranging from 10 to 5,200 per g. soil. It may be significant that the frequency of positives was much higher in soils sampled during June - August 1969, when conditions were moist (9/19 positive), than in the April 1970 samples, when the soil was very dry (2/11 positive). The majority of samples were taken from the top soil, at depths of 0 - 4 inches, and at distances within 6 inches of the cane.

The two samples of filter-cake mud, taken from piles in the field prior to application as fertiliser, both yielded very high counts of *L. mesenteroides*.

It was concluded that the soil of cane fields contains small numbers of *L. mesenteroides* near the surface adjacent to growing cane; this probably contributes to infection of cane at harvest. The practice of applying filter-cake mud to newly planted cane fields ensures that the soil is enriched with *Leuconostoc* once every 6 - 7 years. The survival of *L. mesenteroides* in the soil may be adversely affected by dry climatic conditions.

Future research into the ability of *L. mesenteroides* to survive for long periods in the soil of cane fields would be of interest.

Date	Field	Location of sample: Depth (inches)	Location of sample: Distance from stalk(inches)	Field Treatment	*Leuconostoc* count per g. soil
13.6.69	A	0 - 4	6	Burnt 12th	10
		0 - 1	6	June.	5,000
		0 - 4	12	Harvesting	N.D.
		0 - 1	12	in progress	30
24.6.69	B	0 - 1	6	Burnt 23rd	N.D.
		0 - 1	6	June.	50
		0 - 1	6	Harvesting	N.D.
		0 - 1	6	in progress	1,300
14.7.69	C	0 - 1	0 - 3	Burnt 13th	5,200
		0 - 1	0 - 3	July.	95
		0 - 1	0 - 3	Harvesting	N.D.
		0 - 1	0 - 3	in progress	N.D.
26.7.69	D	0 - 1	0 - 3	Pre-burn	N.D.
		0 - 1	0 - 3	Pre-burn	N.D.
		0 - 1	0 - 3	Post-burn	130
		0 - 1	0 - 3	Post-burn	N.D.
1.8.69	E	0 - 1	0 - 3	Not burnt	10
	F	Soil shaken from roots of cane			N.D.
	G	Soil from newly planted field, no filter-cake			N.D.
3.4.70	H	0 - 3	3	Freshly	N.D.
		3 - 6	3	harvested	N.D.
		6 - 9	3	by hand	N.D.
3.4.70	I	0 - 3	3	Freshly	60
		4 - 8	3	harvested	390
		9 - 12	3	by machine	N.D.
3.4.70	J	0 - 3	3	Freshly	N.D.
		3 - 6	3	harvested	N.D.
		6 - 9	3	by hand	N.D.
3.4.70	K	0 - 3	3	Young, green,	N.D.
		0 - 3	3	standing cane	N.D.
1.8.69	G	Filter cake mud, applied as fertiliser			110,000
3.4.70	L	Filter cake mud, applied as fertiliser			50,000

Table VI.1. The *Leuconostoc* content of soil from cane fields. Frome Estate, 1969 - 1970.

N.D. : Not detected; count less than 5 per g. soil

b) Cane

Attempts were made to isolate L. mesenteroides from the epiphytic flora of the sugarcane.

Selected sites on the cane were swabbed with sterile calcium alginate wool swabs (Grahams Medical Products Ltd., Farrington, Berks.). No attempt was made to define the size of the sample area. The swabs were placed in 10 ml. sterile 'Calgon' Ringer solution (= 10^{-1} dilution) (Oxoid Ltd.), shaken till dissolved, and examined for Leuconostoc within two hours of sampling.

In the first experiment, four stalks of mature, green, standing cane were examined; each was selected from a different field on the same farm. Each stalk was sampled at several sites along its length. The results are summarised in Table VI,2.

In a second series of experiments, the effect of pre-harvest burning on the Leuconostoc content of the epiphytic flora of sugarcane was studied. Swabs were taken from similar sites on adjacent stalks of standing cane shortly before and after a normal pre-harvest burn. The results are summarised in Table VI,3.

In a third experiment, the effect on the epiphytic flora of 'sterilising' the stalk with 70% ethanol was examined. 24 healthy stalks of mature cane (variety B4362, burnt the previous day) were cut by machete and taken immediately to the small two-roller mill. The stalks were mixed and divided into two bundles of 12 stalks each. One bundle was crushed without treatment; the other was first stripped of its leaves and roots and the stalks were 'sterilised' by swabbing with cotton wool swabs soaked in 70% ethanol. The alcohol was allowed to evaporate without flaming. The hands of the operator were washed in alcohol to minimise contamination during handling of the cane. The mill was 'sterilised' by washing and flaming with alcohol before and between crushing of the cane. The juice from each treatment was collected in sterile containers and examined immediately. Microorganisms were enumerated on a range of selective media as described in Chapter II.

Sample No.	Stalk No.	Sample Site		Leuconostoc count per swab
1	1	2nd internode & leaf axis above ground;	ants	N.D.
2		4th " " " " "	borer	N.D.
3		6th " " " " "	moist	N.D.
4		8th " " " " "	split	N.D.
5		11th " " " " "	moist	N.D.
6		Interior of stalk, at split in stem		N.D.
7		Ants taken from base of stem, from nest		N.D.
8	2	2nd internode, with rootlets		N.D.
9		" " " "		5
10		4th internode, and stalk; dry		N.D.
11		7th-9th " " "		535
12		10th-11th " " " ; wet & dirty		1,000
13		12th-13th " " " " "		ca.10,000
14		Interior of stalk, at 8th node		N.D.
15		" " , at split		N.D.
16	3	2nd node and rootlets		N.D.
17		4th-5th node		N.D.
18		6th-7th node, moist		N.D.
19		Interior of stalk, split at 3rd node		N.D.
20	4	1st node and rootlets		N.D.
21		5th node; moist & dirty		ca.50,000
22		8th node; borer hole & borings		55
23		Interior of stalk at borer hole		N.D. (105 lactobacilli)

Table VI.2. Detection of Leuconostoc in the epiphytic flora of mature, green, standing sugarcane. Frome Estate, 1.8.69.

N.D. : Not detected; count less than 5 per swab

Date	Experimental Details	Pre- or Post-Burn	Sample Site	*Leuconostoc* count per swab
13th June 1969	Variety B4362 Burnt 2 pm No rainfall	Pre-; 12 noon	2 bare stalks; 2 leaf axils	N.D.
		Post-; 4 pm	4 " " "	N.D.
		9 am on 14th	2 " " "	N.D.
24th June 1969	Variety B4362 Burnt 1 pm Rainfall at 3 pm	Pre-; 11.45am	4 swabs, all from leaf axils	2 swabs N.D. 1 swab 2,500 1 " 1,250
		Post-; 4 pm	3 swabs, from bare stalks, height 2-6" above soil	2 swabs 10 1 swab 5
26th July 1969	Variety B51129 Burnt 2.30 pm 2" rainfall at 3 pm	Pre-; 2 pm	4 swabs, height 10-15" above soil, separate stalks. 3 of leaf axils, 1 of bare stem.	N.D.
		Post-; 6.45pm	1 swab of leaf axil	10
			1 " " "	70
			1 swab of bare stem	200
			1 " " "	40
3rd April 1970	Variety B4362 Burnt previous day. No rainfall	Post-burn	3 swabs from different stalks of sticky drops exuding from nodes	N.D.

Table VI.3. The effect of burning on *Leuconostoc* in the epiphytic flora of mature, standing sugarcane. Frome Estate, 1969 - 70.

N.D. : Not detected, count less than 5 per swab.

Microorganisms	Count/ml. juice		% reduction in count on 'sterilisation'
	Untreated cane	'Sterilised' cane	
Total spoilage organisms	6.1×10^4	3.3×10^4	47
Leuconostoc & lactobacilli	3.8×10^4	1.4×10^4	63
Mesophilic bacteria	1.3×10^4	1.2×10^4	8
Yeasts	3.1×10^4	1.3×10^4	58
Moulds	9.0×10^4	1.0×10^2	99.9

Table VI.4. The effect of surface 'sterilisation' of cane stalks with 70% ethanol on the microflora of aseptically extracted, fresh cane juice.

The results are summarised in Table VI,4.

Thirty five examinations of green cane and twenty examinations of burnt cane were made. In general, Leuconostoc was not frequently detected either on green cane (8/35 swabs positive) or burnt cane (7/20 swabs positive). The numbers of stalks yielding Leuconostoc were 3/14 for green cane and 7/16 for burnt cane. Combined totals for green and burnt cane were 15/55 swabs positive, and 10/30 stalks positive. Leuconostoc counts per swab ranged from 5 to 50,000.

The organism was not associated with any particular site on the cane; contrary to expectations it was not isolated more frequently at sites nearer the ground, but was randomly distributed over the bare stems and in the moist leaf axils. The highest counts, however, were found on moist, dirty areas of stem (swab nos. 13 and 21, Table VI,2.).

Swabs taken from the interior of the stalk adjacent to splits did not yield Leuconostoc (swab nos. 6, 15 and 19, Table VI,2). The interior of the stalk at a borer hole contained lactobacilli but not Leuconostoc (swab no. 23), although the borer dust was positive (swab no. 22). Ants taken from a nest at the base of a stalk, and the infested stalk itself did not reveal Leuconostoc.

The effects of burning are not clear from the results shown in Table VI,3. In the experiment of 13th June, Leuconostoc was not isolated either before or after the burn; on 24th June, the post-burn counts were lower than pre-burn, whilst on 26th July the pre-burn swabs were all negative and the post-burn swabs all positive. The latter result may have been caused by contamination of the stalks with soil splashes due to extremely heavy rainfall. Contrary to expectations, samples of sticky exudate from the nodes of cane burnt 24 hours previously were not contaminated with Leuconostoc.

In Table VI,4, it is assumed that the organisms present in the expressed juice were derived from the epiphytic flora of the cane, since the interior of healthy cane is thought to be sterile. Contaminants from handling and

crushing the cane were probably in small enough numbers to be ignored. The approximate composition of the epiphytic flora was 50% lactobacilli and *Leuconostoc*, 40% yeasts, and 10% other bacteria, mainly *Bacillus* species and Gram-negative, yellow-pigmented rods (*Erwinia* species?). Mould spores were present in very high numbers, but were not included in the total spoilage organism count. *Monilia sitophila* was the predominant mould.

Surface 'sterilisation' was not very effective, since the count of spoilage organisms was only reduced by about half. The survival of mesophilic bacteria was probably due to heat-resistant spores of *Bacillus* species. The mould count was drastically reduced. These results show that the initial count of spoilage organisms in the juice of healthy cane milled immediately after harvest is less than 10^5 per ml. In view of the practical difficulties and inefficiency of surface sterilisation of stalks, it is suggested that the contribution of the epiphytic flora to the count of expressed juice from stored cane should be ignored. This error is small when the juice of *Leuconostoc*-infected, stored cane is examined, since counts here usually exceed 10^6 per ml.

It was concluded that *L. mesenteroides* occurs in the epiphytic flora of green standing cane, but its incidence is low (between 20 and 35 per cent of stalks). Certain locations on the plant may have high *Leuconostoc* counts, but mechanical damage to the stems, such as splitting and borer holes, is not necessarily associated with *Leuconostoc* infection of the internal tissues. *Leuconostoc* is probably absent from the inside of healthy standing cane. Burning does not appear to 'sterilise' the surface of the stalk.

These results are similar to those of Egan (1964; 1965a; 1965c) in Queensland. They show that the epiphytic flora of cane is a potential source of *Leuconostoc* infection of the cut ends at harvest.

2. Vectors of L. mesenteroides infection

a) Cutting instruments

The objective of the study was to determine the degree of contamination of cutting instruments with L. mesenteroides, in order to assess their role in the infection of cane at harvest.

At the time of the investigation, all cane in Jamaica was harvested by manual labour using machetes. These are heavy steel knives with blades between 12 and 18 inches long, fitted with wooden handles (for photograph see "Appendix"). No cleaning or sterilisation of the machetes is practised.

During the 1970 crop, trials were carried out at Frome with a Massey-Ferguson 201 mechanical harvester. This machine automatically tops the cane and cuts it into billets of average length 10.5 inches; the billets are loaded directly into a cane cart. Since mechanical harvesters may be gradually introduced into the island, it was decided to include this machine in the investigations.

Each machete was examined by swabbing the whole blade with a calcium alginate wool swab. The samples were taken during normal harvesting operations.

The two chopper-blades of the mechanical harvester were similarly examined by swabbing whilst the machine was at a temporary halt.

The Leuconostoc count of the swabs was determined by the method previously described for cane (Section VI 1,b). The results of the machete examination are shown in Table VI,5 and the mechanical harvester in Table VI,6.

A total of 65 machetes from ten different fields was examined. Half of these (32/65) were contaminated with L. mesenteroides but the incidence of 'positives' appeared to be dependent upon the weather. 100% contamination was observed when the conditions were damp and sticky soil adhered to the machetes (fields D, F and H), but in dry conditions only a few of the machetes were infected. Very high counts of Leuconostoc occurred on the machetes in damp weather.

In contrast, the six swabs taken from the chopper blades of the mechanical harvester were all heavily contaminated even in dry weather

Date	Field	Climatic conditions	No. of machetes examined	No. of machetes 'positive'	*Leuconostoc* counts per swab of 'positive' machetes *
13.6.69	A	dry	3	1	160
18.6.69	B	dry	6	1	35
24.6.69	C	dry	8	0	-
1.7.69	D	recent rainfall	8	8	1100; ca.5000; ca.5000; 75; ca.5000; 600; ca.5000; ca.5000
14.7.69	E	dry	8	1	175
19.7.69	F	recent rainfall	8	8	505; 3500; 25000; 80; 1800; 15000; 135; 2800
22.7.69	G	dry	5	0	-
29.7.69	H	dry, but field waterlogged	8	8	1010; ca.10000; ca.5000; ca.10000; ca.10000; ca.25000; 700; ca.5000
1.4.70	I	dry	8	3	190; 60; 40
3.4.70	J	dry	3	2	1125; 965

Table VI,5. *Leuconostoc* counts of swabs from cane cutters' machetes at Frome

* 'Positive' swabs contained 5 or more *Leuconostoc* per swab.

Date	Field	Sample details	*Leuconostoc* count per swab
1.4.70	K	4 swabs taken 1 minute after halt	210; 150; 1200; 410
3.4.70	L	2 swabs taken 30 mins. after halt	1800; 15000

Table VI,6. *Leuconostoc* counts of swabs from the chopper blades of a mechanical harvester at Frome.

It was concluded that machetes may be vectors of infection of manually-cut cane with L. mesenteroides at the moment of harvest, but they are probably only significant when wet weather prevails. The mechanical harvester, however, seems likely to infect cane heavily at all times. The latter result confirms evidence obtained by Egan (1965a).

b) Air

Since harvested cane is normally laid on the ground in the field overnight prior to loading, it was thought that some airborne infection of cane with Leuconostoc may occur.

Attempts were made to assess the degree of infection of the air in cane fields with L. mesenteroides. An apparatus for quantitative air sampling was not available, so the simple exposure plate technique was used.

The fields selected for the experiment were freshly harvested, the same day. In each experiment, eight or nine 4 inch petri-dishes of pre-poured STATA medium were spaced at approximately 20 yard intervals along the rows of cut cane. The dishes were mounted on stands at a height 1 foot above the ground. Each plate was exposed to the atmosphere for 60 minutes, commencing in the cool early evening (5.30 pm) in order to minimise drying-out of the medium. The dishes were then covered, incubated 48 hours at 30°C, and examined for L. mesenteroides colonies.

The results are shown in Table VI,7.

The first experiment (Field A) was invalidated because the 'positive' plates had been overrun with ants; the Leuconostoc colonies may have originated from either the insects or the air. In the three valid experiments 5/26 plates developed Leuconostoc colonies; each had only one isolate per plate.

These results suggest that airborne transmission of L. mesenteroides is unlikely to be a significant vector of infection in the post-harvest biodeterioration of cane.

Date	Field	No. of plates exposed	No. of plates 'positive'	Leuconostoc counts of 'positive' plates
1.7.69	A	8	4*	*
5.7.69	B	8	2	1; 1
2.4.70	C	9	3	1; 1; 1
3.4.70	D	9	0	-

Table VI,7. Enumeration of L. mesenteroides on plates exposed to the atmosphere in canefields for one hour periods.

* These plates were infested with 'sugar ants' which invalidated the results.

c) Insects

In a field of freshly-burnt standing cane it was observed that numerous wasps were feeding on the sticky drops exuded from the nodes. Six wasps were captured, placed in 10 ml. sterile Ringer solution, and shaken vigorously. After one hour the suspension was examined for Leuconostoc.

Two of the six wasps were contaminated with L. mesenteroides, at levels of 20 and 60 per ml. of suspension. No attempt was made to locate the area of the insect which contained the organism.

It was concluded that wasps may play a minor role in the post-harvest infection of cane with Leuconostoc. Other insects, such as ants, may also act as vectors, but the little evidence obtained from samples 1 and 7 in Table VI,2, and sample A, Table VI,7, is inconclusive.

3. Colonisation of cane tissue by L. mesenteroides.

The experiments were carried out at Tate & Lyle Ltd., Research Centre.

a) 'In vitro' experiment

The rate of penetration and multiplication of L. mesenteroides in harvested cane was studied in the laboratory.

Sugar cane (variety B57150) was grown in a greenhouse in pots of vermiculite at the Research Centre. Its age was 11 months, but it was not mature in terms of sucrose content. The leaves were removed and the stalks were swabbed and flamed with IMS. The stalks were then aseptically 'harvested' with sterile shears and cut into 15 inch lengths. The cut ends were sealed in sterile polythene bags.

The test organism was a strain of L. mesenteroides (L16) isolated from sour West Indian cane, grown for 24 hours at 30°C in MRS broth.

4 cane pieces were inoculated with the test organism by immersion of one cut end in 10 ml. culture in a petri dish for five minutes. A fifth, control piece was similarly dipped in sterile Ringer solution.

After inoculation, the ends were re-sealed in sterile polythene bags and the cane was stored in a glass tank at 30°C over saturated KNO_3 solution, to provide a relative humidity of 91%. One infected stalk was removed after storage periods of 15 minutes, 2 hours, 5 hours, and 3 days, and examined for Leuconostoc count.

The stalks were split longitudinally, and approximately one gram samples of cane tissue were excised with a scalpel and weighed into 9 ml. volumes of sterile Ringer solution. This was added to 90 ml. sterile Ringer solution in a Waring blender and blended for one minute. Aseptic technique was used throughout. Leuconostoc counts were determined on the blended solution. The results were expressed as no. of organisms per g. cane tissue.

The 'control' piece was examined at 15 minutes storage time. Control counts were also taken of the 'sterilised' surface of the stalk, and of the inoculum.

An initial experiment showed that when the test organism was diluted in sterile Ringer solution to give an inoculum containing 1,000 organisms per ml., the cane did not become infected. In the second experiment neat broth culture was used, containing 5×10^8 Leuconostoc per ml.

The results are summarised in Table VI,8.

The pattern of results is very similar to those of Egan (1965a). Despite the slight drop in count at 2 hours, which was probably due to between stalk variation, the Leuconostoc count at a given sampling site was proportional to storage time. Similarly, at a given storage time, the Leuconostoc count was inversely proportional to the distance of the site from the inoculated end. The rate of penetration and multiplication of the organism was very rapid. Counts of 10^7 per g. tissue and above are probably sufficient to cause significant economic losses of sugar in the cane.

Distance of sample site from inoculated end (inches)	Leuconostoc count per g. cane tissue at stated storage time			
	15 mins.	2 hours	5 hours	3 days
0.5	$> 10^5$	$> 10^5$	$> 10^5$	3×10^7
3	2.4×10^4	8.7×10^3	7×10^4	4.5×10^6
6	2.2×10^3	6.2×10^2	2.3×10^4	1.2×10^5

Table VI.8. The rate of penetration and multiplication of L. mesenteroides in artifically inoculated cane.

'Control' results :

1 No Leuconostoc detected (less than 100 per g.) at 0.5, 3 or 6 inches site after 15 minutes storage.

2 No Leuconostoc detected (less than 10 per swab) on flamed surface of stalk.

3 Inoculum count: 5×10^8 Leuconostoc per ml.

No adequate explanation can be suggested for the requirement of such a large inoculum to initiate infection. It is very unlikely that inocula of this size occur in the field.

The results suggest that Leuconostoc may spread passively through the stalk via the xylem, but the mechanism of parenchymal colonisation was not examined.

b) The use of TTC to detect L. mesenteroides in cane.

Many microorganisms can reduce the colourless compound 2,3,4-triphenyl-tetrazolium chloride (TTC) to the deep red formazan. Preliminary studies showed that when L. mesenteroides was inoculated into YGC broth containing 0.01% TTC, a red coloration developed after four days, but not in one day's incubation. The possible use of TTC to detect Leuconostoc in cane stalks was examined.

An aseptically harvested cane stalk was cut into two 5 inch pieces. One end of each piece was dipped into seitz-filtered 0.01% TTC solution for five minutes; the 'test' piece was then dipped in a 1:1,000 dilution of overnight broth culture of L. mesenteroides (strain L20). The ends were sealed in aluminium foil, and the stalks were incubated at 30°C for 24 hours over saturated KNO_3 solution. They were then removed, split longitudinally, and examined for red coloration of internal tissues. The stalks were then re-assembled, re-incubated, and re-examined after four day's storage. Samples of red tissue were examined bacteriologically on several media by methods described in Chapter II.

After one day's incubation, no colour changes were detected. At four days, the infected stalk was a homogeneous deep red colour, whilst the control stalk showed irregular patches of red (Figure VI,1.).

The red coloration of the test tissue was associated with very high counts of Leuconostoc only. Red tissue in the control piece contained a high coliform bacteria count, and a few Leuconostoc, thus indicating accidental contamination.

Low-power microscopic examination of the Leuconostoc infected stalk showed that the colour was evenly distributed throughout the tissue and

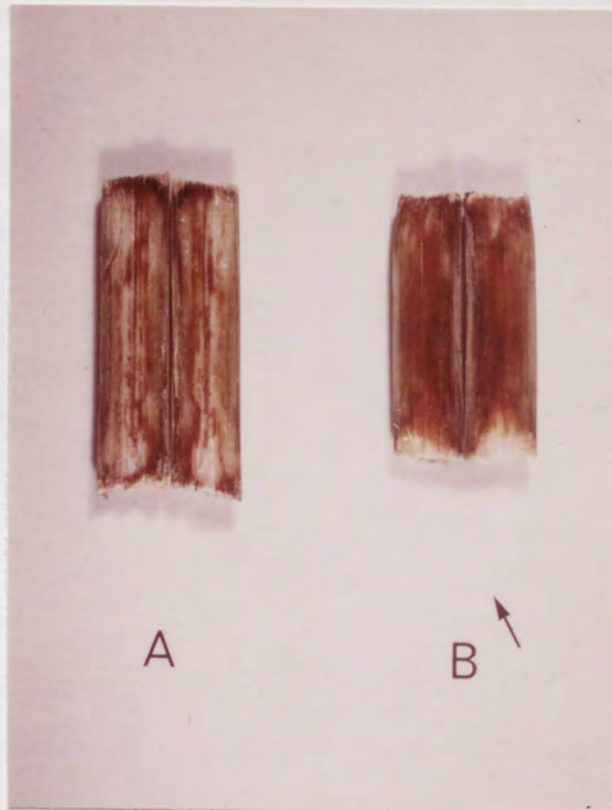


FIG. VI.1.

The use of TTC for detection of Leuconostoc mesenteroides in cane stalks. Stalks incubated 4 days at 30°C. Longitudinal section of stalks (¼ natural size).

Key A. Control stalk. TTC in absence of L. mesenteroides, but in presence of coliform contamination.

B. Test stalk. TTC in presence of inoculated L. mesenteroides L20.

The arrow indicates the inoculated end.

not confined to the xylem. This indicated that the organism had colonised the parenchyma.

It was concluded that TTC is only of limited use to detect areas containing large numbers of Leuconostoc within cane stalks, since it is non- specific and colour development is slow.

4. Factors influencing infection of cane by L. mesenteroides.

a) Harvest damage

A comparison was made between the degree of L. mesenteroides infection of freshly-harvested cane cut manually and by machine. Swabs were taken of the cut ends of cane lying on the ground shortly after harvest and examined for Leuconostoc.

The results are given in Table VI,9.

Date	Field	Sample Details	Swab No.	Leuconostoc count per swab
3.4.70	A	Mechanically-harvested, chopped-up cane, within one minute of harvest	1	2,000
			2	1,500
			3	1,400
		As above, 30 minutes after cutting	4	2,400
			5	820
			6	1,400
3.4.70	B	Manually harvested, whole-stalk cane, two hours after cutting	7	N.D.
			8	N.D.
			9	N.D.
			10	N.D.

Table VI,9. The Leuconostoc content of cut ends of freshly-harvested cane cut by machine and by hand.

N.D. : Not detectable - less than 10 organisms per swab.

Weather conditions were dry and hot.

The billets of chopper-harvested cane were all heavily infected with L. mesenteroides at the cut ends. The ends were jagged, split, moist and dirty in appearance. In contrast, the cut ends of hand-cut, whole-stalk cane were neat, clean and dry and were not infected.

It was concluded that mechanical chopper-harvesters cause heavy infection of the cane billets.

b) Pre-harvest burning

Some experiments on the effect of burning on the Leuconostoc content of the epiphytic flora of standing cane were reported in this chapter, section 1b, Table VI,3.

In another experiment, known volumes of a pure culture of L. mesenteroides were spread on marked areas of sterile microscope slides and allowed to dry. Test slides were attached to standing cane stalks in a field immediately prior to burning, at different heights and locations. After the burn, the slides were recovered and attempts made to count numbers of surviving organisms by a swab and plate technique. No viable organisms were detected, but the experiment was invalidated because the organisms also died out on the untreated control slides. It was observed, however, that the treatment slides were all blackened by smoke, at heights of 1, 5 and 10 feet above the ground. They must, therefore have been exposed to high temperatures during the burn.

c) Disease

The effect of borer damage in cane on the degree of infection of cane with L. mesenteroides was examined. This work was in collaboration with a study by the Research Department of the Sugar Manufacturer's Association of Jamaica Ltd., into the effect of cane-borer damage on juice quality of cane.

Twelve stalks each of healthy and borer-damaged cane were selected at random from a field of mature, green cane. The bundles were crushed on a small 2-roller mill and the Leuconostoc content of the extracted juice was determined. The results of seven experiments from different fields are

summarised in Table VI,10.

Contrary to expectations, the Leuconostoc count of juice from damaged cane was not significantly greater than that of healthy cane; in fact the reverse was true in 5/7 experiments. On this evidence, it does not seem that borer damage contributes to Leuconostoc infection of cane.

Other evidence to support this is found in Table VI,2, samples No. 2, 22 and 23.

Date	Field	Leuconostoc count per ml.juice		Ratio of bored/clean Leuconostoc count
		Healthy cane	Bored cane	
10.3.70	A	210,000	> 400,000	> 2.00
	B	170,000	110,000	0.65
	C	100,000	22,000	0.22
	D	90,000	58,000	0.65
1.4.70	E	17,000	200,000	11.80
	F	190,000	39,000	0.21
	G	90,000	29,000	0.32

Table VI,10. Comparison of Leuconostoc count of juice from healthy and borer-damaged cane.

d) Climate

Controlled experiments on the effect of temperature, rainfall and humidity on the infection of cane by L. mesenteroides were not carried out. However, some indications of the effect of rainfall were obtained. For instance, the incidence of Leuconostoc in soil (Table VI,1) and on machetes (Table VI,5) was much higher in wet weather than in hot dry weather. This evidence favours the widely-held opinion that sour cane is a more serious problem in wet climatic conditions.

C. CONCLUSIONS

1. Primary sources of infection of *Leuconostoc mesenteroides*.

Attempts were made to detect *L. mesenteroides* in the soil of cane fields and in the epiphytic flora of standing cane. The organism was detected in 11/30 samples of soil taken from 11 fields. Counts ranged from 10 to 5,000 per g. soil. The soils were surface samples taken within 12 inches of growing cane.

The organism was detected in the epiphytic flora of 10/30 stalks of mature standing cane, both green and burnt. Counts ranged from 5 to 50,000 organisms per swab. It was not associated with any particular location on the cane, but the highest count was obtained from a moist, dirty area at the 5th node. The organism was not detected inside stalks of healthy cane even where the rind was split, but it was present in the borings of the cane borer insect.

Filter-cake mud from the cane factory was highly contaminated with *Leuconostoc*. Its application as a fertiliser to newly-planted cane fields must lead to regular enrichment of the soil with *L. mesenteroides*.

It was concluded that the soil of cane fields and the epiphytic flora of standing cane contain small numbers of *L. mesenteroides* and therefore are a potential source of infection of the cut ends of cane at harvest.

2. Transmission of *L. mesenteroides* to the cut ends of harvested cane.

Cane-cutters' machetes appeared to be a principal vector of infection. *L. mesenteroides* was detected on the blades of 32/65 machetes taken from ten fields during normal harvest operations. Counts ranged from 30 to 25,000 organisms per machete. The incidence of contamination was much higher in wet weather conditions when moist soil particles adhered to the blades.

The chopper-blades of a mechanical harvester were heavily contaminated with *L. mesenteroides* in dry conditions.

The organism was detected in small numbers in the air of 2/4 freshly-harvested cane fields by the exposure plate technique.

2/6 wasps captured in a burnt cane field revealed the presence of *L. mesenteroides*, and indirect evidence suggested that ants may also carry this organism. It was concluded that insects and airborne dust may be minor vectors of infection of cut cane whilst it lies on the ground overnight prior to loading.

Direct physical contact between the cut ends of cane and the soil after harvest is probably a significant method of contamination.

3. Penetration and colonisation of harvested cane by *L. mesenteroides*

An *in vitro* experiment showed that a pure culture of *L. mesenteroides* was able to penetrate cut cane rapidly and multiply within the parenchyma. *Leuconostoc* counts in the stalk increased with time of storage and were inversely proportional to the distance of the sample site from the inoculated end. The *Leuconostoc* count per g. cane tissue reached a maximum of 3×10^7 organisms at a distance $\frac{1}{2}$ inch from the inoculated end after 3 days storage time. A large inoculum (10^8 organisms per ml.) was required to initiate infection under these conditions. This is probably much greater than inoculum levels '*in vivo*', but an adequate explanation cannot be suggested.

The mechanisms of transport of the organism within the stalk and colonisation of the parenchyma were not studied. A possible method for detection of *Leuconostoc* within cane stalks by application of tetrazolium salt was not satisfactory.

4. Factors influencing the infection of harvested cane by *L. mesenteroides*

The results of several experiments on the effect of pre-harvest burning on the *Leuconostoc* content of standing cane were inconclusive. However, burning did not sterilise cane stalks and *L. mesenteroides* was recovered from several stalks of burnt cane.

L. mesenteroides was not detected on the clean-cut ends of 4 stalks of freshly harvested, manually-cut, whole-stalk cane, but the bruised ends of 6 mechanically-harvested billets were heavily contaminated. It was concluded that the degree of mechanical damage of cut cane may influence

the degree of infection by L. mesenteroides.

A comparison of the L. mesenteroides content of juice from healthy cane and from cane damaged by the cane borer insect did not reveal any significant differences in the seven fields examined.

The incidence of L. mesenteroides in soil and on machetes was much higher in wet weather than in hot, dry weather. This evidence supports the view that wet climatic conditions enhance the degree of infection of harvested cane and hence increases the incidence of sour cane.

5. Recommendations for future research

A study of the ability of L. mesenteroides to survive in the soil of cane fields would be of interest.

The mechanisms of penetration of cane stalks by L. mesenteroides and its ability to colonise the parenchyma remain to be investigated. The results should resolve the question of whether L. mesenteroides is a plant pathogen or a saprophyte.

Further work on the effect of pre-harvest burning on the Leuconostoc content of cane stalks is desirable.

CHAPTER VII ECONOMIC ASSESSMENT

A. INTRODUCTION

The economic losses caused by post-harvest deterioration of sugarcane are conveniently classified into field losses and factory losses.

1. Field Losses

In both stale and sour cane the principal losses in the field (i.e. between harvest and milling) are a reduction in the weight of cane due to evaporation of moisture, and a reduction in the sucrose content of the juice due to inversion and other metabolic changes; the latter is probably the most important. The overrall effect is a reduction in yield of tons of sugar per acre. These losses are borne by the grower, who is usually paid on the basis of the estimated weight of recoverable sugar in the tonnage of cane delivered at the factory.

Despite many papers in which the loss of recoverable sucrose in stored, harvested cane was determined (reviewed in Chapter III), no assessments of actual economic losses appear to have been made. However, it is widely recognised that these losses can be very significant. For instance, Guilbeau, Coll & Martin (1955,1956) calculated that an average 11% of recoverable sugar was lost from whole-stalk cane in Louisiana when stored for 8 days after harvest; in monetary terms this was a loss of $ 17.68 from an original price of $ 183.18 per acre of cane. Lauden (1963) estimated that the Louisiana sugar industry lost 200,000 and 100,000 tons of cane in 1962 and 1963 respectively because of deterioration in cane quality after a freeze, presumably aggravated by Leuconostoc mesenteroides infection. The results of six years research on deterioration of chopped-up cane in Queensland were recently summarised by Egan (1968b). During storage over weekends in 1962, 1963 and 1966, chopped cane lost 6.0, 8.8 and 11% of its original sucrose content respectively, compared with 1-2% for whole stalks.

Assessment of field losses requires not only a knowledge of the rate of deterioration of the principal varieties in a given area throughout the

season, but also an estimate of the frequency distribution of age of cane post-harvest when delivered to the mill. This information was determined during the period March - July 1969 at Frome Estate.

2. Factory Losses

Juice from deteriorated cane possesses three main characteristics which adversely affect the process of sugar manufacture in the factory; these are an increase in acidity, a reduction in sucrose content and purity, and an increase in the soluble polysaccharides or 'gums'. An additional harmful effect may be an increase in the population of microorganisms in the mill due to the processing of heavily infected, sour cane. This could accelerate sucrose loss by inversion in the mill, and lead to the formation of dextran gum or slime which in turn can cause physical blockage of pipes, tanks, strainers, etc. The latter effects have been extensively reported and are adequately reviewed by Thaysen & Galloway (1930); Perquin (1940); Hucker & Pederson (1942); and Owen (1949). Since this problem is intimately related with mill hygiene or 'good housekeeping' and cannot be studied in isolation it was considered to be outside the scope of this thesis.

The harmful effects of increased juice acidity are twofold; firstly, acidic inversion of sucrose in the mill juice is accelerated, and secondly, additional lime is required to neutralise acidity prior to clarification. However, the economic significance of these effects is probably minor.

The reduction in sucrose content and purity of juice is of greater significance, for two reasons. Firstly, as the sucrose content of the juice decreases the time taken to manufacture unit weight of raw sugar increases. Secondly, as the purity or ratio of sucrose to non-sucrose solids decreases, the amount of molasses produced per ton of sugar increases (Serbia,1966). Although molasses is a valuable by-product of sugar manufacture it contains a high sucrose content (30-40% w/w) and its retail value to the factory is only £7-10 per ton compared with £45-50 per ton of raw sugar.

The most harmful effects of deteriorated cane on the factory process arise from its increased 'gum' content. These effects are manifold and have led to numerous investigations.

Early work in Louisiana was stimulated by the deterioration of frozen cane which was associated with severe processing difficulties. Walton & Fort (1931) found that 'jellying' of molasses derived from this cane was due to dextran and mannitol, whilst McCalip & Hall (1938) showed that Leuconostoc mesenteroides infection was responsible for dextran production in frost-damaged cane. The effects of this 'viscous fermentation' on the viscosity of process materials and retardation of crystallisation were so severe that factory operations were almost stopped.

In Egypt Tantaoui (1952) reported similar effects which were due to dextran from sour cane, presumed to be infected with L.mesenteroides. The factory capacity was reduced to 70% and the milling rate had to be retarded. The economic losses were calculated as 3 Kg. sucrose and an extra 7 Kg. of fuel, (coal), per ton of cane, together with the consequences of a reduced milling rate.

One effect of dextran in process materials is to interfere with analytical results in process control. (McCalip & Hall,1938; Tantaoui,1952; Stewart & Rehbein,1964; Anon,1969b). Dextran is dextrorotatory ($(\alpha)^{25}_{D}$ + 200° to 230°) and therefore inflates the direct polarisation ('pol') reading of samples; this results in an artifically high estimate of the purity of materials, i.e. the estimated sucrose content is higher than the true sucrose content. Since chemical control in Jamaican factories is normally based on direct pol measurements rather than true sucrose determinations, significant errors are introduced in calculations. Dextran also affects determinations of true sucrose by conventional methods, and necessitates special modifications to these methods when sour cane is analysed.

In the last decade research on the soluble polysaccharides (loosely termed 'dextran' by many workers) of cane products and their effect on processing has mainly emanated from Australia, stimulated in part by the problem associated with sour storage rot of mechanically-harvested, chopped-up cane. The effects of 'dextran' on sugar processing are summarised by Cross (1966) and Foster (1969c).

Effects are first observed at the clarification stage, where dextran causes poor clarification, cloudy juice, and large volumes of filter cake mud which are difficult to filter (Davis,1959; Cross,1966; Seip,1967; Foster,1969c).

During the stages of evaporation and crystallisation the primary effects of dextran are an increase in the viscosity of syrups and a decrease in the rate of crystallisation of sucrose (Foster, Davies & Sockhill,1957; Davis, 1959; Sutherland,1960a; Smythe,1967; Moritsugu & Tu,1969). The crystallisation rate of sucrose in syrups containing dextran may be reduced by as much as 20% (Moritsugu,1966) or even 50% (Foster,1969c). The most important economic secondary effect is an increase in the time required to manufacture unit weight of sugar; this reduces the capacity of the factory so that the milling rate has to be reduced. Other secondary effects are observed; the walls of the heaters and evaporators scale up more rapidly and lead to a poor heat transfer, further aggravated by the increased viscosity; the increase in processing time leads to greater loss of sucrose by inversion.

In addition to its effect on overall rate of sucrose crystallisation, dextran also alters the shape of the crystal. Normal sucrose crystals are monoclinic, sphenoidal and slightly elongated along the b-axis (ratios a=b=c = 1.26:1.00:0.88), but crystals derived from deteriorated cane juice in raw sugar factories are often needle-shaped and elongated along the c-axis (Allen,1966-67; Moritsugu,1966; Keniry,Lee & Davis,1967a; Sutherland,1968; Leonard & Richards,1969). Apart from the undesirability of this crystal shape from the view point of customer acceptability, it also reduces the efficiency of purging the massecuite in the centrifugals to separate molasses from crystals.

The increased viscosity of massecuites leads to an extension of time required for crystallisation and centrifugation and a reduction in factory capacity. The exhaustability of massecuites is reduced, which causes an increase in the purity and amount of sugar lost in final molasses (Foster et al,1957; Boyes & Wilson,1964; Cross,1966; Keniry et al,1967a).

The accumulation of gums in molasses is thought to be responsible for the phenomenon of 'stickiness' and occasional gelling of molasses in transit, which causes severe handling problems (Roth,1968). Furthermore, the efficiency of molasses as a raw material for alcohol production may be reduced (Cross, 1966).

Raw sugar produced from deteriorated cane is sticky, and difficult to handle, dry and pack (Tantaoui,1952). The presence of 'gums' reduces its filtrability (Davis,1959; Boyes & Wilson,1964; Foster,1969c), which is highly undesirable from the viewpoint of the sugar refiner. Recent evidence from Japan reports that many of the aforementioned effects, observed in the manufacture of raw sugar from deteriorated cane, may be duplicated in the refinery when such sugar is processed (Shirasaki & Kamoda,1966; Kamoda,Onda, Ito,Shirasaki,Miki & Ando,1968). It was shown that these effects could be due to occlusion of gums in the raw sugar crystal (Yamane,Suyuki,Kaga & Takamigawa, 1968). Raw sugars which produced these effects were obtained from Australia, Cuba and S.Africa.

With the current trend towards establishment and enforcement of a system for standards for sugar, and the incurrment of penalties for failure to comply with these standards, manufacturers of raw sugar are becoming very conscious of the need to improve the quality of their product. This has stimulated recent interest in the nature, cause and effects of soluble polysaccharides in sugar products.

3. Soluble Polysaccharides in Cane Sugar Materials

Foster et al (1957) isolated a polysaccharide from viscous Australian molasses and showed that its hydrolysate contained glucose 73%, xylose 26%, and nitrogenous matter 1.1%. This material constituted 1.4% of the original molasses, and when a further 1.5% of polysaccharide was added to the molasses the viscosity of the latter was doubled.

Nicholson & Lilienthal (1959) isolated and characterised a polysaccharide from stored whole-stalk, cut cane. The polysaccharide was highly dextroratory ($(\alpha)^{25}_{D}$ + 193° to 202°) and contained only glucose after hydrolysis; it contained the following α-glucosidic linkages :- 1-6, 29%; 1-4, 60%; 1-3,0-19%.

This 'dextran' was significantly different from L.mesenteroides dextran and was not associated with growth of this organism; it tended to occur late in the harvesting season, after rain. It was attacked by salivary amylase but not by bacterial α-amylase.

Two polysaccharides were isolated from sugar mill syrups and investigated by Sutherland (1960a,b). One was present in all syrups and was found to be a hemicellulose, probably derived from the cane plant cell walls. It contained principally galactose, glucose and arabinose, with small amounts of xylose and rhamnose; its optical rotation was $+ 57^{\circ}$ to $+ 98^{\circ}$. The other polysaccharide was only isolated from syrups of high viscosity, and contained principally glucose with a trace of arabinose; its optical rotation was $+ 198^{\circ}$ to $+ 240^{\circ}$. Its electrophoretic mobility was close to that of L.mesenteroides dextran but differed from the dextran of Nicholson & Lilienthal (1959).

In Louisiana the soluble polysaccharides of sugarcane and its products were studied by Roberts, Jackson & Vance (1964). Juice from freshly-cut cane contained only one type of polysaccharide, which possessed a negative specific rotation ($(\alpha)^{20}_{D}$ - 45° to 47°), and was assumed to be the natural polysaccharide of cane. It was insoluble in 75% ethanol and its molecular weight was ¼-2 million. Sugarcane products (juice, syrup, molasses and sugar) contained a mixture of polysaccharides which exhibited specific rotations ranging from $+ 38^{\circ}$ to $+ 161^{\circ}$; about two-thirds of this complex was alcohol-precipitable and non-dialysable, whilst one-third was not alcohol-precipitable but was dialysable. Upon hydrolysis all the polysaccharides yielded glucose, fructose, arabinose, xylose, rhamnose, fucose and galactose.

Keniry et al (1967a,b) studied the formation of 'dextran' in mechanically-harvested, chopped cane in Queensland, using the 'haze analysis' technique. 'Dextran' was not detected in fresh cane, but was formed rapidly in cane infected by L.mesenteroides. Significant correlations were obtained between the 'dextran' content of syrups and purity of final molasses made from it, and 'dextran' content of low-grade massecuites and c-axis crystal elongation.

However, their method was not specific for dextran. It has since been reported by Foster (1969a) that 'dextran' is sometimes detectable in fresh cane by this method; this appears to be due to formation of a polysaccharide not of bacterial origin, possibly caused by burning or overmaturity of the cane.

Sutherland (1968) and Sutherland & Paton (1969) made a detailed study of the effects of dextran from various sources on crystal elongation of both pure sucrose solutions and process materials. It was clearly shown that L.mesenteroides dextran and dextran from sour, chopped cane promote marked crystal elongation; a concentration of only 0.25% dextran on sucrose doubled the c-axis elongation. Other polysaccharides, including starch and a dextran with a low percentage of α,1-6 linkages isolated from stored, whole-stalk cane, did not promote elongation. The latter, however, produced a marked increase in viscosity of syrups.

Leonard & Richards (1969) isolated and characterised the polysaccharides present in syrups and molasses derived from deteriorated, chopped cane, and studied their effects on crystal elongation. Molasses derived from burnt cane contained two polysaccharide fractions, separated by precipitation with 45% ethanol and gel-permeation chromatography. The low molecular weight fraction was not precipitated by 45% ethanol and did not cause crystal elongation; its hydrolysate contained sugars in the following relative amounts :- glucose 5; galactose 4.4; arabinose 1.8 and xylose 1.0. The major component was a high molecular weight fraction insoluble in 45% ethanol, which caused significant crystal elongation; its hydrolysate contained glucose with only trace amounts of galactose, arabinose and xylose. This fraction was further separated into three fractions, A, B1 and B2, all of which produced crystal elongation. Fraction A contained mainly glucose with trace amounts of galactose, mannose and arabinose, whilst B1 and B2 contained only glucose. None contained starch when tested with iodine. It was concluded that B1 and B2 were glucans, probably dextrans, and that oligosaccharides did not by themselves cause crystal elongation.

A crude, alcohol-precipitable, dextrorotatory polysaccharide was

responsible for the inflation of pol purity readings of cane products in India during cold climatic conditions (Anon,1969b). The crude hydrolysate contained glucose, galactose and xylose, with traces of amino-acids.

Dextran was present in Hawaiian cane juice at a period when elongated crystals were observed in low-grade massecuites (Moritsugu & Tu,1969).

Bruijn (1966a,b;1970) investigated the polysaccharide formed in harvested whole-stalk cane in Natal. It was shown to be a straight-chain glucose polymer having 25% 1-6 and 75% 1-4 α-glucosidic bonds; its molecular weight was between 8,000 and 34,000 and its specific rotation was + 160°. Enzymic degradation with pullulanase revealed that it contained nearly equal ratios of the oligosaccharides maltotriose and maltotetrose. It was also attacked by salivary amylase, but not by bacterial α-amylase. Formation of the polysaccharide, named 'sarkaran', was enhanced by dry storage conditions, and it was not associated with growth of L.mesenteroides.

Finally, Kamoda et al (1968) studied the relationship between crystal shape of raw sugars and their content of various impurities, including gum, starch, silica, phosphate and oligosaccharides. It was shown that occluded oligosaccharides and 'gums' were the cause of c-axis crystal elongation in Japanese refineries.

It is apparent from these results that sugarcane and its products can contain a complex mixture of soluble polysaccharides which varies according to external conditions. Further research is needed to clarify the identity of these polysaccharides, the conditions under which they are formed, and their effects on the process of sugar manufacture. However, the evidence suggests that there are at least five principal types.

Fresh, healthy cane appears to contain two types of gum; one is a high molecular weight compound with negative optical rotation (Roberts et al, 1964); the other may be a high molecular weight hemicellulose type, derived from the plant cell walls, which shows positive optical rotation (+57-98°), but does not cause crystal elongation (Sutherland,1960; Leonard & Richards, 1969). Deteriorated cane may contain in addition three other types of gum. Firstly, a high molecular weight glucan, with a low proportion of 1-6

α-glucosidic linkages, and a high positive specific rotation (+160-202°), appears to be formed in overmature or burnt cane, and harvested whole-stalk cane in the absence of L.mesenteroides. It is named 'sarkaran' by Bruijn (1970) and is similar to the polysaccharide described by Nicholson & Lilienthal (1959). 'Sarkaran' increasesviscosity but does not cause crystal elongation. Secondly, infection of cane with L.mesenteroides and some lactobacilli results in the formation of true dextrans. These possess a high molecular weight, a high proportion of 1-6 α-glucosidic linkages, and high positive specific rotation (+198-240°) (Sutherland,1960; Keniry et al,1967a,b; Leonard & Richard,1969). They increase viscosity and cause crystal elongation. Thirdly, an intermediate complex of polysaccharides may be formed; these yield other sugars in addition to glucose upon hydrolysis, and show intermediate molecular weights and positive specific rotations (Roberts et al,1964). They could be produced either by the cane itself or by microorganisms.

It is extremely difficult to assess economic losses due to deteriorated cane in the factory. Sugar manufacture is a continuous operation and it is not possible to trace the progress of juice from a particular sample of cane through the process. At Frome Estate the problem is rendered even more difficult because it operates two milling tandems simultaneously, and the mixed juice from both tandems is combined prior to clarification. Furthermore, the process is not automated and hence it is subject to operator errors which cannot be controlled. The incoming cane is taken from many sources within a short period and its history is not accurately known, so that wide variations occur in quality, variety, age post-harvest, etc. Certain qualitative effects of deteriorated cane may be noted e.g. poor clarification, slow boiling and viscous materials, crystal elongation, poor exhaustability, etc. However, it does not appear possible to assess these effects quantitatively in economic terms. The only solution would be to process cane of known history in a pilot plant under controlled conditions, but such facilities were not available here.

Attempts were made to assess the losses qualitatively by measuring the dextran content of mill juice composites and other process materials over a period of time, and correlating these with crystal elongation in C-massecuites, final molasses purity and syrup viscosity.

B. EXPERIMENTAL

1. Field Losses

a) Calculation of loss in yield of recoverable sugar from harvested cane during storage.

At Frome the formula used for calculation of yield of recoverable sugar from cane, expressed as tons sugar per ton cane (TS/TC), is derived from the Winter-Carp formula (Meade, 1963) :-

$$\text{Available sugar \% cane (ASC)} = \text{pol \% cane} \left(1.4 - \frac{40}{\text{purity of crusher juice}}\right)$$

In the present case, analyses were based on pol % juice, i.e.

$$\text{Available sugar \% juice (ASJ)} = \text{pol \% juice} \left(1.4 - \frac{40}{\text{apparent purity}}\right) \quad (1)$$

The ASC can be calculated from (1) if the weight of juice % cane is known. This was determined in the storage experiments reported in Chapter III, section B,2. Furthermore, a conversion factor (F) is needed to correct the results obtained on the small mill to the results that would be obtained on the large mill. At Frome this factor was calculated to be x 1.2

The complete equation is therefore :-

$$\text{ASC} = \text{ASJ} \times \text{juice \% cane} \times F \quad (2)$$

In order to express the yield of sugar after any given storage period as a percentage of yield of sugar from freshly-harvested cane, a knowledge of the weight lost by the cane during storage is required. This was also determined in Chapter III, B,2.

The data chosen for calculation of loss in yield of sugar during storage was that for variety B4362, mean of experiments throughout the season, in Chapter III,B,2; this variety constituted 73% of the total cane ground in 1969. The relationships between time of storage and % pol in juice, apparent purity, weight of juice % weight of cane at grinding, and % weight loss on original weight of cane, were analysed statistically and the regression lines plotted in graphs 'E', figures III,9,10,8 and 6 respectively. From these graphs the results for storage times of 0, 0.5, 1.5, 2.5, 3.5 and 4.5 days

were calculated. This data was substituted in formula (2) to enable yields ofrecoverable sugar per 100 tons cane to be calculated.

The results are summarised below :-

Storage time post-harvest (days)	Tons of recoverable sugar per 100 tons of original cane	% loss of original recoverable sugar
0	9.60	0
0.5	9.29	3.24
1.5	8.82	8.12
2.5	8.40	12.5
3.5	7.98	16.9
4.5	7.56	21.2

The mean daily loss in recoverable sugars, expressed as a percentage of the original, was 4.75% These results were used to calculate the economic losses caused by post-harvest deterioration of cane.

b) <u>'Bill-tomill' time studies</u>

Since 1963 the Research Dept. at Frome Estate have executed a series of time studies throughout each crop to measure the time taken between cutting a field of cane and milling of cane from that field in the factory. This is referred to as the 'bill-to-mill' time (BTM). In addition, analysis of the time taken in each component unit operation was made. This data is essential for estimation of the economic losses caused by post-harvest deterioration of cane, and was kindly made available to the author by the Research Officer at Frome.

Results for 1968, 1969 and 1970 are summarised in Table VII,1. The data represents the mean of all time-studies throughout the crop; the studies refer to Estate-grown cane only, since it was impossible to obtain satisfactory results for farmers' cane.

The mean BTM during 1969 exceeded that of 1968 and 1970; this may have been caused by the extension ~~of the extension~~ of the 'crop' or 'grinding season' into the wet months of May, June and July, when muddy field conditions hampered transport operations.

Unit operation	Time in hours		
	Crop		
	1968	1969	1970
Time between burning and cutting	NT	NT	17.02
Total time for cutting	7.21	8.32	7.65
Time between cutting and loading	20.66	24.04	21.14
Time between loading and milling	6.95	8.87	7.42
Total 'bill-to-mill' time, rail transport only	38.27	42.26	36.13
Total 'bill-to-mill' time, road transport only	33.87	39.09	34.45
Mean total 'bill-to-mill' time	34.82	41.14	36.21

Table VII,1. Breakdown into components according to unit operations of 'bill-to-mill' times, Frome Estate.

NT = Not Tested

The greatest reduction in BTM could be made by reducing the time-lag between cutting and loading.

The frequency distribution of BTM in ten-hour class intervals is shown for 1969 and 1970 in Fig.VII,1.

The frequency distribution of BTM in approximately one-day class intervals is given in Table VII,2.

Class intervale (hours)	Mid-mark (hours)	% Frequency of BTM	
		1969	1970
$<$ 25	12.5	15.5	16.3
25 - 49.9	37.5	57.7	60.7
50 - 74.9	62.5	16.9	20.8
75 - 99.9	87.5	8.4	2.2
$>$ 100	-	1.4	0

Table VII,2. Frequency distribution of BTM in one-day class intervals. Frome, 1969 - 1970.

The frequency distribution of BTM is similar in both crops, but that for 1969 shows a 'tail' with higher frequencies of BTM in excess of 3 days, again probably explained by the extension of the crop into the rainy season.

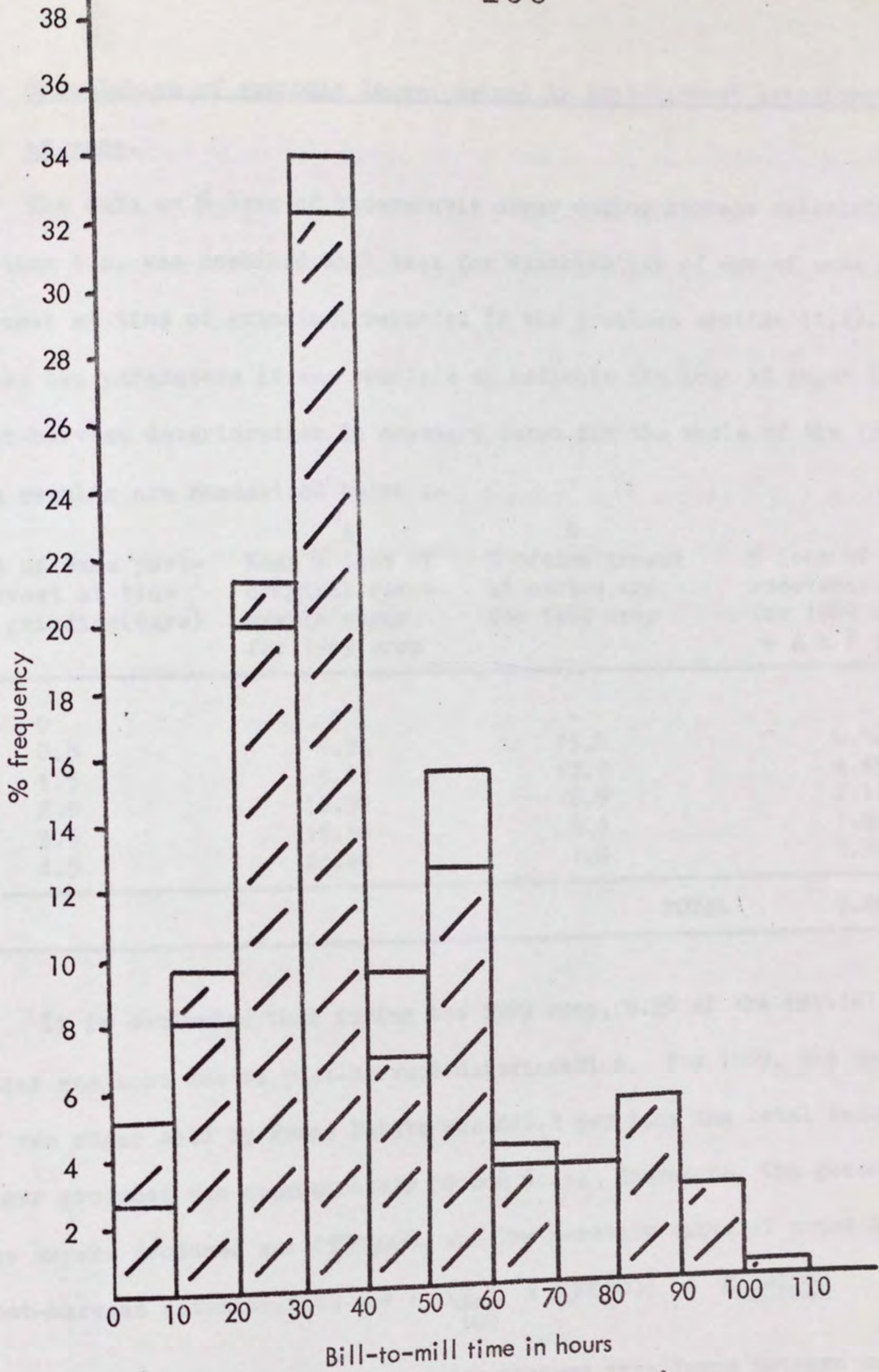


Fig.VII,1. Frequency distribution of bill-to-mill time, Frome Estate.

1969 - cross-hatched (71 samples)

1970 - plain (135 samples)

c) <u>Calculation of economic losses caused by post-harvest deterioration of cane.</u>

The data on % loss of recoverable sugar during storage calculated in section 1,a, was combined with that for distribution of age of cane post-harvest at time of grinding, reported in the previous section (1,b). From these two parameters it was possible to estimate the loss of sugar due to post-harvest deterioration in monetary terms for the whole of the 1969 crop. The results are summarised below :-

Age of cane post-harvest at time of grinding(days)	A Mean % loss of original recoverable sugar for 1969 crop	B % ofcane ground at stated age for 1969 crop	% loss of initial recoverable sugar for 1969 crop = A x B ÷ 100
0	0	-	-
0.5	3.24	15.5	0.50
1.5	8.12	57.7	4.69
2.5	12.50	16.9	2.11
3.5	16.90	8.4	1.60
4.5	21.20	1.4	0.30
		TOTAL	9.20

It is estimated that during the 1969 crop, 9.2% of the initial recoverable sugar was lost due to post-harvest deterioration. For 1969, the mean value of raw sugar sold by Frome Estate was £46.2 per ton; the total tonnage of sugar produced was approximately 80,000 tons. Therefore, the gross value of the sugars produced was £3,700,000, and the monetary value of sugar lost due to post-harvest deterioration was : $\left(\frac{9.2}{100} \times 3{,}700{,}000\right) = £\ 340{,}000$

If it is accepted that a certain minimum time lapse between cutting and grinding of cane is necessary to bring the cane to the factory, the value of these losses is reduced. Taking 1.5 days as the maximum desirable BTM, consistent with the logistics of cane transport, the monetary losses which arise due to BTM's in excess of 1.5 days are :-

$\left(\frac{4.01}{100} \times 3{,}700{,}000\right) = £149{,}000$ (4.01 %) of net value of sugar

These figures for loss are probably conservative estimates, because they are based on BTM data obtained for Estate cane only; the mean BTM of farmers'

cane is almost certainly in excess of the former.

In the case of Estate cane, these losses are borne by the Estate, but private farmers are paid on the basis of an estimate of the recoverable sugar in their cane when delivered at the mill; obviously their income suffers severely if they supply sour or stale cane.

To a slight extent the monetary loss which is caused by deterioration of harvested cane is compensated by the extra volume of molasses produced, but since molasses was only sold for about £7 per ton at Frome in 1969, this is insignificant when compared with loss of sugar at £46 per ton.

The economic losses described are due to all post-harvest deterioration. It was not possible to determine the proportion of these losses caused specifically by microorganisms (biodeterioration, or 'sour cane'), as distinct from normal staling of cane. The results reported in Chapter III showed that nearly all the cane became infected to varying degrees with L.mesenteroides, so it was impossible to distinguish the effects of souring from staling. In the rainy season, however, when cane became very heavily infected with this organism, the mean losses of recoverable sugar appeared to increase.

2. Factory Losses

For reasons previously discussed it was not possible to obtain quantitative data on the economic losses in the factory due to the processing of sour and stale cane. However, some qualitative effects were studied at Frome using dextran content as an indicator of sour cane. Levels of dextran entering the factory in mixed juice were determined, and attempts were made to assess the effect of dextran on crystal elongation of C-massecuites, syrup viscosity, and final molasses purity. The physical properties of some pure dextrans obtained from sour cane isolates of *Leuconostoc mesenteroides* were also examined.

a) Dextran content of mill juices 100 'catch' samples of crusher juice were taken at random from both milling tandems at Frome between April and July, 1969, and the dextran content determined by haze analysis. The results are summarised in Table VII,3.

The mean dextran content of 64 samples from No.1 tandem (farmers' cane) was 0.44 % Bx, and for 36 samples from No.2 tandem (Estate cane) it was 0.35% Bx. The overrall mean was 0.41% Bx. The higher mean result for No.1 mill may be due to a greater BTM of farmers' cane.

Results for 170 samples of mixed juice taken at Frome during February - March, 1970, are summarised in Table VII,4.

Again the mean dextran content of juice from No.1 tandem (0.365% Bx) exceeded that of No.2 tandem (0.316% Bx). The overrall mean dextran content (0.341% Bx) was significantly lower than that obtained in 1969.

Since the overrall mean dextran content of freshly harvested cane (var. B4362) was 0.18% Bx in 1969, it is apparent that the cane ground at the mill is not fresh. The mean age of cane ground at the factory may be estimated from the relationship between dextran content and post-harvest storage time (graph'E', Fig.III,13). These calculations show that the mean age of cane at milling was 58 hours and 48 hours in 1969 and 1970 respectively. In both cases this estimate exceeds the figures obtained for mean BTM (41.14 and 36.21 hours respectively). However, BTM was only determined for Estate cane; therefore it

Month	Week Ending (Mon-Sat)	No. of Samples tested	Dextran % Bx Mean	Dextran % Bx Min^{m}	Dextran % Bx Max^{m}	Total weekly rainfall (inches) (Sat.-Fri.)
April	26	3	0.087	0.058	0.128	0
May	3	0	NT	NT	NT	1.41
	10	15	0.114	0.005	0.450	0.38
	17	10	0.208	0	0.483	4.75
	24	6	0.576	0.011	1.820	3.59
	31	8	0.823	0.326	2.200	7.70
	Monthly	39	0.355	-	-	17.83
June	7	11	0.400	0.011	1.820	2.24
	14	6	0.276	0	0.756	0.86
	21	5	0.310	0.066	0.700	2.08
	28	8	0.296	0.007	0.848	1.18
	Monthly	30	0.332	-	-	6.36
July	5	10	0.629	0.133	1.200	1.46
	12	5	0.552	0.091	1.290	0.67
	19	6	0.693	0.240	2.060	2.11
	26	5	0.294	0.028	0.601	3.26
	Aug.2	2	1.042	0.834	1.250	4.01
	Monthly	28	0.599	-	-	11.51

Table VII.3. Dextran content of crusher juices, Frome, April to July, 1969.

NT = Not Tested

Week Commencing	Tandem No.1 No. Tested	Tandem No.1 Mean Dextran % Bx.	Tandem No.2 Tested	Tandem No.2 Mean Dextran % Bx.	Overrall Results Tested	Overrall Results Mean Dextran % Bx.
Feb. 23rd	8	.287	8	.131	16	.209
March 2nd	14	.209	12	.160	26	.186
March 9th	24	.547	23	.354	47	.454
March 16th	26	.280	25	.366	51	.322
March 23rd	15	.409	15	.397	30	.403
Overrall	87	.365	83	.316	170	.341

Table VII.4. Dextran content of mixed juice samples, Frome, February to March, 1970.

is postulated that the differences are due to the effect of a higher mean BTM of farmers' cane, which constitutes 50% of the crop.

The frequency distributions of dextran content of mill juices in 1969 and 1970 are shown in Figs.VII,2 and VII,3 respectively. The broad pattern of distribution is similar, except that 1969 results exhibit a 'tail' of high dextran contents. In 1969, 14% of the juice samples contained more than 0.8% dextran on brix, compared with only 5% in 1970. This was probably due to the extension of the 1969 crop into the rainy season, resulting in a higher incidence of sour and overmature cane.

Keniry et al (1967a) showed that C-massecuites containing 0.2 dextran % Bx exhibited double the c-axis crystal elongation of dextran-free samples. Similar results were obtained by Sutherland & Paton (1969) for purified dextran. Therefore it seems likely that the dextran present in Frome mill juices is sometimes sufficient to cause severe harmful effects in the factory.

It was observed that in 1969 high mean weekly dextran contents occurred during the May rainy season. Since it was thought that rainfall facilitates infection of cane with L.mesenteroides, the relationship between total weekly rainfall and the corresponding mean weekly dextran content was examined (Fig.VII,4). A broad, positive correlation was evident, but an additional factor appeared to be present in July, since the dextran contents in this month were consistently higher than at other months for similar rainfall. This may have been due to overmaturity of the cane. The results for 1970 were too few in number to enable this correlation to be examined, but in general dry weather prevailed and dextran contents were lower than the wet periods of 1969.

It was postulated that if the 'dextran' in cane juice is due to infection of cane with Leuconostoc mesenteroides there may be a positive correlation between Leuconostoc count and dextran content of mixed juice.

This hypothesis was tested by examining 62 samples of mixed juice over a 2-month period. The juices were either random 'grab' samples or short-term composites. 'Dextran' was determined by haze analysis, and Leuconostoc

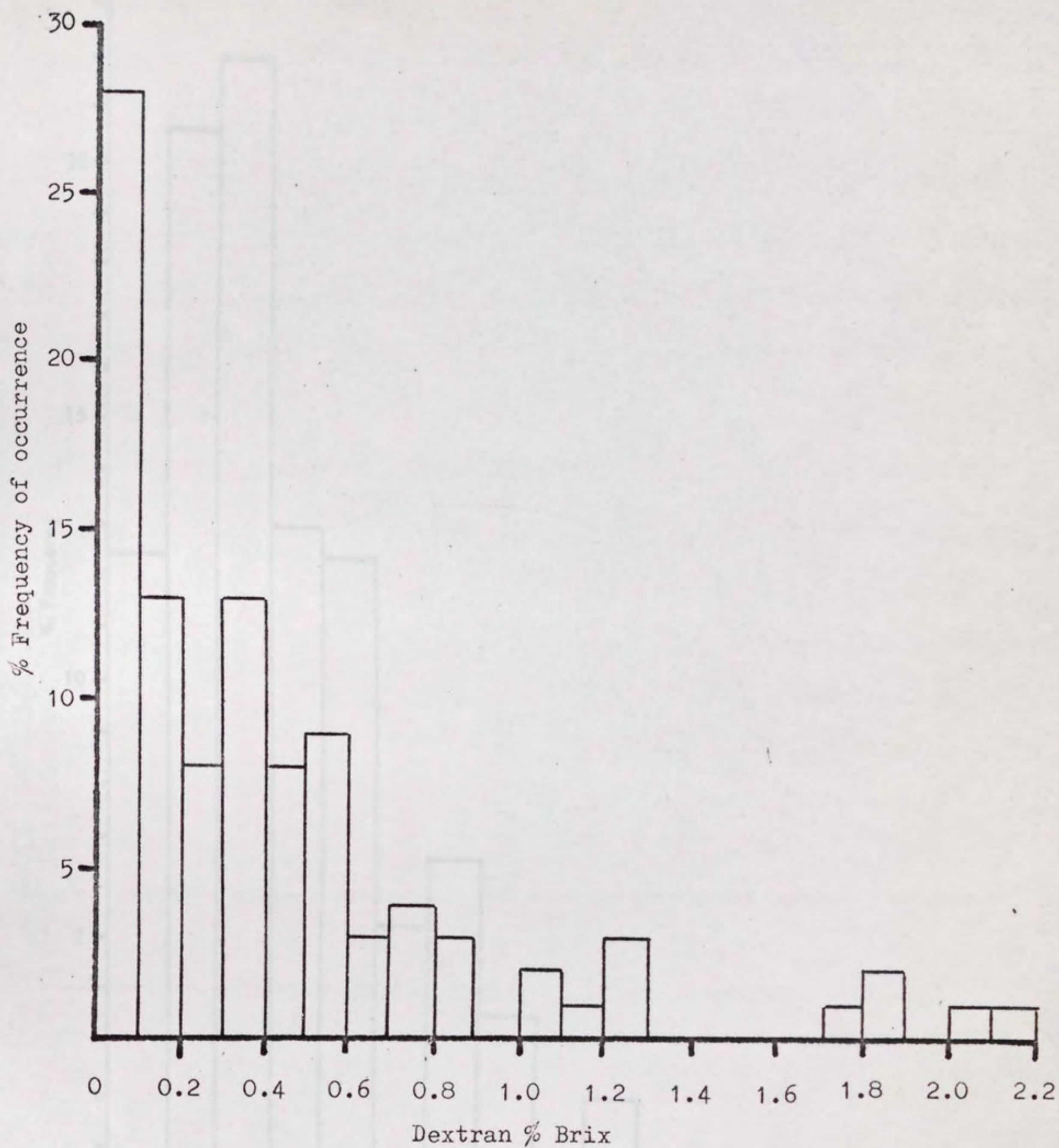


Fig. VII,2. Frequency distribution of dextran content of 100 crusher juice samples, Frome, April-July 1969.

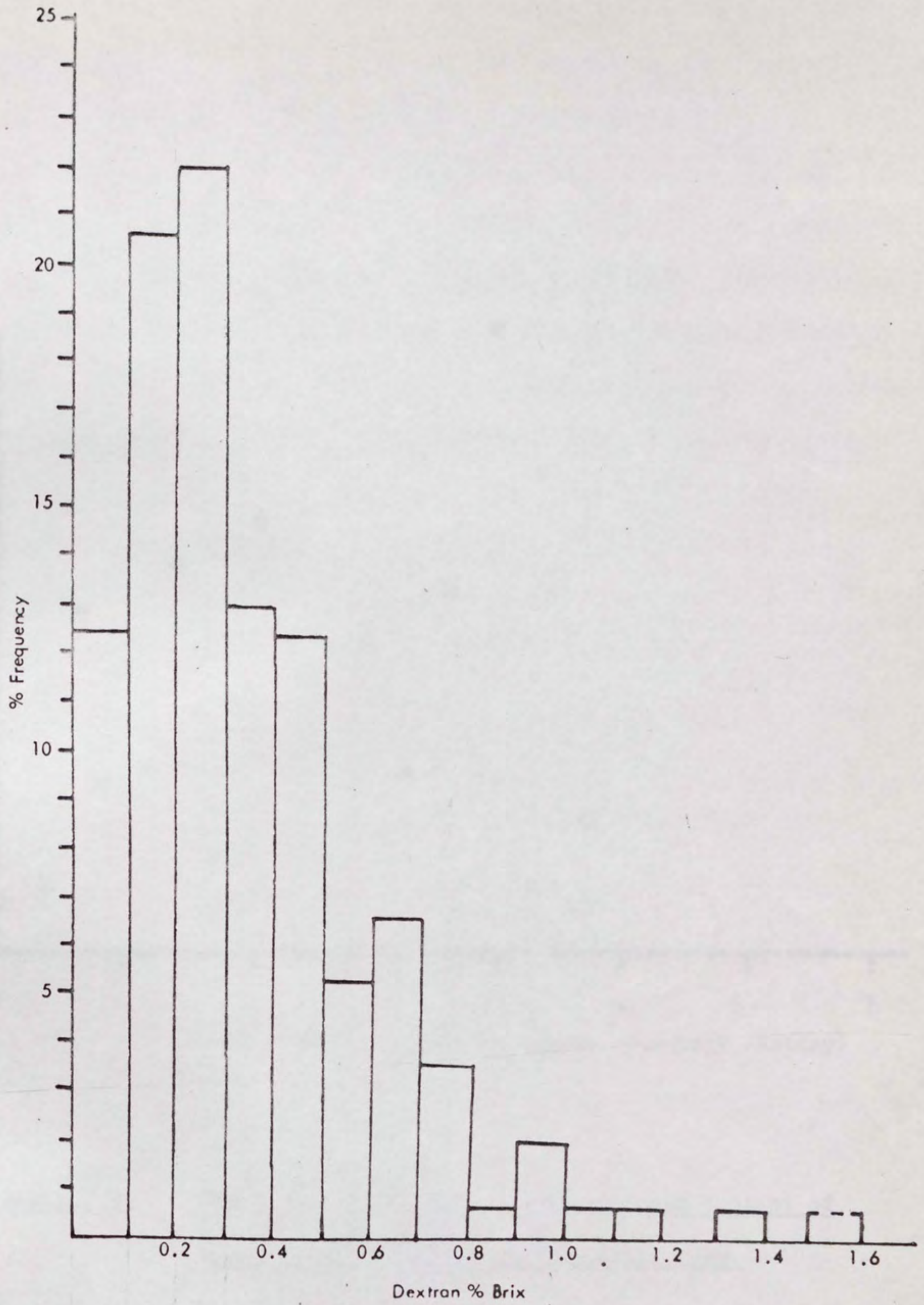


Fig.VII,3. Frequency distribution of dextran content of 170 samples of mixed juice, Frome, February 23rd to March 26th, 1970.

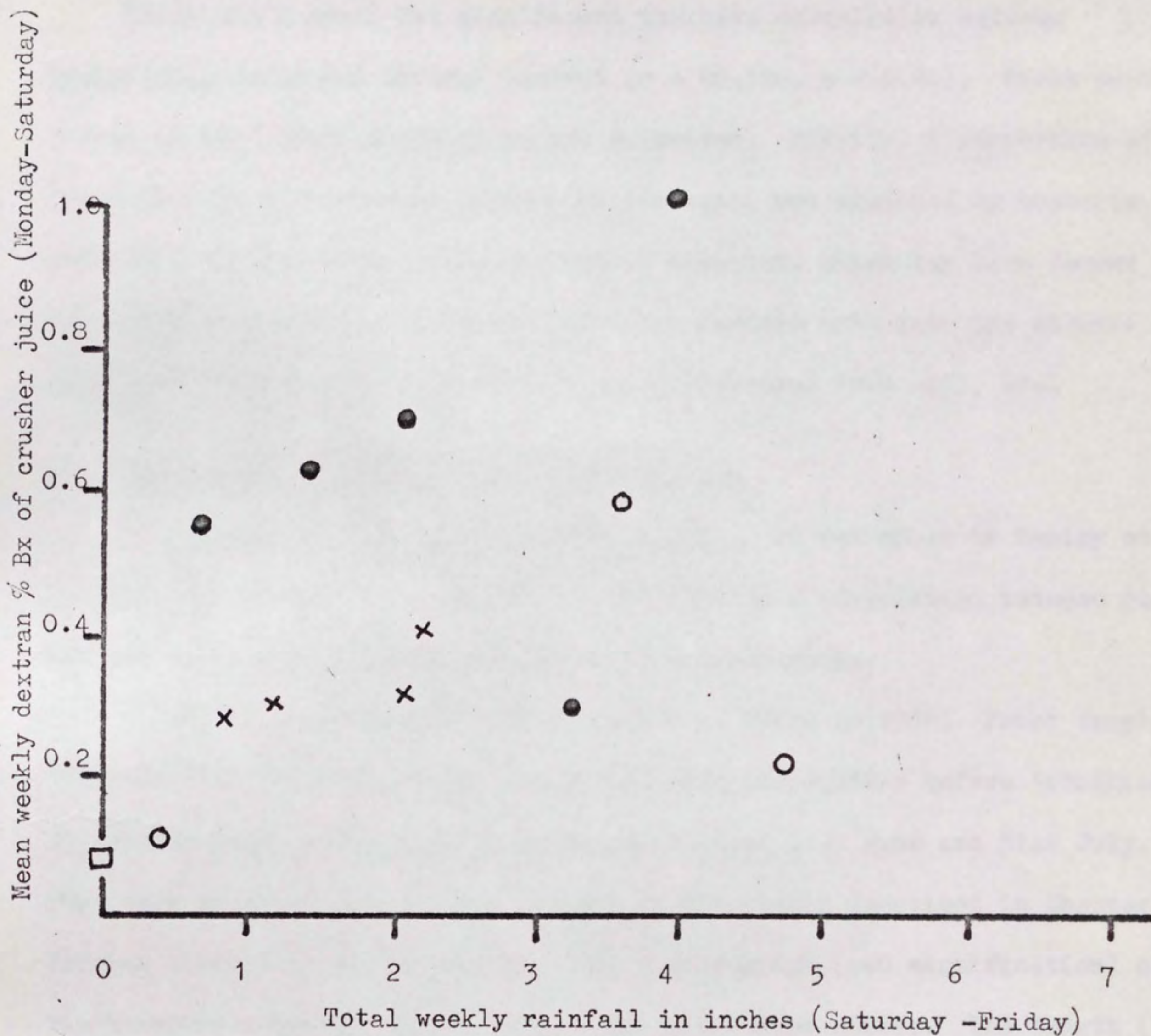


Fig.VII,4. The effect of rainfall on the dextran content of crusher juice, Frome, April - July, 1969.

Key
□ April
○ May
× June
● July

mesenteroides was enumerated by viable plate counts on STATA medium.

The results are expressed graphically in Fig.VII,5.

There was a small but significant positive correlation between Leuconostoc count and dextran content ($r = +0.320$, $p = 0.01$). Three possible causes of this small correlation are suggested. Firstly, a proportion of the 'dextran' may be a natural product of the cane, not produced by bacteria. Secondly, a viable count does not detect organisms which may have formed dextran but then died. Finally, organisms derived from cane are diluted during milling, due to contamination with organisms from soil, etc.

b) Effects of dextran on the factory process

(i) Crystal elongation of C-massecuites It was shown by Keniry et al (1967a) that in Queensland there was a significant correlation between dextran content and c-axis crystal elongation of C-massecuites.

A similar investigation was conducted at Frome in 1969. Proof samples of C-massecuites were taken from the vacuum pans immediately before 'striking'. 23 samples were taken at daily intervals between 11th June and 31st July. They were examined for dextran content by the method described in Chapter II. Crystal elongation was determined from a photograph (x40 magnification) of the maseecuite spread thinly between two microscope slides. The length (l) and breadth (b) of 20 crystals was measured to the nearest millimeter, and the elongation was calculated from the ratio :-

$$\frac{l_1 + l_2 + l_3 \ldots\ldots\ldots\ldots l_{20}}{b_1 + b_2 + b_3 \ldots\ldots\ldots\ldots b_{20}}$$

The statistical analysis of the results showed that the correlation coefficient 'r' was only 0.016, which is highly non-significant. It was concluded that there was no correlation between dextran content and crystal elongation in these samples. However, the dextran content ranged from 0.4 to 2.7% Bx and marked crystal elongation was present in every sample (Fig.VII,6b). The mean dextran content was 1.30% Bx and the mean crystal elongation was 2.32.

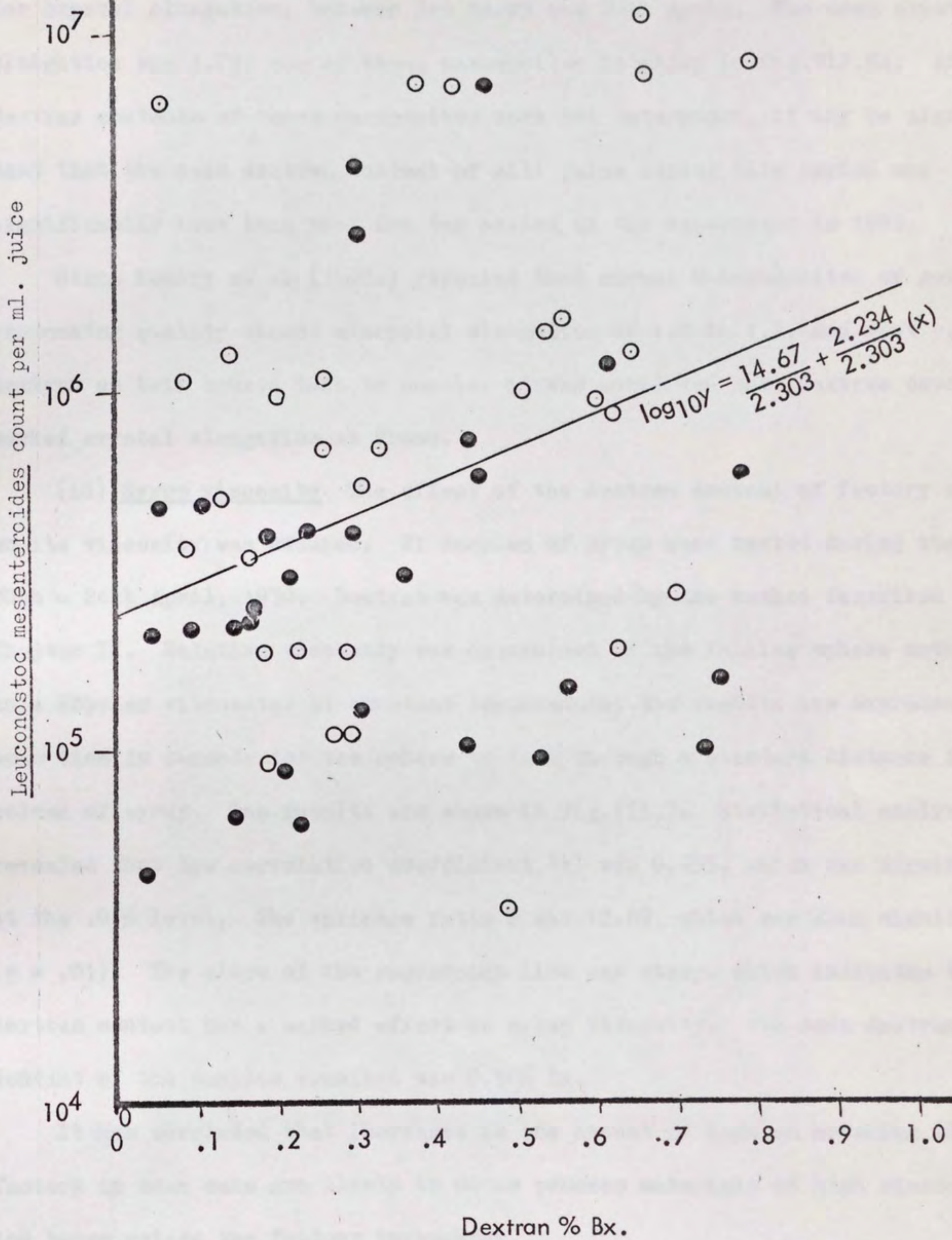


Fig.VII,5. Correlation between Leuconostoc mesenteroides count and dextran content of 62 samples of mixed juice, Frome, 1970. Tandems 1 and 2, 24th Feb.- 24th April.

Key ● Tandem No.1
o Tandem No.2

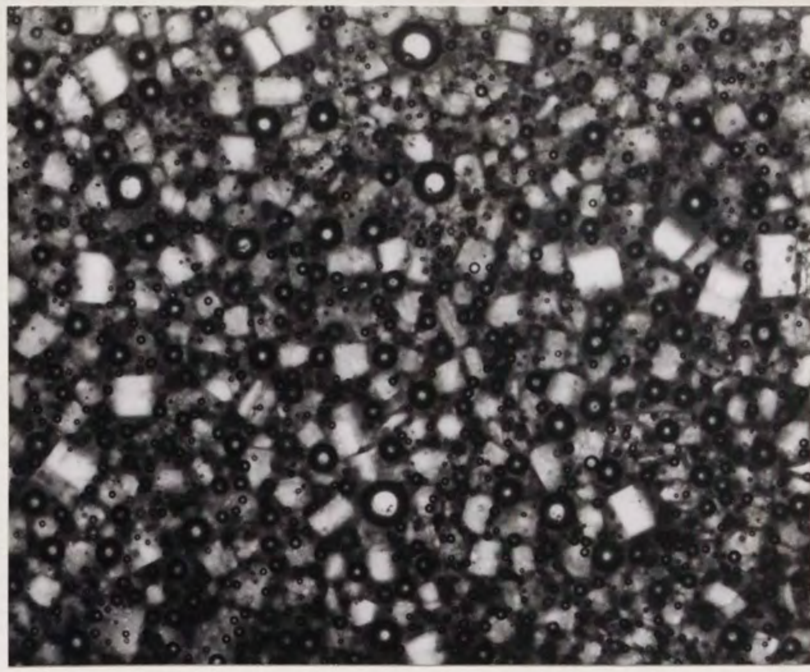
During the 1970 crop a further 36 samples of C-massecuite were examined for crystal elongation, between 3rd March and 27th April. The mean crystal elongation was 1.79; one of these massecuites is shown in Fig.VII,6a. Although dextran contents of these massecuites were not determined, it may be significant that the mean dextran content of mill juice during this period was significantly less than that for the period of the experiment in 1969.

Since Keniry et al (1967a) reported that normal C-massecuites of good processing quality showed a crystal elongation of 1.0 to 1.2, and that o.2% dextran on brix caused this to double, it was concluded that dextran caused marked crystal elongation at Frome.

(ii) Syrup viscosity The effect of the dextran content of factory syrup on its viscosity was studied. 21 samples of syrup were tested during the week 20th - 24th April, 1970. Dextran was determined by the method described in Chapter II. Relative viscosity was determined by the falling sphere method in a Höppler viscometer at constant temperature; the results are expressed as mean time in seconds for the sphere to fall through a standard distance in a column of syrup. The results are shown in Fig.VII,7. Statistical analysis revealed that the correlation coefficient 'r' was 0.633, which was significant at the .01% level. The variance ratio F was 12.69, which was also significant ($p = .01$). The slope of the regression line was steep, which indicates that dextran content has a marked effect on syrup viscosity. The mean dextran content of the samples examined was 0.56% Bx.

It was concluded that increases in the amount of dextran entering the factory in sour cane are likely to cause process materials of high viscosity, and hence retard the factory throughput.

(iii) Final molasses purity Keniry et al (1967a) found a significant positive correlation between the dextran content of syrup and the purity of the final molasses derived from it. This relationship was examined at Frome by comparison of the mean weekly dextran content of mill juice and the final molasses purity for the corresponding week, displaced by four days to allow for pro-



(a)

(b)

FIG.VII, 6

The effect of dextran on crystal elongation in C-massecuites (x 10 magnification)

(a) Normal C-massecuite, 11th March 1970.
Crystal elongation, c/b 1.40; Dextran % Brix < 0.5

(b) C-massecuite derived from sour cane, 3rd July 1969.
Crystal elongation, c/b 2.74; Dextran %Brix 1.48.

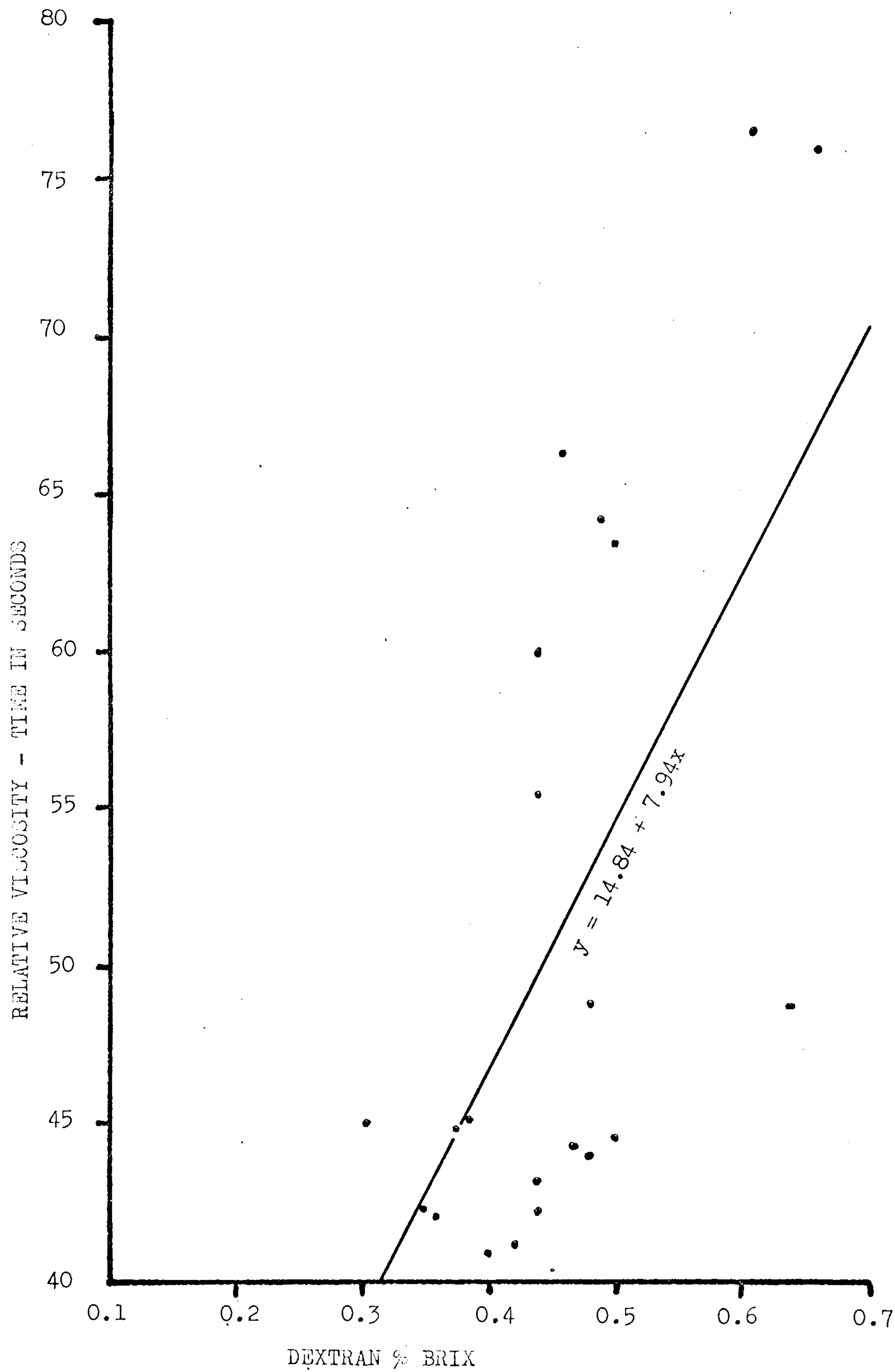


FIG. VII,7. The effect of dextran content of syrup on viscosity.

From 20th - 24th April, 1970

cessing time (normal processing time is 2-3 days). The results for the periods 26th April - 19th July, 1969, and 28th February to 28th March, 1970, are shown in Fig.VII,8. Statistical analysis showed that there was a significant positive correlation ($r = 0.557$, $p = .02$). The variance ratio F was 6.75, which was significant at the 0.02% level.

It was concluded that high dextran levels may cause significant losses of sugar to final molasses. However, during 1969 the cane quality deteriorated due to overmaturity duringthe period studied, which may also have contributed to the correlation.

c) Physical properties of gums from sour cane organisms Dextrans produced by *Leuconostoc mesenteroides* are known to show wide variations in physical properties such as viscosity, water solubility and optical rotation (Jeanes, 1965). These differences are ascribed to variations in molecular weight and degree of cross-linking. Since many different strains of *L.mesenteroides* and other gum-producing lactic acid bacteria were isolated from sour Jamaican cane, it was desirable to study the properties of gums obtained from some of these organisms, in order to estimate their relative effect on factory processing qualities, particularly viscosity and reaction to the 'haze analysis' technique of 'dextran' determination.

The following organisms were selected :- *L.mesenteroides* strains 16b, G7, 70a and L20; *Lactobacillus confusus*, 123a. The methods of gum production, extraction and purification were those of Jeanes (1965). Cultures were grown in a sucrose liquid medium until high viscosity was observed. The gums were precipitated from centrifuged supernatants with 3 volumes of ethanol, purified by re-precipitation and washing in ethanol, concentrated by vacuum evaporation and finally freeze-dried. Moisture contents were determined, and standard aqueous solutionsof pure gum were prepared for testing. Absolute viscosity was determined by the method described in Chapter II, and the optical density of 50% ethanol suspensions was determined at 720 nm by the 'haze analysis' technique of Keniry et al (1967a,b).

The specific rotation of the gum from L20 was found to be $(\alpha)^{25}_{D}$ + 198.2°.

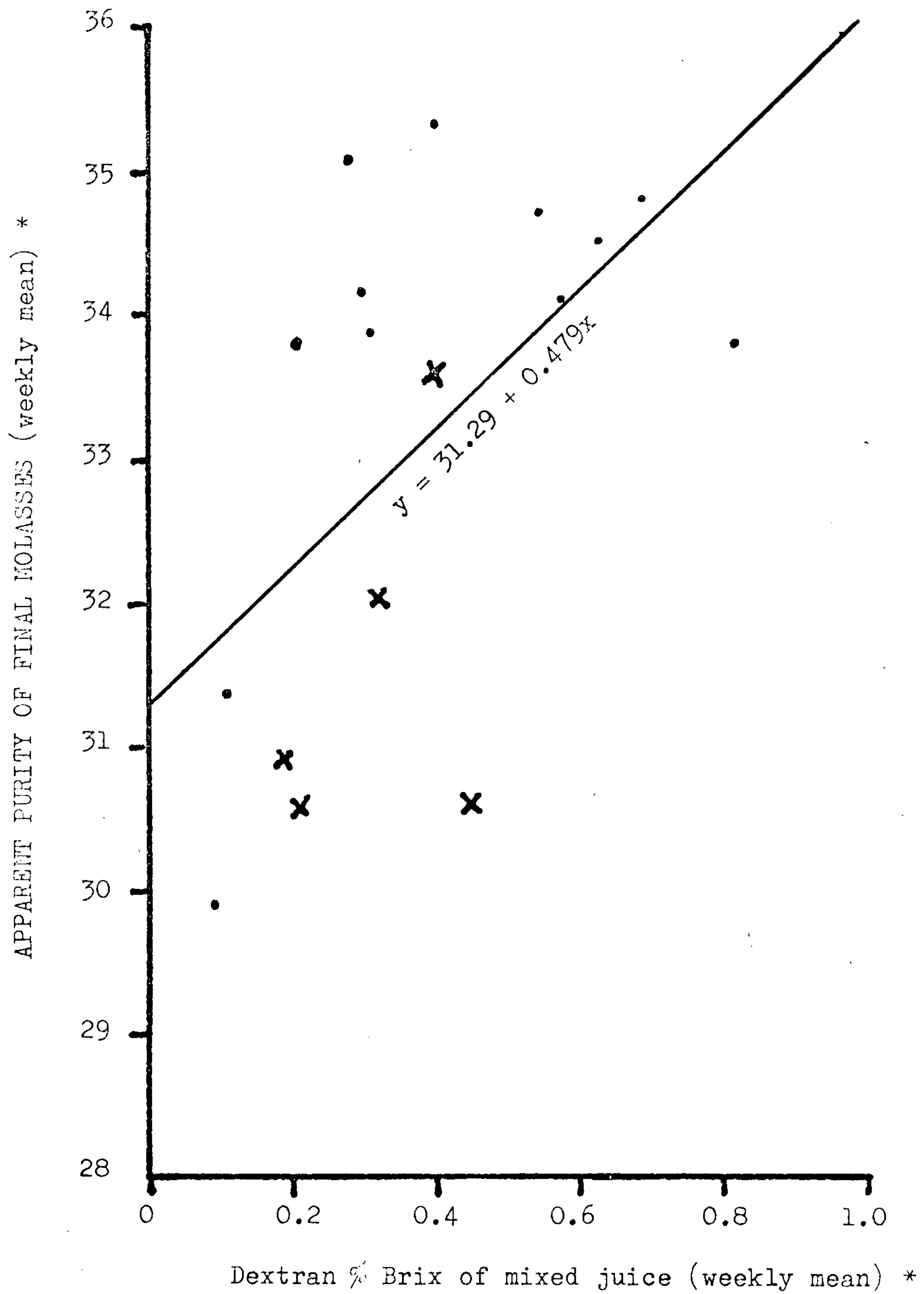


FIG. VII,8. Relationship between dextran content of mixed juice and apparent purity of final molasses made from it. Frome.

Key

• 1969, 26th April - 19th July

x 1970, 28th Feb. - 28th March

* Weekly period of apparent purity is displaced 4 days beyond that of dextran content.

Acid hydrolysis of this gum followed by paper chromatography showed that glucose was the sole detectable monosaccharide. It is likely that this gum is a dextran.

Three of the L.mesenteroides isolates produced a water-insoluble gum which was also separated, purified and examined; these samples carry the suffix 'x'.

The results for viscosity are shown in Table VII,5, and for haze analysis in Fig.VII,9.

Wide variations occurred between different strains in viscosity and optical density by 'haze analysis' at identical gum concentrations. This variation probably explains the low correlation coefficients obtained between dextran, gum and visoosity in Chapter III. The haze anlalysis results indicate that the use of this technique for estimation of dextran content may lead to errors, since the response of dextran from different strains of L.mesenteroides is not identical. The standard curve for use in this test must be prepared with a dextran of known, standard properties in order to obtain meaningful results. However, Keniry et al (1969) found that a reduction in molecular weight of dextran from 2 million to 110,000 did not alter the response to 'haze analysis'.

Organism	No.	Absolute viscosity cp(25°C)	
		0.5% w/v gum	0.1% w/v gum
Leuconostoc mesenteroides	L20	1.522	0.983
" "	16b	1.106	0.937
" "	16bx	NT	0.977
" "	G7	1.273	0.962
" "	G7x	1.146	0.933
" "	70a	1.151	0.932
" "	70ax	1.008	0.947
Lactobacillus confusus	123a	NT	0.940
Bacillus subtilis	F5	NT	0.923
Coliform bacterium	110	NT	1.361

(absolute viscosity of pure water at 250°C = 0.895 cp)

Table VII,5. Viscosities of aqueous solutions of pure gums obtained from various sour cane organisms.

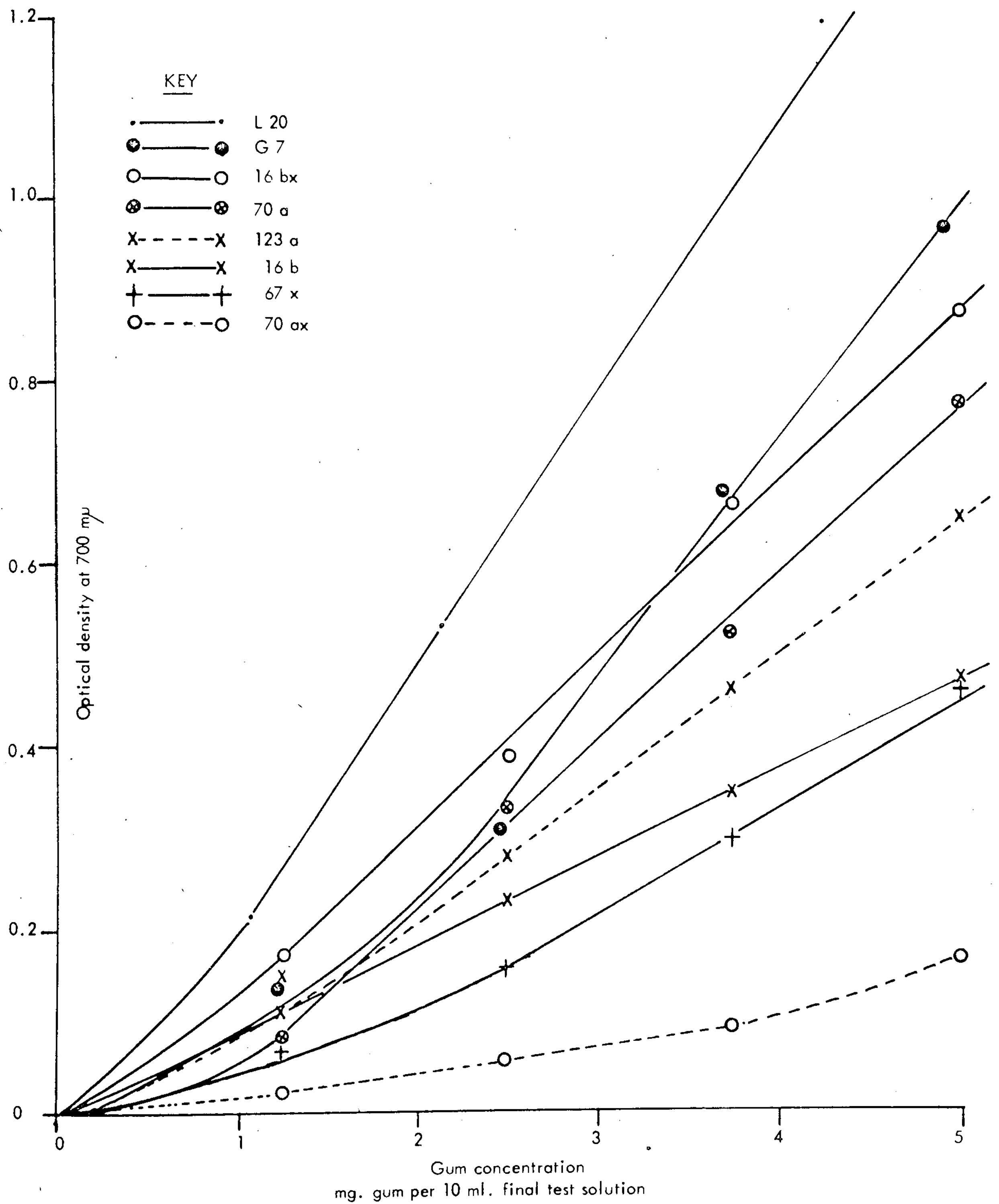


Fig.III,9. The relationship between gum concentration and optical density by the method of haze analysis with gums obtained from lactic acid bacteria

Each point represents the mean of two determinations

L20,G7,16b,70a - *Leuconostoc mesenteroides*

123a - *Lactobacillus confusus* (new species)

It was concluded that the effects of sour cane on viscosity of factory process materials will vary with differences in strain of the infecting organism.

C. CONCLUSIONS

1. Attempts were made to estimate the qualitative and quantitative economic losses caused by post-harvest deterioration of sugar cane, and in particular, losses due to sour cane.

2. The rate ofloss of recoverable sugar in harvested cane was calculated from data obtained in Chapter III. The mean daily loss in recoverable sugar, expressed as a percentage of the original, was 4.75 % for variety B4362, averaged over the period April - July 1969.

3. Bill-to-mill times were assessed for the Frome 1969 crop, and mean times taken in component unit operations were calculated. The most delay occurred between cutting and loading. The mean bill-to-mill time was 41.1 hours. Frequency distributions of age of cane at harvest were plotted for both the 1969 and 1970 crops.

4. The loss of recoverable sugar in cane due to post-harvest deterioration was estimated to be 9.2% of the original for the Frome 1969 crop. In monetary terms the gross value of these losses was £340,000. When allowance was made for normally acceptable losses due to transport of cane from the field to the factory, these losses were reduced to £149,000 (4.01%). Losses due to souring and staling of harvested cane could not be separated.

5. The mean dextran content of mill juices at Frome during 1969 and 1970 was determined and the frequency distributions plotted. This figure was higher in April - July 1969 (0.41% Bx) than in March 1970 (0.34% Bx). Farmers' cane had a higher dextran content than Estate cane.

6. A positive correlation existed betweeen mean weekly rainfall and mean weekly dextran content of mill juice at Frome in the 1969 crop.

7. A slight but significant correlation existed between the Leuconostoc count of mixed juice and its dextran content at Frome during March 1970.

8. Marked c-axis crystal elongation was observed in C-massecuites during June-July 1969, but it was not correlated with dextran content. During March - April 1970 little crystal elongation was detected when dextran contents were low.

9. There was a significant positive correlation between the dextran content and viscosity of syrup.

10. A significant positive correlation existed between the dextran content of mill juice and the purity of final molasses derived from it.

11. The dextran content of cane entering the factory at Frome in 1969 was considered to be sufficient to cause harmful effects on the process, leading to reduced throughput and recovery of sugar. Quantitative data was not obtained.

12. Some physical properties of gums obtained from pure cultures of Leuconostoc mesenteroides isolated from sour cane were examined. They exhibited significant variations in viscosity and reaction to the 'haze analysis' method of dextran determination.

CHAPTER VIII CONTROL METHODS

A. INTRODUCTION

Research on the biodeterioration of harvested sugarcane caused by *L. mesenteroides* has provided information which is essential for the logical development of control methods. It was shown that *L. mesenteroides* occurs naturally in the soil of cane-fields, and on the outer surface of standing cane stalks. Infection of the cut ends occurs at harvest by contact with contaminated cutting instruments and the soil; the degree of infection is increased by rainfall and mechanical damage. The organism multiplies within the stalk during the transport and storage period prior to milling, and causes undesirable changes in juice composition. The extent of these changes is directly proportional to the delay between cutting and grinding of the cane. The principal economic effects are loss of sucrose within the stalk and a reduction in factory efficiency due to dextran in the juice.

The objectives of control methods should be one or more of the following:- to prevent or minimise initial infection; inhibit or minimise growth of the organism after infection; prevent the milling of deteriorated cane of poor processing quality, and remove harmful substances from the juice before processing.

The fundamental requirements of any control method are that it should be effective; physically practical on an industrial scale; economic, and not detrimental to the product, the consumer, the process or the mechanical plant.

These facts are considered in the brief, critical survey of possible control methods given below.

1. Biological Methods

a) Resistant Varieties

The selection and breeding of disease resistant varieties is widely practised in the sugar industry (Martin, Abbot & Hughes, 1961). However, since it is not known whether *L. mesenteroides* is a plant pathogen or a saprophyte, the validity of this approach to prophylaxis of sour cane remains to be established.

In Louisiana it was observed that varietal differences exist in susceptibility to freezing and post-freeze deterioration (Irvine, 1968). Similarly, it is well known that some cane varieties deteriorate more rapidly than others after normal harvest. However, it is not known whether these differences are due to variations in resistance to infection, mechanical damage, drying-out, or autolysis.

Research on this topic was not attempted here.

b) Enzymic Treatment

One of the major economic losses caused by sour cane is a reduction in factory efficiency, due to the presence of certain polysaccharides (Chapter VII). Two main types of glucan polysaccharide are thought to be responsible; one is dextran, containing 80-95% (1-6) and 5-20% (1-4) α-glucosidic linkages, derived from the action of L. mesenteroides on sucrose in sour cane; the other is "sarkaran" (Bruijn 1966b; 1970), containing 25% (1-6) and 75% (1-4) α-glucosidic bonds, derived from natural enzymic changes in the stalks of stale cane.

These polysaccharides are not removed by the normal juice clarification process in the raw sugar factory. They can be hydrolysed by heating with acids or alkalis, but this treatment would also invert sucrose. Dextran can be slightly broken down by ultrasonic waves and ultraviolet light (Watson & Wolff, 1955), but this is not efficient. Removal of polysaccharides by molecular sieves, dialysis or reverse osmosis is theoretically possible, but at present these techniques are not suitable for application in a raw sugar factory.

Many polysaccharides are rapidly hydrolysed by specific enzymes, e.g. the action of various amylases on starch. Some enzymes are commercially available in large quantities at low cost, and are finding increased application in industry. In the sugar industry, bacterial amylases are used in South Africa to remove starch from cane syrups (Bruijn & Jennings, 1968). The advantages of enzymes are that they usually have mild optimum conditions of temperature and pH; they are specific; they are effective at low concentrations, and are usually non-toxic. The main disadvantages are that enzymes are easily 'poisoned' by certain chemicals; they act only within narrow limits of

temperature and pH, and they may be expensive.

The literature reveals that the extracellular enzymes of certain moulds are able to attack dextran (Tsuchiya, Jeanes, Bricker & Wilham, 1952). Interest has recently been renewed in these enzymes ("dextranases", endo-α-1-6 glucanase, E.C.3.2.1.11.), because they appear useful in the prevention of dental caries (Bowen 1968). No prior work has been reported on the use of dextranase to depolymerise dextran in sugar factories. Davies (1959) speculated on this possibility, but did not investigate the subject because it was thought to be uneconomic. However, since then the possibility of dental application has led to large-scale manufacture of cheaper enzyme. The mode of action of various microbial dextranases on dextran was recently discussed by Bretschneider and Copikova (1969). They attempted unsuccessfully to remove dextran from beet sugar diffusion juice with bacterial α-amylase, but did not try dextranases. Research on dextranase was therefore initiated.

Enzymic hydrolysis of 'natural' polysaccharide ("sarkaran") was first examined by Nicholson & Lilienthal (1959); they found that it was completely hydrolysed by salivary amylase but was not attacked by bacterial α-amylase. Bruijn (1970) recently confirmed this, but showed that pullulanase was even more effective.

2. Physical Methods

Many methods of food preservation are based on a modification of the environment which inhibits undesirable microorganisms, e.g. temperature, pH, relative humidity, oxygen content. A knowledge of the structure and physiology of the microorganism aids selection of the best control method.

L. mesenteroides has been studied in detail by many workers interested in the commercial production of dextran from sucrose (Jeanes et al. 1948; Jeanes 1965). *L. mesenteroides* grows in the temperature range 5 - 40°C, the optimum for dextran formation being about 22°C (Whittenbury 1966). Most strains survive exposure to a temperature of 55°C for 15 minutes, and some survive after 30 minutes (Garvie 1960). The pH range for growth is about 7.5 to 3.5; the optimum for dextran formation is 6.5 - 4.5. Little information is

available on the effect of equilibrium relative humidity (ERH) or water activity (a_w) on Leuconostoc. It grows well in a 10% sucrose solution, and tolerates 3% NaCl but not 6.5% NaCl (Garvie 1960). Spores are not formed. It is microaerophilic and catalase negative, but can grow aerobically and anaerobically.

The physical conditions of cane juice inside the cut stalk are almost ideal for growth of Leuconostoc, and the chemical composition of the juice is nutritionally adequate.

Inhibition of growth of Leuconostoc inside harvested cane by modification of environmental conditions during storage does not appear feasible. This is because treatments would need to be applied at each field where cane is harvested, prior to storage and transport; there is no central storage depot. At Frome the factory grinds about 3,000 tons of estate-grown cane per day, continuously day and night for seven months. This cane is harvested from about eight cane fields per day of average size 10 acres, distributed evenly over an area of about 50 square miles. These considerations preclude the storage of cut cane at low or high temperatures or the use of hot-water dips on site. pH modification would require drastic treatment because cane juice is well buffered; such treatment would also accelerate sucrose inversion.

It did seem feasible, however, to inhibit Leuconostoc by dipping the cut ends of freshly-harvested cane into a solution of low water activity. This was studied, using dilute molasses.

Sterilisation or 'pasteurisation' of harvested cane by irradiation is excluded for practical reasons similar to those described above. However, in view of evidence that alternating electromagnetic waves of high intensity were selectively inhibitory to Leuconostoc (Laser 1968), some work was carried out on this topic.

3. Chemical methods

The application of bactericidal or bacteriostatic chemicals active against L. mesenteroides was considered. These could be used to prevent infection by killing Leuconostoc in its primary sources, (i.e. soil or on the cane plant) or on the vectors of infection (cutting instruments). Alternatively, they

could be applied to the harvested stalks to inhibit growth after infection. The use of biocides in the mill to retard inversion in extracted juice was considered to be a problem in factory hygiene outside the scope of this thesis.

A major constraint on the use of bactericides is that they could persist throughout the factory process to the final products, raw sugar and molasses. These are foods and hence subject to strict legislation; therefore, low human toxicity is a fundamental requirement of any chemical under consideration.

Treatment of soil in cane fields or the surface of standing cane stalks prior to harvest could only be achieved by aerial spraying of bactericides. This technique is routinely used for application of fertilisers, fungicides, weed-killers and insecticides to cane fields, but is only effective on the cane leaves. It was not considered to be a practical proposition for bactericides because the dense surface leaf area would prevent adequate penetration of soil or cane stalks.

Egan (1965b) investigated several germicides and antibiotics applied as a dip or soak to the cut ends of freshly-harvested, chopped-up cane billets. Screening tests showed that quaternary ammonium compounds (Q.A.C) and penicillin were most active against Leuconostoc 'in vitro'. The Q.A.C. were not effective in the 'in vivo' trials, however, due to failure to penetrate the stalk. Penicillin was effective, but industrial use of this antibiotic is precluded on ethical grounds. However, penicillin is used in India to control inversion in extracted juice in the mill (Anon. 1969a).

Fungicidal dips of machetes and cane setts prior to planting are used to control certain diseases of cane (Martin et al 1961). However, these fungicides act as prophylactics in the prevention of infection, whereas in post-harvest control of Leuconostoc, infection has already occurred.

Application of Q.A.C. sprays to the chopper blades of a mechanical harvester during operation and cane in the machine did not retard spoilage of the billets, probably because the film of chemical was wiped off as the blades chopped the stalks (Egan, 1965b).

Partial success was achieved by formaldehyde fumigation of chopped-cane stored in bins (Egan 1968a), but the treatment was regarded as uneconomic. This method is not applicable to manually-cut, whole-stalk cane in Jamaica.

Finally, recent work by Alexander (1968) showed that silicon in the form of sodium meta-silicate inhibited the invertases of the cane plant both '*in vitro*' and '*in vivo*'. It also inhibited microbial inversion of sucrose in extracted cane juice, and on this basis it was recommended for use as a sanitation aid in the mill. Alexander suggested that this chemical may be of use for inversion control in cane both before and after harvest, since it is easily absorbed by plants, safe to handle and inexpensive. Unfortunately this publication was too recent to enable this suggestion to be evaluated in the present thesis.

4. Modification of harvest, storage and transport practices

The most obvious solution to the problem of post-harvest biodeterioration of cane is simply to mill the cane before significant deterioration occurs. This solution was found to be the only practical method of minimising 'sour storage rot' in mechanically-harvested, chopped-up cane in Queensland. (Allen 1967; Egan 1968b, 1969; Fost 1969b). Storage of chopped cane over the weekend was discontinued, and the logistics of cane transport were improved by computer control. The objective was to ensure that chopped cane was milled within 12-24 hours of harvest.

In Jamaica, where cane is manually-cut in whole stalks, the rate of post-harvest deterioration is slower; this cane should be milled within 24-36 hours of harvest to prevent significant economic losses. (Chapters III and VII). At Frome Estate time studies carried out since 1964 show mean annual "bill to mill" times ranging from 34 to 45 hours, but a significant proportion of the cane was more than 48 hours old post-harvest (Anon 1968). Analysis of unit operations revealed that the greatest saving in time could be made by reducing the time lag between cutting and loading (about 21 hours on average). The logistics of these operations are extremely complex, however, and plans are constantly disrupted by unforeseeable events such as mechanical breakdowns,

heavy rainfall, labour disputes, etc. The introduction of mechanical harvesters will stress the need for even closer control of bill-to-mill times.

Various other practices in the growth, harvest, storage and transport of cane may help to minimise deterioration losses. These include prevention of disease, minimisation of mechanical damage, and elimination of trash and soil in storage piles. Elimination of pre-harvest burning of cane is also desirable. Recent emphasis on the prevention of atmospheric pollution in the United States suggests that burning of cane-fields may become illegal.

5. Penalty Systems

A possible method of reducing bill-to-mill times and the degree of post-harvest biodeterioration is to introduce a penalty system based on cane quality. This necessitates a routine quality control test on each batch of cane entering the mill which estimates either its degree of "sourness" or processing quality. Unsuitable cane would be rejected, and the grower would be penalised by loss of income. This would provide positive incentive to the grower to supply fresh cane. Furthermore, a graduated scale of penalties could be imposed, related to the degree of estimated deterioration.

In Jamaica and many other countries, cane payment is based on a formula which is computed from the sucrose content of the cane as received at the factory. Growers would receive additional incentive to provide fresh cane if it was fully realised that cane deterioration causes loss of sucrose and hence loss of income.

Many papers have been published on the search for a quality control test that accurately estimates either the age of cane post-harvest, its degree of deterioration, or its processing quality. The additional requirements of such a test are that it should be rapid; acurate; reproduceable; sensitive; simple and inexpensive.

The changes that occur in juice composition during post-harvest storage were discussed in Chapter III. Briefly, these are a reduction in sucrose content, purity, and pH, and an increase in reducing sugars, acidity, "gums", dextran, and microorganisms.

Early work in Louisiana favoured the use of titratable acidity (Fort & Lauritzen 1938a) with freeze-damaged cane. This was also studied by Irvine & Davidson (1963) and Irvine (1964). Fort & Lauritzen (1938a) recommended "excess acidity", which determined volatile acids, because this overcame variations in natural pre-freeze titratable acidity; acetic acid was the principal volatile acid accumulating during post-freeze deterioration. Later Friloux, Cashen & Cangemi (1965) demonstrated that lactic and acetic acids were the sole cause of this increase in acidity. pH was a less sensitive indicator of deterioration in these conditions.

Increases in "gum" content were found to be mainly responsible for processing difficulties in juices from frozen cane (Fort & Lauritzen 1938b; McCalip & Hall 1938; Irvine 1964; Friloux et al 1965). Irvine & Friloux (1965) found that gum content was a better indicator of deterioration than titratable acidity. They used a method based on alcohol precipitation followed by colorimetric determination of total sugars (Roberts & Friloux 1965). Similar conclusions were reached by Egan (1966; 1967b) for chopped-cane in Queensland and Bruijn (1966a) for whole-stalk cane in Natal; the latter used a gravimetric method of gum determination. In India, Gupta, Juneja & Shukla (1968) also reported favourably on the use of gum content as a deterioration indicator.

"Gums" include all alcohol-precipitable polysaccharides, many of which are found in fresh cane in amounts which vary with variety, age, soil and season. Another disadvantage is that the methods of analysis are time-consuming. Most recently, Keniry, Lee & Davis (1967a; 1967b) studied the dextran content of chopped cane and found that it was superior to either gum or lactic acid content as an indicator of processing quality. They used a rapid, simple turbidimetric technique based on the "haze analysis" method of Nicholson & Horsley (1959). "Dextran" was defined as the material precipitated by 50% ethanol from a starch- and protein-free solution of cane juice. Its main advantage was the claim that it was produced solely as a metabolite of _L. mesenteroides_ and hence was not present in fresh cane. Unfortunately,

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more recent evidence by Foster (1969a) showed that fresh cane occasionally contained high levels of "dextran" when determined by haze analysis. It seems probable that this "dextran" is the natural cane polysaccharide "sarkaran" previously discussed. If so, the value of the test is not necessarily reduced, because sarkaran is also thought to interfere with processing quality (Sutherland & Paton 1969).

Various other parameters have been investigated but were found to be less suitable, e.g. alcohols, non-volatile organic acids, and amino acids (Bruijn 1966a) and pH (Egan 1966; 1967b).

Sucrose and reducing sugar content are of little value in estimating degree of deterioration unless their original values in fresh cane are known, but this is seldom the case. Furthermore, sucrose determinations based on polarisation measurements become unreliable in badly deteriorated cane, due to interference by other optically active metabolites, (Tantaoui 1952). However, cane juice of low purity is generally regarded as undesirable. At Frome, the minimum acceptable purity is 79.0. At present this is the sole basis for rejection of poor quality cane. Since apparent purity is usually higher than the true purity in sour juice, due to the dextrorotatory properties of dextran, poor quality cane frequently passes this standard. This fact stimulated research to find a better alternative quality control test.

A recent paper by Serbia (1966) describes a formula called the 'deterioration index' (D.I.) which is used to estimate the degree of deterioration of incoming cane in Puerto Rico. It is calculated from normal process control analysis of crusher juice pol and brix. The D.I. showed a very good correlatinn with final molasses purity, and enabled accurate estimates to be made of yield of sugar from cane. It therefore seems promising and would justify further investigation.

The best estimate of degree of microbiological deterioration of cane is probably a viable count of spoilage organisms, using selective media such as STATA for Leuconostoc and lactobacilli. This technique is valuable for confirmation of spoilage, but is not suitable for quality control because 48 hours incubation is required. Total counts of microorganisms by direct microscopic examination do not distinguish between spoilage and non-spoilage

organisms, or between viable and dead organisms. Rapid methods of estimation of microbial activity, such as dye reduction, are widely used in the milk industry to gauge the bacterial quality of fresh milk; the methylene blue reduction test was therefore considered in this work. This application was first suggested by Owen (1949). A similar test based on T.T.C. reduction is reported to be in use in a Soviet sugar beet factory (Khodurskii & Chechel 1969).

B. EXPERIMENTAL

1. Biological Methods : Enzymatic Hydrolysis of Polysaccharides in Mill Juices with Dextranase.

Unless otherwise stated, all experiments were carried out with samples of freeze-dried dextranase obtained from cultures of *Penicillium funiculosum*. The enzyme was kindly donated by Dr W.H.Bowen (Royal College of Surgeons, Dental Caries Research Unit, Downe, Kent). Its activity was 12 units/mg., where 1 unit is defined as the quantity of enzyme which liberates 1 mg. isomaltose per hour from dextran at 40°C, pH 5.1 - 5.4.

a) Laboratory experiments (Tate & Lyle Ltd., Research Centre)

(i) Effect of dextranase on pure *L. mesenteroides* dextrans. Initially it was thought that dextranase might be used to assay the dextran content of cane juice in a rapid quality control test. Its effect on six different pure dextrans from four strains of *L. mesenteroides* was therefore studied.

The pure dextrans were obtained from growing cultures of *L. mesenteroides*, isolated from cane juice, by the method of Jeanes (1965); 1% (w/v) stock solutions in 0.2 M acetate buffer, pH 4.6, were prepared.

The dextranase was a crude, liquid culture filtrate, activity 20 units/ml.

8 ml. dextran solution were added to 50 ml. 0.2 M acetate buffer, pH 4.6. 2 ml. enzyme was added, mixed, and 30 ml. removed for reducing sugar (R.S.) determination (Luff-Schoorl). The remaining solution was incubated in a water bath at 43°C for one hour, and again analysed for R.S. Solutions were inactivated by heating for ten minutes at 100°C prior to analysis.

The results are shown in Table VIII,1.

L. mesenteroides strain no.	% R.S. formed by hydrolysis	% hydrolysis of dextran (maximum yield = 0.133%) RS
L20	0.052	39.1
G7	0.029	21.4
G7 x*	0.003	2.3
16 b	0.004	3.0
16 bX*	0.010	7.5
70 a	0.019	14.3

Table VIII,1. The degree of hydrolysis of pure dextrans of *L. mesenteroides* treated with dextranase.

* dextran fraction insoluble in original SCJ culture medium.

The enzyme is very specific, because its activity varies widely between dextrans from different strains of *L. mesenteroides*. Similar results were reported by Tsuchiya et al (1952), and Bourne, Hutson & Weigel (1962). The method cannot therefore be used to assay dextran content of cane juice, but since it hydrolysed nearly 40% of one dextran, it may be of value for removal of dextran from cane juice.

(ii) Determination of optimum temperature and pH for dextranase activity.

Various values of temperature and pH have been reported as optimal for *P. funiculosum* dextranase activity. Some of these are given below:-

Optimum temperature °C	Optimum pH	Reference
40	4.0 - 5.5	Tsuchiya et al (1952)
NT	5.25 - 6.5	Kobayashi (1954)
45 - 50	4.5 - 5.5	Bourne et al (1962)
30 - 40	5.1 - 5.4	Anon (1970)

The value of these cardinal points was determined for Dr Bowen's enzyme in two experiments.

Effect of pH on velocity of enzyme reaction : Preliminary tests showed that dextranase was most active in acetate rather than citrate buffer. All subsequent work was therefore carried out in 0.2 M acetate buffer.

Dextran (high molecular weight, food grade, Koch-Light Ltd.) was dissolved in acetate buffers at pH values of 4.7 - 5.7 to give 0.222% w/v solutions. The enzyme was similarly dissolved in the range of buffers to give a concentration of 50 units/ml. 1 ml. enzyme was mixed with 9 ml. of the corresponding dextran solution in test tubes held at 40°C in a water bath. Replicate tubes were withdrawn after incubation periods of 2, 5 and 10 minutes and the enzyme inactivated by immersion in a boiling water bath for two minutes. The residual dextran content was determined by the haze analysis technique of Keniry et al (1967a). Controls were run with additions of buffer instead of enzyme. The amount of dextran hydrolysed versus time was plotted at each pH, and the initial velocity of hydrolysis was calculated from the slope of the curve at zero time. The initial velocity was then plotted against pH. The results are shown in Figure VIII,1. Each point is the mean of three determinations.

Effect of temperature on enzyme velocity. The experiment was similar to that described for pH, except that all reactions were in acetate buffer at pH 5.0, and the temperature of incubation was varied between 30 and 60°C.

The results are shown in Figure VIII,2.

It was concluded that the optimum pH for dextranase activity is 4.9 - 5.1, but good activity is obtained between pH 4.8 - 5.5. The optimum temperature at pH 5.0 is 50°C, with good activity over the range 40 - 55°C. These conclusions are in general agreement with the figures given previously.

(iii) Effect of dextranase and its conditions of use on sucrose.

P. funiculosum dextranase is specific for α1-6 linked glucosidic bonds; it is an endo-enzyme which cleaves the dextran molecule at random. The hydrolysis products are isomaltose (45-53%); isomaltotriose (7-22%); glucose (4-11%) and higher oligosaccharides in a homologous series in small amounts (isomaltotetraose, etc.) (Jeanes et al 1953; Bourne et al 1963). These products all possess the β configuration. Theoretically dextranase should not attack sucrose, but it is possible that the reaction conditions used in its commercial application in mill juice might cause some inversion of sucrose.

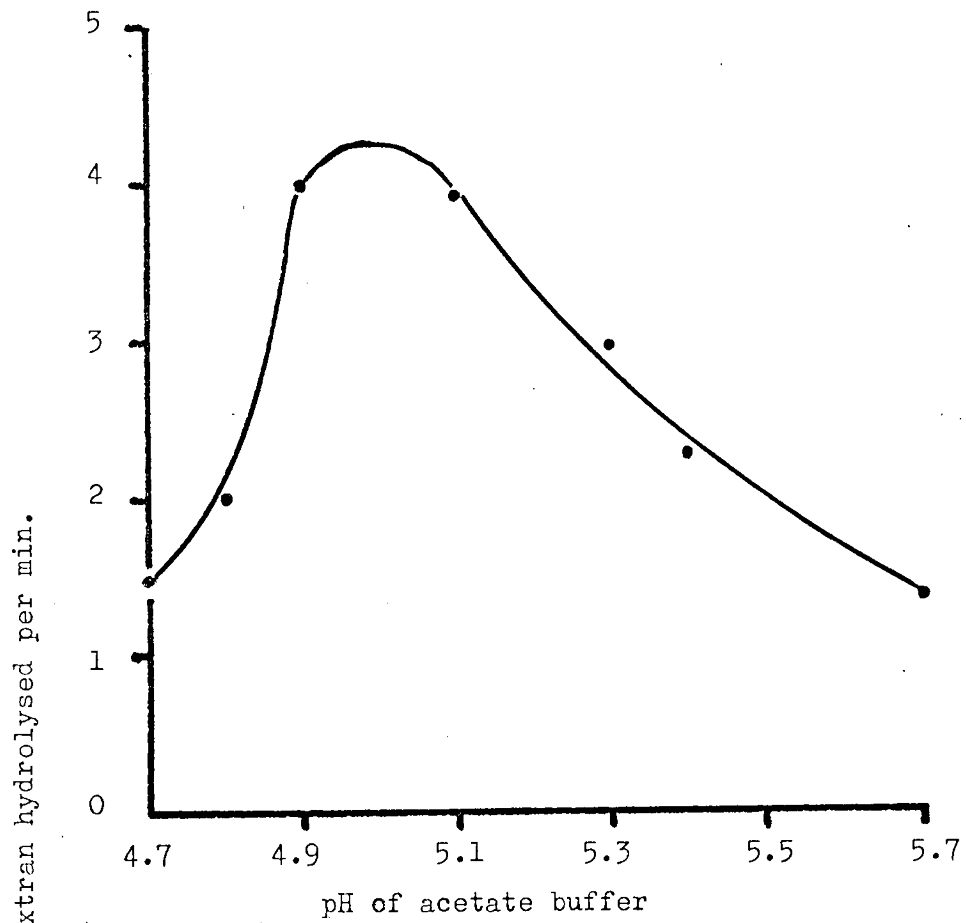

FIG. VIII,1. The effect of pH on activity of dextranase at 40°C.

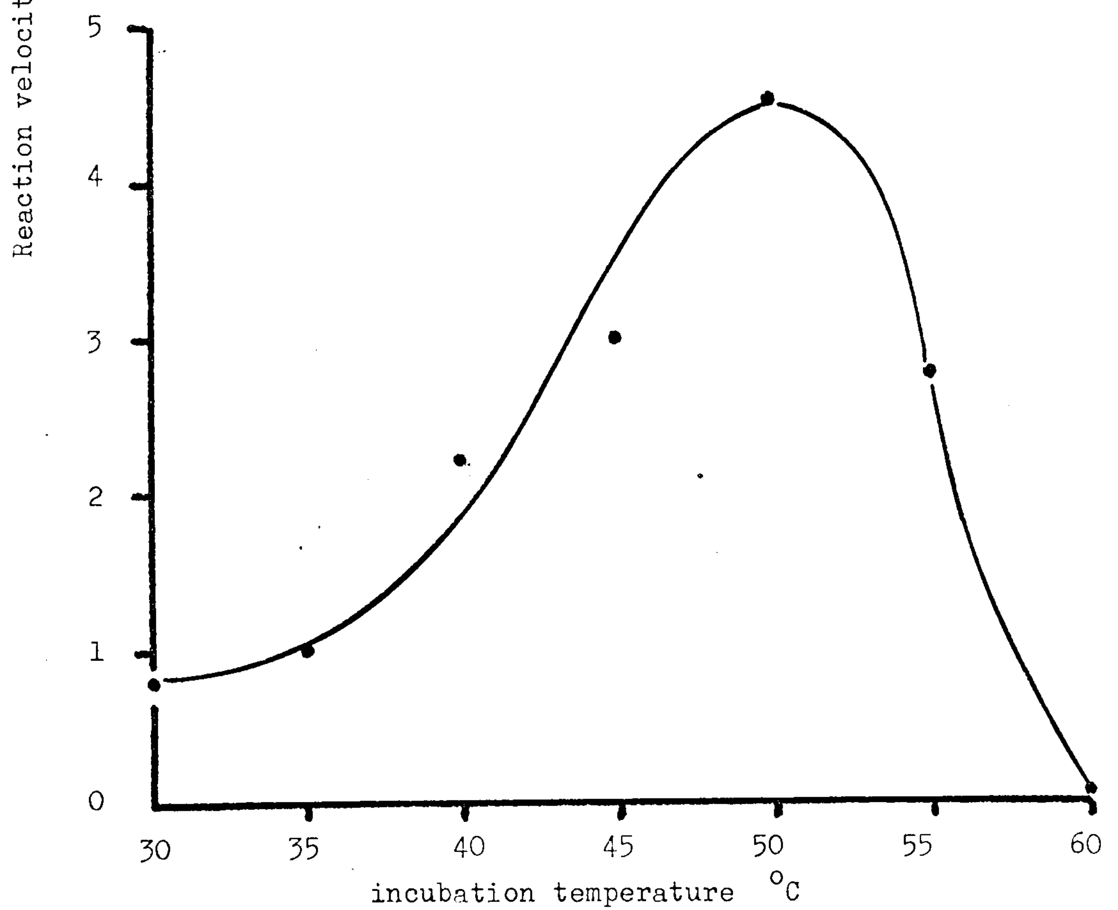

FIG. VIII,2. The effect of temperature on activity of dextranase at pH 5.0.

The probable use conditions (discussed later) are:-

Enzyme concentration - 3-12 units/100 ml juice;

Reaction time - 15-60 minutes;

Temperature - 40-45°C; pH - 5.0-5.5

Since inversion of sucrose would be a disadvantage, the effect of these factors was studied in the following experiment:-

A synthetic 15°Brix 'cane juice' was made up by dissolving 15 g. sucrose (mineral water sugar, R.S. content less than 0.002%) in 85 ml. 0.1M acetate buffer at pH 5.3 and distributed in 9 ml. aliquots into seven test tubes. At zero time, 1 ml. dextranase solution (in acetate buffer) was added to three of the tubes, to give final enzyme concentrations of 12 units /100 ml. juice. 1 ml. acetate buffer was added to each of the other four tubes. One of these (= 'control') was immediately placed on ice. The other six tubes were placed in a 40°C water bath. One tube of each treatment was removed after incubation for periods of 15, 30 and 60 minutes, and placed on ice. Reducing sugar formation was detected by paper chromatography using the silver nitrate method of Trevelyan, Proctor & Harrison (1950). 40 microlitre spots of test solutions were used; the standards contained 10 microlitres.

The chromatograms are shown in Figure VIII,3.

Reducing sugars were not detected in any of the test solutions or in sucrose standards, theoretical maximum R.S. in the spots was 0.12 micrograms and 0.03 micrograms in test and standard respectively.

It was concluded that treatment of sucrose with dextranase under the given conditions does not cause significant formation of reducing sugars. Therefore the probable method of commercial application of dextranase to remove dextran from mill juice does not cause significant sucrose loss.

The effects of isomaltose and related homologous oligosaccharides on sucrose recovery and crystallisation have not been studied, but they seem unlikely to cause any harmful effects (for discussion see Chapter VII).

b) <u>Factory Experiments</u> (Frome Estate, Jamaica).

A series of trials was carried out at Frome in 1969 and 1970. The objectives were to assess the efficiency of dextranase in removal of dextran

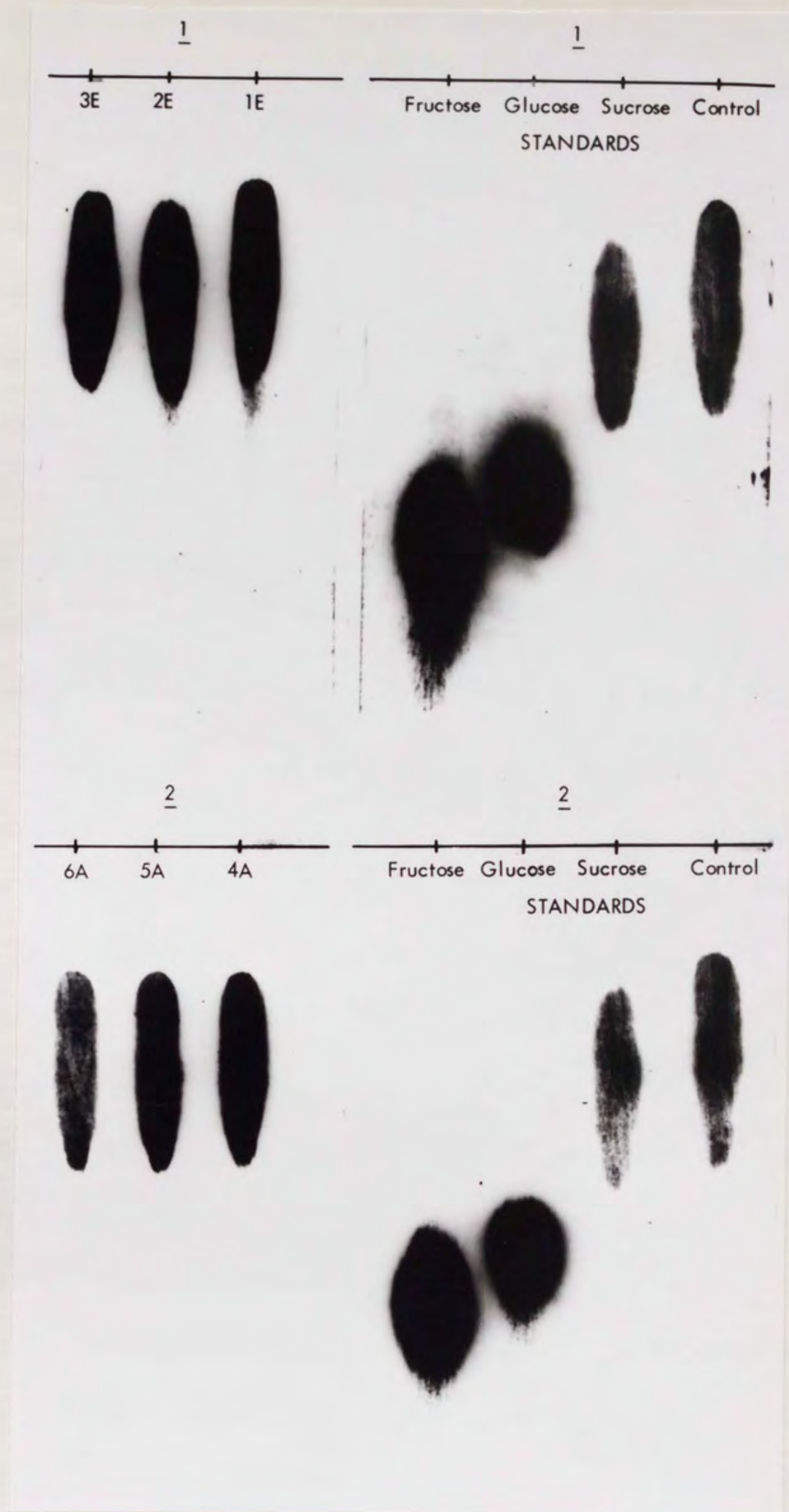

FIG. VIII,3.

The effect of dextranase and reaction conditions on sucrose. Paper chromatographic separation of reaction products.

Key				
Paper 1.	1E	Enzyme + buffer + sucrose;		15 mins.
	2E	" " "		30 "
	3E	" " "		60 "
Paper 2.	4A	No enzyme, " "		15 "
	5A	" " "		30 "
	6A	" " "		60 "
Control		Enzyme " "		0 "

from extracted cane juice prior to processing, and to determine the optimum reaction conditions for commercial application.

The most suitable stage in the factory process at which to add the enzyme is the freshly extracted crusher or mixed juice, prior to clarification. Here the conditions in the juice are nearest to optimal for the enzyme reaction, i.e. temperature about 30°C, pH 5.0-5.6, sucrose content 12-20%. Also, dextran interferes with the next process stage, clarification, in which the juice is heated to 220°F and limed to pH 7.8. In the subsequent stages of evaporation and crystallisation, the viscosity, temperature and pH are too high. Therefore, all experiments were carried out on random samples of crusher or mixed juice taken from the milling tandems and filtered to remove coarse suspended matter.

(i) Preliminary trials, 1969 season. Dextranase was freshly prepared in 0.2M acetate buffer, pH 4.7, and added to the substrate (crusher juice, C.J., or mixed juice, M.J.), in the ratio of 1 to 10 parts by volume. Acetate buffer only was added to the untreated controls. The solutions were incubated with occasional shaking in a water bath at 43°C, for periods ranging from 30 minutes to 1 hour. The enzyme reaction was then stopped by the addition of 0.2 volumes 10% trichloracetic acid and dextran was determined by the haze analysis method of Keniry et al (1967a).

In the first series, the final enzyme concentration was 300 units/100 ml. juice; this was reduced to 30 units/100 ml. in the second series, with a constant reaction time of 30 minutes. Some juices extracted from stored cane in experiments reported in Chapter III were also included.

The results are shown in Tables VIII,2 and VIII,3.

The mean percentage of dextran removed at an enzyme concentration of 300 units/100 ml. was 78.0%; this was reduced to 76.3% when the enzyme concentration was lowered to 30 units/100 ml. and the reaction time shortened to 30 minutes. The enzyme efficiency did not appear to be influenced by initial substrate concentration in the samples tested. The mean result in Table VIII,3 is reduced by three results which show less than 12% dextran removal, compared with 19 results which exceed 50%. The Leuconostoc count

Date	Substrate	Incubation time in minutes	Dextran % Brix		% removal of dextran
			Control	Test	
7.7.69	CJ	60	1.310	0.574	56.2
9.7.69	CJ	45	0.308	0	100.0
	MJ	45	0.186	0	100.0
10.7.69	CJ	60	0.286	0.101	64.7
	MJ(i)	60	0.349	0.018	94.8
	MJ(ii)	60	0.364	0.040	89.0
14.7.69	MJ(i)	40	2.080	0.431	79.3
	MJ(ii)	40	0.286	0.127	55.6
	MJ(iii)	40	1.430	0.047	96.7
	MJ(iv)	40	0.142	0.081	43.0
Mean	(10 samples)		0.674	0.142	78.0

Table VIII,2. The effect of dextranase on the dextran content of mill juices. Frome, 1969. Enzyme concentration 300 units/100 ml. juice.

CJ = crusher juice
MJ = mixed juice.

Date	Substrate	Dextran % Brix		% removal of dextran
		Control	Test	
18.7.69	Exp.50,day 10	0.365	0.354	3.0
	" 52, " 4	0.549	0.554	0.0
	" 53, " 3	0.482	0.107	77.8
	" 54, " 2	1.740	0.525	69.8
22.7.69	" 54, " 6	1.440	0.304	78.9
	" 56, " 0	0.268	0.238	11.9
	CJ	0.714	0.171	76.0
	MJ	1.390	0.250	82.0
23.7.69	MJ	1.350	0.240	82.0
	MJ	0.807	0.096	88.1
	MJ	0.517	0.050	90.3
	MJ	0.268	0.029	89.2
24.7.69	MJ	0.500	0.009	98.2
	MJ	0.318	0.010	97.0
29.7.69	MJ	0.467	0.046	90.2
	MJ	0.980	0.483	50.7
	MJ	0.677	0.153	77.4
	MJ	1.044	0.388	62.8
30.7.69	MJ	0.754	0.247	67.3
	MJ	1.260	0.380	69.8
	MJ	1.038	0.363	65.0
	MJ	0.608	0.043	92.9
Mean	(22 samples)	0.797	0.229	76.3

Table VIII,3. The effect of dextranase on the dextran content of mill juices, in a reaction time of 30 minutes. Frome, 1969. Enzyme concentration 30 units/100 ml. juice.

CJ = crusher juice

MJ = mixed juice

of these three juices was very low; it is therefore postulated that the 'dextran' in these samples may have been sarkaran, rather than L. mesenteriodes dextran, in which case the dextranase would not attack it so rapidly, if at all.

(ii) Further trials, 1970 season. More extensive trials were carried out at Frome during March/April, 1970. This period was dry, and the mean dextran content of crusher juice was 0.34% Brix; this is lower than the result of 0.41% Brix obtained during the previous crop, May/July 1969, when the rainy season was in progress. (Chapter VII).

The test method was similar to that of 1969, except that the enzyme was suspended in 0.2M acetate buffer, pH 5.3, and was added to the juice substrate in a ratio of 1 to 100 parts by volume. The reaction temperature was slightly reduced to 40°C.

The effect of a reduction in reaction time to 15 minutes on the efficiency of dextran removal: 38 samples of mixed juice were selected at random from the mill and treated with dextranase at a final enzyme concentration of 30 units/100 ml.juice. The reaction time was reduced to 15 minutes. The results are summarised in Table VIII,4.

The mean % removal of dextran was 78.7%, which exceeds the 1969 result of 76.3% at the same enzyme concentration. It was concluded that the reaction time can be reduced to 15 minutes without any loss of enzyme efficiency.

Again, enzyme efficiency did not appear to be influenced by substrate concentration. Only four samples showed less than 12% removal of dextran; the remaining 34 samples all exceeded 50% dextran removal. The variation in degree of 'dextran' hydrolysis and occasional weak activity may be explained by the high specificity of P. funiculosum dextranase previously reported here, and in the work of Tsuchiya et al (1952) and Bourne et al (1962). The enzyme specificity is probably related to the degree of cross linking in branched dextrans. This is known to vary widely amongst different strains of L. mesenteriodes (Jeanes et al (1954)). The proportion of α1-3 linkages is probably the controlling factor, since this link is not attacked by dextranase (Bourne et al (1962)). Cane juice contains a variety

Date	Initial Dextran % Brix	% Removal of Dextran
10.3.70	0.183	50.0
	0.215	100.0
	0.322	60.4
12.3.70	0.393	0.0
	0.306	100.0
	0.744	86.1
	0.793	83.3
13.3.70	0.175	100.0
	0.468	100.0
16.3.70	0.145	12.5
	0.339	82.5
	0.535	11.0
	0.310	7.5
17.3.70	0.270	93.7
	0.441	91.6
	0.446	71.0
	0.760	64.5
18.3.70	0.488	100.0
	0.185	100.0
	0.058	100.0
	0.241	92.5
19.3.70	0.194	67.8
	0.276	100.0
	0.169	100.0
	0.224	100.0
23.3.70	0.882	91.0
	0.312	80.0
	0.352	79.0
	0.566	82.4
24.3.70	0.062	100.0
	0.230	100.0
	0.423	93.4
	2.020	74.7
25.3.70	0.091	100.0
	0.630	72.5
	0.194	100.0
	0.143	100.0
26.3.70	0.660	72.1
Mean	0.400	78.7

Table VIII.4. The effect of dextranase on the dextran content of 38 samples of mixed juice, Frome 1970.

Enzyme concentration : 30 units/100 ml. juice

Reaction conditions : 15 mins. at 40°C

of strains of L. mesenteroides (see Chapter IV) and hence the type of dextran present will also be variable.

The effect of a reduction in enzyme concentration on efficiency of dextran removal at two reaction times; Fourteen samples of mixed juice with dextran contents in excess of 0.4% Brix were treated with dextranase at final enzyme concentrations of 12, 6 and 3 units/100 ml. juice; reaction times were 10 and 20 minutes.

The results are given in Table VIII,5.

The mean efficiency of dextran removal was slightly less at 10 minutes than at 20 minutes reaction time; this effect was more pronounced at the lowest enzyme concentrations. In a reaction time of 20 minutes the mean efficiency of dextran removal was independent of enzyme concentration down to 3 units/100 ml.

At an enzyme concentration of 3 units/100 ml. juice the mean % removal of dextran was 68.5% in 20 minutes reaction time. This result compares favourably with commercial processes for enzymic removal of starch in S. African sugar factories, using amylases (Bruijn & Jennings 1968).

The effect of some amylases and dextranase on the dextran content of mixed juice. Cane juice may contain several types of polysaccharide that will produce a 'haze' with 50% ethanol in the method of 'dextran' determination of Keniry et al (1967a) (Chapter VII). In a few samples of mixed juice the 'dextran' content was not significantly reduced by treatment with dextranase (Tables VIII,3 and VIII,4); this indicated the possible presence of polysaccharides other than L. mesenteroides dextran.

It was not possible to study the nature of these polysaccharides at Frome, due to lack of suitable facilities. However, it seemed likely that the polysaccharide 'sarkaran' was present in some juice samples. Sarkaran was shown to be a glucan containing 75% 1-4 and 25% 1-6 α-glucosidic bonds; it was hydrolysed by salivary α-amylase but not by bacterial α-amylase (Bruijn, 1970). Therefore it was decided to examine the effect of some microbial amylases of different type on the 'dextran' content of Frome mixed juice.

Date	Initial Dextran % Brix	% Removal of Dextran at stated Enzyme Concentration (units/100 ml. juice)					
		12		6		3	
		10 mins.	20 mins.	10 mins.	20 mins.	10 mins.	20 mins.
2.4.70	.757	79.9	68.8	84.7	75.7	41.0	73.6
3.4.70	.222	92.3	87.4	100.0	100.0	100.0	100.0
3.4.70	.529	93.7	83.7	100.0	87.0	94.5	93.7
4.4.70	.510	57.9	69.4	13.5	73.8	87.2	100.0
7.4.70	.803	54.0	48.5	46.3	37.0	44.0	56.2
9.4.70	.476	69.0	62.2	45.0	45.0	34.0	30.5
13.4.70	.621	56.5	58.5	64.9	74.1	79.7	79.7
14.4.70	.566	47.0	(60.7*)	NT	NT	65.6	(69.7*)
15.4.70	.548	(58.8*)	72.8	NT	NT	(49.0*)	62.1
16.4.70	.676	(65.3*)	73.1	(75.6*)	80.9	(69.6*)	78.3
20.4.70	.654	73.0	69.6	59.2	56.5	47.8	56.5
21.4.70	.610	51.4	57.7	48.7	55.1	39.6	(42.3*)
23.4.70	.669	36.5	39.2	30.5	39.2	20.0	21.6
24.4.70	.672	72.0	77.5	68.0	69.2	62.5	69.2
Mean	.594	65.3	66.8	60.0	66.1	59.7	68.5

Table VIII,5. The effect of dextranase on the dextran content of 14 samples of mixed juice, Frome 1970.

Enzyme concentrations : 12, 6 and 3 units/100 ml. juice

Reaction conditions : 10 and 20 minutes at 40°C

* Result obtained at 15 minutes reaction time, excluded from calculations of the mean % removal of dextran.

NT = Not tested.

Amylases catalyse the hydrolysis of α 1-4 glucosidic links. α-amylases (α 1, 4-glucan-4-glucanohydrolase, E.C.3.2.1.1.) produce products which have the α configuration at c_1 of the reducing group; they possess an endo-attack mechanism and can by-pass α 1-6 links. (Robyt and Whelan, 1968). A fungal α-amylase produced from Aspergillus oryzae (Novo II) and a bacterial α-amylase of Bacillus subtilis (Nervanase MT) were chosen for study. Another type of amylase is glucoamylase (α-1, 4-glucan-glucohydrolase, E.C.3.2.1.3.), which splits successive α 1-4 bonds from the non-reducing ends of starch chains, yielding glucose. (Reed, 1966). In addition it can hydrolyse α 1-6 glucosidic bonds, although at a slower rate than 1-4 links. An enzyme produced by Aspergillus awamori (Ambazyme PC25) was selected.

The effect of the enzymes on 12 samples of mixed juice with 'dextran' contents in excess of 0.4% Brix was determined. The method was similar to that described previously; the enzymes and reaction conditions are summarised in the table below; optimal conditions for each enzyme were selected.

Type of Enzyme	Trade name and manufacturer	Enzyme Conc.PPM on juice	Reaction time in minutes	Reaction Temp.°C	pH of acetate buffer
1. Dextranase	Dr Bowen	10*	10	40	5.3
2. Fungal α-amylase	Novo II;Globe Products Ltd	10	60	50	5.0
3. Glucoamylase	Ambazyme PC25;ABM	10	60	50	5.0
4. Bacterial α-amylase	Nervanase MT;ABM	10	60	80	5.6

* equivalent to 12 units/100 ml. juice.

The results are shown in Table VIII,6.

Dextranase removed a greater percentage of 'dextran' on average than either of the amylases; fungal α-amylase was marginally more effective than the other amylases. In one sample (9th April) dextranase was much more effective than the amylases, but the reverse was true on 23rd April. Quantitative comparisons cannot strictly be made, however, due to the variety of enzyme concentrations (in terms of activity units) and reaction conditions which were of necessity used.

Date (April)	Initial Dextran % Bx	% Removal of Dextran with stated enzyme			
		dextranase	Novo II	Ambazyme PC25	Nervanase MT
6	.573	NT	61.0	53.9	65.8
7	.803	54.0	76.2*	53.9*	48.5*
9	.476	69.0	22.0*	22.0*	18.4*
11	.796	81.3*	54.1*	NT	NT
13	.621	56.5	44.5	50.1	53.8
15	.548	58.8*	51.1	48.9	51.1
16	.676	65.3*	56.5**	61.0**	69.6**
17	.635	NT	64.7**	64.7**	63.0**
20	.654	73.0	69.8	65.3	67.0
21	.610	51.4	72.2	57.7	48.9
23	.669	36.5	60.8	59.2	53.1
24	.672	72.0	NT	64.0	60.2
Mean	.644	61.8	57.5	54.6	54.5

Table VIII.6. The effect of some amylases and dextranase on the 'dextran' content of 12 samples of mixed juice, Frome, 1970.

*	=	15 mins. reaction time
**	=	30 mins. reaction time
NT	=	Not tested

The observed high percentage of "dextran" removal by the amylases is difficult to explain. It is possible that the α-amylases contained impurities which were capable of hydrolysing α 1-6 links in dextran, since it was shown by Tsuchiya et al (1952) that a crude amylase of _A. niger_ attacked dextran slowly; this could explain the results with Novo II enzyme. The known ability of glucoamylase to hydrolyse α 1-6 links slowly might explain the results with ambazyme PC25, since a comparitively long incubation time (60 minutes) was used. Another possibility is that the dextrans contained a relatively high proportion of α 1-4 links. Subsequent experiments in the laboratory, however, showed that the three amylases did not attack a pure _L. mesenteroides_ dextran (Koch-Light, food grade) in 30 minutes reaction time. The observed activity of the bacterial α-amylase (Nervanase MT) suggests that sarkaran was not a major component of the 'dextrans' in the samples tested. Finally, it is unlikely that starch was present in the juices because they were filtered prior to test and the filtrate did not give a blue colour with the iodine reaction.

Research on the polysaccharides present in cane juice is being continued, both here and in Australia. In addition, the activity of the enzyme pullulanase will be studied. This enzyme is specific for α 1-6 glucosidic bonds which are in conjunction with α 1-4 glucosidic bonds (Price 1968).

c) _Economics of commercial application of dextranase_.

Previous results showed that addition of 3 units dextranase to 100 ml. mixed juice at its natural pH and a temperature of 40°C reduced the 'dextran' content by 60-70% on average in a reaction time of 10-20 minutes.

At Frome, the maximum intake of cane is 320 tons per hour. This would yield about 72 tons of mixed juice per 15 minutes; at 14° Brix the volume of this juice is about 15,000 gallons. The suggested method of application of dextranase is to add it to mixed juice in a vessel of minimum capacity 20,000 gallons at a dose rate of 3 units/100 ml. juice; the vessel would be located between the mixed juice scale and the weighed juice tank, where primary liming occurs. A suitable vessel is readily available in the form

of a Dorr clarifier, which could easily be modified to heat the juice to 40-50°C by means of steam coils. Batch or continuous flow treatment could be devised to guarentee a minimum retention time of 15 minutes. The enzyme would only be used when quality control tests on incoming cane revealed high dextran levels, e.g. in excess of 0.5% Brix. Discussions held with senior factory staff at Frome suggested that such a process would be acceptable from engineering and process aspects. Capital outlay required for these modifications would be approximately £3,000, excluding the cost of the Dorr clarifier, which is already installed and available.

The depolymerisation of dextran by dextranase would probably benefit the process in several ways. Firstly, juice clarification would be improved. Secondly, the viscosity of the process materials would be reduced, thus leading to a faster throughput and greater exhaustibility of molasses. Finally, the harmful effect of dextran on sucrose crystal elongation would be reduced, thus leading to easier centrifugation and an improved product appearance. The raw sugar would also be of better refining quality. The overrall economic benefit is therefore an increase in sucrose recovery and more rapid rate of production. The treatment does not cause significant loss of sucrose by inversion, and the products of dextran hydrolysis are not thought to exert any harmful effects on the process.

It is stressed that these improvements are only predictions; the enzyme was not available in sufficient quantities to enable even pilot-scale trials to be carried out. Furthermore the test selected for measurement of dextran removal did not yield information on viscosity changes in the juice or the hydrolysis products. Facilities for determination of the degree of de-polymerisation or the identification of hydrolysis products were not available. The 'haze analysis' technique was chosen for assay of dextranase activity in the factory tests because it was rapid, simple, accurate and suitable for a routine quality control test. In these respects it was considered superior to assay procedures based on viscosity decrease or increase in reducing sugars.

Keniry, Lee & Mahoney (1969) showed that reductions in the molecular weight of dextran down to 100,000 did not influence the degree of 'haze' formed in 50% ethanol, but no 'haze' was formed with dextran of M.W. 10,000. Since natural Leuconostoc dextrans are normally of high M.W. (1,000,000 or higher) the test can measure a considerable degree of depolymerisation, but does not indicate the extent of this below a M.W. of 10,000. However, workers in other fields have shown that P. funiculosum dextranase rapidly reduces the viscosity of L. mesenteroides dextran solutions (Hultin & Nordstrom (1949); Kobayashi (1954); Charles & Farrell (1957)). Similar results were reported with dextranases of bacterial origin (Ingelman (1948); Sery & Hehre (1956)). The rapid breakdown of dextran into homologous low molecular-weight oligosaccharides was demonstrated by Jeanes et al (1953) and Bourne et al (1963). In view of this evidence it is most likely that the predicted improvement in the process would occur in practice if 70% of the 'dextran' were removed.

The economic losses caused by 'dextran' in the process are very difficult to assess (see Chapter VII); accurate assessment must await the results of future research. Application of dextranase would not, of course, replace sucrose lost by deterioration in the cane prior to grinding in the factory. An attempt has been made, however, to calculate the maximum cost of dextranase which would be compatible with various levels of economic loss due to dextran in the process. These calculations are given for a range of values of the price of the product raw sugar, because this figure varies continuously. It is governed by a complex set of factors which involve the world price of sugar, the Commonwealth Sugar Agreement, and quotas.

The rate of addition of dextranase in the commercial process is assumed to be 3 units/100 ml. mixed juice.

Therefore, 1 l. of mixed juice requires 30 units of enzyme

Now 1 ton of raw sugar is obtained from approximately 10 tons of cane, and 1 ton of cane yields about 2,000 lb. of mixed juice.

Therefore, 1 ton of sugar is obtained from 20,000 lbs. of mixed juice.

Since 1 lb. $\equiv$ 0.454 litres,

1 ton of sugar is obtained from approximately 9,000 litres of mixed juice

Therefore amount of enzyme required to treat 1 ton of raw sugar

$$= (9{,}000 \times 30) \text{ units}$$

$$= 270{,}000 \text{ units}$$

The maximum cost of enzyme (shillings per million units) for economic treatment of mixed juice, at different percentage losses due to dextran and at various prices of sugar, is shown in Table VIII,7. This figure excludes capital costs of plant and operating costs.

% Loss in sugar production due to dextran	Value of raw sugar per ton £	Value of losses-£ per ton sugar	Maximum economic cost of enzyme in shillings per million units
10	20	2.0	148
	30	3.0	222
	40	4.0	296
	50	5.0	370
5.0	20	1.0	74
	30	1.5	111
	40	2.0	148
	50	2.5	185
2.5	20	0.50	37.0
	30	0.75	55.5
	40	1.00	74.0
	50	1.25	92.5
1.0	20	0.2	14.8
	30	0.3	22.2
	40	0.4	29.6
	50	0.5	37.0

Table VIII,7. Maximum cost of dextranase for economic treatment of mixed juice, at different % losses due to dextran and various prices of sugar.

At present, the average value of raw sugar produced at Frome is £46-48 per ton. If the maximum loss in sugar production due to dextran is 10%, the maximum enzyme cost is 340-355 shillings per million units.

At least two manufacturers are known to sell dextranase in 1970; these are Dextran Products Ltd., Scarborough, Ontario, Canada and Globe Products Ltd., Accrington, Lancs. (UK agents for 'Novo' enzymes). Current prices quoted for industrial grade enzyme are:-

Dextran Products Ltd.,	-	500 shillings per million units
Globe Products Ltd.	-	236 " " " "

Therefore at current prices the commercial application of dextranase in the sugar industry is an economic proposition at a sugar loss of 10% but not at 5%. However, it seems probable that growing interest in the dental application of dextranase will reduce prices to a more economic level.

This research has demonstrated the technical and economic feasibility of removal of dextran in mixed juice by treatment with dextranase. This application has been registered in a British Patent Application, No. 55403/69 dated 12.11.69.

2. Physical Methods

a) Use of a molasses dip to reduce the water activity in cut ends of cane.

It was thought that brief immersion of the freshly-cut ends of cane stalks in a solution of low water activity (high osmotic pressure) might inhibit growth of *Leuconostoc mesenteroides*. Molasses was considered suitable for this purpose because its high sugar content is inhibitory to bacterial growth; it is readily available on sugar estates; it could be largely recovered as sugar in the process, thus material costs would be minimal; it is non-toxic and non-corrosive.

The effect of molasses on *Leuconostoc* was studied in two experiments at the Research Centre.

(i) 'In vitro' determination of minimum inhibitory concentration (M.I.C.)

Jamaican blackstrap molasses (80° Brix) was diluted in tapwater to a range of concentrations from 60° Brix to 1.5° Brix. The solutions were

sterilised in 10 g. aliquots in 1 oz 'universal' bottles in an autoclave for 15 minutes at 15 lbs/sq.in. Each bottle was inoculated with 0.1 ml. overnight MRS broth culture of L. mesenteroides strain L20 (isolated from sour cane), and incubated for 48 hours at 30°C. The solutions were then examined for growth of Leuconostoc by microscopic examination and by plate counts in STA medium. The inoculum level was also determined.
The M.I.C of molasses for L. mesenteroides was approximately 30° Bx.

The results are given in Table VIII,8.

Molasses °Brix	Growth observed microscopically	Leuconostoc count/ml (plate count)	Verdict - growth or death of organism
60	0	2.4×10^4	some death
54	0	8.8×10^4	some death
48	0	49	considerable death
42	0	14	considerable death
36	0	1.7×10^6	slight death
30	1	1.0×10^7	no significant change
24	2	6.0×10^8	slight growth
18	3	8.0×10^9	moderate growth
12	4	NT	good growth
6	4	NT	good growth
3	4	NT	good growth
1.5	4	NT	good growth

Table VIII,8. The effect of pure molasses solutions on growth of L. mesenteroides 'in vitro'.

Key to microscopic examination:- 0 = no growth

4 = good growth

NT = Not tested

Inoculum count per ml. molasses = 1×10^8/ml.

(ii) 'In vivo' test: A mature cane stalk was aseptically harvested from the greenhouse and cut into four 3-inch pieces. The billets were 'infected' by immersing one end in a 1:1,000 dilution of overnight culture of L. mesenteroides L20 for five minutes. Slight vacuum was applied to the distal end during infection, by means of a rubber tube attached to a water vacuum pump. This simulated the transpiration pull in a whole stalk and facilitated infection. Five minutes after 'infection', the infected ends of three billets were treated by immersion for five minutes in sterile molasses solutions of 60°, 50° and 40° Brix respectively. Vacuum was again applied during treatment. The fourth stalk was the untreated control. The stalks were incubated for 48 hours at 30^oC and 92% R.H., then removed and examined for Leuconostoc growth. Samples of pith, (ca. 1-2 g.), were aseptically removed at a distance of two inches from the infected end. The pith was weighed, blended in 100 ml. Ringers' solution in a Waring blender for one minute, and plated in STA agar.

The results showed that Leuconostoc counts exceeded 10^6/gm tissue in all stalks; there was no detectable difference between the treatment and the control.

It was concluded that immersion of the ends of infected cane stalks in high Brix molasses was unlikely to retard growth of Leuconostoc in the stalk. This may be due to failure of the molasses to penetrate the stalk, because of its high viscosity. The method was therefore abandoned.

b) Effect of alternating electromagnetic wave energy on L. mesenteroides.

A communication from Dr. H. Laser (University of Cambridge, Molteno Institute of Biology and Parasitology) reported that growth of L. mesenteroides was inhibited by exposure to alternating electromagnetic waves generated in an instrument of his own design. Attempts were made to verify this finding in this laboratory, using an instrument supplied by Dr Laser. Four strains of L. mesenteroides and two Lactobacillus sp., isolated from sour cane, were tested. A loopful of overnight broth culture was inooulated into fresh, sterile medium and distributed into two thin, flat, glass cuvettes. Both

cuvettes were incubated overnight at 30°C, one being exposed to the magnetic waves. A visual comparison of growth revealed that only one organism, a strain of L. mesenteroides, showed any inhibition. It was concluded that the method did not justify further study.

3. Chemical Methods: Bactericides

Treatment of cut ends of stalks with a bactericidal dip or spray was considered to be a possible method of inhibition of Leuconostoc growth inside freshly harvested cane. The essential requirements of a suitable bactericide are that it is inhibitory to L. mesenteroides; can be absorbed by and can penetrate the stalk; retains its activity in the presence of organic matter, especially cane juice and plant tissue; active at pH 5.0-5.5; low mammalian toxicity; colourless and odourless at use concentrations; low cost; easily applied, and removable in the factory process to give non-significant concentrations in the products.

A wide range of bactericides representing the main chemical types was screened for activity against L. mesenteroides strain L20 by 'in vitro' and 'in vivo' techniques in the laboratory. These are listed in Table VIII,9.

a) 'In vitro' tests

(i) M.I.C. tests in S.C.J. medium: The approximate minimum inhibitory concentration (M.I.C.) of each bactericide was determined. Ten serial doubling dilutions of bactericide over a wide range of concentration were prepared in 5 ml. volumes of sterile synthetic cane juice (S.C.J.) medium. The dilutions were inoculated with o.1 ml. overnight Y.G.C. broth culture of organism and incubated at 30°C. Growth was recorded turbidimetrically after 24 and 48 hours incubation. The lowest concentration which inhibited growth was the approximate M.I.C.

One ml. aliquots of broths which failed to show growth in the M.I.C. test were added to fresh, sterile S.C.J. broth, after neutralisation of inhibitory carry-over of bactericide. The broths were examined for growth after 72 hours incubation at 30°C; the lowest original concentration which failed to show growth was the approximate minimum bactericidal concentration (M.B.C.).

Type	No.	Bactericide	Manufacturer
A. Bis-phenols and chlorinated phenols	1	By-Prox BD55	B.P.Chemicals Ltd
	2	Hexachlorophene(G11)	Givaudin Ltd
B. Parahydroxybenzoic a acid esters	3	Nipasol M	Nipa Laboratories Ltd
	4	Nipabutyl sodium	" " "
	5	Nipacombin A	" " "
	6	Nipacombin SK	" " "
C. Aldehydes	7	Formaldehyde(Formalin)	May and Baker Ltd
D. Quaternary ammonium compounds	8	Cetrimide B.P.	Glovers Chemicals Ltd
	9	Gloquat C	" " "
	10	Gloquat SD	" " "
	11	Deciquam 222	British Hydrological Corpn
	12	Vantoc CL	I.C.I.
E. Halogens	13	Sodium hypochlorite	B.D.H.
	14	Diversol BX	Diversey-Deosan Corpn
	15	Chloramine T	B.D.H.
F. Ampholytes	16	Amphionic G	Glovers Chemicals Ltd
	17	Tego 51	Hough,Hoseason & Co. Ltd
	18	Tego 51B	" " " "
G. Amidines	19	Chlorhexidine acetate "Hibitane"	I.C.I.
H. Quinolines & isoquinolines	20	Dequadin chloride	Allen & Hanbury Ltd
I. Organic Mercurials	21	Phenylmercuric nitrate	B.D.H.
J. Organic sulphur compounds	22	Busan 881	Buckman Laboratories Inc
K. Food preservatives	23	Sodium nitrite	B.D.H.
L. Miscellaneous	24	Vantocil I.B.	I.C.I.

Table VIII,9. Bactericides screened for chemical control of sour cane

(Some bactericides were excluded from the screen because of unacceptable toxicity, colour, cost or on ethical grounds. These included alcohols; phenols and cresols; dyes; iodophors; heavy metals and antibiotics.)

The accurate M.I.C. of the most active bactericides was determined by an M.I.C. test similar to that described above except that arithmetic dilutions over a narrow concentration range were used.

The accurate M.I.C. of formaldehyde was determined for 12 strains of L. mesenteroides.

(ii) M.I.C. tests in blended cane tissue: Bactericides which had shown reasonable activity in the M.I.C. tests in S.C.J. medium were selected for further tests to determine the effect of cane tissue on M.I.C. The limited supply of cane stalks precluded the use of 'in vivo' M.I.C. tests. Instead, attempts were made to simulate 'in vivo' conditions by 'in vitro' experiments.

Tests in universal bottles: Several cane stalks were chopped, sliced and treated in a Waring blender to give finely divided 'blended' tissue. This was mixed and distributed in 5 g. aliquots in 1 oz. universal bottles. 5 ml. SCJ medium, pH 5.0, with brom-cresol green indicator, was added to each bottle and these were sterilised in an autoclave for 10 mins. at 10 p.s.i. 5 ml. bactericide was added to give the desired final concentration. The bottles were inoculated with 0.2 ml. of 1:1,000 dilution of overnight broth culture of organism, incubated for 48 hours at 30°C, and examined for growth as indicated by acid production. Each bactericide was tested at three concentrations with two replicates.

Tests in 'artificial cane stalks': A more refined technique was developed in which 'artificial cane stalks' of standard composition were prepared. 4 g. blended cane tissue was packed into 4" x 5/8" glass tubes, whose distal ends were fused to short lengths of narrow glass tubing. 5 ml. S.C.J. medium and indicator, pH 5.0, was added to each tube and allowed to drain out through the narrow tube. Both ends were then plugged with cotton wool and the 'stalks' were sterilised in an autoclave for 15 mins. at 15 p.s.i. 'Infection' was simulated by the addition of 2.5 ml. of 1:1,000 dilution of overnight broth culture of organism whilst vacuum was applied to the distal ends through attachment to a water vacuum pump. When the culture liquid had been sucked through, 2.5 ml. of bactericide at the desired concentration was similarly applied. The tubes were then re-plugged, incubated at 30°C for two days

No.	Germicide	S.C.J. Medium Approx. M.I.C.	S.C.J. Medium Accurate M.I.C.	S.C.J. Medium Approx. M.B.C.	Blended Cane Universal Bottles Approx. M.I.C.	Blended Cane Artificial stalks Approx. M.I.C.	Blended Cane Artificial stalks Accurate M.I.C.
1.	By-Prox BD55	2	NT	>250	125	>125	NT
2	G11	4	NT	62.5	125	250	150
3	Nipasol M	500	NT	1000	NT	NT	NT
4	Nipabutyl sodium	125	165	1000	1000	> 750	NT
5	Nipacombin A	1000	NT	2000	NT	NT	NT
6	Nipacombin SK	250	NT	500	NT	NT	NT
7	Formaldehyde	62.5	80	2000	<125	<125	<75
8	Cetrimide B.P.	31	NT	250	NT	NT	NT
9	Gloquat C	2	NT	> 250	250	>500	NT
10	Gloquat SD	10	NT	NT	NT	NT	> 800
11	Deciquam 222	2	NT	> 250	NT	NT	NT
12	Vantoc CL	31	NT	> 250	NT	NT	NT
13	NaOCl	>1000	NT	>1000	NT	NT	NT
14	Diversol BX	500	NT	> 500	NT	NT	NT
15	Chloramine T	1000	NT	>1000	NT	NT	NT
16	Amphionic G	250	NT	> 250	>500	NT	NT
17	Tego 51	1250	NT	>2500	NT	NT	NT
18	Tego 51B	62.5	50	>2500	250	NT	NT
19	Hibitane	31	NT	>250	> 250	NT	NT
20	Dequadin chloride	62.5	50	>250	>250	NT	NT
21	Phenylmercuric nitrate	0.5	NT	NT	< 62.5	<16	NT
22	Busan 881	>1000	NT	>1000	> 500	>1000	NT
23	Sodium nitrite	500	450	>2000	750	< 500	>700
24	Vantocil 1B	250	150	>250	>500	NT	NT

Table VIII.10 Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of bactericides for *L. mesenteroides* strain L20 estimated by different '*in vitro*' techniques. Bactericide concentrations are expressed as P.P.M. active constituent.

NT = Not tested

and examined for growth as indicated by acid production (indicator colour change from blue-green to yellow). The bactericides were tested at three concentrations with two replicates under standard conditions.

The results of all the M.I.C. tests are summarised in Table VIII,10.

The results of one experiment using 'artificial cane stalks' are shown in Figure VIII,4.

Several compounds were obviously inactivated by organic matter and were ineffective in both SCJ medium and blended cane; these included the three
except Tego 51B, Busan 881, & the parahydroxbenzoic acid esters,
halogen compounds; the ampholytic compounds,/except Nipabutyl sodium. Others were very effective in SCJ but lost most of their activity in blended cane, e.g. the Q.A.C.'s, Hibitane; Dequadin chloride, and Vantocil IB. The phenol derivatives retained some activity in blended cane. Sodium nitrite and Tego 51B were moderately effective, but the best bacteriostats were formaldehyde and phenylmercuric nitrate.

The result of an accurate determination of the M.I.C. of formaldehyde in S.C.J. medium for 12 strains of <u>L. mesenteroides</u> was:-

No. of strains	M.I.C. :- P.P.M. Formaldehyde
3	60 - 70
8	70 - 80
1	90 - 100

b) Simulated '*in vivo*' tests

Attempts were made to simulate field conditions using cane stalks grown in a greenhouse. Unfortunately, only a few stalks were available, so the scope of the test was limited.

The stalks were harvested aseptically, cut into 6-inch billets and infected by immersing one end for 5 minutes in a 1:1,000 dilution of overnight broth culture of <u>L. mesenteroides</u>. One minute after removal the same end was immersed for 5 minutes in bactericide at the desired concentration. The stalks were then removed, the ends wrapped in sterile foil, and incubated at $30^{o}C$ and 92% R.H. for 48 hours. 1g. samples of pith were removed aseptically from the stalk centre, 4 inches from the treated end; the pith was weighed

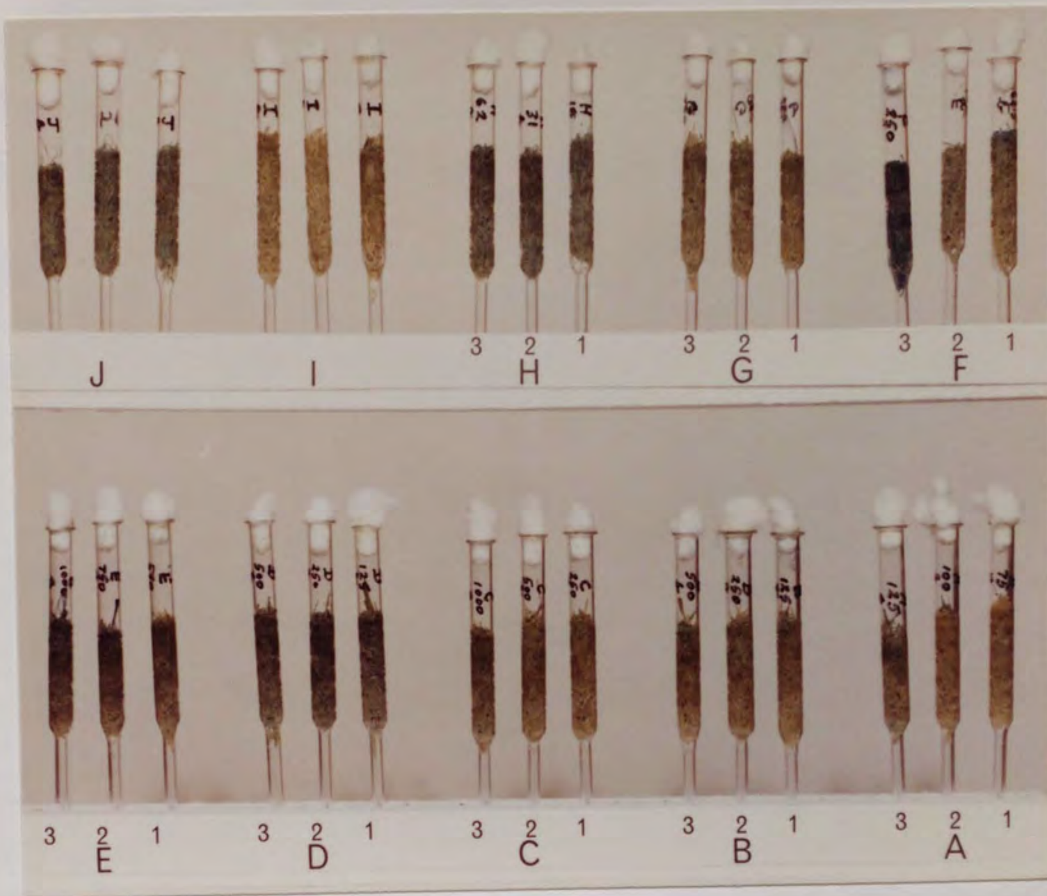

FIG. VIII,4.

Determination of approximate M.I.C. of six bactericides for
L. mesenteroides, strain L20 in 'artificial cane stalks'.
'Stalks' incubated 48 hours at 30°C.

Key   Blue-green = no growth   Yellow = growth and acid production

Key to bactericides		Bactericide concn. as PPM active ingredient		
Code	Bactericide	Tube 1	Tube 2	Tube 3
A	By-Prox BD55	75	100	125
B	Gloquat C	125	250	500
C	Busan 881	250	500	1,000
D	Formaldehyde	125	250	500
E	Sodium nitrite	500	750	1,000
F	Hexachloraphene	62.5	125	250
G	Nipabutyl Na	250	500	750
H	Phenylmercuric nitrate	16	31	62.5
I	Positive Control - Inoculated, bactericide absent			
J	Negative Control - Sterile, bactericide absent			

into Ringer solution, blended, and plated out on Rogosa agar. *Leuconostoc* colonies were counted after three days incubation of the plates at 30°C; counts were expressed per gm. cane tissue, and compared with controls not treated with bactericide.

Four experiments were carried out, using selected bactericides at several concentrations. Valid results were only obtained from the first experiment, shown in Table VIII,11.

Bactericide	Conç, of active ingredient in PPM	*Leuconostoc* count per g. tissue	% Survival
Untreated Control	-	2.0×10^5	-
By-Prox BD 55	20	4.7×10^4	23.2
Cetrimide	40	$> 2 \times 10^5$	100
Gloquat C	20	$> 2 \times 10^5$	100
Deciquam 222	20	$> 2 \times 10^5$	100
Vantoc CL	80	$> 2 \times 10^5$	100
NaOCl	2,000	1.3×10^5	66.6
Diversol BX	1,000	1.1×10^5	55.5
Busan 881	2,000	4.2×10^3	2.1
Formaldehyde	125	3.6×10^3	1.8
Sodium nitrite($NaNO_2$)	1,000	5.5×10^3	0.3

Table VIII,11. The effect of bactericidal dips on the count of *L. mesenteroides* inside artificially-infected cane stalks.

By-Prox, Busan 881, Formaldehyde and $NaNO_2$ were effective in retarding growth of the organism in cane tissue. However, in the second experiment, higher concentrations of these bactericides failed to inhibit Leuconostoc. In the third test, results were invalidated because the untreated control remained sterile, whilst in a fourth experiment, the lowest control count was below the counts recorded for test bactericides. It was concluded that the cane was too variable in size and quality to enable accurate comparisons to be made; furthermore, the degree of infection in the controls was variable and uncertain. Insufficient material was available to ensure adequate replication or improvement of the test method.

However, supplies of sweet sorghum(variety Sart), a grass closely related to sugarcane, were more plentiful in the greenhouse. An experiment similar to that described above was carried out with whole stalks of 15 week old sorghum. The principal modification was that vacuum from a water pump was applied to the distal end of the stalk during 'infection'. The most promising compound, formaldehyde, was tested at three concentrations, with three replicates. The results are shown in Table VIII,12.

Formaldehyde Conc. PPM	Replicate No.	Leuconostoc count per g. tissue
None: untreated control	1	9.0×10^4
	2	6.5×10^3
	3	5.0×10^2
80	1	2.0×10^4
	2	ND
	3	ND
125	1	ND
	2	1.5×10^2
	3	ND
250	1	1.6×10^4
	2	8.0×10^2
	3	ND

Table VIII,12. The effect of a formaldehyde dip on the Leuconostoc count inside artificially-infected sweet sorghum stalks.

ND = Not detected; less than 100 per g. tissue

The results were again variable, but less so than in the experiments on cane. Formaldehyde reduced the *Leuconostoc* count significantly in seven samples, but it was ineffective in two samples out of nine tested. 80 PPM appeared to be as effective as 250 PPM formaldehyde.

Estimates of the relative 'use costs' of 14 of the most effective bactericides were calculated in Table VIII,13. These were based on cost of product in bulk quantities (1 cwt. or more), combined with the probable use concentration, deduced from the results of the screening tests. The five most economical bactericides, in order of increasing 'use cost' were formaldehyde, Gloquat C, hexachlorophene (G11), Busan 881, and phenylmercuric nitrate (PMN). Of these Busan 881 was excluded because it is dangerous to handle, and PMN because mercury compounds are undesirable in foodstuffs. Formaldehyde was selected as the most promising bactericide for a field trial in Jamaica, because it was most economic and showed good activity in both '*in vitro*' and '*in vivo*' screening tests. No major disadvantages were envisaged in its use.

c) *Field trial with formaldehyde, Frome Estate, 1969*.

60 adjacent stalks of mature, whole, undamaged cane, burnt the previous day, were harvested aseptically from the same row in a cane field. The cutting area of each stalk and machete were 'sterilised' prior to harvest with alcohol swabs, and the cut ends were immediately wrapped in sterile paper. The stalks were mixed and divided into three samples, each consisting of five bundles of four stalks.

The three samples were treated as follows:-

'*Sterile controls*'	:	No treatment
'*Inoculated*'	:	The cut ends were dipped for 5 seconds into a pure culture of *L. mesenteroides* containing approximately 10^8 viable cells/ml.
'*Formaldehyde*'	:	After inoculation as above, the cut ends were dipped for 5 seconds into a freshly-prepared 200 PPM formaldehyde solution.

Bactericide	Product cost shillings/Kg	% Active ingredient	Active ingredient cost shillings/Kg	Probable use conc PPM	*Relative use cost in shillings
By-Prox BD55	2.3	2.2	105.5	125	13,190
Gloquat C	4.4	50.0	8.8	250	2,188
Busan 881	11.0	100.0	11.0	500	5,500
Formaldehyde	1.2	40.0	3.0	125	375
Sodium nitrite	15.0	100.0	15.0	750	11,250
Hexachlorophene	40.0	100.0	40.0	125	5,000
Vantocil IB	11.0	20.0	55.0	750	41,250
Dequadin chloride	580.0	100.0	580.0	500	290,000
Tego 51B	24.3	25.0	97.0	500	48,500
Nipabutyl sodium	49.5	100.0	49.5	750	37,175
Phenylmercuric nitrate	510.0	100.0	510.0	16	8,150
Hibitane acetate	400.0	100.0	400.0	500	200,000
Amphionic G	21.5	30.0	72.0	750	54,000
Diversol BX	6.2	3.3	186.8	1000	187,000

Table VIII,13. Estimate of relative use cost of bactericides for control of sour cane.

*Relative 'use cost' in shillings = probable use concn. x cost of active ingredient.

After treatment the paper was replaced on the cut ends and the bundles were laid on grass, in the open. Initially, and on successive days up to four days storage time, a bundle of each treatment was crushed in a 'sterile' 2-roller laboratory mill. The *Leuconostoc* count of each juice was determined by plate counts on STATA medium.

The results are shown in Table VIII,14.

Despite careful aseptic precautions, gross contamination with *Leuconostoc* occurred in the 'sterile' controls, which were infected to the same degree as the 'inoculated' samples. Formaldehyde treatment did not significantly reduce the *Leuconostoc* count of the test stalks. Cross infection of controls

was a problem also experienced by Egan & Rehbein (1963).

Treatment	*Leuconostoc* count per ml. juice at stated storage time in days				
	0	1	2	3	4
'Sterile' control	6.4	190	84	120	56
Inoculated	8.8	82	130	69	54
Inoculated + 200 PPM formaldehyde	6.6	160	62	83	43

Table VIII.14. The effect of a formaldehyde dip on the *Leuconostoc* count of juice from artificially-inoculated, stored cane.

Counts are expressed as count per ml x 10^6

Further tests were abondoned as a result of personal observations on the practice of manual cane-cutting. It was apparent that the application of a bactericidal dip to the freshly cut ends of harvested cane would be extremely impractical in terms of time and labour, even if an effective bactericide were available. Spray application would be quicker, but it would be very inefficient due to the protection of the inner stalks in a pile by the surface stalks.

It was concluded that the control of sour cane with bactericides is not a suitable method.

4. Modification of harvest, storage & transport practices.

Studies on 'bill-to-mill' times were reported in Chapter VII.

It is apparent from the results of experiments on the control of sour cane by biological, physical and chemical methods that none of these approaches are satisfactory. Therefore, the only practical solution is to mill the cane before post-harvest biodeterioration becomes significant, i.e. within 24-[illegible] hours of harvest.

5. <u>Penalty systems : Quality control tests</u>

The changes in various parameters that occur during storage of burnt, manually-cut cane in Jamaica were described in Chapter III. None of these parameters were considered to be completely satisfactory for use as a routine quality control test for assessment of the age of cane post-harvest, its degree of deterioration, or its processing quality.

Despite some disadvantages which have been previously discussed, the most promising test appeared to be the determination of 'dextran' content by the method of Keniry et al (1967a; 1969). This test is more sensitive than viscosity determination, and several determinations can be carried out in a shorter time (about 30 minutes). It was concluded that at the present time the determination of dextran in cane juice by 'haze analysis' is the most satisfactory indicator of sour cane for quality control purposes.

a) <u>A rapid method for estimation of 'dextran' content of cane juice in the field</u>.

A need exists for a test which can estimate harvested cane quality in the field or at some stage prior to milling. This could prevent the unnecessary transport to the factory of cane below an acceptable processing quality. The requirements of such a test are similar to those of a quality control test in the factory; in addition, however, it must be very rapid, and simple enough to be performed by a semi-skilled operator without sophisticated equipment.

The following test was developed, based on an adaptation of dextran determination by 'haze analysis'.

Several stalks of 'suspect' cane are crushed in a portable 2-roller, hand-operated mill; this can be bolted to the back of a Land-Rover. The juice is collected, and after mixing, 10 ml. is pipetted into a beaker with 2 ml. of 10% trichloracetic acid and a little Filter-Aid. This suspension is placed in the barrel of a 20 ml. plastic syringe, attached to a 'Swinnex-25' filter unit (Millipore U.K. Ltd.); it is then filtered through Whatman No.5 filter paper by hand pressure. 5 ml. of clear, filtered juice is

mixed with 5 ml. ethanol, and after two minutes the opacity or 'haze' is estimated by comparison with a set of Brown's Standard Opacity Tubes (Burroughs Wellcome Ltd.). The Opacity Tubes are calibrated against 20° Brix sucrose solutions containing known concentrations of dextran. The calibration and interpretation of results is shown in Table VIII,15.

Opacity Tube No.	Dextran content % w/v	Dextran % Brix at 16° Brix	Remarks and Action
1	.030	.188	Accept
2	.060	.375	Accept
3	.080	.500	Accept
4	.095	.593	Warning
5	.110	.690	Severe warning
6	.125	.780	) Danger level. Confirm by lab.
7	.140	.875	) test. Reject if dextran exceeds 0.8% Brix
8	.160	1.000	Reject
9	.180	1.125	Reject
10	.200	1.250	Reject

<u>Table VIII,15.</u> Calibration of Brown's Opacity Tubes for a rapid field test for dextran content of cane juice.

The mean dextran content of mixed juice at Frome during April-July 1969 was 0.41% Brix. Significant effects of dextran on processing begin to occur at 0.5% Brix, and would be severe at 0.8% Brix. Application of this test would have resulted in rejection of about 14% of the cane tested in the factory in this period, and 5% of cane during March-April 1970.

b) <u>Evaluation of the Methylene Blue Reduction Test for estimation of microbial count of cane juice</u> (Tate & Lyle Research Centre)

In initial experiments the Standard Methylene Blue Reduction Test, as used for estimating the microbial quality of pasteurised milk, was studied.

10 ml. aliquots of test cane juice were added to each of three 6" x 5/8" test tubes; two tubes were immersed in boiling water for three minutes to kill the organisms present. 1 ml. of sterile aqueous methylene blue

solution was added to one boiled tube (= 'blue' control) and the unboiled tube (= 'test') to give a final dye concentration of 3.3 PPM. The remaining tube was the 'white' control. The tubes were fitted with sterile rubber bungs, mixed by inversion, and incubated at 37°C and 30°C in a water bath. They were examined at 30 minute intervals for dye reduction. The juices were either cane juices extracted from frozen Jamaican cane which had been thawed and stored for 48 hours at 30°C, or SCJ medium inoculated with L. mesenteroides (strain L20) overnight broth culture to give counts from 10^{10} to 10^{12} per ml.

The results showed that natural cane juices with a Leuconostoc count of 10^{10}/ml did not reduce the dye within 3½ hours; pure cultures in SCJ medium reduced the dye slightly at 3 hours when the inoculum was 10^{12}/ml, but at 10^{10}/ml., 5 hours were required.

It was concluded that the standard test is unsuitable for routine use as an estimate of the microbial count of cane juice in the factory, because the incubation period is too long, even when viable counts are very high.

A modified test was devised to accelerate the time required for dye reduction. The organisms in 10 ml. juice were concentrated by centrifugation and re-suspended in a small volume of Ringer solution (1.3 ml). The methylene blue test was repeated on the concentrated juice, but capillary tubes were used in place of test tubes. The results showed that complete reduction of the dye occurred within 30 minutes at an original inoculum of 10^{11} organisms per ml. cane juice; at 10^{9}/ml. 1½ hours was required. This micro-method appeared promising as a routine control test, but it could not be evaluated at Frome because a centrifuge was not available.

C. CONCLUSIONS

1. Biological Methods

The dextran content of mill juice was reduced to a level unlikely to interfere with sugar manufacture by enzymatic treatment with dextranase from P. funiculosum. The optimum conditions for economic application were established in laboratory-scale trials. Addition of 3 units dextranase to

100 ml. mixed juice at its natural pH and a temperature of 40°C reduced the dextran content by an average of 68.5% in 20 minutes reaction time. The suggested treatment did not result in detectable loss of sucrose by reducing sugar formation.

The process appeared to be technically feasible and economic at a 10% loss of sugar production due to dextran, but not at 5%.

A British Patent Application No. 55403/69 (12th November 1969) has been filed for the process.

Results obtained with several amylase enzymes indicated the presence of polysaccharides other than L. mesenteroides dextran in some cane juices. Further research on this topic is recommended.

2. Physical Methods

The minimum inhibitory concentration (M.I.C.) of blackstrap molasses for a strain of L. mesenteroides was 30° Brix, but application of molasses to the cut ends of artificially-infected cane stalks did not inhibit growth of Leuconostoc.

Alternating electromagnetic wave energy was found to have little inhibitory effect on the growth of L. mesenteroides 'in vitro'.

3. Chemical Methods

24 bactericides and bacteriostats of different chemical type were screened for activity against L. mesenteroides by 'in vitro' and simulated 'in vivo' techniques. The most effective bactericides in the presence of blended cane tissue were formaldehyde and phenylmercuric nitrate, but tests on artificially-infected cane stalks did not give reproduceable results.

Estimates of the relative cost of effective bactericides at their probable use concentrations showed that formaldehyde was the most economic.

The M.I.C. of formaldehyde in a synthetic cane juice medium was 80 PPM for 11/12 strains of L. mesenteroides.

In a field trial with artificially-infected cane at Frome, formaldehyde at 200 PPM did not inhibit Leuconostoc growth when applied as a dip to the ends of freshly-harvested stalks.

Bactericidal dips or sprays were not considered to be a practical solution to the control of sour cane.

4. Modification of harvest, storage & transport practices

It was concluded that the only effective control method for sour cane is to mill the cane before the onset of significant post-harvest biodeterioration (within ~~48~~ 24-36 hours of harvest for whole-stalk, manually-cut cane). The use of regular 'bill-to-mill' time studies was recommended to provide information leading to improvements in the logistics of cane harvest, storage and transport.

5. Penalty Systems and Quality Control

Despite some disadvantages the determination of 'dextran' content by the 'haze analysis' method of Keniry et al (1967a, 1970) was the most suitable test for routine estimation of sour cane and cane quality in the factory. A simple, rapid modification of this test was developed for application in the field.

The standard methylene blue reduction test was not suitable for routine estimation of the microbial quality of cane juice in the factory, but a 'micro-method' modification appeared to warrant further evaluation.

The adoption of a penalty system, based on dextran analysis, was recommended in order to provide an incentive to growers to supply fresh cane to the factory.

CHAPTER IX GENERAL DISCUSSION AND CONCLUSIONS

The results of storage experiments on harvested cane, both in the U.K. and Jamaica, show that it is invariably infected with Leuconostoc mesenteroides. Although the rate of growth and maximum count of this organism in harvested cane shows some variation, in the majority of cases it reaches a maximum count of between 10^7 and 10^8 organisms per ml. of expressed juice within 3 - 4 days of harvest. This population is capable of producing significant changes in juice composition. Other microorganisms are present in juice from freshly-harvested cane, but in general they do not appear to increase in numbers to a level capable of causing significant changes in juice composition. The exceptions are members of the genus Lactobacillus, including a new dextran-forming species, which sometimes predominate in juice from cane stored for periods in excess of 5 - 7 days.

In vitro screening tests in a synthetic cane juice showed that L.mesenteroides is potentially the most deteriogenic organism, but attempts to prove that this organism was the etiological cause of sour cane were inconclusive. However, this was largely due to difficulties in experimental technique, since it proved impossible to compare the deterioration rate of artificially inoculated cane with sterile controls. In the absence of such data; it is not possible to distinguish between changes due to staling of non-infected cane, and changes due to biodeterioration. Some effects, however, are almost certainly caused by growth of L.mesenteroides in cane, notably the observed increase in dextran content and juice viscosity during storage of harvested cane, and especially in wet weather. Dextran content appears to be a useful indicator of degree of biodeterioration and juice processing quality, but a more specific test than 'haze analysis' is needed for its accurate determination. Some evidence suggests that polysaccharides other than Leuconostoc dextran, probably of non-microbial origin, may be important in the staling of cane and effect on factory processing quality;

further research is needed to define the nature, origin and effects of polysaccharides in harvested cane.

The economic effects of post-harvest deterioration of cane may be severe. Losses ofrecoverable sugar were nearly 10% in the Frome 1969 crop, but again, no distinction could be made between losses due to staling and souring. The effects of deteriorated cane on the factory process are manifold, but exceedingly difficult to quantitate, owing to the continuous and uncontrollable nature of sugar manufacture. However, the qualitative effects of dextran, presumably formed by growth of L.mesenteroides in harvested cane, were readily observed at Frome, in the form of increased syrup viscosity, increased final molasses purity and crystal elongation of massecuites.

An ecological study of L.mesenteroides in relation to cane fields revealed that it is present in low numbers in the soil and the epiphytic flora of the cane plant. The most likely vector of transmission of the organism to the cut ends of cane is the cane cutter's machete, especially in moist climatic conditions. Evidence was obtained which confirmed the observations of Egan and his colleagues in Queensland, that mechanically-harvested, chopped-up cane deteriorates at a much greater rate than whole-stalk, manually-cut cane. Here the deterioration was more readily ascribable to massive infection of cane with L.mesenteroides than to staling. The high probability of a rapid change from manual to machine harvesting in Jamaica in the near future suggests that L.mesenteroides infection may become an even more serious problem.

Rainfall appears to be a significant factor in the post-harvest deterioration of cane in Jamaica. High rainfall is associated with a definite sour cane problem, evidenced by factory processing difficulties and more rapid deterioration of cane quality. Variety B4362 is more susceptible to post-harvest deterioration than B51129. Overmaturity of cane also enhances its deterioration rate, but this is probably an effect on staling rather than souring.

A thorough examination of possible methods of control for sour cane showed that it is not practical to prevent infection of cane at harvest with L.mesenteroides, nor to prevent its subsequent multiplication within the stalk, by means of chemical treatment or modification of the physical environment. The only practical solution is to mill the cane within 24 hours of harvest to minimise economic losses; this result confirms the conclusions reached by Queensland workers. However, a promising new approach was developed for removal of dextran in the mill juice prior to clarification, by treatment with the enzyme dextranase. This appears to be technically and economically feasible in the near future, and is the subject of a British Patent and further research.

It is concluded that under Jamaican conditions Leuconostoc mesenteroides plays a major role in post-harvest biodeterioration of sugar cane, and significantly increases the economic losses due to staling alone.

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FIG.X,1.

External appearance of the mature
sugarcane plant

(approx. 1/10 actual size)

parenchyma tissue of mature internodes.

The internal structure of the stem is shown in Fig.X,2. The outer part or rind contains the epidermis and layers of thick-walled lignified cells. Fibrovascular bundles are scattered throughout the ground tissue or parenchyma, being more numerous toward the periphery, but the larger bundles occur toward the centre. Each bundle contains sclerenchyma, xylem and phloem tissue. The parenchyma cells are circular in cross-section and rectangular in longitudinal section; they are large, soft and thin-walled, and contain most of the sucrose-rich juice which is extracted by crushing for the production of sugar.

2. <u>Cultivation</u>

In Jamaica sugar cane is propagated by planting short pieces of stalk, containing 2 or 3 buds, at intervals along the ridges of 'Louisiana banks', whose inter-furrow distance is 5' 6". A typical field is about 10 acres in area and is divided into "quarters" and "laterals" by drainage and irrigation ditches. Each bud may germinate to produce a shoot. After germination, each shoot becomes independent of the 'seed-piece' and may form an individual plant which tillers to become a multi-stalked 'stool'.

The cane is grown until it becomes mature or 'ripe', when its sucrose content reaches a maximum (determined by analysis of sample stalks). In Jamaica this growth period is about 12 months. During growth suitable fertilisers, herbicides and insecticides are applied when required; irrigation is also practised in dry areas. The ripening of cane depends on many factors, chief of which at Frome is the amount and distribution of rainfall. Maturity is normally reached in dry weather, but it may be controlled artificially by irrigation. The average rainfall distribution at Frome over the 10-year period 1956-1966 is given below :-

	Jan.	Feb.	March	April	May	June
Monthly rainfall	2.04	2.75	3.74	6.83	11.55	10.48
No.of rainy days	1	1	2	6	8	10

	July	Aug.	Sept.	Oct.	Nov.	Dec.
Monthly rainfall	10.37	10.11	8.46	10.28	4.52	3.67
No.of rainy days	10	7	9	5	3	2

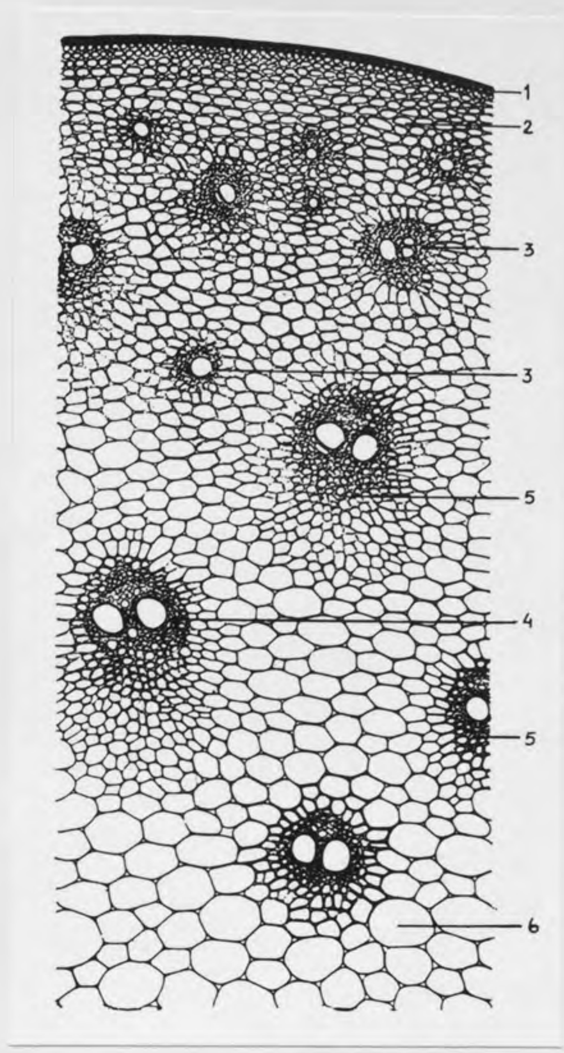


FIG. X.2.

Diagrammatic transverse section through outer part of a cane stem (x 45)
Reproduced from Martin, Abbot and Hughes (1961)

Key
1. Epidermis
2. Thick-walled cells of the rind
3.) Vascular bundles of different sizes
4.)
5. Sclerenchyma
6. Parenchyma (ground tissue or storage cells)

'Rainy days' are defined as days receiving more than 0.5" rain per 24 hours.

The normal harvest season or 'crop' at Frome extends from early December to middle or late May, but occasionally it may be extended in the June rainy season. Planting and harvesting operations are planned so that the proportion of cane reaching maturity is phased equally throughout the crop.

Re-growth from the stubble after harvest becomes the subsequent crop, known as a ratoon crop. Each ratoon crop yields successively less sugar per acre, and at Frome becomes uneconomic to process after six or seven ratoons. Therefore fields are re-planted at intervals of six or seven years.

B. HARVEST AND FIELD OPERATIONS

In 1969 approximately 900,000 tons of cane were ground at Frome factory. Approximately half of this cane was grown by the Estate whilst the rest was supplied by more than one thousand private farmers. Frome Estate is subdivided into ten farms whose total acreage of cane is 14,106 acres.

1. Burning

All Estate cane and about 50% of farmers' cane is burnt prior to harvest, in order to facilitate cutting and reduce the amount of green leaves ('trash') transported to the factory. Selected fields of mature cane are normally burnt in the evening of the day before harvest. In dry conditions the fire spreads rapidly through a field leaving the exterior of the stalks bare and blackened, except for a few green leaves at the top. Figures X,3,4 and 5 show the appearance of cane immediately before, during and after the burn respectively.

2. Cutting

Cane cutting begins at 7 am on the day following the burn. An average size field is normally reaped in one day, but larger fields may take two days. At present all cane in Jamaica is manually cut by means of machetes (Fig.X,6). The cane cutters work in pairs and each pair cuts four adjacent rows. The stalks are cut at the nearest internode above the ground, and the green tops of the stalks are removed just above the highest coloured internode. The whole stalks, 8-12 feet in length, are laid horizontally in small piles across the peaks of the middle two rows,

FIG.X,3.

Field of mature, green cane prior to burning (approx. 1/70 natural size)

FIG.X,4.

Above field during the burn

FIG.X,5.

Cane from above field after the burn

behind the cutters (Fig.X,7). One man may cut 5 to 7 tons of cane per day, and is paid according to the tonnage reaped. In many countries mechanised harvesters have wholly or partially replaced manual cutters, and in Jamaica this trend seems likely to be followed in the near future. During the 1970 crop a Massey-Ferguson 'Cane Commander' (MF201) mechanical harvester underwent trials at Frome Estate; it was the first such machine permitted in Jamaica by the Government. This machine automatically tops the cane and chops the stalks into short billets of average length 10.5 inches; the billets are loaded directly into carts drawn behind or alongside the harvester, (Fig.X,8). Under Frome conditions this machine achieved a harvesting rate of 25 tons of cane per hour, although its maximum rate is double this, and 30-35 tons/hour is estimated to be a good commercial rate. The mean percentage of extraneous matter (tops, roots, trash and soil) included in the cane was 7%

3. Loading, transportation and storage.

Harvested estate cane usually remains on the ground in the field until the following morning, when it is loaded into tractor-drawn transport carts. About 50% of the cane is manually loaded into carts of 4-ton capacity, (Fig.X,9), whilst half is mechanically loaded by means of Toft grab-loaders into carts of 8-ton capacity, (Fig.X,10). The carts are either taken directly to the factory by road, or are taken to central hoists where the cane is off-loaded and weighed into rail-cars; these are transported by diesel locomotive to the factory. Farmers' cane is usually loaded into lorries and taken to the factory by road. Much of the farmers' cane comes from outlying districts which may be up to 20 miles distance from the factory.

At the factory the cane is unloaded and stored in large piles of several hundred tons capacity for periods which do not normally exceed 6 hours. When required the cane is then weighed and fed into the mill.

In 1969 the average yields for Estate cane were :- tons cane per acre - 34.30; tons cane per ton sugar - 10.52; tons sugar per acre - 3.26.

12 inches

FIG.X,6. Cane cutter's machete

FIG.X,7.

Manual harvesting of cane

FIG.X,8.

Massey-Ferguson MF201 mechanical harvester in operation

FIG.X,9.

Manual loading of harvested cane

FIG.X,10.

Mechanical loading of harvested cane

C. RAW SUGAR MANUFACTURE : FACTORY OPERATIONS

The aim of the factory is to manufacture relatively pure, crystalline sucrose (raw sugar) from the juice of sugar cane. The composition of sugar cane and its juice is shown in Chapter I, Table I,1. The cane stalk consists of 11-16% insoluble fibre, whilst the remainder is juice, which is an aqueous solution containing 10-16% soluble solids. Sucrose comprises 70-88% of these soluble solids.

The stages in manufacture of raw sugar are shown diagrammatically in Fig,X.11.

1. Extraction

The weighed cane is cut into small pieces by passage through a set of revolving blades, and fed into a series of large, 3-roller, crusher mills, which together comprise the milling tandem. As the cane passes through the tandem it is sprayed with hot water,('inbibition'water), to aid juice extraction. The first-expressed juice is named crusher juice and the total extracted juice is known as mixed juice. The residual fibre, or bagasse, is stored for subsequent use as boiler fuel.

At Frome there are two milling tandems which operate simultaneously, one for Estate cane, the other for farmers' cane. Each can grind 150 tons cane per hour.

2. Clarification and Filtration

Mixed juice is acid, cloudy in appearance, and contains many undesirable impurities. Many of these impurities are removed in the clarification process, in which the juice is limed to a pH of 7.5-8.0 and heated under pressure to 220°F. The coagulated matter or 'mud' so formed is removed from the clear juice by sedimentation in a continuous, closed-tray clarifier. The mud is passed through a rotary-drum vacuum filter, and the expressed juice is returned to the process. The residual product, known as 'filter-cake mud' is utilised as a fertiliser in the cane fields.

3. Evaporation

Clarified juice contains 12-15% w/w soluble solids, mainly sucrose.

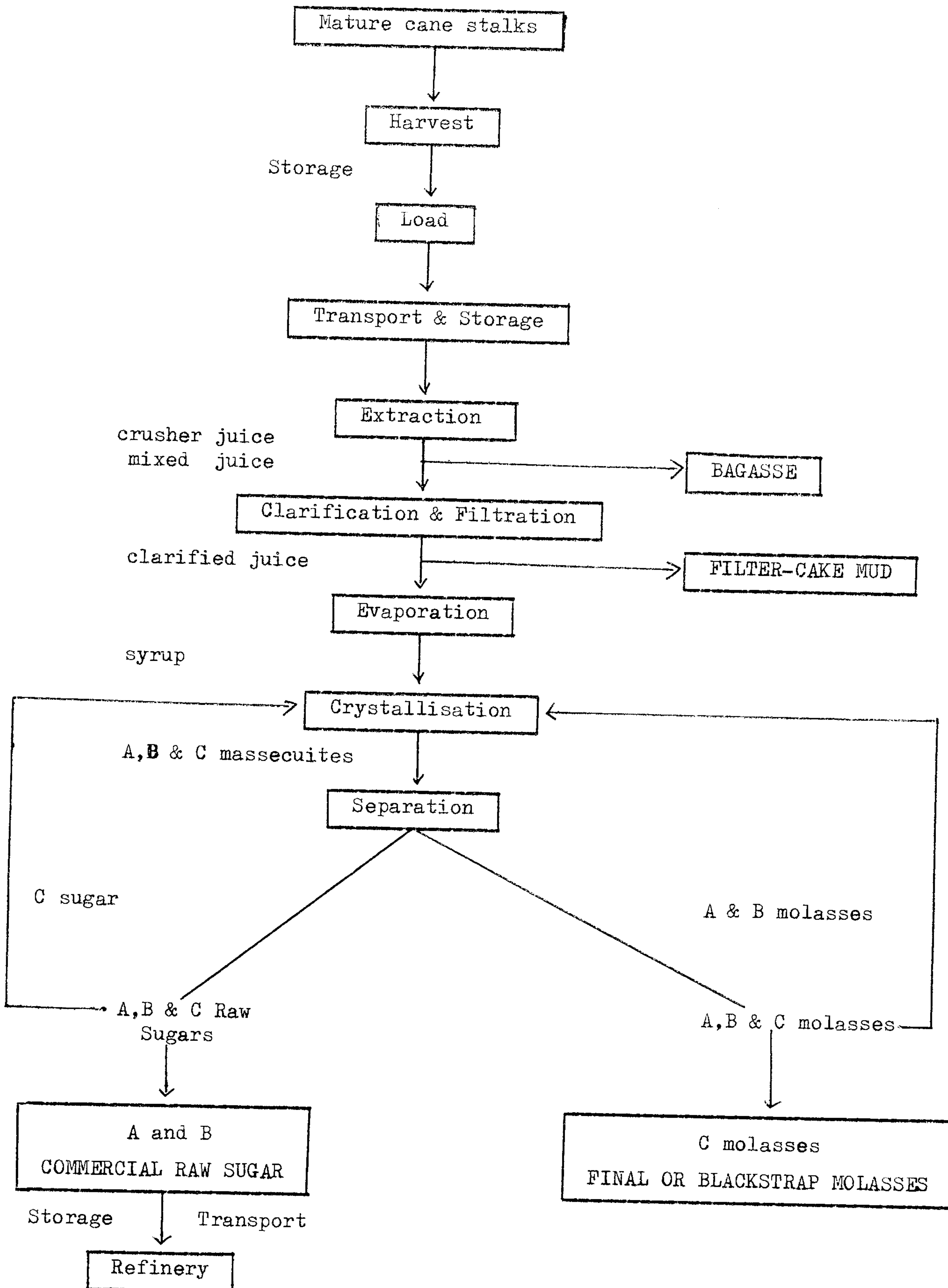


Fig.X.11. Flow diagram of manufacture of raw cane sugar

Before crystallisation, much of the water is removed by boiling the juice under successively reduced pressure in a series of multiple-effect evaporators. The evaporated juice is known as syrup and contains 50-60% w/w soluble solids.

4. Crystallisation

Syrup is boiled under reduced pressure in single-effect vacuum pans until it becomes supersaturated with sucrose. Seed grain is then introduced and the sucrose is allowed to crystallise out under carefully controlled conditions until a suitable grain size is reached. The mixture of mother liquor and crystals, which is named 'massecuite', is then run out or 'struck' into stirred receivers known as crystallisers, where it is mixed and allowed to cool.

5. Separation or Purging

Massecuite from the crystallisers is fed into centrifugal machines which consist of rotating drums with perforated walls, named 'baskets'. The basket is spun at 1,200 - 1,500 R.P.M. for two to three minutes, forcing the liquor through the perforated screen, which retains the crystals. The separated liquor is known as 'molasses', whilst the crystals constitute raw sugar. The raw sugar contains 96-98% w/w sucrose, 0.5 to 1.5% moisture and 1.0 to 2.5% non-sucrose solids, principally reducing sugars and ash.

At Frome a 3-massecuite system of crystallisation and separation is operated. The first boiling of syrup results in an 'A' massecuite which yields 'A' sugar and 'A' molasses on separation. 'A' molasses is re-boiled to give a 'B' massecuite which yields 'B' sugar and 'B' molasses on separation. 'B' molasses is re-boiled to give 'C' massecuite which yields 'C' sugar and 'C' molasses on separation. A mixture of 'A' and 'B' sugar constitutes the commercial raw sugar which is stored, transported to sugar refineries, and refined to produce white sugar and other products. 'C' sugar is used as 'seed' in the vacuum pans, or is sold for local consumption. 'C' molasses is fully exhausted, i.e. it is uneconomic to crystallise more sucrose from it. The by-product is known as final molasses or 'blackstrap' and is widely used

as a substrate for fermentation or in animal fodder. It contains 82-88% solids, of which 40-45% w/w is sucrose, 30-40% is reducing sugar and the remainder is inorganic ash and other impurities. Between 5 and 8 gallons of final molasses are produced per ton of cane.

Sugar manufacture is a continuous process. The factory grinds cane throughout the crop except for a weekly nine-hour stop to enable the plant to be cleaned. The complete process of sugar production from a particular batch of cane normally requires between 34 and 45 hours.

APPENDIX 2. DEFINITIONS OF SPECIAL TERMS USED IN THE SUGAR INDUSTRY

1. Brix

Brix is the percentage by weight of solids in a pure sucrose solution. By general acceptance the Brix represents the apparent solids in a sugar solution as determined by the Brix hydrometer.

Refractometer solids or Refractometer Brix is the percentage by weight of solids determined in a refractometer, calibrated for pure sucrose solutions.

In this thesis all values of Brix were determined by refractometer, but are conveniently abbreviated to 'Brix'.

Brix is expressed in degrees, e.g. a 60% w/w sucrose solution is written as 60° Brix, abbreviated to 60°Bx.

Since the refractometer calibration is based on the refractive indices of pure sucrose solutions, values of the apparent percentage solids become increasingly inaccurate as the proportion of non-sucrose solids in the solution increases.

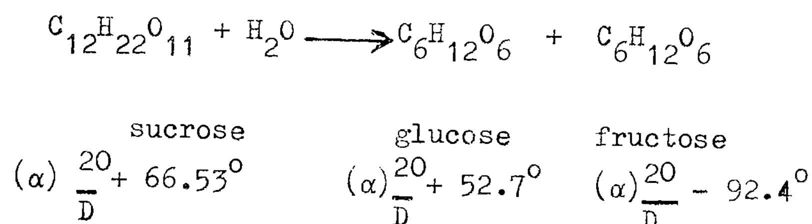
2. Polarisation, Pol and Sucrose

Pure sucrose is dextrorotatory; its specific rotation is $(\alpha)\frac{20}{D} + 66.53^{o}$ at a concentration of 26 g. per 100 ml. aqueous solution (= 'normal weight'). Use is made of this property to estimate the concentration of sucrose in a sugar solution, by measuring the direct or single polarisation of a normal weight solution in a polarimeter (saccharimeter). The result of this polarisation is termed the pol, expressed in degrees. Pure sucrose has a pol of 100.00^{o}. Pol is identical with the percentage w/w of sucrose in a pure solution, but is only an estimate of sucrose content when impure solutions containing other optically active compounds are tested. The true sucrose content of impure solutions may be determined by a double polarisation technique known as the Clerget method, in which the pol is determined before and after inversion (q.v.) and hence the effect of impurities is removed by difference.

At Frome, and most other sugar factories, pol rather than true sucrose is the basis of all chemical control calculations, because its advantage of speed of determination is considered to outweigh its disadvantage of inaccuracy.

3. Reducing Sugars, Invert and Inversion

Hydrolysis of sucrose yields equimolecular amounts of its constituent monosaccharides, glucose and fructose :-



$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

sucrose $(\alpha)^{20}_{D} + 66.53^{\circ}$ glucose $(\alpha)^{20}_{D} + 52.7^{\circ}$ fructose $(\alpha)^{20}_{D} - 92.4^{\circ}$

This reaction is termed 'inversion' because it results in a change in optical rotation from dextrorotatory sucrose to the net laevorotatory activity $(\alpha)^{20}_{D} - 39.7^{\circ}$ of the products. The equimolecular mixture of glucose and fructose is known as 'invert sugar' or simply 'invert'.

Inversion is catalysed by acid and also by the enzyme invertase. In acid conditions the rate of hydrolysis is proportional to the temperature and degree of acidity.

In the sugar industry, reducing sugars are defined as "the reducing substances present in a solution, calculated as invert sugar"; often reducing sugars are simply termed 'invert'.

4. Purity

Purity is defined as the sugar content expressed as a percentage of solids. Since the sugar content can be expressed as pol or sucrose, and the solids as Brix, refractometer solids, or solids by drying, the purity relationship can be expressed in several forms. The most widely used is :-

$$\text{Apparent purity} = \frac{\text{Pol}}{\text{Brix}} \times 100$$

In this thesis, the Refractometer-Pol Purity is used, in which Brix is substituted by refractometer solids.