Towards the Development of Integrated Cultural Control of Tomato Late Blight (*Phytophthora infestans*) in Uganda

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Towards the Development of Integrated Cultural Control of Tomato Late Blight (*Phytophthora infestans*) in Uganda

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Propositions (Stellingen)

1. Sanitation alone can reduce late blight disease but has a deleterious effect on tomato growth and production; when used in combination with other cultural methods the adverse effects can be alleviated to some extent.

   This thesis.

2. Integrated pest management which includes control of late blight will become important for tomato cultivation in Uganda.

   This thesis.

3. Tomatoes in Uganda are fighting aerial and soil battles, and it seems only agricultural research can come to their rescue.

   This thesis.

4. Fungicides do not provide the complete solution for late blight (*Phytophthora infestans*) control.

   This thesis.

5. If you consider vegetables in Uganda, tomatoes must definitely be included.

   This thesis.

6. The changes in world food production over the coming decades must both address the population increase and make up the food deficit of the most impoverished countries.

   Rerat, A; Matassino, D (ed.); Boyazogui, J. (ed.) and Cappuccio, A., 1997. International symposium on Mediterranean animal germplasm and future human challenges.

7. Countries should use science and technology in order to transform agriculture into an instrument of continuous green revolution.

   Scarascia-Mugnozza, G.I. and Swaminathgan, M.S, 1997. Agricultura, 45.

8. Industrialization is important but will never be more important than agriculture.

9. We eat to live but we do not live to eat - just as we practise agriculture to live rather than living to practise agriculture.

10. 'God made man, man made Holland': this is a good reflection of what man has contributed in reclaiming land for the Netherlands.

11. 'No gain without pain' is true indeed.

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AUTHOR'S ABSTRACT

Tumwine. J., 1999. Towards the development of integrated cultural control of tomato late blight (*Phytophthora infestans*) in Uganda.

Tomato (*Lycopersicon esculentum*) is a major vegetable crop in Uganda. Moneymaker, Marglobe, Heinz and Roma are the major commercial varieties grown in the country, the first two being the most popular. Late blight (*Phytophthora infestans*) is the most important disease of Ugandan tomatoes. Tomato cultivation in Uganda is mostly done continuously throughout the year which perpetuates *P. infestans* survival and sources of inoculum. Late blight in Uganda is predominately controlled by fungicides and the most commonly used fungicide is Dithane M-45 (mancozeb). However, there are virtually no data available on the effectiveness of control of the disease on tomatoes in the country. This research was aimed at developing integrated disease management practices, which avoid where possible the use of fungicides, for late blight disease in Uganda. In the Ugandan situation, this effectively means a range of cultural control practices. New isolation techniques which make use of healthy, and diseased tomato fruits to isolate the pathogen from tomatoes were developed and used in the research. Ugandan isolates of *P. infestans* were found to be of A1 mating type but this was on the basis of a very limited number of samples. The study indicated that sanitation can reduce late blight disease incidence and severity but causes adverse effects on the crop in terms of height, flower formation, fruit numbers and yield. The use of polythene shelters, intercropping, or high tomato planting densities each combined with sanitation helped to alleviate the adverse effect of sanitation and reduced the disease levels even further. The fungicide (Dithane M-45) was only partially effective in controlling late blight by delaying epidemics for about 2 weeks. Nevertheless the fungicide consistently gave higher numbers of fruits and yield than any of the cultural practices, with or without sanitation. If cultural practices are to substitute for fungicides within an integrated disease management system, then further development work is required.

Key words: Tomato, *Lycopersicon esculentum*, varieties, cultivation, late blight, *Phytophthora infestans*, disease, isolation, pest, pesticides, fungicide, Dithane M-45, polythene shelter, intercropping, sorghum, sunflower, soybean, sesame, sanitation, planting density, integrated control, survey, agro-climatic, Uganda.

Dedicated to my children: Colette, Mike, Rodney, Rita, Scovia, and my entire family.

Preface

This research was initiated in 1994, and with the assistance of Prof. J.C. Zadoks and Ir. H.D. Frinking of the Department of Phytopathology, Wageningen Agricultural University (WAU), I was able to secure funding for the research through a WAU PhD sandwich fellowship programme. I am very grateful for their initial contributions and I highly appreciate the WAU PhD sandwich fellowship.

The study project was later developed under the guidance of my promoter, Prof M.J. Jeger to whom I sincerely appreciate very much his tireless efforts towards this work. Practical research was done in Uganda and was at different stages physically supervised by Ir. H.D. Frinking and Prof. M.J. Jeger,. To both, I say thank you very much for enduring those long and tiresome journeys to Uganda. I also extend my sincere gratitude to Ir. Frinking and Prof. Jeger for their continued efforts in the supervision of this research work up to the end. Dr. Francine Govers of the Department of Phytopathology WAU has advised especially on aspects of the pathogen and for this I am sincerely grateful. I further thank Dr. Govers and Dr. Maarten Zwankhuizen for carrying out compatibility tests on the Ugandan *P. infestans* isolates. Technical assistance, especially in computer skills, was provided by Corrie Geerds of the Department of Phytopathology WAU to whom I extend my appreciation. Deans Office for International Students WAU arranged for my travel and accommodation, I am very grateful for their services.

Data collection in Uganda was done with the help of Mr. Mayanja-Kasirye, Chief Technician, Kawanda Agricultural Research Institute (KARI), his dedication was of paramount importance and I thank him for this. I further extend my gratitude to the Director of KARI, Dr. S. Nahdy, for making it possible for me to use the institute research facilities and for his administrative support of this project.

I wish to extend my sincere gratitude to the family of Jan Bakker at Ede, the Netherlands, for their extraordinary hospitality accorded to me. In the same spirit, my appreciation goes to my mother Mrs Didia Katuromunda and my family for their support.

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This project was co-funded by the National Agricultural Research Organisation, Uganda. I am sincerely grateful for the financial support.

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Contents

Chapter 1

General Introduction

General Introduction

Tomato production in Uganda

Tomato (*Lycopersicon esculentum*, Mill.) is a major vegetable crop grown in Uganda. Tomato fruits, cooked, as salads, in processed form as ketchup or paste, are a major source of nutrients especially vitamin A and C, and provide income for farmers. At present, tomato processing in Uganda is done on a limited scale with two major factories in production. Considering the good Ugandan soils and environment for tomato production, there is considerable potential for expansion. According to a research survey carried out in Uganda (Tumwine *et al.*, unpublished), the crop is attacked by many pests with the most damaging being bollworm (*Helicoverpa armigera*), thrips (*Thrips* spp), cutworms (*Agrotis* ssp), aphids (*Aphis* spp) and whiteflies (*Bemisia tabaci*). The major diseases of tomato in Uganda include late blight (*Phytophthora infestans*), bacterial wilt (*Pseudomonas solanacearum*), early blight (*Alternaria solani*) and root knot (*Meloidogyne* spp) nematodes. Viral diseases such as tomato yellow leaf curl virus (TYLCV) are of economic importance. Most farmers consider it impossible to grow tomatoes without the use of fungicides. The most commonly used fungicide to control foliar tomato diseases in Uganda is Dithane M-45 (Mancozeb: 62% Ethylenebisdithiocarbamate ions, 16% Manganese, 2% Zinc, and 20% inert ingredient). According to the survey carried out in Uganda (Tumwine *et al.*, unpublished) the fungicide is very expensive for farmers, and quite often it does not effectively control the disease due to label expiry, adulteration and under dosage. In addition, this fungicide has no systemic activity but is a protectant which is easily washed off by heavy tropical rains and therefore is not always effective under these conditions. In some of the areas visited, farmers have been forced to increase both the dosage and application rate. It is therefore imperative to develop integrated and environmentally friendly control measures for tomato diseases with special emphasis on late blight. These measures should mainly be cultural practices such as sanitation, intercropping, polythene shelters (physical barriers), resistant cultivars, suitable crop density and appropriate planting time.

Tomato plant

Tomato belongs to the family Solanaceae. This family contains other crops of economic importance such as potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), pepper (*Capsicum* spp) and tobacco (*Nicotiana* spp). Tomato originated from Central and South America (Jenkins, 1948; Rick, 1960; Cobley 1963; Purseglove, 1969). Tomato cultivars may be determinate or indeterminate with various colours, sizes, shapes, flavours, vitamin contents and maturity periods (Purseglove, 1969). According to Cobley (1963), the plants are annuals or short lived perennials mostly treated as annuals in cultivation. Tomato stem length range is 0.7-2 m bearing numerous alternate leaves, unevenly pinnate structures with variously indented and lobed margins (Cobley, 1963; Purseglove, 1969). In the nursery, tomato seedlings are ready for transplanting in 4-6 weeks at a height of 12-16 cm. The crop is almost exclusively self-pollinated (Cobley, 1963; Purseglove, 1969). Depending on cultivar, spacing is usually about 1 m between rows and 0.3 m within rows, and maturity period range is 4-5 months with a harvest period of 3-4 weeks (Purseglove, 1969).

The pathogen *Phytophthora infestans*

According to recent taxonomic studies, *P. infestans* (Mont.) de Bary is not a fungus but 'fungus like', related to algae (Erwin and Ribeiro, 1996). The pathogen belongs to a separate kingdom Chromista (Cavalier-Smith, 1986, 1987; Barr 1992; Dick, 1969, 1995a, b). The pathogen is grouped in the phylum Oomycota, subclass Peronosporomycetidae, order Pythiales and family Pythiaceae (Dick, 1990a, 1995a, b). Characteristics of oomycetes are: formation of oospores, a coenocytic mycelium, a diploid life cycle, cell walls containing predominately cellulose rather than chitin and inability to synthesize sterols (Hendrix, 1964; Bartnicki-Garcia, 1968; Sansome and Brasier, 1973). The genus *Phytophthora* forms several sporangia at the terminal branches of tree-like sporangiophores and it is a hemibiotroph. The pathogen is heterothallic with mating types A1 and A2 (Fry *et al.*, 1993).

Origin and distribution of *Phytophthora infestans*

The centre of origin of *P. infestans* is considered to be Mexico (Turkensteen, 1973, Niederhauser, 1991; Goodwin *et al.*, 1994a, b; Legard *et al.*, 1995; Erwin and Ribeiro, 1996). It was in France where late blight on tomato was first described (Tulasne, 1854). Late blight is one of the most devastating diseases of tomatoes (AVRDC, 1993) and potatoes (Drenth *et al.*, 1995) both in temperate and tropical regions. Before 1980, the A1 mating type was well distributed throughout the world, while A2 was reported in central Mexico only (Niederhauser, 1956; Hohl and Iselin, 1984). After 1980, A2 has been discovered in Switzerland (Hohl and Iselin, 1984), UK (Tantius *et al.*, 1986), The Netherlands (Frinking *et al.*, 1987) and Germany (Dagget *et al.*, 1993). At present, the two mating types of *P. infestans* occur in all continents except Australia (Spielman *et al.*, 1991; Kato *et al.*, 1992; Fry *et al.*, 1992, 1993; Drenth *et al.*, 1993a, b, 1995; Shattock, 1995; Umaerus, 1996). First reports of A2 in Africa (Fry *et al.*, 1993) were reported on Egyptian potato in 1984 (Shaw *et al.*, 1985) and in Rwanda in the 1980s (Goodwin *et al.*, 1991).

Infectivity of *Phytophthora infestans*

The pathogen has a limited host range, mainly solanaceous plants (Turkensteen, 1973, 1978; Erwin and Ribeiro, 1996). Reported hosts include *Capsicum annum* (Red pepper), *Datura* spp, *Galinsoga parviflora*, *Gerunium nepalense* (Cranebill), *Ipomoea* spp (Morning-glory), *Lycopersicon esculentum* (Tomatoes), *Nicotiana* spp, *Solanum nigrum* (Black nightshade), and *Solanum tuberosum* (Potato) (Erwin and Ribeiro, 1996). Pathogenicity to potato appears universal in *P. infestans* but not all isolates infect tomato (De Bruyn, 1952; Graham *et al.*, 1961; Wilson and Gallegly, 1955; Goodwin *et al.*, 1995). There is host specialisation with tomato-aggressive and tomato-nonaggressive *P. infestans* isolates, and aggressiveness to tomato may be a recently acquired trait (Legard, *et al.*, 1995). It is now known that some potato strains behave differently as tomato strains when inoculated onto tomato plants (Erwin and Ribeiro, 1996). The fungus affects leaves, stems, branches and fruits (Sherf and Macnab, 1986; Eckert and Ogawa, 1988). Green tomatoes are more susceptible to *P. infestans* than ripe ones (Castellanos-Linares and Garoia-Correoso, 1986; Eckert and Ogawa, 1988). According to Turkensteen (1973), colouring or ripe fruits are very resistant to the *P. infestans*. Strongly pathogenic isolates of *P. infestans* form water-soaked lesions of about 4 mm diameter, which extend to 10-15 mm within 3-4 days (Turkesteen, 1973). Typical lesions with the appearance of darkened concentric rings, are present on stems and fruits (Turkesteen, 1973; Erwin and Ribeiro, 1996). After infection under favourable conditions, the pathogen begins secondary cycles of sporulation in about 5 days (Sherf and Macnab, 1986; Agrios, 1988; Fry *et al.*, 1992; Drenth, 1994).

Factors affecting *Phytophthora infestans* growth and reproduction

The minimum temperature for growth is 4 C, optimum 20 C and maximum 26 C (Erwin and Ribeiro, 1996). The growth of *P. infestans* and sporangia production are favoured by temperatures between 16 C and 21 C and relative humidity approaching 100% (Sherf and Macnab, 1986; Agrios, 1988; Marco and Brunelli, 1989; Erwin and Ribeiro, 1996). The potential duration of sporulation in a lesion is 13 days at a relative humidity of more than 90% for 9-10 hours (Xiao and Xiao, 1991). Temperatures above 29 C can stop late blight disease development (Sherf and Macnab, 1986). At high temperatures (12 C and above), sporangia can germinate directly to form a germtube. At low temperatures (12 C and below) the sporangia differentiate into 3 to 8 (and more) biflagellate zoospores (Turkensteen, 1973). The optimum temperature for direct germination is about 24 C and for indirect germination it is 12 C (Turkensteen, 1973). A film of water is necessary for spores to infect tomatoes (Sherf and Macnab, 1986; Agrios, 1988). A minimum dew duration of 3-4 hours (optimum 24 hours) and temperatures of around 17 C are necessary for tomato infection and epidemic development (Marco and Brunelli, 1989; Xiao *et al.*, 1992). According to AVRDC (1993), the disease can cause losses of 100% in tomato under a conducive environment of wet and cool conditions if not controlled with fungicides. The pathogen can be serious in unheated greenhouses (Fletcher, 1984) which is one of the factors enhancing disease severity in greenhouses in the near East (Alebeek van and Lenteren van, 1992). Sporangia of *P. infestans* lose viability within 5 hours (Sherf and Macnab, 1986; Agrios, 1988; Fry *et al.*, 1992).

Sexual reproduction, resulting in the formation of oospores, is controlled by sex hormones *a*1 and *a*2 produced by A1 and A2 mating types of *P. infestans* isolates respectively (Ko, 1978, 1980, 1988). Temperature and the level of resistance in cultivars also have an influence on sexual reproduction (Drenth, 1994; Drenth *et al.*, 1995). A continuous supply of moisture is essential for oospore production (Cohen *et al.*, 1997). According to Fry *et al* (1992), mere occurrence of both mating types in a location does not necessarily ensure sexual recombination.

In the laboratory, potato cultivar Bintje is used for isolation because it is very susceptible (Zwankhuizen personal communication, 1995; Andrivon and Lucas, 1998). *In culture, the best media for growth of P. infestans* is Rye A agar (Snieszko *et al.*, 1947; Hartman and Huang, 1995; Erwin and Ribeiro, 1996). There are few reports of similar isolation and culturing procedures for tomato isolates.

Survival and dispersal of *Phytophthora infestans*

The pathogen survives in various crops such as potatoes, tomatoes and other susceptible plants (Turkensteen, 1973, 1978). According to Drenth (1994), mycelia can survive in plant debris under natural conditions and are able to cause infection for short periods. Oospores are the main form in which the pathogen can survive for long periods of 8 months and above in absence of plant materials (Drenth, 1994; Drenth *et al.*, 1995). Oospores are formed mostly in stems of potato and tomato (Frinking *et al.*, 1987; Mosa *et al.*, 1991). Tomatoes support more oospore production than potato (Cohen *et al.*, 1997). According to Turkensteen (personal communication, 1995), oospores produced by *P. infestans* can be non-viable, thus not all contribute to the problem of late blight. Oospores in the soil are of significant importance in *P. infestans* infection and late blight epidemiology (Fry *et al.*, 1992; Drenth, 1994, Drenth *et al.*, 1995; Pittis and Shattock, 1994). Oospores remain viable and infectious in infested soil for 35 weeks (Drenth *et al.*, 1995), 8 months (Pittis and Shattock, 1994) and 2 years (Perhes and Galindo, 1969). In vitro, oospore germination of up to 13.4% was obtained (Pittis and Shattock, 1994). Light is essential for germination (Chang and Ko, 1991).

Long distance dispersal of *P. infestans* is associated with infected plants or plant parts transported by man (Fry *et al.*, 1992; Shattock, 1995). Short distance sporangia dispersal is through wind, dew and rain splash (Gretna, 1983; Sherf and Macnab, 1986; Fry *et al.*, 1992; Erwin and Ribeiro, 1996). Zoospores can contribute to local spread of the disease (Drenth, 1994).

Control of late blight in tomato

Fungicides

Sprays of metalaxyl once or twice per week can reduce late blight and yield loss and increase fruit numbers (Sherf and Macnab, 1986). Fungicides have at times been combined in order to overcome the challenges of late blight. Mancozeb and Ridomil plus (metalaxyl + cuprous oxide) proved effective against late blight in Cameroon (Fontem and Aighewi, 1993). Metalaxyl combined with mancozeb was also effective in Germany (Gutsche, 1994). However, *P. infestans* has shown resistance to metalaxyl in Russia (Volob-eva-Yu *et al.*, 1992), USA (Deahl *et al.*, 1995), Canada (Deahl *et al.*, 1995; Chycoski and Punja, 1996) and Morocco (Sedegui *et al.*, 1997) calling for more research on how the resistance problems can be avoided. In Uganda, Dithane M-45 (mancozeb) is the only fungicide used extensively by tomato farmers (Tumwine *et al.*, unpublished).

Resistant cultivars

Some lines of tomatoes have shown resistance to late blight (Guseva *et al.*, 1978; An *et al.* 1984; Kaur *et al.*, 1988; Zhang, *et al.*, 1988; Markovic *et al.*, 1990). Systemic acquired resistance can be induced in tomato against *P. infestans* by pre-inoculation with tobacco necrosis virus (Anfoka and Buchenauer, 1997) or fungicide fosetyl-AL (Chahal, 1990). However, there are difficulties in developing effective resistance for late blight in tomato and therefore the number of resistant lines is still low (AVRDC, 1993; Khalid-Majid, 1995; Laterrot, 1996). According to Hartman and Huang (1995), strains of *P. infestans* can infect tomato lines containing resistance genes thus there is need for more research on the nature of this resistance and its durability. Crop protection strategies based on late blight resistant tomato cultivars and fungicides need to be developed to counteract the new strains A1 and A2 of *P. infestans* (Deahl, 1995; Shattock, 1995). In Uganda, all tomato varieties grown by farmers are susceptible to late blight (Tumwine *et al.*, unpublished).

Biological control

A number of biological control agents such as *Trichoderma, Penicillium, Microdochium, Botryodiplodia, Bacillus, Streptomycetes* and *Actimycytes*, show potential for control of *Phytophthora* spp (Erwin and Ribeiro, 1996). These control agents work as parasites or antagonists but their ability to control *P. infestans* in the field has not been adequately tested.

Cultural control methods

Excluding early inoculum of *P. infestans* by sanitation and growing tomatoes far from potatoes is recommended for late blight management (Sherf and Macnab, 1986). Sanitation was attempted for late blight control in tomato and potato (Inglis *et al.*, 1996). Plastic shelters reduced the level of late blight, fungicide sprays were minimal, and tomato yields and fruit size were improved (Hanada, 1988). Polythene-film covered greenhouses reduced tomato infection by *P. infestans* (Sunarjono and Hardinah, 1972; Chee *et al.*, 1988). Shelter belts on outdoor irrigated tomatoes increased plant growth, and late blight disease was only enhanced where warm and humid conditions were created (Jebari, 1989). Intercropping faba beans and corn with potatoes reduce the incidence and severity of late blight (Sharaiha, *et al.* 1989). Using the fungicide benlate in tomatoes at high plant densities, diseases including late blight were not aggravated (AVRDC, 1992). According to AVRDC (1987), yield responses of tomato to plant and row spacings can be expected. High tomato (Peto 86 and PT 4121) density of 2 plants per 20 cm^2 gave the greatest numbers of green fruit and least unmarketable yields, compared with 1 plant per 15, 20 and 30 cm^2 (AVRDC, 1992). Experiments to investigate cultural methods of controlling late blight are the topic of this thesis.

Integrated control

Integrated control of late blight on tomato has been recommended in the USA (Bolkan, 1997, Bauske *et al.*, 1998). Integrating environmental information into schemes to minimize chemical use has been long advocated by many scientists (Rebelo, 1991; Alebeek van and Lenteren van, 1992; Raposo *et al.*, 1983). Forecasting reduced the number of chemical sprays on tomato in a season in Emilia Romagna, Italy, (Bugiani *et al.*, 1993) and can reduce fungicide sprays against tomato late blight by 30% (Govoni and Bugiani *et al.*, 1993). Monitoring of airborne concentrations of sporangia of *P. infestans* can provide a more rational late blight control strategy in tomatoes (Picco, 1992; Poti and Cavanni, 1992; Bugiani *et al.*, 1995). Antifungal plant extracts such as the phytoalexin capsidol from pepper fruits could be used against *P. infestans* (Sherf and Macnab, 1986; Schmitt, 1996). Although diluted cattle slurry was not effective (Holm, 1997), compost extracts could be used (Ketterer and Schwager, 1992). These are promising late blight disease control methods which could be further enhanced in an integrated disease management system.

Goal and objectives of late blight research in Uganda

The general goal of research on late blight of tomato in Uganda was to determine whether cultural control methods offered a realistic alternative to the use of fungicides in reducing late blight in tomato and increasing tomato yields. As part of this general goal, the following objectives for the research project discussed in this thesis were:

1. to evaluate sanitation (removal of diseased plant parts) as a cultural control method to reduce dependence on fungicides;
2. to increase sanitation efficiency by combining with other cultural practices for control of late blight;
3. to take the first steps in developing an integrated management system for tomato late blight.

It is important to note that in all experiments, sanitation with and without additional cultural practices were compared with the commonly-used fungicide (Dithane M-45/mancozeb) in Uganda and not with more recently developed and effective compounds. Similarly all experiments were conducted with MT55, a tomato line which is susceptible to late blight but resistant to bacterial wilt.

Chapter 2

Tomato Late Blight (*Phytophthora infestans*) in Uganda

(Submitted to International Journal of Pest Management)

Tomato Late Blight (*Phytophthora infestans*) in Uganda

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Abstract

Late blight (*Phytophthora infestans*) infects tomatoes (*Lycoperscon esculantum*), potatoes (*Solanum tuberosum*) and other solanaceous plants. The pathogen survives in plant debris but oospores are the main structure by which the pathogen can survive for long periods in the absence of plant material. Dispersal of *P. infestans* is associated with infected plants or plant parts transported by man, and through wind, dew and rain splash or by zoospore movement. The main aim of the agro-climatical research survey in Uganda was to obtain information on the current tomato late blight situation, and practices for disease management. Ten districts from different agro-climatic zones were selected for the late blight survey. *Phytophthora infestans* isolates from tomatoes were obtained from some of the agro-climatic zones of Uganda and compatibility tests were done and only A1 mating type was recovered. The study revealed that tomato growing is mostly done throughout the year and the major commercial varieties are Moneymaker, Marglobe, Heinz and Roma. Late blight (*P. infestans*) and bacterial wilt (*Pseudomonas solanacearum*) are the main diseases, and bollworm (*Helicoverpa armigera*) and thrips (*Thrips* spp) the major insect pests on tomatoes in Uganda. All tomato varieties grown are susceptible to late blight. Chemical control mostly by Dithane M-45 (mancozeb), is the predominant strategy used by farmers for late blight control. Sanitation is practised by some farmers, but in association with fungicides, to control late blight and also to reduce the amount of pesticides applied per plant. Plant nutrition did not appear to play a major role in late blight management. Problems associated with pesticides and poor tomato marketability were identified as the major constraints in tomato production in Uganda.

Introduction

Uganda is a country located in East Africa, between 4^0 N and 1^0 S latitudes, and 30^0 E and 35^0 E longitudes. It shares borders with Sudan in the north, Kenya in the east, Tanzania in the south, Rwanda in south-west and Zaire (Democratic Republic of Congo) in the west. The country, with a human population of approximately 20 million, is at an altitude of about 1,500 m (above sea level). Uganda has 2 peak rain periods with most parts of the country receiving annual rainfall above 1,000 mm, and the temperature ranges between 16 C and 30 C. Tomato is one of the main vegetable crops grown in Uganda and is consumed cooked, as salads, ketch up and provides income to farmers. Late blight (*P. infestans*) is one of the major diseases on the crop which limits its cultivation and quite often increases production costs because of the use of fungicides to control the disease.

The centre of origin of *P. infestans* is considered to be Mexico (Turkensteen, 1973; Goodwin *et al.*, 1994a, b; Erwin and Ribeiro, 1996). The pathogen was first reported on tomato in France (Tulasne, 1854). Since then, the pathogen has been one of the most devastating diseases of tomatoes both in temperate and tropical regions (AVRDC, 1993; Drenth, 1995). Prior to 1980 the A1 mating type of *P. infestans* was the only strain distributed worldwide. Mating type A2 was only reported in central Mexico (Niederhauser, 1956; Hohl and Iselin, 1984) where mating and oospore formation took place. Since 1980, the A2 mating type and oospores have been discovered in Switzerland (Hohl and Iselin, 1984), UK (Tantius *et al.*, 1986), the Netherlands (Frinking *et al.*, 1987) and Germany (Dagget *et al.*, 1993). To date, the A2 mating type occurs in all continents except Australia (Spielman *et al.*, 1991; Kato *et al.*, 1992; Fry *et al.*, 1992, 1993; Drenth *et al.*, 1993a, b, 1995; Shattock, 1995; Umaerus, 1996). First reports of the A2 mating type in Africa were made on Egyptian potatoes in 1984 (Shaw *et al.*, 1985) and Rwanda in the 1980s (Goodwin *et al.*, 1991).

The pathogen has a limited host range mainly solanaceous plants (Turkensteen, 1973, 1978; Erwin and Ribeiro, 1996). The pathogen survives in various crops such as potatoes and tomatoes (Turkensteen, 1973, 1978). According to Drenth (1994), mycelia can survive in plant debris under natural conditions and continues to cause infection for short periods if contact is made with susceptible plants. Tomatoes support more oospore production than potatoes (Cohen *et al.*, 1997). Oospores are the main structure by which the pathogen survives for long periods of 8 months and more in the absence of it host tissue (Perhes and Galindo, 1969; Drenth, 1994; Pittis and Shattock, 1994; Drenth *et al.*, 1995). Oospores in the soil are important in *P. infestans* infection and late blight epidemiology (Fry *et al.*, 1992; Drenth, 1994; Pittis and Shattock, 1994; Drenth *et al*,. 1995). Long distance dispersal of *P. infestans* is associated with infected plant materials transported by man (Fry *et al.*, 1992; Shattock, 1995). Short distance dispersal of sporangia is through wind, and rain splash (Gretna, 1983; Sherf and Macnab, 1986; Fry *et al.*, 1992; Erwin and Ribeiro, 1996). Zoospores can contribute to local spread of the disease (Drenth, 1994).

In Uganda, late blight disease control is practised almost entirely by fungicides, mainly Dithane M-45 (mancozeb). However, the fungicides have the disadvantages of being expensive, lack of availability, adulteration, label expiry and poor application techniques. In addition, the most popular fungicide Dithane M-45 is a protectant and is easily washed off by heavy tropical rains. This makes the fungicides not always effective against late blight in the country. In India, differences were observed between fungicides such as Dithane M-45 and Ridomil MZ-72 (metalaxyl) on their effectiveness against late blight (Verma *et al.*, 1994). Sprays of metalaxyl once or twice per week can reduce late blight and yield loss, and increase fruit numbers (Sherf and Macnab, 1986), as also found in Taiwan (Hartman and Huang, 1995). In Indonesia, Difolatan (Captafol) was the most efficient fungicide against late blight in tomatoes in the rainy season and Dithane-M45 was more effective in the dry season (Suhardi *et al.*, 1977). Late blight disease was found to be most severe in wet and cool seasons in Malaysia (Mohd-Shukor *et al.*, 1987), Costa Rica (Calvo-Domingo *et al.*, 1990) and Cameroon (Fontem, 1993). These seasonal differences can aggravate the late blight disease situation. Preventive sprays with mancozeb which has largely protectant properties can still be useful against late blight as proved in Israel (Cohen, 1987) and India (Maheshwari and Mehta, 1993). Crop protection strategies need to be developed to counteract the threats posed by the presence of both mating types A1 and A2 of *P. infestans* and their progenies, and also by the development of fungicide resistance (Deahl *et al.*, 1995; Shattock, 1995; Bolkan, 1997; Bauske *et al.*, 1998). These strategies would include mainly cultural practices such as sanitation, intercropping, physical barriers (polythene covers), suitable planting time and appropriate crop density. The main aim of the tomato late blight research survey was to establish a baseline of information on disease management practices of small farmers and to ascertain the inoculum and disease pressure in different parts of Uganda.

Methodology

Ten districts from different agro-climatical zones were selected for a late blight research survey. The zones were classified on the basis of temperature, rainfall, and sunshine differences; ranging from cool and wet to hot and dry conditions. The districts involved were Kabale and Mbale (cool and wet), Bushenyi (warm and wet), Mpigi and Mukono (warm, wet and humid), Luwero and Masindi (warm and dry), Kasese, Kumi and Tororo (hot and dry) (Fig. 1). In each district the aim was to interview at least 10 respondents including agricultural extension staff, farmers and agrochemical retailers. A total of 93 respondents from 10 districts were interviewed using a structured questionnaire. The questionnaire also included some open free choice questions related to tomato production in general. A collection of *P. infestans* isolates for characterisation was made in some of the districts.

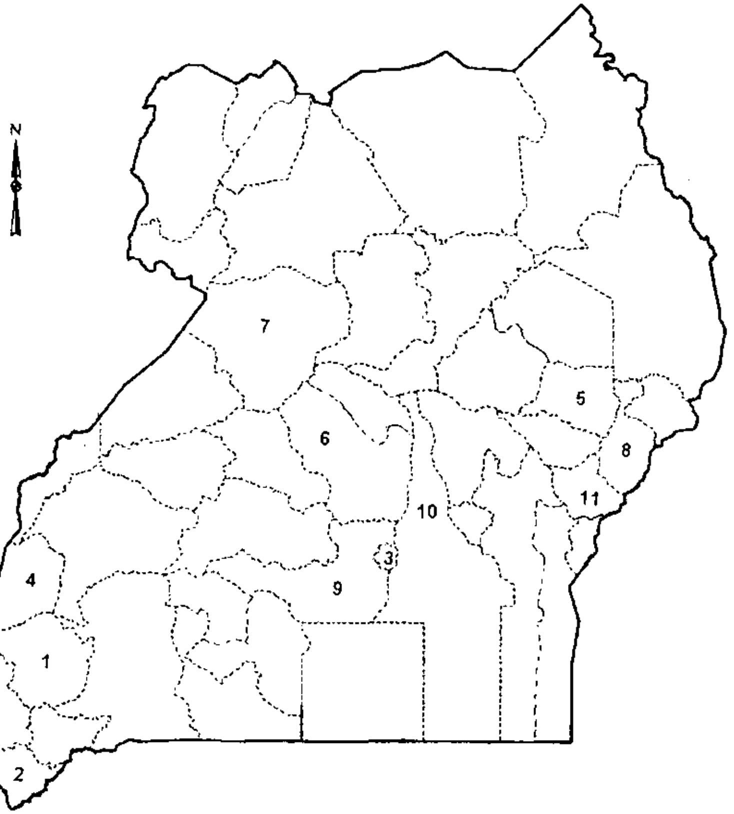

Fig. 1: Map of Uganda showing districts in which the survey and *P. infestans* isolate collection were carried out (1. Bushenyi, 2. Kabale, 3. Kampala, 4. Kasese, 5. Kumi, 6. Luwero, 7. Masindi, 8. Mbale, 9. Mpigi, 10. Mukono, 11. Tororo)

Compatibility test for Ugandan *Phytophthora infestans* isolates

In 1996, a total of 8 *P. infestans* isolates from Mbale, Mpigi, Kampala, Kumi and Tororo districts were successfully obtained but three of these were lost through contamination. The isolates were sent to Wageningen Agricultural University for characterisation, in 1996. The compatibility tests were carried out by placing known *P. infestans* mating types A1 and A2 isolates in proximity with the Ugandan isolates on clarified Rye A agar medium. After 1 week oospores were observed between Ugandan *P. infestans* and the A2 mating type only. These results indicated that the *P. infestans* isolates from Ugandan tomatoes were mating type A1 (Table 1). This was the first time isolates of *P. infestans* from Uganda, on tomatoes, had been characterised.

Table 1: *Phytophthora infestans* isolates from Ugandan tomatoes

Isolate number	Date of isolation	Mating type	Location	
			County	District
UG96001	June 96	A1	Kyadondo	Mpigi
UG96002	June 96	A1	Kyadondo	Mpigi
UG96003	June 96	A1	Nakawa	Kampala
UG96007	August 96	A1	Mbale central	Mbale
UG96008	August 96	A1	Kwapa	Tororo

Major commercial tomato varieties grown in Uganda

Figure 2 indicates the popularity of the major commercial tomato varieties grown in Uganda. Overall Moneymaker and Marglobe were the most popular tomato varieties grown, although there were some regional differences. Moneymaker is preferred by many farmers because of its indeterminate growth characteristics. Farmers harvest over a long period thereby reducing market-associated risks. At the end of the season the total amount of fruit harvested is substantial. Marglobe is preferred by farmers because of its large fruit, which is attractive to customers. The disadvantage of Marglobe is that because it is determinate, there are high risks in marketing, as the fruits mature at the same time. This problem is aggravated by a lack of storage and insufficient processing facilities in the country. Heinz, which bears big fruits, was grown in the western and central regions, and not in the eastern parts of the country. There was also a cultivar known as Enganda (land-race similar to Moneymaker, with medium size fruits) which was grown in the western and central regions only. In Kasese district, in the west of the country, there is a tomato processing factory which has led to the increased popularity of Roma (a processing variety). Cultivation of Roma was encouraged in Kasese district because of its low water content and high brix (sugar content), factors important in tomato ketchup and paste production. There is another tomato processing factory in Tororo district in the eastern part of the country. However, these processing factories do not provide a large enough market for the tomatoes during peak harvesting periods in Kasese, Tororo and neighbouring districts.

The survey revealed that the major usages for tomatoes grown in Uganda were home consumption and income generation.

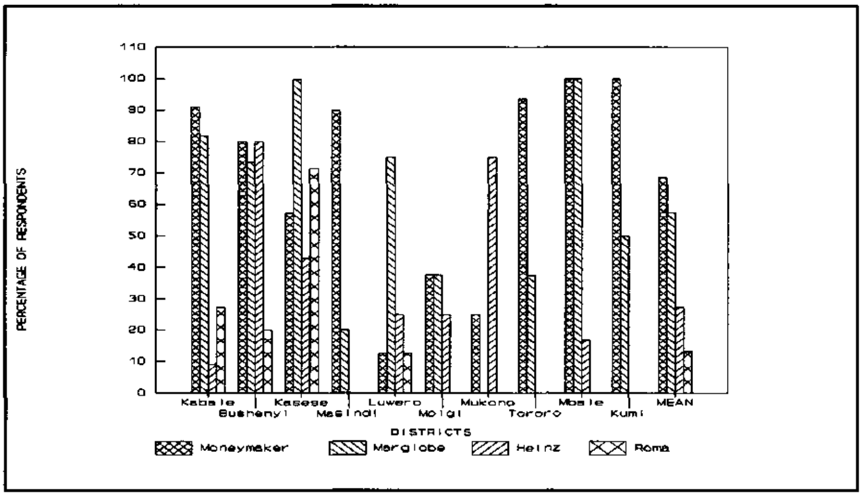

Fig. 2: Popularity of four tomato varieties in Uganda, as indicated by questionnaire responses

Major tomato diseases and other pests in Uganda

Table 2 summarizes the major diseases and insect pests encountered in tomato cultivation in Uganda in descending order of importance for each category. Late blight was identified as the most serious disease in tomato cultivation in the country. Bacterial wilt (*Pseudomonas solanacearum*) ranks as the second major disease in tomatoes. Although not always effective, some farmers found late blight easier to control than bacterial wilt because of the availability of late blight fungicides. There is no effective control measure for bacterial wilt in commercial varieties grown in Uganda. Bollworm was the most troublesome insect pest in the country. The insect bores and hides inside the fruits, making it difficult to control by insecticides and continues to inflict much damage by attacking various fruits. Blossom end rot, flower abortion, fruit rots, wild birds, monkeys and human beings were also mentioned to be causing serious problems in some areas. *Commelina* spp, *Galinsoga parviflora*, *Digitaria* spp, *Bidens pilosa* and *Oxalis latifolia* were reported as the most troublesome weeds in tomato fields in Uganda.

Table 2: Major tomato diseases and insect pests in Uganda

Category	Common name	Scientific name	Percentage of respondents citing
Diseases	Late blight	*Phytophthora infestans*	49
	Bacterial wilt	*Pseudomonas solanacearum*	41
	Viral diseases	TYLCV and others	20
	Root knot	*Meloidogyne* spp	18
	Early blight	*Alternaria solani*	15
	Damping off	*Pythium* spp	3
Insect pests	Bollworm	*Helicoverpa armigera*	72
	Thrips	*Thrips* spp	42
	Cutworm	*Agrotis* spp	23
	Aphids	*Aphis* spp	20
	Whiteflies	*Bemisia tabaci*	8

Varietal susceptibility to *Phytophthora infestans*

The susceptibility to late blight of the major tomato varieties grown in Uganda as perceived by the respondents is shown in Figure 3. All are perceived as susceptible to late blight with the most popular tomato cultivars being the most susceptible to the disease. Some farmers reported some degree of variation in the level of susceptibility for Moneymaker depending on whether the seeds were imported from Kenya, Denmark or The Netherlands. In some districts, local tomato land-races (cultivars with very small fruits) were reported to be resistant to late blight although the fungus was seen growing and sporulating on some of them. The high level of susceptibility perceived makes the farmers heavily biased towards fungicides for the control of late blight.

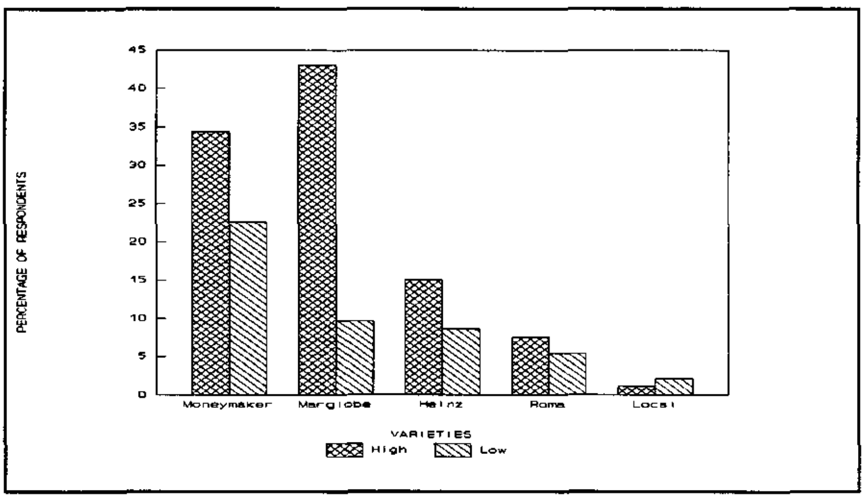

Fig. 3: Tomato varietal susceptibility to late blight as indicated by questionnaire responses (the level of susceptibility for each variety was assessed independently)

Field establishment

Tomato growers in Uganda indicated that they use imported packed seeds, collect seeds from mature fruits, or both. Kenya, Denmark, and The Netherlands were the major sources of packed certified tomato seeds. The majority of the farmers used nurseries in which the tomato seedlings spend 4-5 weeks, including 1 week for hardening, before transplanting into the main field at a height of about 15 cm high. Some farmers carried out direct planting with about 5 seeds per hole which after germination were thinned to 1-2 plants per hole. Two plants per hole were left in order to increase the chances of crop survival. Spacing varied with soil fertility, season (water availability), and number of plants per hole but ranged from 30-90 cm within rows to 60-120 cm between rows. Mulching with grass, staking, or both were practised. Intercropping of tomatoes with bananas and cauliflower was seen in some districts, though not widely practised.

Survival and dispersal of *Phytophthora infestans* in Uganda

In most parts of the country, March-May and September-November are the rainy seasons while December-February and June-August are dry periods. The survey revealed that tomatoes are grown during the rainy season (about 3 months), dry season (about 3 months) and all-year around (above 60% in most districts), mostly as relay cropping (Fig. 4). This practice enables tomatoes of different ages to coexist in the same field, which provides susceptible tissue throughout the year. In some districts, potato cultivation follows the same pattern as tomatoes. The pathogen is able to disperse to the new crop generation, hence surviving adverse drought conditions. However late blight is known to be most severe during the rainy seasons as was reported in Malaysia (Mohd-Shukor *et al.*, 1987), Costa Rica (Calvo-Domingo *et al.*, 1990) and Cameroon (Fontem, 1993). Some farmers, especially in Kasese and Tororo districts, had a habit of hanging diseased or insect-damaged tomato fruits in the fields. The damaged fruits were used as indicators of the extent of damage (threshold) in order to apply pesticides. If such fruits are infected with *P. infestans*, under favourable conditions, the pathogen can sporulate and this can enhance short distance dispersal (Gretna, 1983; Sherf and Macnab, 1986; Fry *et al.*, 1992; Erwin and Ribeiro, 1996). In some districts, for example Kumi, tomatoes were grown in isolated areas widely separated from each other, but late blight was still a threat. Based on this, availability of alternative host for *P. infestans* in Uganda, other than potato (*Solanum tuberosum*) can not be ruled out and research should take it into account. Potential alternative host for *P. infestans* reported elsewhere (Erwin and Ribeiro, 1996), which are available in Uganda as weeds, include *Datura stramonium* (Datura), *Galinsoga parviflora*, and *Solanum nigrum* (Black nightshade).

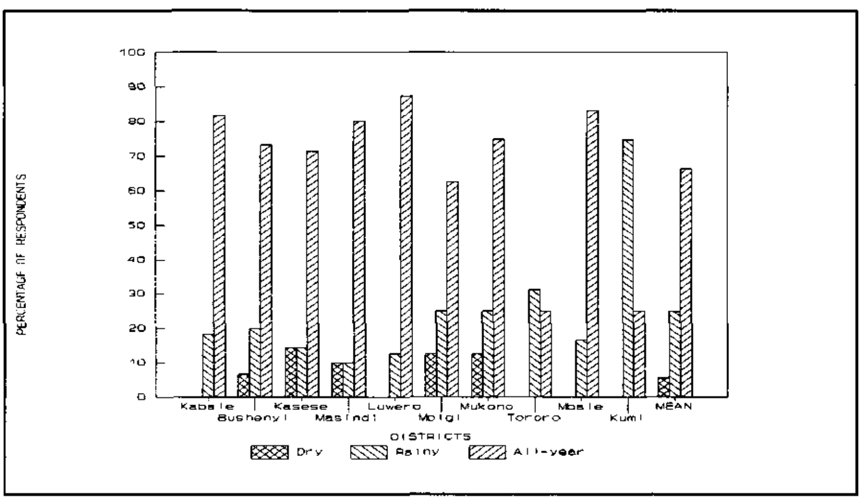

Fig. 4: Major periods of tomato cultivation in Uganda as established by questionnaire responses

Crop protection and sources of nutrients

The survey revealed that chemicals play a major role in current practices for the control of late blight in Uganda (Fig. 5). Dithane M-45 (mancozeb: 16% Manganese, 2% Zinc, 62% Ethylenebisdithiocarbamate ions, and 20% inert ingredient) was the most widely used fungicide constituting more than 90% of usage in most districts. This suggests that the chemical is still seen as effective, as reported in other countries (Cohen, 1987; Maheshwari and Mehta, 1993). In some parts of the country, the systemic fungicide ridomil (MZ 63.5 WP: mancozeb + metalalyx) was known to be very effective against late blight as shown previously (Sherf and Macnab, 1986; Chahal *et al.*, 1990; Hartman and Huang, 1995). However, the survey indicated that ridomil was not commonly used in the country because of lack of availability and awareness by most farmers. Copper based compounds (Cu_2O and $CuSO_4$) were also used on a very small scale in some areas. The survey showed that some farmers have in most cases increased the Dithane M-45 (2-3 times) beyond the recommended dosage of 2.5 grams per litre. When there is a lot of rainfall, the farmers also increase the frequency of fungicide application to 2-3 times per week instead of once per week. In addition, the commonly used insecticides such as Ambush super (Cypermethrin/Peremithrin), Rogor (40% Dimethoate), Dursban (48% Chlorpyrifos), and Sumithion (50% Fenitrothion) were applied in a mixture with fungicides without any consideration of pest economic thresholds. These practices increase the amounts of pesticide entering the environment. In some districts, due to the high cost of pesticides, farmers used reduced doses of fungicides which might encourage the build-up of resistant *P. infestans* strains (Sherf and Macnab, 1986).

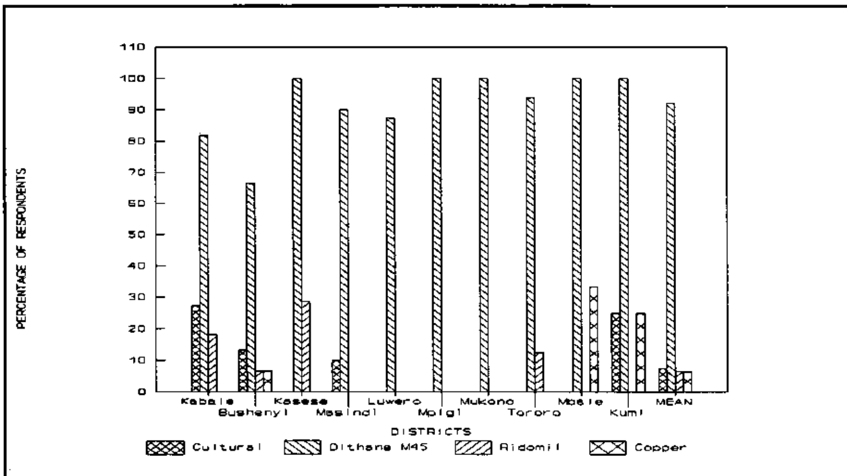

Fig. 5: Tomato late blight control methods as reported in the questionnaire responses

Cultural control methods against late blight were rarely used (Fig. 5). However, growing tomatoes on hills, pruning, and detrussing (sanitation), combined with fungicide application, were reported to reduce late blight severity. This type of integration of control measures for late blight has been recommended in the USA (Bolkan, 1997; Bauske *et al.*, 1998). Where sanitation was practised in Uganda, there was a reduction in the amount of pesticides applied to the plants. However, some farmers reported that sanitation had a deleterious effect in terms of reducing continuous flowering of the plants.

According to farmers' perceptions, none of the forms of fertilizer applied (Fig. 6) influenced late blight outbreaks. However, the nutrients supplied could be contributing to late blight resistance by increasing plant vigour and physical defences. Investigations on this point are needed.

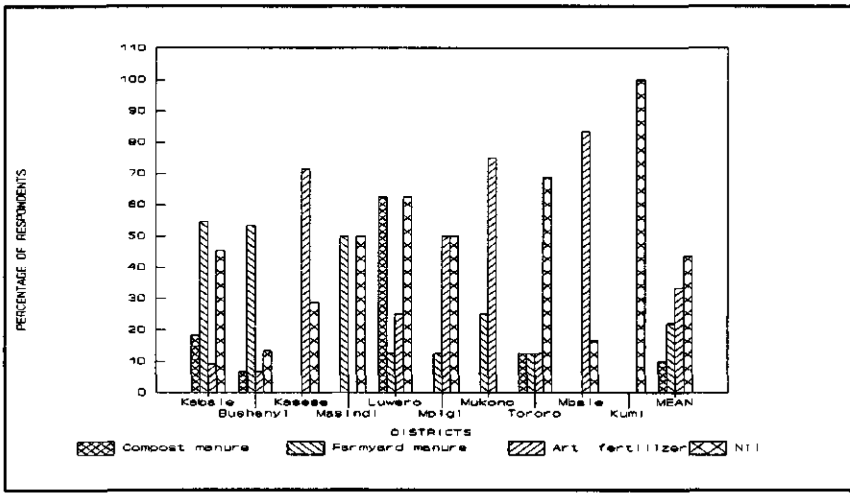

Fig. 6: Sources of applied nutrients for tomato cultivation as reported in the questionnaire responses

Major constraints in tomato production in Uganda

Overall, problems associated with pesticides were identified as the most serious constraint to tomato production in Uganda, followed by marketability (lack of market, infrastructure and storage facilities) (Table 3). Although late blight as a single constraint was the third ranked, it is more significant because of the high usage of fungicides, the most serious perceived constraint. At the district level, late blight was still featuring as constraint number one in tomato cultivation for example in Bushenyi, Mpigi Masindi and Mbale. The disease could also be affecting marketability by causing postharvest rotting of tomatoes.

Table 3: Major constraints in tomato production in Uganda obtained by free and open questionnaire

CONSTRAINT	PERCENTAGE OF RESPONDENTS BY DISTRICT										MEAN
	Kabale	Bushenyi	Kasese	Masindi	Luwero	Mpigi	Mukono	Tororo	Mbale	Kumi	
Lack/high cost of pesticides	91	67	86	80	75	50	75	88	50	100	76.08
Poor marketability (market, transport, storage)	36	67	86	90	63	38	63	88	67	75	67.04
Late blight disease	82	73	43	80	63	63	25	44	67	75	61.34
Lack/high cost of other inputs (sprayers, hoes)	64	60	71	60	38	38	75	69	83	0	55.71
Pests and other diseases (nematodes, bacterial, viral)	9	53	43	40	75	13	13	50	67	0	36.19
Lack/high cost of labour	27	20	43	10	50	0	13	38	33	50	28.35
Poor pesticide efficacy (fake, expired, adulterated)	36	13	29	40	13	38	25	6	50	0	24.95
Environmental hazards (sub burn, hailstone)	0	47	14	20	38	25	0	44	17	0	20.39
Land scarcity and low fertility	18	13	0	0	0	38	0	19	83	0	17.11
Lack of know-how	0	20	0	10	13	13	0	13	17	75	15.92
Lack of capital	0	0	0	10	38	38	38	0	0	0	11.25

Conclusions

According to the research survey made in Uganda there is no evidence that A2 mating type has entered the country, although the testing of *P. infestans* was very limited in scale. Researchers and quarantine personnel should be more vigilant to prevent movements of infected potato or tomato into the country since A2 is already reported in Africa (Shaw *et al.*, 1985; Goodwin *et al.*, 1991; Frey *et al.*, 1993,) including the neighbouring nation of Rwanda (Goodwin *et al.*, 1991). The most popular tomato varieties grown in Uganda are Moneymaker and Marglobe but unfortunately the varieties are highly susceptible to late blight and bacterial wilt. Late blight and bacterial wilt rank first and second respectively as the major diseases in tomatoes in Uganda. Other commercial tomato cultivars grown in Uganda are susceptible to these major diseases. Tomato cultivation in Uganda is done throughout the year thus providing a continuity of susceptible plant tissue and enabling pathogen survival. Bollworm and thrips rank first and second respectively as the major insect pests of tomatoes in Uganda. Problems associated with pesticides, marketability and late blight disease are major constraints to tomato production in Uganda. Although not always effective, chemical control dominates pest and disease management strategies in tomato cultivation in Uganda.

On the basis of this survey, the priorities for research on tomatoes in Uganda are to investigate effective control methods for late blight disease. These would include cultural control methods such as sanitation, intercropping, polythene shelters as barriers, resistant varieties through identifying sources of resistance and breeding, and avoidance of alternative hosts or volunteer plants. Integration of these various control measures should be developed for late blight disease in tomatoes in Uganda. Multidisciplinary research should also start to encompass other major constraints identified such as bacterial wilt disease, bollworm and thrip problems, and postharvest handling and marketing.

Chapter 3

Isolation Techniques and Cultural Media for *Phytophthora infestans* from Tomatoes

Short communication

(Submitted to The Mycologist)

Results and discussion

For both techniques, *P. infestans* rapidly colonized and formed a dense mass of mycelia on the tomato slices (Fig. 1). The pathogen continued to sporulate on the slices for at least 15 days (Fig 1). However, if sterilisation is not effected, the tomato slices can be invaded by other microorganisms. The fungus when transferred onto rye A agar was able to form colonies that expanded to cover the plates. Results using sorghum, finger millet and maize as replacement ingredients for culturing media were preliminary and although the pathogen was able to grow, this was not so profusely as with rye. Maize seemed to be the most promising grain but this needs further experimentation to optimise the procedure.

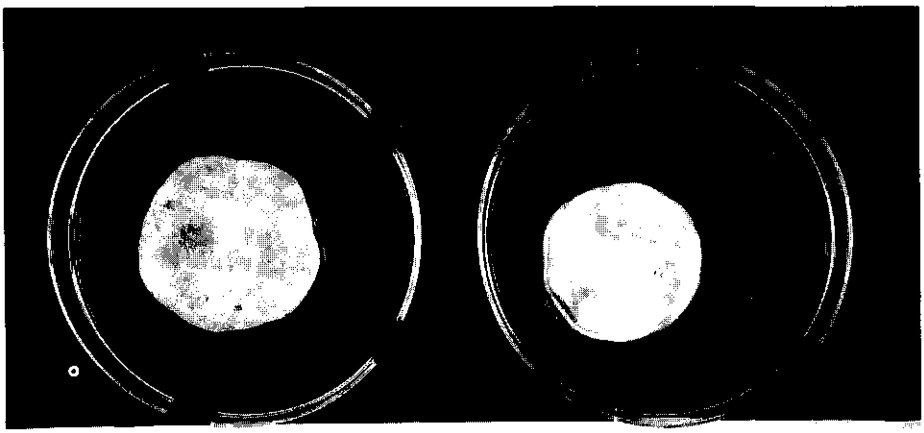

Fig. 1: Tomato slices (Moneymaker) colonized by *Phytophthora infestans*; (left, 8 days; right, 15 days)

The technique of isolating *P. infestans* from diseased leaves has the advantage that it can be adapted for other infected parts of the tomato plant. Also the tomato slices used are from healthy fruits, hence are not readily colonized by other microorganisms which cause rotting. The disadvantage of this technique is that isolation has to be done within a short time because of problems caused by dehydration.

The technique for isolating *P. infestans* directly from infected fruits extends the shelf life of the samples without risks of losing the pathogen. The main disadvantage is that if the fruit is already infected with other pathogens, these can grow and make isolation of *P. infestans* very difficult. Fully-grown fruits can rot rapidly which encourages contamination and interferes with the colonisation of the slices by *P. infestans*.

The two described techniques have a major advantage of being able to aseptically isolate *P. infestans* from tomatoes without the use of antibiotics or selective media. However, culturing of the hyphal tips should be done as early as possible to avoid contamination. According to these results, *P. infestans* can readily be isolated directly using tomato materials alone, with the technique of using diseased leaves being the most successful.

Chapter 4

The Effect of Sanitation in Cultural Control of Late Blight (*Phytophthora infestans*) Disease in Tomatoes

(Submitted to Plant Pathology)

The Effect of Sanitation in Cultural Control of Late Blight (*Phytophthora infestans*) Disease in Tomatoes

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Abstract

Late blight (*Phytophthora infestans*), bacterial wilt (*Pseudomonas solanacearum*), early blight (*Alternaria solani*), root knot (*Meloidogyne* spp) nematodes and some viruses are the major tomato diseases in Uganda. Sanitation combined with other cultural measures such as polythene shelters, intercropping or high density cropping was done in six experiments over 3 years. The main aim of the experiments was to establish the effect of sanitation on late blight on tomatoes in Uganda. Each experiment included sanitation, fungicide treatment, and an untreated control, as standard treatments in the experimental design. Tomato plant height, numbers of healthy and diseased leaves, disease incidence, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and weight of harvested fruits were assessed during the course of the experiments. The results showed that late blight disease incidence and severity were greatly reduced by sanitation. On average sanitation resulted in the same number of healthy leaves as the control, but had an advantage in lowering the disease level in the field. Tomato growth and production were adversely affected by extensive sanitation. Fungicide treated plots retained the highest number of healthy leaves and ultimately gave the highest yields. The fungicide always gave the highest numbers of flowers and attached fruits. The study showed that fungicides do not always contain late blight epidemics, which can result in total yield loss. The study indicated that sanitation has the potential of reducing late blight, and therefore could be integrated with other cultural practices to achieve more effective disease control.

Introduction

Late blight (*Phytophthora infestans*), bacterial wilt (*Pseudomonas solanacearum*), early blight, (*Alternaria solani*), root knot (*Meloidogyne* spp) nematodes and some viruses are major diseases in tomato cultivation in Uganda. These diseases, as with most other plant diseases, can be controlled by chemicals or cultural means such as sanitation (removal of diseased plants or plant parts). Field sanitation has been a major component of cultural control practices for pests and diseases (Cazelles, 1992; Emebiri and Obiefuna, 1992; LaMondia, 1997). Cultural control methods involving sanitation may reduce disease incidence and severity (Emebiri and Obiefuna, 1992). Sanitation measures have been recommended for virus control in potato (Souza-Dias, 1996), citrus (Su *et al.*, 1991; Terranova, 1995) and orchids (Hu and Ferreira, 1980). The use of resistant cultivars combined with sanitation has been used to control African cassava mosaic disease (Ogbe *et al.*, 1996). Bacterial diseases can be controlled by sanitation measures (Cazelles, 1992). Roguing blackleg diseased (*Erwinia*) potato plants increased yield (Ciampi *et al.*, 1997). Sanitation can be beneficial in supervised control programmes for fire blight (*Erwinia amylovora*) on apples and pears (Lecomte *et al.*, 1996). Field sanitation measures are recommended for the control of cylindrocarpon dieback (*Nectria radicicola*) of fruit trees (Kanchaveli, 1997) and leaf blight in mulberry (Tomy-Philip *et al*., 1994). Sanitation and other cultural measures effectively controlled target spot and stem rot (*Rhizoctonia solani*) in tobacco (Gutierrez, 1997) and reduced *Alternaria* blight on *Brassica campestris* (Begum *et al*., 1995). Combining sanitation with fungicides led to a significantly lower incidence of grey mould fruit rot in strawberry (Legard *et al*., 1997). Sanitation was reported to be a protective measure against dieback (*Ceratocystis/Ophiostoma*) of oak (Kryukova and Balder, 1993) and in control of Moniliosis (*Monilia* spp) of peach (Pratella, 1996). Sanitation is essential in bean (*Phaseolus vulgaris*) cultivation in Africa (Trutmann, 1993) and forms an integral part in the management of *Botrytis* in greenhouse crops and geranium diseases (Honda and Yunoki, 1980; Hausbeck and Moorman, 1996).

Sanitation was attempted for late blight control in tomato and potato (Inglis *et al*., 1996). Excluding early *P. infestans* inoculum and primary foci by field sanitation and growing tomatoes far from potatoes is recommended for late blight management (Sherf and Macnab, 1986; Cohen, 1987). Destroying foliage and harvesting after 2 weeks prevents tuber infection by *P. infestans* (Cohen, 1987). Removal of diseased leaves weekly controlled late blight in potatoes (Fontem, 1995). Sanitation was shown to delay late blight disease development in potato for about 8 days behind untreated field (van der Plank, 1963). According to van der Plank (1963), a delay of 5-10 days resulting from destruction of potato cull piles was considered important, but did not stop epidemics. Removing diseased plants or plant parts as a sanitation measure can in some cases be compensated for by enhanced growth of adjacent plants (Ricker and Riedal, 1993), although deleterious effects on individual plant growth and productivity may be expected.

Research on sanitation in combination with other cultural measures such as the use of polythene shelters, intercropping or high density cropping was done in Uganda over a three year period. The main aim of the research described in this paper was to establish whether sanitation as a sole means of control for late blight gave results as good as and could be substituted for commonly-used fungicides in Uganda. The additional effects obtained by combining sanitation with other cultural measures will be reported separately.

Materials and methods

Experimental lay-out

Six experiments were conducted at different times during 1996-1998. In each experiment there were 3 standard treatments: sanitation, fungicide treatment (Dithane M-45/mancozeb) and untreated control, each replicated 5 times. Experimental units were plots of 20 m^2 (5m x 4m) with an average planting density of 55 tomato plants per plot.

Soil characteristics

Prior to setting the experiments, soil samples were taken and sent to the Faculty of Agriculture, Makerere University for analysis (Table 1). The field was divided into 2 major areas, on the basis of slope and soil appearance. Based on the results of the soil analysis, 80 kg, 200 kg and 60 kg per hectare of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) respectively were recommended for area A. All the P_2O_5 was applied at planting time in area A. Only half of N and K_2O were applied before planting with remainder applied 7 weeks after transplanting. In area B, 80 kg, 100 kg and 40 kg per hectare of N, P_2O_5 and K_2O respectively were recommended applied in the same procedure as for are A.

Table 1. pH and nutrient levels at Kawanda experimental site.

Field area	pH	Organic matter (%)	N (%)	P (ppm)	K (mg/100 g)	Na (mg/100g)	Ca (mg/100g)	Mg (mg/100g)
A	5.8	3.90	0.21	3.12	0.31	0.06	5.20	1.87
B	6.0	4.10	0.24	10.77	0.46	0.11	6.40	2.04

Field establishment

In all experiments, tomato line MT55 seedlings were raised in nurseries for approximately 4 weeks. MT55 was used because it is susceptible to late blight, but resistant to bacterial wilt. The tomato nurseries were prevented from late blight infection by spraying them with Dithane M-45 at a recommended dosage of 2.5 grams per litre, at intervals of 7 days. The seedlings were raised during dry weather which together with chemical control ensured healthy and uniform seedlings for the experiments. The main experimental fields were marked by use of 30 cm pieces of reeds and sisal string at the time of transplanting. Four week old tomato seedlings with an average height of 15 cm were transplanted into the experimental field. Tomato planting densities were 44, 55 and 66 plants per plot, each in two of the six experiments. The corresponding spacings were 1.2 m, 1 m and 0.9 m between rows and 0.35 m within rows. A total of 48 tomato spreader plants were also transplanted at every corner of the experimental units, 2 m distant. All experimental fields were enclosed within a guard maize (*Zea mays*) crop with 4 m space between the two. Mulching with spear grass (*Imperata cylindrica*) was carried out in all the experimental plots. Table 2 summarises the planting and harvesting dates for the 6 experiments.

Table 2: Experimental periods for the effect of sanitation

Experiment	Planting date	Harvesting time
1	24 March 1996	May 1996
2	14 April 1996	May 1996
3	28 October 1996	January 1997
4	04 November 1996	February 1997
5	04 April 1997	July 1997
6	10 October 1997	January 1998

Field inoculation

Pathogen inoculum was prepared by collecting late blight infected tomato leaves and incubating them for 48 hours at room temperature in 5 petri dishes of 94 mm diameter. The petri dishes contained moist cotton wool to enhance further sporulation. The spores were washed off with 500 ml of water and put in a 1.5 litre capacity hand sprayer for inoculation. Spore count using a haemocytometer gave a concentration of 2.5×10^5 spores per ml. Inoculations were done 2 weeks after transplanting and in the evening to facilitate infection.

Field management

In the sanitation treatment, diseased leaves, flowers, fruits, branches and exceptionally entire plants were removed using scissors every 2 days throughout the experimental period. Strict measures were observed to prevent the shaking of diseased tomato parts over healthy plants as this would enhance spread of *P. infestans* spores. The diseased materials were contained and disposed of far from the field. In the fungicide plots, Dithane M-45 was sprayed to run-off at a dosage of 2.5 grams per litre at intervals of 7 days, using a 15 litre capacity knapsack sprayer. In case of insect pest attack, Dursban (Chlorpyrifos 400g/litre) broad spectrum insecticide at a dosage of 18.5 ml per 15 litres of water was applied to run-off. Weeding of the experimental fields was done regularly as needed.

Assessment

Two weeks after transplanting, 6 plants per plot were marked for field assessment. The variables assessed in the study were tomato plant height, numbers of healthy (in 4 experiments) and diseased leaves, disease incidence, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and plot weight of harvested fruits (in 4 experiments). Disease incidence was observed on 30 randomly chosen plants per plot and the other variables were scored on the main stem of the 6 marked plants, at intervals of 14 days. Fruits from the 6 marked plants and those from the remaining tomatoes per plot were harvested, taken to the laboratory and weighed separately.

Statistical analyses

The data were analyzed using analysis of variance and pre-determined linear contrasts at the 5% significance level to compare the sanitation effect against both the fungicide treatments and the control. Because there was replication within each experiment, it was possible to extract separate error terms for experiments (A) and treatments (B) and to test for the significance of the interaction (AxB).

Results

Average tomato plant height

In terms of the mean plant height over all experiments, fungicide treatment gave the tallest plants and sanitation the shortest (Fig. 1). Sanitation gave significantly shorter tomato plants than the fungicide treatment ($P < 0.01$) and control ($P < 0.05$), (Table 3). There were significant differences across experiments (Table 4). In some experiments (3 and 5) the fungicide treated plants were shorter than the untreated control, thus giving the significant interaction (Table 4).

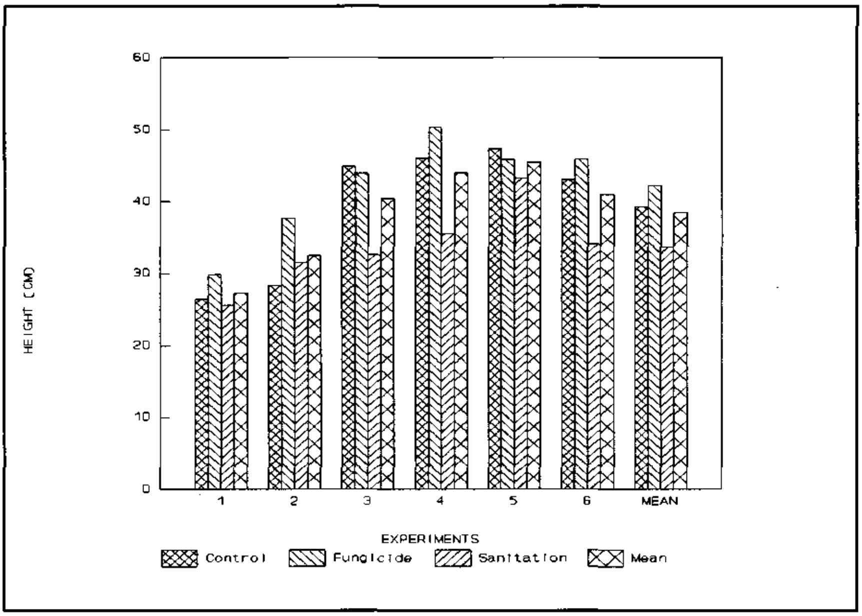


Fig. 1: Average tomato plant height for treatments and mean

Healthy leaves

Fungicide treatment always gave the highest numbers of healthy leaves on the main stem. Sanitation gave significantly fewer numbers of healthy leaves than the fungicide ($P < 0.001$) but did not significantly differ from the control (Fig. 2, Table 3). There were significant differences between experiments (across treatments), and the interaction between treatments and experiments was significant, depending on the relative magnitude of the fungicide effect in any given experiment (e.g. experiment 4 and 6) (Fig. 2, Table 4).

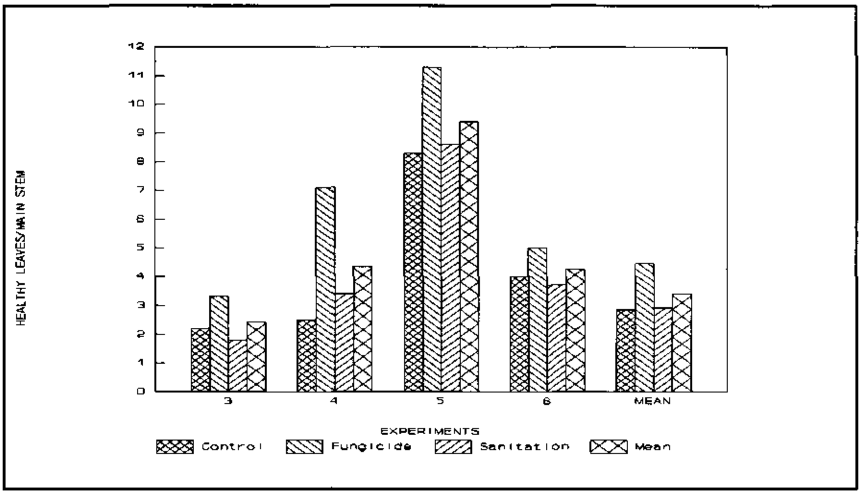
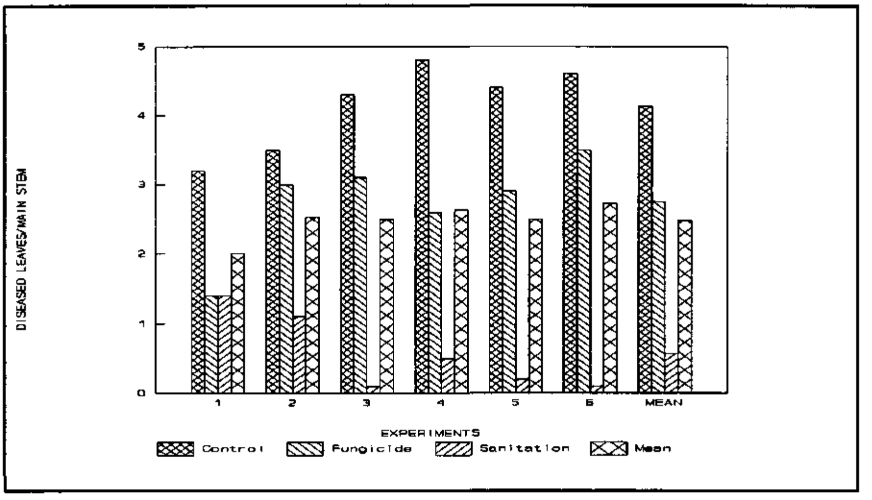


Fig. 2: Healthy leaves on the main stem in experiments 3 to 6

Late blight disease incidence

Figure (3a) shows the mean number of diseased leaves on the main stems of the marked tomato plants during the assessment period. In all the experiments, sanitation resulted in very small numbers of diseased leaves, significantly ($P < 0.001$) less than the fungicide treatment and control (Table 3). There were no significant differences between experiments (across treatments) but the interaction of treatments with experiments was significant reflecting the very low numbers of diseased leaves in the sanitation treatments in some experiments (cases 3, 5 and 6), (Table 4). Sanitation plots had the least late blight incidence in terms of plants per plot (Fig. 3b) but not significantly less than the fungicide treated plots (Table 3). There were significant effects on plot incidence with experiments, treatments and their interaction (Table 4). This is revealed by the inconsistent effects of sanitation compared to the fungicide across the experiments (Fig. 3b).

(a)



(b)

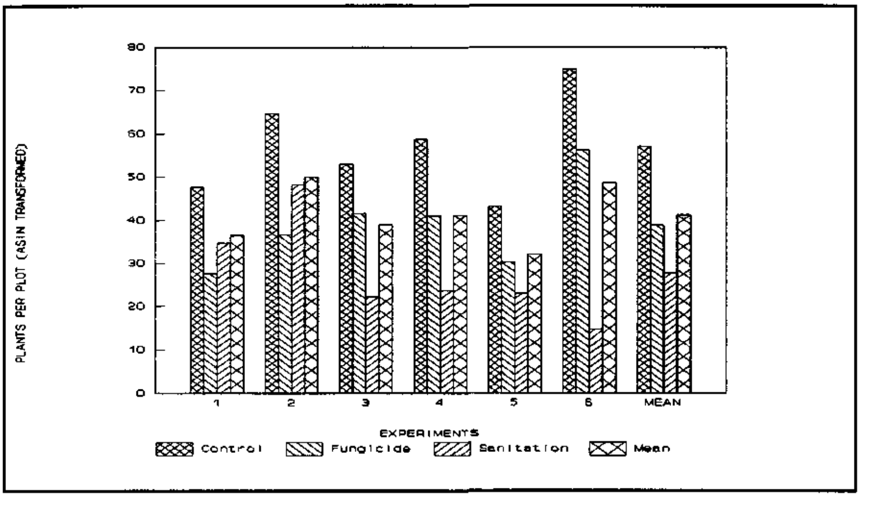
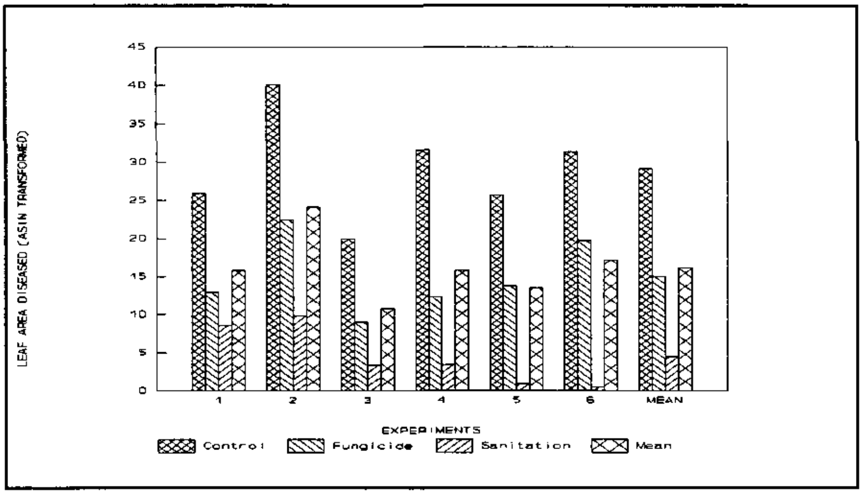


Fig. 3: Late blight incidence; (a) on leaves on the main stem, (b) on plants per plot (data transformed into angles)

Late blight disease severity

The mean late blight disease severities on the 5th and 9th leaves, over all experiments, during the period of assessment were least in the sanitation treatments and highest in the control treatment (Fig. 4). The disease severity was significantly lower in sanitation compared with the fungicide treatments ($P < 0.001$). The disease was marginally more severe on the 9th than 5th leaf. There were significant differences in severity across experiments on the 5th but not the 9th leaf (Table 4). The significant interaction arises again because of the variable effects of fungicide treatment and sanitation across experiments.

(a)



(b)

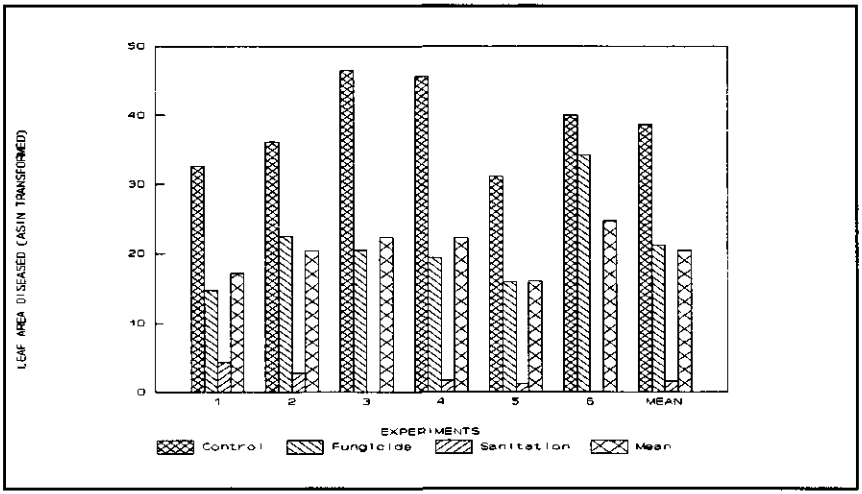
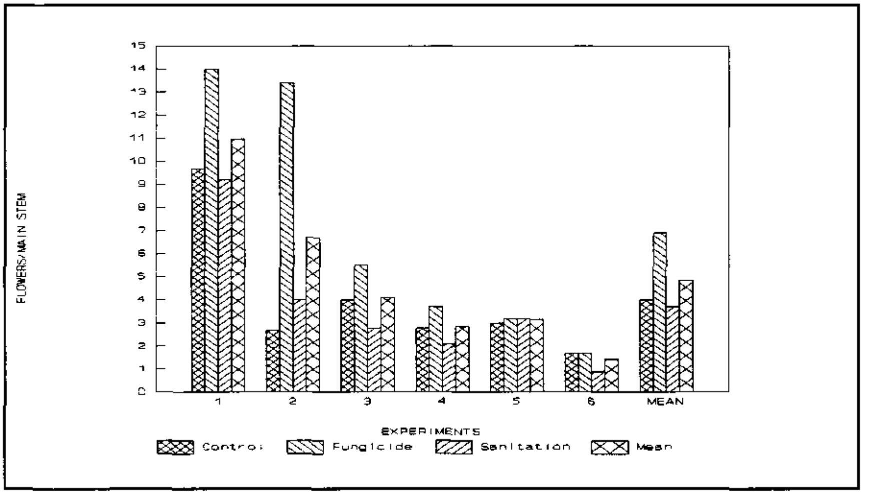


Fig. 4: Late blight severity (angles); (a) on 5th leaf, (b) on 9th leaf

Tomato production

Fungicide treated plots overall had the highest number of flowers and attached fruits in most experiments (Fig. 5). In all the experiments, tomato yield was highest in fungicide plots and least in sanitation plots (Fig. 6). The mean number of flowers, attached fruits and weight of harvested fruits in the sanitation treatments was significantly lower than the fungicide treated plots but did not differ significantly from the control (Table 3). In all production aspects, there were highly significant differences between experiments, although not consistently for each variable (Fig. 5, 6; Table 4).

(a)



(b)

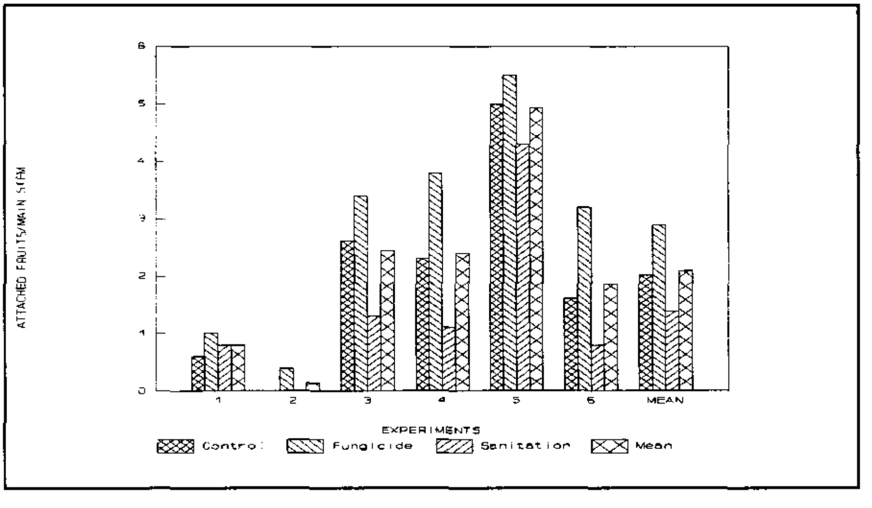


Fig. 5: Tomato production; (a) flowers on the main stem, (b) attached fruits on the main stem.

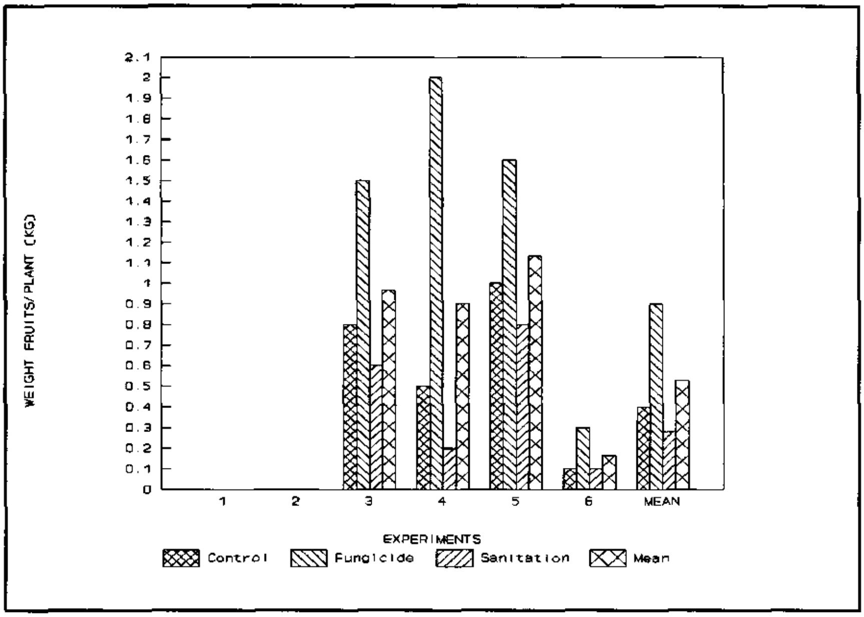


Fig. 6: Weight of tomato harvested in the 6 different experiments

Weather conditions during the experiments

Figure 7 and 8 show the weekly total amount of rainfall, and average minimum and maximum temperatures during the course of the experiments respectively. In all the experiments, rainfall was available and temperature was within the range for late blight disease development.

Table 3: Orthogonal contrast for sanitation effect

Contrast	a=5%	Height	Healthy leaves	Diseased leaves	Disease incidence	Severity 5th leaf	Severity 9th leaf	Flowers	Attached fruits	Weight fruits
Sanitation vs Fungicide	 F: P:	S 17.044 0.002	S 14.353 0.009	S 26.135 0.000	NS 3.104 0.109	S 26.744 0.000	S 39.633 0.000	S 6.284 0.031	S 22.791 0.001	S 8.835 0.014
Sanitation vs Untreated	 F: P:	S 7.456 0.021	NS 0.020	S 68.046 0.000	S 20.869 0.001	S 133.756 0.000	S 133.453 0.000	NS 0.050	NS 4.051 0.072	NS 0.064

Legend: F = F value, P = Probability, S = significant, NS = Not significant

Table 4: Summary of ANOVA indicating sources of variation

Source	a=5%	Height	Healthy leaves	Diseased leaves	Disease incidence	Severity 5th leaf	Severity 9th leaf	Flowers	Attached fruits	Weight fruits
Experiment (A)	 F: P:	S 24.7428 0.0000	S 232.2060 0.0000	NS 1.8473 0.1492	S 13.4750 0.0000	S 19.0811 0.0000	S 4.6550 0.0056	S 17.5410 0.0000	S 32.3000 0.0000	S 30.2520 0.0000
Treatment (B)	 F: P:	S 24.6332 0.0000	S 57.8315 0.0000	S 193.0254 0.0000	S 162.3209 0.0000	S 260.3686 0.0000	S 266.1093 0.0000	S 22.9256 0.0000	S 19.3213 0.0000	S 22.4959 0.0000
Interaction (AxB)	 F: P:	S 2.7919 0.0083	S 5.8673 0.0003	S 5.3294 0.0000	S 14.7366 0.0000	S 3.9258 0.0006	S 3.3486 0.0023	S 5.9561 0.0000	NS 1.6811 0.1129	S 4.8333 0.0001

Legend: F = F value, P = Probability, S = significant, NS = Not significant

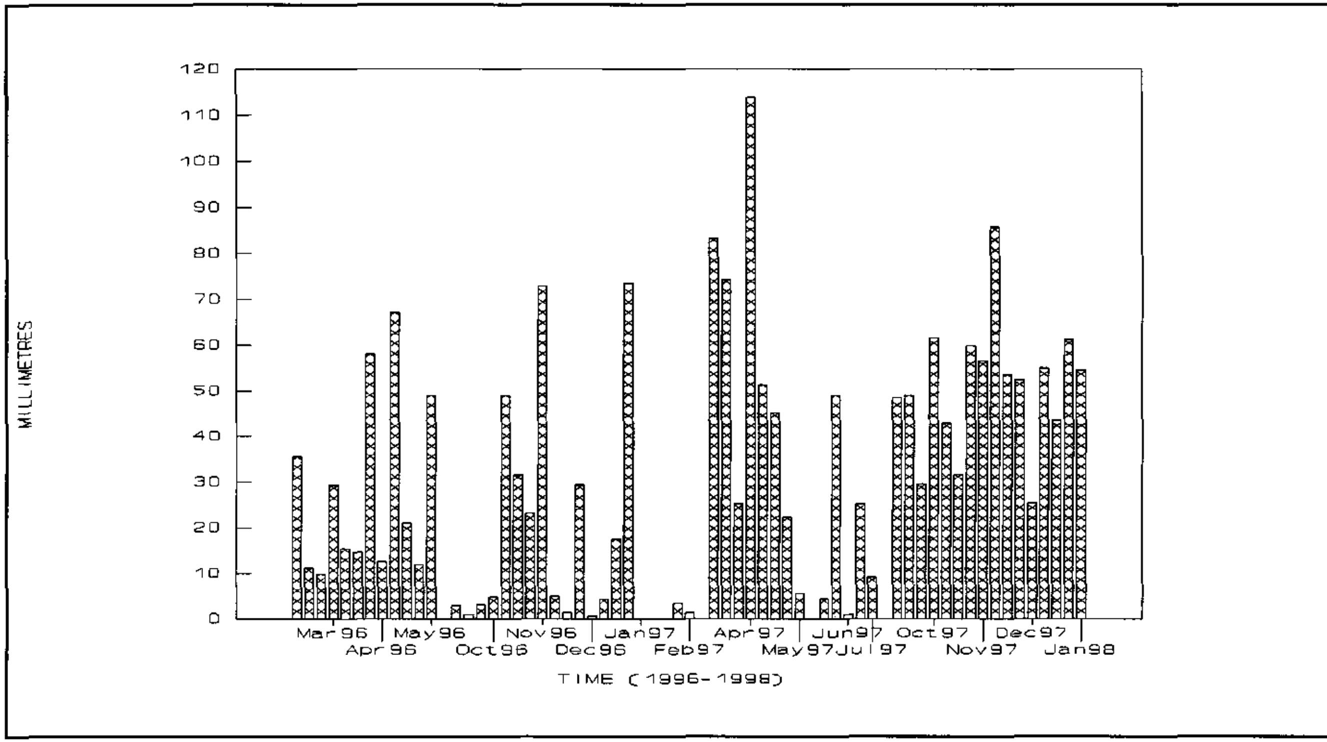


Fig. 7: Weekly total rainfall during the experiments (March 1996-January 1998)

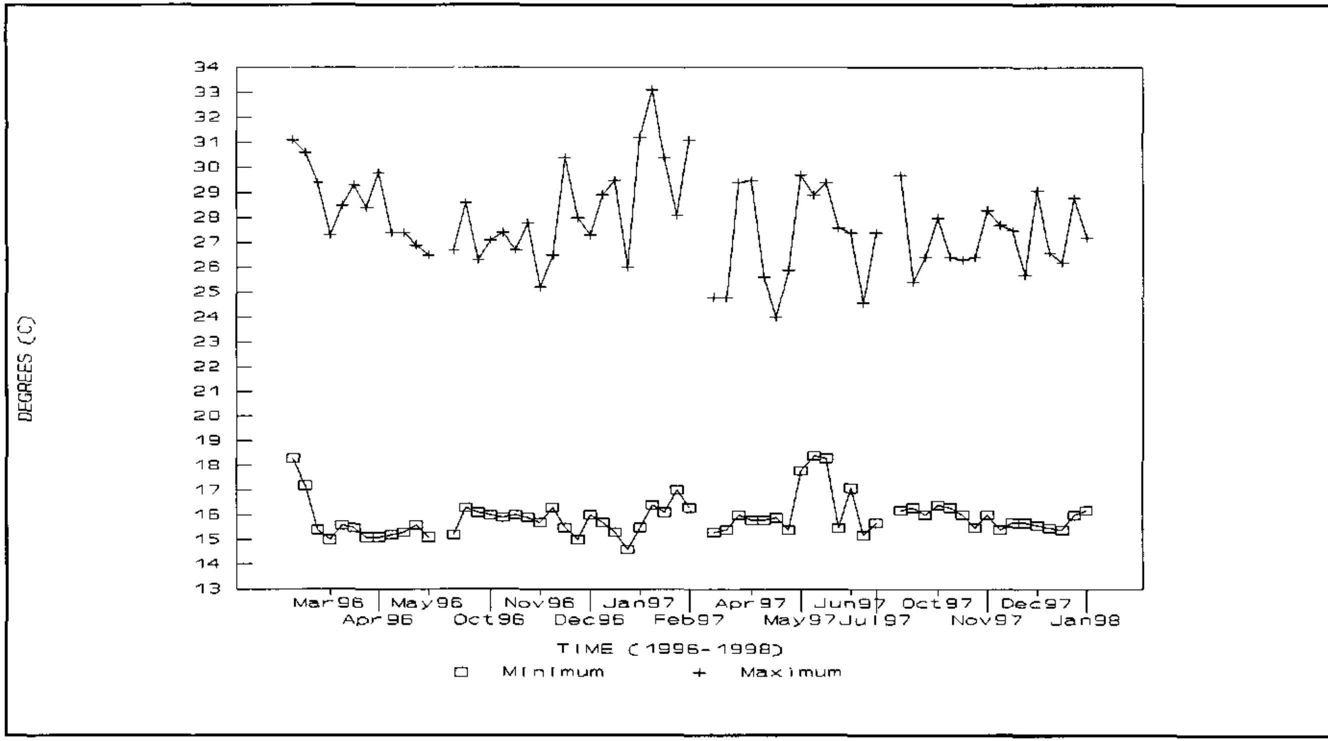


Fig. 8: Weekly average minimum and maximum temperatures during the experiments (March 1996-January 1998)

Discussion

This research has shown that sanitation can significantly lower late blight disease incidences in terms of leaves per plant and plants per plot compared with fungicide. Therefore sanitation can reduce late blight disease incidence as already proved for other diseases such as leaf blight in mulberry (Tomy-Philip *et al.*, 1994) and cylindrocapon dieback of fruit trees (Kachaveli, 1997). The high numbers of diseased leaves in fungicide treated and control plots means there is a high *P. infestans* potential especially under favourable conditions. However, the fungicide treated plots retained the highest number of healthy leaves in all the 4 experiments where it was measured. This could have significant consequences in terms of yield. On average, sanitation plots had the same numbers of healthy leaves as the control plots but had an advantage in lowering disease levels in the field. Removal and destruction of the diseased materials 5 times per week reduced late blight in potato (Fontem, 1995) and could contribute substantially in late blight control in tomato. Late blight severity was least in sanitation plots confirming its importance in reducing initial inoculum and preventing the establishment of primary foci of *P. infestans* (Sherf and Macnab, 1986; Cohen, 1987).

Sanitation as sole means of controlling late blight had a growth retardation effect on tomato plants as reflected in plant height. This could be due to plant reaction to the physical damage inflicted through removal of the diseased parts. Integrating sanitation with other control measures such as the use of fungicides as shown in other diseases (Legard *et al.*, 1997) could possibly reduce its intensity and frequency thus minimizing the deleterious effect caused. The adverse effect of sanitation on plant growth and production was also reflected through fewer flowers, attached fruits and harvested fruits than plants in fungicide plots. The higher performance of the fungicide could be attributed to the higher numbers of healthy leaves which are responsible for carbohydrate assimilation that is vital for production. Although the fungicide was the best in terms of numbers of flowers, attached fruits, and harvested tomatoes, the research indicated that in some cases the fungicides are not able to contain late blight epidemics. This can result into total destruction of the crop and 100% yield loss as earlier reported (AVRDC, 1993). In all the 3 treatments, there were fewer attached fruits than flowers, which means not all flowers form fruits. The low numbers of flowers and fruits in the control could be attributed to the late blight adverse effect on tomato development and production. Weather can have a major effect on late blight epidemiology. It can affect yield as was the case for the first 2 experiments where there was high amounts of rainfall and relatively low temperatures.

Although sanitation as sole means of late blight control had an adverse effect on tomato growth, development and production, it reduced the level of late blight disease on tomatoes (Sherf and Macnab, 1986), as earlier suggested on potatoes (Cohen, 1987; Fontem, 1995). Introducing other cultural control measures, such as polythene shelters and intercropping, can reduce the amount of dispersing *P. infestans* spores in the field. There may be an added effect when sanitation is combined with these cultural measures in integrated control of late blight. Combining sanitation with other cultural measures was proved effective for *Alternaria* blight on *Brassica campestris* (Begum *et al.*, 1995) and stem rot in tobacco (Gutierrez *et al.*,

1997) and could also apply to late blight disease in tomatoes. Other control strategies such as the use of resistant varieties can play an important role in integrated control of late blight in tomatoes. This could possibly reduce the frequency of sanitation which in turn reduces the adverse effect of sanitation on the crops. According to this research, sanitation is a promising control measure against late blight in tomato production as earlier suggested (Inglis *et al.*, 1996). However, it is important to neutralize the deleterious effect of sanitation on the crop in order to utilize its positive effect of reducing late blight disease. In future, sanitation is likely to gain popularity for control of late blight in tomatoes especially if it is integrated with other late blight control practices.

The Effect of Polythene Shelters and Sanitation on Late Blight (*Phytophthora infestans*) Disease in Rain-fed Tomato Cultivation

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Abstract

Late blight disease (*Phytophthora infestans*) is one of the major diseases of tomatoes (*Lycopersicon esculentum*) in Uganda. Tomato growers entirely rely on fungicides in their attempts to control the disease. The fungicides, predominantly Dithane M-45, are expensive, adulterated, have expired label dates or have lost efficacy. Elsewhere, large plastic tunnels have been used for vegetable production under irrigation systems but these are usually expensive to install and are unaffordable by small-scale farmers in Uganda. Research on polythene shelters was directed at finding an integrated, alternative, cheap and environmentally-friendly method of controlling late blight disease. Small polytunnels combined with sanitation, used at different times of the growth period were evaluated against Dithane M-45, sanitation alone and untreated control. The experimental design was a randomized complete block with 5 replications. The results indicated that fungicide treated plants had a high incidence and severity of late blight disease but tomato growth and yield were relatively high. Where fungicide was applied, it delayed epidemics for about 2 weeks. Tomatoes in sanitation alone and late polythene shelters had the shortest plants, and fewer numbers of healthy leaves than those in early shelters and fungicide-treated plots. The numbers of diseased leaves and disease severity were very low in the sanitation alone treatment and sanitation combined with polythene shelters. Late blight disease progress was also low in early shelters, sanitation alone and fungicide treated plots. Sanitation alone had an adverse effect on tomato growth and yield. Flower and fruit production was significantly higher in early polythene sheltered tomatoes than with sanitation alone and a significant increase in yield was obtained. Early polythene shelters reduced the need for and intensity of sanitation, and alleviated its adverse effect in the control of late blight. Early sheltering combined with sanitation could be used to substitute for the fungicide Dithane M-45.

Introduction

Polythene (plastic) tunnels have been used in agriculture for various purposes (Al-Maslamani and Suwwan, 1987; Besri, 1991; May, 1991; Alebeek van and Lenteren van, 1992; Porporato, 1993), mostly under irrigation conditions (Randhawa, 1990). Rain-shelters can enable a more efficient use of fertilizer in tomato cultivation (AVRDC, 1987, 1994). A combination of rain-shelters, fruit-setting hormone and raised beds led to a threefold increase in tomato yield above the control (AVRDC, 1994). Polythene shelters can be used to protect tomatoes from direct rain effects (AVRDC, 1987) and as suntraps in crop production (Varga, 1984). *Nepeta cataria* grown in polythene shelters were taller, with higher fresh and dry matter, and more neral and geranial content of volatile oils than those grown outside (Svoboda *et al.*, 1996). Polythene shelters did not increase production of the endogenous growth regulator IAA (Campen *et al.*, 1996). The growth of *Pinus pinea, Pinus brutia* and *Cupressus sempervirens* in nurseries in Syria was better in polythene tunnels than in the open (Akhmed, 1990). The yield of strawberry was higher under plastic tunnels than in the open (Economides and Gregoriou, 1988). Seedlings of tomato emerged and developed faster in polythene tunnels than in the open (Mittra *et al.*, 1990).

Tomato seedlings raised under Sarkada thatch, (*Saccharum arundinum*), polyethylene bags or polyethylene sheets had better yields than those raised without cover protection (Randhawa, 1990). Unheated tall plastic tunnels could be more economical than heated ones for tomato and pepper production (Gent, 1991).

Damage of Chinese cabbage by *Plutella xylostella* was reduced when grown under plastic covers (Hanada, 1988). The efficiency of the parasitoid *Edovum puttleri* against *Leptinotarsa decemlineata* was enhanced in eggplant under plastic tunnels (Maini, 1990). Leaf blight and *Phytophthora nicotianae* on *Capsicum* and *Phaeoisriopsis griseola* on French bean were low inside polythene greenhouses (Bhatnagar, 1990). Cucumber downy mildew (*Pseudoperonospora cubensis*) was less severe, yields were higher and maturity periods shorter in plastic tunnels than in the open (Guney and Celik, 1986; Milevij, 1991). Muslin tunnels and colour traps delayed the build-up of whitefly (*Bemisia tabaci*) transmitting tomato yellow leaf curl virus (TYLCV) (Al-Musa, 1986; El-Serwiy *et al.*, 1987; Rosset, 1988). Ultraviolet blocking by polythene films reduced insects population and virus diseases in tomatoes and cucumbers (Antignus *et al.*, 1996, 1996). Development of *Sclerotinia sclerotiorum, Botrytis cinerea, Alternaria dauci, A. porri* and *A. solani* on tomato and other vegetables was lower in glasshouses covered with ultraviolet-absorbing vinyl (Honda and Yunoki, 1980; Sasaki *et al.*, 1985). Ultraviolet-blocking films could reduce the use of chemicals in vegetable production (Honda and Yunoki, 1980; Antignus *et al.*, 1996). Sanitation has been combined with ultraviolet-absorbing films to reduce diseases in vegetables (Honda and Yunoki, 1980). Tomatoes grown in polythene greenhouses were protected against frost, and had low disease incidence of *Alternaria solani* and *Septoria lycopersici* (Bhatnagar *et al.*, 1990). Tomato disorders such as fruit cracking were low in polythene covered greenhouses (Chee *et al.*, 1988).

Particular attention has been given to polythene covers in late blight control. Plastic shelters kept tomato infection by late blight low, fungicide sprays were minimal, tomato yields and fruit size were improved (Hanada, 1988). Polythene-film covered greenhouses reduced tomato infection by *P. infestans* (Sunarjono and Hardinah, 1972; Chee *et al.*, 1988). Shelter belts on outdoor irrigated tomatoes increased plant growth and late blight disease was only enhanced where warm and humid conditions were created (Jebari, 1989).

It was hypothesised that transparent polythene shelters introduced at various times in the cropping cycle, combined with sanitation, could protect tomatoes against air-borne *P. infestans* spores. This would in turn reduce the level of late blight disease development and damage on tomatoes. Research was done with the major objectives of evaluating polythene shelters and establishing the best period for their use in late blight management in rain-fed tomato cultivation. Sheltering was also intended to reduce the requirement for sanitation in controlling late blight in tomatoes and to alleviate any side effects as a result of removing diseased leaves and other parts.

Table 2: Orthogonal contrasts for first polythene shelter experiment

Contrast	a=5%	Variable								
	F&P	Height	Healthy leaves	Diseased leaves	Incidence on 30 plants	Severity on 5th leaf	Severity on 9th leaf	Flowers	Attached fruits	Yield
Fungicide vs Untreated		NS	S	S	S	S	S	NS	S	S
	F	1.707	70.098	53.061	45.390	164.733	158.886	3.466	7.510	51.976
	P	0.204	0.000	0.000	0.000	0.000	0.000	0.075	0.011	0.000
Sanitation alone vs Fungicide		S	S	S	S	S	S	S	S	S
	F	19.880	45.675	53.856	42.630	34.257	73.569	10.394	24.678	69.169
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000
All shelters vs Sanitation alone		S	S	NS	S	NS	NS	S	S	S
	F	24.495	11.824	1.154	5.671	0.127	0.004	10.310	15.076	6.105
	P	0.000	0.002	0.293	0.026	-	-	0.004	0.001	0.021
Early shelter (1 & 2) vs Sanitation alone		S	S	NS	S	NS	NS	S	S	S
	F	30.628	14.153	0.779	6.454	0.501	0.579	10.228	15.145	6.862
	P	0.000	0.001	-	0.018	-	-	0.004	-	0.015
Early shelter (1) vs Late shelter (3)		S	NS	NS	NS	NS	NS	S	S	NS
	F	27.419	4.233	0.009	3.225	1.090	1.542	13.088	8.154	4.184
	P	0.000	0.051	-	0.085	0.307	0.226	0.001	0.009	0.052
Early shelter (1 & 4) vs No early shelter (3 & 5)		S	S	NS	S	NS	NS	S	S	S
	F	57.083	18.434	0.824	12.511	0.678	0.191	30.078	30.438	12.352
	P	0.000	0.000	-	0.002	-	-	0.000	0.000	0.002

Legend: F = F value, P = Probability, S = Significant, NS = Not significant; Numbers in brackets refer to treatments

Weather conditions during the first polythene shelter experiment

Figure (8a) shows the weekly amount of rainfall at Kawanda during the first polythene shelter experiment. The rainfall was sufficient for both tomato production and late blight disease development. Figure (8b) shows the Kawanda weekly average minimum and maximum temperatures during the first polythene sheltering experiment. The data indicate that during the course of the experiment, the temperature range was conducive for *P. infestans* infection, growth and sporulation. The temperatures in the tunnels increased far beyond 30 C, which places the tunnel conditions outside the optimum range (4-26 C) for *P. infestans* (Fig. 8c). The high temperatures in the tunnel did not appear to damage tomato plants.

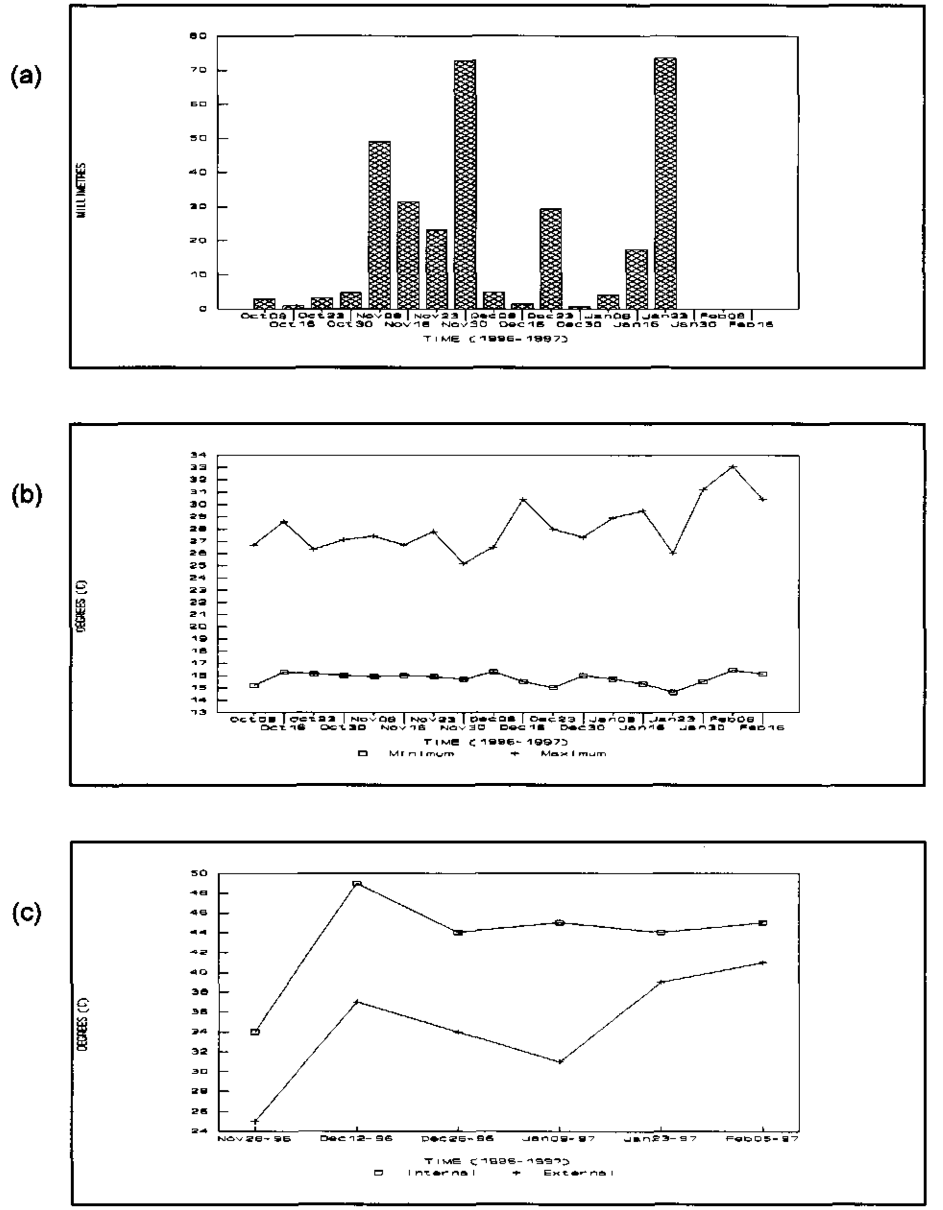

Fig. 8: Weather conditions during the first polythene shelter experiment at Kawanda 1996/97; (a) weekly total rainfall, (b) weekly maximum and minimum ambient temperature, (c) internal and external maximum polythene shelter temperature.

Second polythene shelter experiment (April - July 1997)

Tomato plant height

Mean tomato plant height for the second polythene shelter experiment is shown in Figure 9. There was no significant difference between fungicide-treated plants and those subjected to sanitation alone or in control plots (Table 3). Sanitation alone led to tomato plants significantly shorter ($P < 0.01$) than plants in all shelters combined and early shelters (Table 3). Early shelters (0-2, 1-3, and 0-4 months) gave tomato plants significantly taller than those in late shelter (2-4 months).

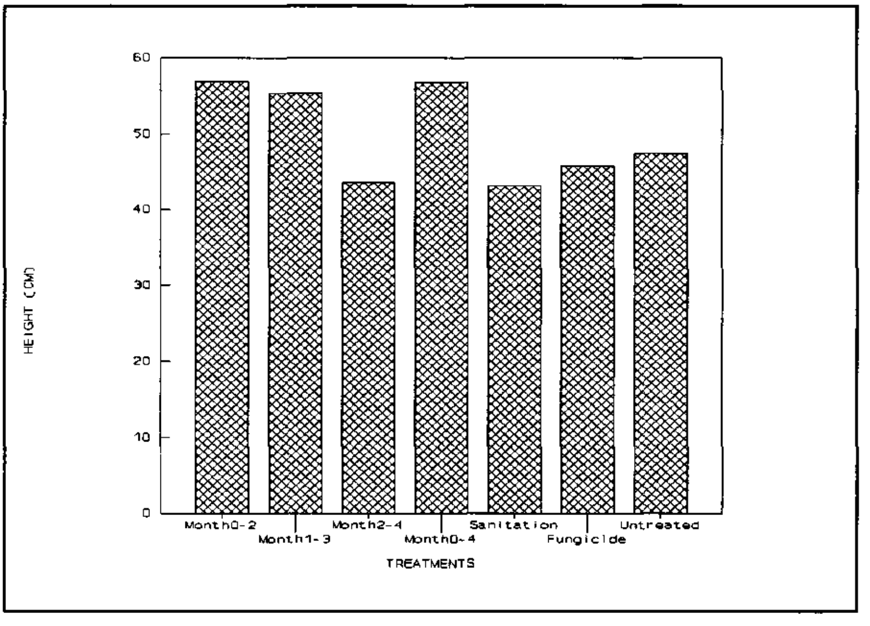

Fig. 9: Mean tomato plant height in the second polythene experiment; significance of pre-determined linear contrasts is shown in table 3.

Late blight disease incidence

Figure 10 shows the mean numbers of healthy and diseased leaves on the main stem. Fungicide treated plants had significantly more healthy and fewer diseased leaves than the control (Fig. 10, Table 3). Tomato plants in the sanitation treatment alone had significantly fewer healthy and diseases leaves than plants in the fungicide treatments ($P < 0.001$). In this experiment the numbers of healthy leaves on early sheltered plants (treatment 1 and 4) was about the same as in the fungicide treatment (Fig. 10). Early sheltered tomato plants had significantly more healthy leaves than late sheltered plants (Fig. 10, Table 3). There were no significant differences in diseased leaves among the shelter treatments (Table 3).

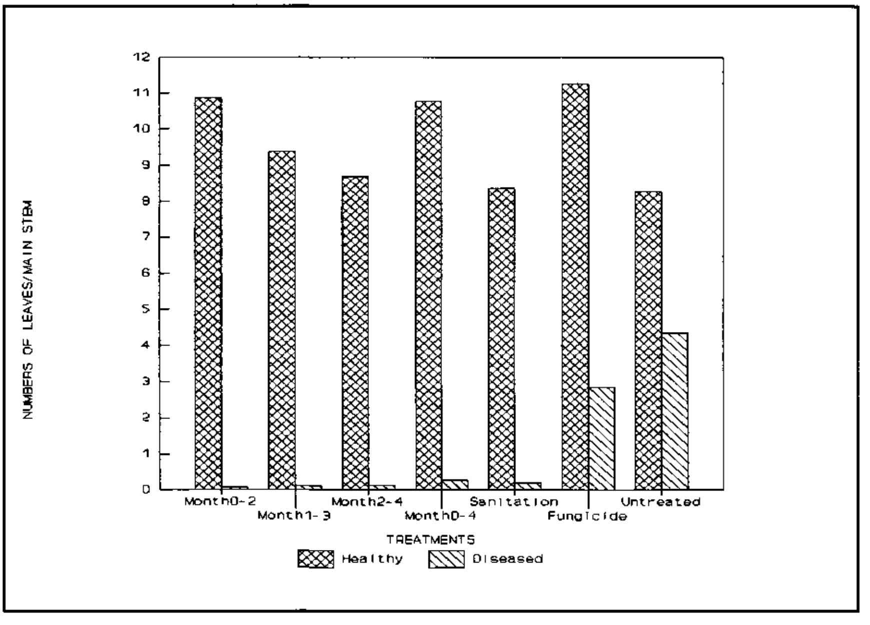

Fig. 10: Late blight effect on leaves (indicating healthy and diseased leaves on the main stem); significance of pre-determined linear contrasts is shown in table 3.

Late blight disease progress

The season was dry a few weeks after transplanting but towards the end of the experiment, it became conducive for late blight epidemics (Fig. 15a). Control plots had the highest incidence of late blight infected plants from the beginning up to the end of the experiment (Fig. 11). Late blight incidence was low during the first part of the season, later resulting in an epidemic indicated by a sharp increase in disease in the control and fungicide plots from June 25 to July 10 1997. At the end of experiment, the control plots were showing 100% late blight incidence. Fungicide plots also rapidly increased from 0 to 40% in the last 2 weeks. At the beginning of the experiment up to the 4th week, early sheltering, late sheltering, and sanitation alone gave similar results to fungicide treatment. Towards the end of the season, there were slight differences in incidence in the shelter plots.

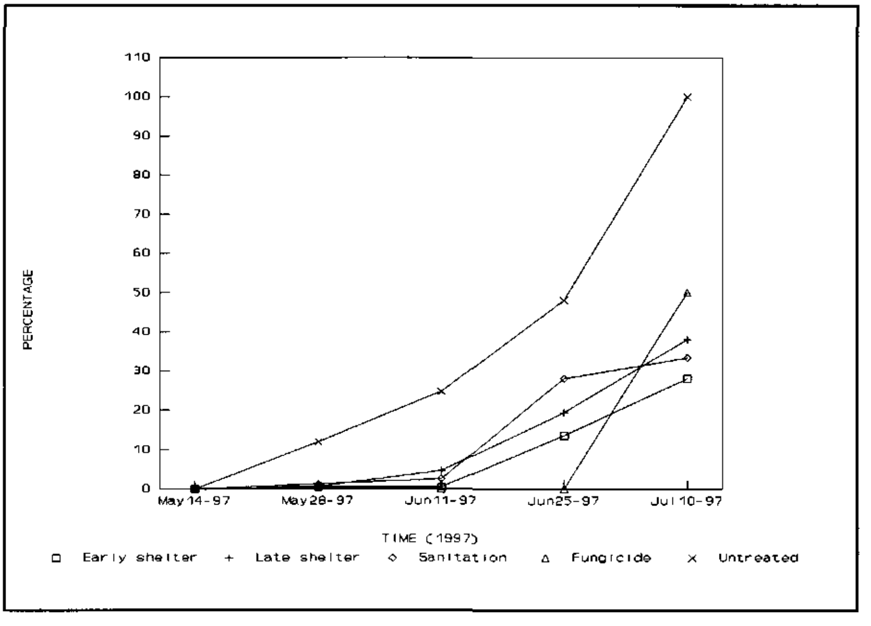

Fig. 11: Late blight progress (% of plants infected per plot) during the second polythene shelter experiment.

Late blight disease severity

Fungicide treatment significantly reduced disease severity (Table 3) compared with the control (P < 0.001) but was less effective than the treatments involving sanitation (Fig. 12, Table 3). Disease severity assessed on the 5th and 9th leaves on the main branch remained very low for all treatments involving sanitation (Fig. 12) and there were no significant differences between these treatments (Table 3). Disease severity was greatest in control plots, and overall the 9th leaf was more severely affected than 5th leaf.

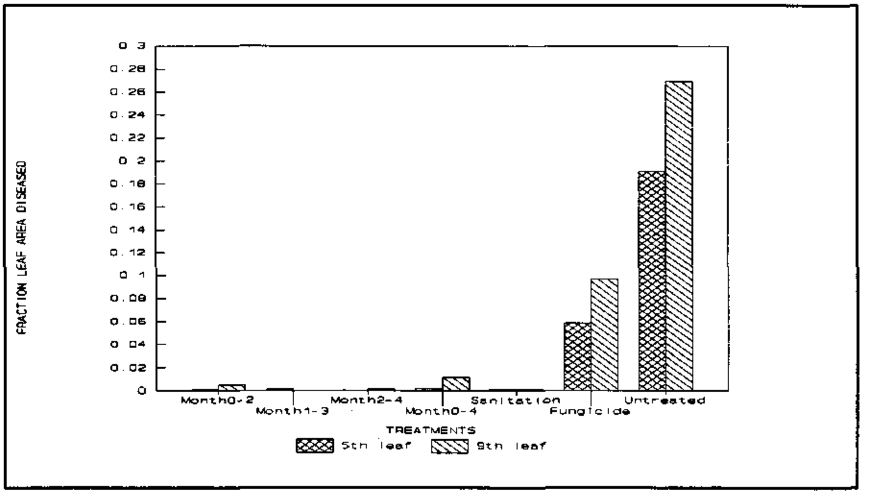

Fig. 12: Late blight severity on the 5th and 9th leaves; significance of pre-determined linear contrasts is shown in table 3.

Tomato production

There were no significant differences in flower production between the fungicide treated plots and the control or sanitation plots (Fig. 13, Table 3). Flower production was significantly higher in treatments with early shelters compared with sanitation alone (P < 0.05), late shelters (P < 0.05), and no early shelters (P < 0.01) (Fig. 13, Table 3). The only significant differences in attached fruits (P < 0.05) was between early shelters and sanitation alone (Table 3). However, the fungicide was again significantly superior to other treatments in terms of plot yield (Fig. 14, Table 3). The weight of harvested tomato was significantly higher for the mean of all sheltered treatments, and early shelters, compared with sanitation alone (P < 0.05).

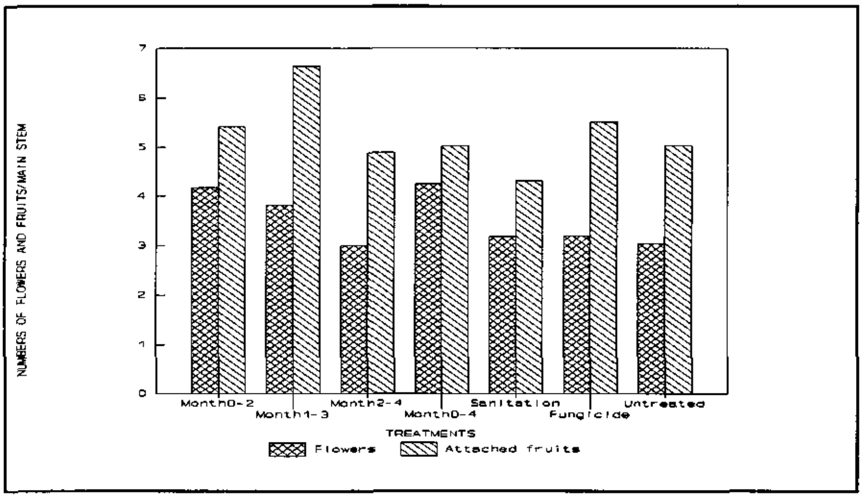

Fig. 13: Late blight effect on tomato production (flower and attached fruits on the main stem); significance of pre-determined linear contrasts is shown in table 3.

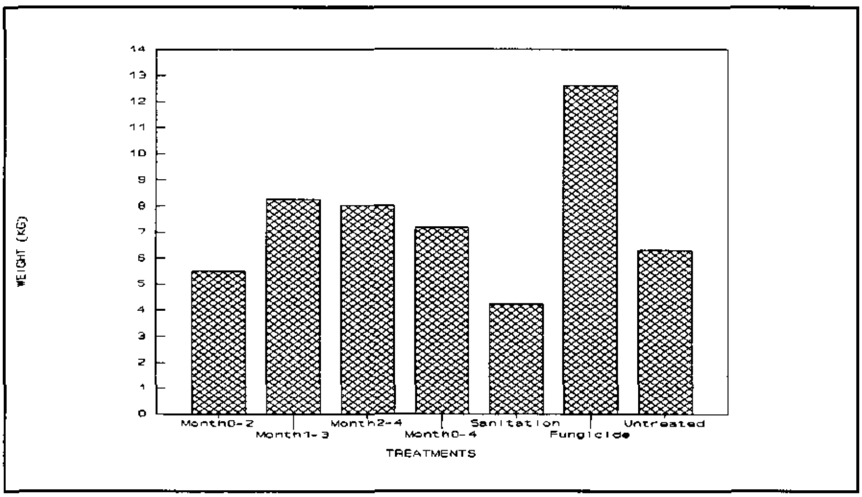

Fig. 14: Weight of harvested tomato per plot of 66 plants in the second shelter experiment; significance of pre-determined linear contrasts is shown in table 3.

Table 3: Orthogonal contrasts for second polythene shelter experiment

Contrast	a=5%	Variable								
	F&P	Height	Healthy leaves	Diseased leaves	Incidence on 30 plants	Severity on 5th leaf	Severity on 9th leaf	Flowers	Attached fruits	Yield
Fungicide vs		NS	S	S	S	S	S	NS	NS	S
Untreated	F	0.235	21.997	14.523	8.055	63.544	26.835	0.108	0.319	5.732
	P	-	0.000	0.001	0.009	0.000	0.000	-	-	0.025
Sanitation alone vs		NS	S	S	NS	S	S	NS	NS	S
Fungicide	F	0.585	20.674	44.549	0.787	63.544	25.941	0.000	1.969	11.235
	P	-	0.000	0.000	-	0.000	0.000	-	0.173	0.003
All shelters vs		S	S	NS	NS	NS	NS	NS	NS	S
Sanitation alone	F	13.812	9.550	0.045	0.653	0.034	0.092	3.523	3.022	6.047
	P	0.001	0.005	-	-	-	-	0.072	0.095	0.022
Early shelters (1 & 2) vs		S	S	NS	NS	NS	NS	S	S	S
Sanitation alone	F	19.360	10.031	0.124	0.009	0.011	0.017	4.965	5.348	6.820
	P	0.000	0.004	-	-	-	-	0.035	0.030	0.015
Early shelters (1) vs		S	S	NS	NS	NS	NS	S	NS	NS
Late shelters (3)	F	15.328	11.477	0.022	0.926	0.039	0.040	7.513	0.389	0.013
	P	0.001	0.002	-	-	-	-	0.011	-	-
Early shelters (1 & 4) vs		S	S	NS	NS	NS	NS	S	NS	NS
No early shelter (3 & 5)	F	31.391	25.678	0.000	0.485	0.360	0.957	13.681	1.055	1.700
	P	0.000	0.000	-	-	-	-	0.001	0.315	0.205

Legend: F = F value, P = Probability, S = Significant, NS = Not significant; Numbers in brackets refer to treatments

Weather records in the second polythene shelter experiment

There was sufficient rain in May during tomato seedling transplanting and field establishment (Fig. 15a). Thereafter, rainfall dwindled up to late May, rendering the conditions too dry for late blight epidemic. However, during the latter part of June and again in July, there were large quantities of rain. During those periods, the weather conditions were again favourable for late blight development. Temperatures were in a suitable range (optimum 20 C) for late blight disease development (Fig. 15b). For much of the experimental period, internal shelter temperatures were higher than external ones, both in the morning and afternoon (Fig. 15c). Around mid June, the morning temperatures decreased and increased thereafter while the afternoon temperatures remained high, with internal temperatures far beyond that which inhibits *P. infestans* growth (29 C). This lasted till the end of June. Both morning and afternoon temperatures dropped at the end of June and increased at the beginning of July. Relative humidity both inside and outside the shelters was too low to favour late blight infection (R.H. close to 100%), especially in the afternoon (Fig. 15d). Ambient temperature was high during the first part of the experiment (Fig. 15c), and relative humidity in the shelters was lower than outside (Fig. 15d), therefore not favourable for disease development. This was reversed at the end of June and in July, especially for morning relative humidity.

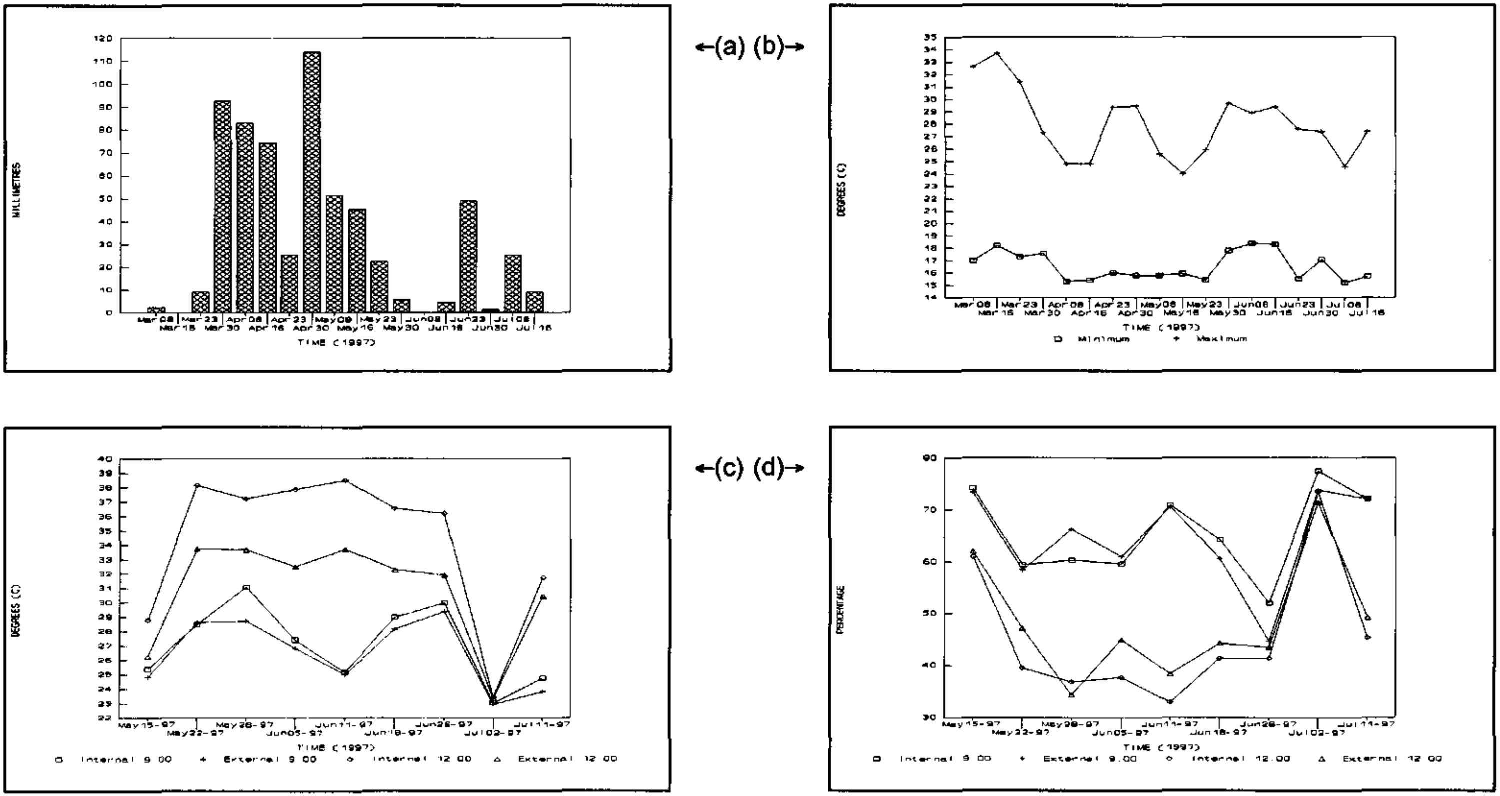

Fig. 15: Weather conditions during the second shelter experiment at Kawanda 1997; (a) weekly total rainfall, (b) weekly minimum and maximum ambient temperature (c) mean field temperature recorded by a thermohygrometer, (d) relative humidity.

Discussion

In both experiments, the fungicide treatment delayed late blight epidemics by about 2 weeks compared with the control and resulted in more late blight than any of the sanitation combined with sheltering treatments. However, yields per plot in fungicide treatment were superior in both experiments. The numbers of healthy leaves in sheltered treatments also remained relatively high (especially in the second experiment). This suggests that diseased leaves continue to contribute to yield accumulation in fungicide treated plots, and that, combined with the delayed epidemic, accounts for its performance.

Sanitation alone, even though maintaining low disease incidence and severity, stunted plant growth, and resulted in lower yields per plot than the control. However, sanitation could be combined with shelters to overcome the adverse effect of sanitation (Honda and Yunoki, 1980). Beneficial results of combining rain-shelters with other factors such as fruit set hormone and raised beds for tomato production was previously reported (AVRDC, 1994). Late sheltering combined with sanitation maintained low disease incidence and severity, but resulted in reduced plant growth, and low numbers of flower, attached fruits and yield, especially in the first experiment. This means that *P. infestans* spores had access to the plants because of the absence of a barrier in the early growth stages, necessitating a high intensity of sanitation. This resulted in similar effects as sanitation alone on crop growth and production. Late shelters led to better good yields in the second experiment, possibly because of environmental factors that did not favour early late blight disease development.

Early shelters combined with sanitation had a positive effect on plant growth compared to sanitation alone, and maintained relatively high numbers of healthy leaves on plants especially in the second experiment. Diseased leaves and severity were always lowest in these treatments. Early polythene shelters could have excluded *P. infestans* spores (Hanada, 1988; Bhatnagar *et al.*, 1990). This shows that shelters can effectively reduce late blight disease on tomatoes as previously reported (Sunarjono and Hardinah, 1972; Chee *et al.*, 1988; Hanada, 1988). Statistically, combined sheltering with sanitation, and early shelters were always significantly better than sanitation alone except for diseased leaves and severity. In most cases, there were no significant differences within the sheltered plant treatments, presumably because of the effect of sanitation on disease development and progress. The most remarkable aspect on the use of shelters to control late blight is the significantly better effect of early shelters on plant growth and production compared with sanitation alone. Early shelters combined with sanitation were equally as good as the fungicide treatment in numbers of flowers and attached fruits, although tomato plot yields were always highest in fungicide treatments. However, latent infections were not tested in this study and possibly some of the fruits from plants in the open, surrounded by high *P. infestans* inoculum (including fungicide-treated plots), could have been infected. Latent infection can affect postharvest quality of the tomatoes.

There was also a general observation of less insect damage in sheltered plots than those in the open. The fruits from sheltered plots appeared cleaner but were slightly smaller than those from the plants which were not sheltered. This suggests that possibly the sheltered plants did receive as much water as those in the open. High temperatures recorded inside the shelters could have had an effect in reducing

P. infestans performance as anticipated. Relative humidity under shelters was high in the morning decreased by noon far below the optimum (close to 100%) for *P. infestans* infection. Therefore the shelters did not create humid conditions that could enhance late blight disease in tomatoes (Jebari, 1989). This could mean that during sunny days, late blight development is retarded because of high temperatures (beyond 29 C) and low relative humidity. The polythene shelters could also have other beneficial attributes, such as blocking ultra-violet radiation, known to reduce levels of other diseases (Honda and Yunoki, 1980; Sasaki *et al.*, 1985). Therefore, simple shelters can be used to protect tomatoes from air-borne *P. infestans* spores (AVRDC, 1987; Picco, 1992).

According to the results of the polythene shelter experiments in Uganda, the following conclusions can be made.

(a). The fungicide (Dithane M-45) did not stop late blight development but delayed the epidemic for about 2 weeks.

(b). Fungicides provided the highest yields per plot despite high disease levels and not necessarily the highest numbers of flowers and attached fruits.

(c). Sanitation alone had an adverse effect on tomato plant growth.

(d). Shelters may help to alleviate the adverse effects of sanitation and could substitute for fungicide in the control of late blight, especially when shelters are used early in the growing season.

Chapter 6

The Effect of Intercropping and Sanitation on Tomato Late Blight (*Phytophthora infestans*) Disease Epidemiology

Submitted to Plant Pathology

The Effect of Intercropping and Sanitation on Tomato Late Blight (*Phytophthora infestans*) Disease Epidemiology

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Abstract

Intercropping is widely practised in subsistence agriculture in the tropics. Intercropping reduces plant disease severity by limiting inoculum dispersal through decreasing wind velocity, reducing susceptible tissue and increasing distances between plants of the same species. The major aim of intercropping experiments in Uganda was to identify crops which were compatible with and reduced late blight in tomato polyculture. The experiments compared the effect of sanitation on tomatoes intercropped with sorghum (*Sorghum bicolor*), sunflower (*Helianthus annuus*), soybean (*Glysine max*) and sesame (*Sesamum indicum*), and tomato (*Lycopersicon esculentum*) monoculture, with sanitation alone, fungicide treatment (Dithane M-45) and an untreated control. The results indicated that tomato plants were always shorter than intercrops, which provided a barrier against dispersing spores of *P. infestans*. Control plots had the highest numbers of diseased leaves, disease incidence and severity. Fungicide treatment gave the highest numbers of healthy leaves, flowers, attached fruits and yield, and delayed late blight epidemics for 2-4 weeks. Tomato production was also substantial with soybean and sesame intercropping in comparison with the fungicide treatment. Soybean and sesame were compatible with tomatoes at 60 cm tomato-intercrop between-row spacing, and late blight severity was limited to some extent. Sorghum and sunflower had a suppressive effect on tomato growth and production at the used tomato-intercrop row spacing. According to the experiments, soybean and sesame could be intercropped with tomatoes, as part of an integrated late blight management practice in polyculture cropping system.

Introduction

Intercropping of crop plants is a common cultural practice in many developing countries in the tropics. Amongst its advantages are the effects on the population dynamics of pests that may minimise crop damage (Perrin *et al.*, 1978). Intercropping also ensures food security, and additional income to farmers by reducing the risk of crop failure. Although intercropping can cause yield reductions (Afifi and Haydar, 1990), arthropod pests in various crops (Lawani, 1982; Rathore and Lal, 1994) and weeds (Bridgemohan, 1993) can be controlled. Intercropping reduces insect damage due to diverse sources of food and shelter which is advantageous for the build up of natural enemies (Huis van, 1990). According to Huis van (1990), the distances between plants of the same species is increased in polyculture, reducing the chances of the insects to move to similar plants. Intercropping may also favour biological control by enhancing the performance of insect pathogenic fungi (Maniania, 1991). Row orientation plays an important role as a barrier against pests migrating towards host crop (Leon *et al.*, 1997). Greater height of maize was suggested to shelter soybean from insect infestation and dry winds (Cervancia and Rejesus, 1984). Community diversity reduced *Empoasca kraemeri* and *Hortensia similis* in *Phaseolus vulgaris* and tomatoes (Power *et al.*, 1987). Tomatoes and other crops when intercropped with cabbage reduced diamondback moth (*Plutella xylostella*) on cabbage (Sivapragasam, 1982; Chelliah and Srinivasan, 1986; Talekar *et al.*, 1986;

AVRDC, 1987; Bach and Tabashnik, 1990). Intercropping tomatoes with onion or garlic reduced the levels of *Bemisia tabaci, Myzus persicae* and *Phthorimaea operculella* on tomatoes (Afifi *et al.*, 1990) and thrips (*Thrips tabaci*) on onion and garlic (Afifi and Haydar 1990). Potato intercropped with tomato, onion, maize, soybean or bean (*Phaseolus*) had less tuber damage by *Phthorimaea operculella* (Raymundo and Alcazar, 1983). Infestation of cowpea (*Vigna unguiculata*) by *Empoasca dolichi* and *Heliothis armigera* was significantly reduced by intercropping with tomato (Ofuya, 1991). Intercropping tomato with beans largely reduced the damage of tomatoes by *Heliothis* spp, *Spodoptera* spp and *Liriomyza satiae* (Rosset *et al.*, 1987; Rosset, 1988). The incidence of pests and diseases was low in tomato intercropped with maize (Pino *et al.*, 1994; Picanco *et al.*, 1996). Intercropping proved to be useful in integrated control of pests in sugar cane (Liu *et al*,. 1985) and other graminaceous crops (Reddy, 1990). Intercropping tomatoes with sorghum controlled whiteflies (the vector of golden mosaic virus) and had the best yields and effects on arthropod predators (Gravena *et al.*, 1984). Tomatoes grown with cucumber or *Capsicum* delayed the build-up of whitefly (*Bemisia tabaci*) transmitted tomato yellow leaf curl virus (TYLCV) (Al-Musa, 1986; El-Serwiy *et al.*, 1987).

Intercropping can affect disease incidence and severity (Boudreau and Mundi, 1992; Emebiri and Obiefuna, 1992). The intercropping effect on plant diseases is attributed to a reduced wind dispersal of spores due to barrier effects (Boudreau, 1993) or increased distance between plants of the same species (Huis van, 1990) which also limits inoculum dispersal (Gretna, 1983). Disease can be greatly influenced by intercrop phenology and chemicals produced by the intercrop especially for vector transmitted diseases.

Intercropping *Tagetes* spp with tomato can be effective in controlling the nematodes; *Meloidogyne* spp, *Rotylenchulus reniformis* and *Tylenchorynchus brassicae* on tomatoes (Siddiqui and Alum, 1987; Salem and Osman, 1988; Abid and Maqbool, 1990; Jain *et al.*, 1990; Ali *et al.*, 1995). *Zinnia elegans* (Tiyagi *et al.*, 1986; Owino and Mousa, 1994; Yassin and Ismail, 1994, 1995), *Azadirachta indica* and *Melia azedarach* (Siddiqui and Saxena 1987) intercrops can be effective against *Meloidogyne* spp and *Rotylenchulus reniformis* on tomatoes. *Meloidogyne* spp on tomatoes were reduced by intercropping with *Concanavalia ensiformis, Macuna deeringiana, Pueraria phaseoloides, Arachis pintoi* (Marban-Mendoza *et al.*, 1989, 1992) and garlic (Ali *et al.*, 1995). Intercropping Pepper with *Tagetes patula, Crotalaria* or maize reduced the reproduction factor of *Meloidogyne* spp (Martowo and Rohana, 1987). Intercropping tomato with cucumber and late sowing can control *Meloidogyne* spp (Hanna *et al.*, 1996).

Intercropping tomato with cowpea reduced the area under the disease progress curve of bacterial wilt (*Pseudomonas solanacearum*) on tomato and its incidence on potato (AVRDC, 1994; Michel *et al.*, 1997). According to AVRDC (1994), intercropping tomato with cowpea could be used in integrated pest management in the tropics to control bacterial wilt. Bud necrosis disease in groundnuts by tomato spotted wilt virus was reduced when intercropped with pearl millet (Ghaneker, 1980). The incidence of ring mosaic, late leaf spot (*Mycosphaerella barkeleyi*) and rust (*Puccinia arachidis*) were reduced on ground nuts intercropped with sorghum (*Sorghum bicolor*) and cumbu (*Pennisetum glaucum*) but not sunflower (Ganapathy and Narayanasamy, 1991). The incidence and severity of *Alternaria* leaf spot (*Alternaria alternata*) on faba bean, and rust (*Puccinia sorghi*) on maize, were reduced in a maize/bean intercrop

(Shairaha *et al.*, 1989). Intercropping tomato with *Tagetes erecta* reduced foliar damage and incidence of *Alternaria solani* (Zavaleta-mejia and Gomez, 1995). Tomato intercropped with cauliflower had better yields than those not intercropped (Randhawa, 1990). The incidence and severity of late blight on potato (*Solanum tuberosum*) were reduced when potatoes were intercropped with faba bean and corn (Sharaiha *et al.*, 1989).

Intercropping experiments were carried out in Uganda with the aim of determining the effect on late blight in tomatoes by introducing non-host plants in the polyculture. Intercropping was also intended to reduce the requirements for sanitation in controlling late blight and to alleviate any side effects as a result of removing diseased tomato leaves and other parts.

Materials and methods

Experimental design

The experiment consisted of 7 treatments (Table 1) in a randomised complete block design, with 5 replications, and was carried out twice. Experimental units were plots of 5 m by 4 m surrounded by a 3 m space with a tomato spreader plant at each corner, 2 m distant. The intercrops could be separated into tall (sorghum and sunflower) and short (sesame and soybean) classes.

Table 1: Treatments used in intercropping experiments

Treatment	Description
1	Tomato + sorghum (*Sorghum bicolor*) + sanitation
2	Tomato + sunflower (*Helianthus annuus*) + sanitation
3	Tomato + soybean (*Glysine max*) + sanitation
4	Tomato + sesame (*Sesamum indicum*) + sanitation
5	Tomato + sanitation
6	Tomato + fungicide (Dithane M-45/mancozeb)
7	Tomato, untreated (control)

Field establishment

Tomato cultivar MT55 was used in the experiments because it is susceptible to late blight but resistant to bacterial wilt. Tomato seedlings were raised in nurseries for approximately 4 weeks. The tomato nurseries were protected against late blight infection by spraying them with Dithane M-45 at a recommended dosage of 2.5 grams per litre, at intervals of 7 days. The seedlings were raised during dry weather which together with the fungicide treatments ensured healthy and uniform seedlings for the experiments. The main experimental fields were marked out by use of 30 cm pieces of reeds and sisal string at the time of transplanting. The intercrops were planted 3 weeks before transplanting tomatoes at 120 cm between rows. The within-row spacings were 10 cm, 25 cm, 5 cm and 10 cm for sorghum (Sekedo), sunflower (New Sunfola), soybean (Nam 1) and sesame respectively. Four week tomato seedlings were transplanted with an average height of 15 cm to the middle of the intercrop rows (Fig. 1). Spacings for tomatoes were 0.35 m intra-row and 1.2 m inter-row. A total of 44 tomato seedlings per plot and 48 spreader plants were transplanted into the experimental field. The entire experimental field was surrounded by a guard maize (*Zea mays*) crop. Mulching with spear grass (*Imperata cylindrica*) was carried out in all the experimental plots. The first experiment was planted on 14 April 1996 and the second one on 28 October 1996.

Fig 1: Tomato plants intercropped with sesame (*Sesamum indicum*)

Field inoculation

Two weeks after transplanting, late blight infected leaves were obtained from a tomato plant at Kawanda Agricultural Research Institute. The diseased leaves were detached from the plants and taken to the laboratory. Infected leaflets were incubated in 5 petri dishes of 94 mm diameter for 48 hours at room temperature. The petri dishes contained moist cotton wool which provided high humidity to enhance sporulation. The spores were washed off with 500 ml water and placed in a 1.5 litre capacity hand sprayer. Tomato spreader plants were artificially inoculated with *P. infestans* spores using the hand sprayer. The field inoculation was carried out in the evening to ensure cool conditions facilitating infection. Five days after inoculation, the spreader plants started showing late blight symptoms and sporulation.

Field management

Diseased leaves, flowers, fruits or branches in treatments 1-5 were carefully removed with scissors into a 10 litre capacity plastic bucket and disposed of far from the field. Strict measures were observed when removing the infected materials to avoid shaking the diseased parts during sanitation as this would enhance spread of the disease. Sanitation was done every 2 days from the beginning to the end of each experiment. In the fungicide plots Dithane M-45 at a dosage of 2.5 grams per litre was applied to run-off at intervals of 7 days using a 15 litre capacity knapsack sprayer. To prevent insect pests, Dursban at a dosage of 18.5 ml per litres of water was sprayed to run-off using a knapsack sprayer, and weeding was done regularly.

Assessment

Two weeks after transplanting, 6 plants per plot were randomly chosen and marked for field assessments. The variables assessed were plant height, numbers of healthy (second experiment) and diseased leaves, disease incidence, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and weight of harvested fruit. Disease incidence was assessed on 30 randomly chosen tomato plants per plot while the other variables were assessed on the main stem of the marked plants, at intervals of 14 days. Environmental conditions during the experiments were recorded at the Kawanda Agricultural Research Institute weather station.

Statistical analyses

The data were analyzed using two way analysis of variance and pre-determined linear contrasts at 5% significance level. The contrasts chosen were: fungicide versus control, fungicide versus sanitation, mean of all intercrops combined with sanitation versus sanitation, and tall versus short intercrops (Table 2 and 3).

Results

First intercropping experiment (April - May 1996)

Tomato and intercrop development

Crop development was studied through time (Fig. 2). At any stage of crop development, sunflower and sorghum (tall intercrops) were taller than tomato plants, in terms of stem length. Sesame and soybean (short intercrops) had plants of about the same height as tomato. Maturing tomato plants had a growth habit of laying on the ground, a factor which could contribute to protecting the plants from dispersing spores. Fungicide treated tomatoes were taller than those in the control or sanitation treatments. Tomato plant height was not significantly affected by any of the intercrops (Table 2).

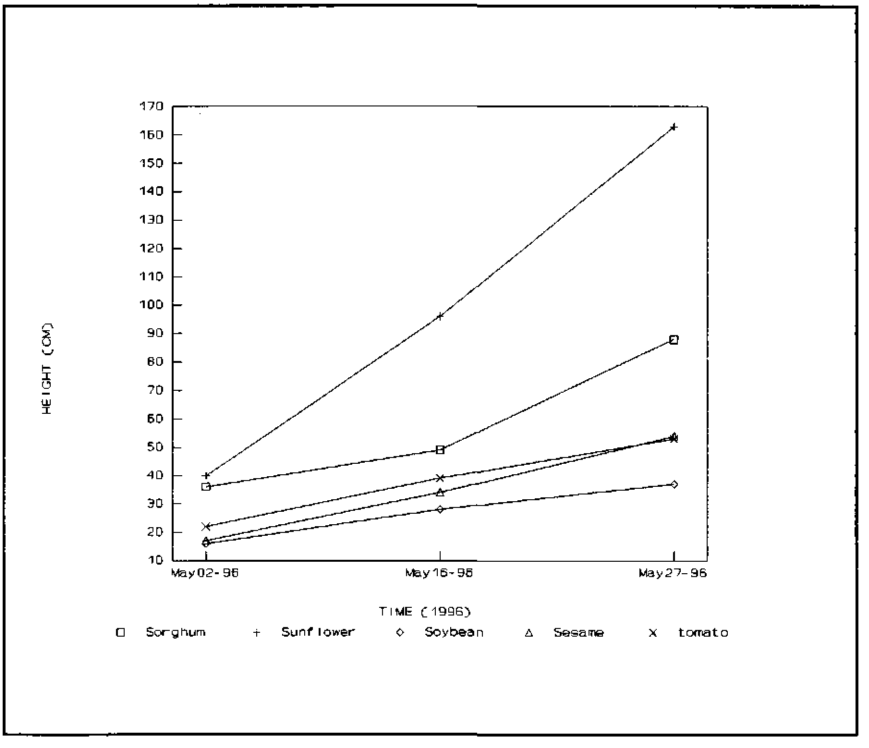

Fig. 2: Crop growth measured by progressive plant height during the experimental period.

Late blight disease incidence

There was no significant difference between the fungicide treatment and the control but the number of diseased leaves was significantly higher than in the sanitation treatment. Healthy leaves were not assessed in the first experiment. There were no significant differences in the mean numbers of diseased leaves between sanitation alone and sanitation combined with intercrops (Fig. 3, Table 2).

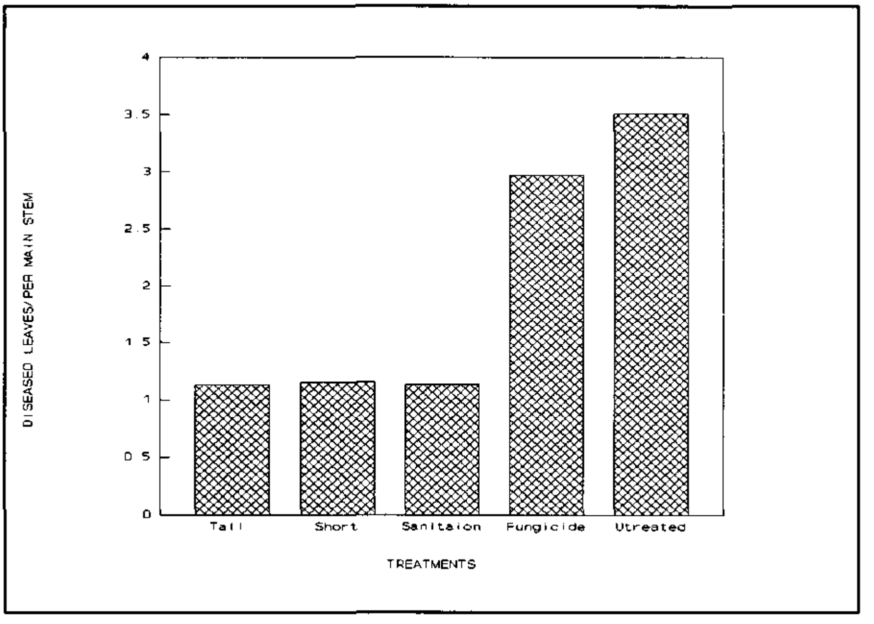

Fig. 3: Late blight effect on leaves (mean numbers of diseased leaves on the main stem during the period of assessment); significance of pre-determined linear contrasts is shown in Table 2.

Late blight disease progress

Fungicide treatment was significantly better than the control and sanitation alone ($P < 0.001$). Late blight progress in terms of plot incidence indicated that the fungicide treatment was superior to the other treatments (Fig. 4). There were no significant differences between sanitation alone and the intercrops, or between tall and short intercrops (Table 2). Short intercrops had significantly fewer diseased plants per plot than the control. Apart from fungicide treated plots, disease incidence reached 100% by May 30th 1996. Figure 4 shows that the fungicide treatment did not stop the late blight epidemic but delayed it for about 4 weeks. The weather became very favourable to late blight development in May, due to considerable rain and an optimum temperature range (Fig. 7). This culminated in conditions that were so conducive that the recommended fungicide dosage could not contain the disease and the experiment was terminated.

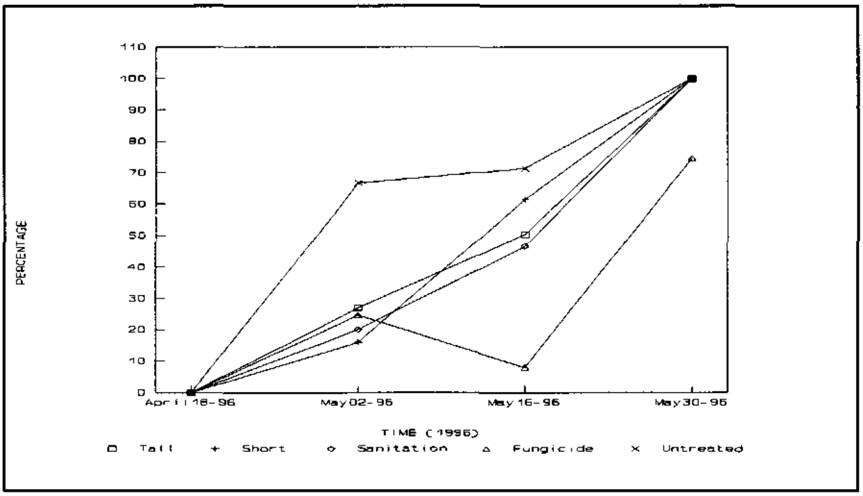

Fig. 4: Late blight disease progress (% of on tomato plants infected per plot) during the growth period in the first intercropping experiment.

Late blight disease severity

Tomato plants in the control plots had significantly ($P < 0.001$) higher disease severity on the 5th and 9th leaves than the fungicide treatment (Fig. 5, Table 2). Sanitation gave significantly lower disease severity on the 5th ($P < 0.001$) and 9th ($P < 0.05$) leaves than the fungicide treatment (Table 2). However, there were no significant differences in leaf severity on either leaf between sanitation alone and sanitation combined with intercropping (Table 2).

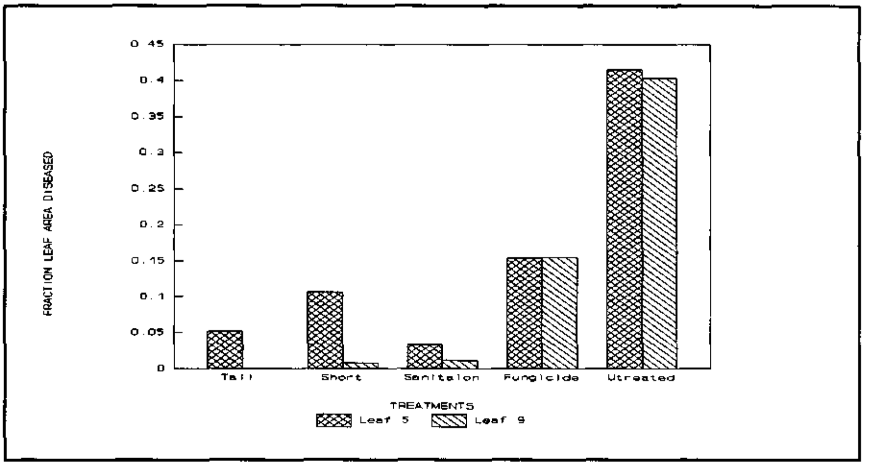

Fig. 5: Late blight disease severity on the 5th and 9th leaves on the main stem of tomato plants; significance of pre-determined linear contrasts is shown in Table 2.

Tomato production

Fungicide treated plots had the highest numbers of flowers (Fig. 6), significantly ($P < 0.001$) more than the control or sanitation plots (Table 2). Tomatoes intercropped with short plants had significantly more flowers than those with tall intercrops ($P < 0.05$). Only tomato intercropped with short intercrops and those treated with fungicide managed to set fruit. No plot yield data were obtained.

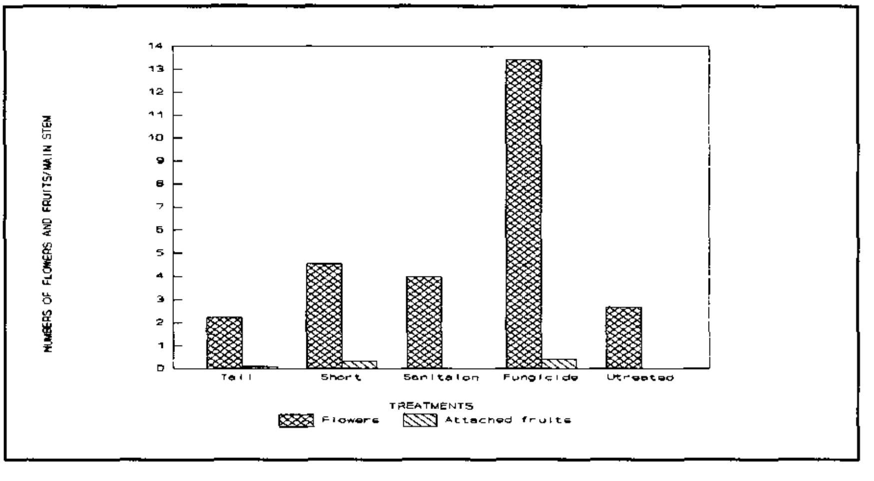

Fig. 6: Late blight effect on tomato flower and fruit production; significance of pre-determined linear contrasts is shown in Table 2.

Table 2: Orthogonal contrasts for the first intercropping experiment

Contrast	*a* = 5%	Variable					
	F & P	Height	Diseased leaves	Disease incidence	Severity on 5th leaf	Severity on 9th leaf	Flowers
Fungicide vs		S	NS	S	S	S	S
Untreated	F	15.994	3.214	101.451	53.562	6.234	65.865
	P	0.001	0.086	0.000	0.000	0.020	0.000
Fungicide vs		S	S	S	S	S	S
Sanitation alone	F	6.872	36.531	17.326	27.084	12.662	51.045
	P	0.015	0.000	0.000	0.000	0.002	0.000
Intercrops/sanitation vs		NS	NS	NS	NS	NS	NS
Sanitation alone	F	0.140	0.002	0.935	3.415	0.102	0.305
	P	-	-	-	0.077	-	
Tall vs Short intercrops		NS	NS	NS	NS	NS	S
	F	0.011	0.011	0.000	0.874	0.240	6.076
	P	-	-	-	-	-	0.021

Legend: F = F value, P = probability, S = Significant, NS = Not significant

more flowers than tall plants which implies a suppressive effect of tall plants shadowing tomatoes. The tall intercrops (sorghum and sunflower) suppressed tomatoes at the tomato-intercrop spacing of 60 cm used in the study presumably because of shading effect of the tall intercrops. This suggests that short intercrops (soybean and sesame) were more compatible with tomatoes at the used spacing. Row spacing between intercrops is therefore important as this influences plant competition for light and nutrients. Therefore intercropping tomatoes with a wrong crop or at a wrong spacing can have suppress growth and this can affect tomato yield as previously identified (Afifi and Haydar, 1990). Multiple rows can also be practised to minimize such competition, and maximize barrier effect and yield.

Although the fungicide treated plots had the highest numbers of flowers and attached fruits, short intercrops plots, gave better results than tall intercrops, and slightly better than sanitation alone. This means short intercrops were somehow effective in reducing the effect of late blight (Shairaha *et al.* 1989; Zavaleta-mejia and Gomez, 1995). The study showed that intercropping combined with sanitation was not significantly different from sanitation alone, which emphasises the effect of sanitation (Emebiri and Obiefuna 1992). Tomato intercropped with short plants had higher tomato yield compared with those intercropped with tall plants, presumably due to suppression. In addition to the beneficial effects of managing late blight spread and severity, intercrops provide some yield which could ensure food security and provide additional income. Since significantly better tomato yields were obtained with short intercrops this means that if further studied, the intercrops could give as good results as the fungicide treatment. However, intercropping alone has not yet been tested to establish its effect alone, when compared with sanitation alone.

According to this study of intercropping short and tall crops with tomatoes, the following conclusions can be derived.

(a) Soybean and sesame were compatible for intercropping with tomatoes.

(b). Tall plants such as sorghum and sunflower have a suppressive effect on tomato growth and productivity when intercropped.

(c). Relatively short plants could be intercropped with tomatoes as part of an integrated late blight management scheme.

(d) Row spacing between intercrops is crucial to minimize side effects such as shading and competition for nutrients.

(e). Sanitation alone has an adverse effect on tomato growth and production.

Chapter 7

The Effect of High Planting Density and Sanitation on Tomato Late Blight (*Phytophthora infestans*) Disease

(Submitted to Plant Pathology)

The Effect of High Planting Density and Sanitation on Tomato Late Blight (*Phytophthora infestans*) Disease

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Abstract

Crop planting density varies according to cultivar, soil factors and farmer's objectives. Manipulating crop density can be achieved through changing between row, or within-row spacing, or number of plants/hole. The aim of the high planting density experiments in Uganda was to investigate the effect of high planting density on late blight management in tomato cultivation. Seven levels of high to low tomato population densities combined with sanitation, fungicide treatment and untreated control were studied in a randomized complete block design. The results indicated that the fungicide had little effect on late blight development, but delayed the epidemics for about 2 weeks. Late blight disease levels were highest in the control plots and least in plots where sanitation was done. Sanitation had an adverse effect on tomato growth and production irrespective of planting densities. Numbers of healthy leaves, flowers, attached fruits and yield were highest in the fungicide plots, and were almost identical in other treatments. The study indicated that spacing could be manipulated without significant effects both on late blight and tomato development. Therefore, increased tomato densities could be integrated into late blight management; however it does not give yields as good as conventional fungicide treatments.

Introduction

Crop planting density is determined by different factors eg. cultivar, soil fertility, soil moisture and the purpose of the crop (Purseglove, 1968, 1969, 1972). Changes in host density may change disease incidence (Burdon and Chilvers, 1982). According to Burdon and Chilvers (1982), increasing host density where disease is transmitted does not necessarily change the absolute quantity of disease but the proportion of infected plants. The relationship between host and pathogen density may be confounded by environment, vector behaviour and presence of other species (Burdon, 1993). Depending on the crop and disease, high crop density can reduce or increase disease levels. Higher crop densities increased seed yield without increasing the risk of white mould occurrence on *Phaseolus vulgaris* (Saindon *et al.*, 1993, 1995). A combination of higher application rates of sulphur and an increase in plant density reduced the incidence of anthracnose (*Colletotrichum graminicola*) and increased crop yield (Gowily, 1995). Alteration of between-row spacings had a greater effect on stem rot (*Erwinia carotovora*) disease than change of within-row spacings (Cappaert and Powelson, 1990). *Stemphylium* blight incidence on onion was lowest at higher density coupled with appropriate planting date (Jakhar *et al.*, 1994). Early planting and high density reduced the incidence of bud necrosis disease in groundnuts (Ghanekar, 1980). Crop density of rape in the range of 30-150 seeds/m^2 did not affect fungal and insect attack (Masnicki *et al.*, 1994). Higher sowing rate of faba bean had no effect on chocolate spot (*Botrytis cinerea and B. fabae*) and increased yield (McEwen and Yeoman, 1990).

High density plant populations can also aggravate disease outbreaks. In the case of foliar diseases, high crop density and irrigation increased average air temperature and extended dew duration periods (Cappaert and Powelson, 1990; Sentelhas *et al.*, 1993), which affected micro-climate and disease development. According to Sentelhas *et al.* (1993), high crop density and irrigation increased infection rates of spot blotch (*Helminthosporium sativum*) and powdery mildew (*Erysiphe graminis*) on wheat. Although smut (*Ustilago zeae*) infection on maize was more pronounced at higher density, yield was improved (Kostandi, 1992). Crop density is correlated with grey mould (*Botrytis cinerea*) disease development in straw berry (Wang, 1997). High tomato plant density increased incidence of *P. infestans, Septoria lycopersici and Alternaria solani* (Silva-Junior *et al.*, 1992). For soil-borne diseases, reduction in number of plants/plot was the most critical factor influencing yield loss in soybean and *Phytophthora* root rot development (Moots *et al.*, 1988). Increased levels of white rot (*Sclerotium cepivorum*) on onion was associated with a higher host density (Littley and Rahe, 1987). Charcoal rot (*Macrophomina phaseolina*) disease incidence on sunflower increased with density (Zazzerini *et al.*, 1985). The risk of *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) attack in rape was linked to inoculum level, crop density and weather factors (Twengstrom, 1996).

When the fungicide benlate was used to control tomato diseases, there was no disease aggravation caused by close spacings (AVRDC, 1992). According to AVRDC (1987), yield responses of tomato to plant and row spacing are expected. Tomato fruit numbers and weight per plant increased more with wider plant spacings than with wider row spacings (AVRDC, 1987). High tomato (Claudia) density spacings at 80 x 30, 80 x 20, and 80 x 10 cm resulted in increased yields (Al-Maslamani and Suwwan, 1987). According to AVRDC (1992), high tomato density of 2 plants per 20 cm^2 with Peto 86 and PT 4121 gave the greatest green fruits and least unmarketable yield compared with densities of 1 plant per 15, 20 and 30 cm^2. According to AVRDC (1991), tomato (FMTT 33) at inter-row and intra-row spacings of 30, 40 and 50 cm had the highest yield at closest spacing. Increased tomato high yields were attributed to high plant population and high fruit numbers (AVRDC, 1991).

The aim of tomato planting density experiments was investigate whether high planting density can help to alleviate possible effects of sanitation practices on plant growth and productivity, while maintaining low levels of late blight.

Materials and methods

Experimental design

The experiment consisted of 7 treatments (Table 1) which were randomly allocated to each of 5 blocks. Plots of 5 m by 4 m, surrounded by borders of 3 m wide with one spreader tomato plant at each corner, 2 m distant, formed experimental units.

Table 1: Treatments applied in high tomato planting density experiment

Treatment	Practice	Spacing (m)	Population (per plot)	Density (plants/m^2)
1	Sanitation	0.35 x 0.70	77	4.1
2	Sanitation	0.35 x 0.50	110	5.7
3	Sanitation	0.35 x 0.35	154	8.2
4	Sanitation	0.25 x 1.0	80	4.0
5	Sanitation	0.35 x 1.0	55	2.9
6	Fungicide (Dithane M-45)	0.35 x 1.0	55	2.9
7	Untreated (control)	0.35 x 1.0	55	2.9

Field establishment

Tomato seedlings (MT55) were raised in nurseries for approximately 4 weeks. MT55 was used because it is resistant to bacterial wilt, but susceptible to late blight. The tomato nurseries were protected against late blight infection by spraying them with Dithane M-45 at a recommended dosage of 2.5 grams per litre, at intervals of 7 days. The seedlings were raised during dry weather which together with the fungicide treatment ensured healthy and uniform seedlings for the experiments. The main experimental fields were marked out by use of 30 cm pieces of reeds and sisal string at the time of transplanting. Four week old tomato seedlings were transplanted with an average height of 15 cm. Table 1 gives the various spacings used and the corresponding densities of tomato plants per m^2. A total of 48 spreader plants were also transplanted into the experimental field. The experimental field was enclosed within a guard maize (*Zea mays*) crop, with a 4 m border space. Mulching with spear grass (*Imperata cylindrica*) was carried out in all the experimental plots. The first experiment was planted on 24 March 1996 and the second one on 10 October 1997.

Field inoculation

Two weeks after transplanting, late blight infected leaves were obtained from a tomato plant at Kawanda Agricultural Research Institute. The diseased leaves were detached from the plants and taken to the laboratory. Late blight infected leaflets were incubated in 5 petri dishes of 94 mm diameter for 48 hours at room temperature. The petri dishes contained moist cotton wool to ensure high humidity to enhance sporulation. The spores of *P. infestans* were washed off with 500 ml water and placed in a 1.5 litre capacity hand sprayer and used to artificially infect the tomato spreader plants. The field inoculation was carried out in the evening to ensure cool conditions facilitating infection. Five days after inoculation, the spreader plants started showing late blight symptoms and sporulation.

Field management

Diseased leaves, flowers, fruits or branches in treatment 1-5 were carefully removed with scissors into a 10 litre capacity plastic bucket and disposed of far from the field. Strict measures were observed when removing infected material to avoid shaking the diseased parts during sanitation as this would enhance spread of the disease. Sanitation was done every 2 days from the beginning to the end of each experiment. In the fungicide plots Dithane M-45 at a dosage of 2.5 grams per litre, was sprayed to run-off at intervals of 7 days, using a 15 litre capacity knapsack sprayer. In case of insect pest attack, Dursban (Chlorpyrifos 400g/litre) broad spectrum insecticide was sprayed to run-off at a dosage of 18.5 ml per 15 litres of water in all the plots. Weeding of the experimental fields was done regularly.

Assessment

Two weeks after transplanting, 6 plants per plot were randomly chosen and marked for field assessments. The variables assessed were plant height, numbers of healthy (second experiment) and diseased leaves, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and weight of harvested fruits. Disease incidence was assessed on 30 randomly chosen plants per plot, and the other variables on the main stem of the 6 marked plants at intervals of 14 days. Environmental conditions during the experiments were recorded at the Kawanda Agricultural Research Institute weather station.

Statistical analyses

The data was analyzed by two way analysis of variance and pre-determined linear contrasts at the 5% significance level. The comparisons chosen were: fungicide compared with control, fungicide compared with sanitation alone, and high planting density combined with sanitation versus sanitation alone (Table 2 and 3).

Results

First high tomato planting density (March 1996 - May 1996)

Tomato plant height

There were no significant effects of high density planting on plant height, nor of fungicide treatment compared with the control, but fungicide treated plants were taller than those subjected to sanitation (Table 2). The mean height for fungicide treated plants, and those in sanitation were 29 cm and 26 cm respectively.

Late blight disease incidence

There was a significantly higher number of diseased leaves in the control pots compared to treated plots (Fig. 1, Table 2). There was no significant difference between sanitation and fungicide treatment (Table 2). Healthy leaves were not assessed in the first experiment.

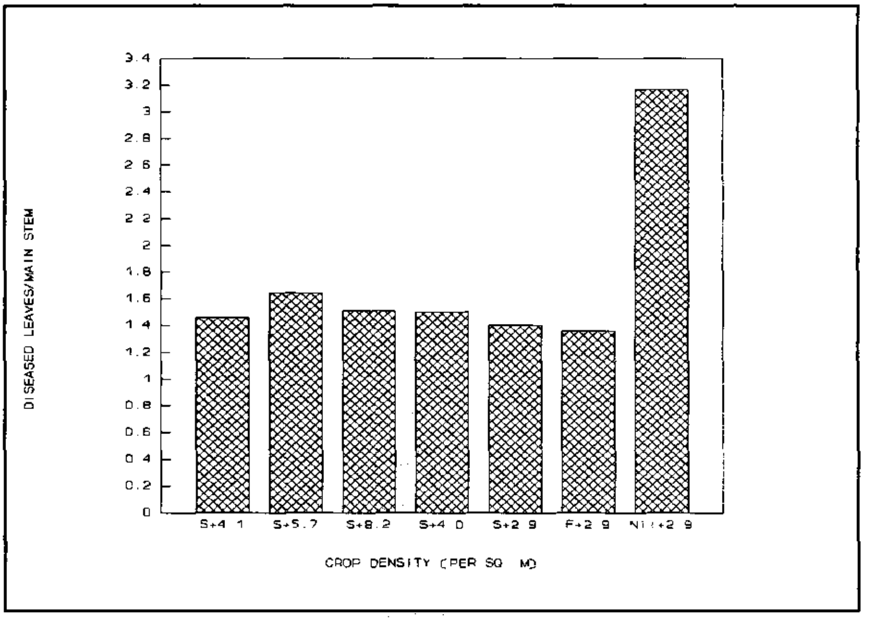


Fig. 1: Late blight disease effect on leaves on the main stem; significance of pre-determined linear contrasts is shown in Table 2.

Late blight disease progress

Disease incidence was highest in control plots from the first few weeks to the end of the experiment (Fig. 2). Sanitation combined with high planting density was slightly better than sanitation alone, but not significantly. Fungicide treated plots had a significantly lower proportion of diseased plants per plot than control or sanitation alone (Table 2). Although level of disease in fungicide treated plots remained low (Fig. 2) late blight epidemics increased tremendously after May 13 and the experiment was terminated before the next assessments were made.

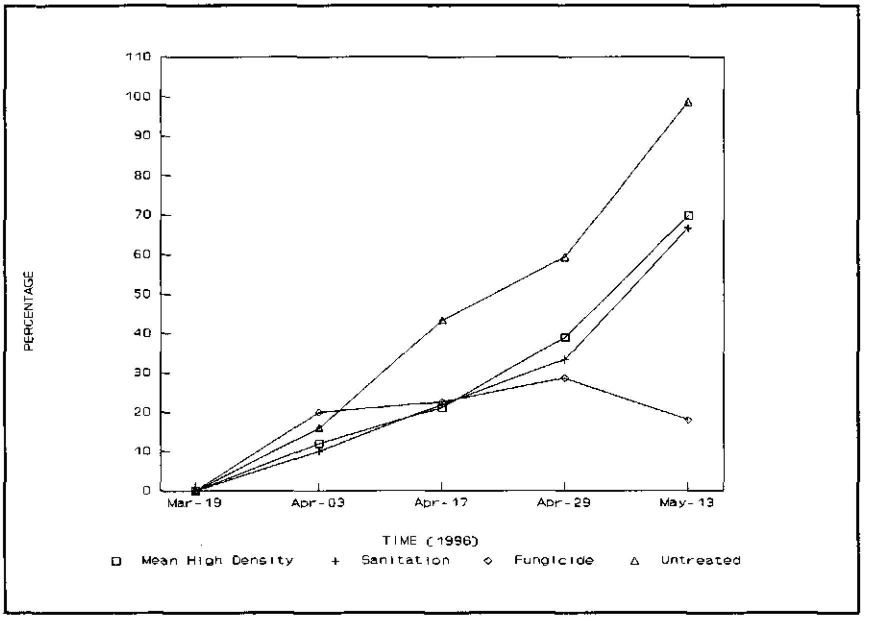


Fig. 2: Late blight progress (% diseased plants per plot) during the first high density experiment.

Late blight disease severity

Fungicide treated plants had significantly ($P < 0.001$) less disease severity on both leaves than the control. There was no difference between high density spacings and sanitation alone on either leaf in terms of late blight severity (Fig. 3, Table 2). There were also no significant differences among the high density treatments. Disease severity on the 9th leaf only was significantly higher in fungicide plots than in sanitation plots ($P < 0.01$).

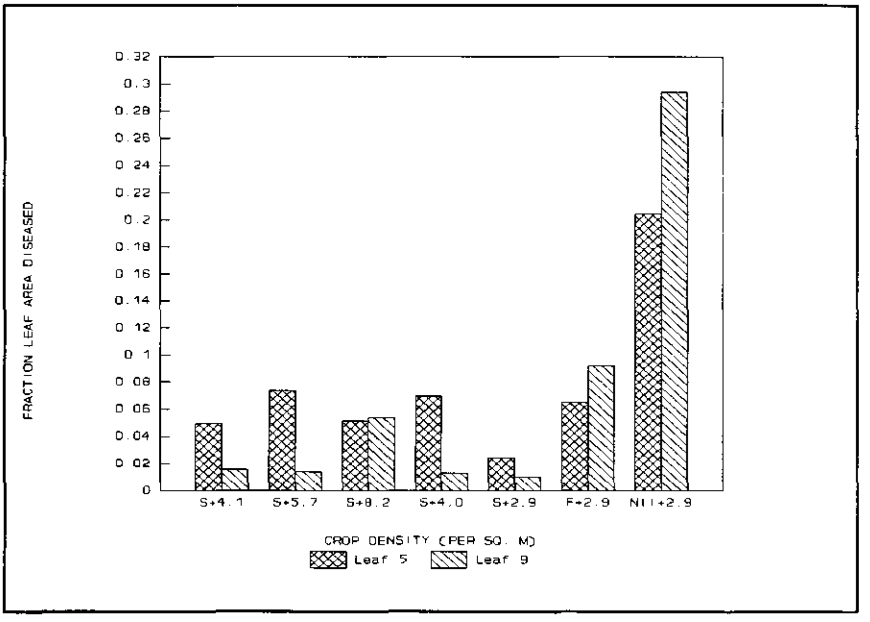


Fig. 3: Late blight severity on the 5th and 9th leaves on the main stem; significance of pre-determined linear contrasts is shown in Table 2.

Tomato production

Tomatoes flowered more in the fungicide treated plots than in other treatments but not significantly (Fig. 4, Table 2). A tomato plant population density of 8.2 plants per m^2 (spacing of 35 cm by 35 cm) was the second best in terms of flower formation. No significant differences were observed among the contrasts in terms of flower or fruit production (Table 2). Plants in all treatments were able to produce a few fruits which were later affected equally due to favourable weather conditions for late blight at the end of May (Fig. 5). Harvestable tomatoes were negligible in all treatments due to the severe late blight epidemics.

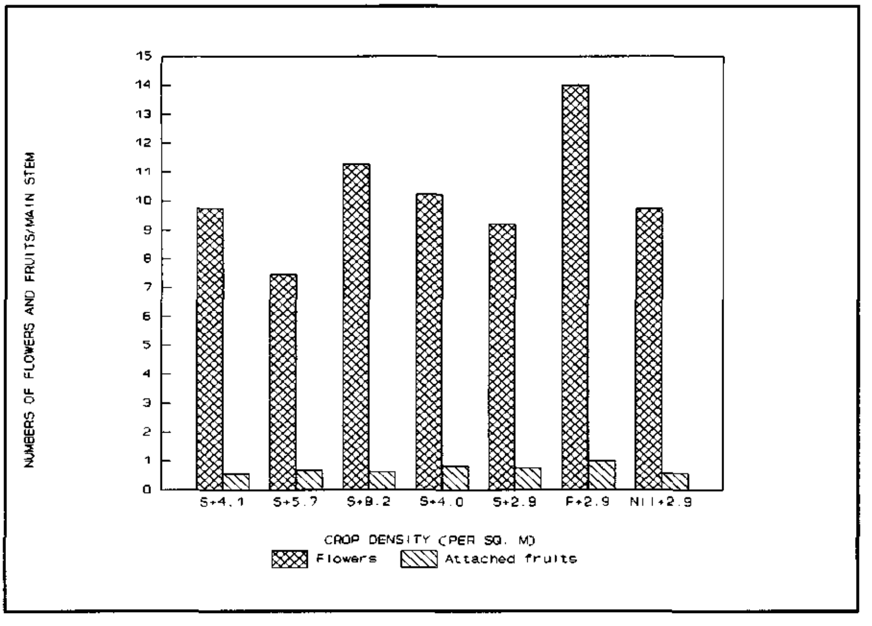


Fig. 4: Late blight effect flower and fruit production on the main stem; significance of pre-determined linear contrasts is shown in Table 2.

Table 2: Orthogonal contrast for the first high density experiment

Contrast	a = 5%	Variables						
	F & P	Height	Diseased leaves	Disease incidence	Severity on 5th leaf	Severity on 9th leaf	Flowers	Attached fruits
Fungicide vs Untreated	F P	NS 3.448 0.076	S 61.592 0.000	S 92.960 0.000	S 14.364 0.001	S 23.889 0.000	NS 2.736 0.111	NS 1.148 0.295
Fungicide vs Sanitation	F P	S 5.450 0.028	NS 0.033 -	S 11.890 0.002	NS 1.469 0.211	S 8.352 0.008	NS 3.439 0.076	NS 0.504 -
High density/sanitation vs Sanitation	F P	NS 1.316 0.263	NS 0.487 -	NS 1.096 0.305	NS 3.610 0.070	NS 0.922 -	NS 0.050 -	NS 0.017 -

Legend: F = F value, P = Probability, S = Significant, NS = Not significant

Weather conditions during the first high density experiment

There was little rainfall at the Kawanda experimental site in March but considerable rain fell in April and May 1996 (Fig. 5a), providing suitable relative humidity for *P. infestans* infection. The temperature range (15-28 C) was favourable for *P. infestans* infection and spread. The high rainfall (Fig. 5a) coupled with a sharp drop in maximum temperature in May (Fig. 5b) caused a late blight epidemic which wiped out all the tomato plants and the experiment was terminated prematurely with a few harvested fruits.

(a)

(b)

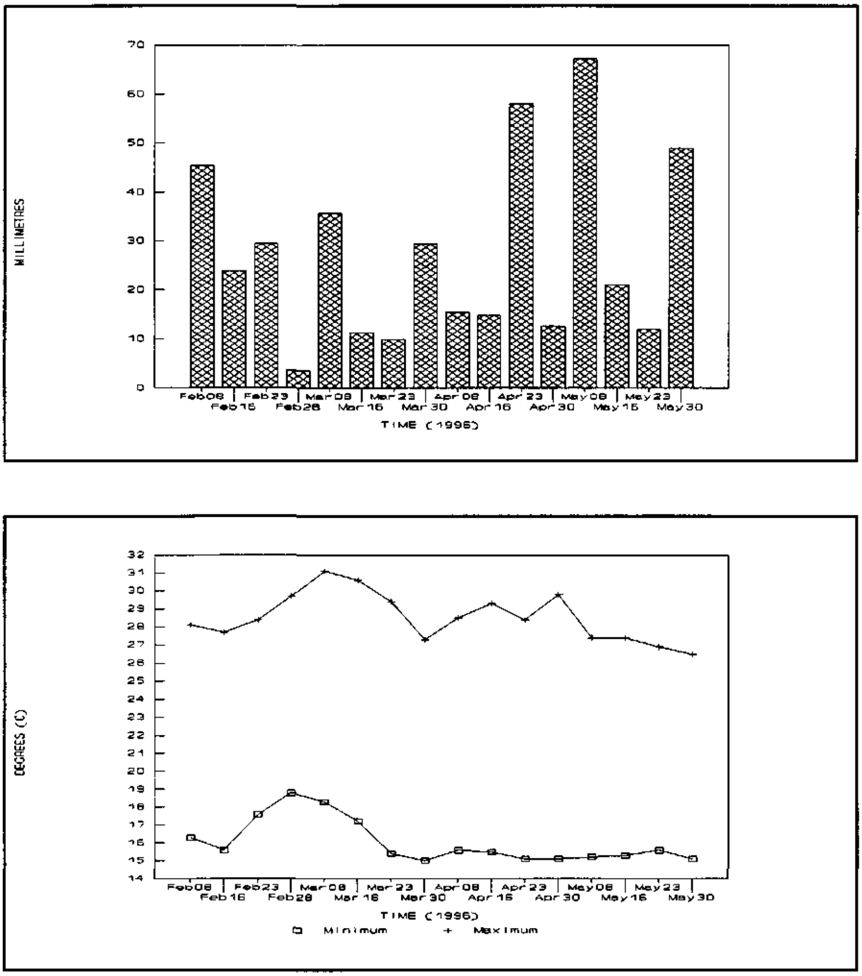


Fig. 5: Weather conditions at Kawanda in the first half of 1996; (a) total weekly rainfall (b) minimum and maximum ambient temperature.

Second high density experiment (October 1997 - January 1998)

Tomato plant height

Fungicide treated plants were significantly taller than those subjected to sanitation, or in the control (Table 3). The mean height for fungicide treated plants, and those in sanitation were 46 cm and 34 cm respectively. There were no significant differences for mean tomato height in the high density plots.

Late blight disease incidence

There were no significant differences in healthy or diseased leaves when comparing sanitation alone with the high density plantings (Table 3). Control plots had a significantly higher number of diseased and a lower number of healthy leaves than fungicide-treated plots (Fig. 6). The fungicide treatment plots had a significantly higher number of diseased and healthy leaves than sanitation alone.

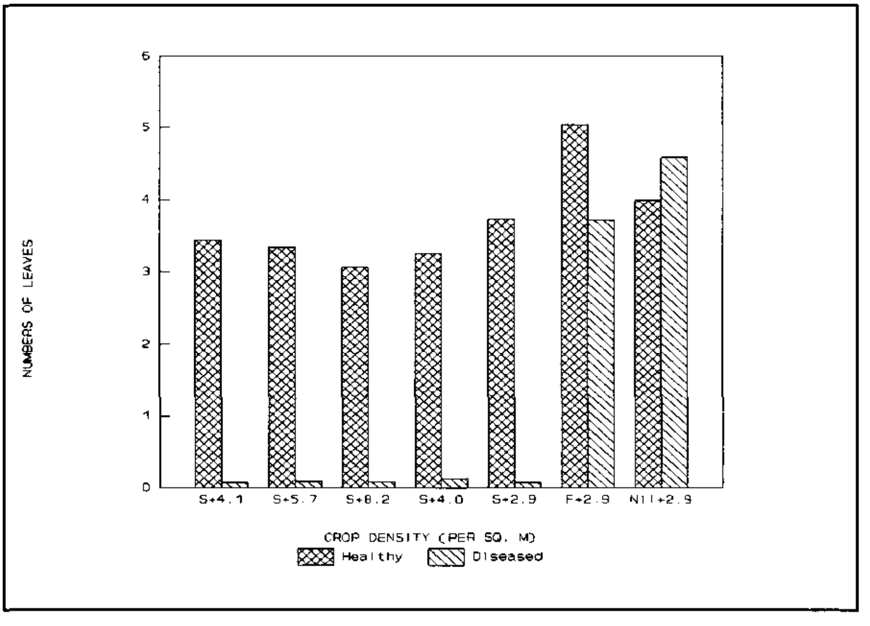


Fig. 6: Late blight effect on leaves on the main stem in the second high density experiment; significance of pre-determined linear contrasts is shown in Table 3.

Late blight disease progress

In plots where sanitation was practised, the level of late blight was always very low (Fig. 7, Table 3). Fungicide treatment had little effect on the disease compared with control. The incidence of late blight per plot in both control and fungicide plots reached 100% by the end of the experiment (Fig. 7). The fungicide delayed late blight epidemics for 1-2 weeks but had a significantly (P 0.001) lower mean incidence on plants per plot during November. Fungicide treated plots also had a significantly (P 0.001) higher disease incidence per plot than sanitation alone (Table 3). Disease incidence was not significantly different comparing sanitation alone to the high density plots.

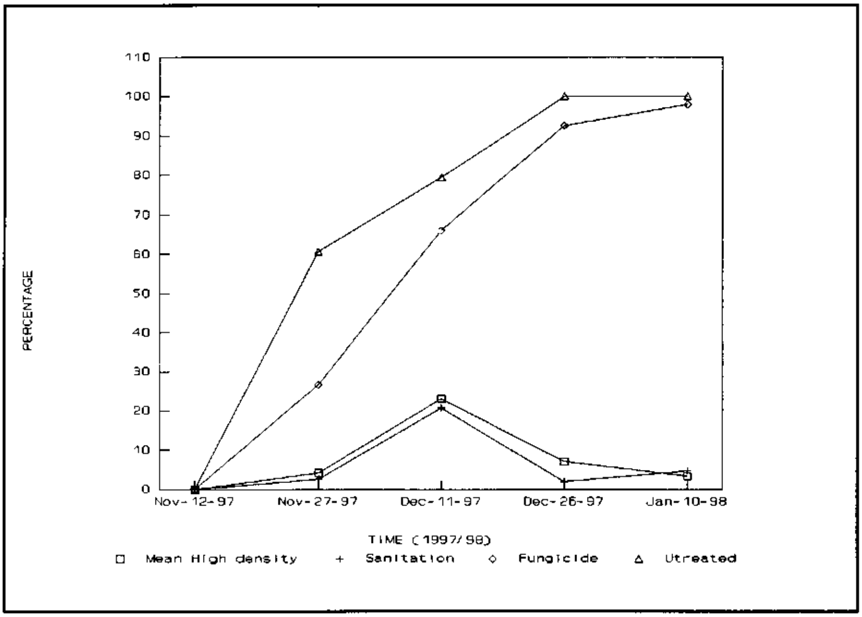


Fig. 7: Late blight progress (% diseased plants per plot).

Late blight disease severity

Late blight disease severity remained extremely low in all plots involving sanitation compared with the fungicide treatment and the control (Fig. 8, Table 3). Fungicide treatment gave significantly lower severities than the control on leaf 5 (P < 0.001) and leaf 9 (P < 0.05).

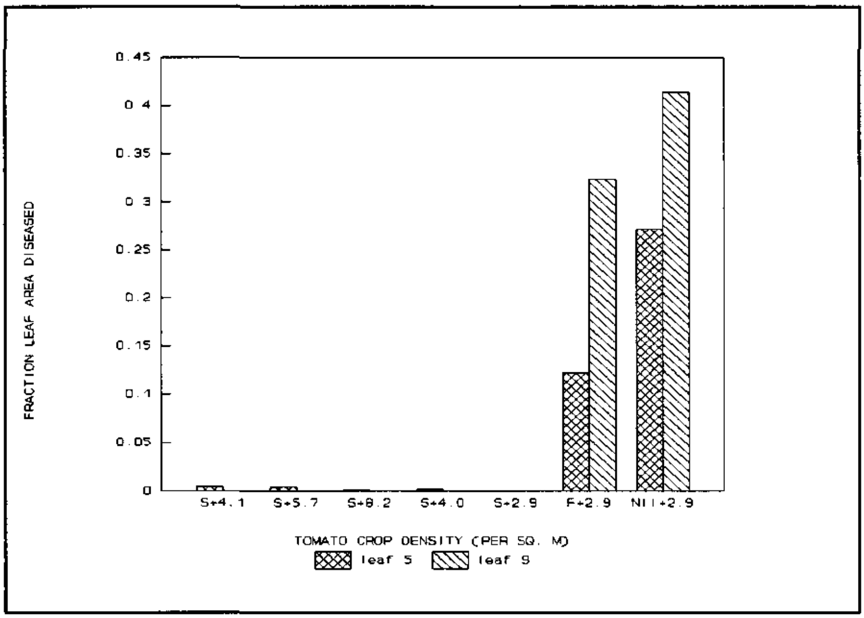


Fig. 8: Late blight severity on 5th and 9th leaves on the main stem; significance of pre-determined linear contrasts is shown in Table 3.

Tomato production

Although there were no significant differences for flower production, fungicide treatment gave a high fruit production ($P < 0.001$) and yield ($P < 0.01$) (Fig. 9 and 10, Table 3) than the control. There were no significant differences between treatments where sanitation was done (Table 3). Sanitation with or without high density planting had a major deleterious effect on yield, reducing it to below that of the control.

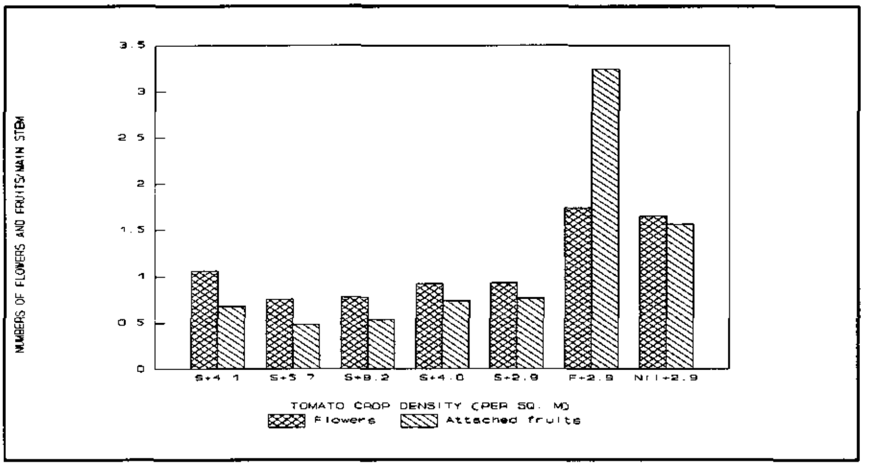


Fig. 9: Late blight effect on tomato production (flowers and attached fruits on the main stem); significance of pre-determined linear contrasts is shown in Table 3.

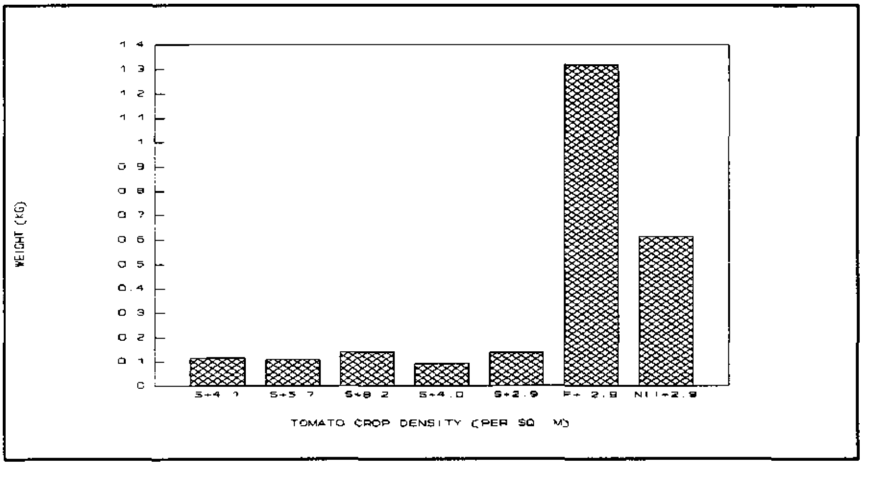


Fig. 10: Yield (weight of harvested tomatoes); significance of pre-determined linear contrasts is shown *in* Table 3.

Table 3: Orthogonal contrast for the second high density experiment

Contrast	*a* = 5%	Variables								
	F & P	Height	Healthy leaves	Diseased leaves	Disease incidence	Severity on 5th leaf	Severity on 9th leaf	Flowers	Attached fruits	Weight fruits
Fungicide vs Untreated		NS	S	S	S	S	S	NS	S	S
	F	0.958	10.257	8.027	23.282	23.038	5.562	0.159	22.410	11.821
	P	-	0.004	0.009	0.000	0.000	0.027	-	0.000	0.002
Fungicide vs Sanitation		S	S	S	S	S	S	S	S	S
	F	16.063	16.132	139.043	114.624	63.881	193.784	12.296	48.645	22.382
	P	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.000	0.000
High density/sanitation vs Sanitation		NS	NS	NS	NS	NS	NS	NS	NS	NS
	F	0.001	2.986	0.005	0.570	0.559	0.000	0.082	0.310	0.678
	P	-	0.097	-	-	-	-	-	-	-

Legend: F = F value, P = Probability, S = Significant, NS = Not significant

Weather conditions during the second high density experiment

Figure 11 shows the weather conditions at Kawanda during the second high density experiment. There were considerable rainfall for both plant growth and disease development. The temperature range of 16 to 27 C was favourable for *P. infestans* infection and spread.

(a)

(b)

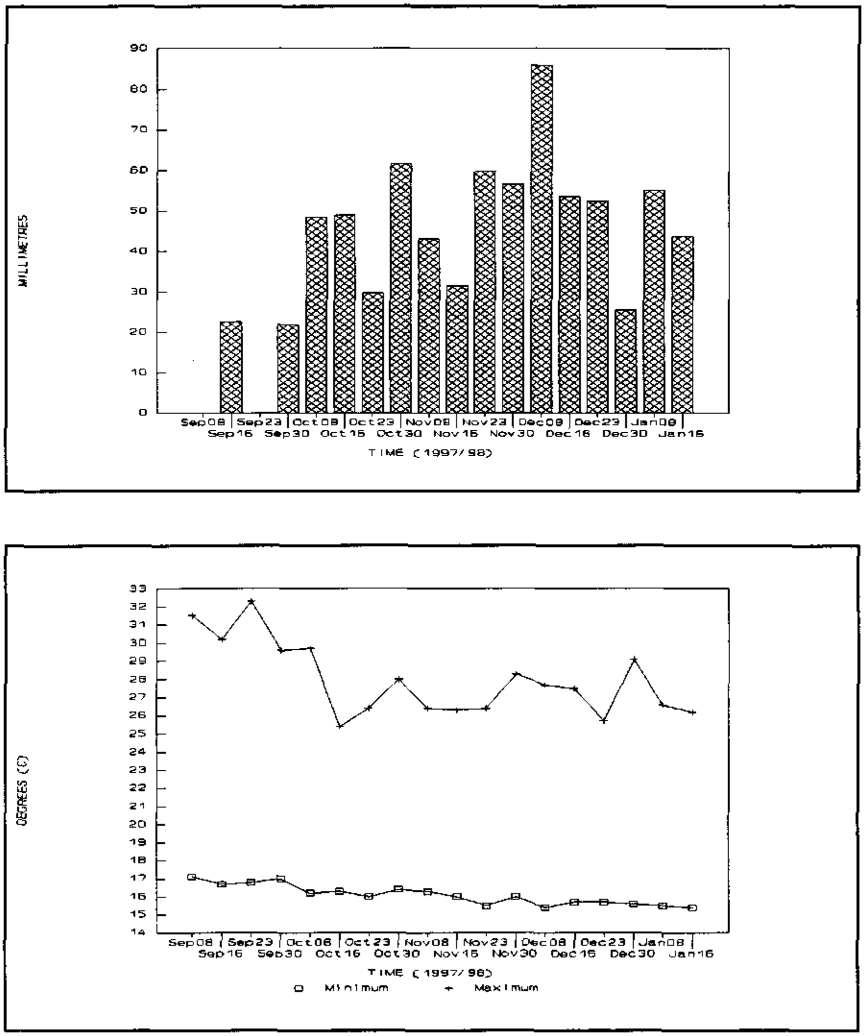


Fig. 11: Weather conditions at Kawanda, 1997/98; (a) total weekly rainfall (b) minimum and maximum ambient temperature.

Discussion

In both experiments, temperatures and rainfall were adequate for late blight development and tomato production. The observed differences in the results can therefore be attributed to the effect of different treatments. Late blight disease levels were higher in fungicide treated-plots than in plots where sanitation was practised in conjunction with high planting densities. This indicated that sanitation can limit the disease levels in the field, at various high plant populations. Increased tomato densities, though combined with sanitation, did not aggravate late blight as previously reported (AVRDC, 1992, Silva-Junior *et al.*, 1992). The fungicide treatment could not stop late blight epidemics but delayed it for about 2 weeks in the second experiment.

The study indicated that sanitation at various spacing had almost the same effect on tomato plant height. This means that the spacing effect may not be significant as previously reported (AVRDC, 1992), but it was rather sanitation which reduced plant height relative to fungicide treated plots. Tomato plants in the control plots were shorter than in the fungicide treated plots possibly due to the effect of late blight. Flower and fruit formation was almost the same at the various tomato crop densities combined with sanitation. Since flower and fruit formation were not significantly different at the various high planting densities, it means that tomato density as a variable could be increased and integrated in late blight management (Ghanekkar 1980; Cappaert and Powelson, 1990; Gowily, 1995;). For all the variables, high density combined with sanitation was not significantly different from sanitation alone at normal spacing. The effect of high planting densities alone on yield was not established in these experiments because the emphasis was on integrated control involving sanitation. Integrated control including high crop density was proved possible in Uganda by integrating polythene shelters, intercropping and high planting densities (Tumwine *et al.*, unpublished). Fungicide treatment gave significantly better results than sanitation alone in terms of growth and production, and showed that sanitation alone had an adverse effect on tomato plants.

According to the experiments on tomato high density combined with sanitation in late blight management, the following conclusions can be drawn.

(a). The fungicide (Dithane M-45) did not stop late blight epidemics but delayed it for about two weeks.

(b). The tomato population density could be increased and integrated in late blight management practices, but there is no indication of effectiveness without sanitation.

(c). Sanitation could reduce *P. infestans* levels in the field. It could be part of an integrated management system for late blight, but the intensities used in this experiment had an adverse effect on tomato growth and production.

Chapter 8

The Effect of Integrated Cultural Control Practices on Tomato late Blight (*Phytophthora infestans*)

(Submitted to Crop Protection)

and 5 holes of 3 cm diameter were made on each side of the tunnel for ventilation and to reduce resistance to wind. Figure 1 shows the inside of the shelters in the experiment.

Table 1: Treatments used in the integrated control for late blight experiment

Treatment	Description
1	Polythene shelter, sesame, sanitation
2	Polythene shelter, sesame, no sanitation
3	Sanitation alone
4	Fungicide (Dithane M-45/mancozeb)
5	Untreated (control)

Fig. 1: Integrated control practices for late blight; (left) tomato plants inside the shelters showing flowers and attached fruits, and (right) sesame intercrop inside the shelters.

Field establishment

Tomato seedlings (MT55) were raised in nurseries in March 1998 for 4 weeks. Tomato line MT55 was used because it is susceptible to late blight but resistant to bacterial wilt. The seedlings were protected from late blight infection by spraying with Dithane M-45 at a recommended dosage of 2.5 grams per litre, at intervals of 7 days.

The seedlings were raised in dry weather which had the added advantage of ensuring healthy and uniform seedlings for the experiment. The intercrop was planted in March 1998. When the intercrops were 3 weeks old, 15 cm tomato seedlings, including spreader plants, were transplanted into the experimental field. Two rows of tomatoes were planted at a spacing of 1.2 m inter-row and 0.25 m intra-row, which was the same as in open plots, giving a total of 96 plants per plot. This was at a high planting density as in the previous experiment in order to maximize the tunnel space. The sesame (*Sesamum indicum*) sown in the middle of the 2 tomato rows, was at an intra-row spacing of 10 cm. After transplanting the crops were mulched with grass (*Imperata cylindrica*) and the polythene shelters were erected in the integrated plots. The entire experiment was surrounded by maize (*Zea mays*), 4 m distant, as a guard crop. The experiment was planted on 27 March 1998 and harvested in June, 1998.

Inoculation

Two weeks after transplanting, late blight infected leaves were obtained from a tomato plant at Kawanda Agricultural Research Institute. The late blight infected leaves were detached from a late blight infected plant and taken to the laboratory. Leaflets from the diseased leaves were incubated in 5 petri dishes of 94 mm diameter for 48 hours at room temperature (around 25 C). Moist cotton wool was placed in the petri dishes to enhance further sporulation. The spores were washed off and placed in a 1.5 litre capacity hand sprayer to make a 500 ml spore suspension. Tomato spreader plants were inoculated using a hand sprayer, in the evening to facilitate infection. Five days after inoculation, the spreader plants had started to show late blight symptoms and sporulation.

Field management

Diseased plant parts in treatments 1 and 3 were carefully removed with scissors every 2 days throughout the experimental period. Strict measures were taken when removing the infected material to avoid shaking the diseased tomato parts as this would enhance spread of the disease. The diseased materials were contained in a 10 litre capacity plastic bucket and disposed of far from the field. In the fungicide plots, Dithane M-45 at a dosage of 2.5 grams per litre was sprayed to run-off, at intervals of 7 days using a 15 litre capacity knapsack sprayer. In case of insect pest attack, Dursban (Chlorpyrifos 400g/litre) broad spectrum insecticide was applied to run-off at a rate of 18.5 ml per 15 litres of water in all the plots. Weeding of the experimental field was done regularly as required.

Assessment

Two weeks after transplanting, 6 tomato plants per plot were randomly chosen and marked for subsequent disease assessments on the main branch. Disease incidence was assessed on 30 randomly chosen tomato plants per plot. The variables assessed were tomato plant height, numbers of healthy and diseased leaves, disease incidence, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and weight of harvested fruits. Field assessment was done at intervals of 14 days. During harvesting, fruits from the marked plants and those from the remainder were handled separately, and taken to the laboratory for weighing. A thermometer was used to record minimum and maximum temperatures in the integrated treatments. A digital thermohygrometer was used to measure the average internal temperatures and relative humidity in the shelter treatments.

Statistical analyses

The data were analyzed by two way analysis of variance at the 5% level of significance. Pre-determined linear contrasts were set. The integrated treatment combined with sanitation was compared with the integrated treatment alone to determine the overall effect of sanitation. The integrated treatment combined with sanitation was compared with sanitation alone to determine the overall effect of the integrated treatments. The fungicide treatment was compared with both the integrated treatment with sanitation, and the integrated treatment alone to establish the effectiveness of these treatments compared with the standard fungicide used in Uganda to control tomato late blight. No contrast was made with the untreated control as the important comparison is with fungicide treatment, the standard practice in Uganda.

Results

Tomato plant height

The mean tomato plant height in integrated plots with sanitation was not significantly less than integrated plots alone, nor different from fungicide treated plots (Fig 2, Table 2). Plants in integrated plots with sanitation were significantly higher than in sanitation plots alone ($P < 0.001$). Integrated plots without sanitation had significantly taller tomato plants than those in fungicide treated plots ($P < 0.01$). Sanitation alone has a deleterious effect on tomato growth which is ameliorated by the integrated treatment.

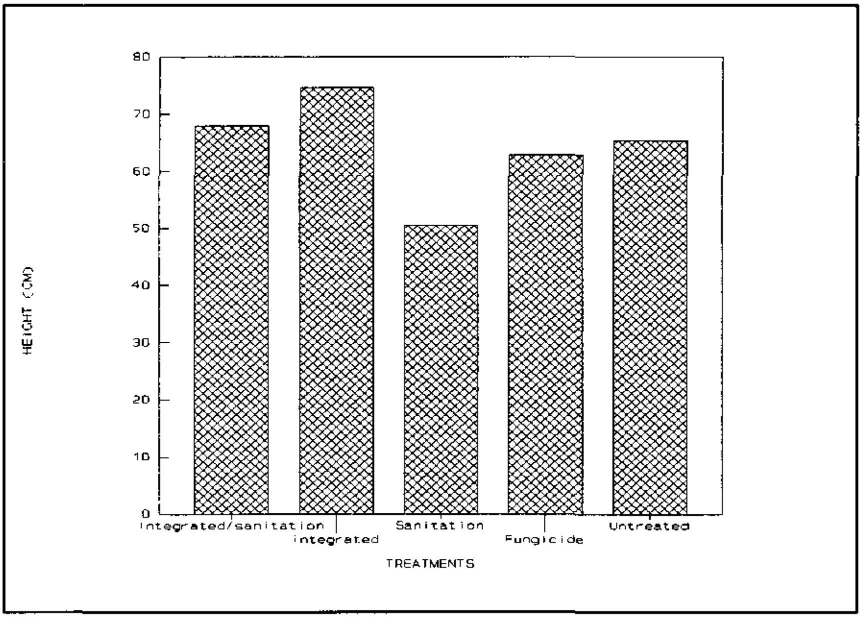


Fig. 2: Mean tomato plant height during the experimental period; significance of pre-determined linear contrasts is shown in table 2.

Late blight disease incidence

Plants in integrated plots with or without sanitation had almost the same numbers of healthy leaves, on average about seven per main stem (Fig. 3). There were no significant differences in numbers of healthy leaves between integrated plots with and without sanitation, and fungicide treated plots (Table 2). Integrated treatment with sanitation gave significantly more healthy leaves than sanitation alone ($P < 0.05$). Again the deleterious effect of sanitation can be ameliorated by the integrated treatment.

Where sanitation was practised, there were very few diseased leaves (Fig. 3). Integrated plots alone had considerably more diseased leaves than integrated plots with sanitation ($P< 0.001$) (Fig. 3, Table 2). Integrated plots combined with sanitation had significantly fewer diseased leaves than the fungicide treated plots ($P< 0.001$) with no penalty in terms of numbers of healthy leaves. There were no significant differences in terms of diseased leaves between integrated plots with sanitation compared with sanitation alone, nor between integrated plots alone and fungicide treated plots (Table 2). Thus the integrated treatment with sanitation was clearly the best in terms of maintaining a high number of healthy leaves on the plants while reducing markedly the number of diseased leaves.

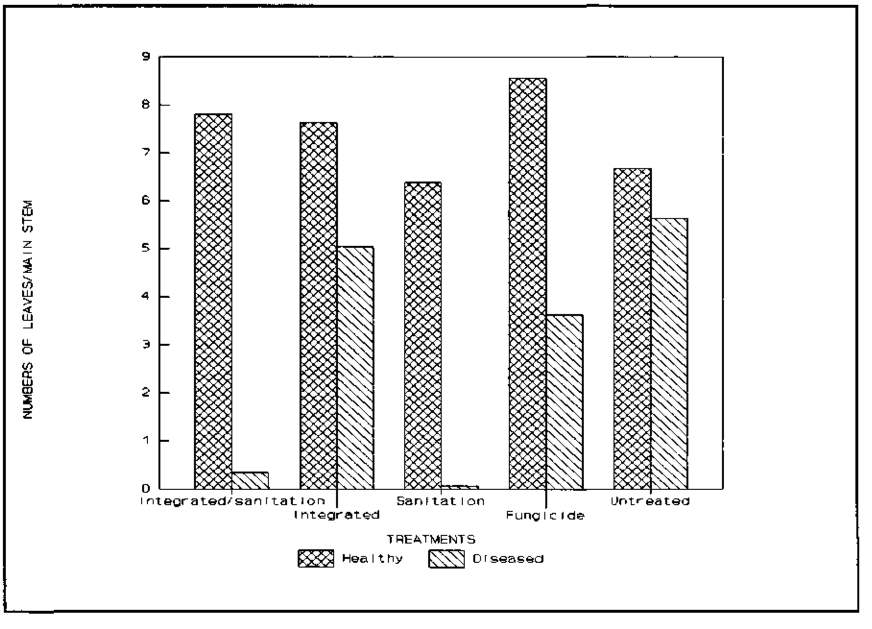


Fig. 3: Late blight effect on leaves on the main stem; significance of pre-determined linear contrasts is shown in table 2.

Late blight disease progress

Late blight disease progress on plants per plot was highest in control plots, followed by the integrated treatment alone, and the fungicide treatment plots (Fig. 4). At the end of the experiment, there was almost 100% late blight incidence in each of these treatments. Disease progress in plots where sanitation was practised was the same irrespective of whether it was combined with the integrated treatment or not.

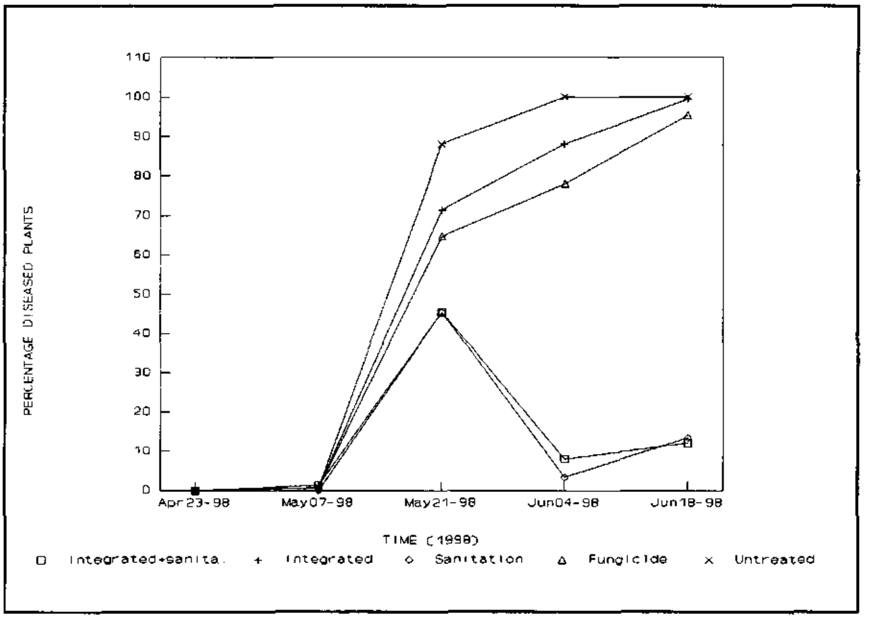


Fig. 4: Late blight progress (% diseased plants per plot)

Late blight severity

Where sanitation was practised, late blight disease severity was very low (Fig. 5). Disease severity was significantly higher in integrated treatment alone than integrated with sanitation plots (P < 0.001). Late blight severity was only significantly less on the 9th leaf in integrated treatment with sanitation plots (P < 0.001) compared to fungicide treated plots. Integrated treatment alone had significantly more severe late blight than fungicide treated plots (P < 0.05) on both leaves. Clearly the integrated without sanitation does not offer an alternative to the fungicide treatment, in terms of reducing disease severity.

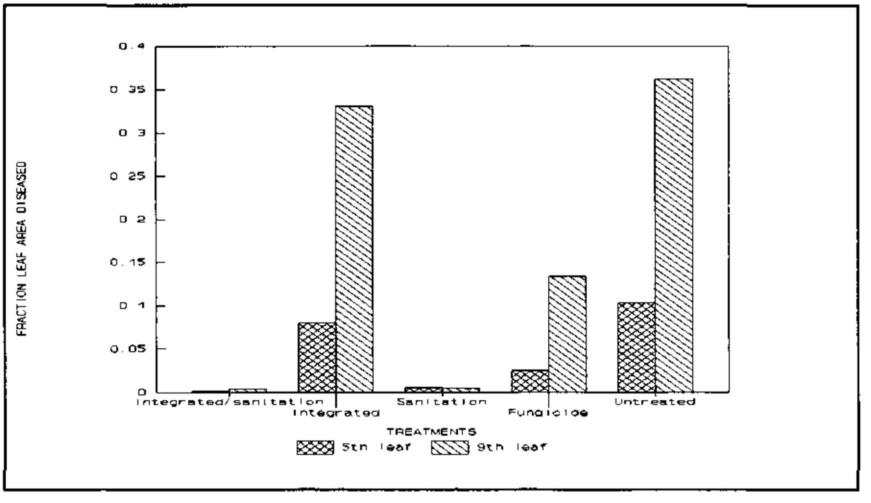
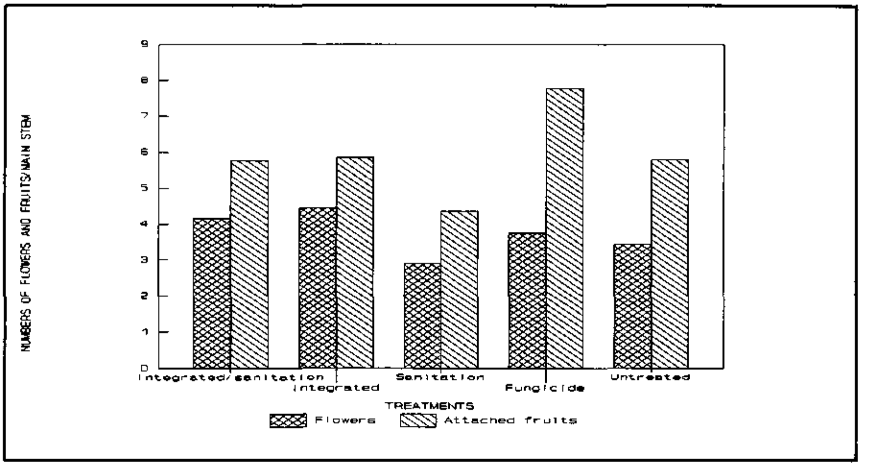


Fig. 5: Late blight severity on the 5th and 9th leaf level; significance of pre-determined linear contrasts is shown in table 2.

Tomato production

The late blight effect on plant production was assessed by numbers of healthy flowers, attached fruits on the main stem, and weight of harvested fruits per plot. In terms of flower production, both integrated alone and integrated with sanitation treatments were equally as good as the fungicide treatment (Fig. 6a, Table 2). The integrated with sanitation treatment was significantly better (P < 0.05) than sanitation alone. For numbers of attached fruits, the fungicide treatment was significantly better than either of the integrated treatments (P < 0.01). The integrated with sanitation treatment had significantly more attached fruits than sanitation alone (P < 0.05). There were no differences between the integrated treatments in terms of numbers of flowers and attached fruits. Tomato plants in integrated without sanitation plots gave yields which were not significantly less than those from fungicide treated plots (Fig 6b, Table 2). However, the integrated with sanitation treatment gave significantly lower yields than the fungicide treatment (P < 0.01). The integrated with and without sanitation plots gave about the same yield, 140 kg per hectare of sesame.

(a)



(b)

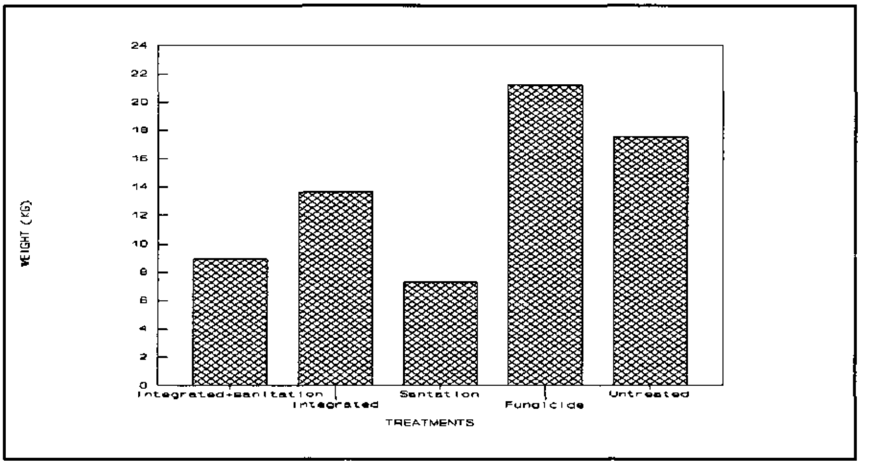


Fig. 6: Late blight effect on tomato production, (a) flowers and attached fruits on the main stem, (b) harvested fruits per plot; significance of pre-determined linear contrasts is shown in table 2.

Weather conditions during the experiment

Rainfall was conducive for late blight epidemic development at the beginning but was less so towards the end of the experiment (Fig. 7a). The minimum temperatures were suitable for late blight infection but maximum temperatures exceeded the conducive range (4-26 C) throughout the experiment (Fig. 7b). Maximum and average temperatures were always higher inside the polythene shelters than outside (Fig. 7c). Relative humidity was higher inside the shelters than outside (Fig. 7d) during periods of high rainfall (Fig. 7a) but when rainfall intensity reduced, the external relative humidity increased above that inside the shelters.

Table 2: Summary of orthogonal contrast for integrated disease management experiment

Contrast	a = 5%	Variables								
	F & P	Height	Healthy leaves	Diseased leaves	Disease incidence	Severity on 5th leaf	Severity on 9th leaf	Flowers	Attached fruits	Weight fruits
Integrated+Sanitation vs Integrated alone		NS	NS	S	S	S	S	NS	NS	NS
	F	3.871	0.084	33.858	84.488	17.437	51.196	0.520	0.1016	0.066
	P	0.067	-	0.000	0.000	0.001	0.000	-		-
Integrated+Sanitation vs Sanitation alone		S	S	NS	NS	NS	NS	S	S	NS
	F	26.066	5.101	0.123	0.073	0.144	0.003	9.684	4.654	0.518
	P	0.000	0.038	-		-	-	0.007	0.047	-
Integrated+Sanitation vs Fungicide		NS	NS	S	S	NS	S	NS	S	S
	F	2.256	1.257	16.466	68.038	4.167	16.739	1.020	9.278	9.263
	P	0.153	0.257	0.001	0.000	0.058	0.001	0.328	0.008	0.008
Integrated alone vs Fungicide		S	NS	NS	NS	S	S	NS	S	NS
	F	12.037	2.150	3.101	0.890	4.555	9.387	2.996	8.520	7.768
	P	0.003	0.162	0.097	-	0.049	0.007	0.103	0.010	0.013

Legend: F = F value, P = Probability, S = Significant, NS = Not significant

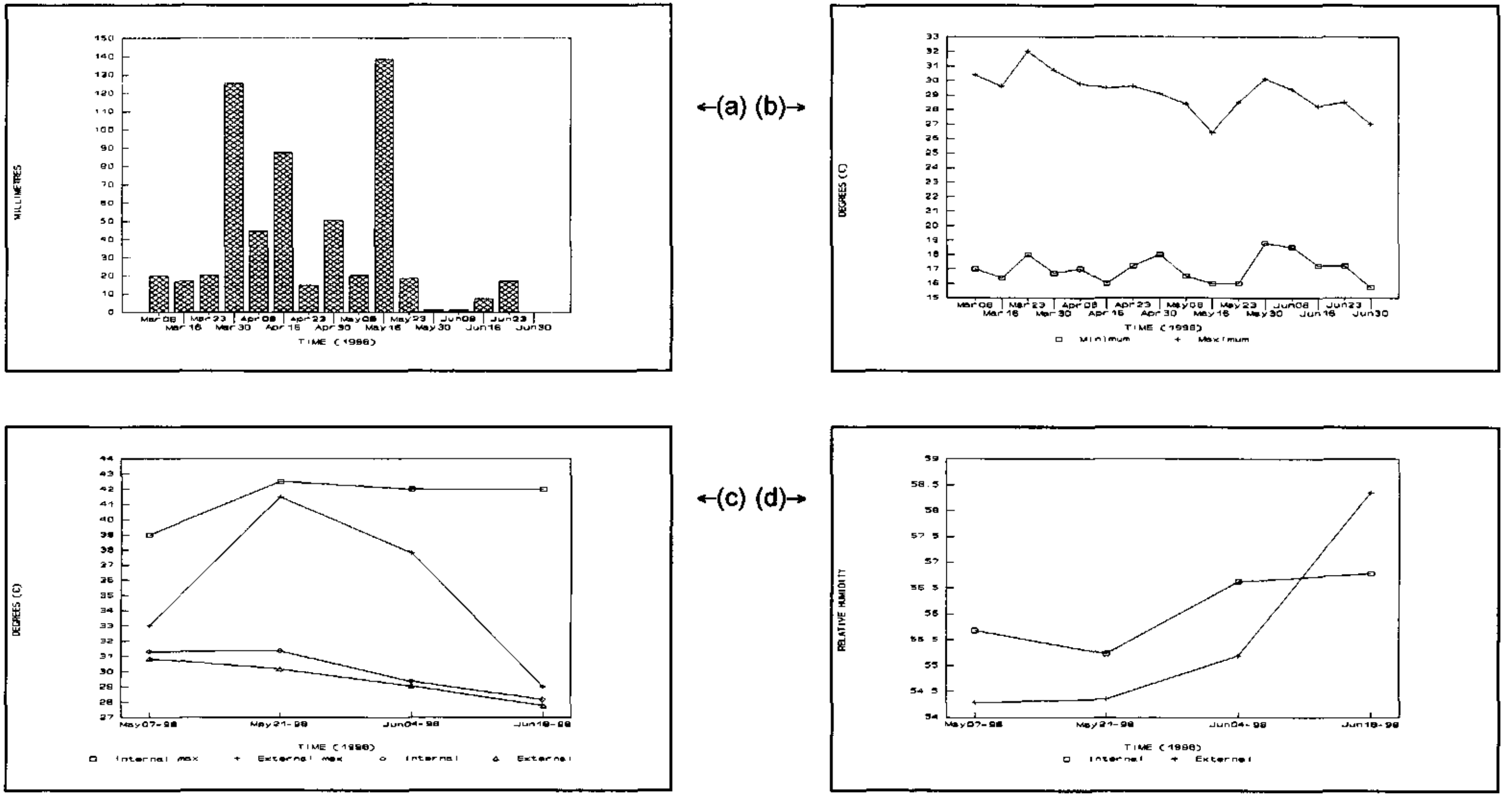


Fig. 7: Environmental conditions during the experiment; (a) weekly total rainfall, (b) weekly average minimum and maximum temperatures, (c) temperatures inside and outside the shelters, and (d) relative humidity inside and outside the shelters.

Discussion

Late blight disease incidence and disease severity were least where sanitation was carried out and highest where there was no sanitation. This means that sanitation alone or in combination with other cultural measures has the same effect of reducing late blight disease incidence and severity due to reducing the inoculum pressure. The fungicide treatment and integrated (sheltering/sesame intercrop) without sanitation plots had very high disease level throughout the season because of high levels of inoculum generated. There was a significant difference between integrated with sanitation and sanitation alone plots in terms of flower and fruit production because of the shelter and intercropping effects. This indicates that the combination of sheltering and intercropping were effective in providing a barrier to *P. infestans* spores which disperse readily in the environment (Gretna, 1983; Sherf and Macnab, 1986; Fry *et al.*, 1992; Drenth, 1994).

Integrated with or without sanitation plots, and fungicide treated plots gave tomato plants of about the same height. Healthy tomato leaves were maintained at high levels in integrated with or without sanitation treatments as was flower and fruit production. This implied that integrated control was comparable to fungicide. Sanitation alone plots had the shortest tomato plants, lower numbers of healthy leaves, and reduced flower and fruit production which confirms the deleterious effect on plant growth and production. The integrated method for late blight control limited the adverse effect of sanitation presumably by reducing the intensity of leaf removal, which resulted in reasonable yields (Bolkan, 1997; Bauske *et al.*, 1998). The integrated treatment without sanitation had the tallest tomato plants possibly due to some etiolation resulting from a lot of leaves which included diseased ones. This could be amplified if the shelters become dirty and reduced the amount of light passing through.

Fruit production was reasonably high in the integrated without sanitation treatments possibly due to the effect of polythene shelter which increased the temperature inside the tunnels beyond the optimum range for late blight disease development. *P. infestans* infection especially on flowers and fruits were limited, without seriously damaging flower and fruit production. Although the fungicide treatment gave the highest weight of harvested fruits per plot, the integrated with or without sanitation plots also gave reasonable yields. The tomatoes harvested from the integrated plots appeared very clean without blemishes but seemed to be reduced in size which in turn reduced the plot yields. This could possibly mean that plants inside the shelters do receive sufficient water for yield assimilation as those in the open, especially when there is little rain. This problem could be overcome by irrigation when necessary. The plot yield data may also be misleading as some of the fruits from the control plots and fungicides plots could have been in a latent stage of infection. Detection of latent infection at the time of harvesting was not possible but could have serious post harvest repercussions in terms of marketing. There was also a general observation that insect damage especially due to bollworm was reduced in the integrated treatments. Integrated control of late blight in tomatoes should be developed further as suggested by other authors for potato (Perley, 1994; Mackay, 1996; Duvauchelles and Dubois, 1998; Schepers and Bouma, 1998).

According to these results, it can be concluded that:

(a). Sanitation alone or combined with sheltering and intercropping prevented late blight epidemics hence disease incidence and severity were greatly reduced.

(b). Sanitation alone adversely reduced tomato growth and production.

(c). Integrated methods of control combining polythene shelters and sesame intercropping alleviate the adverse effect of sanitation, while reducing late blight disease.

(d). Integrated control measures promoted good tomato growth and reasonable yields at high tomato population density.

(e). The fungicide Dithane M-45 did not prevent late blight epidemics but achieved the highest yields.

(f). Sanitation must be included in integrated control, but it must be less stringent and subjected to a threshold to avoid deleterious effects.

(g). Aspects such as ensuring sufficient water, effects of cultural practices on individual fruit size, and the possibility of latent infections must be studied further before integrated control without using fungicides can be achieved.

Chapter 9

General Discussion

General Discussion

The original goal of this research was to develop integrated cultural control practices for late blight on tomatoes in Uganda. Such practices would aim to reduce disease levels and crop losses due to late blight, minimize fungicide applications and increase tomato production. Prior to this research, it was expected that different cultural measures such as sanitation, use of polythene shelters, intercropping, intensive planting densities, and their interaction would play a major role in developing the integrated control methods. However it was not clear what effect each of the measures, singly or integrated, would have on late blight disease epidemiology, and tomato development and production. The experiments were aimed to separate out the effect of sanitation, and that of other practices to be combined with and supplement sanitation. The research was focused on combining cultural control methods for plant diseases with the major emphasis on sanitation, excluding the use of fungicides.

The research on integrated cultural control practices for late blight on tomatoes in Uganda investigated the effect of each cultural practice in combination with sanitation, and tried to establish their effectiveness against the disease. New information has been generated as a result of this study. This research has proved that sanitation as a sole cultural practice can reduce the levels of late blight disease incidence and severity on tomatoes as previously reported (Sherf and Macnab, 1985; Fontem, 1995; Inglis *et al.*, 1996). This positive effect of sanitation is probably achieved through reduced inoculum production by removing diseased plants and plant parts. However, sanitation alone at the intensity studied has side effects of interfering with crop development and production.

Introduction of other control measures such as polythene shelters (Sunarjono, 1972; Chee *et al.*, 1988; Hanada, 1988) can reduce late blight disease incidence and severity, and can help alleviate the adverse effect of sanitation on the crop. This complimentary effects of polythene shelters and sanitation was proved relatively effective on tomato late blight in Uganda (Tumwine *et al.*, unpublished). Polythene shelters can act as barriers (Jebari, 1989) against dispersing spores of *P. infestans*, hence reducing late blight disease spread and development. In addition, the environmental conditions inside the polythene shelters can retard disease development through increased temperatures especially during sunny days as established in Uganda (Tumwine *et al.*, unpublished). The polythene shelters can also exclude rain water to some extent, therefore not favouring infection (Marco and Brunelli, 1989; Xiao *et al.*, 1992). Compatible intercrops such as sesame and soybean, included in this research, have the potential for reducing late blight incidence and severity as earlier established for potato (Shairaha *et al.*, 1989). Intercrops can act as barriers (Jebari, 1989) against dispersing spores of *P. infestans*, which can reduce late blight disease spread and development. Intercropping can be supplemented by sanitation to achieve some good results on reducing late blight disease on tomatoes. However, in the experiments performed, it was clear that the major component of disease control was achieved through sanitation. The study also showed that tomatoes can be grown under intensive crop density without aggravating late blight disease situations as indicated before (AVRDC, 1992). The cultural practices alone may not necessarily reduce the late blight disease level, but reasonable yield can be realised as proved in the integrated control experiment. In fact, this can be equated to the use of the fungicide Dithane M-45 which by delaying late blight epidemics for 2-4 weeks, achieves considerable benefits in Uganda (Tumwine *et al.*, unpublished).

As the major goal of this research, it is possible to integrate all the mentioned cultural practices with sanitation to control late blight as previously advocated (Bolkan, 1997; Bauske *et al.*, 1998). Integrated control of late blight in tomato should gain favour as was the case for late blight in potato (Perley, 1994; Macaky, 1996; Duvauchelles and Dubois, 1998; Schepers and Bouma, 1998). The knowledge generated from this study was not sufficiently conclusive to be used by farmers at present. The research was mainly exploratory and has made some progress towards developing various cultural practices for late blight control in Uganda and perhaps elsewhere. The study has formed a solid foundation on which further research should be based to refine and expand the established knowledge of cultural effects on late blight and tomato crop growth and production.

Further research to define economic thresholds for sanitation by determining the numbers of healthy leaves to be maintained on a plant, and numbers of diseased leaves per plant to be tolerated. Such thresholds investigations should include tolerant and resistant cultivars as part of an integrated control for late blight. Additional research work should be devoted to the appropriate design and size of low-cost polythene shelters for tomato production under rain-fed cultivation. Sheltering could be combined with time of planting, which can make it possible to produce tomatoes during dry periods, to reduce the amount of fungicides. In this case, supplementary watering should be incorporated in the design, research and technology development. However, under such practices, closed season to reduce the sources of *P. infestans* inoculum need to be determined. The effects of polythene shelters on other diseases and pests, and quality improvement should be studied. Further research to establish the appropriate spacing for both tomatoes and intercrops should be performed. Further candidate intercrops should be identified and tested. In particular the effects of intercrops on the whole range of pests and diseases, and the added value provided by the intercrops should be evaluated. Identifying sources of and breeding for durable resistance against late blight is also an important area of research for Uganda (Guseva *et al.*, 1978; An *et al.*, 1984; Kaur *et al.*, 1988; Zhang *et al.*, 1988; Markovic *et al.*, 1990). Investigations on latent infection and its postharvest repercussions should be carried out. Research work on alternative hosts of *P. infestans* in Uganda need to be initiated in order to develop an integrated control practice that also aims at removing such plants from where tomatoes are cultivated. This would also help to establish thorough weed control practices. Cultural control practices which are designed to utilize the positive effects of sanitation in plant disease have high potential in the management of late blight in tomato cultivation. What remains, through adaptive research, is to establish the gaps and shortcomings of cultural control, and devise means of overcoming them. This would pave the way for future adoption of cultural control practices, mainly in combinations, for late blight disease on tomato production in Uganda. Farmers will continue to use fungicides if they perceive associated increase in yield, and they need to be convinced that similar results can also be achieved through improved integrated cultural control practices.

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Summary

Chapter 1

Tomato (*Lycopersicon esculentum*, Mill.) is a major vegetable crop grown in Uganda. Tomato fruits are consumed cooked, as salads, or in processed form as ketchup or paste. Tomato cultivation provides income for farmers. The crop in Uganda is attacked by pests such as bollworm (*Helicoverpa armigera*), thrips (*Thrips* spp), cutworms (*Agrotis* spp), aphids (*Aphis* spp) and whiteflies (*Bemisia tabaci*). Major diseases include late blight (*Phytophthora infestans*), bacterial wilt (*Pseudomonas solanacearum*), early blight (*Alternaria solani*), root knot (*Meloidogyne* spp) nematodes and some viruses such as TYLCV. Most farmers consider it impossible to grow tomatoes without the use of fungicides. The most commonly used fungicide to control foliar tomato diseases in Uganda is Dithane M-45 (mancozeb). It is therefore imperative to develop integrated and environmentally friendly control measures for tomato diseases with special emphasis on late blight. These measures would be mainly cultural practices such as sanitation, intercropping, polythene shelters (physical barriers), resistant cultivars, suitable crop density and planting time.

Tomato belongs to the family Solanaceae together with other crops of economic important such as potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), pepper (*Capsicum* spp) and tobacco (*Nicotiana* spp). The crop originated from Central and South America which is also the centre of origin of *P. infestans*. The pathogen belongs to the kingdom Chromista, phylum Oomycota, subclass Peronosporomycetidae, order Pythiales and family Pythiaceae. Characteristics of oomycetes are: formation of oospores, a coenocytic mycelium, a diploid life cycle, predominantly cellulose cell walls (not chitin) and lack of sterols. The pathogen is a hemibiotroph and is heterothallic with mating types A1 and A2. The pathogen has a narrow host range with some strains exhibiting specific pathogenicity to potato or tomato. The temperature range for *P. infestans* growth is 4-26 C (optimum 20 C) and a relative humidity approaching 100%. Temperatures above 29 C can stop *P. infestans* development. Sporangia remain viable for about 5 hours, mycelia can survive in plant debris and cause infection for short periods, while sexual oospores are the main structures for long-term pathogen survival. Long distance dispersal is associated with transportation of infected plant parts by man. Short distance dispersal is by sporangia dispersed through wind, dew, rain splash or as zoospores.

Chemical control by fungicides such as Dithane M-45 and Ridomil MZ-72 (metalaxyl) is the major way of managing the pathogen. However, in Uganda fungicides have some disadvantages: high prices, lack of availability, adulteration, label expiry and environmental pollution. Elsewhere, some strains of *P. infestans* have shown resistance to fungicides such as metalaxyl. Resistant tomato cultivars against late blight are few and are not cultivated in Uganda. The use of cultural control methods is not well developed.

The general goal of late blight research on tomato in Uganda, is to reduce damage through an integrated disease management approach, minimizing fungicide treatments, and subsequently increasing tomato production. A tomato line MT55 was used because of its susceptibility to late blight and resistance to bacterial wilt.

Chapter 2

Ten districts from different agro-climatical zones of Uganda were surveyed for late blight control and tomato cultivation practices. Using a structured questionnaire information was obtained from agricultural extension staff, farmers and agrochemical retailers. A collection was made of isolates of *P. infestans* on tomato from some districts of Uganda. The isolates were characterised at Wageningen Agricultural University, the Netherlands. Compatibility tests were carried out by putting known *P. infestans* strain A1 and A2 in proximity with the Ugandan isolates on a clarified Rye A agar medium. After one week oospores were observed between Ugandan *P. infestans* and A2 only, indicating that the *P. infestans* isolates from Ugandan tomatoes were of mating type A1.

'Moneymaker', 'Marglobe', 'Heinz' and 'Roma' are the major commercial tomato cultivars grown in Uganda, but the first two are the most popular. Late blight and bacterial wilt are the most serious diseases in tomato cultivation in Uganda. Bollworm and thrips are the most troublesome insect pests. All the tomato varieties grown in Uganda are susceptible to *P. infestans* and the most popular tomato cultivars are the most susceptible. The survey revealed that tomato growing is mostly done all-year around, which makes it possible for the pathogen to disperse to a new crop generation and survive. Late blight is most severe during the rainy season. Fungicides play a major role in the control of late blight in Uganda and Dithane M-45 (mancozeb) was the most used, constituting more than 90% of total usage in most districts. Fungicides are not always successful and this has prompted farmers to double or triple the application frequency per week and to raise the dosage. Growing tomatoes on top of hills, pruning and detrussing (sanitation) combined with fungicides were reported to reduce late blight severity and amount of fungicide used in Uganda. Insecticides were always applied in a mixture with fungicides. According to farmers perceptions, fertilisation practices were found not to contribute to late blight management, but could be beneficial by enhancing resistance, increasing plant vigour and physical defences. Problems associated with pesticides, marketability and late blight were the most serious constraints.

Chapter 3

Phytophthora species are often difficult to isolate from decaying tissue or soil. Isolation of the pathogen has generally been by use of susceptible potato (*Solanum tuberosum*) cultivars such as 'Bintje'. Direct plating of small amounts of diseased tissue on Rye A agar containing an antibiotic cocktail has also been tried. In the absence of a recommendable susceptible potato cultivar, a new method for the isolation of the pathogen from tomato was an urgent need. Culturing is also a problem in the tropics where ingredients such as rye grain, the recommended media Rye A agar, or V8 juice agar, have to be imported.

Young green and healthy tomato fruits ('Moneymaker') were surface disinfected in 96% ethanol for 10 minutes and aseptically sliced into 5 mm thick slices. Pieces of tomato leaves of approximately 25 mm^2 were cut from a leading edge of a late blight lesion. The infected discs were then covered by the fruit slices in 94 mm diameter sterile petri dishes and incubated at room temperature (25 C) for 5 days.

Another technique made use of parts of infected young green fruits, following the above procedure except that no leaves were involved. *P. infestans* rapidly colonized the tomato slices to form a dense mass of mycelia on the tomato slices. The pathogen continued to sporulate on the slices for at least 15 days. The hyphal tips of the fungus were aseptically transferred onto Rye A agar and cultured. Further purification was achieved by adding pimaricine, rifampicine and oxytetracycline antibiotics, and the fungicide carbendazim into Rye A agar.

The technique of isolating from diseased leaves has the advantage that it can be adapted for other infected parts of the tomato plant. Since a healthy tissue is used, the slices are not readily invaded by other rot causing microorganisms. The disadvantage of this technique is that isolation has to be done with in a short time to avoid dehydration. The technique for isolating directly from infected fruits extends the shelf life of the samples. The main disadvantage is that if already infected by other pathogens, they can confound the isolation. Work on sorghum, finger millet and maize as replacement ingredients for culturing media was preliminary. The pathogen was only able to grow sparsely. Maize was the most promising cereal but this needs further experimentation.

Chapter 4

The main aim of the study was to establish whether sanitation could substitute for fungicides as a sole means of controlling late blight on tomatoes. The experiments were set up as a factorial design with 6 experiments over a 3 year period as the main factor and 3 treatments (sanitation, fungicide and untreated) as second factor within experiments. Other treatments in these experiments are reported separately in chapter 5-7.

Tomato seedlings were raised in nurseries for approximately 4 weeks during dry periods. The tomato nurseries were protected against late blight infection by spraying them with Dithane M-45 at a recommended dosage of 2.5 grams per litre, at intervals of 7 days. Tomato seedlings (MT55) of 15 cm average height were transplanted into the field together with spreader plants on each corner of the experimental units, 2 m distant. All experimental fields had a 4 m border, and were enclosed in a guard maize (*Zea mays*) crop. Mulching with spear grass (*Imperata cylindrica*) was carried out in all the experimental plots. Pathogen inoculum was prepared by collecting late blight infected leaves and incubating them for 48 hours at room temperature. Incubation was done in 5 petri dishes of 94 mm diameter containing wet cotton wool to enhance further sporulation. Using a 1.5 litre capacity hand sprayer, 500 ml suspension of $2.5x10^5$ spores per ml was sprayed on the spreader plants 2 weeks after transplanting, and in the evening to facilitate infection. Diseased plant parts were removed at intervals of 2 days. Strict measures were observed to prevent the shaking of diseased tomato parts over healthy plants so as not to enhance spread of *P. infestans* spores. The fungicide (Dithane M-45) was applied at a dosage of 2.5 grams per litre, at intervals of 7 days, using a 15 litre capacity knapsack sprayer. In case of insect pest attack, Dursban at a dosage of 18.5 ml per 15 litres of water was sprayed to run-off, and weeding was done regularly. Two weeks after transplanting, 6 plants per plot were marked for field assessment. The variables assessed in all the experiments were tomato plant height, numbers of diseased and healthy leaves, disease severity on the 5th and 9th leaves, numbers of flowers and attached fruits, and weight of harvested fruits. Disease incidence was observed on 30 randomly chosen plants per plot. Other variables were scored on the main stem of the 6 marked plants. Field assessment was done at intervals of 14 days. Environmental conditions were recorded at the Kawanda weather station. The data were analyzed using analysis of variance and pre-determined linear contrasts.

Late blight disease severity and incidence per plot were lowest in sanitation plots. This could be due to reduced sources and subsequently low amounts of inoculum in the sanitation plots. Sanitation as sole means of controlling late blight had a growth retarding effect on tomato plants which was reflected in reduced plant height, flower formation and fruit production. Although sanitation alone had an adverse effect on tomato development and production, it reduced the level of late blight disease on the crop and as such, it is a promising control measure against late blight. Although disease levels were relatively high, fungicide-treated tomato plants gave the highest numbers of healthy leaves, flowers, fruits and yields.

Chapter 5

It was hypothesised that polythene shelters when introduced early in the crop cycle and combined with sanitation could protect tomatoes against air-borne *P. infestans* spores. Research was done with major objectives of evaluating polythene shelters and establishing the best period for their use in late blight management in rain-fed tomato cultivation. Sheltering was done during 0-2, 1-3, 2-4 and 0-4 months of the growth period, combined with sanitation, and evaluated against the three standard treatments; sanitation alone, fungicide and untreated control. The treatments formed a randomised complete block design with 5 replications. Transparent polythene shelters (tunnels) 4 m long, 1.2 m wide and 1.5 m high each were constructed after transplanting the tomato seedlings. Shelters were constructed using eucalyptus poles covered by two transparent pieces of polythene sheet. Plots of 5 x 4 m surrounded by a 3 m border and with an infected tomato spreader plant at every corner (2 m distant) formed the experimental units. Three tunnels, separated by 0.7 m spaces, were constructed for each experimental plot in the polythene shelter treatments. Temperature and relative humidity inside and outside the tunnels were recorded by a digital thermohygrometer.

The results indicated that early shelters combined with sanitation had high numbers of healthy and the lowest numbers of diseased leaves. Late blight disease incidence and severity were high in control and fungicide treated plots, and consistently low where sanitation was practised. Fungicide did not stop late blight epidemics but delayed them for about 2 weeks. Early shelters combined with sanitation did not affect tomato plant growth when compared to fungicide treated plots. Sanitation alone or with late shelters had an adverse effect on plant growth and production and early polythene shelters could help alleviate the adverse effect. Flower and fruit production was as good in early shelters as in fungicide-treated plots, but yield per plot was always highest in fungicide plots. The study showed that polythene shelters, especially if introduced early in the crop cycle, could substitute for Dithane M-45 fungicide to control late blight in tomatoes.

tomatoes in Uganda was intended to investigate the effect of each of the control measures and to establish their effectiveness against the disease. Much knowledge has been generated as a result of this study. Sanitation as a sole cultural practice can reduce late blight disease incidence and severity on tomatoes. However, sanitation alone has undesirable side effects in interfering with crop development and production. Introduction of other control measures such as polythene shelters, compatible intercrops such as sesame and soybean, and high tomato density can reduce late blight disease incidence and severity and help to alleviate these adverse side effects. Polythene shelters and intercrops can be effective barriers against dispersing spores of *P. infestans* and hence reducing late blight disease.

This research was mainly exploratory and has taken the first steps towards the development of cultural control practices for late blight in Uganda and elsewhere. The study has formed a sold foundation on which further research can be based. Further research to define economic thresholds for sanitation and how this could be combined with resistant cultivars need to be ascertained. Additional research work should be devoted at the appropriate design and size of low-cost polythene shelters for tomato production under rain-fed cultivation. Further research to ensure other factors such as adequate water for tomato plants inside the shelters need to be carried out. Identifying other crops that are compatible for intercropping with tomatoes and further research on these intercrops is worth undertaking. Identifying sources of and breeding for durable resistance against late blight is also an important area of research in Uganda, not covered in this thesis. Cultural control practices which are designed to utilize the positive effects of sanitation in plant disease have high potential in the management of late blight in tomato cultivation. What remains, through adaptive research, is to establish the gaps and shortcomings of cultural control, and devise means of overcoming them. This would pave the way for future adoption of cultural control practices, mainly in combination, for late blight disease on tomato production in Uganda and possibly in other countries. Although fungicide treatment gave the highest yields, integrated cultural control, if further developed, can also provide an effective protection against tomato late blight to enable farmers to realise substantial yields.

Samenvatting

Hoofdstuk 1

Tomaat (*Lycopersicon esculentum* Mill.) behoort tot de belangrijke groentegewassen in Uganda. Tomaten worden gekookt of als salade gegeten, maar ook in bewerkte vorm, zoals ketchup en puree, geconsumeerd. De tomaatcultuur verschaft een belangrijk inkomen aan de boeren. Het gewas wordt in Uganda belaagd door insectenplagen zoals bolworm (*Helicoverpa armigera)*, thrips (*Thrips* spp.), aardrups (*Agrotis* spp.), bladluis (*Aphis* spp.) en witte vlieg (*Bemisia tabaci*). Belangrijke ziekten zijn de *Phytophthora*-ziekte (*Phytophthora infestans)*, ringrot (*Pseudomonas solanacearum*), de *Alternaria*-ziekte (*Alternaria solani*), wortelknobbelaaltjes (*Meloidogyne* spp) en enkele virussoorten zoals het tomatengeelkrulbladvirus (TYLCV). De meeste boeren achten het onmogelijk om tomaten te telen zonder gebruik van fungiciden. Dithane M-45 (mancozeb) is in Uganda het meest gebruikte fungicide om bladziekten van tomaat te bestrijden. Het is daarom noodzakelijk om geïntegreerde en milieuvriendelijke bestrijdingsmaatregelen te ontwikkelen tegen ziekten van tomaat, met speciale aandacht voor *P. infestans*. Deze maatregelen zouden voornamelijk cultuurmaatregelen moeten zijn, zoals sanitatie, *intercropping,* barrières (kunststof tunnels), resistente cultivars, geschikte plantdichtheid en planttijdstip.

Tomaat behoort, samen met andere economisch belangrijke gewassen zoals aardappel (*Solanum tuberosum*), aubergine (*Solanum melongena*), hete en zoete pepers (*Capsicum* spp.) en tabak (*Nicotiana* spp.) tot de familie Solanaceae. Het gewas komt oorspronkelijk uit Midden- en Zuid-Amerika dat ook het oorsprongsgebied is van *P. infestans*. Dit pathogeen behoort tot het rijk Chromista, het phylum Oomycota, de sub-klasse Peronosporomycetidae, de orde Phytiales en de familie Phytiaceae. Karakteristieken van oomyceten zijn: vorming van oosporen, een coenocytisch mycelium, een diploïde levenscyclus, cellulose in de celwand (geen chitine) en het ontbreken van sterolen. Het pathogeen is een hemibiotroof en is heterothallisch met paringstypen A1 en A2. Het pathogeen heeft een kleine gastheerreeks met enkele stammen welke een specifieke pathogeniteit vertonen voor aardappel en tomaat. Het temperatuurstraject voor groei van *P. infestans* is 4-26 C (optimum 20 C) en een relatieve luchtvochtigheid welke de 100% benadert. Een temperatuur boven de 29 C kan de ontwikkeling van *P. infestans* stoppen. Sporangiën blijven gedurende 5 uur levensvatbaar, mycelium kan overleven in plantafval en gedurende korte perioden infectie veroorzaken, terwijl de geslachtelijke oosporen de belangrijkste structuren zijn voor de overleving van het pathogeen gedurende langere perioden. Lange afstandsverspreiding wordt in verband gebracht met het transport van infectieuze plantdelen door de mens. Korte afstandsverspreiding gebeurt door middel van sporangiën, verspreid door wind, dauw of regendruppels of door middel van zoösporen.

Chemische bestrijding met behulp van fungiciden zoals Dithane M-45 (mancozeb) en Ridomil MZ-72 (metalaxyl) is de meest voorkomende manier om het pathogeen in de hand te houden. In Uganda echter hebben fungiciden veel nadelen: hoge prijzen, slechte beschikbaarheid, vervalste produkten, slechte houdbaarheid en milieuverontreiniging. Op sommige plaatsen hebben stammen van *P. infestans* metalaxyl-resistentie vertoond. *Phytophthora*-resistente tomaatcultivars zijn er weinig en worden niet in Uganda geteeld. Het gebruik van gewijzigde cultuurmethoden als bestrijdingswijze is niet goed ontwikkeld.

Het algemene doel van onderzoek aan de *Phytophthora*-ziekte op tomaat in Uganda is de vermindering van schade door middel van een geïntegreerd ziektebestrijdingsplan, waarbij het gebruik van fungiciden is geminimaliseerd en de tomatenproductie vervolgens wordt verhoogd. In deze studie werd gebruik gemaakt van de tomatenlijn MT55 omdat deze vatbaar is voor de *Phytophthora*-ziekte en resistent tegen de bacterieziekte veroorzaakt door *P. solanacearum*.

Hoofdstuk 2

Tien districten uit verschillende agro-klimatologische zones in Uganda werden bekeken op de daar gebruikte *Phytophthora*-bestrijdingsmethoden en tomatenteeltwijzen. Gebruikmakend van een van tevoren opgestelde vragenlijst werd informatie verkregen van de landbouwvoorlichtingsstaf, boeren en verkopers van landbouwchemicaliën. Er werd een collectie aangelegd van isolaten van *P. infestans* van tomaat uit enkele districten van Uganda. Deze isolaten werden gekarakteriseerd aan de Landbouwuniversiteit Wageningen (Nederland). Compatibiliteitstoetsen werden uitgevoerd door het samenbrengen van bekende *P. infestans*-stammen A1 en A2 met de Ugandese isolaten. Dit gebeurde op helder Rye A agarmedium. Na een week werden slechts bij de combinaties *P. infestans* uit Uganda en A2 stammen oosporen waargenomen. Dit geeft aan dat de *P. infestans* isolaten afkomstig van tomaat uit Uganda van het paringstype A1 waren.

'Moneymaker', 'Marglobe', 'Heinz' en 'Roma' zijn de meest commerciële tomaatcultivars welke in Uganda worden geteeld, maar de eerste twee zijn het meest populair. De *Phytophthora*-ziekte en ringrot zijn de meest serieuze ziekten van tomaat in Uganda. Bolworm en thrips zijn de meest storende insectenplagen. Alle tomaatvariëteiten welke in Uganda worden geteeld, zijn vatbaar voor *P. infestans* en de meest populaire tomaatcultivars zijn de meest vatbare. Het onderzoek bracht aan het licht dat de tomaatcultuur meestal het jaar rond plaats vindt, wat het voor het pathogeen mogelijk maakt om zich te verspreiden naar een nieuwe generatie gewassen en dus te overleven. De *Phytophthora*-ziekte is het ernstigst gedurende het regenseizoen. Fungiciden spelen in Uganda een belangrijke rol in de bestrijding van de ziekte en Dithane M45 (mancozeb) werd het meest gebruikt. In de meeste districten was dit meer dan 90% van het totale gebruik. Fungiciden zijn niet altijd succesvol in de bestrijding en dit heeft boeren ertoe gebracht de wekelijkse spuitfrequentie te verdubbelen of te verdrievoudigen en de dosering te verhogen. Het telen van tomaat op de top van heuvels, het snoeien en sanitatie gecombineerd met fungiciden werden in de literatuur vermeld als een middel om de *Phytophthora*-aantasting en het fungicidengebruik te verlagen. Insecticiden werden altijd in combinatie met fungiciden toegepast. Volgens het inzicht van de boeren zou bemesting niets bijdragen aan een beheerssysteem voor de *Phytophthora*-ziekte, maar zou het gunstig kunnen zijn bij de ontwikkeling van resistentie door versterking van de groeikracht en fysieke afweer. Problemen verbonden aan pesticiden, de verkoopbaarheid van het produkt en de *Phytophthora*-ziekte bleken de meest serieuze beperkingen te zijn.

Hoofdstuk 3

Phytophthora-soorten zijn dikwijls moeilijk uit rottend weefsel of grond te isoleren. Isolatie van het pathogeen vindt in het algemeen plaats met gebruik van vatbare aardappel (*Solanum tuberosum*) cultivars, zoals 'Bintje'. Ook is getracht om kleine stukjes ziek weefsel rechtstreeks uit te platen op Rye A agar aangevuld met een cocktail van antibiotica. Bij afwezigheid van een vatbare aardappelcultivar was het vinden van een nieuwe methode voor de isolatie van het pathogeen uit tomaat noodzakelijk. Het in cultuur brengen van het pathogeen is ook in de tropen een probleem daar ingrediënten als roggezaad, het aanbevolen Rye A agarmedium of V8 agar geïmporteerd moeten worden.

Van jonge, groene en gezonde tomaten ('Moneymaker') werd de buitenkant gedurende 10 minuten in 96% ethanol gedesinfecteerd. De vruchten werden vervolgens aseptisch in schijven van 5 mm gesneden. Uit de rand van een *Phytophthora*-kolonie op een tomatenblad werden stukjes van ongeveer 25 mm^2 gesneden. De geïnfecteerde stukjes werden vervolgens in steriele petrischalen van 94 mm diameter gelegd, bedekt met een schijf tomaat en gedurende 5 dagen geïncubeerd bij kamertemperatuur (25 C). Een andere techniek maakte gebruik van delen van geïnfecteerde groene vruchten. De bovenbeschreven procedure werd daarbij gevolgd zonder dat bladdelen werden gebruikt. De tomatenschijven werden snel door *Phytophthora* gekoloniseerd en vormden een dichte massa mycelium op de tomatenschijven. Het pathogeen bleef gedurende tenminste 15 dagen op de schijven sporuleren. De uiteinden van hyfen van de schimmel werden aseptisch overgebracht op Rye A agar en in cultuur gebracht. Verdere zuivering werd bereikt door toevoeging van de antibiotica pimaricine, rifampicine en oxytetracycline en het fungicide carbendazim in Rye A agar.

De techniek van het isoleren uit zieke bladeren heeft het voordeel dat die aangepast kan worden voor andere aangetaste delen van de tomatenplant. Omdat gezond weefsel wordt gebruikt, worden de schijven niet meteen gekoloniseerd door andere rot veroorzakende microorganismen. Het nadeel van deze techniek is dat de isolatie snel moet gebeuren om uitdroging te voorkomen. De techniek waarbij isolatie direkt uit aangetaste vruchten plaats vindt, verlengt de levensduur van de monsters. Het grootste nadeel is dat de isolatie in de war wordt gebracht wanneer infektie al heeft plaats gevonden door andere pathogenen. Preliminair onderzoek werd gedaan aan het gebruik van sorghum, gierst en maïs als vervangende ingrediënten voor media. Het pathogeen groeide hierop slechts matig. Maïs was het meest veelbelovend, maar verdere experimenten zijn noodzakelijk.

Hoofdstuk 4

Het belangrijkste doel van deze studie was de vaststelling of het gebruik van fungiciden zou kunnen worden vervangen door sanitatie als enige methode om de *Phytophthora*-ziekte op tomaat te bestrijden. Het onderzoek werd opgezet als factoranalyse met 6 experimenten verdeeld over een periode van 3 jaar als de primaire factor en 3 behandelingen (sanitatie, fungicide en onbehandeld) als secundaire factoren binnen de experimenten. Andere behandelingen binnen deze experimenten zijn afzonderlijk vermeld in hoofdstuk 5-7.

Zaailingen van tomaat werden in droge perioden gedurende ongeveer 4 weken opgekweekt in kweekbedden. De kweekbedden werden iedere 7 dagen tegen de *Phytophthora*-ziekte beschermd door bespuiting met Dithane M-45 met de aanbevolen dosering van 2.5 gr per liter. Tomatenzaailingen (MT55) van 15 cm gemiddelde lengte werden naar het veld overgebracht samen met geïnfecteerde bronplanten die op iedere hoek van de experimentele eenheden werden geplaatst op een afstand van 2 meter. Alle experimentele velden hadden een rand van 4 m en werden omsloten door maïs (*Zea mays*). In alle plots werd *mulching* toegepast met *Imperata* gras (*Imperata cylindrica*). Een spore inoculum werd verkregen door geïnfecteerde bladeren te verzamelen en gedurende 48 uur bij kamertemperatuur te incuberen. De incubatie vond plaats in 5 petrischalen van 94 mm diameter waarin zich natte watten bevonden om verdere sporulatie te bevorderen. Een hoeveelheid van 500 ml sporesuspensie van 2.5×10^5 sporen per ml werd 2 weken na overplanting met behulp van een handspuit met een capaciteit van 1.5 liter over de bronplanten verneveld. Dit gebeurde in de avond om infectie te vergemakkelijken. Zieke plantendelen werden iedere 2 dagen verwijderd. Strikte maatregelen werden genomen om te voorkomen dat zieke plantendelen *Phytophthora*-sporen konden verspreiden over gezonde planten. Het fungicide werd toegepast in een dosering van 2.5 gram per liter met een interval van 7 dagen. Hiervoor werd een rugspuit gebruikt met een capaciteit van 15 liter. Als insectenplagen voorkwamen werd Dursban gespoten in een dosering van 18.5 ml per 15 liter water. Regelmatig werd gewied. Twee weken na overplanting werden er voor het doen van waarnemingen 6 planten per plot gemerkt. De variabelen welke in alle experimenten werden gescoord waren: plantlengte, aantallen zieke en gezonde bladeren, aantastingsgraad van het 5^e en 9^e blad, aantallen bloemen en vruchten en het gewicht van de geoogste vruchten. De ziekte-incidentie werd waargenomen aan 30 *at random* gekozen planten per plot. Andere variabelen werden gescoord op de hoofdstengel van de 6 gemerkte planten. Veldwaarnemingen werden iedere 14 dagen verricht. De weersomstandigheden werden vastgelegd op het Kawanda weerstation. De gegevens werden geanalyseerd met behulp van ANOVA.

De ziektegraad en incidentie per plot waren het laagst in de plots met sanitatie. Dit kan zijn veroorzaakt door een verminderd aantal bronnen en dus door lage hoeveelheden inoculum in de plots met sanitatie. Sanitatie als enige middel ter bestrijding van *Phytophthora*-ziekte had een groeiremmend effect op tomatenplanten. Dit werd weerspiegeld in de gereduceerde planthoogte, bloemvorming en vruchtproduktie. Ofschoon sanitatie alleen een averechts effect had op de tomatenontwikkeling en -produktie, reduceerde het wel het *Phytophthora*-niveau in het gewas en was het als zodanig een veelbelovende bestrijdingswijze van de *Phytophthora*-ziekte. Ofschoon de ziekteniveaus relatief hoog waren, gaven de met fungicide behandelde tomatenplanten de hoogste aantallen gezonde bladeren, bloemen en vruchten en de hoogste opbrengst.

Hoofdstuk 5

Er werd verondersteld dat afscherming van tomatenplanten met een polyethyleendek, indien vroeg in de gewascyclus aangebracht en gecombineerd met sanitatie, tomaten kan beschermen tegen luchtverspreide *P. infestans* sporen. Er werd onderzoek gedaan met als belangrijkste doelen het evalueren van het gebruik van polyethyleentunnels en het vaststellen van de beste periode voor het gebruik ervan in de beheersing van *Phytophthora*-ziekte in een regenafhankelijke cultuur. Er werden polyethyleentunnels toegepast gedurende 0-2, 1-3, 2-4 en 0-4 maanden van de groeiperiode. Dit werd gecombineerd met sanitatie en vergeleken met de 3 standaard behandelingen: alleen sanitatie, fungicidenbehandeling en een onbehandelde controle. De behandelingen vormden een zgn. *randomised complete block design* met 5 herhalingen. Na het overplanten van de tomatenzaailingen werden transparante polyethyleentunnels van 4 m lang, 1.2 m breed en 1.5 m hoog aangebracht. De tunnels werden gemaakt van eucalyptuspalen bedekt met 2 stukken transparant polyethyleen. Experimentele eenheden werden gevormd door plots van 5 x 4 m met een geïnfecteerde tomatenbron op iedere hoek (afstand 2 m) en omringd door een rand van 3 m. Voor de tunnelbehandelingen werden er in ieder plot 3 tunnels geplaatst, 0.7 m van elkaar. Temperatuur en relatieve luchtvochtigheid binnen en buiten de tunnels werd vastgelegd door een digitale thermohygrometer.

De resultaten lieten zien dat tunnels in een vroeg stadium en gecombineerd met sanitatie de hoogste aantallen gezonde en de laagste aantallen zieke bladeren gaven. *Phytophthora*-incidentie en -aantastingsgraad waren hoog in de controleplots en in de met fungicide behandelde plots en waren steeds laag wanneer sanitatie werd toegepast. Een fungicidenbehandeling stopte de *Phytophthora*-epidemieën niet maar vertraagde ze ongeveer 2 weken. Tunnels aangebracht in een vroeg stadium en gecombineerd met sanitatie hadden geen effect op de plantengroei, indien vergeleken met de fungicidenbehandeling. Sanitatie alleen of in combinatie met laat aangebrachte tunnels hadden een nadelig effect op de plantengroei en -produktie. Vroeg aangebrachte tunnels konden het nadelige effect verminderen. Bloem- en vruchtproduktie waren even goed bij de plots met vroeg aangebrachte tunnels als in de met fungicide behandelde plots, maar de opbrengst per plot was altijd het hoogst in de met fungicide behandelde plots. De studie toonde aan dat polyethyleentunnels, vooral indien vroeg in de gewascyclus aangebracht, een Dithane M-45 behandeling tegen *Phytophthora* konden vervangen.

Hoofdstuk 6

Er werden *intercropping*-experimenten uitgevoerd met als belangrijkste doel de *Phytophthora*-ontwikkeling in tomaat te reduceren door het introduceren van niet-gastheer planten in een polycultuur. *Intercropping* verzekert de boeren ook van voedsel door verlaging van het risico van een misoogst en de verschaffing van een andere bron van inkomsten. De experimenten werden uitgevoerd in een zgn. *randomised complete block design* met 5 herhalingen. Sorghum (*Sorghum bicolor*), zonnebloem (*Helianthus annuus*), sojaboon (*Glycine max*) en sesam (*Sesamum indicum*) werden elk gebruikt als tussengewas bij tomaat en gecombineerd met sanitatie. Deze behandelingen werden vergeleken met de standaardbehandelingen: alleen sanitatie, fungicidenbehandeling en een onbehandelde controle.

Er waren meer zieke bladeren in de met fungicide behandelde en controleplots dan in de plots met tussengewas en alleen sanitatie. Tomaten die groeiden samen met lage tussengewassen (sojaboon en sesam) hadden grotere aantallen gezonde bladeren dan die samen met hoge tussengewassen (zonnebloem en sorghum).

Phytophthora-incidentie en -aantastingsgraad waren altijd lager in plots met een tussengewas gecombineerd met sanitatie dan in met fungicide behandelde plots en controleplots. Tomatenplanten met een tussengewas waren gedurende hun hele ontwikkeling altijd lager dan het tussengewas. Dit betekent dat een tussengewas in staat is: (1) een barrière te vormen die de verspreide *Phytophthora*-sporen kan tegenhouden, (2) de afstanden tussen weefsel dat vatbaar is voor *Phytophthora* en gezond weefsel te vergroten, en (3) de windsnelheid af te remmen die nodig is om de sporen van het blad los te maken en te verspreiden. De hoge tussengewassen (zonnebloem en sorghum) vertoonden een storend effect op de groei van tomatenplanten bij een rijafstand van 60 cm, vermoedelijk door schaduwwerking. Tomatenplanten met een laag tussengewas gaven meer bloemen en vruchten en een hogere opbrengst dan die welke hoge planten als tussengewas hadden of die welke alleen een sanitatiebehandeling hadden. De met fungicide behandelde plots gaven de hoogste aantallen bloemen en vruchten en ook de hoogste opbrengst. De studie toonde aan dat sojaboon en sesam (lage gewassen) geschikt zijn als tussengewas van tomaat.

Hoofdstuk 7

Het hoofddoel van de experimenten met hoge tomatenplantdichtheden was de mogelijkheden te onderzoeken om de potentiële neveneffecten van sanitatie te reduceren in de ziektenbestrijding. Het experiment was een zgn. *randomised complete block design* met 5 herhalingen. Tomatenplots met plantafstanden van 0.35 x 0.70 m, 0.35 x 0.50 m, 0.35 x 0.35 m en 0.25 x 1.0 m, elk gecombineerd met sanitatie, werden vergeleken met alleen sanitatie, een fungicidenbehandeling en een onbehandelde controle, allen met een standaard plantafstand van 0.35 x 1.0 m.

Phytophthora-incidentie en -aantastigsgraad waren het hoogst in de controleplots en het laagst in plots waar sanitatie werd toegepast. Het fungicide stopte de *Phytophthora*-epidemieën niet maar stelde ze weer ongeveer 2 weken uit. De *Phytophthora*-ziekte veroorzaakte in belangrijke mate bladval in de controleplots. Sanitatie had, ongeacht de gewasdichtheid, steeds ongeveer hetzelfde negatieve effect op de planthoogte. Bloem- en vruchtvorming waren ook ongeveer gelijk bij de verschillende gewasdichtheden. Dit betekent dat de verschillende plantafstanden geen negatieve agronomische effekten hadden, zodat verhoging van de gewasdichtheid als factor kan worden gebruikt in een geïntegreerd bestrijdingsprogramma voor *P. infestans.* Met fungicide behandelde plots gaven de hoogste opbrengst. Waar sanitatie werd toegepast was er geen significant verschil in opbrengst ongeacht de plantdichtheid.

Hoofdstuk 8

Het onderzoek aan *P. infestans* op tomaat in Uganda had tot doel eerder bestudeerde bestrijdingswijzen te combineren. De in dit proefschrift beschreven experimenten met polyethyleentunnels, intercropping van tomaat met andere gewassen en hoge plantdichtheden, elk gecombineerd met sanitatie, gaven daartoe veelbelovende resultaten. Het hier beschreven experiment bestudeerde het collectieve effect van polyethyleentunnels, intercropping, hoge plantdichtheid en sanitatie op de epidemiologie van *P. infestans* in tomaat in regenafhankelijke gebieden. De behandelingen betroffen: polyethyleentunnels plus sesam (*Sesamum indicum*) als tussengewas met en zonder sanitatie, alleen sanitatie, een fungicide (Dithane M-45) behandeling en een onbehandelde controle. Het experiment werd uitgevoerd in een *randomised complete block design* met 5 herhalingen. De experimentele eenheden waren plots van 6 x 4 m met op iedere hoek een bronplant (op 2 m afstand) en omringd door een open ruimte van 3 m. De plots met polyethyleentunnels hadden 3 tunnels van 4 m lengte, 1.5 m breedte en 1.5 m hoogte. Ze stonden 0.75 m van elkaar. De tunnels werden gebouwd met behulp van palen van eucalyptushout en 2 stukken polyethyleen. Temperatuur en relatieve luchtvochtigheid binnen en buiten de tunnels werd vastgelegd door een digitale thermohygrometer.

De incidentie en aantastingsgraad van *P. infestans* waren het laagst waar sanitatie werd toegepast en het hoogst zonder sanitatie. Dit betekent dat sanitatie alleen of toegepast in combinatie met andere cultuurmaatregelen een gelijk effect hadden op de verlaging van *P. infestans*-incidentie en -aantastingsgraad. De controleplots, de met fungicide behandelde plots en de geïntegreerde plots zonder sanitatie hadden gedurende het hele seizoen erg hoge ziekteniveaus. Dit was vermoedelijk te wijten aan hoge inoculumniveaus. De fungicidenbehandeling was effectiever in de reductie van *P. infestans*-niveaus dan de geïntegreerde behandeling zonder sanitatie. De fungicidenbehandeling en de geïntegreerde behandeling zonder sanitatie stopten de *Phytophthora*-epidemie niet, maar verlaagden eerder de ontwikkelingssnelheid ervan. De tomatenplanten werden zowel in de geïntegreerde plots met en zonder sanitatie als in de met fungicide behandelde plots even groot. Het aantal gezonde tomatenbladeren was in de geïntegreerde plots met en zonder sanitatie hoog, evenals de bloem- en vruchtproduktie. Sanitatie alleen gaf de laagste planten en laagste aantallen gezonde bladeren, bloemen en vruchten. Het experiment bevestigde eens te meer dat sanitatie op het niveau zoals hier toegepast een nadelig effect had op de plantengroei en -produktie. De methode van geïntegreerde bestrijding van de *Phytophthora*-ziekte scheen hier het nadelige effect van sanitatie te hebben gelimiteerd en gaf goede opbrengsten. De fungicidenbehandeling gaf echter ook hier het hoogste gewicht aan geoogste vruchten per plot.

Hoofdstuk 9

Het doel van dit onderzoek was de ontwikkeling van methoden voor geïntegreerde bestrijding van de *Phytophthora*-ziekte in tomaat in Uganda. Dit zou hopelijk leiden tot lagere ziekteniveaus en oogstverliezen, tot verminderd gebruik van fungiciden en tot een hogere tomatenproduktie. Verwacht werd dat verschillende cultuurmaatregelen, zoals sanitatie, het gebruik van polyethyleentunnels, *intercropping,* een verhoogde plantdichtheid en een combinatie van deze methoden, een belangrijke rol zouden spelen bij de ontwikkeling van geïntegreerde bestrijdingsmaatregelen. Het was echter niet duidelijk welk effect deze maatregelen, afzonderlijk of in combinatie, zouden hebben op de epidemiologie van de *Phytophthora*-ziekte en op de tomatenontwikkeling en -produktie. Het hier beschreven onderzoek had tot doel het effect te bestuderen van elk van de genoemde maatregelen op de ziekte. Sanitatie, als cultuurmaatregel alleen toegepast, kan de ziekte-incidentie en -aantastingsgraad op tomaat verlagen. Sanitatie alleen heeft echter ongewenste bijeffecten op de ontwikkeling van het gewas en zijn produktie. De introduktie van andere bestrijdingsmethoden zoals polyethyleentunnels, compatibele tussengewassen (bv sesam en sojaboon) en een grotere plantdichtheid kunnen de ziekte-incidentie en -aantastingsgraad ook verlagen en tevens deze nadelige effecten verlagen. Polyethyleentunnels en tussengewassen kunnen effectieve barrières zijn voor de verspreiding van *P. infestans* sporen en op deze wijze het ziekteniveau verlagen.

Dit onderzoek was hoofdzakelijk explorerend van aard en bedoeld als eerste stap in de richting van de ontwikkeling van een bestrijdingspraktijk met behulp van cultuurmaatregelen voor de *Phytophthora*-ziekte in Uganda en elders. Deze studie heeft de grondslag gelegd waarop verder onderzoek kan worden gebaseerd. Verder onderzoek moet worden gedaan om economische drempelwaarden voor sanitatie vast te leggen en vast te stellen hoe deze kunnen worden gecombineerd met resistente cultivars. Aanvullend onderzoek moet worden verricht om de meest geschikte constructie te vinden van goedkope polyethyleentunnels voor tomaat in een regenafhankelijke cultuur. Aanvullend onderzoek moet worden gedaan aan andere faktoren zoals een voldoende watergift aan de tomatenplanten in de tunnels. Het is ook van belang om andere gewassen te identificeren als tussengewas en verder onderzoek hieraan te verrichten. Andere belangrijke terreinen van onderzoek in Uganda, welke niet in dit proefschrift worden behandeld zijn de identificatie van bronnen van resistentie en resistentieveredeling, beide nodig voor het verkrijgen van duurzame resistentie tegen *P. infestans.* Cultuurmaatregelen welke erop gericht zijn de positieve effecten van sanitatie te gebruiken in de bestrijding van plantenziekten hebben grote mogelijkheden bij de beheersing van de *Phytophthora*-ziekte in de tomatencultuur. Wat blijft is om door toegepast onderzoek vast te stellen welke de witte vlekken en tekortkomingen zijn van cultuurmethoden voor ziektenbestrijding. Dit zou, in Uganda en mogelijk ook andere landen, de weg effenen voor een toekomstige aanvaarding en toepassing van bestrijdingsmethoden met behulp van cultuurmaatregelen, voornamelijk in combinatie. Ofschoon fungicidenbehandelingen de hoogste opbrengst gaven, kunnen geïntegreerde cultuurmaatregelen, indien verder ontwikkeld, een effectieve bescherming geven tegen de *Phytophthora*-ziekte van tomaat, om zo boeren in staat te stellen substantiële opbrengsten te realiseren.

Publications

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2. Tumwine, J.; Frinking, H.D. and Jeger, M.J., 1999. Isolation techniques and culturing media for *Phytophthora infestans* on tomatoes (Short communication). The Mycologist.

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Author's curriculum vitae

The author was born on 8 July 1962 at Kagazi village, Kabarole district, Uganda. He received his junior education at Kiceece, Bunena and Kitagata primary schools. He went to Ibanda secondary school and obtained an ordinary certificate of education in 1982. He proceeded to Ntare school where he obtained an advanced certificate of education in 1985. He enroled at Makerere University in 1985 from where he obtained a B.Sc. in Agriculture (Crop Protection) in June 1988. He started working as a Research Officer (Plant Pathologist) at Kawanda Agricultural Research institute in November 1988. He went for in-service training courses in: Plantain Research and Technology Transfer, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria in September 1989; Integrated Pest Management, Mukono DFI, Uganda in April 1991; Biological Control of Food Crop Pests, IITA, Cotonou, Benin in October 1991; Identification of Microfungi of Importance in Crops, Kabeta campus University of Nairobi, Kenya in November 1991; and Diagnostic Techniques in Plant Pathology, Chiromo campus University of Nairobi, Kenya in May 1996. The author started a Masters degree course at Wageningen Agricultural University (WAU), The Netherlands in August 1992 and graduated with an M.Sc. (Crop Protection/Plant Pathology) in January 1994. He embarked on a PhD research project in February 1995 in the Department of Phytopathology WAU, of which this thesis is a product, defended in public on 1 February 1999. He is married to Immaculate Mbabazi Tumwine, and he has five children.

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