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Monitoring and diagnostic aids for predicting and managing soil-borne diseases in fresh tomatoes

Dr Graham Stirling
Biological Crop Protection Pty Ltd

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Level 1
50 Carrington Street
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Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

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Graham R. Stirling *et al.*

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Principal Investigator

Dr. Graham Stirling
Biological Crop Protection Pty. Ltd.
3601 Moggill Road
Moggill QLD 4070

Telephone: (07) 3202 7419
Facsimile: (07) 3202 8033
Email: biolcrop@powerup.com.au

Other key personnel

Mr. Dale Griffin, Biological Crop Protection
Dr Marcelle Stirling, Biological Crop Protection
Dr Kathy Ophel-Keller, SARDI
Dr Alan McKay, SARDI
Mr Chris Monsour, Bowen Crop Monitoring Services
Mr Bill Hardie, Crop Tech
Mr Julian Winch, Valley Crop Monitoring Service

Purpose of this report

To report on research aimed at improving the monitoring and diagnostic aids available for predicting and managing soil-borne diseases of tomatoes.

Cover Photograph

Project participants inspecting soil-borne diseases in a Bundaberg tomato-field.

Funding Sources and Acknowledgment of contributions

This project was funded by Horticulture Australia, with voluntary contributions from Queensland Fruit and Vegetable Growers Ltd. Graham Stirling led the project while Dale Griffin coordinated the monitoring component and did much of the technical work. Chris Monsour, Bill Hardie and Julian Winch located appropriate monitoring sites, conducted field monitoring, collected samples and provided feedback to growers. Kathy Ophel-Keller and Alan McKay collaborated with CSIRO in the development of specific PCR primers for root-knot nematode and *Fusarium oxysporum* f. sp. *lycopersici*. They also optimised DNA tests and analysed soil samples. Marcelle Stirling counted free-living nematodes and did much of the soil health work, while Heidi Martin (Queensland DPI) kindly provided several cultures of *F. oxysporum* f. sp. *lycopersici*.

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1. MEDIA SUMMARY

In Australia's main tomato growing areas around Bowen and Bundaberg in Queensland, soil-borne diseases often cause heavy crop losses. Many growers cope with these disease problems by fumigating soil with methyl bromide or metham sodium, or by applying nematicides such as fenamiphos or oxamyl. However, the decision to use these chemicals is not always made on a rational basis. Fumigants and nematicides are often applied routinely as an insurance against losses, even though the causal pathogens are not always present at levels that will cause economic damage.

In a project funded by the Queensland tomato industry and Horticulture Australia, Dr Graham Stirling from Biological Crop Protection and a team of pest management consultants from all tomato-growing areas of Queensland developed procedures that will enable growers to reliably predict whether particular diseases will cause problems in their next tomato crop. Information on cropping history, previous disease incidence, soil texture and planting date was used to calculate a Hazard Index for each of the diseases that are likely to occur in a field. The research team then went on to show that this index provided a useful numerical assessment of disease risk. Losses from root-knot nematode and fusarium wilt, for example, were only observed in fields with a Hazard Index of more than 40 and 30, respectively.

Another way of improving the prediction process is to collect soil samples prior to planting, determine the levels of pathogens in the sample and then use the results to make an informed management decision. In this component of the work, Dr Stirling collaborated with Dr Kathy Ophel-Keller and Dr Alan Mc Kay from the South Australian Research and Development Institute in Adelaide, who have used DNA technologies to develop a root disease testing service for the Australian cereal industry. The aim was to see whether similar procedures could be used in horticulture. The results were encouraging, as it was possible to detect and quantify root-knot nematode and the fungal pathogens responsible for fusarium and verticillium wilt in soil from tomato fields using DNA techniques. The amount of pathogen DNA was related to disease incidence in the field, demonstrating that the test will be useful for predictive purposes.

Growers interested in using the Hazard Index to predict disease risk should contact their local pest management consultant. However, the DNA tests will require further

development before they are available to growers, but Dr Stirling is hopeful that they will be commercialised within a few years. In the meantime, he is confident that the work done to date provides the basis for improving risk management systems for soil-borne diseases in the tomato industry.

2. TECHNICAL SUMMARY

Soil-borne diseases caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Verticillium dahliae*, and root-knot nematode (*Meloidogyne* spp.) are three of the main constraints to tomato production in Australia's main winter tomato-growing areas. Many growers use soil fumigants such as methyl bromide and metham sodium, and nematicides such as fenamiphos or oxamyl as control measures, but the decision to use them does not always have a rational basis. In many cases, the chemicals are applied routinely prior to or immediately after planting as an insurance against losses, even though pathogens may or may not be present at levels that will cause economic damage.

The objective of this project was to improve the monitoring and diagnostic aids available to growers so that they can confidently and reliably predict whether particular pests or pathogens will cause problems in the next tomato crop.

The first step in improving the predictive process is to recognise that historical and environmental factors are important in determining whether a particular pathogen will occur in a particular field. Records of cropping history, notes on disease incidence in previous crops, details of the soil texture and knowledge of the temperatures likely to be experienced by the forthcoming crop all provide information of predictive value, and this information is always available before a crop is planted. Numerical values were therefore assigned to various scenarios based on an understanding of the factors that influence disease severity, and the points allocated were then collated to produce a single number between 1 and 50 known as the Hazard Index. The risk assessment process was therefore formalised and the end result was a numerical estimate of disease risk for a particular field.

During the 2001 and 2002 growing seasons, collaborating pest management consultants in all tomato-growing areas of Queensland collected soil samples from more than 100 commercial fields prior to planting. They also obtained historical records from growers and used them with relevant environmental information to determine a Hazard Index for both root-knot nematode and FOL in all fields. Later in the season they returned to the fields and recorded the incidence and severity of soil-borne diseases.

During the monitoring process, consultants observed that root-knot nematode did not cause economic damage unless the mean gall rating at harvest was greater than about 6 (on

a 0-10 scale). The Hazard Index proved to be useful for identifying fields where there was some risk of nematode damage because high gall ratings only occurred in fields with a Hazard Index greater than 40. About 50% of fields had a Hazard Index of less than 40 and very low gall ratings, suggesting that root-knot nematode is not a universal problem in the Queensland tomato industry.

The predictive value of the Hazard Index for FOL could only be evaluated in fields planted to varieties that are susceptible race 3 of FOL. Nevertheless, the results from these fields indicated that the Hazard Index had predictive value, as unacceptable levels of fusarium wilt were never observed in fields with a Hazard Index of less than 30.

The second step in improving the prediction process involved quantifying key pathogens in pre-plant soil samples and using the data to predict disease risk. The results for root-knot nematode showed that when nematodes were extracted using standard extraction techniques, the presence or absence of the nematode at planting had predictive value. When root-knot nematode was present in pre-plant samples, there was a chance that losses would occur. When the nematode was absent, roots were either not galled or gall ratings were too low to cause economic damage.

Experiments in large pots and in the field showed that there was a good relationship (R^2 = 0.64-0.73) between numbers of root-knot nematode detected in soil using traditional nematode-extraction methods and numbers obtained using a DNA technique. Detection limits for both methods were about the same, which opens up opportunities to use DNA techniques to quantify root-knot nematode in vegetable-growing soils. As with manual techniques, there were difficulties in detecting and quantifying very low nematode densities. However, DNA readings were clearly positive in fields where the initial nematode density was high enough to produce a final gall rating greater than 4. Since levels galling must be higher than this before there is any likelihood of economic damage, the DNA test will therefore be useful for predictive purposes.

Because there are no reliable techniques for quantifying inoculum of fungal pathogens such as FOL, a molecular primer was developed that is specific for this pathogen. When the primer was used to quantify FOL DNA in tomato-growing soils, very high readings (100-550 pg FOL DNA/g soil) were obtained in fields that had grown tomatoes for many years. However, the results of pot experiments and observations in the field showed that disease incidence was relatively high (4-32% of plants affected by fusarium wilt) in soils with less

than10 pg FOL DNA/g soil. A similar DNA test for *Verticillium dahliae* (VD), the cause of verticillium wilt of tomato, showed that when temperatures were suitable for the pathogen, disease incidence was high in soils with less than10 pg VD DNA/g soil. These results indicate that unacceptably high levels of both fusarium and verticillium wilt can occur in soils in which the causal pathogens are just detectable with DNA techniques.

The work done in this project provides the basis for developing a root disease risk management service for the tomato industry similar to the Root Disease Testing Service operated by SARDI for the cereal industry in Southern Australia. However, before such a service can be established, the new DNA tests must be commercialised and an accreditation program developed for agronomists and pest management consultants so that they are able to interpret test results and provide reliable advice to growers. Additional research will also be needed to further validate the DNA tests for fusarium and verticillium wilts in the field, and to widen the range of pathogens that can be quantified with DNA methods.

3. INTRODUCTION

The Queensland tomato industry produces about 75% of Australia's fresh-market tomatoes. This makes it one of the state's most significant horticultural industries. Production is concentrated within three main areas: Bowen, Bundaberg, and southeast Queensland (i.e. the Lockyer Valley and Granite Belt). Each area is located within a different climatic region, enabling the industry to supply markets with a year-round supply of tomatoes. Most of the Queensland production occurs during winter and spring, when low temperatures limit production in the southern states.

Tomato crops in Queensland are grown using a specialised production system. Wire trellising, subterranean trickle-irrigation and plastic mulch have been introduced to increase yields and improve fruit quality, mainly by reducing the incidence of foliar diseases and fruit rots that are common in conventional, ground-grown cropping systems. Fruit is picked by hand onto large machines that straddle several rows and is graded and packed in highly mechanised packing sheds. This production system is expensive, which means that a limited number of large growers now produce most of the tomatoes grown in Queensland. The production system is also intensive, which means that land is often cropped to tomatoes every 1–3 years.

Intensive production systems such as those used in the tomato industry provide an ideal environment for soil-borne diseases. Indeed, losses from soil-borne diseases occur in all tomato-growing areas. The most important pests and pathogens vary from region to region, but the heaviest losses are caused by root-knot nematode (RKN) (*Meloidogyne* spp.), the fungal pathogens *Fusarium oxysporum* f.sp. *lycopersici* (FOL), *Sclerotium rolfsii* (SR) and *Verticillium dahliae* (VD); and the bacterial-wilt pathogen, *Ralstonia solonacearum* (RS).

Many tomato growers routinely use soil fumigation to insure against losses caused by these pests and pathogens. However, due to the health and environmental impacts of fumigants like methyl bromide and metham sodium, soil fumigation has a limited future. Methyl bromide, for example, is being phased from use in 2005 because of its ozone- depleting properties. Furthermore, soil fumigants are broad-spectrum biocides, and while they are used primarily to control pests and pathogens, they also eliminate beneficial soil organisms, including the natural enemies of nematodes and fungal pathogens.

Integrated pest management (IPM) offers an alternative to soil-fumigation and is currently used by some tomato growers. However, the IPM strategies employed are quite rudimentary and are mainly used in situations where there is a single key pest or pathogen (e.g. RKN or FOL). Relatively few control options are used (e.g. crop rotation, resistant cultivars and target-specific chemicals such as non-volatile nematicides) and decisions on control measures are made on the basis of grower experience or are determined by trial and error.

A more sophisticated IPM program for soil-borne diseases of tomatoes would involve quantifying pathogen levels in a field, using the data to predict the likely impact of those pathogens and then deciding on appropriate control measures. Tomato growers are already familiar with this concept and use it to minimise the impact of aboveground pests such as mites and *Helicoverpa*. Because the practice of IPM requires knowledge of pest biology and an understanding of the interactions between pest species, the environment and the pests' natural enemies, many growers employ professional crop consultants to do the monitoring and provide control recommendations. This existing infrastructure could therefore be used to provide monitoring, diagnostic and prediction services for soil-borne pathogens.

Despite their potential, IPM approaches for soil-borne diseases of tomatoes are currently limited by our inability to confidently predict which pests or pathogens are likely to cause problems in the next crop. Population densities of RKN can be quantified using conventional nematode-extraction techniques and economic thresholds for tomato are known (Stirling, 1999), but similar risk-prediction systems are not available for other pathogens. The main obstacle is our inability to reliably and inexpensively quantify soil-borne pathogens with currently available techniques. Recent developments in molecular diagnostics offer a possible solution to this problem, with DNA-based tests now being used to quantify the main nematode and fungal pathogens of cereals in southern Australia (Ophel-Keller *et al.*, 1999). If similar methodologies were available for tomatoes, it would be possible for consultants to monitor soil-borne pests and pathogens in tomato fields and provide growers with a range of control options.

This report describes work aimed at developing the monitoring protocols and diagnostic tests necessary for predicting losses from the main soil-borne diseases of tomato in Queensland. It consists of four main components: 1. Use of information on cropping history, disease history, soil type and temperature to make an interim assessment of the risk

from soil-borne diseases in a particular field. 2. Development of molecular techniques to reliably detect and quantify RKN and FOL in pre-plant soil samples. 3. Use of data on the levels of various pathogens prior to planting together with an interim assessment of disease risk to predict the extent of crop losses due to soil-borne disease in particular fields. 4. Evaluation of the utility and accuracy of field-specific predictions by comparing predicted risk with the actual levels of disease observed in crops at harvest.

4. INTERIM RISK ASSESSMENT FOR SOIL-BORNE PESTS AND DISEASES

Introduction

Historical factors play an important role in determining the likelihood that a soil-borne disease will occur in a particular tomato field. Cropping history is particularly important because some pathogens (e.g. FOL) have a relatively narrow host range and are only hosted by tomatoes and other closely related crops. Thus, prior cultivation of tomatoes increases the risk that host-specific diseases will occur. The previous occurrence of a disease also provides a warning that the disease could recur in the future. Not only does it demonstrate that the environment in that field is suitable for the disease, but it also indicates that inoculum of the pathogen may still be present.

An understanding of the environment in which the crop is to be grown also provides clues as to the likelihood that certain soil-borne diseases will occur in a field. Temperature is probably the most important environmental factor, as it has a major impact on the incidence and severity of all the important soil-borne diseases of tomato. FOL, RKN, SR and RS have relatively high optimum temperatures, whereas VD is favoured by low temperatures (Jones *et al.*, 1991). Thus the incidence of diseases caused by the first four pathogens could be expected to be greater in spring and summer crops than those grown in winter. In the case of Verticillium wilt, the situation is likely to be reversed. A second environmental factor that warrants consideration from the point of view of prediction is soil texture. It is particularly important for RKN, which is more common and causes more serious crop losses in coarse-textured than fine textured soils (Van Gundy, 1985).

The above observations highlight the fact that where accurate records of cropping and disease history are available and the environment is known, it should be possible to make a preliminary assessment of disease risk. This chapter describes the development of a simple numerical index that could be used by consultants to make such an assessment.

Materials and Methods

Early in the year 2000, crop consultants located in Bowen, Bundaberg and SEQ selected fields that were to be planted to tomatoes during that year. Fields were included if they had previously grown tomatoes, if they had a history of soil-borne disease problems and if they were unlikely to be fumigated prior to planting. The aim was to obtain a

representative sample of fields in which soil-borne diseases were likely to occur in the subsequent tomato crop.

Once fields were selected, a standard recording sheet was used to record details of cropping history and disease history. Depending on the size of the field, between one and three representative 0.4 ha sampling units were then defined in each field and all further observations were made in these areas. The soil texture was classified into five categories using the "ribbon" method of Northcote (1971). Briefly, a sample of field soil was moistened to near field capacity and squeezed between the thumb and forefinger, with texture being determined according to the length of intact ribbon.

The incidence of various soil-borne diseases was determined in each field during the final stages of harvest. Within each sampling unit, four random points were selected and at each of these points, all plants in the four adjacent panels were inspected. The number of plants affected by various soil-borne diseases was recorded and the incidence of root-knot nematode was determined by digging two plants at each point and checking roots for galling. The number of plants inspected in each sampling unit was dependent on plant spacing but ranged from 120 – 200 plants. In situations where consultants could not confidently identify diseases based on symptomology, samples were collected and the causal pathogen was isolated. Large pieces of tissue were surface sterilized with 95% methylated ethanol followed by flaming, whereas small pieces of tissue were immersed in a solution of 1% sodium hypochlorite for 2 min and then washed twice in sterile distilled water. The tissue was then plated onto potato dextrose agar (PDA) amended with streptomycin sulphate (streptomycin sulfate (Sigma) 120 mg L^{-1}).

Results

About 75% of the selected fields had a history of tomato production (Table 4-1) and most of these had grown tomatoes during the last 4 years. Crop rotation was most common in Bundaberg, as 43% of the fields had never previously been used for tomatoes, compared with only 11% and 13% in Bowen and SEQ respectively.

Table 4-1. Production histories of fields monitored in three regions of Queensland during the 2000 season.

Region (# fields)	The number of fields with each production history			
	Never used for tomato production	Used for tomato production more than 4 years ago	Used for tomato production during the last 4 years	
			1 crop	2–4 crops
Bowen (18)	2	4	5	7
Bundaberg (14)	6	1	7	0
SEQ (8)	1	1	4	2
Total (40)	9	6	16	9

The reported incidence of soil-borne diseases in previous tomato crops in each region is presented in Table 4-2. These historical data suggest that FOL is the most important soil-borne pathogen of tomato in Queensland. Fusarium wilt had previously been recorded in 50% of the fields that were selected, but its incidence varied from region to region. The disease had been detected in 80% of the fields around Bowen and only 36% of the fields in Bundaberg. It had never previously been observed in SEQ.

Table 4-2. Historical incidence of various soil-borne diseases in fields planted to tomatoes in 2000.

Region (# fields)	The number of fields where the disease had previously been observed				
	Fusarium wilt	Root-knot nematode	Sclerotium base-rot	Bacterial wilt	Verticillium wilt
Bowen (18)	15	5	6	0	0
Bundaberg (14)	5	5	1	1	0
SEQ (8)	0	5	3	0	1
Total (40)	20	15	10	1	1

Root-knot nematode and Sclerotium base-rot were relatively common in previous crops and had occurred in all regions (Table 4-2). In contrast, verticillium wilt and bacterial wilt had previously been reported from only 5% of fields. The selected fields contained a representative cross section of soil textures and planting times (Tables 4-3 and 4-4).

Table 4-3. The soil textural classes of fields planted to tomatoes during the year 2000

	The number of fields of each soil texture				
Region (# fields)	**Sand**	**Sandy loam**	**Sandy clay-loam**	**Clay loam**	**Clay**
Bowen (18)	1	5	6	6	0
Bundaberg (14)	0	8	0	3	3
SEQ (8)	2	1	0	5	0
Total (40)	3	14	6	14	3

Table 4-4. The planting time of tomato crops monitored during the year 2000

	The number of fields planted in each season			
Region (# fields)	**Spring**	**Summer**	**Autumn**	**Winter**
Bowen (18)	0	0	8	10
Bundaberg (14)	2	7	2	3
SEQ (8)	1	7	0	0
Total (40)	3	14	10	13

Observations in the selected fields at the time crops were harvested showed that four diseases were present. Fusarium wilt was found only in Bowen and Bundaberg, while Verticillium wilt was restricted to SEQ (Table 4-5). Disease history had a significant impact on disease incidence, as most diseases were more common in fields where the disease had previously been observed than in fields that had never had the disease (Table 4-6). This was particularly apparent for Fusarium wilt, root-knot nematode and Sclerotium base rot. The exception was Verticillium wilt, which was sometimes detected in fields where it had never previously been observed, probably because its symptoms are relatively inconspicuous and had not been previously recognised by growers or consultants.

Cropping history also had an impact on the incidence of both Fusarium wilt and Sclerotium base-rot (Table 4-7). Both these diseases were more common in fields that had previously grown tomatoes than in fields that had not. In contrast, previous cropping of tomatoes did not increase the incidence of root-knot nematodes.

Root-knot nematode was more common in coarse than fine-textured soils (Table 4-8), but incidence of other diseases was not affected by soil texture. The effect of planting time on disease incidence was difficult to determine because our data were compromised by the fact that different regions tended to have different planting times (Table 4-4). Thus the high incidence of Fusarium wilt in autumn and winter-planted crops and Verticillium wilt in crops planted during spring and summer (Table 4-9) probably reflects the dominance of these planting times in Bowen and SEQ respectively. The main conclusion that could be drawn about planting time is that the incidence of root-knot nematode was highest in crops growing during the hottest time of the year.

Table 4-5. The incidence of soil-borne diseases in selected Queensland tomato fields during the year 2000

	Number of fields in which the disease was detected				
Region (# fields)	**Fusarium wilt**	**Root-knot nematode**	**Sclerotium base-rot**	**Bacterial wilt**	**Verticillium wilt**
Bowen (18)	9	1	8	0	0
Bundaberg (14)	2	8	2	0	0
SEQ (8)	0	8	5	0	8
Total (40)	11	17	15	0	8

Table 4-6. The relationship between previous disease history and disease incidence in selected Queensland tomato fields

Soil-borne disease		Disease detected historically		Not detected historically	
		Present in monitored crop	Absent in monitored crop	Present in monitored crop	Absent in monitored crop
Fusarium wilt[1]	Resistant cultivar grown	0	10	0	2
	Susceptible cultivar grown	9	1	2	8
Root-knot nematode		11	4	6	19
Sclerotium base-rot		9	1	6	24
Bacterial wilt		0	1	0	39
Verticillium wilt		1	0	7	32

[1] Figures for Fusarium wilt disease exclude fields monitored in SEQ as this disease is not known to occur in this region.

Table 4-7. The relationship between cropping history and disease incidence in selected Queensland tomato fields

		Present in crop	Not present in crop	Present in crop	Not present in crop	Present in crop	Not present in crop	Present in crop	Not present in crop
		History of tomato production							
Soil-borne disease		Never		>4 yr ago only		1 crop in last 4 yr		2 – 4 crops in last 4 yr	
Fusarium wilt[1]	Resistant cultivar grown	0	1	0	0	0	6	0	6
	Susceptible cultivar grown	1	6	5	0	4	2	1	0
Root-knot nematode		5	4	1	5	9	7	2	7
Sclerotium base-rot		1	8	1	5	5	11	8	1
Bacterial wilt		0	9	0	6	0	16	0	9
Verticillium wilt		1	8	1	5	4	12	2	7

[1] Figures for Fusarium wilt disease exclude fields monitored in SEQ as this disease is not known to occur in this region.

Table 4-8. The relationship between soil texture and disease incidence in selected Queensland tomato fields

	% of fields in which a disease was detected	
	Coarse-textured soils[1]	**Fine-textured soils[2]**
Fusarium wilt[3]	44	64
Root-knot nematode	53	35
Sclerotium base-rot	29	61
Bacterial wilt	0	0
Verticillium wilt	18	22

[1] Coarse-textured soils include those classified as either sands or sandy loams.

[2] Fine-textured soils include those classified as either sandy clay-loams, clay loams or clays.

[3] Figures for Fusarium wilt disease exclude fields monitored in SEQ and fields in which a resistant cultivar was grown.

Table 4-9. The relationship between planting time and disease incidence in selected Queensland tomato fields

	% of fields in which a disease was detected			
Planting season	**Spring**	**Summer**	**Autumn**	**Winter**
Fusarium wilt[1]	0	20	100	50
Root-knot nematode	67	79	0	31
Sclerotium base-rot	0	43	60	23
Bacterial wilt	0	0	0	0
Verticillium wilt	33	50	0	0

[1] Figures for Fusarium wilt disease exclude fields monitored in SEQ and fields in which a resistant cultivar was grown.

Discussion

The observations made on selected fields during the 2000 season confirmed expectations that cropping history, disease history, soil type and temperature affected the incidence of all the important soil-borne diseases of tomato in Queensland.

Data for Fusarium wilt were compromised by the widespread use of resistant varieties in Bowen. Nevertheless, both cropping history and disease history had a major impact on the incidence of this disease. When susceptible varieties were grown in Bowen or Bundaberg, the disease was common in fields cropped frequently with tomatoes and was usually present in fields in which the disease had previously been detected. A clear effect of planting time on disease incidence was not apparent in this study. However, previous disease surveys in Bundaberg (Stirling and Ashley, 1999) demonstrated that Fusarium wilt was most common in crops grown during the warmer months of the year. Such a result was anticipated, as temperatures around 28°C favour this disease (Jones *et al.*, 1991).

In contrast to Fusarium wilt, Verticillium wilt was clearly favoured by cool weather. The disease was most common in SEQ, the southernmost and therefore the coolest tomato-growing region in Queensland.

The parameters of most predictive value for root-knot nematode were soil texture and planting time. Crops planted in coarse-textured soils during the warmest time of the year were most likely to be infested. Our results therefore confirm those of others (Van Gundy, 1985) who have shown that the heaviest infestations of root-knot nematodes occur in coarse-textured soils and that the optimum temperature for growth and reproduction is 25-30°C. The number of previous tomato crops did not affect incidence, probably because the nematode has a wide host range and can survive between tomato crops on weeds and other crops that are included in the rotation.

Sclerotium base-rot was similar to root-knot nematode and Fusarium wilt in that its incidence was highest in crops planted during summer and autumn. Cropping history also had an impact on disease incidence, as fields in which tomatoes had previously been grown and fields where the disease had previously been detected had the greatest chance of having the disease.

Since cropping history, disease history, soil texture and temperature clearly affected disease incidence, we developed a hazard index (Table 4-10) to provide a quantitative assessment of risk for the two most widespread diseases of tomato (FOL and RKN). A total of 50 points were allocated for each disease, but the proportion of the points allocated to cropping history, disease history, soil texture and temperature was weighted according to their perceived importance to each disease. Thus cropping history received 10/50 or 20% of the points for Fusarium wilt and only 5/50 or 10% of the points for root-knot nematode. Similarly, soil texture was considered more important for root-knot nematode than for Fusarium wilt. Within each of the four major parameters, the same process was used, with various scenarios allocated points based on their perceived contribution to disease risk.

To determine the disease risk for a particular field, points were allocated to a blank version of Table 4-10. Initially, the risk attributable to each of the major parameters was determined. Points applied to the scenarios listed under each parameter ('Points' column) were then transcribed into the adjacent 'field' column and subtotalled. When the sub-total for a particular parameter was equal to or less than the maximum number of points allocated to that parameter, it was recorded as the total. However, if the sub-total was higher, the maximum point allocation was used. The appropriate number of points for the expected mean temperature parameter was dependent on the anticipated planting date and was allocated for each region and planting time according to Table 4-11. Ultimately, a numerical hazard index was calculated for each field by adding the total points determined for each major parameter.

The main advantage of using a numerical system such as a hazard index is that the process of assessing disease risk is formalised. Thus, when different consultants assess the same field, they should come to the same conclusion about the disease risk. Although the numerical values assigned to various scenarios are arbitrary, the index was thought to have some validity because it was based on an understanding of the main factors that influence disease severity. It was therefore calculated for all fields that were monitored in 2001 and 2002 and its value as a predictive tool was assessed during those seasons (see Chapter 5).

Table 4-10. Values used in calculating a hazard index to quantitatively assess the risk of losses from two diseases of tomato.

PARAMETER	DISEASE			
	Root-knot nematode		Fusarium wilt	
CROPPING HISTORY	Points	Field	Points	Field
Tomatoes grown last year	**5**		**5**	
Tomatoes grown 2 yr ago	**4**		**5**	
Tomatoes grown 3 yr ago	**2**		**5**	
Tomatoes grown 4-10 yr ago	**0**		**3**	
Tomatoes never grown at site previously	**0**		**0**	
RKN-susceptible crop or weeds grown in the last 2 yr	**5**		**N/A**[1]	**N/A**
Forage sorghum green-manure crop prior to planting	**-2**		**N/A**	**N/A**
Cropping history unknown to grower	**5**		**10**	
MAXIMUM/ SUB TOTAL	**5**		**10**	
CROPPING HISTORY TOTAL (adjust down if > max poss.)				
DISEASE HISTORY				
Disease on occasional tomato plant during last 5 yr	**N/A**	**N/A**	**20**	
Disease common on tomatoes during last 5 yr	**N/A**	**N/A**	**25**	
Disease observed on tomatoes >5 yr ago	**N/A**	**N/A**	**15**	
Disease never observed because tomatoes never grown	**N/A**	**N/A**	**0**	
Disease uncommon regionally and never previously observed	**N/A**	**N/A**	**0**	
Heavy RKN galling on tomatoes (other crop/weeds) in last 2 yr	**25**		**N/A**	**N/A**
Light RKN galling on tomatoes (other crop/weeds) in last 2 yr	**20**		**N/A**	**N/A**
Heavy RKN galling on any susceptible crop more than 2 yr ago	**20**		**N/A**	**N/A**
Light RKN galling on any susceptible crop more than 2 yr ago	**15**		**N/A**	**N/A**
Weed hosts of nematodes or pathogen present	**20**		**N/A**	**N/A**
Fumigation, nematicides or resistant cultivars used previously	**15**		**15**	
Previously pasture or virgin soil	**25**		**0**	
Nematode or disease history unknown	**25**		**25**	
MAXIMUM/ SUB TOTAL	**25**		**25**	
DISEASE HISTORY TOTAL (adjust down if > max poss.)				
SOIL TEXTURAL CHARACTERISTICS				
Sand	**10**		**5**	
Sandy loam	**8**		**5**	
Sandy clay loam	**5**			
Well structured and freely draining clay loams or clays	**5**		**3**	
Compacted silty loam or clay loam	**2**		**3**	
Compacted clay	**0**		**3**	
MAXIMUM/ SUB TOTAL	**10**		**5**	
SOIL TEXTURE TOTAL (adjust down if > max poss.)				
TEMPERATURE				
Expected mean temp. first 3 months after planting >23°C	**10**		**10**	
Expected mean temp. first 3 months after planting >21-23°C	**8**		**8**	
Expected mean temp. first 3 months after planting >19-21°C	**5**		**5**	
Expected mean temp. first 3 months after planting >15-19°C	**2**		**2**	
Expected mean temp. first 3 months after planting <15°C	**0**	**0**	**0**	**0**
MAXIMUM/ SUB TOTAL	**10**		**10**	
TEMPERATURE TOTAL (adjust down if > max poss.)				
HAZARD INDEX FOR SITE	**50**		**50**	

[1] **N/A = not applicable.**

Table 4-11. The number of points allocated to the 'temperature' parameter of the hazard index for various regions and planting times. Adapted from weather data published by the Australian Bureau of Meteorology (http://www.bom.gov.au/climate/averages/tables, 2000).

	The number of points allocated when planted during a particular month											
Region	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Bundaberg	10	10	8	5	2	2	2	5	5	10	10	10
SEQ (Gatton)	10	8	5	2	0	0	2	2	5	8	10	10
Bowen	10	10	10	8	5	2	5	8	10	10	10	10

5. VALIDATION OF A PREDICTION SYSTEM FOR SOIL-BORNE DISEASES

Introduction

Preliminary observations made on a representative sample of tomato fields during the 2000 season (see Chapter 4) showed that Fusarium wilt and root-knot nematodes were the most important soil-borne diseases in Queensland. A hazard index was then developed to encapsulate information on cropping history, disease history, soil texture and expected temperature during the growing season because it was thought to be a useful way of providing a quantitative assessment of the likely risk from these diseases in the next tomato crop.

The objective of the work described in this chapter was to determine whether the incidence and severity of Fusarium wilt, root-knot nematode and other soil-borne diseases of tomato could be reliably predicted using information available prior to planting. That process involved calculating a hazard index, counting root-knot nematodes in pre-plant soil samples and enumerating FOL and root-knot nematode using newly developed DNA techniques (see Chapters 6 and 7 for details). These parameters were determined in a representative sample of tomato fields prior to planting and their predictive value was determined by relating the measurements obtained to the incidence and severity of the diseases actually observed in those fields when the crop was harvested.

Materials and Methods

Field selection and pre-plant sampling. During the 2001 and 2002 growing seasons (2000/2001 and 2001/2002 seasons in south-east Queensland), collaborating consultants selected a representative range of fields to be monitored. Details of each field were recorded and this information was then used to calculate a hazard index using Table 4-10 as a template. Two 0.4 ha sampling units were then defined in each of the selected fields and a soil sample was collected from each of the sampling units prior to planting. Soil samples consisted of 30 cores taken at random with a 2 cm-diameter sampling tube at depths of 0-25 cm.

Processing of pre-plant samples. When soil samples (usually about 1.5 L of soil) were received at the laboratory, the soil was gently mixed and subdivided into three sub-samples for analysis. The first sub-sample was processed for nematodes using a Baermann

tray (Whitehead and Hemming 1965). Two 200 mL samples were placed on trays, water was added and the trays were incubated at 22-28 °C for 4 days. The contents of the trays were then combined and nematodes were retrieved by sieving the suspension twice through a 38 μm sieve. Root-knot nematodes in each sample were counted. Previous studies had shown that the extraction efficiency of this method was approximately 50%, which meant that the number of RKN juveniles recovered from a 400 mL sample approximated the number of nematodes actually present in 200 mL of soil. The second sub-sample of 400 mL soil was sent to the SARDI laboratory in Adelaide and specific primers were used to quantify RKN and FOL at some sites using methods detailed in chapters 6 and 7, respectively. *Verticillium dahliae* was also quantified in the same sub-sample using methods developed by CSIRO and SARDI (Ophel-Keller and McKay 2002). The third sub-sample was used in a bioassay for FOL. Ten replicate 200 mL pots were filled with 25 mL of field soil mixed with 175 mL of pasteurised peat/sand potting mix and planted with bare-rooted seedlings of tomato (cv. Indy). Plants were grown in the glasshouse at temperatures of 22-30°C until disease symptoms were observed or for a maximum of 2 months. The number of plants that died due to diseases such as damping off was recorded and the presence of FOL in the remaining plants was determined by examining them for vascular staining.

Disease incidence and severity in the field. At some time during the harvest period (usually 12-16 weeks after planting), consultants interviewed the grower and recorded management and agronomic information (e.g. fumigants or nematicides used, variety) that may have affected the incidence or severity of diseases in the selected fields. Sampling units that were identified prior to planting were located and disease incidence and severity was assessed at 10 randomly-selected points within each sampling unit. At each of these points, 2 plants were dug and rated for galling on the 0-10 scale of Zeck (1971). The number of diseased plants in the four panels closest to each sampling point was also counted and probable causes were identified by noting symptoms. When diseased plants were present, the extent of yield loss was estimated by counting the number of plants in four categories: none (no yield loss), low (<20% yield loss), medium (21-50% yield loss) and high (>50% yield loss). The average % yield loss on diseased plants in the sampling unit was then calculated by assuming 0, 10, 30 and 60% yield loss respectively in each of the categories listed above.

Results and Discussion

Root-knot nematode. The results for RKN (detailed in Appendix 1 and summarized in Table 5-1) showed that the pest is not a major problem under the management practices currently used in the Queensland tomato industry. Of the 114 sites monitored, crops at only 4 sites had widespread and relatively severe root galling. Observations at harvest suggested that infestations of this magnitude did not cause yield losses. The data-set for 59 sites planted to susceptible varieties without a nematicide also indicated that RKN usually did not cause significant damage. Galling was observed in 39% of these untreated sites at harvest, but the gall ratings were relatively low (Table 5-1). This dearth of nematode problems is partly due to the fact many soils in Bowen have a heavy texture which does not suit the nematode. In Bundaberg, where most soils are suitable, beds used for tomatoes are often prepared and covered with plastic 2-3 months before planting and so some nematode control is achieved through a fallow and solarisation effect. Initial nematode population densities are therefore reduced to the point where significant galling often does not occur by the time the crop is harvested.

Table 5-1. Relationship between presence or absence of root-knot nematode (RKN) in pre-plant soil samples and the incidence and severity of galling at 100 sites where susceptible tomato varieties were grown in either nematicide-treated or untreated soil.

Nematicide treatment	Occurrence of RKN in pre-plant samples	No. of sites	No. sites with galls	No. sites heavily galled [8]	Gall rating (overall)	Gall rating (infested plants, infested sites)
Untreated	Not detected	51	20	0	0.46	3.55
	Detected	4	1	0	0.24	2.34
Nematicide	Not detected	29	15	2	0.93	1.99
	Detected	16	13	2	0.84	3.72

[8] Incidence of galled plants >50% and an overall gall rating >5.0 or gall rating of infested plants >6.0

Forty-five of the monitoring sites, mainly in Bundaberg, parts of the Lockyer Valley and the Granite Belt, were treated with a nematicide, probably because the growers felt they were particularly prone to nematode problems. Metham sodium at 800 L/ha the most

common treatment. Vydate (oxamyl®) was also used, sometimes in addition to metham sodium, but it was always applied after planting at relatively low rates (2-6 L/ ha). The results for these sites (Table 5-1) suggested that the nematicide treatments had some effect, because gall ratings were generally low, despite the fact that the nematode was common at these sites.

Nematode-resistant cultivars are not normally used in the Queensland fresh tomato industry because they lack the agronomic characteristics desired by growers. However, one resistant cultivar (Commando) was planted at 14 of the sites surveyed. Since it was mainly used in the heavy soils at Bowen, where root-knot nematode is often not detected, it was probably chosen for reasons other than nematode resistance. However, its nematode-resistance proved to be effective, as roots were not galled at the two sites where root-knot nematode was detected in pre-plant samples.

One objective of the monitoring work was to determine whether pre-plant nematode counts had predictive value. The results indicated that the presence of RKN in samples collected prior to planting was a useful indicator of potential nematode problems, as galling was almost always observed at these sites at harvest (Table 5-1). A nematode count of zero was also a useful indicator, as roots were either not galled or gall ratings were low at most of these sites. However, the predictive value of a pre-plant nematode count was tempered by the fact that significant and widespread galling was observed at two sites where root-knot nematode was not detected. Also, the incidence and severity of galling was not closely related to the number of RKN found in pre-plant samples.

The hazard index was useful in identifying sites where significant and widespread galling occurred. Overall gall ratings increased as the hazard index increased and all sites with a mean gall rating greater than 4 at harvest had a hazard index of more than 40 (Table 5-2).

Table 5-2. Relationships between the hazard index and the incidence and severity of galling caused by root-knot nematode at sites where susceptible tomato varieties were grown.

Hazard index	No. of sites	% of sites with galls	No. sites with overall gall rating >4	Gall rating (overall)	Gall rating (infested plants, infested sites)
11-30	10	50	0	0.52	1.64
31-35	8	38	0	0.39	4.41
36-40	29	34	0	0.32	3.80
41-45	37	54	4	1.18	3.93
46-50	16	56	3	1.54	4.58

From a practical point of view, the monitoring results indicate that determining the hazard index is a useful first step in making a prediction for RKN. In our study, about half the sites had a hazard index of <40 and could be eliminated as sites where RKN is likely to cause problems. A pre-plant nematode count then helps to predict which of the remaining sites are most likely to be affected by root-knot nematode. However, even when this is done, it was apparent from our results that situations where overall gall ratings range from 4 to 6 at harvest may occasionally be missed. This level of damage is sometimes a concern to growers, but our observations and the results of field trials on crops with higher gall ratings and much higher nematode densities (Vawdrey and Stirling, 1996; Stirling and Smith, 1998) suggest that provided water and nutrient management is adequate and the weather is not too hot, economic losses are minimal in such situations.

Fusarium wilt. Because FOL occurs in some tomato-growing regions of Queensland and not others, monitoring results for this pathogen were collated by region. Data for Bowen and Bundaberg, where the disease is common, were kept separate from data for south-east Queensland, where the disease does not occur (Appendix 2 a, b).

The widespread distribution of race 3 of FOL in Bowen and Bundaberg meant that varieties resistant to this pathogen were grown at 23 of the 60 monitoring sites. Most of these sites had a history of fusarium wilt and the disease was often detected by bioassay, but the disease was not observed in the field at any site (Table 5-3). This indicates that varieties with resistance to FOL race 3 provide effective control.

Table 5-3. Occurrence of fusarium wilt on bioassay plants grown in soils from Bowen and Bundaberg, and on tomato varieties resistant or susceptible to *Fusarium oxysporum* f.sp. *lycopersici* grown in fields from which bioassayed soil was collected.

Resistance status	No. of sites	No. of sites with positive bioassay	No. of fields with fusarium wilt	
			Incidence 1-3%	Incidence >3%
Resistant	23	10	0	0
Susceptible	37	7	13	8

Varieties susceptible to FOL race 3 were grown at 37 sites in Bundaberg and Bowen. Fusarium wilt was present in the field at 21 of these sites, generally at a low incidence (Table 5-3). Only 8 sites had more than 3% of plants affected, but there was a very high incidence (28-54% of plants diseased) at 4 of these sites.

The bioassay was a poor indicator of the presence of FOL, as it only detected the pathogen in 7 of the 21 fields where the disease was observed at harvest. The fact that some of these fields were heavily infested suggests that the problem is with the bioassay itself rather than sampling issues associated with the relatively small quantity of field soil added to bioassay pots. One problem in some soils was that as many as four of the ten replicate plants died due to damping-off and other seedling diseases. Another possible deficiency was the temperature regime in the glasshouse. Minimum temperatures remained at about 22°C throughout the year, but maximum temperatures were 28-30°C in summer and only 25-27°C in winter. *Fusarium oysporum* f.sp. *vasinfectum* has an optimum temperature for infection of about 21°C on cotton (Dr. J. Kochman, pers. comm.), and if FOL on tomato responds in the same way, the temperatures which occurred during summer may have been sub-optimal for infection.

The hazard index was useful for predicting whether fusarium wilt would cause significant losses on susceptible varieties, as seven of eight sites where more than 3% of plants succumbed to the disease had hazard indexes of more than 35 (Table 5-3). The other site was an exception, as 54 % of plants had fusarium wilt, but the hazard index was only 31.

The disease risk at this site was probably underestimated because there was no record of a previous infestation in the field.

There were two reasons why low levels of fusarium wilt were observed in fields with a hazard index of less than 15 (Table 5-3). In one case, the field was relatively new land that had never previously grown tomatoes. Since the disease was only found in association with stakes used for trellising, the pathogen was almost certainly introduced from elsewhere. Such situations could never be predicted using a hazard index. The other case involved relatively new land that had previously grown only two tomato crops. When determining the hazard index, the previous crops were thought to have been free of fusarium wilt when in fact they probably had very low levels of disease. Both these situations highlight the fact that disease records need to be detailed and accurate if they are to be useful for predictive purposes.

Table 5-3. Relationship between the hazard index and the incidence of fusarium wilt caused by race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at sites where resistant and susceptible tomato varieties were grown.

	Resistant varieies		Susceptible varieties		
Hazard index	**No. of sites**	**No. sites with fusarium wilt**	**No. sites**	**No. sites with fusarium wilt**	**No. sites with >3% fusarium wilt**
<15	0	0	10	5	0
16-20	0	0	4	0	0
21-25	0	0	0	0	0
26-30	1	0	4	1	0
31-35	2	0	3	2	1
36-40	8	0	10	7	5
41-45	12	0	6	6	2

DNA data are not summarized because quantification methods were gradually improved during the two-year monitoring period and early DNA readings may not be comparable with later readings. Nevertheless, RKN was detectable using the DNA test, as the four samples with more than 10 nematodes/400 mL soil all had relatively high DNA readings (Appendix 1). Levels of FOL DNA were generally less than 5 pg DNA/g soil, but five

sites at Bowen and two sites at Bundaberg had more than 20 pg DNA/g soil. The highest reading was 233 pg DNA/g soil.

Verticillium wilt. Verticillium wilt caused by *V.dahliae* (VD) was not a major focus of this project, as the disease tends to be restricted to a few winter crops in Bundaberg, and some Granite Belt and Lockyer Valley crops planted during winter and early spring. DNA data from 87 sites showed that six sites had 10-30 pg VD DNA/g soil, while another three sites had 31-71 pg VD DNA/g soil. Nevertheless, verticillium wilt was only observed at two of these sites, presumably because most of the crops were planted between December and February.

Other soil-borne diseases. Sclerotium base rot caused by *S. rolfsii* and bacterial wilt caused by *Ralstonia solanacearum* were the only other significant diseases observed at the monitoring sites. Sclerotium base rot was found at 33 of 114 sites, including some in every tomato-growing region. Disease incidence was generally relatively low, but there were 7 sites where 3-13% of plants were killed. Bacterial wilt occurred at 7 sites. However incidence data are not reported because disease distribution within a field was erratic and data collected from the relatively small sampling units used in this project did not necessarily reflect the actual importance of the disease.

6. VALIDATION OF A QUANTITATIVE DNA ASSAY FOR *MELOIDOGYNE* IN SOIL

Introduction

Root-knot nematode (RKN) is the most widely distributed soil-borne pest in the Queensland fresh tomato industry. Data presented in Chapter 5, for example, indicated that galled roots were observed at harvest in about 50% of our monitoring sites. The nematode is found most commonly in light-textured sands and well-structured clay loam soils (Van Gundy 1985) and is therefore widespread in Bundaberg, the Granite Belt and parts of the Lockyer Valley. It is less common in the predominantly heavy soils of the Bowen region.

Although nematicides are often used in nematode-infested soils, there is evidence to suggest that routine nematicide treatment is rarely necessary. Only 4 of 94 of our monitoring sites had plants with heavily galled root systems at harvest (Chapter 5) and results of field experiments (Vawdrey and Stirling 1996: Stirling and Smith 1998) showed that such infestations do not necessarily result in yield loss. This suggests that if better prediction methods were available, nematicide usage could be reduced considerably.

Since losses due to RKN nematodes increase as nematode population density increases, the relationship between pre-plant nematode density and yield has been used to determine the threshold levels above which economic damage is likely to occur. Such work (Barker et al. 1975; Aochi and Baker 1990) has shown that the economic threshold on tomato is in the order of 10-20 RKN/200 mL soil (after correction for extraction efficiency). For methods commonly used to extract nematodes, which are about 50% efficient, this equates to 10-20 nematodes extracted from a 400 mL sample. Recent studies in Queensland (Stirling 1999) indicated that population densities of this magnitude can be detected using standard nematode extraction techniques. However, the variability in nematode populations within fields means that a reliable estimate of initial nematode density can only be obtained by processing several soil samples from each field.

An alternative way of quantifying nematode populations is to use DNA techniques. This technology has been used for several years in the Australian cereal industry to quantify cyst

nematode, *Heterodera avenae*, and two species of lesion nematode, *Pratylenchus thornei* and *P. neglectus* (Ophel-Keller 1999). The work described in this chapter aimed to determine whether similar technologies could be used to quantify RKN in tomato-growing soils. Since the nematodes that attack cereals have threshold levels of 1-5 nematodes/g soil (Fisher and Hancock 1991; Taylor *et al.* 1999) and RKN may damage tomato and other vegetable crops at initial densities of 0.025 nematodes/g soil, another important objective was to ascertain whether DNA methods could reliably detect and quantify very low nematode densities in soil.

Materials and methods

Quantification of nematode populations. All soil samples were passed through a 3 mm sieve, mixed gently and sub-divided into sub-samples that were then used for nematode extractions, DNA tests or bioassays. One 400 mL sub-sample (containing 360-400 g dry weight of soil, depending on the source of the soil) was spread on two 30 x 20 cm extraction trays (Whitehead and Hemming 1965) and left at temperatures of 22-26 °C for 4 days. The contents of the trays were then combined, nematodes were recovered by sieving twice on a 38μm sieve and second-stage juveniles of root-knot nematode were counted at a magnification of 40X.

RKN was quantified in a second 400 mL sub-sample using DNA methods. Soil was dried at 40°C overnight, DNA was extracted using a commercially confidential protocol developed by SARDI/CSIRO, and amplified using primers specific to *Meloidogyne* spp. supplied by D. Hartley, CSIRO Entomology. Amplified DNA was quantified using a fluorescently-labelled DNA probe specific for *Meloidogyne* spp. Soil to which known numbers of RKN juveniles had been added was processed in the same manner and the number of RKN in each sample was determined using the standard sample as a reference.

The third sub-sample was used in a bioassay for RKN. Nematodes in roots were either quantified by counting galls or rating roots for galling, as detailed in the methods sections of individual experiments.

Comparison of quantification methods with seedling bioassays

The first batch of soil samples was collected in May 2001 from two adjacent fields on a potato farm located 30 km north of Bundaberg, Queensland. Previous sampling had shown that one field was infested with RKN and the other was not. Different proportions of the sandy loam soil from the two fields were mixed to create twenty-one 2L samples with a range of nematode densities. Each sample was then sub-sampled so that RKN could be quantified using extraction trays and DNA methods (see above), and a seedling bioassay. For the bioassay, a 1 L sub-sample of soil was added to a pot and a tomato seedling (cv. Tiny Tim) was planted. Plants grew in a glasshouse for 30 days at temperatures of 22-28° C and then roots were washed free of soil, floated in water and the number of galls on each root system was counted. Provided there was less than about 100 galls/root system, each gall was found to contain a single RKN. To enable comparisons with data obtained from extraction trays and the DNA method, the number of galls/pot was divided by 2.5 to give the number of viable nematodes/400 mL soil.

A second set of thirty two 2 L soil samples (batch 2) were collected in February 2002 from sampling points spaced 30 x 30 m apart in a 4 ha field located 50 km north of Brisbane, Queensland. The soil was a sandy loam from a site that had recently been cleared of native vegetation and prepared for planting. Additional soil was collected from the site so that nematodes in four 2 L samples could be killed by either heating the sample at 48° C for 2 days or fumigating it in plastic bags using methyl iodide at 160 μg/L soil. Thus there were a total of 40 soil samples in which root-knot nematode was quantified using extraction trays, DNA and a seedling bioassay as described previously.

Soil for the third batch of samples was collected in April 2002 from 12 fields on the potato farm where the first batch of samples was obtained. The fields were known to be infested with various population densities of RKN. About 10 L of soil was taken from each field after it was cultivated in readiness for planting. The soils were then mixed together in various proportions to create 36 samples (2 L each) with a range of nematode densities. Nematodes in a further four 2 L samples were killed by autoclaving the soil for 30 min. Root-knot nematodes in all 40 samples were then quantified using extraction trays, the DNA test and a bioassay. Since individual galls could not be counted on some of the bioassay seedlings because roots were too heavily infested, galling on all plants was assessed using the 0-10 gall rating system of Zeck (1971).

Comparison of quantification methods with mature plant bioassays

The natural variability in numbers of RKN within two adjacent fields near Gatton, about 80 km east of Brisbane, Queensland was used to obtain 40 soil samples with various nematode densities. Soil was collected from different areas of these fields in August 2002, at a time when they were fallowed prior to the next tomato crop, and added to 10 L plastic pots. Ten soil cores were then taken from each pot with a 2 cm-diameter sampling tube, the soil was mixed, two 400 mL sub-samples were retrieved and RKN was quantified using extraction tray and DNA methods. Pots were then moved to a greenhouse and a tomato seedling (cv. Tiny Tim) was planted in each pot. When plants had reached maturity (i.e. after growing for 12 weeks), roots were washed free of soil and rated for galling using the 0-10 scale of Zeck (1971).

The above experiment was repeated with sandy loam and sandy clay loam soils from two fields near Bundaberg, except that the soil used to fill 50 pots was obtained by mixing differing proportions of the soil from the two fields. Pots were sampled as for the previous experiment, planted to tomato (cv. Redcoat) and gall ratings were taken after plants had grown in a shade-house for 12 weeks.

The relationship between initial population densities of RKN (measured by the DNA method) and the severity of galling at harvest was obtained from a field with a well-structured clay loam soil located near Childers, Queensland. In July 2002, the field had been cultivated, raised beds had been prepared, trickle tubing had been laid and beds were covered with plastic in preparation for planting a tomato crop. Twenty four plots each 20 m long were identified in randomly-selected areas of the field by marking sections of the newly-prepared beds with paint. Soil collected from 8 points in each plot to a depth of about 15 cm was then mixed in a bucket and a 400 mL sample taken for DNA analysis. In September 2002, the field was planted by the grower to a commercial crop of tomato (cv. Grenade). When harvest was almost finished (i.e. 14 weeks after planting), ten plants were dug from each plot and rated for galling as described previously for the pot experiments.

In all experiments, relationships between variables were assessed using regression analysis. Nematode counts, DNA readings and gall counts were transformed [$(\log_{10}(x+1)$] prior to analysis. In the field experiment, mean gall ratings for each plot were used in the analysis.

Results

Comparison of quantification methods with seedling bioassays.

The number of RKN in the batch 1 samples was relatively low (Table 6-1), but there were galls on 18 of the 21 bioassay plants. Juveniles of root-knot nematode were detected by extraction in 17 of the 18 positive samples, and by DNA in 13 of the same samples. The regressions of J2 on galls, DNA on galls and J2 on DNA were significant but the relationships ranged from relatively weak to moderate (Table 6-1).

One sample in batch 2 had a high nematode count (extraction trays and DNA methods giving counts of 512 and 403 J2/400 mL soil, respectively), while the bioassay plant was too heavily galled to count individual galls. The other 39 samples had relatively low numbers of RKN and regression analyses showed a significant but low to moderate relationship between the variables measured (Table 6-1). Galls caused by RKN were observed in 22 of the 39 samples and the nematode was detected by extraction and DNA methods in 16 and 13 of these galled samples, respectively. No J2 or galls were observed in fumigated or heated samples, but there was a small background reading with the DNA test in some of these samples.

Only 4 of the 40 samples in batch 3 were not infested with root-knot nematodes (as evidenced by lack of galls on bioassay seedlings). Most samples had high numbers of nematodes and 12 of the 40 bioassay plants had gall ratings of 6 or 7. There was a moderate and significant relationship between J2 and gall rating, and J2 and DNA, but a relatively weak relationship between DNA and gall rating (Table 6-3).

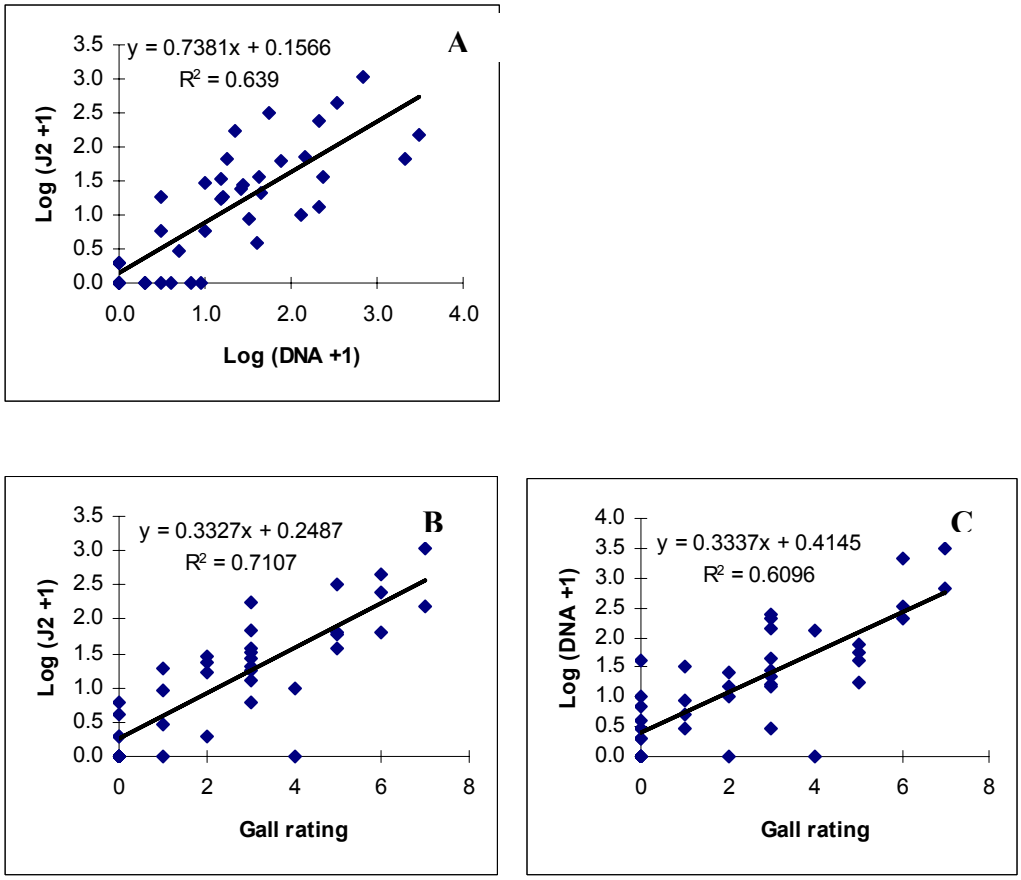
Table 6-1. Relationships between numbers of root-knot nematode in three batches of soil samples as assessed by a DNA test, extraction of second-stage juveniles (J2), and by gall counts or gall ratings (GR) on seedling bioassay plants.

Batch no.	No. of samples	Range of nematode densities		Relationship between variables (R^2)		
		J2/400 mL soil	Galls/400 mL soil	J2 v. Galls	DNA v. Galls	J2 v. DNA
1	21	0-22	0-25.6	0.62	0.46	0.40
2	39	0-13	0-13.2	0.32	0.58	0.32
3	40	0-830	GR 0-7	0.57	0.40	0.63

Comparison of quantification methods with mature plant bioassays

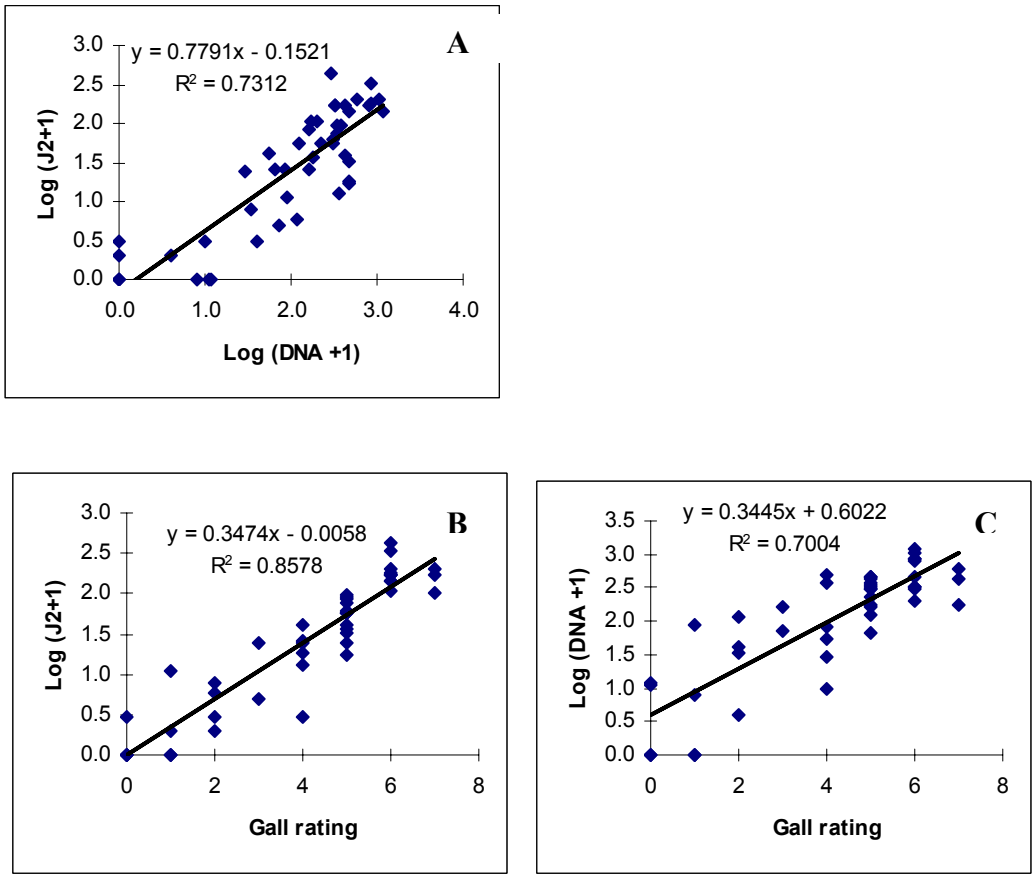
In the experiment with soil from Gatton, initial nematode population densities measured using extraction trays ranged from 0 to 1070 J2/400 mL soil. There was a good relationship (R^2 =0.64) between these pre-plant nematode counts and measurements obtained using DNA (Figure 6-1A). Gall ratings at harvest ranged from 0 to 7 and were related to initial nematode density, whether the nematode density was measured by counting J2 extracted on trays or by the DNA method (Figures 6-1B, 6-1C).

Figure 6-1. Relationships between various variables in a pot experiment with soil from Gatton



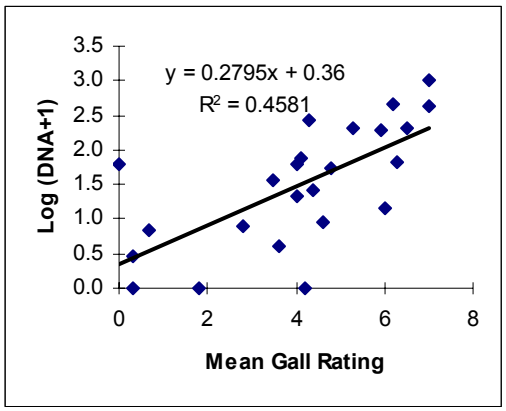
Three plants in the Bundaberg pot experiment were discarded because they died or grew poorly for reasons other than nematode damage. Initial nematode densities in the remaining 47 pots ranged from 0 to 436 J2/400 mL soil, and this resulted in gall ratings at harvest ranging from 0 to 7. The relationships between J2 and DNA readings, J2 and gall ratings, and DNA and gall ratings were all stronger than in the previous experiment (Figures 6-2 A-C).

Figure 6-2. Relationships between various variables in a pot experiment with soil from Bundaberg



Initial nematode densities in 24 field plots (estimated using DNA) ranged from 0 to 1022 RKN/400 mL soil. At harvest, plants in 16 of the plots showed moderate to heavy galling (mean gall ratings greater than 4), and two plots had a mean gall rating of 7.0 Plants in the latter plots were showing above-ground effects of nematode damage, as they were growing poorly and showed signs of moisture stress. The relationship between DNA readings and galling (Figure 6-3) showed that the variation in gall ratings between plots was related to initial nematode density (R^2 =0.46).

Figure 6-3. Relationship between initial nematode density of root-knot nematodes (measured using DNA) and gall rating of field-grown tomatoes at harvest



One observation made in all three experiments was that the regression of DNA readings against gall rating did not intercept the y axis at zero. Thus there was often a positive DNA reading in soils where there was no galling on bioassay plants. This did not occur to the same extent when the initial nematode population was estimated by extracting J2 on trays.

Discussion

In the experiments described, the nematode population density was determined using three different methods. However, the bioassay was the only one of these methods to indicate the number of root-knot nematodes in a soil sample that were able to invade a tomato root and then multiply and cause damage. The utility of extraction tray and DNA methods for quantifying nematode populations was therefore assessed by comparing nematode counts or DNA readings with gall counts or gall ratings obtained from bioassays.

Experiments using soils with nematode population densities ranging from 0-25 RKN/400 mL soil (as determined with a seedling bioassay) showed that there were significant but moderate to weak relationships between DNA readings and galling, and J2 and galling. In samples where a bioassay subsequently confirmed that the nematode was present, neither method consistently detected the nematode. This suggests that the detection limit for both extraction tray and DNA methods is somewhere between 0 and 25 root-knot

nematodes/400 mL soil. At these population densities, extraction trays have an advantage over DNA methods because in situations where a single J2 is extracted, it can be positively identified. Since background readings inevitably occur with the DNA method, one can never be certain that a low reading is actually a positive reading.

One problem in experimenting with soils that have very low nematode densities is that sub-samples processed by different methods may not contain the same number of nematodes. Even when a sample is mixed and sub-sampled thoroughly, there is a good chance that nematode numbers will vary between sub-samples. This is particularly a problem with RKN, as some nematodes are likely to be aggregated in portions of egg masses. Consequently, at low nematode densities, it may never be possible to achieve a close relationship between the counts obtained from sub-samples, regardless of the quantification method used.

Experiments in which plants were grown to maturity, either in large pots or the field, showed that the relationship between DNA readings and the bioassay ranged from moderate to strong (R^2 =0.46, 0.61 and 0.70). The results also indicated that the DNA method was useful from a practical point of view, because at sites with gall ratings greater than 4 at harvest, there was always a clearly positive DNA reading in soil samples taken prior to planting.

Regardless whether seedling or mature plant bioassays were used, the intercept on the Y axis for the regression of DNA readings against gall ratings was consistently positive. This indicates that the DNA method often detected low numbers of RKN in soils where the gall ratings on bioassay plants were zero. The reasons for this are not clear, but perhaps the DNA method detects J2 that have died recently, J2 that have insufficient food reserves to invade roots, or eggs that are incapable of hatching.

The difficulties involved in detecting and quantifying low nematode numbers with any method raises questions about the nematode population density that must be detected if growers are to be given reliable advice on the need for control measures. Previous work in the USA suggests that the economic threshold for RKN on tomato is in the order of 10-20 nematodes extracted/400 mL soil (Barker et al. 1976; Aochi and Baker, 1990). However, more recent work in Australia (Vawdrey and Stirling 1996) demonstrates that there are strong environmental effects on the relationship between initial nematode density and yield. Yield losses from nematodes were not observed in heavily-galled tomato crops that

were harvested during winter, the main vegetable production season in the subtropics. Similar observations were made in the monitoring component of this project, as above-ground symptoms indicative of nematode damage were never seen in the field, despite relatively heavy galling at some sites. This suggests that under the management practices used in the Australian subtropical tomato industry, economic losses do not occur unless the root-gall index at harvest is greater than about 6. Even in situations where this level of galling does occur, yield losses are not inevitable. They are most likely to occur when a crop carrying a heavy fruit load is stressed by hot, dry weather.

The regression equations from the Gatton and Bundaberg pot experiments show that an initial nematode population at planting (measured using extraction trays) of 37 and 23 RKN/400 mL soil, respectively, resulted in a gall rating of 4 at harvest. The corresponding figure for the field experiment (measured using DNA) was 29 root-knot nematodes/400 mL soil. The nematode populations that resulted in gall ratings of 6 were much higher than this. Since root gall ratings of at least 6 seem to be needed before there is any chance of yield losses in the field, this suggests that the economic threshold for RKN on tomato in subtropical Australia is higher than has been reported elsewhere. Probable reasons for this are that tomato crops grown with modern management practices are able to cope with some root damage because they are rarely stressed for nutrients or water. Sap nutrient levels are monitored regularly and used to schedule fertiliser applications, while irrigation frequency is determined by monitoring soil moisture with computerised sensors and data loggers.

Because tomato is a high-value crop, growers can incur losses worth thousands of dollars per hectare if the advice they are given on nematode control is inaccurate. It is therefore imperative that the relationships between nematode counts and root gall ratings reported here are confirmed under modern crop management practices for a range of soil types and planting times. The economic impact of various levels of galling also must be determined, as heavy galling may have little effect on yield but may reduce fruit size and therefore the number of marketable fruit. However, regardless of the actual threshold level, our results demonstrate that DNA methods are an alternative to manual extraction as a means of estimating nematode populations for predictive purposes.

As this study progressed, we reduced the nematode population densities that could be detected with the DNA method to levels that can be achieved manually. This opens up

opportunities to use the technology more widely for quantifying nematodes in soil. The dearth of trained people capable of identifying plant-parasitic nematodes and the cost advantages of automated, high-throughput analytical systems for DNA are good reasons for using this new technology for diagnostic services. However, the main advantage of DNA technology is that it can be used to quantify several pathogens in the same sample. In the cereal industry in southern Australia, for example, DNA tests for six important nematode and fungal pathogens have been available commercially since 1999 at a price of about $160/sample. Since DNA tests for FOL and VD, the most important fungal pathogens of tomato, have already been developed (see Chapters 5 and 7), a similar suite of DNA tests will eventually be available to the tomato industry.

7. DEVELOPMENT OF PCR PRIMERS SPECIFIC FOR *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* AND THEIR USE IN QUANTIFYING THE PATHOGEN IN SOIL

Introduction

In the monitoring component of this project (chapter 5), we presented results to show that glasshouse bioassays did not reliably detect FOL in field soil. When FOL-susceptible tomato seedlings were planted in soil from Bowen that was known to be heavily infested with the pathogen, they often did not show symptoms of Fusarium wilt after growing in the glasshouse for about 8 weeks. When the disease was observed, it usually occurred in less than 20% of plants. Attempts to reliably detect and quantify FOL using other methods have also proved unsuccessful. A selective medium for *F. oxysporum* in soil is available (Komada, 1975), but our tests with tomato-growing soils showed that it was laborious and not very selective. Considerable experience was required to differentiate the colony types that occurred on plates and *Fusarium* colonies were difficult to identify to species level using morphological characters.

Because all organisms contain unique DNA sequences in their genomes, it is now possible to use molecular technologies to detect and quantify specific organisms. The process involves designing sets of primers that hybridize with a unique sequence of DNA in the target organism and then amplifying the sequence using the polymerase chain reaction (PCR). The successful use of such technologies in southern Australia to quantify nematode and fungal pathogens in cereal soils (Ophel-Keller, 1999) demonstrates that it should be possible to develop similar systems for pathogens of horticultural crops.

This chapter describes the development of a set of molecular primers to detect FOL, the most important soil-borne pathogen of tomatoes in Queensland, and demonstrates that it is specific for the organism of interest. In the case of FOL, this means that it must be able to separate FOL from other species and form-species of *Fusarium* that occur in tomato-growing soils. One issue of particular importance was the race specificity of the primers. Race 3 of FOL, which was first detected in Bowen about 20 years ago (Grattidge and O'Brien, 1982), has since spread to Bundaberg and is the most important race to detect from an economic point of view. However, we also needed to know whether the primers

detected races 1 and 2 of FOL, as they may continue to survive as saprophytes in Queensland tomato-growing soils, despite the widespread use of varieties with resistance to these races.

In the third section of this chapter, we determined whether the primers would be useful as a routine diagnostic tool. The level of FOL DNA in soil samples taken before and after planting was determined and then tomato varieties susceptible to FOL race 3 were grown either in pots or in the field. Relationships between the incidence and severity of fusarium wilt and levels of FOL DNA in soil were examined.

Materials and Methods

Development of FOL-specific primers. Single spore isolates of *Fusarium* (BCPM60, BCPM70 and BCPM82) were generated from cultures obtained in 1997 and 1998 from infected tomatoes growing near Bundaberg (Elliot Heads, Farnsfield and Childers respectively). Their identity as FOL was confirmed in pathogenicity tests. Additional isolates of *Fusarium* were obtained from Dr. S Bentley, CRC for Tropical Plant Pathology. DNA was extracted from all isolates using a commercially-available DNA extraction kit (MoBio Laboratories, Ca, USA). The intragenic spacer (IGS) region of the following form species of *F. oxysporum* was then amplified and sequenced by CSIRO Entomology (form species, with number of isolates in parentheses): *lycopersici* (4), *cubense* (3), *basilicum* (2), *dianthi* (2), *vasinfectum*, *pisi*, *medicagenis*, *passiflora*, *niveum*, *fragariae*, *zingiberae* and *gladioli*.

A specific set of FOL PCR primers and a hybridisation oligo probe specific to *F. oxysporum* were developed in the IGS region of the ribosomal DNA. Specificity was initially confirmed by amplifying DNA from all fusaria and visualising the PCR product under UV light on an agarose gel (2%) stained with ethidium bromide. The PCR conditions were then optimised by adjusting the concentration of magnesium chloride and the DNA annealing temperature so that the FOL primers only amplified DNA from FOL cultures.

Validation of specificity. Thirty-one isolates of *Fusarium oxysporum* were isolated from diseased plant material collected from the field, from bioassay plants grown in infested soils and from fungal collections maintained by other plant pathologists (Table 7-1). The aim was to obtain a reasonably comprehensive collection of isolates from Queensland that included non-pathogenic *Fusarium* spp. associated with roots, several different form-

species of *Fusarium oxysporum* and isolates of races 1, 2 and 3 of *Fusarium oxysporum* f.sp. *lycopersici*.

Isolates were stored on filter paper or as lyophilised cultures. When required, each fungus was retrieved by placing pieces of filter paper or lyophilised agar onto PDA. Once hyphal growth was evident, isolates were sub-cultured onto carnation leaves overlain with water agar, a low nutrient medium that reduces the chance of mutation. Cultures were maintained at room temperature and 7-10 days before fungi were required for pathogenicity tests and DNA extraction, they were sub-cultured onto PDA.

The form species and race designation of each isolate was determined by pathogenicity tests using the following differential set of tomato cultivars: Yellow pear (susceptible to all races of FOL); Fire Fox (resistant to race 1 of FOL); Floradade (resistant to races 1 and 2 of FOL); and Guardian (resistant to race 1, 2 and 3 of FOL). Isolates suspected of belonging to form species *melonis*, *pisi*, *basilicum* and *niveum* were tested using watermelon (cv. War Paint), snow pea (cv. Stir Fry), basil (cv. Bush Basil) and rockmelon (cv. Planters Jumbo) respectively, and were also tested on the differential set of tomato cultivars.

Pathogenicity tests were done by dipping roots in a conidial suspension. Conidia of each isolate were produced by adding sterile phosphate-buffered saline (PBS) to a PDA plate culture, removing the surface mycelia and microconidia with a scraper and filtering the resulting suspension through 4 layers of muslin cloth. The suspension was then adjusted to a concentration of 1.0×10^6 conidia mL^{-1} using sterile PBS. Three replicate seedlings of each cultivar grown in pasteurised potting mix for 21-28 days were root-dipped for 15 min in a conidial suspension of each isolate and transplanted into foam cups containing 200 mL of pasteurised potting mix. Transplants were grown in the glasshouse until aboveground symptoms (wilting and unilateral leaf-chlorosis) developed, or for a maximum of 4 weeks. Stems were then cut longitudinally and those that showed vascular staining were considered to be infected.

The specificity of the primers to various form-species of *Fusarium oxysporum* and races of FOL was examined independently in two laboratories. At SARDI, DNA was extracted from the 31 isolates listed in Table 7-1 using a commercial plant DNA extraction kit (MoBio Laboratories). The DNA was amplified using the FOL-specific primers and the amount of product was measured using a fluorescent labelled oligo probe specific to *F. oxysporum*. The fluorescent signal was measured and compared to standards prepared from

soil with a known amount of FOL DNA. At the other laboratory (BCP), aerial mycelium was scraped from PDA plates, DNA was extracted using a protocol suitable for plant tissue (Promega, 1999) and amplified using the same technique as SARDI. The products were separated using agarose gel (1.5 %) electrophoresis (100 V for 60 min in 0.5 % TBE buffer), gels were stained using EtBr and visualised under UV light.

Relationship between DNA levels and disease. FOL DNA was quantified in soil by extracting DNA from soil (SARDI Root Disease Testing Service) and amplifying it using the FOL-specific primers described above. Amplified DNA was quantified by hybridisation to a fluorescently-labelled oligo probe. A DNA standard was made by adding FOL DNA to DNA extracted from soil containing no detectable FOL.

The relationship between FOL DNA levels and the incidence and severity of fusarium wilt was examined at two field sites and in two pot experiments. At field site 1, about 25 km north of Childers, a crop of capsicum had been grown at the northern end of a 3 ha field in 2001 while the southern end was left to weeds and volunteer tomato seedlings. In March 2002, both ends of the field were planted to a tomato variety susceptible to FOL race 3. When the mature crop was inspected on 22 June 2002, fusarium wilt was patchily distributed in the southern end of the field, whereas the northern end that had previously grown capsicum was almost free of the disease. Thirty plots, each 20 m long and containing 40 plants, were selected in various areas of the field so that plots with no disease and low, medium and high levels of fusarium wilt were available for study. The number of plants affected by fusarium wilt was counted in each plot and FOL DNA was quantified in a 400 mL soil sample collected with a 2-cm diameter sampling tube from ten points in each plot.

Field site 2, about 10 km north of Childers, consisted of five adjacent fields ranging in size from 2-5 ha. Fusarium wilt had been observed in tomato crops planted in these fields in 1997, but they were then planted to sugarcane for 4 years. In July 2002, the sugarcane crop had been harvested and ploughed out and beds were covered with plastic mulch in preparation for another tomato crop. Sixty plots, each 20 m long, were randomly selected across the five fields and the location of each plot was marked with paint on the plastic. A soil sample (500 mL) was then taken with a trowel from eight points in each plot at depths of 0-10 cm. In twenty plots, a second sample was taken in the same way and all samples were processed for FOL DNA as described previously. Levels of RKN and VD DNA

were also determined for all samples. The beds were planted to tomatoes between 28 July and 30 September 2002. Varieties resistant to FOL race 3 were planted in all fields, but with the cooperation of the grower, susceptible varieties (cvv. Redcoat and Samba) were planted in 15 and 9 plots respectively in two fields. These plots, which contained 40 plants, were checked regularly for the presence of fusarium wilt during the period 8-14 weeks after planting and disease incidence was recorded.

Pot experiment 1 was established at Bowen using soil collected from six sites. Soil A was from a field that had recently been cleared of native vegetation and then cultivated in preparation for planting a tomato crop. Soils D, E and F were from different areas of a field where a low level of fusarium wilt had been observed previously, while soils G and H were from an adjacent heavily-infested field. At the time soil was collected (August 2002), fields had been cultivated in preparation for planting tomatoes. Each soil was mixed in a concrete mixer, with soils with the lowest levels of FOL processed first to avoid cross contamination. Additional quantities of three of the soils were mixed in the proportions 75%A + 25%D, and 75% A + 25% E, to create two additional soils designated B and C respectively. All eight soils were added to 10 replicate 10 L pots and 500 mL soil samples were then collected from each pot for DNA analysis. Tomato seedlings (cv. Daniella) planted on 7 August 2002 were then grown in the open and watered and fertilised according to normal district practice. On 12 November 2002, the incidence of fusarium wilt was assessed and disease severity was scored using a rating of 0-3, where 0= no symptoms, 1= slight above-ground symptoms and limited vascular staining near the base of the stem, 2= moderate above-ground symptoms and extensive vascular staining up the stem and evident in petiole scars, and 3= severe symptoms (wilting and plant death).

Pot experiment 2 was established with soils from a relatively new tomato farm at Bundaberg, where incidence of Fusarium wilt was low. A total of 38 soil samples, each of about 7L , were collected from an area of about 40 ha that was sub-divided into eight fields. For each sample, a 500 mL sub-sample was collected for DNA analysis and the remaining soil was used to fill 5.8 L pots. A tomato seedling (cv. Redcoat) was planted in each pot and plants were grown in a glasshouse until assessed for FOL wilt 12 weeks later.

Results

Validation of specificity. Pathogenicity tests (Table 7-1) confirmed that 21 of the 31 isolates tested were FOL, as they caused vascular staining in tomato. Twelve isolates were designated FOL race 3, as they produced disease in cv. Floradade but not in cv. Guardian. Two isolates caused Fusarium wilt only in cv. Yellow Pear and were therefore designated FOL race 1. Isolate GRS709 was identified as FOL race 2 because it caused disease in cultivars Yellow Pear and Fire Fox, but not in the cultivars Floradade or Guardian. Interestingly, the other 6 pathogenic isolates produced vascular staining in the race 3-resistant cultivar Guardian. Although this observation suggests that these isolates should be designated FOL race 4, further confirmation is required because there is no evidence that cultivars resistant to FOL race 3 have succumbed to the disease in the fields from where these isolates were obtained.

Six of the isolates obtained from tomato did not cause any symptoms on tomatoes in pathogenicity tests. This suggests that these isolates were not FOL, or that they had lost pathogenicity during storage. The latter problem is common with highly variable species such as *F. oxysporum* and has been well documented in the literature (Burgess *et al.*, 1994). The other isolates were other form-species of *F. oxysporum*, as they incited disease in various host species but failed to produce symptoms when inoculated onto tomatoes.

The molecular work suggested that the PCR primers were specific to FOL, as only DNA sequences from isolates shown to be FOL in pathogenicity tests were amplified (Table 7-1). Furthermore, a negative result was obtained with other form-species of *Fusarium oxysporum*, *Fusarium solani* and other non-pathogenic fusaria. There were three inconsistencies between laboratories, but unfortunately these isolates could not be re-examined because of time constraints and the loss of viability of one isolate (BCPDG061).

The primers showed no specificity at the race level. A positive result was obtained with all isolates, whether they were designated as FOL races 1, 2 or 3.

Only FOL cultures hybridised to the *F. oxysporum* probe when a standardised amount of DNA from each *Fusarium* isolate was added to the soil assay and amplified using the FOL-specific primers. This confirmed the specificity of the DNA hybridisation test used for the soil assay.

Table 7-1. Identity of 31 *Fusarium* isolates (as determined by pathogenicity on various cultivars of tomato or other crops), and their reaction to DNA primers specific for *F. oxysporum lycopersici* (FOL) in two laboratories.

Isolate code[1]	Origin	Identity[2]	Reaction to primers	
			BCP	SARDI
BCPDG009	Field, Gillens Crk. Rd., Bundaberg	*FOL 3*	+	+
BCPDG018	Field, glasshouse-grown crop, Bundaberg	*FOL 3*	+	+
BCPDG022	Bioassay, Collinsville Rd, Bowen	*FOL 3*	+	+
BCPDG026	Bioassay, Delta area, Bowen	*FOL 3*	+	+
BCPDG027	Field, Bruce Hwy, Bowen	*FOL 3*	+	+
BCPDG026	Bioassay, Willcox, Bowen	*FOL 3*	+	+
BCPDG027	Field, East Euri Crk Rd, Bowen	*FOL 3*	+	+
BCPDG031	Field, Bundaberg	*FOL 3*	+	+
BCPDG061	Field, near Elliot River, South Bundaberg	*FOL 3*	+	-
BCPDG055	Field, East Euri Crk Rd, Bowen	*FOL 3*	+	+
GRS 897	Field, Ashfield Rd, Kalkie	*FOL 3*	+	+
GRS 1021	Field, Ashfield Rd, Kalkie	*FOL 3*	+	+
GRS 709	Field, Goodwood Rd, Bundaberg	*FOL 2*	-	+
BCPDG044	Bioassay, Springfield Rd, Bundaberg	*FOL 1*	+	+
BCPDG046	Bioassay, Wises Rd, Bundaberg	*FOL 1*	+	-
BCPDG053	Field, Springfield Rd, Bundaberg	*FOL 3?*	+	+
GRS 652	Field, Bundaberg	*FOL 3?*	+	+
GRS 695	Field, Bundaberg	*FOL 3?*	+	+
GRS 712	Field, Bundaberg	*FOL 3?*	+	+
GRS 894	Field, Ashfield Rd, Kalkie	*FOL 3?*	+	+
GRS 1002(1)	Field, glasshouse-grown crop, Bundaberg	*FOL 3?*	+	+
BCPDG042	Field, Mahogany Crk Rd, Bundaberg	*F. oxysporum* f.sp. *pisi*	-	-
BCPDG051	Field, Atherton	*F. oxysporum* f.sp. *basilicum*	-	-
BCPDG060	Field, Bundaberg	*F. oxysporum* f.sp. *niveum*	-	-
BRIP 16511	DPI, Indooroopilly, Herbarium	*Fusarium solani*	-	-
BCPDG023	Bioassay, Goodwood Rd, Bundaberg	Non pathogenic	-	-
BCPDG029	Bioassay, Springfield Rd, Bundaberg	Non pathogenic	-	-
BCPDG035	Bioassay, Springfield Rd, Bundaberg	Non pathogenic	-	-
BCPDG037	Bioassay, East Euri Crk Rd, Bowen	Non pathogenic	-	-
BCPDG052	Bioassay, Springfield Rd, Bundaberg	Non pathogenic	-	-
BCPDG059	Bioassay, Springfield Rd, Bundaberg	Non pathogenic	-	-

[1] Isolates coded GRS… were obtained from Ms. Heidi Martin, Queensland Department of Primary Industries, Gatton Research Station.

[2] Isolates designated FOL 3? caused vascular staining in the cultivar 'Guardian' (a race 3 resistant cultivar). However, their status as a distinct race has not been confirmed.

Relationship between DNA levels and disease. There was a significant relationship (R^2 = 0.65) between the incidence of fusarium wilt at field site 1 and measurements of FOL DNA taken just after the crop was harvested. A summary of the data (Table 7-2) showed that low levels of fusarium wilt were observed in the 15 plots where capsicum had grown the previous year and FOL DNA was virtually non-detectable. Disease incidence was higher in the area where volunteer tomatoes and weeds had grown previously, and severe disease was always observed in plots with relatively low levels of FOL DNA. Thus plots with 5-10 pg FOL DNA/ g soil had a mean disease incidence of 32%.

Table 7-2. Relationship between FOL DNA levels in soil taken from 40-plant plots at Childers (site 1) just after a tomato crop was harvested, and incidence of fusarium wilt.

FOL DNA (pg DNA/g soil)	No. of plots	% plants with fusarium wilt	
		Mean	Range
0-1	15	1.3	0-5
2-4	0	-	-
5-10	7	32	15-78
11-30	5	43	15-91
31-75	3	100	100

At site 2, the data for soil samples collected from 80 plots in five fields showed that FOL DNA levels in four fields ranged from 5-35 pg DNA/g soil. Levels of FOL DNA varied randomly within fields and mean FOL DNA readings in each field were similar. In contrast, there was considerable variation across the fifth field. The mean DNA reading for 9 plots on the northern side of the field was 6 pg DNA/ g soil (range 1-21), whereas the mean of 6 plots on the southern side was 170 pg DNA/g soil (range 65-307).

Results from duplicate samples taken in some plots showed that the variability in DNA readings within plots was relatively small (CV=9%) compared with the very large amount of variation (CV=162%) that occurred across the field.

In one of the above fields, nine plots were planted on 3 July 2002 with tomato cv. Redcoat, a cultivar susceptible to race 3 of FOL. Within 2-3 months of planting, severe symptoms of verticillium wilt were observed throughout the field, despite the fact that levels of

Verticillium DNA in pre-plant samples were relatively low (mean 4.8 pg DNA/g soil, range 0-9). FOL was isolated from vascular tissue of some of these plants later in the season, but the severity of the verticillium wilt made it impossible to clearly identify plants infected with FOL.

In the field at site 2 planted to cv. Samba, there was a relationship between the incidence of fusarium wilt and FOL DNA levels in soil prior to planting ($R^2 = 0.38$). Disease incidence was low in plots with 1-2 pg FOL DNA/g soil prior to planting, a level which is below the reliable detection limit for the DNA assay, while more than 17% of plants were affected by fusarium wilt in plots with more than 5 pg FOL DNA/g soil (Table 7-3).

Table 7-3. Relationship between FOL DNA levels in soil collected prior to planting 40 tomato plants in plots 20 m long at site 2, and incidence of fusarium wilt at harvest.

FOL DNA (pg DNA/g soil)	**No. of plots**	**% plants with fusarium wilt**	
		Mean	Range
1-2	4	3.8	0-5
2-5	0	-	-
6-10	2	20	17-23
10-20	5	38	20-78
21-33	4	38	25-65

In pot experiment 1 at Bowen, the incidence and severity of fusarium wilt increased as pre-plant FOL DNA levels increased (Table 7-4). The disease was observed in pots filled with soil containing 0-2 pg FOL DNA/g soil, while a large percentage of plants were severely affected in pots filled with more heavily infested soils.

Table 7-4. Incidence and severity of Fusarium wilt in tomatoes planted in pots filled with soil from eight fields at Bowen with various levels of FOL DNA prior to planting.

Field/sample	No. plants	FOL DNA (pg/g soil)		Fusarium wilt		
		Mean	Range	Incidence (%)	Mean severity	Severity (affected plants)
A	10	0	0	0	0.00	0.00
B, C	20	0.6	0-2	10	0.10	1.00
D, E, F	30	19.7	13-30	43	0.60	1.38
G, H	20	145.6	84-226	90	1.85	2.06

Most of the FOL DNA readings in the 38 soil samples used for pot experiment 2 were relatively low. However, there were occasional readings greater than 100 pg FOL DNA/g soil, and fusarium wilt was observed in bioassays of some of these samples (Table 7-5).

Table 7-5. FOL DNA readings and bioassay results from 38 soil samples collected from a farm in Bundaberg with a low but scattered infestation of fusarium wilt.

FOL DNA (pg/g soil)	No. of samples	No. of bioassay plants with fusarium wilt
0	16	0
1-10	11	0
11-20	4	0
20-100	2	0
101-200	3	1
>200	2	2

Discussion

Laboratory tests with a range of *Fusarium* isolates from vegetable fields in Queensland showed that the primers developed in this project was specific for *Fusarium oxysporum* f. sp. *lycopersici* (FOL). It did not detect other form species of *Fusarium* likely to be associated with vegetable crops grown in rotation with tomato, or non-pathogenic fusaria that are present in all soils. Results of race-specificity tests suggested that the primers detected all three races of FOL. However, only three cultures of FOL races 1 and 2 were tested and two of these gave conflicting results in different laboratories, suggesting that the situation with regard to race specificity needs to be checked more fully. Whether this is necessary from a practical point of view is a moot point, as our tests with soil samples from the field showed a consistently good correlation between FOL DNA readings and the incidence of fusarium wilt on race 3-susceptible varieties. Races 1 and 2 of FOL may occur in Queensland tomato-growing soils, but race 3 appears to be the predominant race detected by our test.

Evidence obtained from field and pot experiments demonstrates that the FOL-specific primers not only detected the pathogen in soil but also provided a broad indication of inoculum density and likely disease incidence in the next tomato crop. In every case in which the DNA test was used in the field or in soil in which bioassay plants were grown to maturity, severe fusarium wilt was observed when readings were greater than about 30 pg FOL DNA/g soil. Readings as high as 100-550 pg FOL DNA/g soil were observed in some fields, suggesting that there is a chronic FOL infestation on farms that have grown tomatoes for many years.

From a commercial perspective, a DNA test is likely to have limited predictive value on farms with a heavy FOL infestation, as the growers involved are aware of the situation, having experienced losses from fusarium wilt in the past. Thus the critical question is whether a DNA test can provide useful information in fields where the incidence of fusarium wilt is less than about 2.5%. The reason for this is that growers are unlikely to accept greater losses, as an infestation of this magnitude will cost them between $1500 and $4000/ha. Our results suggest that the sensitivity of the DNA test will need to be improved if it is to reliably detect such infestations. Disease incidence was 3.8% and 10% in soil samples with FOL DNA readings that were below the reliable detection limit for the DNA assay (Tables 7-3, 7-4), and was 32% and 20% when readings ranged from 5-10

pg FOL DNA/g soil (Tables 7-2, 7-3). This demonstrates that unacceptably high levels of fusarium wilt can occur in soils with very low inoculum densities.

FOL inoculum densities are most likely to be low in newly-cleared land and in soils that have rarely been used previously for tomato production. When tomatoes are grown in these situations, the pathogen is normally introduced on machinery or on trellis posts and disease incidence increases to unacceptable levels after two or three tomato crops. However, disease distribution is often patchy. If DNA tests are to be used in such situations, multiple samples will need to be collected from each field, and until better information is available, results should be interpreted conservatively. Thus when any FOL DNA is detected in a sample, it should be assumed that there is a risk of economic losses from fusarium wilt.

Given the paucity of data on the economic impact of infestation levels from 0-20 pg FOL DNA/g soil on the incidence and severity of fusarium wilt, any move to provide commercial DNA tests for FOL is premature. The accuracy of the predictions that can be made from pre-plant samples must be improved before commercialisation occurs, and this requires further work in the following areas: 1), improvements in the DNA quantification process to reduce detection limits and minimise the number of false positive readings. 2), studies on the variation in FOL DNA levels within fields with low inoculum densities and 3), field experimentation to examine the relationship between FOL DNA levels and disease incidence over a greater range soil types and planting times than were done in this project.

One interesting observation made in a field near Childers was that severe verticillium wilt occurred in soil where VD DNA readings ranged from 0-9 pg/g soil. This suggests that when environmental conditions are suitable for the pathogen, VD behaves in much the same way as FOL, causing relatively high levels of disease at very low inoculum densities.

8. THE EFFECT OF MOISTURE, ORGANIC MATTER AND A NON-HOST CROP ON MICROBIAL ACTIVITY AND LEVELS OF FOL DNA IN SOIL

Introduction

FOL is widespread in both Bowen and Bundaberg and growers must consider the pathogen when planning a crop rotation program or deciding whether to plant a resistant variety. However, when crop rotation is used as a control measure, the number of years required to reduce inoculum density to non-damaging levels is not known. Since the rate of decline of the pathogen is likely to be influenced by environmental factors such as soil moisture, reliable predictions can only be made if the factors that influence inoculum density are understood.

New molecular techniques that enable fungal pathogens to be quantified in soil (see Chapter 7) provide opportunities to study the decline in FOL over time. This chapter describes an experiment in which these techniques were used to measure changes in FOL DNA when infested soil was subjected to various treatments. The effect of soil moisture and amending soil with organic matter were studied because both factors were likely to have indirect effects on FOL through their impact on the soil microflora. A sugarcane rotation crop was also included because tomatoes are often rotated with sugarcane in Bundaberg and a non-host crop grown for 5 years was likely to have a major impact on levels of FOL in soil.

Materials and Methods

The experiment was done at Moggill in micro-plots cut from 30 cm-diameter PVC drainage pipe. Thirty micro-plots capable of holding 15 L of soil were set into the ground to a depth of approximately 25 cm and filled with clay loam soil from a Bundaberg tomato field. The field had a long history of tomato production and a high incidence of Fusarium wilt disease in previous tomato crops. The soil was mixed thoroughly in a cement mixer to disperse FOL inoculum evenly and then 15 L of soil was added to each of 20 micro-plots. Further batches of soil were amended with either sugarcane trash (20 g L^{-1} soil) or sugarcane trash (10 g L^{-1} soil) plus pelletised poultry manure (20 g Organic X-tra® L^{-1} soil) and added to 5 replicate plots.

To simulate a sugarcane rotation, five replicate micro-plots of non-amended soil were planted with single eye setts of sugarcane (variety Q151). The cane was irrigated weekly and plants were fertilised twice during the growing season with ammoniacal fertiliser (160 kg N ha^{-1}). The remaining non-amended micro-plots were maintained as a bare fallow, and three soil moisture treatments were imposed: a moist treatment that was open to rainfall and was also irrigated weekly, an intermediate moisture treatment that was irrigated once every two months and a dry treatment that was irrigated once every 6 months. Between irrigations, the latter treatments were kept dry by covering micro-plots with clear plastic A-frame shelters. The sides of shelters remained open to encourage airflow and prevent increases in soil temperature. Thus, the experiment consisted of 6 treatments (Table 8-1) each replicated 5 times in a randomised complete block design.

Soil microbial activity and the level of FOL DNA were assessed in soil samples taken from micro-plots 28, 90, 180, 270 and 360 days after establishment (DAE). Samples of approximately 400 mL were collected by taking several cores from the top 15 cm of soil using a sampling tube. Microbial activity was estimated by measuring the rate hydrolysis of fluorescein diacetate using a protocol developed by Schnurer and Rosswall (1982). FOL DNA was quantified using a PCR based assay (see Chapter 7). Samples collected at 360 DAE were also assayed for Fusarium wilt disease using the bioassay procedure described in Chapter 5.

Results

The results clearly showed that microbial activity increased when soil amended with cane trash plus poultry manure was kept moist, and the effect lasted for at least 360 days (Table 8-1). Addition of cane trash also increased microbial activity, but the magnitude of the increase was not as great and it took 90 days for the effect to become apparent. Soil planted to sugarcane was similar to bare fallow for the first 270 days, but at 360 days microbial activity was similar to the two amended treatments. Microbial activity was not affected by differences in soil moisture in non-amended soil kept bare fallow.

Table 8-1. Effect of soil moisture, organic amendments and sugarcane on soil microbial activity measured by hydrolysis of fluorescein diacetate (FDA) at five sampling times and reported as μg FDA min^{-1} g^{-1} soil.

Treatment			Time after establishment (days)				
Amendment	**Rotation**	**Moisture**	**28**	**90**	**180**	**270**	**360**
Nil	**Fallow**	**Moist**	0.422^{ab}	0.323^{a}	0.634^{b}	1.051^{a}	0.523^{a}
Nil	**Fallow**	**Intermediate**	0.496^{b}	0.263^{a}	0.510^{ab}	1.130^{a}	0.405^{a}
Nil	**Fallow**	**Dry**	0.414^{ab}	0.365^{a}	0.518^{ab}	0.929^{a}	0.398^{a}
Nil	**Sugarcane**	**Moist**	0.387^{a}	0.349^{a}	0.356^{a}	1.150^{a}	0.693^{b}
Cane trash	**Fallow**	**Moist**	0.321^{a}	0.569^{b}	1.042^{c}	1.605^{b}	0.669^{b}
Cane trash/PM	**Fallow**	**Moist**	1.091^{c}	0.894^{c}	1.261^{d}	2.333^{c}	0.669^{b}
		LSD $_{(5\%)}$	0.115	0.130	0.197	0.239	0.132

Measurements of FOL DNA at 28 days are not presented because there was unusual variability within treatments. However, the data for other sampling times (Table 8-2) suggested that DNA levels were affected in the same way as microbial activity. There were no differences in bare fallow non-amended soil due to moisture, but the addition of sugarcane trash or sugarcane trash plus poultry manure produced a significant increase in FOL DNA at some sampling times. FOL DNA also increased in plots planted to sugarcane at the 360-day sampling time.

Table 8-2. Effect of soil moisture, organic amendments and a sugarcane crop on levels of FOL DNA (pg/g soil) measured at various times after microplots were established.

Treatment			Time after establishment (days)				
Amendment	**Rotation**	**Moisture**	**28**	**90**	**180**	**270**	**360**
Nil	**Fallow**	**Moist**	-	54 ^{a}	61^{a}	81^{a}	42^{a}
Nil	**Fallow**	**Intermediate**	-	72 ^{a}	59^{a}	67^{a}	38^{a}
Nil	**Fallow**	**Dry**	-	38 ^{a}	91^{a}	56^{a}	44^{a}
Nil	**Sugarcane**	**Moist**	-	52 ^{a}	143^{a}	154^{ab}	127^{b}
Cane trash	**Fallow**	**Moist**	-	121 ^{b}	201^{ab}	140^{ab}	79^{a}
Cane trash/PM	**Fallow**	**Moist**	-	295 ^{b}	309^{b}	246^{b}	140^{b}
		LSD $_{(5\%)}$	-	51	145	117	43

When soil was bioassayed at 360 days, between 18 and 32% of bioassay plants were affected by fusarium wilt. However, the number of affected plants did not differ significantly between treatments (Table 8-3).

Table 8-3. Effect of soil moisture, organic amendments and sugarcane on the incidence of Fusarium wilt in bioassay plants 360 days after treatments were established.

Treatment			Incidence
Amendment	**Rotation**	**Moisture**	**(% Bioassay plants affected)**
Nil	**Fallow**	**Moist**	30
Nil	**Fallow**	**Intermediate**	24
Nil	**Fallow**	**Dry**	18
Nil	**Sugarcane**	**Moist**	28
Cane trash	**Fallow**	**Moist**	22
Cane trash/PM	**Fallow**	**Moist**	32
		LSD $_{(5\%)}$	n.s.

Discussion

The results of this experiment clearly show that when soil is fallowed, soil moisture content has no effect on the survival of FOL. In contrast, DNA data suggested that the pathogen multiplied on organic materials that are added to soil. Multiplication occurred on both sugarcane trash and a mixture of sugarcane trash and poultry manure, but was more marked on the latter amendment and the effect lasted longer. The pathogen also multiplied on the roots of sugarcane, a non-host plant. These results have implications for the tomato farming system in Bundaberg, where tomatoes are often grown in rotation with sugarcane. The presence of roots of sugarcane and the return of large quantities of sugarcane trash to the soil following each sugarcane harvest suggests that there is ample opportunity for FOL to survive and even increase during the break between tomato crops. This observation needs to be confirmed with follow-up field studies, but it highlights the fact that new DNA methods of monitoring FOL will enable us to undertake ecological studies that have never been possible in the past.

Although FOL DNA readings at 360 days were significantly higher after the sugarcane crop and in soil amended with sugarcane trash plus poultry manure, the incidence of

fusarium wilt in bioassay plants was no greater than in the other treatments. This observation raises the possibility that there is not always a direct relationship between FOL DNA levels and disease incidence. Since the background soil biology was affected by treatment (as evidenced by changes in microbial activity in both cropped and amended soils), other soil organisms may have ameliorated the effects of the pathogen. Such observations need to be followed up, as they may lead to an understanding of how natural suppressive mechanisms can be enhanced to achieve better disease control.

9. SOIL BIOLOGICAL HEALTH AND ITS POSSIBLE IMPACT ON DISEASE EXPRESSION

Introduction

One of the main objectives of this project was to develop better methods of quantifying the key soil-borne pests of tomato in samples collected prior to planting. Once the population density of a pest is known, it has predictive value because disease incidence and severity for many soil-borne pests is markedly influenced by inoculum density at the time a crop is planted. Relationships between pest density and crop losses are best understood for nematodes, as techniques for quantifying them have been available for many years. However, it is well known that the threshold level at which nematodes cause economic damage is markedly influenced by environmental factors such as soil moisture or nutrient supply (McSorley and Phillips, 1993). Thus, a low nematode population density may cause losses in a crop when it is stressed for moisture, but a much higher density may be needed to produce the same effect when soil moisture is not limiting.

In the past, most research has been directed towards understanding the effects of the physical and chemical environment on damage thresholds. There have been few studies on the effects of the soil biological environment on the relationship between pest density and yield, but it is likely that biology has both direct and indirect effects. Thus, the background soil biology may directly affect the capacity of a pest to multiply and cause damage through mechanisms of biological control such as competition, antibiosis and predation. Biology also affects soil properties such as soil structure and water-holding capacity. Therefore, it will indirectly influence a plant's response to the pest.

In the absence of any previous work on the biological status of Queensland tomato-growing soils, we sought to establish whether soil biology was likely to influence the damage caused by the key pests and pathogens of tomatoes. We did this by measuring several biological parameters in a representative sample of tomato-growing soils and comparing the results to those obtained from a reference soil under permanent pasture. We also compared the biology of a tomato-growing soil that was managed in a standard way was to a site where tomatoes were grown in a permanent bed system that was likely to have a beneficial effect on the soil biology.

Materials and Methods

Survey of soil biology. During the 2000 growing season, soil samples were collected from 11 typical tomato fields. Six of the fields were located in the Bundaberg/Childers region; four were from Bowen and two from SEQ. All fields were sampled prior to planting and some were sampled again during the harvest period. Three assays were used to provide a general indication of the biological status of the soils at both sampling times. Nematodes were extracted from a 200 mL sample on a Baermann tray for 4 days, separated into five trophic groups (fungal feeding Tylenchida and Aphelenchina, fungal feeding Dorylaimida, bacterial feeding Rhabditida, omnivorous Dorylaimida and predatory Mononchina) and counted. Numbers of culturable microorganisms were determined by dilution plating onto 1/5 strength tryptic soy agar for bacteria, Kings medium B (Simon and Ridge, 1974) for fluorescent pseudomonads, Martin's medium (Martin, 1950) for fungi and Cellulose Congo Red agar for actinomycetes (Hendricks *et al.*, 1995). Microbial activity was estimated by determining the rate of hydrolysis of fluorescein diacetate (FDA) using the method of Schnurer and Rosswall (1982).

An additional assay was done on the Bundaberg soils to determine whether any of them were suppressive to *Pythium*. The fungus was grown on Corn meal sand (Corn meal, 3.0 g; Sand, 97.0 g; distilled water, 15.0 mL) for 2 weeks and added to 200 mL pots at either 1.0 or 10.0 g L^{-1}. Ten bean seeds (cv. Labrador) were then planted in each pot and the number that did not germinate due to damping-off were counted after 7 days.

Biology under different soil management systems. Two fields were selected at Bowen on a property where the grower was experimenting with sustainable systems of tomato culture. In one field, a green manure crop was grown on a raised bed for most of the year and this crop was sprayed with herbicide when the field was required for tomatoes. Tomato seedlings were then planted into the undisturbed bed. This management system had been in place for two years. The second field was farmed using a conventional system of tomato production in which land was left as a weedy fallow between tomato crops and the soil was cultivated, beds were formed and plastic mulch was laid prior to planting tomatoes.

The selected fields were planted to tomatoes in 2000 and 2001 and in both years, soil was collected from each field prior to planting and at harvest. Samples consisted of soil taken from depths of 5-15 and 15-25 cm using a trowel. The biological status of all samples was

compared in both years by measuring microbial activity and by counting free-living nematodes as described previously. Samples collected in 2001 were also assessed for suppressiveness to *Pythium* by adding the soil to two 200 ml pots, inoculating with *Pythium* in corn meal sand (10 g L^{-1}) planting 10 bean (cv. Labrador) seeds in each pot and counting the number of seeds that did not germinate. Suppressiveness to root-knot nematode was also measured in the same samples by inoculating five replicate 200 ml samples with 4000 nematode eggs, incubating the samples for 13 days and then counting the number of second-stage juveniles extracted. Pasteurised washed-river sand was included as a non-suppressive standard. Suppression was quantified by determining the percentage reduction in the number of nematodes recovered from the test soil in comparison to the non-suppressive standard.

Results

Survey of soil biology. The pasture reference site had approximately equal proportions of fungal and bacterial-feeding nematodes whereas bacterial-feeding species were predominant in most of the tomato-growing soils when they were sampled prior to planting (Table 9-1). Nematode population densities in tomato soils had often changed by harvest, but the predominance of bacterial-feeding nematodes was still apparent. Omivorous and predatory nematodes did not occur in high numbers in either the reference site or in any of the tomato-growing soils. The number of culturable soil microorganisms was also similar in the tomato-growing soils and in the reference site. Counts generally did not vary more than 1.5 log units between sites (Table 9-1) and there were no clear relationships between numbers of specific groups of microorganisms and region, cropping history or soil type.

Microbial activity was high in soil from the pasture reference site but was very low in all tomato-growing soils. The one exception was the site at Hapsburg Rd., Childers, which had previously grown sugarcane for at least 5 years. Interestingly, only 8 and 31% of plants respectively died from damping-off when *Pythium* inoculum was added at 10g/L to the pasture and Hapsburg Rd. soils. In contrast, 50-69% of plants died in most of the tomato-growing soils.

Biology under different soil management systems. The results of microbiological analyses done during the 2000 season (Tables 9-2 and 9-3) showed that numbers of culturable microorganisms was usually higher in the permanent bed than in a

field that was managed in a conventional manner. This effect was apparent for total bacteria, Gram-positive bacteria, fluorescent pseudomonads and total fungi and was observed at both sampling depths and at two sampling times. However, actinomycetes were an exception, as their population densities were generally higher in the conventional field than in the permanent bed.

In the surface soil during the 2000 season, microbial activity was much higher in the permanent bed than in the conventional field (Tables 9-2 and 9-3). However, this difference disappeared with depth. The situation was reversed in 2001, as microbial activity in the two fields was similar in the surface soil and higher in the conventional field at depth (Tables 9-4 and 9-5).

Differences in populations of free-living nematodes in the two management systems were apparent in both years, particularly in the surface soil (Tables 9-2, 9-3, 9-4 and 9-5). The effect of cultivation was very obvious with the omnivorous nematodes, as numbers were much higher in the permanent bed than in the conventionally-cultivated field. There were also more bacterial-feeding nematodes in the permanent bed at all sampling times and at both depths in the profile. In contrast, numbers of fungal-feeding nematodes were usually lower in the permanent bed.

Results of assays for suppression to *Pythium* and root-knot nematode are presented in Tables 9-4 and 9-5. The former assay showed that both soils were conducive to *Pythium*, as most bean seedlings died from damping-off at both sampling times. Some suppressiveness towards root-knot nematode was evident in both soils, but the level of suppression did not differ between the two soils.

Table 9-1. Soil biological measurements[1] in typical Queensland tomato fields that were sampled prior to planting (P) and at harvest (H) during the 2000 season.

Site	Sampling time	No. nematodes/200 ml soil					FDA	Log_{10} cfu microorganisms/g dry wt. soil				
		FF	FF (Dory)	BF	Dory.	Mon.		Total bacteria	G+ bacteria	FP	Act.	Fungi
Bucca Rd., Bundaberg	P	470	33	1807	1	0	0.397	7.1	6.0	4.6	6.9	5.3
	H	94	13	703	0	0	0.127	7.2	-	5.2	6.2	5.7
Burnett Heads, via Bundaberg	P	356	20	693	0	0	0.272	7.9	7.3	3.9	6.8	5.1
	H	77	2	285	0	0	0.103	8.7	-	5.9	6.7	5.1
Kalkie, via Bundaberg	P	97	38	108	0	0	0.116	7.4	7.0	2.6	6.6	4.9
	H	210	60	1170	2	0	0.057	-	-	-	-	-
Alloway, via Bundaberg (1)	P	64	4	457	4	0	0.478	7.4	6.6	4.6	6.5	4.7
	H	41	14	763	1	0	0.648	7.9	-	5.9	6.8	5.5
Alloway, via Bundaberg (2)	P	133	13	887	0	0	0.491	7.6	6.7	3.8	7.4	5.7
	H	-	-	-	-	-	-	-	-	-	-	-
Hapsburg Rd., Childers	P	95	35	147	0	0	1.023	7.2	6.1	4.1	6.7	4.6
	H	430	130	1375	0	0	0.648	7.9	-	5.7	9.9	4.9
E. Euri Creek Rd., Bowen	P	840	5	375	0	0	0.435	8.5	7.2	5.7	7.2	6.0
	H	75	4	34	5	100	0.230	8.0	-	5.7	9.9	4.9
Collinsville Rd., Bowen	P	370	60	805	8	0	0.258	7.4	6.8	5.9	5.8	5.1
	H	-	-	-	-	-	-	-	-	-	-	-
Bruce Hwy., Bowen	P	290	45	595	0	0	0.220	7.5	6.5	5.6	7.0	5.7
	H	700	10	1529	5	0	0.090	7.9	7.5	5.7	7.0	5.8
Delta, via Bowen	P	210	200	700	10	0	0.149	7.7	7.3	4.2	6.8	5.1
	H	470	20	1640	3	0	0.090	7.8	7.6	5.0	5.1	5.9
Flagstone Crk, Lockyer Valley	P	580	40	670	0	0	0.089	7.5	7.3	4.7	7.4	5.5
	H	-	-	-	-	-	-	-	-	-	-	-
Gatton, Lockyer Valley	P	210	10	400	0	0	0.208	8.3	6.4	5.2	7.6	6.5
	H	-	-	-	-	-	-	-	-	-	-	-
Pasture reference site, B'berg		513	47	717	0	12	2.12	7.5	6.8	5.5	7.3	5.5

[1] F = fungal feeding Aphelenchida and Tylenchida, FF (Dory) = fungal feeding Dorylaimida, BF = bacterial-feeding nematodes, Dory = Dorylaimida, Mon = Mononchida, FDA = microbial activity (e.g. FDA hydrolyzed min^{-1} g^{-1} soil), G+ = gram-positive bacteria, FP = fluorescent psuedomonads, Act = Actinomycetes.

Table 9-2. Biological status of tomato-growing soils managed under a conventional or permanent bed system. Pre-plant samples collected August 2000.

	Permanent bed		Conventional	
	10 cm	**20 cm**	**10 cm**	**20 cm**
Culturable microorganisms (log cfu/g soil)				
Total bacteria	7.24	7.81	7.39	6.93
Gram positive bacteria	6.93	6.67	6.39	6.39
Fluorescent pseudomonads	5.60	4.81	3.81	4.2
Actinomycetes	6.85	5.93	7.43	6.54
Total fungi	5.39	4.85	4.86	4.20
Microbial activity (µg FDA hydrolysed/g/min)	0.348	0.253	0.193	0.312
Free-living nematodes (numbers/200 ml soil)				
Fungal feeding nematodes	940	840	550	846
Bacterial feeding nematodes	1340	2040	550	1800
Omnivorous nematodes	88	20	0	9

Table 9-3. Biological status of tomato-growing soils managed under a conventional or permanent bed system. Post-plant samples collected October 2000.

	Permanent bed		Conventional	
	10 cm	**20 cm**	**10 cm**	**20 cm**
Culturable microorganisms (log cfu/g soil)				
Total bacteria	8.30	7.39	7.39	7.22
Gram positive bacteria	7.30	5.93	6.74	7.22
Fluorescent pseudomonads	5.30	5.08	4.93	4.85
Actinomycetes	6.39	6.59	6.39	6.74
Total fungi	5.22	5.53	5.36	5.04
Microbial activity (µg FDA hydrolysed/g/min)	0.339	0.268	0.276	0.259
Free-living nematodes (numbers/200 ml soil)				
Fungal feeding nematodes	200	150	160	240
Bacterial feeding nematodes	860	760	400	450
Omnivorous nematodes	7	2	6	0

Table 9-4 . Biological status of tomato-growing soils managed under a conventional or permanent bed system. Pre-plant samples collected July 2001.

	Permanent bed		Conventional	
	10 cm	**20 cm**	**10 cm**	**20 cm**
Microbial activity (µg FDA hydrolysed/g/min)	0.38	0.18	0.37	0.51
Free-living nematodes (numbers/200 ml soil)				
Fungal feeding nematodes	140	60	860	440
Bacterial feeding nematodes	1220	960	800	500
Omnivorous nematodes	66	12	4	16
% seedlings with damping-off	95	70	50	60
% suppression of root-knot nematode	62	30	62	53

Table 9-5. Biological status of tomato-growing soils managed under a conventional or permanent bed system. Post-plant samples collected October 2001.

	Permanent bed		Conventional	
	10 cm	**20 cm**	**10 cm**	**20 cm**
Microbial activity (µg FDA hydrolysed/g/min)	0.25	0.13	0.24	0.24
Free-living nematodes (numbers/200 ml soil)				
Fungal feeding nematodes	200	150	1200	400
Bacterial feeding nematodes	2200	2050	1350	1000
Omnivorous nematodes	75	15	40	0
% seedlings with damping-off	95	90	90	95
% suppression of root-knot nematode	42	71	0	57

Discussion

The resources available for this component of the project enabled us to do no more than take a cursory look at the beneficial component of the biology in tomato-growing soils. Nevertheless, we were able to demonstrate that the microbial activity of most soils was low in comparison to the single pasture reference site, probably because most tomato growers cultivate regularly, fumigate the soil, use herbicides for weed control and do not grow green manure crops or add organic amendments. The only site with relatively high microbial activity was previously planted to sugarcane and its better biological status was probably due to lack of cultivation and continual inputs of organic matter during the previous sugarcane cycle.

Our study of two adjacent fields in which soil was managed in different ways suggested that in the first year samples were taken, soil in the permanent beds was healthier than the soil under conventional cultivation, particularly in the upper 10 cm. The main indicators of improved biological status were higher microbial activity and higher numbers of omnivorous nematodes. However, these differences were not as apparent in the following year. Our initial studies of these samples suggested that neither soil was suppressive to damping-off caused by *Pythium*, or to root-knot nematode.

Methodological problems and resource constraints limited what could be achieved in this component of the project. Although our results suggested that the biological status of tomato-growing soils is generally poor, this conclusion needs to be confirmed, as our study included only one pasture reference site. One way of doing this would be to identify a set of paired sites and measure a series of biological parameters in both tomato-growing soils and pasture soils. Further work is also required to evaluate soil health under different soil management practices. Our study of the biological status of soils under conventional and permanent bed systems was hampered by the fact that replicated plots were not available and so there was no guarantee that the fields that were sampled differed only in the way the soil was managed. This problem can only be overcome by establishing a long-term field experiment that would serve as a study site for anyone interested in understanding the effects of different soil management systems on soil health.

10. TECHNOLOGY TRANSFER

Because pest management consultants who service the Bowen, Bundaberg, Lockyer Valley and Granite Belt regions were involved in the project, technology transfer was a continual process. Fields on most farms in the tomato industry were monitored at some stage in the project, which meant that there was a continual exchange of information between growers and consultants during the monitoring process. Details of monitoring procedures were explained at the time fields were selected, information on cropping history and previous disease incidence was obtained during discussions with growers and results of any tests for nematodes or fungi were passed on to the appropriate grower after they were received.

Two grower meetings were held to explain the aims of the project and present results. The first was held at Bowen on 5 July 2001 and was attended by about 30 people. Dale Abbott (Bowen Crop Monitoring Services) chaired the meeting and introduced the project team. Dale Griffin discussed the main diseases that were being targeted and explained various technologies that could be used to improve the disease recording systems currently used on-farm. Graham Stirling discussed the need for new approaches to soil-borne disease management, highlighting problems with current measures such as fumigation and advocating a predictive approach to managing soil pests and diseases. He also introduced the concept of soil health and presented data on the soil health status of typical tomato-growing soils in Queensland. Kathy Ophel-Keller discussed the use of molecular diagnostics in soil disease management, using the example of the diagnostic service provided by SARDI to cereal growers in southern Australia.

The second meeting was held at Bundaberg on 18 July 2001. More than 50 people attended, including a good cross section of growers, consultants, agribusiness representatives and government extension officers. Graham Stirling presented a talk entitled 'Nematode monitoring as a management tool', Dale Griffin discussed 'Monitoring and recording systems for managing soil-borne diseases' and Kathy Ophel-Keller covered 'New molecular techniques for monitoring soil-borne pathogens'. Feedback received during and since the meeting suggests that there is a high level of interest in improving prediction systems for soil-borne diseases in vegetable crops.

11. RECOMMENDATIONS

Through their involvement in this project, pest management consultants in each of the main tomato-growing areas of Queensland have a good understanding of the principles of managing soil-borne diseases of tomato. They are aware of the records that must be kept before reliable risk assessments can be made, and they have used the hazard index system to make predictions. The next step is for these consultants to use this process to help clients improve their disease management decisions. Growers have different methods of keeping records at a field level, so consultants will have to develop appropriate systems for collecting and recording information on disease incidence and severity. They will also have to collect pre-plant soil samples, and help growers interpret the results of nematode counts and DNA tests.

Although the above approach is relatively simple, adoption rates are likely to be relatively low because consultants have no plant pathological support and will lose confidence in the prediction system when unexpected problems arise. In an attempt to overcome this problem, a Concept Development Proposal seeking a voluntary contribution for a continuing project entitled 'Implementation of a prediction service for soil-borne diseases of fresh tomatoes' was submitted to Queensland Fruit and Vegetable Growers in August 2001. The proposal sought to provide plant pathological support to consultants for two years while they operated a pilot disease management and prediction service. That application was rejected but in the light of our results, we recommend that industry reconsider that decision.

C-Qentec Diagnostics, under licence from SARDI-CSIRO, have first commercial rights to the DNA assays developed in this project. The above parties must decide by December 2003 whether to commercialise the assays. Given that the assay for root-knot nematode gave results which were comparable to manual extraction methods, this test could be marketed as an alternative method of estimating nematode populations for predictive and diagnostic purposes. However, the situation with regard to the assays for FOL and VD is less clear, as unacceptably high levels of both fusarium and verticillium wilt can occur in soils in which the pathogens are just detectable with DNA techniques. The accuracy of the predictions that can be made from pre-plant samples will therefore need to be improved before commercialisation occurs. This is likely to be achievable, but will require further

work in the following areas: 1), improvements in the DNA quantification process to reduce detection limits and minimise the number of false positive readings. 2), studies on the spatial variation in FOL and VD DNA levels within fields with low inoculum densities and 3), field experimentation to examine the relationship between DNA levels and disease incidence over a greater range soil types and planting times than was possible in this project.

The ultimate objective of this work was to establish a risk management service for soil-borne diseases similar to the Root Disease Testing Service now operated by SARDI for the Australian cereal industry. However, before such a service can be established, an accreditation program must be developed for agronomists and pest management consultants so that they are able to interpret test results and provide reliable advice to growers. The range of pathogens that can be quantified using DNA methods should also be widened to include *Sclerotium rolfsii*, the next most important soil-borne pathogen of tomatoes in Queensland.

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Appendix 1. Summary of root-knot nematode (RKN) data obtained from monitoring sites during the 2001 and 2002 seasons

Site	Sampling Unit	Region	Nematicide[1]	RKN Resistance	Hazard Index	RKN J2 (Baermann Tray	RKN DNA (RKN eggs/400 g soil)	Incidence of galling	Mean GR[2] (Overall)	Mean GR (infected plants)
7	B	Bundaberg	Untreated	S	45	0	0	0.75	3.1	4.13
50	A	Bowen	Untreated	S	36	0	0	0.4	2.5	6.25
50	B	Bowen	Untreated	S	36	0	1	0.3	1.95	6.5
57	A	Bundaberg	Untreated	S	43	0	<10	0.45	1.65	3.67
59	A	Bundaberg	Untreated	S	34	0	5	0.35	1.55	4.43
9	B	Bundaberg	Untreated	S	45	0	0	0.5	1.3	2.6
57	B	Bundaberg	Untreated	S	43	0	<10	0.25	1.2	4.8
56	A	Bundaberg	Untreated	S	43	0	<10	0.4	1.15	2.875
11	B	Bundaberg	Untreated	S	45	0	0	0.2	0.95	4.75
7	A	Bundaberg	Untreated	S	45	2	0	0.4	0.95	2.34
10	A	Bundaberg	Untreated	S	45	0	0	0.25	0.75	3
35	A	SEQ	Untreated	S	28	0	-	0.2	0.7	3.5
59	B	Bundaberg	Untreated	S	34	0	1	0.15	0.65	4.3
35	B	SEQ	Untreated	S	28	0	-	0.25	0.65	2.6
10	B	Bundaberg	Untreated	S	45	0	0	0.2	0.55	2.75
27	B	SEQ	Untreated	S	40	0	-	0.416	0.5	1.2
11	A	Bundaberg	Untreated	S	45	0	0	0.2	0.45	2.25
58	B	Bundaberg	Untreated	S	43	0	4	0.1	0.3	3
38	A	SEQ	Untreated	S	39	0	-	0.1	0.3	3
38	B	SEQ	Untreated	S	39	0	-	0.05	0.015	3
58	A	Bundaberg	Untreated	S	43	0	4	0	0	0
51	A	Bundaberg	Untreated	S	40	0	<10	0	0	0
51	B	Bundaberg	Untreated	S	40	0	<10	0	0	0
49	A	SEQ	Untreated	S	34	0	0	0	0	0
49	B	SEQ	Untreated	S	34	0	1	0	0	0
48	B	SEQ	Untreated	S	40	0	<10	0	0	0
45	A	SEQ	Untreated	S	42	0	<10	0	0	0
45	B	SEQ	Untreated	S	42	0	<10	0	0	0
44	A	SEQ	Untreated	S	40	0	<10	0	0	0
44	B	SEQ	Untreated	S	40	0	<10	0	0	0

Site	Sampling Unit	Region	Nematicide[1]	RKN Resistance	Hazard Index	RKN J2 (Baermann Tray	RKN DNA (RKN eggs/400 g soil)	Incidence of galling	Mean GR[2] (Overall)	Mean GR (infected plants)
42	A	SEQ	Untreated	S	40	0	<10	0	0	0
42	B	SEQ	Untreated	S	40	0	<10	0	0	0
39	A	SEQ	Untreated	S	40	0	1	0	0	0
39	B	SEQ	Untreated	S	40	0	1	0	0	0
36	A	SEQ	Untreated	S	45	0	<10	0	0	0
36	B	SEQ	Untreated	S	45	0	<10	0	0	0
27	A	SEQ	Untreated	S	40	0	-	0	0	0
26	A	SEQ	Untreated	S	41	0	-	0	0	0
26	B	SEQ	Untreated	S	41	0	-	0	0	0
24	A	SEQ	Untreated	S	42	0	-	0	0	0
24	B	SEQ	Untreated	S	42	0	-	0	0	0
19	A	Bowen	Untreated	S	32	0	0	0	0	0
17	B	Bowen	Untreated	S	40	0	0	0	0	0
9	A	Bundaberg	Untreated	S	45	0	1	0	0	0
8	A	Bundaberg	Untreated	S	45	0	0	0	0	0
8	B	Bundaberg	Untreated	S	45	0	0	0	0	0
56	B	Bundaberg	Untreated	S	43	1	<10	0	0	0
48	A	SEQ	Untreated	S	40	1	<10	0	0	0
19	B	Bowen	Untreated	S	32	1	0	0	0	0
33	A	SEQ	Untreated	S	15	-	-	0	0	0
33	B	SEQ	Untreated	S	15	-	-	0	0	0
25	A	SEQ	Untreated	S	48	-	-	0	0	0
25	B	SEQ	Untreated	S	48	-	-	0	0	0
23	A	SEQ	Untreated	S	42	-	-	0	0	0
23	B	SEQ	Untreated	S	42	-	-	0	0	0
30	A	SEQ	N	S	45	2	-	1	6	6
32	B	SEQ	N	S	48	0	-	0.75	5	6.67
31	A	SEQ	N	S	48	4	-	0.75	5	6.67
22	B	SEQ	N	S	41	0	-	0.75	4.5	6
31	B	SEQ	N	S	48	18	-	0.75	4.13	5.5
29	A	SEQ	N	S	48	9	-	0.6875	3.25	4.72
32	A	SEQ	N	S	48	0	-	0.25	1	4

Site	Sampling Unit	Region	Nematicide[1]	RKN Resistance	Hazard Index	RKN J2 (Baermann Tray	RKN DNA (RKN eggs/400 g soil)	Incidence of galling	Mean GR[2] (Overall)	Mean GR (infected plants)
47	B	SEQ	N	S	46	0	<10	0.2	0.7	3.5
22	A	SEQ	N	S	41	0	-	0.125	0.5	4
55	B	Bundaberg	N	S	40	0	<10	0.1	0.4	4
47	A	SEQ	N	S	46	0	<10	0.15	0.4	2.67
55	A	Bundaberg	N	S	40	0	<10	0	0	0
43	A	SEQ	N	S	39	0	<10	0	0	0
43	B	SEQ	N	S	39	0	<10	0	0	0
34	A	SEQ	N	S	43	0	0	0	0	0
34	B	SEQ	N	S	43	0	1	0	0	0
21	A	SEQ	N	S	37	0	-	0	0	0
46	B	SEQ	N	S	46	1	<10	0	0	0
3	B	Bundaberg	MSN	S	43	140	88	1	6.35	6.35
3	A	Bundaberg	MSN	S	43	8	71	0.85	4.45	5.2
2	B	Bundaberg	MSN	S	28	0	0	0.6	2.75	4.58
54	B	Bundaberg	MSN	S	43	14	84	0.45	1.85	4.1
12	A	Bundaberg	MSN	S	38	0	0	0.35	1.75	5
54	A	Bundaberg	MSN	S	43	44	143	0.4	1.5	3.75
12	B	Bundaberg	MSN	S	38	2	0	0.25	1.3	5.2
4	B	Bundaberg	MSN	S	33	2	0	0.2	0.9	4.5
5	A	Bundaberg	MSN	S	36	1	0	0.25	0.65	2.6
53	A	Bundaberg	MSN	S	15	0	<20	0.25	0.6	2.4
2	A	Bundaberg	MSN	S	28	0	0	0.15	0.5	3.33
52	A	Bundaberg	MSN	S	38	124	784	0.05	0.1	2
53	B	Bundaberg	MSN	S	15	0	<20	0	0	0
5	B	Bundaberg	MSN	S	36	0	0	0	0	0
4	A	Bundaberg	MSN	S	33	0	0	0	0	0
1	A	Bundaberg	MSN	S	28	0	0	0	0	0
1	B	Bundaberg	MSN	S	28	0	0	0	0	0
52	B	Bundaberg	MSN	S	38	56	1346	0	0	0
28	B	SEQ	MS	S	45	0	-	0.875	3.6	4.1
41	B	SEQ	MS	S	50	0	<10	0.65	2.9	4.46
28	A	SEQ	MS	S	45	2	-	0.875	2.6	3

Site	Sampling Unit	Region	Nematicide[1]	RKN Resistance	Hazard Index	RKN J2 (Baermann Tray	RKN DNA (RKN eggs/400 g soil)	Incidence of galling	Mean GR[2] (Overall)	Mean GR (infected plants)
41	A	SEQ	MS	S	50	0	<10	0.8	2.4	3
40	B	SEQ	MS	S	50	0	<10	0	0	0
37	A	SEQ	MS	S	48	0	<10	0	0	0
37	B	SEQ	MS	S	48	0	<10	0	0	0
17	A	Bowen	MS	S	40	0	10	0	0	0
40	A	SEQ	MS	S	50	1	14	0	0	0
20	A	Bowen	Untreated	R	33	0	0	0	0	0
20	B	Bowen	Untreated	R	33	0	0	0	0	0
16	A	Bowen	Untreated	R	30	0	0	0	0	0
16	B	Bowen	Untreated	R	30	0	0	0	0	0
15	A	Bowen	Untreated	R	30	0	1	0	0	0
15	B	Bowen	Untreated	R	30	0	1	0	0	0
18	B	Bowen	Untreated	R	46	3	0	0	0	0
18	A	Bowen	Untreated	R	46	5	8	0	0	0
14	A	Bowen	Untreated	R	28	-	-	0	0	0
14	B	Bowen	Untreated	R	28	-	-	0	0	0
13	B	Bowen	Untreated	R	35	-	-	0	0	0
6	A	Bundaberg	MSN	R	40	0	0	0	0	0
6	B	Bundaberg	MSN	R	40	0	1	0	0	0
13	A	Bowen	MS	R	35	0	1	0	0	0

[1] MS = metham sodium, N = nematicide
[2] GR = Gall rating.

Appendix 2a. Summary of FOL data obtained from monitoring sites during the 2001 and 2002 seasons (Bowen and Bundabaerg)

Site	Sampling Unit	Region	FOL3 resistance	Hazard Index	FW[1] Incidence (Bioassay)	FOL DNA (pg DNA/g soil)	FW Incidence (Field)	Severity of FW (Field)
58	A	Bundaberg	S	44	0.38	71	1.00	0.47
5	A	Bundaberg	S	31	0.00	0	0.54	0.47
17	A	Bowen	S	40	0.00	0	0.38	0.55
17	B	Bowen	S	40	0.00	0	0.29	0.45
51	A	Bundaberg	S	36	0.00	13	0.20	0.11
51	B	Bundaberg	S	36	0.00	2	0.18	0.03
58	B	Bundaberg	S	44	0.00	5	0.16	0.23
59	A	Bundaberg	S	38	0.00	5	0.08	0.05
12	B	Bundaberg	S	45	1.00	2	0.03	0.00
3	A	Bundaberg	S	41	0.00	1	0.03	0.07
19	B	Bowen	S	5	0.00	0	0.03	0.17
12	A	Bundaberg	S	45	1.00	52	0.03	0.00
59	B	Bundaberg	S	38	0.10	48	0.02	0.00
19	A	Bowen	S	5	0.00	0	0.02	0.08
7	B	Bundaberg	S	33	0.10	6	0.02	0.02
57	A	Bundaberg	S	39	0.00	<10	0.01	0.00
10	B	Bundaberg	S	13	0.00	5	0.01	0.00
11	A	Bundaberg	S	28	0.00	27	0.01	0.00
3	B	Bundaberg	S	41	0.00	8	0.01	0.00
10	A	Bundaberg	S	13	0.00	5	0.01	0.00
53	A	Bundaberg	S	13	0.11	<10	0.01	0.00
7	A	Bundaberg	S	33	0.00	9	0.00	0.00
56	A	Bundaberg	S	11	0.00	<10	0.00	0.00
56	B	Bundaberg	S	11	0.00	<10	0.00	0.00
53	B	Bundaberg	S	13	0.00	<10	0.00	0.00
1	A	Bundaberg	S	39	0.00	0	0.00	0.00
1	B	Bundaberg	S	39	0.00	0	0.00	0.00
57	B	Bundaberg	S	39	0.00	<10	0.00	0.00
11	B	Bundaberg	S	28	0.00	21	0.00	0.00
2	A	Bundaberg	S	26	0.00	1	0.00	0.00

Site	Sampling Unit	Region	FOL3 resistance	Hazard Index	FW[1] Incidence (Bioassay)	FOL DNA (pg DNA/g soil)	FW Incidence (Field)	Severity of FW (Field)
2	B	Bundaberg	S	26	0.00	0	0.00	0.00
8	A	Bundaberg	S	18	0.00	0	0.00	0.00
8	B	Bundaberg	S	18	0.00	1	0.00	0.00
9	A	Bundaberg	S	18	0.00	0	0.00	0.00
9	B	Bundaberg	S	18	0.10	10	0.00	0.00
55	A	Bundaberg	S	13	0.00	<10	0.00	0.00
55	B	Bundaberg	S	13	0.00	<10	0.00	0.00
5	B	Bundaberg	R	31	0.00	0	0.00	0.00
13	A	Bowen	R	43	0.10	66	0.00	0.00
13	B	Bowen	R	43	-	-	0.00	0.00
18	A	Bowen	R	43	0.00	0	0.00	0.00
18	B	Bowen	R	43	0.10	0	0.00	0.00
20	A	Bowen	R	43	0.25	35	0.00	0.00
20	B	Bowen	R	43	0.00	79	0.00	0.00
14	A	Bowen	R	38	0.67	-	0.00	0.00
14	B	Bowen	R	38	1.00	-	0.00	0.00
15	A	Bowen	R	38	0.10	233	0.00	0.00
15	B	Bowen	R	38	0.00	57	0.00	0.00
16	A	Bowen	R	38	1.00	0	0.00	0.00
16	B	Bowen	R	38	1.00	0	0.00	0.00
50	A	Bowen	R	36	0.11	4	0.00	0.00
50	B	Bowen	R	36	0.00	3	0.00	0.00
6	A	Bundaberg	R	44	0.00	0	0.00	0.00
6	B	Bundaberg	R	44	0.00	0	0.00	0.00
4	A	Bundaberg	R	41	0.00	2	0.00	0.00
4	B	Bundaberg	R	41	0.00	1	0.00	0.00
54	A	Bundaberg	R	41	0.00	<10	0.00	0.00
54	B	Bundaberg	R	41	0.00	<10	0.00	0.00
52	A	Bundaberg	R	35	0.00	<10	0.00	0.00
52	B	Bundaberg	R	35	0.11	<10	0.00	0.00

[1] FW = Fusarium wilt, the disease caused by *Fusarium oxysporum* f. sp. *lycopersici*

Appendix 2b. Summary of FOL data obtained from monitoring sites during the 2001 and 2002 seasons (SEQ)

Site	Sampling Unit	Region	FOL3 resistance	Hazard Index	FW[1] Incidence (Bioassay)	FOL DNA (pg DNA/g soil)	FW Incidence (Field)	Severity of FW (Field)
46	A	SEQ	S	38	0.0	<10	-	-
34	A	SEQ	S	40	0.0	0.0	0.00	-
34	B	SEQ	S	40	0.0	1.0	0.00	-
46	B	SEQ	S	38	0.0	<10	0.00	-
47	A	SEQ	S	38	0.0	<10	0.00	-
47	B	SEQ	S	38	0.0	<10	0.00	-
48	A	SEQ	S	36	0.0	<10	0.00	-
48	B	SEQ	S	36	0.0	<10	0.00	-
38	A	SEQ	S	34	-	-	0.00	-
38	B	SEQ	S	34	-	-	0.00	-
28	B	SEQ	S	33	-	-	0.00	-
28	A	SEQ	S	33	-	-	0.00	-
45	A	SEQ	S	33	0.0	<10	0.00	-
45	B	SEQ	S	33	0.0	<10	0.00	-
43	A	SEQ	S	33	0.0	<10	0.00	-
43	B	SEQ	S	33	0.0	<10	0.00	-
35	A	SEQ	S	29	-	-	0.00	-
35	B	SEQ	S	29	-	-	0.00	-
49	A	SEQ	S	28	0.0	0.0	0.00	-
49	B	SEQ	S	28	0.0	1.0	0.00	-
25	A	SEQ	S	25	-	-	0.00	-
25	B	SEQ	S	25	-	-	0.00	-
31	A	SEQ	S	25	-	-	0.00	-
31	B	SEQ	S	25	-	-	0.00	-
32	A	SEQ	S	25	-	-	0.00	-
32	B	SEQ	S	25	-	-	0.00	-
21	A	SEQ	S	23	-	-	0.00	-
36	A	SEQ	S	23	0.0	<5	0.00	-
36	B	SEQ	S	23	0.0	<5	0.00	-
40	A	SEQ	S	20	0.0	<5	0.00	-

Site	Sampling Unit	Region	FOL3 resistance	Hazard Index	FW[1] Incidence (Bioassay)	FOL DNA (pg DNA/g soil)	FW Incidence (Field)	Severity of FW (Field)
40	B	SEQ	S	20	0.0	<5	0.00	-
22	A	SEQ	S	18	-	-	0.00	-
22	B	SEQ	S	18	-	-	0.00	-
23	A	SEQ	S	18	-	-	0.00	-
23	B	SEQ	S	18	-	-	0.00	-
24	A	SEQ	S	18	-	-	0.00	-
24	B	SEQ	S	18	-	-	0.00	-
41	A	SEQ	S	18	0.0	<5	0.00	-
41	B	SEQ	S	18	0.0	<5	0.00	-
44	A	SEQ	S	18	0.0	<10	0.00	-
44	B	SEQ	S	18	0.0	<10	0.00	-
29	A	SEQ	S	15	-	-	0.00	-
37	A	SEQ	S	15	0.0	<5	0.00	-
37	B	SEQ	S	15	0.0	<5	0.00	-
39	A	SEQ	S	13	0.0	2.0	0.00	-
39	B	SEQ	S	13	0.0	3.0	0.00	-
42	A	SEQ	S	13	0.0	<5	0.00	-
42	B	SEQ	S	13	0.0	<5	0.00	-
26	A	SEQ	S	11	-	-	0.00	-
26	B	SEQ	S	11	-	-	0.00	-
30	A	SEQ	S	10	-	-	0.00	-
33	A	SEQ	S	8	-	-	0.00	-
33	B	SEQ	S	8	-	-	0.00	-
27	A	SEQ	S	7	-	-	0.00	-
27	B	SEQ	S	7	-	-	0	-

1 FW = Fusarium wilt, the disease caused by *Fusarium oxysporum* f. sp. *lycopersici*