

Combining an initial risk assessment process with DNA assays to improve prediction of soilborne diseases caused by root-knot nematode (*Meloidogyne* spp.) and *Fusarium oxysporum* f. sp. *lycopersici* in the Queensland tomato industry

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Abstract. A two-step process was used to assess the risk of losses from root-knot nematode and *Fusarium* wilt in fields to be planted to tomatoes. The first step involved deciding well before planting whether the risk of disease was high enough to justify collecting soil samples to determine pathogen inoculum density. This interim assessment was done using information on the major factors likely to affect disease risk (i.e. cropping history, disease history, soil texture and expected temperature during the growing season), in order to calculate a hazard index (score between 0 and 50). Its value as a predictive tool was validated by relating the hazard index to actual disease incidence and severity in representative tomato fields. The usefulness of the hazard index was often found to be limited by a lack of reliable information on disease history. Nevertheless, it had some predictive value, as all sites with moderate infestations of root-knot nematode had hazard indexes greater than 40, and most sites with more than 3% *Fusarium* wilt had hazard indexes greater than 35. The second step in the prediction process involved using DNA tests to estimate inoculum densities of *Fusarium oxysporum* f. sp. *lycopersici* and root-knot nematode in soil collected before planting. Experiments in pots and in the field confirmed that the incidence and severity of both diseases was related to pre-plant inoculum density. The DNA test for root-knot nematode was useful from a practical point of view as it detected nematode populations capable of causing economically damaging levels of galling at harvest. However, the test for *F. oxysporum* f. sp. *lycopersici* was not sensitive enough to always detect the pathogen in soils where 4–10% of plants were diseased.

Additional keywords: disease management, IPM.

Introduction

About 75% of Australia's fresh-market tomatoes are grown within 200 km of the Queensland coast. Due to climatic differences between the four main production regions, tomatoes are grown throughout the year, with the Queensland industry having a dominant place in the market during winter and spring, when low temperatures limit production in southern states. The production system involves trellised crops that are grown in beds covered with plastic and watered by trickle-irrigation. This system results in consistently high yields and minimises the incidence of

foliar diseases and fruit rots that are otherwise common in ground-grown cropping systems.

As in other tomato-growing areas of the world, soilborne diseases are a major factor limiting production. *Fusarium* wilt caused by race 3 of *Fusarium oxysporum* f. sp. *lycopersici* and root galling caused by root-knot nematodes (primarily *Meloidogyne javanica* and *M. incognita*) are widespread and economically important, while outbreaks of Sclerotium base rot (caused by *Sclerotium rolfsii*), Verticillium wilt (caused by *Verticillium dahliae*) and bacterial wilt (caused by *Ralstonia solonacearum*) occasionally result in some losses (Stirling

Table 1. The risk factors considered in calculating a hazard index to estimate the risk of loss from root-knot nematode (RKN) and Fusarium wilt (FW) of tomato

Risk factors	No. of points allocated for particular scenarios	
	Root-knot nematode	Fusarium wilt
Cropping History		
Tomato crop grown last year	5	5
Number of tomato crops 2–3 years ago	2 (one crop), 4 (two crops)	5 (one crop), 10 (two crops)
Tomato crops grown 4–10 years ago	0	3–5, depending on number of crops
RKN-susceptible crops or weeds present in the previous 2 years	5	–
Forage sorghum green manure crop grown before planting	–2	–
Maximum for cropping history	5	10
Disease History		
FW observed more than 5 years ago	0	15
FW observed in the last 5 years	0	25
FW-resistant cultivars or methyl bromide used previously	–	25
Light, medium or heavy galling observed on previous crops or weeds	15, 20 or 25, depending on severity	–
Nematicides, fumigants or nematode-resistant cultivars used previously	15	–
Maximum for disease history	25	25
Soil texture		
Soil texture based on the proportion of sand, silt and clay	2 for compacted clay soils, increasing to 10 for sand	3 for clay and clay loam soils and 5 for sands and sandy loams
Maximum for soil texture	10	5
Temperature		
Expected mean temperature in the three months after planting	2 for 15–19°C, increasing to 10 for > 23°C	2 for 15–19°C, increasing to 10 for > 23°C
Maximum for temperature	10	10
Total points	50	50

and Ashley 2003). Cultivars resistant to Fusarium wilt are invariably grown in fields where the disease has been observed previously, and green manure crops of forage sorghum are sometimes used to reduce root-knot nematode populations. However, chemicals are the primary control tactic. Non-volatile nematicides (e.g. fenamiphos or oxamyl) are applied in situations where root-knot nematode is the key pest, and soil is fumigated with either methyl bromide or metham sodium when broad-spectrum control is required.

Previous studies have shown that growers often use soil fumigants and nematicides as a form of insurance and, therefore, apply them in situations where there is little likelihood of economic damage (Stirling and Ashley 2003). The reason growers use chemicals in this way is that they are unable to confidently predict whether particular soilborne diseases will cause problems in the next tomato crop (Stirling 1999). Disease control strategies that are less dependent on fumigants and nematicides will be adopted only when growers can reliably predict the impact of key diseases before they plant a tomato crop.

The objective of this work was to develop and validate a two-step prediction process that would provide growers with reliable advice on whether *F. oxysporum* f. sp. *lycopersici* and root-knot nematode, the two most economically important soilborne diseases in the Queensland tomato industry, are likely to cause unacceptable losses when a field is next planted to tomatoes. The first step involved deciding well before planting whether the risk of disease was high enough to justify collecting soil samples to determine the pre-plant inoculum density of key pathogens. This interim

assessment of disease risk was done using information on the major factors likely to affect disease incidence in a future tomato crop (i.e. cropping history, disease history, soil texture and expected temperature during the growing season) to calculate a hazard index. Its value as a predictive tool was validated by relating the hazard index to actual disease incidence and severity in a representative sample of tomato fields. The second step in the prediction process involved using DNA tests to estimate the inoculum density of *F. oxysporum* f. sp. *lycopersici* and root-knot nematode in soil collected before planting. The DNA tests were validated in pots and in the field by relating inoculum density to disease incidence and severity later in the season.

Methods

Interim assessment of risk

Cropping and disease history influence the incidence and severity of soilborne diseases by affecting the amount of inoculum that is present in a field before planting. Factors such as soil texture and temperature affect disease expression by influencing pathogen activity and the reaction of the plant to the pathogen. Since information on all four components is available before planting a tomato crop, a scheme was developed to distil this information into a single figure (a hazard index) that provided a quantitative assessment of the likely risk from Fusarium wilt or root-knot nematode in the next crop. Details of how the hazard index was calculated are given in Table 1. Briefly, a total of 50 points were allocated to each disease, with cropping history, disease history, soil texture and temperature allocated a proportion of the total points according to their perceived contribution to disease risk.

Half the points of the hazard index were allocated to disease history because the incidence of diseases in previous tomato crops was considered the most important predictor of disease. Soilborne inoculum

is most likely to be present in fields where symptoms of root-knot nematode or Fusarium wilt have been observed previously, and the previous occurrence of a disease also indicates that environmental conditions suitable for disease expression can occur at that site. The criteria for allocation of points for various risk factors associated with disease history are given in Table 1. Fields where fumigants, nematicides or disease resistant cultivars had been used previously were considered to be at risk of disease because this indicated that disease problems had possibly occurred in the past. Also, these pesticides do not necessarily reduce pathogen inoculum density at the end of the growing season, while pathogenic form species of *Fusarium* can survive on the roots of resistant cultivars and weeds, and as saprophytes (Katan 1971; Ben-Yephet *et al.* 1996).

The cropping history component of the hazard index was based on the premise that the number and timing of previous tomato crops would affect the amount of inoculum likely to be present when the next tomato crop is planted. This component was considered particularly important for *F. oxysporum* f. sp. *lycopersici*, as it is host-specific and survives in the soil for many years. It is less important for a pathogen such as root-knot nematode, which has a broad host range that includes many other crops and weeds. Forage sorghum was considered to reduce the risk of damage from root-knot nematode because nematode populations decline when it is grown as a rotation crop (Gallaher *et al.* 1991; McSorley and Gallaher 1993) and further decline occurs when it is incorporated as a green manure (Widmer and Abawi 2002). Temperature and soil texture contributed to the hazard index because losses from Fusarium wilt and root-knot nematode increase as temperature increases (Jones 1991; Overman 1991), and damage from both pathogens is most severe in coarse-textured soils (Jones 1991; Van Gundy 1985).

In situations where a field had many risk factors and the total number of points for one component of the hazard index was excessive, a maximum number of points was set for each component. When information on cropping or disease history was unavailable, the worst case was assumed and maximum points allocated.

Predictive value of the hazard index

Before planting the 2001 and 2002 tomato crop, 114 sites at Bowen, Bundaberg and in the Lockyer Valley/Granite Belt regions were selected as being representative of the situations in which tomatoes are grown in Queensland. Hazard indexes for root-knot nematode and Fusarium wilt in each field were then calculated as described previously. The contribution of disease history and cropping history to the hazard index was determined using information provided by the grower, while the likely effect of temperature was estimated using regional mean temperatures for the 3 months after the proposed planting date. Soil texture was categorised using the ribbon method of Northcote (1971).

Towards the end of the harvest period or immediately after harvest (i.e. 12–18 weeks after planting, depending on the time of the year), the incidence of both root-knot nematode and Fusarium wilt was determined in all fields. A representative 0.4 ha sampling unit was identified and the roots of 20 plants in this area (two plants from ten randomly-selected points) were rated for galling caused by root-knot nematode using the 0–10 scale of Zeck (1971). The incidence of Fusarium wilt was determined by inspecting all plants between the trellis stakes closest to the above sampling points (i.e. ten replicates of ~40 plants). Wilted plants, plants with dark vascular tissue and plants with leaflets that were yellow on one side of the petiole were counted, as these symptoms are specific for Fusarium wilt (Jones 1991). When there was doubt about the causal agent, the presence of the pathogen was confirmed by isolation. The predictive value of the hazard index was assessed by determining whether it was related to disease incidence.

Deoxyribonucleic acid tests to quantify pathogens

The intragenic spacer (IGS) region of the rDNA was amplified and sequenced for 12 different form species of *F. oxysporum*. Specific PCR primers for *F. oxysporum* f. sp. *lycopersici* and an oligonucleotide hybridisation probe specific to *F. oxysporum* were then developed in the IGS region. Specificity was initially confirmed by amplifying DNA from all isolates of *F. oxysporum* and visualising the PCR product under UV light on a 2% agarose gel 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 primers amplified only DNA from cultures of *F. oxysporum* f. sp. *lycopersici*. For root-knot nematode, rDNA probe sequences specific to the genus *Meloidogyne* were developed by CSIRO Entomology, Canberra, ACT.

To assess the amount of pathogen DNA in soil, samples were forwarded to the South Australian Research and Development Institute. Quantitative PCR assays using rDNA probe sequences specific to *F. oxysporum* f. sp. *lycopersici* and *Meloidogyne* spp. were applied to total DNA extracted from ~400 g of soil (Ophel-Keller *et al.* 1999). For *F. oxysporum* f. sp. *lycopersici*, DNA standards were made from fungal mycelium and quantified. For *Meloidogyne*, DNA from eggs and second-stage juveniles was amplified and standard curves based on known numbers of nematodes were used to convert the DNA results to an estimate of the number of *Meloidogyne* present. Similar methods have been used previously to quantify *Pratylenchus* in soil (Hollaway *et al.* 2003). The assays for both *F. oxysporum* f. sp. *lycopersici* and *Meloidogyne* are available from the Root Disease Testing Service (RDTS), South Australian Research and Development Institute, GPO Box 397, Adelaide, SA, 5001.

Validation of a DNA method of quantifying *Fusarium oxysporum* f. sp. *lycopersici*

The relationship between inoculum density of *F. oxysporum* f. sp. *lycopersici* and the incidence of Fusarium wilt was examined in pots containing soil from six sites at Bowen. Soil A was from a field that had recently been cleared of native vegetation and was then cultivated in preparation for planting a tomato crop. Soils D, E and F were from different areas of a field where low levels 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), all fields had been cultivated in preparation for planting tomatoes. Each soil was mixed in a concrete mixer, with the virgin soil processed first to avoid the possibility of contaminating it with infested soil. Additional quantities of three soils were mixed in the proportions 75%A + 25%D and 75% A + 25% E, and designated B and C, respectively. Ten replicate 10 L pots were filled with each of the eight soils and a 400 mL soil sample was then collected from each pot for DNA analysis (see above). A tomato seedling (cv. Daniella) was planted in each pot on 7 August 2002 and after plants had grown to maturity (12 November 2002), they were checked for Fusarium wilt. Disease severity was assessed using a 0–3 rating scheme, 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).

The relationship between inoculum density and disease incidence was examined in the field at a 3 ha site ~10 km north of Childers. Fusarium wilt was observed in tomatoes grown at the site during 1997 and the land had then been cropped to sugarcane for 4 years. In July 2002, the field was cultivated and plastic mulch laid on beds in preparation for another tomato crop. Fifteen plots, each 20 m long, were established at randomly selected points in the field and then a composite soil sample was collected from eight points in each plot at a depth of 0–10 cm. The soil was mixed, a 400 mL sub-sample was taken,

and DNA of *F. oxysporum* f. sp. *lycopersici* was quantified by RDTS using methods described previously. On 26 September 2002, the field was planted to tomato cv. Grenade, a cultivar with resistance to race 3 of *F. oxysporum* f. sp. *lycopersici*. However, with the cooperation of the grower, 40 plants of the susceptible cultivar Samba were planted in each of the plots that had been sampled previously. Plants in these plots were checked regularly during the period 8–14 weeks after planting and the final incidence of Fusarium wilt was recorded at 14 weeks.

Validation of standard and DNA methods of quantifying root-knot nematode

Relationships between nematode population density before planting and the level of galling at harvest were examined in soil from two locations. In the first experiment, 40 samples of sandy loam soil were collected from different areas of two adjacent tomato fields near Gatton. Each sample was placed in a 10 L plastic pot, a 1 L sub-sample was retrieved with a 2 cm-diameter sampling tube and a tomato seedling (cv. Tiny Tim) was then planted in each pot. Plants were grown in a glasshouse until they reached maturity (~12 weeks) and then roots were washed free of soil and rated for galling using the 0–10 scale of Zeck (1971). Similar methods were used for a second experiment containing 50 pots, except that tomato cv. Redcoat was grown and the soil was obtained by mixing differing proportions of two sandy loam soils from fields near Bundaberg.

In both experiments, the soil collected from each pot was mixed gently and sub-divided into two 400 mL samples. Nematodes were recovered from one sample (containing 360–400 g dry weight equivalent of soil) by spreading the soil on two 30 × 20 cm extraction trays (Whitehead and Hemming 1965). After incubation at 22–26°C for 4 days, the contents of the trays were combined, nematodes were recovered by sieving twice on a 38 µm sieve and second-stage juveniles of root-knot nematode (J2) were counted at a magnification of 40×. The second sample was dried overnight at 40°C and then DNA was extracted by RDTS using methods described previously.

The DNA assay was further validated in a tomato crop grown in a well-structured clay-loam soil at a field site near Childers. In July 2002, raised beds were prepared, trickle tubing was laid and beds were covered with plastic in preparation for planting. 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 eight points in each plot to a depth of ~15 cm was then mixed in a bucket and a 400 mL sample was taken for DNA analysis. In September 2002, the field was planted by the grower to a commercial crop of tomato cv. Grenade. During the harvest period (i.e. ~14 weeks after planting), ten plants were dug from each plot and rated for galling as described previously for the pot experiments.

Statistical analyses

In each experiment, relationships between the following variables were assessed by regression analysis using either Genstat 5 (Rothamsted Experimental Station, UK) or Microsoft Excel. (1) Pot experiment with *Fusarium*-infested soils from Bowen: mean disease incidence or severity in ten replicates of each of the eight soils v. mean inoculum density in those soils estimated using the DNA assay. (2) Field experiment in *Fusarium*-infested soil at Childers: proportion of *Fusarium*-infested plants in each plot v. amount of pathogen DNA/g soil before planting. (3) Pot experiments with root-knot nematode in soils from Gatton and Bundaberg: the number of root-knot nematodes estimated using DNA v. the number extracted. Pre-plant population density in each pot (estimated by either DNA or extraction) v. the gall rating in each pot at harvest. (4) Field experiment at Childers in soil infested with root-knot nematode: initial nematode population density in each plot (estimated using DNA) v. mean gall ratings for ten replicate plants in those plots at harvest.

Nematode numbers obtained using either the standard extraction method or the DNA assay were always transformed [$(\log_{10}(x + 1))$] for analysis.

Results

Predictive value of the hazard index

Tomato cultivars with resistance to root-knot nematode were grown at 14 sites, but results for susceptible cultivars at the other 100 sites showed that heavily galled plants were rarely observed. Of the 2000 plants assessed, only 50 plants at a total of only 12 sites had root gall ratings greater than six. These heavily galled plants showed no above-ground signs of nematode damage during the harvest period, and grower's records indicated that crops at these sites produced acceptable yields.

Forty five of the 100 sites were treated with a nematicide, most commonly metham sodium at 800 L/ha. Oxamyl was occasionally applied via trickle irrigation, sometimes in addition to metham sodium, but it was always used at relatively low rates (0.48–1.44 kg/ha). Results indicated that gall ratings at harvest tended to be higher in nematicide-treated sites than untreated sites (Table 2). Sites with moderate to heavy galling had high hazard indexes, as mean gall ratings greater than 4 occurred only at sites with hazard indexes greater than 40.

Since *F. oxysporum* f. sp. *lycopersici* does not occur in the Lockyer Valley or the Granite Belt, meaningful data could be obtained only from the two regions where the pathogen is found (i.e. Bowen and Bundaberg). However, this dataset (60 sites) was further compromised by the fact that cultivars resistant to race 3 of *F. oxysporum* f. sp. *lycopersici* were grown at 23 sites. Nevertheless, results from the 37 sites where susceptible cultivars were grown (Table 3) showed that Fusarium wilt occurred at more than half the sites. Incidence was usually low, but more than 3% of plants were affected at eight sites, while four sites had a very high disease incidence (28–54% of plants with Fusarium wilt). The hazard index had some predictive value because the index was greater than 35 at seven of the eight sites where more than 3% of plants succumbed to the disease (Table 3). However, 54% of plants at one site were affected by Fusarium wilt despite a hazard index of only 31. Also, occasional infected plants were sometimes found at sites with hazard indexes of less than 15.

Validation of a DNA method of quantifying F. oxysporum f. sp. lycopersici

In the pot experiment with soil from Bowen, regression analyses showed that both the incidence and severity of Fusarium wilt increased as the amount of *F. oxysporum* f. sp. *lycopersici* DNA in pre-plant soil samples increased ($F_{1,6} = 25.2$, $P = 0.002$, $R^2 = 0.81$ for disease incidence; $F_{1,6} = 94.2$, $P < 0.001$, $R^2 = 0.94$ for disease severity). The disease was observed in pots containing soil with less than 3 pg of pathogen DNA/g soil, and 90% of plants were

Table 2. Relationships between a hazard index and the incidence and severity of galling caused by root-knot nematode on susceptible tomato cultivars at nematicide-treated and untreated sites

Treatment	Hazard index	No. of sites	No. of sites with a mean gall rating > 4	Mean gall rating \pm s.e. ^A
Not treated	11–25	2	0	0 \pm 0
	26–35	8	0	0.31 \pm 0.12
	36–40	21	0	0.25 \pm 0.15
	41–45	22	0	0.56 \pm 0.17
	46–50	2	0	0 \pm 0
Nematicide	11–25	2	0	0.30 \pm 0.30
	26–35	7	0	0.61 \pm 0.38
	36–40	11	0	0.37 \pm 0.19
	41–45	11	4	2.85 \pm 0.69
	46–50	14	3	1.77 \pm 0.52

^AMean gall rating from 20 replicate plants at each site.

Table 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 cultivars were grown

Hazard index	Resistant cultivars		Susceptible cultivars		
	No. of sites	No. of sites with <i>Fusarium wilt</i>	No. of sites	No. of sites with <i>Fusarium wilt</i>	No. of sites with > 3% <i>Fusarium wilt</i>
< 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

Table 4. Incidence and severity of *Fusarium wilt* in tomato (cv. Daniella) in eight soils infested with various levels of *Fusarium oxysporum* f. sp. *lycopersici* (FOL), as determined by measuring FOL DNA before planting

Soil	No. of plants	FOL DNA (pg/g soil)		Incidence (%)	Fusarium wilt	
		Mean	Range		Mean severity (all plants) ^A	Severity (affected plants) ^A
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

^ASeverity was rated on a 0–3 scale where 0 = healthy and 3 = severe wilting and/or death.

severely affected when more than 84 pg of pathogen DNA/g soil was present (Table 4).

In the field plots planted to cv. Samba, there was a significant relationship between the incidence of *Fusarium wilt* and levels of *F. oxysporum* f. sp. *lycopersici* DNA in pre-plant soil samples ($F_{1,13} = 7.86$, $P = 0.015$, $R^2 = 0.38$; Fig. 1). Disease incidence was as high as 5% in plots with 1–2 pg of pathogen DNA/g soil, a level which is below the reliable detection limit for the assay. More than 17% of plants were affected by *Fusarium wilt* in plots with more than 5 pg DNA/g soil.

Validation of standard and DNA methods of quantifying root-knot nematode

In the experiment with soil from Gatton, initial nematode population densities (determined by extraction) ranged from 0 to 1070 juveniles of root-knot nematode/400 mL soil. There was a highly significant relationship ($F_{1,38} = 67.3$, $P < 0.001$, $R^2 = 0.64$) between these pre-plant nematode counts and estimates obtained using DNA (Fig. 2a). Gall ratings at harvest ranged from 0 to 7 and were significantly related to initial nematode densities ($P < 0.001$), whether the nematode density was determined by counting juveniles

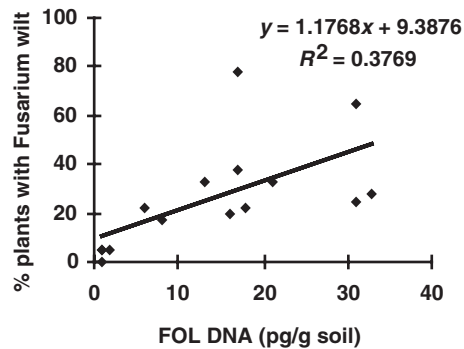


Fig. 1. Relationship between the amount of *Fusarium oxysporum* f. sp. *lycopersici* DNA (FOL DNA) in pre-plant soil samples and the incidence of Fusarium wilt in field-grown tomatoes at harvest.

extracted on trays or estimated by the DNA method (Fig. 2*b, c*).

Three plants in the second 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 numbers of juveniles and DNA readings, numbers of juveniles and gall ratings, and DNA and gall ratings (Fig. 3*a–c*) were all highly significant ($P < 0.001$).

Initial nematode densities in the 24 field plots at Childers (estimated using DNA) ranged from 0 to 1022 juveniles of root-knot nematode/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. Plants in the latter plots were affected by root-knot nematode, as they were growing poorly and showed signs of moisture stress. The relationship between nematode numbers, (estimated using DNA) and galling (Fig. 4) indicated that gall ratings were correlated to initial nematode density ($F_{1,22} = 18.6$, $P < 0.001$, $R^2 = 0.46$).

Discussion

Our hazard index is a simple and objective risk assessment tool that enables growers to identify well before planting which of their fields are most at risk of damage from soilborne disease. Its predictive value for root-knot nematode is shown by the fact that none of the tomato crops planted at sites with low hazard indexes were heavily galled at harvest, whereas seven sites with hazard indexes of more than 40 had gall ratings greater than 4 (Table 1). However, the results in Table 1 also indicate that the hazard index over-estimates the level of risk, as many of the crops grown at sites with high hazard indexes were not heavily galled. This problem is mainly due to the fact that the hazard index at these sites was inflated by the 25 points allocated in situations where growers had never previously checked roots

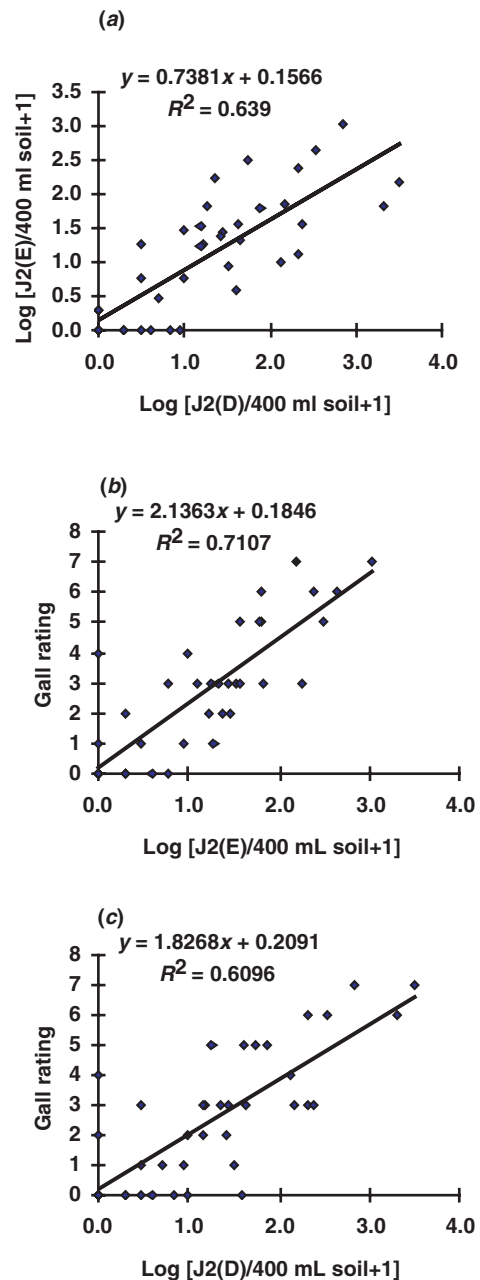


Fig. 2. Relationships between various parameters in a pot experiment with soil from Gatton. (a) Numbers of root-knot nematode juveniles estimated using DNA [J2(D)] v. numbers estimated by extraction [J2(E)]. (b) Numbers of root-knot nematode juveniles estimated by extraction [J2(E)] v. gall ratings on mature plants. (c) Numbers of root-knot nematode juveniles estimated using DNA [J2(D)] v. gall ratings on mature plants.

for galling. We suggest that if systematic nematode monitoring programs had been in operation on these properties, hazard indexes would have been more realistic and this problem would have been minimised.

It is apparent from the data presented in Table 1 that nematicide-treated sites were usually more heavily galled

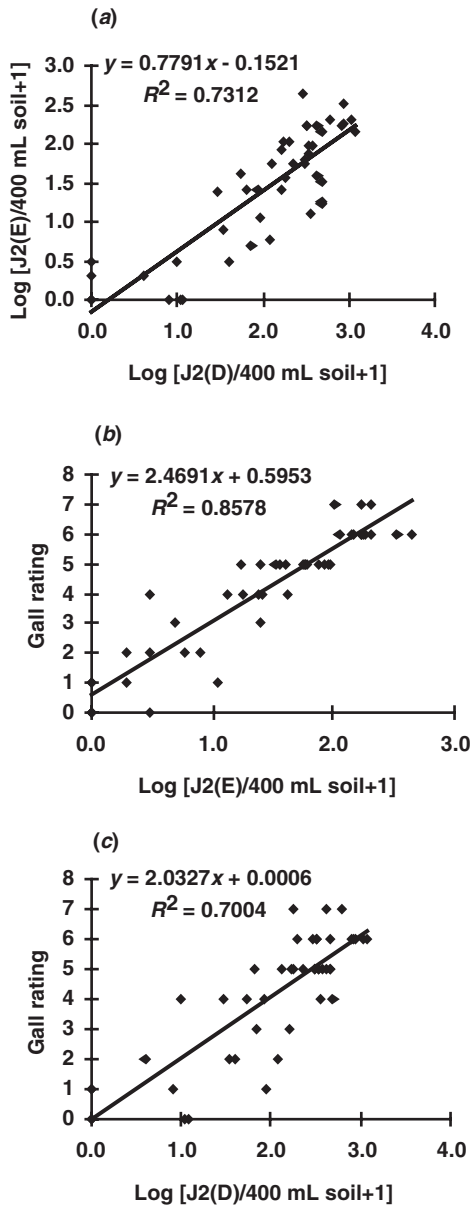


Fig. 3. Relationships between various parameters in a pot experiment with soil from Bundaberg. (a) Numbers of root-knot nematode juveniles estimated using DNA [J2(D)] v. numbers estimated by extraction [J2(E)]. (b) Numbers of root-knot nematode juveniles estimated by extraction [J2(E)] v. gall ratings on mature plants. (c) Numbers of root-knot nematode juveniles estimated using DNA [J2(D)] v. gall ratings on mature plants.

than untreated sites. This indicates that growers had identified these sites as high-risk situations, and justifies our decision to include previous use of nematicides and fumigants as a risk factor in the hazard index.

The survey results showed that root-knot nematode is widespread on tomatoes in Queensland, but that mature plants rarely have gall ratings greater than 5. Of the 114 fields included in this study, plants in the seven fields with

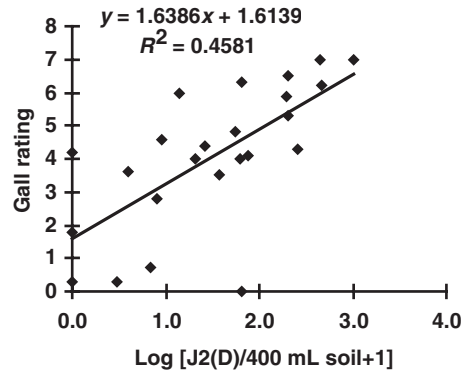


Fig. 4. Relationship between initial numbers of root-knot nematode juveniles estimated using DNA [J2(D)] and gall ratings of field-grown tomatoes at harvest.

the heaviest nematode infestation had only moderate levels of galling. Since previous studies in Queensland have shown that well-managed crops with heavily galled roots usually do not suffer yield losses from nematodes (Vawdrey and Stirling 1996; Stirling and Smith 1998), we conclude that under current management practices, root-knot nematode has minimal economic impact. From the point of view of prediction, this indicates that the hazard index used in this study may over-emphasise the level of risk.

The hazard index proved useful for predicting Fusarium wilt, as situations where disease incidence was greater than 3% were mainly restricted to sites with a hazard index greater than 35. However, there were some anomalies. For example, severe Fusarium wilt was observed in one field with a hazard index of only 31, probably because the grower was unaware of a previous disease infestation in the field. Low levels of Fusarium wilt were also observed unexpectedly in several fields with hazard indexes of less than 15. In one case, the field was new land that had never previously grown tomatoes. Since the disease was found only in association with stakes used for trellising, the pathogen was almost certainly introduced on soil adhering to the stakes. Such situations can never be predicted using a hazard index. The other cases involved relatively new land that had previously grown only one or 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. This situation highlights the fact that disease records need to be detailed and accurate if they are to be useful for predictive purposes.

Our hazard index encapsulates information that is readily available to growers, and could be used well before planting to identify fields where there is risk of loss from soilborne disease. However, disease severity in a short-term crop such as tomato is likely to be related to pathogen density at planting, so the next step is to assay the soil in medium- and high-risk situations to estimate pathogen density.

Relationships between crop yield and initial population density are well understood for nematodes (McSorley and Phillips 1993), but are not well developed for fungal pathogens, which cannot be readily quantified in soil by current techniques. We, therefore, assessed DNA methods and found that *F. oxysporum* f. sp. *lycopersici* could be detected in soil using a pathogen-specific DNA test. Observations in pots and in the field showed that inoculum density (estimated from the amount of *F. oxysporum* f. sp. *lycopersici* DNA in pre-plant samples) was related to disease incidence in the following tomato crop.

Our observations in pots and the field indicated that the incidence of Fusarium wilt was greater than 20% when the inoculum density was more than ~15 pg of *F. oxysporum* f. sp. *lycopersici* DNA/g soil. Since disease incidence of this magnitude is unacceptable to growers, the critical question from a practical perspective is whether the DNA test can provide useful information in fields with a much lower incidence of Fusarium wilt. We found that disease incidence was 10% (Table 4) and 5% (Fig. 1) in soils where pathogen-specific DNA concentrations were below the reliable detection limit for the test, demonstrating that unacceptably high levels of Fusarium wilt can occur in soils with very low inoculum densities. Thus the sensitivity of the DNA test will need to be improved if it is to reliably detect such infestations.

Standard nematode extraction techniques are routinely used to determine nematode population densities for diagnostic purposes (Stirling *et al.* 1999), and our results with root-knot nematode show that similar estimates are obtained using DNA. Furthermore, the DNA test was useful from a practical point of view, as it was sensitive enough to detect the nematode at population densities that resulted in gall ratings greater than 4 at harvest. Neither the DNA test nor the standard nematode extraction method was sensitive enough to always detect populations that resulted in very low gall ratings, but populations of this magnitude probably cause little economic damage.

In conclusion, we suggest that a two-step process in which risk is quantified using a hazard index and pre-plant inoculum density is estimated for key pathogens using a DNA test, is a potentially useful risk assessment tool for the tomato industry. However, our results indicate that reliable information on cropping history and previous disease incidence must be available if accurate predictions are to be made. Thus risk management services are likely to be successful only when pest management consultants are employed to monitor diseases, maintain records and collect soil samples. Deoxyribonucleic acid tests will eventually play a vital role in the provision of such services, because analytical procedures involving DNA can be automated and several pathogens quantified in a single soil sample. A broad-ranging suite of tests for nematode and fungal pathogens is already offered to the Australian cereal industry (Ophel-Keller *et al.*

1999) and it should eventually be possible to develop a similar package of tests for tomatoes. For this to be achieved, the sensitivity of the DNA test for *F. oxysporum* f. sp. *lycopersici* will need to be improved and tests for other key tomato pathogens will have to be developed.

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