Grower Summary

SF 122

Strawberry and raspberry: using soil nematode threshold levels to reduce direct feeding damage on roots and interactions with Verticillium wilt

Final 2013

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Project Title:	Strawberry and raspberry: using soil nematode threshold levels to reduce direct feeding damage on roots and interactions with Verticillium wilt
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Headlines

- High levels of the root lesion nematode *Pratylenchus thornei* have limited impact on growth of strawberry.
- Novel molecular tests allow accurate identification and rapid quantification of nematodes in soil.

Background and expected deliverables

Nematodes are important pests of strawberries and raspberries and can cause crop losses through direct feeding damage on roots, transmission of viruses and possibly increasing susceptibility to Verticillium wilt caused by *Verticillium dahliae*. Nematode problems in strawberry and raspberry are of increasing concern to growers, especially with tighter rotations. However, the relative occurrence of different nematode species in UK soil-grown soft fruit crops is unknown. An improved understanding of these pests is likely to become increasingly important particularly as the availability and use of soil disinfestation treatments for nematode control is decreasing.

There are a number of gaps in our understanding of free-living nematodes. Firstly, it is unclear how many root lesion nematodes (*Pratylenchus* spp.) are needed to cause direct feeding damage. Threshold levels used for assessing risk of direct damage to strawberry are based on anecdotal not experimental evidence. Secondly, there may be an interaction between the presence of *Pratylenchus* species and the incidence of Verticillium wilt. It is known that some Verticillium wilts (e.g. in *Acer* and potato) can be exacerbated by plant pathogenic nematodes, particularly of the genus *Pratylenchus*. Furthermore, despite the risk of serious losses from nematodes in soft fruit, expertise in their extraction, identification and evaluation is limited.

A quantitative molecular (QPCR) test has recently been developed (SF 97) for determination of *Verticillium dahliae* in soil which is able to detect and quantify inoculum of the pathogen within 24 hours with a high level of specificity. DNA extraction from soil to quantify *V. dahliae* offers prospects for rapid determination of any other pests and pathogens present, including nematodes, as the method extracts all DNA present. Recent advances in DNA techniques offer the potential to identify nematode species accurately and quickly without the need for taxonomic expertise.

This project aims to reduce losses in strawberry and raspberry caused by root nematodes through determination of threshold levels that cause direct damage and an increased

understanding of their interaction with *Verticillium dahliae* to cause Verticillium wilt in strawberry.

The specific project objectives were:

1. To determine the nematode species most commonly found associated with soil-grown strawberry and raspberry crops in the UK.
2. To confirm the soil threshold level for direct root damage to strawberry by a *Pratylenchus* species.
3. To determine whether nematode species present in a soil sample can be identified by testing the mass DNA extracted from soil samples when testing for *V. dahliae* by molecular quantification.
4. To determine whether increasing levels of a root lesion nematode species increases the risk of strawberry Verticillium wilt caused by *V. dahliae*.

Summary of the project and main conclusions

Objective 1: Occurrence of nematodes in UK soils used for soft fruit production

The relative frequency of different genera of nematodes found in 92 soil samples submitted by soft fruit growers to ADAS and Fera and processed by ADAS Pest Evaluation Services between 2001 and 2011 was examined. Strawberries were the most frequently sampled crop/prospective crop followed by raspberries; together they accounted for 91% of samples processed.

The most commonly recovered nematodes were stunt/spiral nematodes (e.g. *Tylenchorynchus* spp.) which were present in 98% of samples (Table 1); these are considered one of the least pathogenic groups. Root lesion nematodes (*Pratylenchus* spp.) which can potentially damage soft fruit were the next most common nematode group, being found in 86% of samples, followed by needle nematodes (*Longidorus* spp.) which were present in 58% of samples. Stubby root nematodes (*Trichodorus* spp. were found in 49% of samples and cyst juveniles (*Globodera/Heterodera* spp.) and dagger nematodes (*Xiphinema* spp) in 30% or less of samples.

The current threshold levels for individual nematode groups are shown in Table 2. The proportion of nematode counts above thresholds for individual groups gives an indication of the potential crop area likely to be treated with a nematicide. These data are presented in Table 3.

Table 1. Free-living nematodes recovered from 92 soil samples from fruit farms examined by ADAS 2001-2011: Numbers detected and range

Nematode group	Number/Litre		Proportion of samples with nematode present	Numbers of nematodes/Litre of soil comprising 90% of max-min range
	Min	Max		
Cyst juveniles	0	525	30	72
Dagger nematodes	0	395	23	12
Needle nematodes	0	2,835	58	330
Root lesion nematodes	0	3,025	86	671
Stubby root nematodes	0	3,475	49	221
Stunt/spiral nematodes	0	10,400	98	5309

Table 2. Anecdotal threshold levels for direct feeding damage and virus transmission to soft fruit crops from different nematode groups

Nematode group	Main genera	Threshold level (Number/L) for:	
		Direct damage	Virus transmission
Dagger	*Xiphinema* spp.	50	Any
Needle	*Longidorus* spp.	50	Any
Root lesion	*Pratylenchus* spp.	700	NA
Stubby root	*Trichodorus* spp.	200	NA
	Paratrichodorus spp.		
Stunt/spiral	*Tylenchorynchus* spp.	10,000	NA
	Helicotylenchus spp.		

NA – not applicable; these genera are not known to transmit viruses.

Table 3. Proportion of sites above threshold for different nematode groups in soil samples extracted for fruit/prospective fruit crops, 2001-2011

	% sites over threshold for:	
Nematode group	Direct feeding damage	Virus transmission
Dagger nematodes	5	15
Needle nematodes	29	54
Root lesion nematodes	10	NA
Stubby root nematodes	12	NA
Stunt/spiral nematodes	1	NA

NA – not applicable; these genera are not known to transmit viruses.

Above threshold counts of needle nematodes were more common than for all other nematode groups for both direct feeding damage and virus transmission. Almost 30% of samples had threshold counts for direct feeding damage. Needle nematodes were present in 54% of samples and are potentially the most important virus vector in soft fruit crops.

Soil samples were taken from four strawberry crops and four raspberry crops considered to be at high risk of nematode problems due to their cropping history. Although no symptoms in the growing crop attributable to nematode damage or nematode-transmitted virus were reported at the time of soil sampling, a total of eight and 17 plant parasitic species were identified from the strawberry and raspberry soils respectively. Numbers of nematodes in the strawberry soils were relatively low while those in the raspberry soils were slightly higher. There was a potential for direct feeding damage in one of the strawberry and all of the raspberry crops based on current threshold levels.

Objective 2: Soil threshold levels for direct damage

A range of populations of a root lesion nematode (*Pratylenchus thornei*) was created by soil dilution in both year 1 and year 2 of the project. This involved mixing soil infested with the nematode with the same soil which had been sterilised by oven drying at 60ºC for 45 minutes. A total of 50 target populations were created in 15 cm diameter plant pots. A single strawberry plant (cv. Elsanta) was planted in each pot and maintained in a polythene tunnel. After approximately four months the plants were harvested. Dry matter yield of the foliage, crown, roots and total plant dry weight was assessed. The population of root lesion nematodes in each pot at harvest was also determined.

No nematode species other than *Pratylenchus thornei* were detected in the soil. Soil dilution was effective at providing a range of populations. Despite actual populations being lower than the target population the nematode counts ranged from approximately zero to 1200 root lesion nematodes/L soil in year 1 and from zero to 775 root lesion nematodes/L soil in year 2. Nematode numbers in year 1 were both well below and above the anecdotal threshold of 700 root lesion nematodes/L soil and so provided a good range over which to assess their impact on strawberry growth. Although numbers were lower in year 2 the highest population was still greater than the anecdotal threshold.

Results suggested that populations of *P. thornei* as high as 1200/L soil have limited impact on strawberry growth. In Year 1 there was a slight negative relationship with root dry weight decreasing with increasing nematode numbers at harvest. This relationship was not apparent in year 2. Numbers of nematodes in the created populations were well in excess of the anecdotal threshold (700/L) at both the start and end of the experiment. This suggests

that the current thresholds may be too conservative and below the number of root lesion nematodes which can be tolerated by the crop.

If strawberries are more tolerant of nematodes than previously thought it will have a significant impact on nematicide use and potentially increase the profitability of the crop. However, it should be borne in mind that there are a range of species of root lesion nematodes which may not all exhibit the same degree of pathogenicity towards strawberries.

Objective 3: Identification of nematodes by molecular methods

DNA barcoding techniques were carried out at Fera to determine how well nematode species present in soil samples can be identified by testing the mass DNA extracted from soil samples. Large, moderately variable coding regions of conserved genes are considered useful for providing suitable resolution between taxa. The use of at least two of these barcoding genes are a good basis for a robust and reliable means of identifying free-living nematodes.

In Year 1, total DNA was extracted from 36 single-isolate nematode samples in water. Representative samples were chosen to evaluate the suitability of five candidate barcoding primer sets. Five isolates were initially barcoded. The resulting sequences were aligned using a database to give a 'best match' identification. All 'best match' identifications matched with the visual identification at either genus or species level.

In Year 2, the sequencing data obtained from the barcoding was used to develop specific assays for the detection and quantification for three pathogenic nematode species. Real-time PCR assays were developed to *Pratylenchus penetrans, Pratylenchus thornei,* and *Meloidogyne hapla*. All three free living nematode species tested using the new PCR assays were detected in at least some of the 32 soil samples tested supplied by soft fruit growers. *Meloidogyne hapla* was the most frequently detected, in 18 of the 32 soil samples. *Pratylenchus penetrans* and *P. thornei* were detected in 8 and 10 samples, respectively.

Table 4. Results from comparisons between visual identification and barcoding identification from DNA extracts using SSU sequence analysis.

DNA extract reference number	Visual identification	ADAS reference number	Barcoding closest similarity
2	*Rotylenchus buxophilus*	7965.002	*Rotylenchus* goodeyi
5	*Bitylenchus dubius*	8036.001	*Bitylenchus dubius*
14	*Paratylenchus* sp.	8013.001	*Paratylenchus dianthus*
16	*Paratylenchus* sp.	8013.001	*Paratylenchus dianthus*
36	*Pratylenchus thornei*	8013.001	*Pratylenchus thornei*

Objective 4: To determine whether increasing levels of a root lesion nematode species increases the risk of strawberry Verticillium wilt caused by V. dahliae

An experiment was designed in which pots of soil with four defined levels (nil, low, medium and high) of predominantly one *Pratylenchus* species and four levels of *Verticillium dahliae* (nil, low, medium and high) were prepared and planted with a strawberry variety susceptible to Verticillium wilt (cv. Elsanta). All combinations of the different *Pratylenchus* and *V. dahliae* levels were examined, resulting in 16 treatments. Nematode numbers at the start of the experiment were 0, 42, 517 and 875/L soil. This declined to 0, 42, 192 and 268/L soil after two months but numbers then remained relatively constant for the rest of the experiment. The nematode species was identified as *Pratylenchus thornei,* a species pathogenic to strawberry, raspberry, blackberry and other fruit crops.

Levels of *V. dahliae* at the start of the experiment as determined by qPCR and expressed as cfu/g equivalents were 0.3 (nil), 5.1 (low), 13.1 (medium) and 38.0 (high). Levels as measured by wet sieving followed by selective nutrient agar were 7, 33, 55 and 122 cfu/g. Levels showed little change between the start and end of the experiment and a clear distinction was maintained between the zero, low, medium and high infestations. It is difficult to directly compare the results from the qPCR test and the wet sieving method. However, the two methods did produce comparable relative values. *V. dahliae* was recorded in the zero infestation which was unexpected as a qPCR analysis of the original soil showed it to be negative for both *V. dahliae* and *V. albo-atrum*. In general, qPCR was less effective at detecting *V. dahliae* than wet sieving and culturing using selective nutrient agar.

Plants were examined after 3 and 5 months for occurrence of Verticillium wilt, after 5 months for effect on plant growth (dry matter) and after 10 months for survival over winter. There was no indication that increasing nematode numbers influenced susceptibility to Verticillium wilt. Although treatments showed differences in leaf browning, dry weight and vigour, no plants showed a sudden collapse, typical symptoms of the disease. Leaf browning, reduced vigour and reduced dry weight were taken as possible symptoms of Verticillium wilt. There was a trend for increased leaf browning with increasing levels of *V. dahliae* at the 3 month assessment. There was no consistent increase in leaf browning or reduction in dry weight and crop vigour with increasing nematode levels. It should be borne in mind that in general, 2012 was not a good year for Verticillium wilt development in strawberries (or raspberries). This was probably due to the cool wet summer which failed to stress plants and made them less susceptible to the fungal infection. Under more stressful conditions it is possible that feeding by increasing numbers of nematodes could have an impact on susceptibility to Verticillium wilt.

It was interesting that crop vigour in August, leaf number and number of live plants in September and root dry weight and total dry weight at harvest were highest in the presence of nematodes. This suggests a possible compensatory response from the crop to nematode feeding. However it is also possible that the heat sterilisation procedure used to kill any free-living nematodes had a detrimental impact on the nutrient availability of the soil and consequently the growth of the strawberry plants.

There was no evidence to suggest that *V. dahliae* affects the overwintering survival of strawberry plants. However, as even the lowest level fungal infection in this experiment would be expected to significantly affect the growth of strawberries (cv. Elsanta) it is possible that all levels of *V. dahliae* were equally damaging. Although overwintering survival of strawberries did differ between nematode infestation levels there was no clear trend to the data and this result should be treated with caution.

Financial benefits

No immediate financial benefits from this work have been identified. However, several potential financial benefits are possible given confirmation of our results and further test development:

a) Savings in nematicide use through adoption of a higher threshold level for *P. thornei*;

b) Increased accuracy in the identification of nematode species present in soft fruit soils through use of DNA barcoding techniques;

c) Use of a rapid pre-plant soil test for *P. penetrans* and *P. thornei* in samples where DNA has been extracted for *V. dahliae* determination;

d) Improved risk assessment for nematodes to develop more effective pest and disease control strategies based on barcoding methods and a new, higher threshold for *P. thornei.*

Action points for growers

- Growers should continue to sample land for free-living nematodes to assess the risk for those groups potentially damaging to fruit.
- Growers should adopt a new threshold limit of 1200 root lesion nematodes/L soil to replace the anecdotal threshold of 700 root lesion nematodes/L soil. This could result in potential savings on nematicide use.
- Growers should be aware that needle nematodes (*Longidorus* spp.) appear to be potentially the most damaging to soft fruit in view of the frequency and numbers at which they are recorded in soil samples.
- Growers should continue to use wet sieving followed by selective nutrient agar to detect the presence of *V. dahliae* until the precision of the qPCR analysis can be improved.