Grower Summary

SF 122

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

Annual 2012

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Project Number:	SF 122
Project Title:	Strawberry and raspberry: using soil nematode threshold levels to reduce direct feeding damage on roots and interactions with Verticillium wilt
Project Leader:	Tim O'Neil
Contractor:	ADAS UK Ltd
Industry Representative:	Laurie Adams, Heathlands farm
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Headline

- 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, whilst other early results suggest that reductions can be made on nematicide use, even though current thresholds are at best anecdotal.

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*, although cyst nematodes may also have a synergistic effect in potato wilt. 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 barcoding techniques (methods used for species identification based on the DNA sequence of conserved genes) offers 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 are:

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 the predominant *Pratylenchus* species as identified in Objective 1

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 the soil threshold level for the predominant nematode species which increases the risk of strawberry verticillium wilt caused by *V. dahliae*.

In year one of this project, Objectives 1-3 have been started. Objective 4 will not begin until year 2.

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 soils submitted by soft fruit growers to ADAS and Fera between 2001 and 2011 was examined. The site identification details were removed to retain the confidentiality of the grower submitting the sample. There were a total of 92 samples submitted to ADAS over this period.

In addition, four strawberry crops and four raspberry crops with a history of nematode problems, or sites considered at high risk (e.g. grown strawberries at least twice in last 10 years) were selected by ADAS Soft Fruit Consultants.

Of those soil samples from fruit crops submitted to ADAS Pest Evaluation Services, strawberries were the most frequently sampled crop/prospective crop followed by raspberries. Together these two crops 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.

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

Nematode group	Number/Litre Min	Number/Litre Max	Proportion of samples with nematode present	Numbers of nematodes/Litre of soil comprising 90% of max-min range
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	980	5,309

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

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

Table 3. Proportion of sites above threshold for different nematode groups for both direct feeding damage and virus transmission in soil samples extracted for fruit/prospective fruit crops, 2001-2011

Nematode group	% sites over threshold: Direct feeding damage	% sites over threshold: 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 root lesion nematodes was created by soil dilution. This involved mixing soil infested with nematodes with the same soil which had been sterilised by oven drying at 60ºC for 45 minutes. For example, to achieve a target nematode population of 1,000 root lesion nematodes/L of soil, 1 L of soil containing 2,000 stubby root nematodes/L soil was mixed with 1 L of sterile soil. A total of 50 target populations was 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.

Soil dilution was effective at providing a range of populations of root lesion nematodes. In general the actual population was approximately one third that of the target population. Despite actual populations being lower than the target population the nematode counts ranged from approximately zero to 1,200 root lesion nematodes/L soil. This is 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.

Results suggested that populations of root lesion nematodes as high as 1,200/L soil had limited impact on strawberry growth; there was a slight negative relationship suggesting that root dry weight decreased with increasing nematode numbers at harvest. There is no recognised threshold for root lesion nematodes in strawberries although anecdotal evidence suggests that 700/L soil may be damaging. Numbers of nematodes in the created populations were well in excess of this threshold at both the start and end of the experiment. This suggests that the current thresholds may be too conservative and below the number of 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. Recent advances in DNA barcoding techniques (methods used for species identification based on the DNA sequence of conserved genes) offers the potential to identify nematode species accurately and quickly without the need for taxonomic expertise. Large, moderately variable coding regions are considered useful for providing suitable resolution between taxa. The use of at least two of those barcoding genes are a good basis for a robust and reliable means of identifying free-living nematodes.

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 using the SSU gene. 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. The sequencing data obtained from the barcoding will be used to develop specific assays for the detection and quantification for up to five pathogenic nematode species in Year 2 of the project.

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*

Financial benefits

It is not possible to estimate the potential financial benefits from this work until the project is completed. Benefits may arise from:

a) Reduced losses from nematodes and verticillium wilt;

b) Improved risk assessment for nematodes to develop more effective pest and disease control strategies;

c) Increased accuracy in the identification of nematode species present in soft fruit soils;

d) Development of a rapid pre-plant soil test for nematodes in samples where DNA has been extracted for *V. dahliae* determination;

e) Increased understanding of interaction between nematodes and *V. dahliae* in the development of verticillium wilt in soft fruit;

f) Availability of new information to help improve rational decision-making on planting decisions.

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 continue to use current thresholds (even though they are at best anecdotal) as there are still potential savings to be made 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.