Project title:	Onions: improving risk assessment for free-living nematodes
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Industry Representative:	Nick Walker of PG Rix Ltd
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Pot experiments indicate that populations of needle, root lesion, stubby root and stunt/spiral nematodes well above current guideline thresholds have no effect on onion growth, suggesting that significant savings can be made on nematicide use.

Background

Free-living nematodes are important pests of onions but chemical control options are limited. Therefore it is becoming increasingly important to be able to determine where the crop can be grown without the risk of nematode damage.

The risk from free-living nematodes (FLN) can be assessed by considering field history, previous cropping and representative soil sampling. However, there is little information available on those free-living nematodes that are most damaging to onions and at what level they pose a risk. In addition, there is some confusion over when is best to sample for free-living nematodes due to their potential to move up and down the soil profile.

This project aims to improve risk assessment for free-living nematodes in onions by studying the following:

1. *Infestation levels:* The first and most fundamental component of risk assessment is to understand the nematode infestation level that justifies treatment. Current guideline thresholds for onions have little scientific basis and are based on anecdotal information. Work is required to develop robust thresholds quoted as either numbers per volume or weight of soil.
2. *Historical data from soil analysis:* ADAS Pest Evaluation Services (PES) have several thousand records of free-living nematode analyses between 2000 and 2010. These data will be interrogated to indicate the relative abundance of different nematode groups, their numbers, proportion of samples over threshold and any trends in nematode numbers over a 10 year period.
3. *Soil sampling:* There is a lack of confidence in soil sampling to predict risk from nematodes as they may move up and down the soil profile in response to soil moisture and temperature. This could affect estimates of numbers depending upon when samples are taken and to what depth. Work will determine the optimum soil moisture and temperature ranges at which to sample to get the best estimate of pest numbers. In addition the impact of soil cultivation on nematode numbers will be investigated.

4. *Alternative products*: The final aspect of the project will evaluate potential alternative products for control of free-living nematodes. Alternative chemical control options such as HDCI 036 are available but are not approved for this use on onions.

The specific objectives of this project are listed below:

1. To measure the effect of different populations of needle (*Longidorus* spp.), stubby root (*Trichodorus/Paratrichodorus* spp.), stunt/spiral (*Tylenchorynchus/ Helicotylenchus/Rotylenchus* spp.) and root lesion (*Pratylenchus* spp.) nematodes on the growth of onions, to determine which nematode species are potentially most damaging.
2. To analyse historical sampling data to provide background information on field populations of different free-living nematode groups.
3. To monitor the vertical distribution of nematodes in relation to soil moisture, temperature and before and after cultivation in order to recommend an optimum period and depth for soil sampling.
4. To undertake pot experiments to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

In future, protecting crops from free-living nematode damage will become increasingly reliant on integrated strategies that combine cultural and chemical control. Robust risk assessment will be fundamental to the success of such IPM programmes.

In year one of this project, objectives 1 and 3 were started and objective 2 completed. In year 2 objectives 1, 3 and 4 were completed.

Summary

Objective 1: Pot experiments to establish the most damaging nematode species to onions

A range of populations of needle, root lesion, stubby root, stunt/spiral and needle nematodes were created by soil dilution. The root lesion, stubby root and stunt/spiral nematodes were studied in year 1 and the needle nematodes in year 2. The nematode populations were created by mixing soil infested with nematodes with the same soil that has been sterilised. A total of 30 target populations was created for each nematode group (Table 1). The table also shows the current provisional threshold at which nematicide treatment is advised. These appear to be based on anecdotal evidence only and therefore are of limited value when interpreting the results of FLN analysis.

Table 1. Target nematode populations for pot experiments and provisional thresholds.

Nematode group	Provisional threshold (nematodes/litre soil)	Target population range (nematodes/litre soil)
Needle	50	0 – 1,305
Root lesion	2,500	0 – 3,350
Stubby root	200	0 – 6,902
Stunt/spiral	10,000	0 – 11,600

The target populations were made up in 1.5 L pots and sown with 20 onion seeds (Variety Vision). Pots were maintained in a polythene tunnel and watered as necessary.

Nematode numbers were assessed to determine how the actual populations compared with the target populations. To assess the impact of nematode populations on onion growth seedling emergence was monitored daily and onion dry matter yield measured.

Nematode counts showed that the actual populations were very close to the target population. However, there was no obvious effect of nematode population on onion growth and yield. This result suggests that current guideline thresholds for free-living nematodes are far too conservative and that the crop can tolerate much higher populations of these pests. Also, potentially nematicide used in onions can be significantly reduced which would greatly improve crop profitability.

The results for root lesion and stunt/spiral nematodes were supported by additional work undertaken using a different methodology which are reported in Appendix 1.

Objective 2: Analysis of historic sampling data

A total of 11,733 records for free-living nematode samples was extracted from the PES database for the period 31 October 2000 until 23 September 2010. Summaries were provided on the range of nematode groups and field populations in relation to existing guideline thresholds. The aim was to determine the most frequently recovered nematodes, their numbers, how these compared with the current guideline thresholds and whether there have been any obvious trends in nematode counts over the 10 year period.

The most frequently extracted nematodes between 2000 and 2010 were stunt/spiral nematodes followed by root lesion nematodes, stubby root nematodes, needle nematodes, cyst juveniles, dagger nematodes, stem nematodes and root knot nematodes. Both stem nematode and root knot nematode are both potential important pests of onions but were recovered from less than 1% of samples. There were no clear trends in nematode numbers between years and no indication that the risk from free-living nematodes is increasing or decreasing. Most nematodes were recovered least frequently in 2007 and most frequently in 2004. Stubby root nematodes were the main exception to these trends as they were least common in 2006 and most common in 2003.

The maximum nematode count was 33,975 stunt spiral nematodes/L soil. The next highest individual nematode counts were for stem nematode (20,325/L soil) and root knot nematodes (15,750/L soil). These nematodes were rarely recovered from soil as previously discussed but when present could be found in very high numbers.

The proportion of nematode counts above threshold for individual groups gives an indication of the potential crop area likely to be treated with a nematicide. There is some evidence to suggest that guideline thresholds for stubby root nematodes are too conservative at 200/L soil (Ellis, unpublished data) and that 1000/L soil is a more realistic figure. The impact of increasing the threshold for stubby root nematodes was also considered.

Stubby root nematodes are most likely to exceed current threshold levels. Between 2000 and 2010, 41.5% of samples contained numbers of stubby root nematodes above the 200/L threshold and so would have justified nematicide treatment. If the stubby root nematode threshold is increased to 1000/L soil, as suggested by previous work, then only 9.4% of samples would have exceeded this level. For other nematode groups for which there are guideline thresholds, nematicide treatment would have been justified in 14.1% of samples with needle nematodes, 3.5% of samples with root lesion nematodes and less than 1% of samples with dagger and stunt/spiral nematodes. Therefore, stubby root nematodes are the most important nematode group in determining nematicide use, particularly if the 200/L threshold is retained. If thresholds are far too conservative, as suggested by the pot experiments undertaken as part of objective 1, then there is considerable potential to reduce nematicide treatment in the onion crop.

Objective 3: Monitoring vertical distribution of free-living nematodes and numbers pre and post cultivation.

At three sites regular soil samples were taken at monthly intervals from small plots and the vertical distribution of nematode numbers related to detailed data on soil moisture and soil temperature. These samples were taken from September 2010 until August 2011. The three sites were as below:

1. West Dereham, Norfolk, (sandy loam), field Barshall I in sugar beet
2. Euximoor, Christchurch, Cambs. (silt) field Euximoor 4 in onions
3. Chatteris. (organic) Field Pickle Fen 1 in potatoes

A datalogger (Adcon soil moisture and temperature monitoring system) was used to measure soil temperature (ºC) and soil moisture (% water by volume) at a range of soil depths from 10 – 60 cm. A single probe was used to take measurements at each depth. A total of 10 cores was taken around a semi-circle of radius approximately 10 m centred on the datalogger. The data were used to investigate nematode migration patterns in relation to moisture and temperature in order to determine the optimum conditions for soil sampling to give the best estimate of nematode risk.

The impact of soil cultivation on nematode numbers (achieved by sampling pre- and post-sampling) was assessed at five sites.

1. Swaffham Prior 1, Cambridgeshire
2. Swaffham Prior 2, Cambridgeshire
3. Chatteris, Cambridgeshire
4. Upwell, Norfolk
5. Littleport, Cambridgeshire.

At all monitoring sites soil temperature varied between 1ºC and 16ºC with lowest temperatures in December 2010 and the highest in September 2010 or August 2011. In general, there was little difference in mean soil temperature between the six soil depths. Soil moisture tended to be more variable than temperature and varied between a minimum of approximately 15% and maximum of 35% moisture by volume. There was a maximum difference of approximately 10-15% moisture by volume between different soil depths.

In general results showed that numbers of all nematode groups recorded at the three sites decreased with increasing soil depth. In the majority of cases most nematodes were found at 0-20 cm.

There was a trend for a positive relationship between soil temperature and nematode numbers extracted. Numbers extracted tended to increase with increasing temperature within the range of 1-16ºC. In contrast to soil temperature data, there was generally a trend for a negative relationship between soil moisture and nematode numbers. Numbers decreased with increasing soil moisture within the range of 15-35% moisture by volume. Equations generated to describe the relationship between nematodes and soil temperature and moisture could not be relied upon to give an accurate indication of nematode numbers at a particular value of soil temperature or moisture.

In general, there was little effect of cultivation on nematode numbers.

Objective 4: Pot experiment to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

A pot experiment was set up to compare the efficacy of six novel products, three nematicide standards and an untreated control against free-living nematodes (Table 2).

Table 2. Products evaluated for control of nematodes and their effect on total numbers.

Treatment number	Product	Active ingredient	Rate/unit area	Total nematodes/L soil
1	Untreated control	-	-	396
2	Vydate 10G	Oxamyl 10% w/w	2000 g ai/ha	317
3	Vydate 10G	Oxamyl 10% w/w	4000 g ai/ha	554
4	HDCI 036	Confidential	30 kg/ha	138
5	HDCI 037	Confidential	1kg/5L water	325
6	HDCI 038	Confidential	2000 g ai/ha	342
7	Sesamin EC	Sesame oil 70% g/l	10 L/ha	496
8	Sesamin EC	Sesame oil 70% g/l	20 L/ha	412
9	Biofence meal	De-fatted mustard seed meal	300 g/m^2	304
10	Biofence granules	De-fatted mustard seed meal	300 g/m^2	396

Biofence is classified as a 100% vegetable organic fertiliser.

Soil was collected from a field which was shown to be infested with cyst juvenile nematodes, root lesion nematodes, stem nematode, stubby root nematodes and stunt/spiral nematodes. There were six replicates of each treatment (60 pots in total) and each pot was sown with 20 onion seeds (Variety Vision).

HDCI 036 was the most effective of the standard treatments tested.

Biofence, particularly the meal formulation was most effective of the new products at reducing nematode levels (particularly of root lesion nematodes) in soil but this must be balanced against the fact that it can double the time for seedling emergence. Further work is required to determine how to make best use of this product.

There was also limited evidence that HDCI 037 and HDCI 038 may warrant further evaluation.

Financial Benefits

Results suggest that guideline thresholds for free-living nematodes are far too conservative. If this is the case then growers can be much more confident that most land will not require a nematicide treatment unless it is infested with stem or root knot nematodes. This could mean significant savings of approximately £200/ha

Action Points

- Growers should continue to sample land for free-living nematodes but specifically to assess the risk for stem nematode or root knot nematode. These nematodes are only rarely recovered but can have a significant impact on the crop if present. With the exception of stem nematode and root knot nematode the majority of other free-living species appear to have limited effect on onion growth.
- Growers can have increased confidence that unless numbers of most free-living species are exceptionally high they will not require nematicide treatment. This could have a significant impact on gross margins but further work is required to confirm this.
- Soil sampling advice can be fine-tuned as a result of monitoring nematode numbers and soil moisture and temperature over one year at three soil depths. Most reliable counts are likely to be achieved by sampling at 0-15 cm and avoiding the coldest months and waterlogged soil. For root lesion and stunt/spiral nematodes samples could be taken pre- or post-cultivation.
- Soil sampling for stem nematode should follow the guidelines above until further information is available on how this nematode is distributed through the soil profile and how it reacts to changes in soil temperature and moisture and whether it is affected by soil cultivation.
- When sampling fields for stem nematodes (Project FV 327), individual soil samples (which can be bulked) should be taken from at least 100 uniformly distributed points per 4 ha to give the best chance of detecting the pest and to ensure that an acceptable measure of the average numbers of nematodes present is obtained.

SCIENCE SECTION

Introduction

Free-living nematodes are important pests of onions as they can reduce crop vigour and growth but there is only one available option for chemical control (SOLA 20061890 Vydate 10G (a.i. oxamyl) on bulb onions and shallots). In addition, Vydate 10G is already on the restricted list of certain retailers and its continued commercial acceptability is questionable. It will therefore become increasingly important to be able to determine in which fields onions can be grown without the risk of nematode damage.

The risk to onions from free-living nematodes is currently assessed by considering field history, previous cropping and representative soil sampling. Soil sampling is recommended by Assured Produce protocols but there is little information available on the species of free-living nematodes that are most damaging to onions and at what level they pose a risk. In addition, there is some confusion over when is best to sample for free-living nematodes due to their potential to move up and down the soil profile. With only one nematicide available for use on onions and pressure from retailers to reduce nematicide use, reliable soil sampling will become increasingly important for identifying fields at risk.

Components of risk assessment

This project studied four components of risk assessment as detailed below.

1. *Infestation levels*: The first and most fundamental component of risk assessment is to understand the nematode infestation level that justifies treatment. Currently, guideline thresholds for onions have little scientific basis and are based on anecdotal information. Work is required to develop robust thresholds for UK nematodes and soil types quoted as either numbers per volume or weight of soil.

2. *Historical data from soil analysis*: ADAS Pest Evaluation Services (PES) have several thousand records of free-living nematode analyses between 2000 and 2010 which can provide useful background information on likely field populations of a range of species. These data were interrogated to indicate the relative abundance of different nematode groups, their numbers, proportion of samples over threshold and any trends in nematode numbers over a 10 year period.

3. *Soil sampling*: There is a lack of confidence in soil sampling to predict risk from nematodes due to their potential to move up and down the soil profile in response to soil moisture and temperature. This could affect estimates of nematode numbers depending upon when samples are taken and to what depth. Work is therefore

required to determine the optimum soil moisture and temperature ranges at which to sample to get the best estimate of pest numbers. In addition the impact of soil cultivation on nematode numbers will be investigated.

4. *Alternative products*: The final aspect of the project was to evaluate potential alternative products for control of free-living nematodes. Alternative chemical control options such as HDCI 036 are available and potential biopesticides but not approved for use on onions but may be worthy of investigation.

Rationale for study

The Assured Produce protocol for onions strongly recommends that growers assess the risk of nematode damage by considering field history, previous cropping and representative soil sampling. A nematicide should only be used where 'fully justified'. Growers are supportive of soil sampling as a part of risk assessment but at present do not have the necessary information to be able to relate nematode numbers confidently to the potential risk of damage for the most important free-living species. In addition, they are not necessarily confident that soil sampling will predict damage in all cases.

This project aims to improve the precision with which growers and agronomists sample land for free-living nematodes and their confidence to interpret the results in order to decide on the suitability of the land for cropping with onions and need for nematicide treatment. Additional data will also be generated with which to compare potential alternative products for nematode control with Vydate. In future, protecting crops from free-living nematode damage will become increasingly reliant on integrated strategies that combine cultural and chemical control. Robust risk assessment will be fundamental to the success of such IPM programmes.

The specific objectives of this project are listed below:

1. To measure the effect of different populations of stubby root (*Trichodorus/Paratrichodorus* spp), needle (*Longidorus* spp), stunt/spiral (*Tylenchorynchus/Helicotylenchus/Rotylenchus* spp and root lesion (*Pratylenchus* spp) nematodes on the growth of onions, to determine which species are potentially most damaging.

2. To analyse historical sampling data to provide background information on field populations of different free-living nematode groups.

3. To monitor the vertical distribution of nematodes in relation to soil moisture, temperature, before and after cultivation in order to recommend an optimum period and depth for soil sampling.

4. To undertake pot experiments to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

Objectives 1 – 3 were started in year 1 and objective 4 in year 2. Objective 2 was completed in year 1 and objectives 1, 3 and 4 were completed in year 2. This report will concentrate principally on the completion of objectives 1, 3 and 4.

Materials and methods

Objective 1: Pot experiments to establish the most damaging nematode species to onions

This study on needle nematodes followed on from previous pot experiments on stubby root lesion, stubby root and stunt/spiral nematodes. Approximately 75 kg of field soil was collected from a site in Staffordshire known to be infested with needle nematodes. The soil was collected using a spade to sample to a depth of approximately 15 cm at a range of points across the field and contained in plastic dustbins. The bins were returned to the laboratory and sampled using a 15 cm deep x 2 cm diameter cheese corer. A total of 20 cores was taken from each bin and each sample extracted twice, once using the Seinhorst two flask technique (Seinhorst, 1955) to look for small to medium sized nematodes (root lesion, stubby root & stunt/spiral nematodes) and once using Flegg modified Cobb technique (Flegg, 1967) for large nematodes (needle & dagger nematodes).

A range of 30 nematode populations was created by taking a known volume of nematode infested soil and diluting this with a known volume of sterile soil. Populations were created in 15 cm diameter x 15 cm deep pots. Half of the soil collected for each nematode group was sterilised by autoclaving at 121ºC for 45 minutes. This was done in cotton bags in 5 kg batches. After autoclaving the soil was allowed to cool for at least 24 hours before using it to dilute the nematode infested soil.

As each pot contained approximately 1.5 L soil, the nematode populations were prepared in 2 L soil. This provided enough soil to fill the pot and sufficient spare to check the accuracy of the created population. For example, a target nematode population of 1000 stubby root nematodes/L soil can be prepared by mixing 1 L of soil containing 2000 stubby root nematodes/l soil with 1 L of sterile soil. The exact quantities of soil required to create the populations depended on the number of nematodes in the infested soil. The sterile and infested soil was mixed on a sheet of polythene. This was folded carefully from one side to another to ensure thorough mixing of the soil without damaging the nematodes. The mixed soil was carefully tipped into the pot until approximately 2.5 cm from the rim. The spare soil was retained and stored in a labelled polythene bag in a cold store at approximately 5ºC and was later extracted using Flegg modified Cobb technique to check the population of needle nematodes. The target population range and provisional threshold for needle nematodes in shown in Table 3. The provisional threshold is the level of nematode infestation at which nematicide treatment is advised.

Table 3. Target population ranges for needle nematodes to be achieved by soil dilution.

Nematode group	Provisional threshold (number nematodes/litre soil)	Target population range (number nematodes/litre soil)
Needle	50	0 – 1305

Once all the pots had been filled with soil they were labelled and sown with 20 onion seeds (cv. Vision) and covered to a depth of 1 cm with spare soil from the original mixture. Prior to sowing, the onion seed was subjected to a germination test to ensure it was viable. This was done by placing a batch of 100 onion seeds on to moist filter paper within a covered Petri dish. There were two replicate tests done. Petri dishes were maintained at room temperature within a dark cupboard. The number of germinated seeds was assessed daily and these seeds removed from the dish

Pots were maintained in a polythene tunnel and watered as necessary. The number of seedlings that emerged was assessed daily until there was no change over a period of five days. Once seedling germination was complete the plants were thinned to four per pot and these grown on to monitor whether there was any further impact of nematodes on growth. After approximately six months onion yield was assessed. Plants were harvested and the dry matter yield assessed for both the roots and tops (foliage + bulb) by oven drying at 80ºC for 16 hours. The pot soil was also extracted using the Flegg modified Cobb technique to compare the initial and final needle nematode population.

Regression analyses (in Genstat) were undertaken on three data sets to assess the impact of the actual nematode populations at the start of the experiment on onion growth as listed below:

1. 75 % onion seed emergence - The time taken for 75% of onion seedlings to emerge in each pot was determined and the relationship with actual nematode number investigated. If seedling emergence was inhibited by increasing nematode number then the time to 75% emergence might be expected to increase.
2. Area under the seedling emergence curve (AUC) – The area under the curve of seedling emergence against time was calculated and the relationship with actual

nematode number investigated. If increasing nematode number decreased seedling emergence then the area under the curve would be expected to decrease.

3. Dry matter onion yield – The relationship between mean onion yield per plant and actual nematode number was investigated.

Objective 3: Monitoring vertical distribution of free-living nematodes

At three sites regular soil samples were taken at monthly intervals from small plots and the vertical distribution of nematode numbers related to detailed data on soil moisture and soil temperature. These samples were taken from September 2010 until August 2011. The three sites were located as below:

1. G S Shropshire & Sons, Barshall Farm, West Dereham, Norfolk, (sandy loam), field Barshall I in sugar beet
2. Waldersey Farms Ltd, Well Fen Farm, Euximoor, Christchurch, Cambs. (silt) field Euximoor 4 in onions
3. R A Latta, Pickle Fen Farm, Somersham Road, Chatteris. (organic) Field Pickle Fen 1in potatoes

A standard soil sample was taken from each site in order to determine the free-living nematode species present. This involved taking 50 dibs with a 2.5 cm diameter cheese corer along an extended ‘W’ shaped path to provide approximately 1.5 kg of soil. The sample was extracted using the Seinhorst two-flask and Flegg modified Cobb techniques. These data were used to determine the extraction method for all subsequent samples.

A datalogger (Adcon soil moisture and temperature monitoring system) was used to measure soil temperature (ºC) and soil moisture (% water by volume) at a range of soil depths from 10 – 60 cm (Figure 1). A single probe was used to take measurements at each depth. A total of 10 cores was taken around a semi-circle of radius approximately 10 m centred on the datalogger. To ensure that cores were taken from approximately the same area on each sampling occasion (it was not possible to sample the same auger hole) a stake was located by the logger to which a 10 m length of cord was attached. This was used to describe a semi-circle of radius 10 m around which the 10 sample points were defined. At each point a single core was taken to a depth of 60 cm and this sub-divided into cores from 0-20 cm, 20-40 cm and 40-60 cm. Cores were taken with a Lishman hammerhead core auger 80cm long with a sampling depth of 60 cm, the core radius was 13 mm the corer was hammered into the ground and the sample removed from the marked depth rings separately. Each of these sub-divisions was considered to be a replicate and was bagged and labelled separately.

Figure 1. Data logger in situ at Christchurch, Cambridgeshire

Therefore around each datalogger on each sampling occasion a total of 30 samples was taken (3 depths x 10 sampling points).

Figure 2. Lishman hammerhead core auger used to sample soil at three soil depths.

The data were used to investigate nematode migration patterns in relation to moisture and temperature in order to determine the optimum conditions for soil sampling to give the best estimate of nematode risk.

The impact of soil cultivation on nematode numbers was assessed at five sites. The details for each of these sites is given below.

1. Swaffham Prior 1, Cambridgeshire Lords Ground Field 46. Grid ref: TL524677
2. Swaffham Prior 2, Cambridgeshire Lords Ground Field 47. Grid ref: TL528672
3. Chatteris, Cambridgeshire, West Fen Field. Grid ref: TL 400805
4. Upwell, Norfolk. Grid ref: TF 520014
5. Littleport, Cambridgeshire, Primrose Hill Field. Grid ref: TL 562893

Soil samples were taken pre- and post-cultivation in March 2011 at a range of soil depths as above. At each site an area was defined from which to take samples. This was located via GPS, canes and field margin markers. The defined area was used for both pre and post cultivation samples. Samples were taken in a similar manner to those taken around the dataloggers. A stake was used to describe a semi-circle of radius 10 m around which the 10 sample points were defined. Therefore cores taken pre- and post-cultivation could be taken from virtually the same spot. A total of 10 cores were taken from the defined area at both pre- and post-cultivation sampling.

Objective 4: Pot experiment to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

A pot experiment was set up to compare the efficacy of six novel products, three nematicide standards and an untreated control against free-living nematodes. The full treatment list is given in Table 4.

Table 4. Products evaluated for control of nematodes

Treatment number	Product	Active ingredient	Rate/unit area	Application method
1	Untreated control	-	-	-
2	Vydate 10G	Oxamyl 10% w/w	2000 g ai/ha	Granules
3	Vydate 10G	Oxamyl 10% w/w	4000 g ai/ha	Granules
4	HDCI 036	-	30 kg/ha	Granules
5	HDCI 037	-	1 kg/5L water	Drench
6	HDCI 038	-	2000 g ai/ha	Drench
7	Sesamin EC	Sesame oil 70% g/L	10 L/ha	Drench
8	Sesamin EC	Sesame oil 70% g/L	20 L/ha	Drench
9	Biofence meal	De-fatted mustard seed meal	300 g/m^2	Soil incorporation
10	Biofence granules	De-fatted mustard seed meal	300 g/m^2	Soil incorporation

Biofence is classified as a 100% vegetable organic fertiliser.

Nematode inoculum was prepared as follows. Approximately 80 kg of soil was collected from a field in North Yorkshire which was known to be infested by a wide range of free-living nematode species. On returning to the laboratory a sub-sample of the soil (approximately 1 kg) was taken with a cheese corer and subjected to both the Seinhorst two-flask extraction and the Flegg modified Cobb extraction to determine the baseline nematode population. The results of these extractions are shown in Table 5.

Table 5. Free living nematodes (number/L soil) recovered in bulk field sample.

Site	Nematode group (number/L soil)				
	Stubby root	Stunt/spiral	Cyst juveniles	Root lesion	Stem nematode
Field soil	200	1175	75	175	95

The numbers present gave a wide range of nematode species. In view of the importance of stem nematodes to onions the numbers of this species was supplemented by more individuals collected from infected plant material. The infected material was cut up and left in 1 L water overnight to allow the nematodes to escape. The plant material was then sieved off and the numbers of stem nematodes in the remaining solution counted by examination of three 1 ml samples in a Peter's counting slide. There were 94,000 nematodes per litre or 94 nematodes per ml. Therefore it was decided to apply 2 ml of nematode solution to each pot, this being equivalent to 188 stem nematode per pot.

The experiment was conducted in 15 cm diameter pots which were filled with approximately 1.5 L of soil. There were six replicates of each treatment. Biofence, Vydate 10G and HDCI 036 were added to the soil prior to sowing onion seed. The Biofence meal was applied to the surface of the soil. The soil was then watered until the water was just starting to escape from the base (field capacity). The pot was then covered with Clingfilm for 4 days. The Clingfilm was then removed and the pots left for a further four days before sowing onion seed. The Biofence pellets were added to a polythene bag with approx 1.5 L soil. This was then gently shaken to incorporate the pellets and the mix returned to the pot. The pot was then watered and covered with Clingfilm as with the Biofence meal. Pots were sown with 20 onion seeds (cv. Vision) on the fourth day after the Clingfilm was removed from the Biofence pots. All pots except those with Biofence were filled with soil until about 2.5 cm from the top of the pot. At this stage the Vydate 10G and HDCI 036 were applied. These were weighed out in pot doses and then pepper-potted over the soil surface at drilling depth and lightly incorporated. Once all the pots had been filled with soil they were labelled and sown with 20 onion seeds (cv. Vision). At this stage HDCI 038 was applied as a drench over the seed. All pots except the Biofence treatments were then covered to a depth of 1 cm with spare soil from the original field sample. HDCI 037 was then applied as a drench over the surface of the pots. In the case of the Biofence treatments the seeds were sown on the soil surface and then dibbed in to a depth of about 1 cm with a pencil. This was done so as not to disturb the fungal growth which had developed after application of Biofence. Prior to sowing the onion seed was subjected to a germination test to ensure it was viable.

Pots were arranged in a randomised block design in a polythene tunnel and watered as necessary. The number of seedlings that emerged was assessed daily until there was no change over a period of 5 days. Once seedling germination was complete the plants were thinned to four per pot and these grown on to monitor whether there was any further impact of nematodes on growth. After approximately 6 months onion yield was assessed. Plants were harvested and the dry matter yield assessed for both the roots and tops (foliage + bulb) by oven drying at 80^{o}C for 16 hours. The pot soil was also extracted using the Flegg modified Cobb technique to compare the initial and final needle nematode population.

Results

Objective 1: Pot experiments to establish the most damaging nematode species to onions

Germination test

The germination test of the onion seed (cv. Vision) showed it to be 84% viable. This was considered a sufficiently healthy seed lot with which to conduct the pot experiments.

Comparison of actual and target nematode numbers

Regression analysis was used to compare the target population of each nematode group achieved by soil dilution to the actual population as measured by the Flegg modified Cobb technique. The line was forced to pass through the origin as a target population of zero nematodes was guaranteed by sterilising the soil as previously discussed. The actual population was measured twice once immediately after the population was created and secondly at the end of the experiment. The equation of the regression line and the percentage variation accounted for is given in Table 6. If 100% of variation is accounted for this represents a perfect fit between target and actual nematode populations.

Table 6. Results of regression analyses to compare target and actual nematode populations (y = actual population, x = target population)

Nematode group	Regression line equation		Probability		% variation accounted for	
	At start	At end	At start	At end	At start	At end
Needle	y = 0.63x	y = 0.42x	<0.001	<0.001	90.5	24.3

Regression analyses showed a significant fit between actual and target nematode population for needle nematodes (P<0.001). At the start of the experiment actual populations were approximately 63% of target populations and there was a wide range of counts over which the impact on onion growth could be measured. The maximum needle nematode count was about 20 times greater than the current threshold. At the end of the experiment needle nematode numbers had declined but the range of actual numbers was still sufficiently wide to provide a good test of their impact on onion growth.

A graph of actual against target populations for needle nematodes is given in Figure 3.

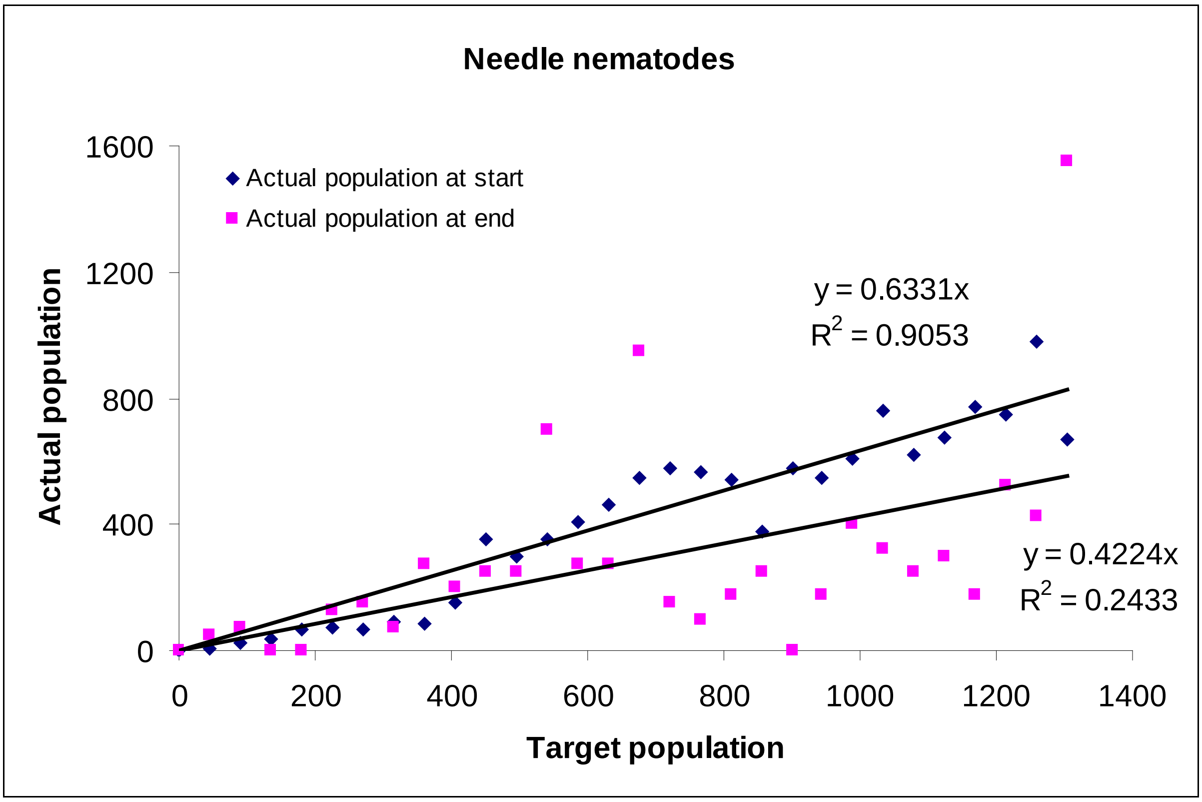


Figure 3. Actual needle nematode populations against target populations (number/litre soil)

In summary the soil dilution method produced a good range of needle nematode populations over which to evaluate the impact on onion growth.

Impact of nematodes on onion growth

Regression analyses (in Genstat) were undertaken on three data sets to assess the impact of the actual nematode populations at the start of the experiment on onion growth as listed below:

1. 75 % onion seed emergence - The time taken for 75% of onion seedlings to emerge in each pot was determined and the relationship with actual nematode number investigated. If seedling emergence was inhibited by increasing nematode number then the time to 75% emergence might be expected to increase.

2. Area under the seedling emergence curve (AUC) – The area under the curve of seedling emergence against time was calculated and the relationship with actual nematode number investigated. If increasing nematode number decreased seedling emergence then the area under the curve would be expected to decrease.
3. Dry matter onion yield. – The relationship between mean onion yield per plant and actual nematode number was investigated.

The results of analyses done using data on the time taken to 75% emergence of onion seedlings and its relationship with actual nematode numbers at the start of the experiment are summarised in Table 7.

Table 7. Results of regression analyses to investigate the relationship between 75% onion seed emergence and actual nematode populations at the start of the experiment (x = actual population, y = time to 75% emergence)

Nematode group	Regression line equation	Probability	% variation accounted for
Needle	y = -0.0349x + 17.58	0.003	24.4

Although the time to 75% emergence showed a trend to decrease slightly with increasing nematode numbers there was no clear relationship and the regression line only accounted for 24.4% of the variation. These data are shown in Figure 4.

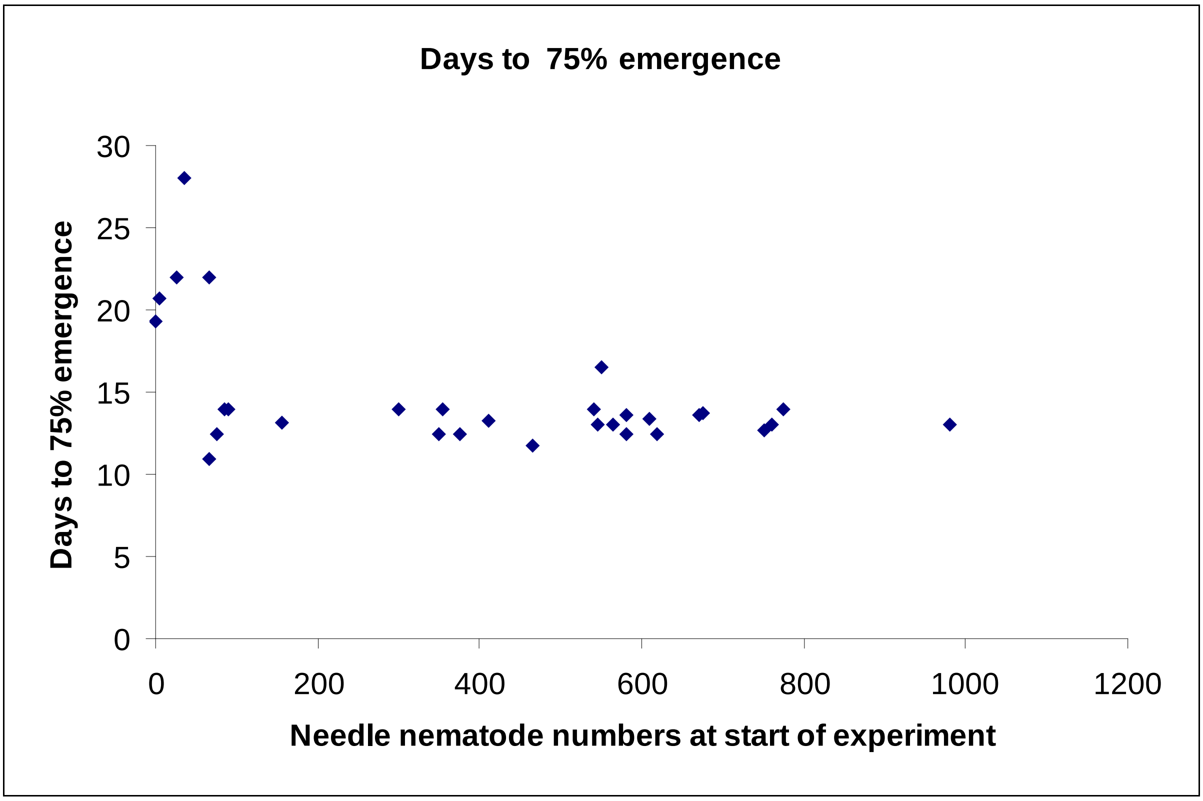


Figure 4. Relationship between time to 75% emergence and actual needle nematode population (number/litre soil) at the start of the experiment.

The results of an analysis done using data on the area under the curve of seedling emergence against time and its relationship with actual nematode numbers at the start of the experiment are summarised in Table 8.

Table 8. Results of regression analyses to investigate the relationship between the area under the seedling emergence against time curve and actual nematode populations at the start of the experiment (x = actual population, y = area under the seedling emergence against time curve)

Nematode group	Regression line equation	Probability	% variation accounted for
Needle	y = 0.38x + 385.4	0.021	14.7

Overall there was no clear relationship between the area under the seedling emergence against time curve and actual nematode population. These data are shown as graphs in Figure 5.

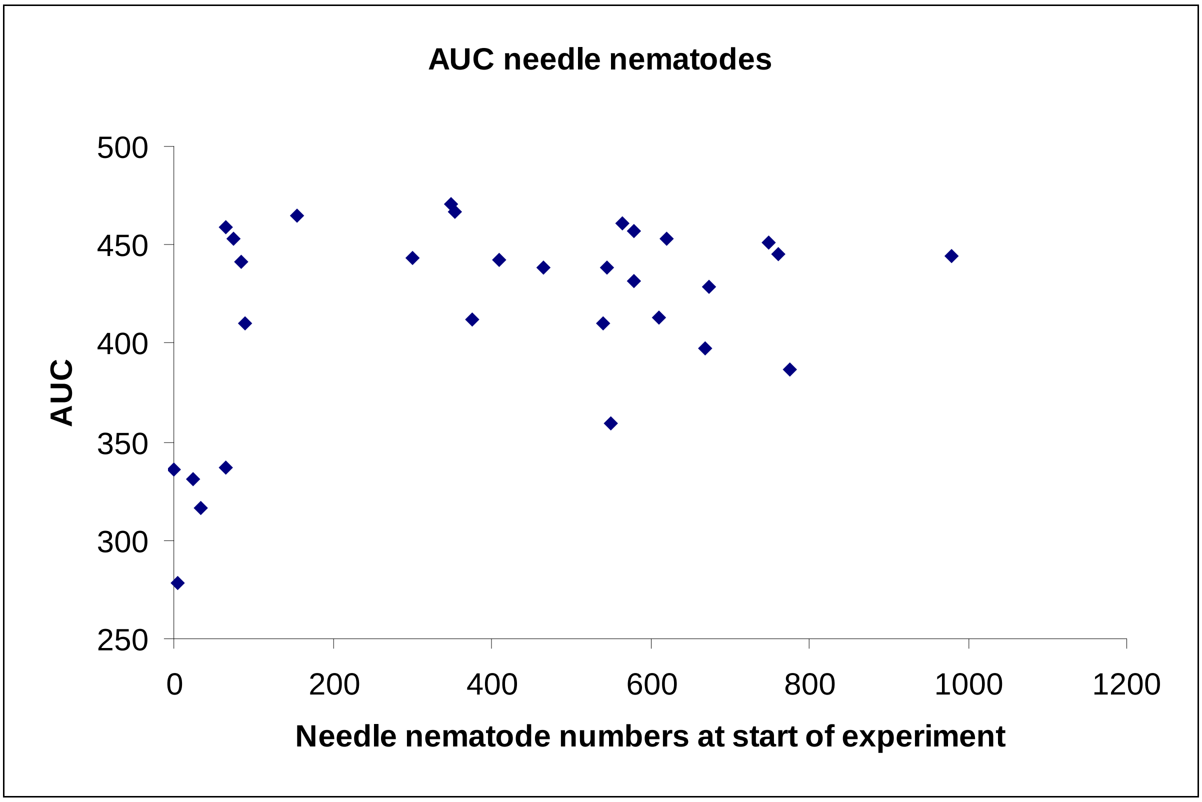


Figure 5. Relationship between area under the seedling emergence against time curve (AUC) and actual needle nematode population (number/litre soil) at the start of the experiment.

The results of an analysis done using data on mean onion dry matter yield/plant and its relationship with actual needle nematode numbers at the start of the experiment are summarised in Table 9.

Table 9. Results of regression analyses to investigate the relationship between onion dry matter yield and actual nematode populations at the start of the experiment (x = actual population, y = onion dry weight)

Nematode group	Regression line equation	Probability	% variation accounted for
Needle	y = -0.00056x + 0.1949	0.017	15.8

Although there was a trend for onion dry weight to decrease with increasing number of needle nematodes there was no clear relationship between onion dry matter yield and actual nematode population (Figure 6).

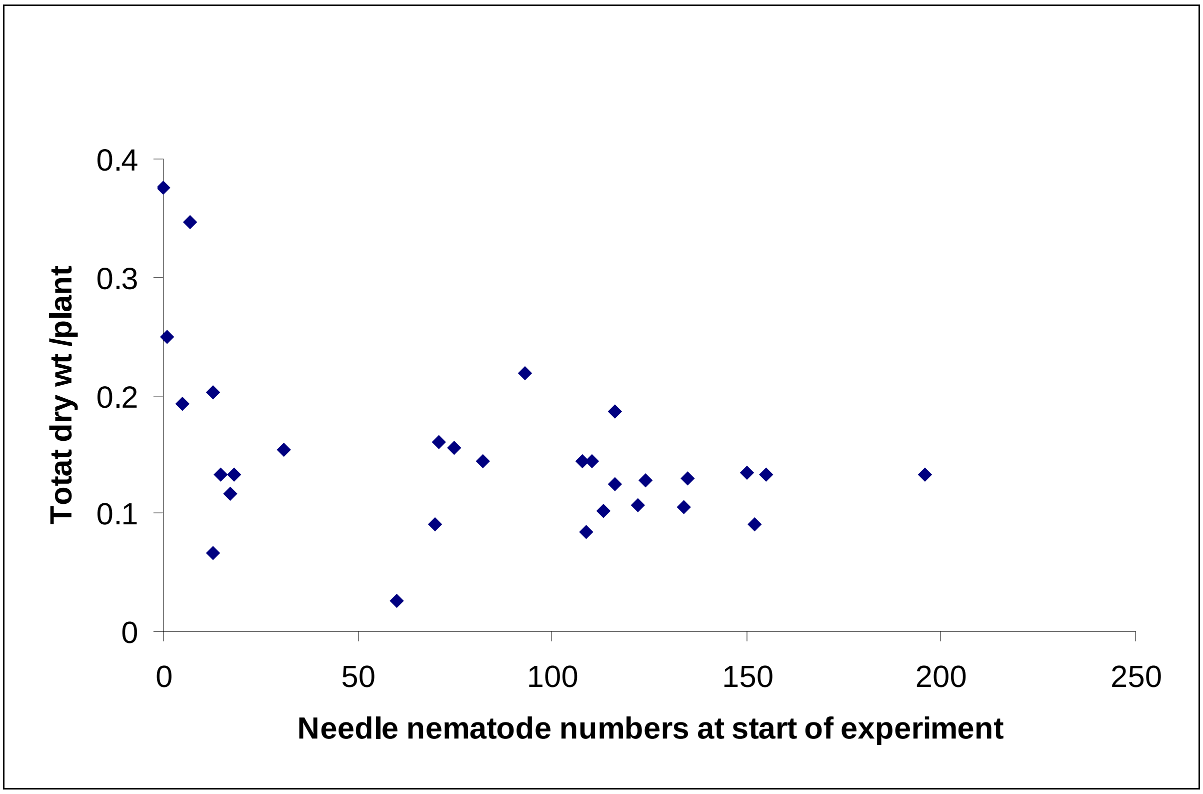


Figure 6. Relationship between onion dry weight and actual needle nematode population (number/litre soil) at the start of the experiment.

Objective 3: Monitoring vertical distribution of free-living nematodes

Monitoring the impact of soil temperature and moisture at a range of soil depths

The results of extractions of soil samples to determine the range of nematodes at each site at which data loggers were located are given in Table 10.

Stubby root nematodes were only recovered from West Dereham. Both stunt/spiral and root lesion nematodes were found at all sites and cyst juveniles at Christchurch and Chatteris. Overall the combination of sites provides a good range of the most commonly recorded nematode groups in UK soil.

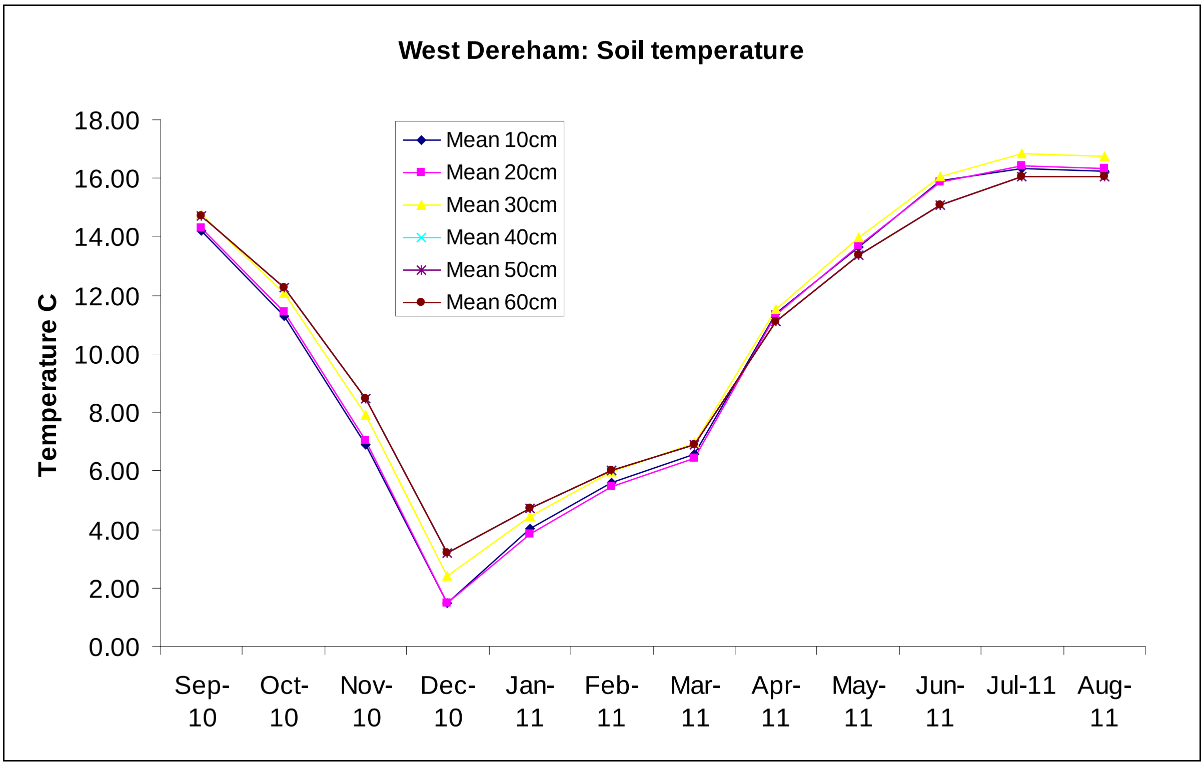
Table 10. Nematode groups and nematode numbers (number/litre soil) in soil at each data logger site in September 2010

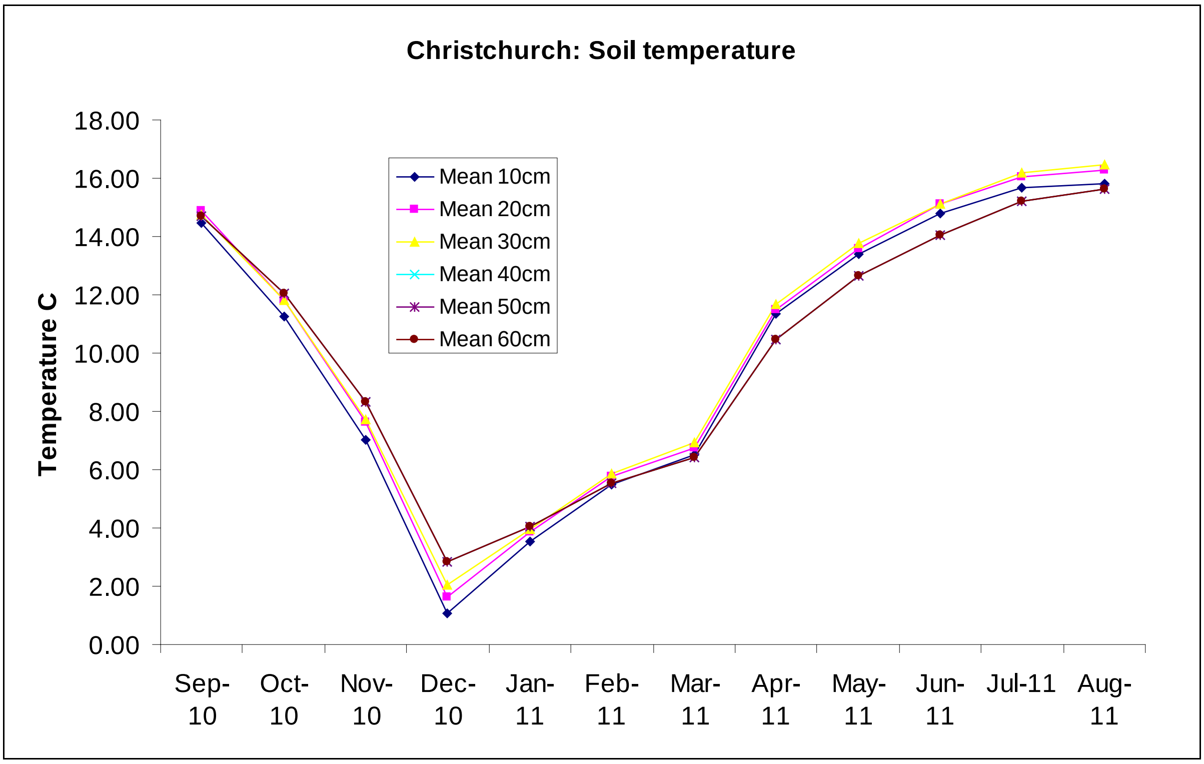
Site	Soil type	Nematode numbers (number/L soil)							
		Stubby root	Stunt/ spiral	Cyst juvenile	Root lesion	Needle	Dagger	Stem nematode	Root knot nematode
Waldersey Farms – Euximoor 4 Christchurch, Cambs	Silt	0	2350	50	1300	0	0	0	0
G A Shropshire – Barshalls 1 West Dercham, Norfolk	Sandy loam	925	1500	0	375	0	0	0	0
R A Latta – Pickle Fen 1 Chatteris, Cambs	Organic	0	400	3275	300	0	0	0	0

The data loggers were located at each site in August 2010 and the first monthly readings and soil samples started in September 2010. In total a year's data was collected and analysed to determine any relationship between nematode numbers and soil moisture and temperature at three soil depths, 0-20 cm, 20-40 cm and 40-60 cm.

Data on nematode numbers/l soil for different nematode groups at each of the three sites were subjected to an analysis of variance (ANOVA). This allowed comparison of numbers between the three soil depths for each month of monitoring from September 2010 until August 2011. In addition, regression analyses were used to investigate the relationship between nematode numbers at each depth and soil temperature and moisture. The percentage variation accounted for in the regression analyses was used as a measure of the goodness of fit of the regression line. At first the regression analysis was constrained by assuming that the slope of each line at each soil depth was identical. Subsequently further analyses were done in which the slope of each line was allowed to vary to determine if this improved the percentage of variation accounted for.

Summary figures of soil temperature and soil moisture are shown in Figures 7 and 8 respectively. Data on nematode numbers for each month are presented in Table 11 and Figure 9. The regression analyses are summarised in Table 12.


West Dereham: Soil temperature
Temperature C
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Mean 10cm
Mean 20cm
Mean 30cm
Mean 40cm
Mean 50cm
Mean 60cm
Sep-10
Oct-10
Nov-10
Dec-10
Jan-11
Feb-11
Mar-11
Apr-11
May-11
Jun-11
Jul-11
Aug-11


Christchurch: Soil temperature
Temperature C
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Mean 10cm
Mean 20cm
Mean 30cm
Mean 40cm
Mean 50cm
Mean 60cm
Sep-10
Oct-10
Nov-10
Dec-10
Jan-11
Feb-11
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May-11
Jun-11
Jul-11
Aug-11

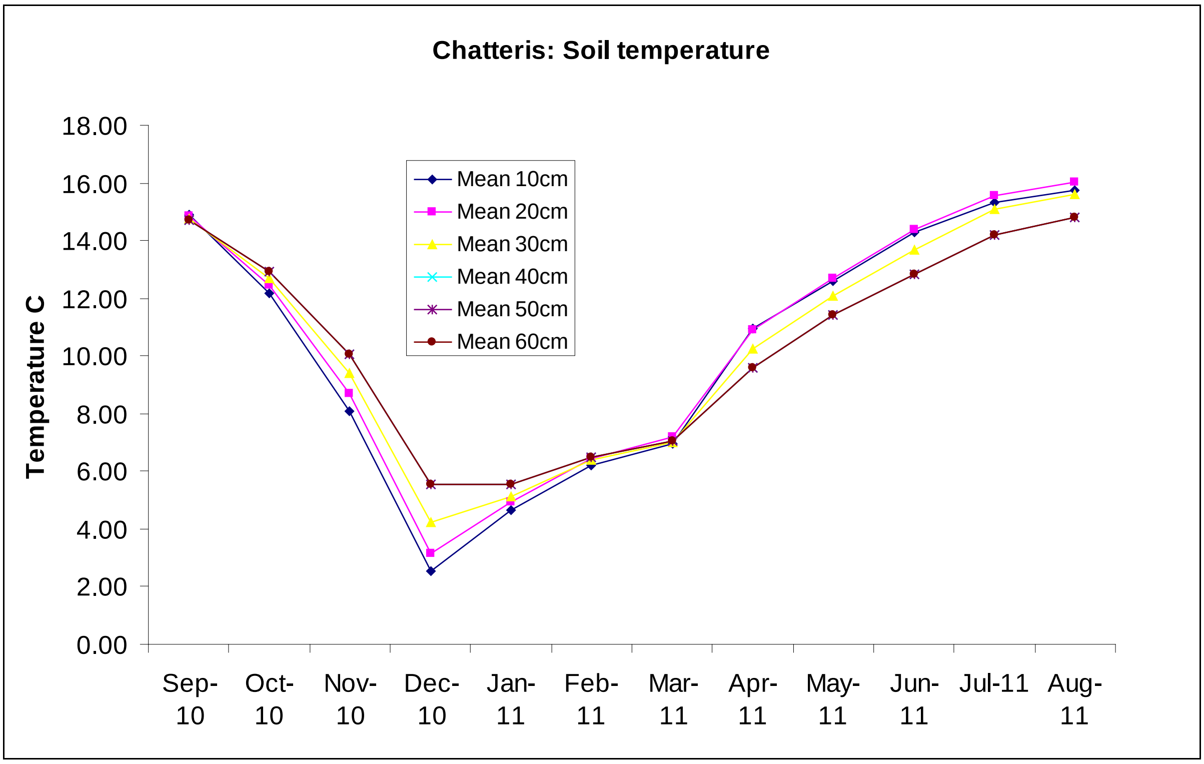
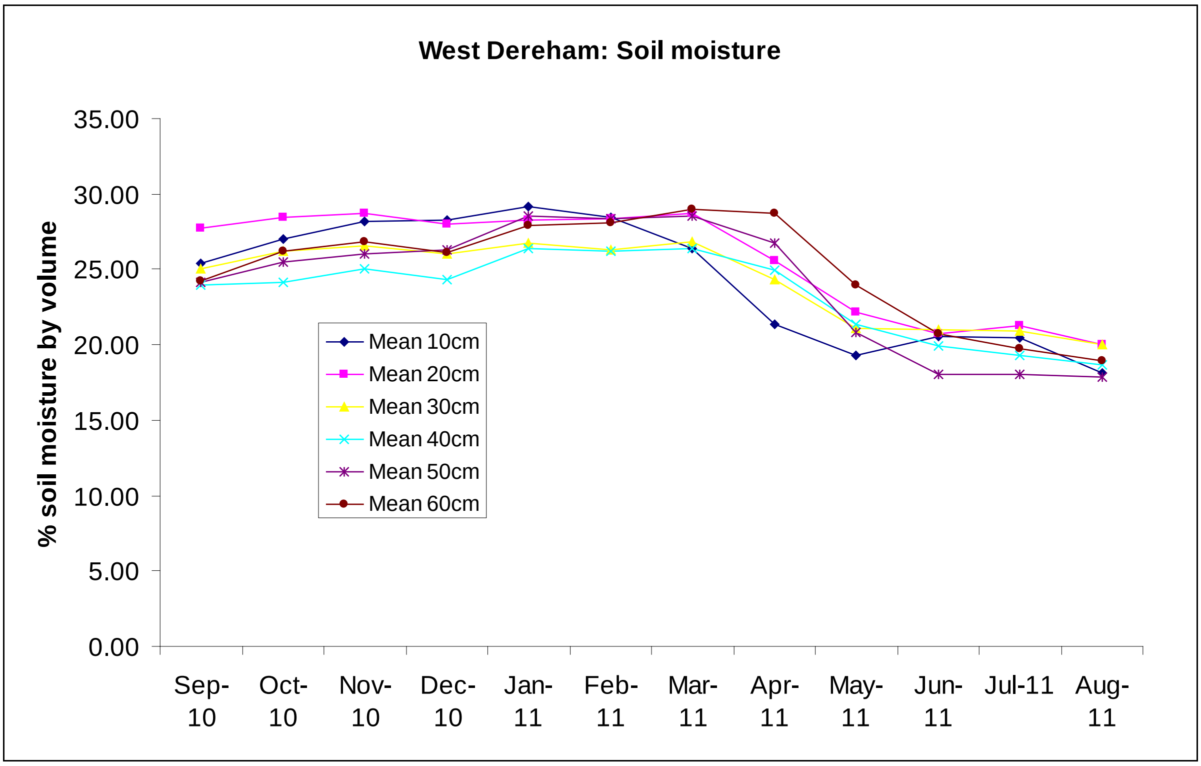
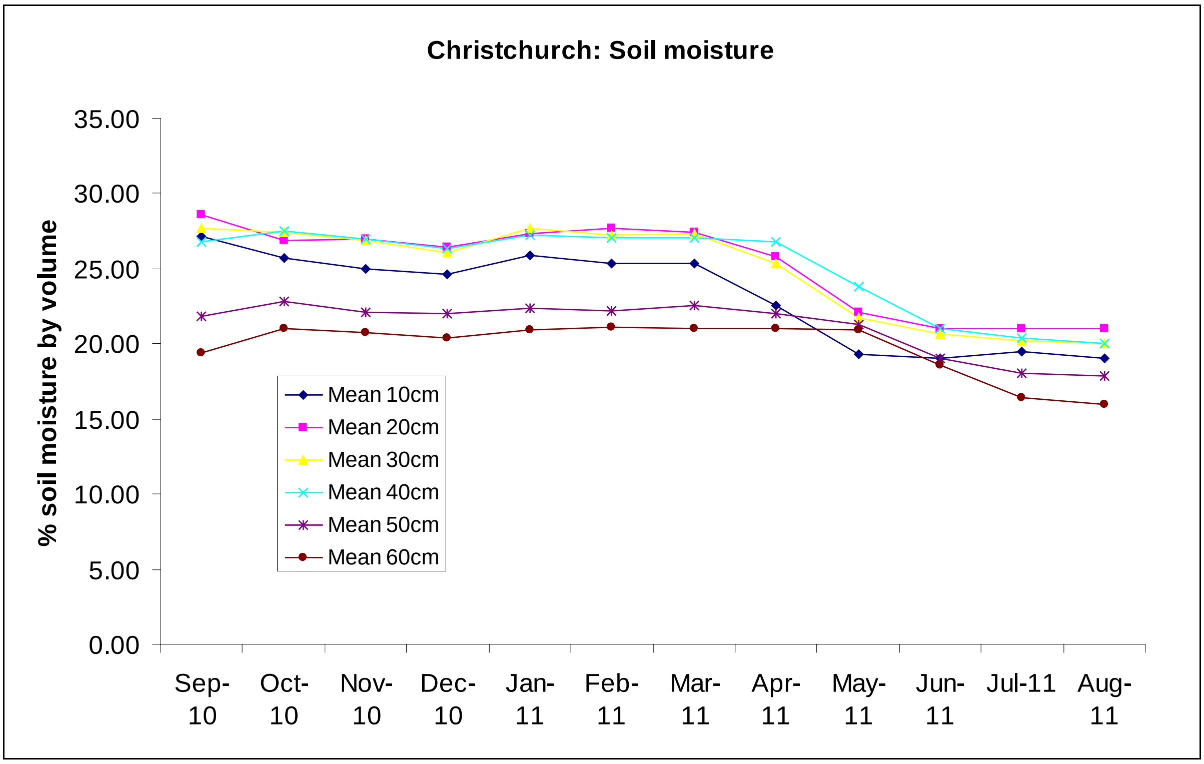


Figure 7. Mean soil temperature (ºC) at six soil depths (0-60 cm) at three monitoring sites from September 2010 – August 2011





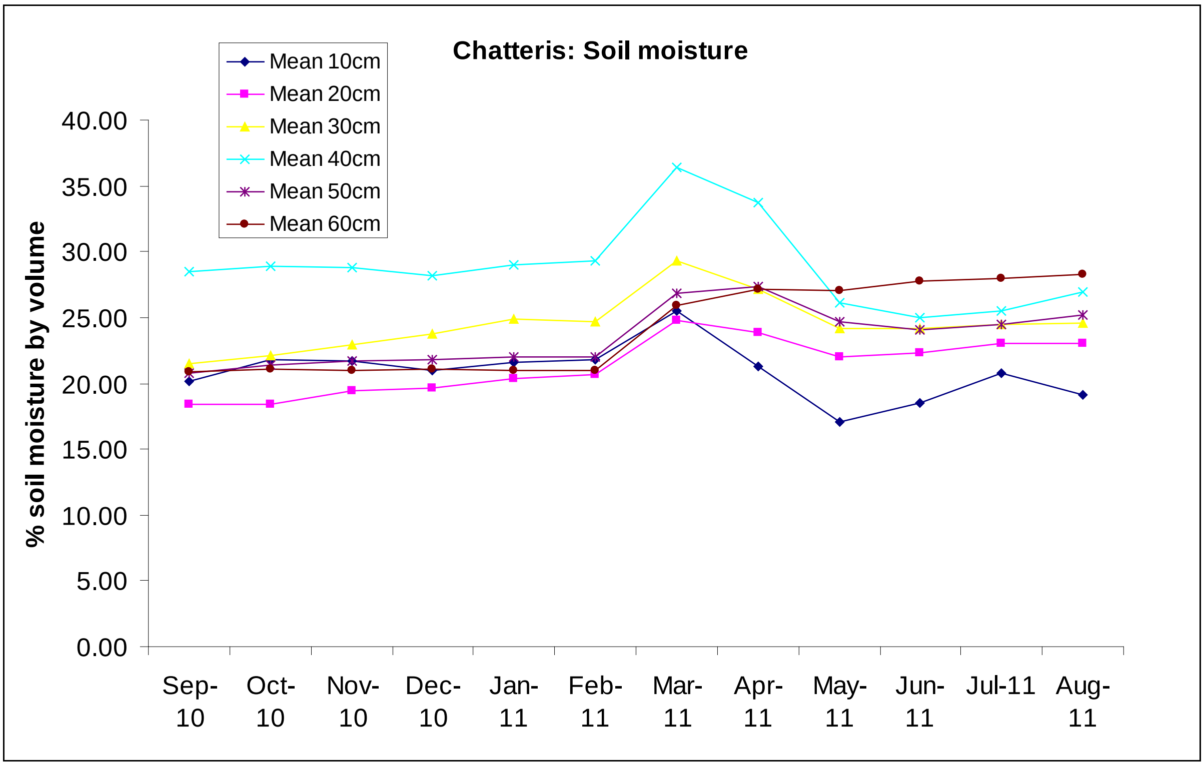


Figure 8. Mean soil moisture (% moisture by volume) at six soil depths (0-60 cm) at three monitoring sites from September 2010 – August 2011

At all monitoring sites soil temperature varied between 1ºC and 16ºC with lowest temperatures in December 2010 and the highest in September 2010 or August 2011 (Figure

7). In general, there was little difference in mean soil temperature between the six soil depths. Soil moisture tended to be more variable than temperature and varied between a minimum of approximately 15% and maximum of 35% moisture by volume (Figure 8). There was a maximum difference of approximately 10-15% moisture by volume between different soil depths. Data from West Dereham and Christchurch showed a similar pattern across the 12 month monitoring period. Soil moisture remained relatively constant within soil depths from September 2010 until March 2011 after which it tended to decline. At Chatteris, the organic site, soil moisture remained relatively constant throughout the year within soil depths with the exception of an increase of about 5% moisture by volume during March 2011.

At West Dereham there was a trend for root lesion nematode numbers to decrease with increasing soil depth from September until May. In some months there were significant differences in root lesion nematode numbers between soil depths (September, October, December, March and April). This was generally due to higher numbers at 0-20 cm than at 40-60 cm. From May onwards there was no significant difference in numbers between depths. Numbers of stubby root nematodes were greater at 0-20 cm than all other depths in all months except May. There were significant differences between soil depths in September and November to March (six months in total). During these months there were significantly more nematodes at 0-20 cm than at 40-60 cm and in November and January there were more at 20-40 cm than at 40-60 cm. From April until August there were no significant differences in nematode numbers between depths. Numbers of stunt/spiral nematodes differed significantly between soil depths in all months except April. Counts at 0-20 cm were always higher than at all other depths and significantly higher than at 40-60 cm in all months and statistically higher than at 20-40 cm in September to December, February, March, May, July and August. Numbers at 20-40 cm were never statistically higher than at 40-60 cm.

At Christchurch, numbers of root lesion nematodes differed significantly between soil depth in nine months (September-March and April and June). During these months there were always significantly more nematodes at 0-20 cm or 20-40 cm than at 40-60 cm. There was little difference in numbers between 0-20 cm and 20-40 cm. Numbers of stunt/spiral nematodes differed significantly between depths in all months. This is similar to West Dereham where there were differences in 11 out of 12 months. As at West Dereham numbers were always highest at 0-20 cm than at all other depths and also higher at 20-40 cm than at 40-60 cm. Significantly more nematodes were present at 0-20 cm than 40-60 cm in all months and at 20-40 cm than at 40-60 cm in all months except April, July and August. In April and August there were significantly more nematodes at 0-20 cm than at 20-40 cm.

At Chatteris, there were significantly different numbers of cyst juveniles between depths at seven out of 12 months (September-January, April and August). Most cyst juveniles were found at 0-20 cm in 10 out of 12 months and there were significantly more at 0-20 cm than at 40-60 cm from September until May and in August. There were also significantly more at 0-20 cm than at 20-40 cm from September to January and in April and July. Generally there was little significant difference in numbers between 20-40 cm and 40-60 cm. Root lesion nematode numbers differed significantly between soil depths in six of 12 months (September, November, December, February, April and August). This was mainly dues to higher numbers at 0-20 cm and 20-40 cm than at 40-60 cm in all these months. In seven out of 12 months (November-January and March to June) numbers of stunt/spiral nematodes differed significantly between depths. In all these months significantly more were found at 0-20 cm than at 40-60 cm. There were also significantly more nematodes at 0-20 cm than 20-40 cm in November March and April and significantly more at 20-40 cm than at 40-60 cm in December. As at Barshall and Euximoor numbers were generally highest at 0-20 cm.

In general, analyses of variance showed that numbers of all nematode groups recorded at the three sites decreased with increasing soil depth. In the majority of cases least nematodes were found at 40-60 cm. Overall this effect appeared to be most marked for stunt/spiral nematodes which were usually most numerous at 0-20 cm than at any other depths. Root lesion nematodes were often found deeper in the soil profile and were likely to be as numerous at 20-40 cm as at 0-20 cm. Stubby root nematodes were only found at West Dereham and from September until March tended to be found in lower numbers as soil depth increased. However, between April and August there was much less distinction between the numbers found at the three depths. Cyst juveniles were only found at Chatteris and for most months were most common at 0-20 cm. Only in May and June were most found at 20-40 cm.

Regression analyses (Table 12) generally showed that there was a trend for a positive relationship between soil temperature and nematode numbers. Numbers tended to increase with increasing temperature within the range of 1-16^{O}C. The only exception to this was at Chatteris where numbers of root lesion nematodes decreased with increasing temperature. In contrast to soil temperature data, there was generally a trend for a negative relationship between soil moisture and nematode numbers. Numbers decreased with increasing soil moisture within the range of 15-35% moisture by volume. Again root lesion nematodes at Chatteris were the only exception where numbers increased with increasing soil moisture. Overall regression analysis was unable to account for more than 50% of the variation in nematode numbers suggesting that other factors were impacting on their behaviour.

Consequently the regression equations could not be relied upon to give an accurate indication of nematode numbers at a particular value of soil temperature or moisture.

Table 11. Mean numbers of nematodes at 0-20, 21-40 and 41-60 cm deep at three monitoring sites over 12 months between October 2010 and September 2011.

Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
West Dereham	September 2010	Root lesion	384	87	33	<0.05	136.4
	October 2010		47	27	0	0.01	14.2
	November 2010		139	102	13	0.180	68.1
	December 2010		78	32	7	<0.001	16.0
	January 2011		39	35	19	0.532	18.0
	February 2011		80	64	11	0.424	54.7
	March 2011		206	106	60	<0.05	55.3
	April 2011		55	73	10	<0.05	24.1
	May 2011		177	114	62	0.487	94.4
	June 2011		0	50	10	0.105	23.9
	July 2011		30	73	40	0.450	35.4
	August 2011		237	362	149	0.134	103.2
	September 2010	Stubby root	1104	156	68	<0.001	265.3
	October 2010		509	402	124	0.089	172.6
	November 2010		681	458	177	<0.01	124.0
	December 2010		272	111	85	<0.001	43.0
	January 2011		399	346	112	<0.05	95.5

Table 11. (cont'd)

Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
	February 2011		547	244	171	<0.001	86.6
	March 2011		1007	345	420	<0.05	235.8
	April 2011		828	669	608	0.764	308.8
	May 2011		517	778	621	0.546	236.4
	June 2011		544	537	470	0.833	134.9
	July 2011		492	325	652	0.180	170.9
	August 2011		899	745	341	0.083	246.5
	September 2010	Stunt/spiral	752	20	0	<0.001	171.5
	October 2010		128	25	20	<0.05	43.2
	November 2010		232	50	11	<0.001	39.8
	December 2010		126	32	2	<0.001	17.4
	January 2011		149	77	10	<0.01	37.0
	February 2011		167	44	16	<0.001	38.0
	March 2011		944	147	74	<0.05	307.7
	April 2011		110	107	0	0.074	52.4
	May 2011		517	147	7	<0.05	184.4
	June 2011		311	140	20	<0.05	109.2
	July 2011		857	412	45	0.001	193.5
	August 2011		1543	298	65	<0.01	396.2

Table 11. (cont'd)

Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
Christchurch	September 2011	Root lesion	1290	1041	27	<0.001	293.3
	October 2010		794	1286	296	<0.05	342.1
	November 2010		2695	2315	340	<0.001	583.8
	December 2010		715	859	69	<0.001	154.8
	January 2011		396	389	65	<0.001	80.1
	February 2011		239	418	74	<0.001	60.9
	March 2011		843	1345	98	<0.01	352.0
	April 2011		370	372	120	0.01	87.3
	May 2011		107	335	120	0.120	119.5
	June 2011		774	1662	283	<0.01	393.4
	July 2011		1377	7320	4673	0.359	4080.1
	August 2011		2595	4143	1543	0.212	1442.4
	September 2010	Stunt spiral	1704	1613	68	<0.01	530.7
	October 2010		1235	1270	125	<0.05	466.4
	November 2010		3655	1675	180	<0.001	690.8
	December 2010		1052	783	53	<0.01	298.9
	January 2011		656	455	34	<0.001	97.3
	February 2011		553	352	35	<0.001	92.2
	March 2011		1374	652	33	<0.001	289.4

Table 11. (cont'd)

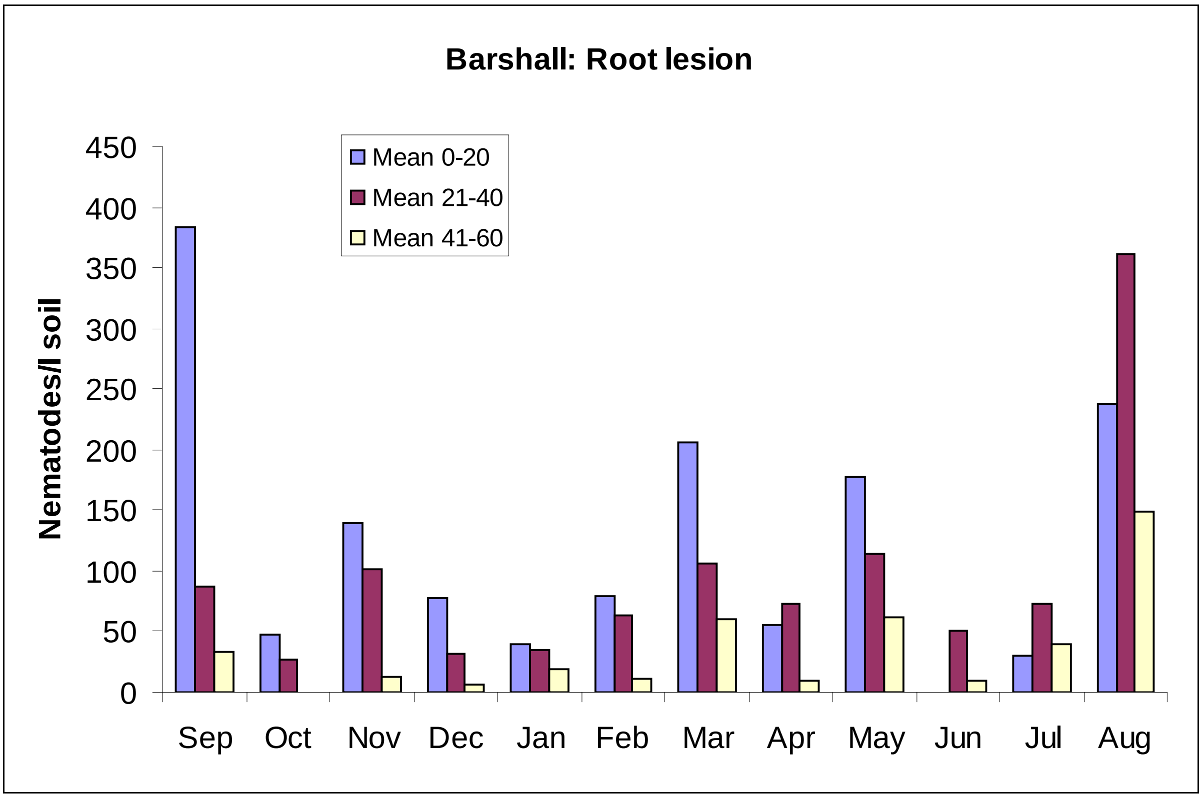
Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
	April 2011		2210	522	148	<0.001	344.3
	May 2011		1092	1063	182	<0.05	355.0
	June 2011		2784	2404	232	<0.001	574.2
	July 2011		4390	2377	1570	<0.05	897.5
	August 2011		9178	1700	970	<0.001	1629.3
Chatteris	September 2010	Cyst juveniles	653	364	17	<0.001	139.1
	October 2010		1739	659	17	<0.01	460.7
	November 2010		337	71	30	<0.01	88.7
	December 2010		430	29	0	<0.05	164.0
	January 2011		98	20	0	<0.01	30.3
	February 2011		67	23	0	0.085	29.1
	March 2011		351	189	20	0.146	162.5
	April 2011		813	206	7	<0.001	135.4
	May 2011		1016	1182	153	0.059	440.5
	June 2011		2408	3130	468	0.337	1829.4
	July 2011		2825	582	364	0.064	1102.3
	August 2011		1978	593	10	<0.05	774.4
	September 2011	Root lesion	636	787	111	<0.05	236.0

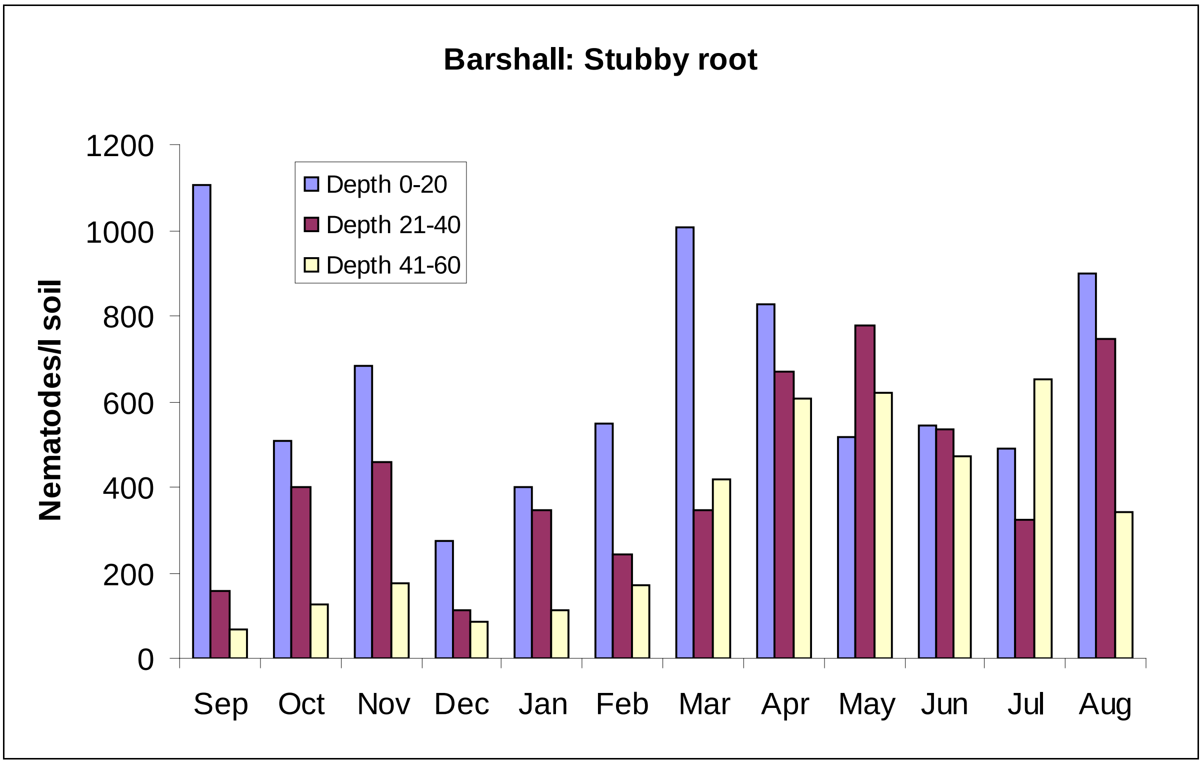
Table 11. (cont'd)

Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
	October 2010		587	621	236	0.139	206.5
	November 2010		1753	1312	357	<0.01	410.4
	December 2010		1380	1255	73	<0.01	359.0
	January 2011		573	757	183	0.145	287.4
	February 2011		640	609	70	<0.01	166.0
	March 2011		1245	1246	407	0.114	445.7
	April 2011		1130	1137	284	<0.05	327.3
	May 2011		1250	409	1213	0.262	567.0
	June 2011		507	570	631	0.883	247.6
	July 2011		810	1195	199	0.067	410.3
	August 2011		908	900	51	<0.01	277.5
	September 2010	Stunt/spiral	36	27	12	0.401	17.6
	October 2010		118	59	40	0.314	52.5
	November 2010		262	52	0	<0.001	42.5
	December 2010		238	256	19	<0.05	91.6
	January 2011		160	73	26	<0.05	45.2
	February 2011		66	49	5	0.106	28.5
	March 2011		96	13	0	<0.001	21.3
	April 2011		210	23	7	<0.001	47.8

Table 11. (cont'd)

Site	Month	Nematode group	Soil depth (cm)			Probability (P)	SED (27 DF)
			0-20	21-40	41-60		
	May 2011		374	197	90	<0.05	106.6
	June 2011		318	60	10	<0.01	86.8
	July 2011		190	61	35	0.098	73.8
	August 2011		453	167	9	0.057	178.3


Barshall: Root lesion
Mean 0-20
Mean 21-40
Mean 41-60
Nematodes/l soil
450
400
350
300
250
200
150
100
50
0
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug


Barshall: Stubby root
Depth 0-20
Depth 21-40
Depth 41-60
Nematodes/l soil
1200
1000
800
600
400
200
0
Sep
Oct
Nov
Dec
Jan
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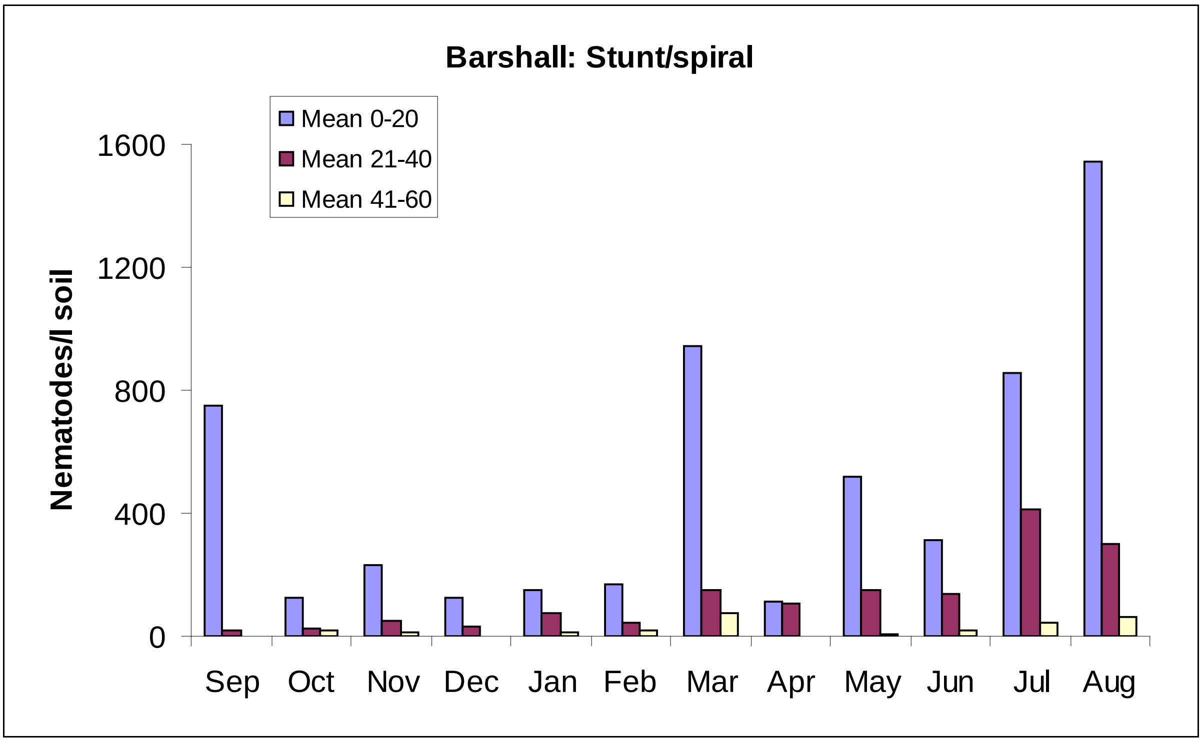
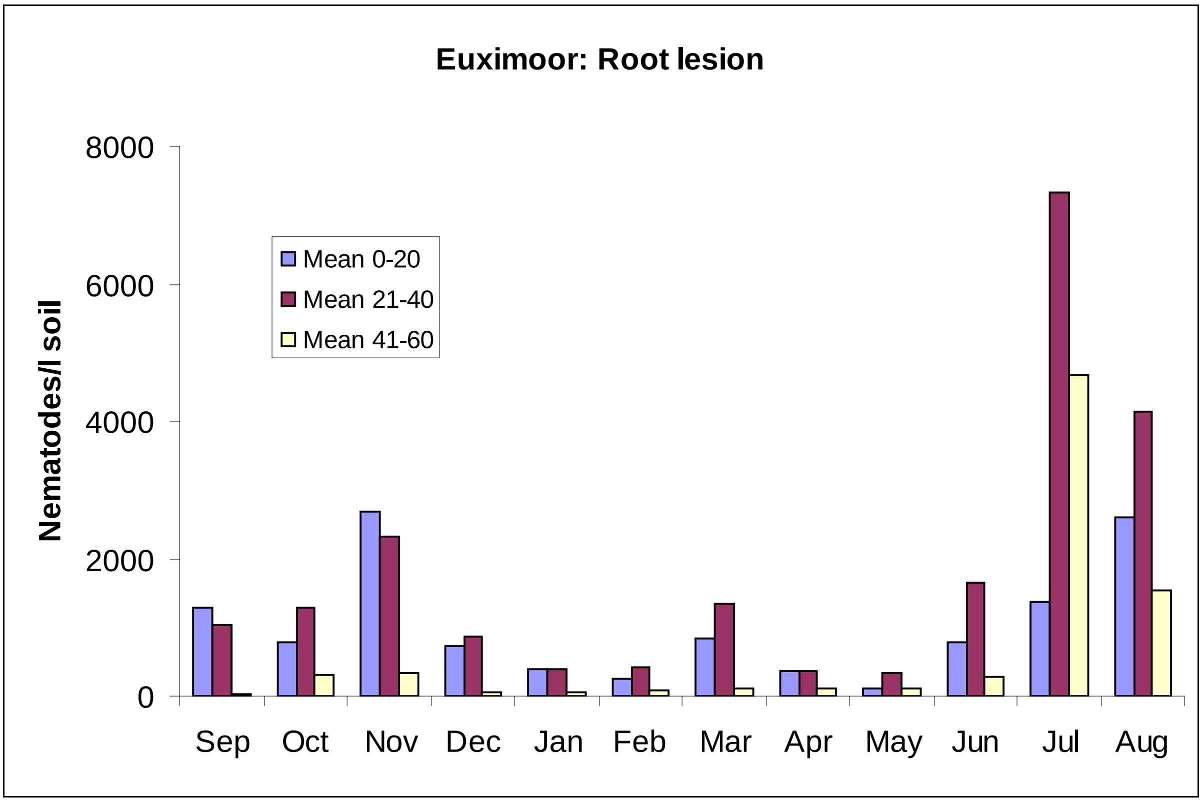
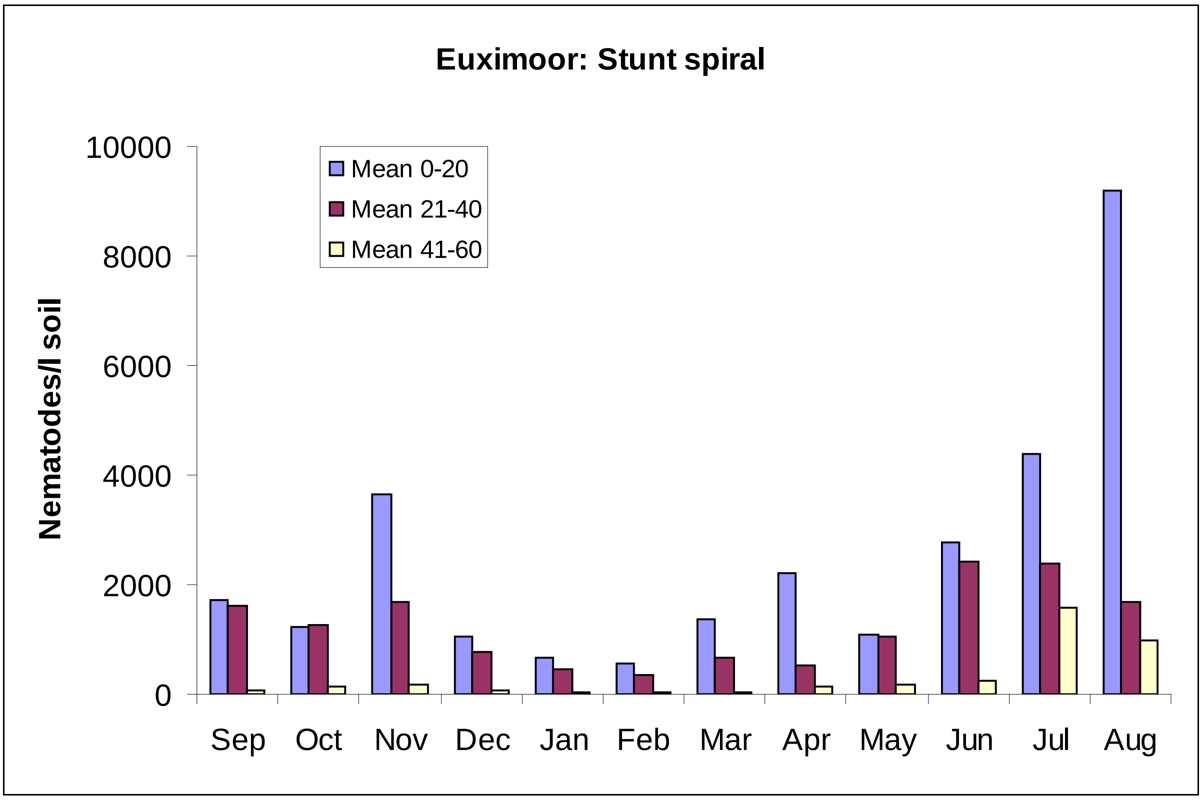

Barshall: Stunt/spiral
Mean 0-20
Mean 21-40
Mean 41-60
Nematodes/l soil
1600
1200
800
400
0
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug


Euximoor: Root lesion
Mean 0-20
Mean 21-40
Mean 41-60
Nematodes/l soil
8000
6000
4000
2000
0
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug


Euximoor: Stunt spiral
Nematodes/l soil
10000
8000
6000
4000
2000
0
Mean 0-20
Mean 21-40
Mean 41-60
Sep
Oct
Nov
Dec
Jan
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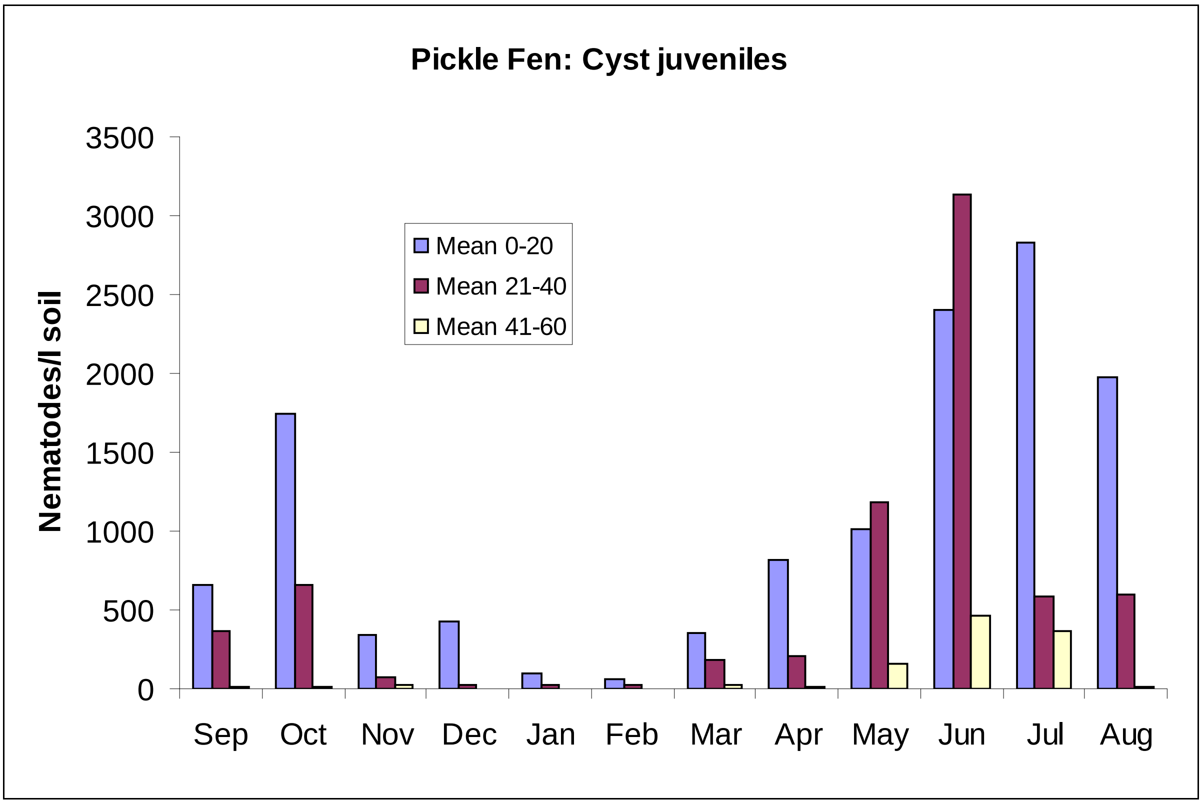
Pickle Fen: Cyst juveniles
Nematodes/l soil
3500
3000
2500
2000
1500
1000
500
0
Mean 0-20
Mean 21-40
Mean 41-60
Sep
Oct
Nov
Dec
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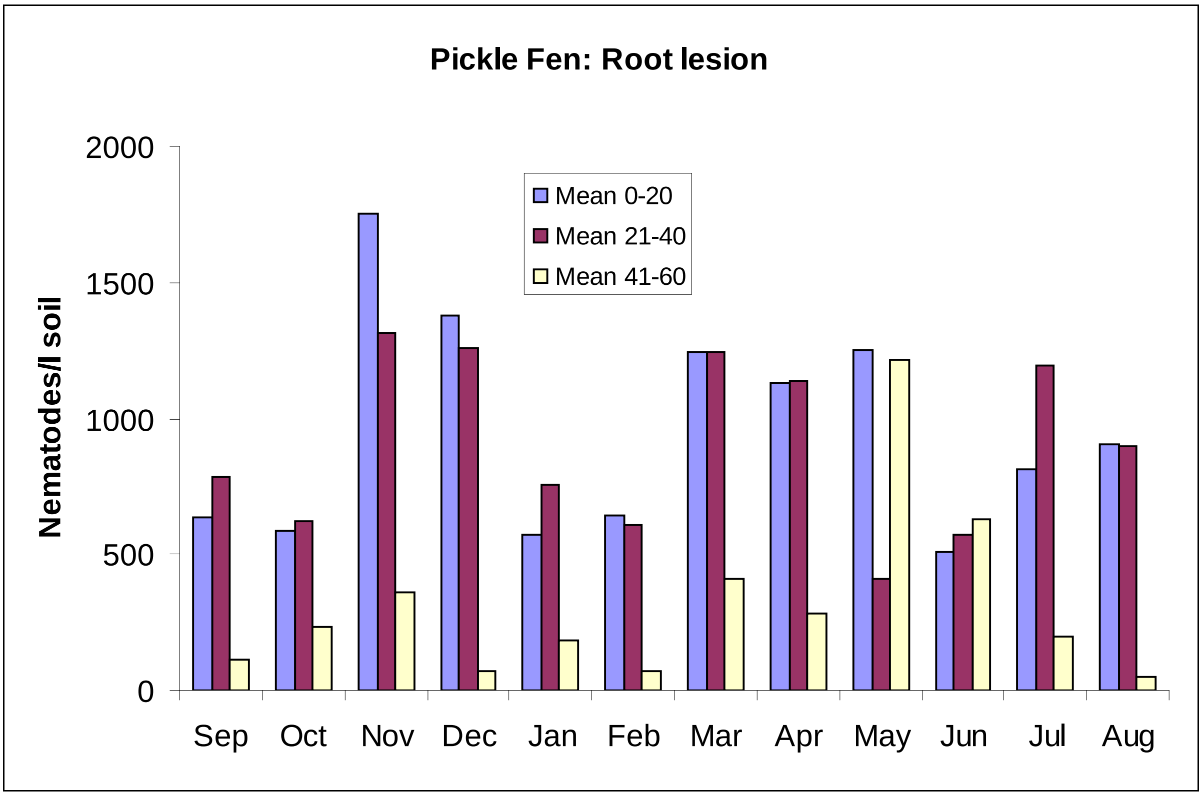

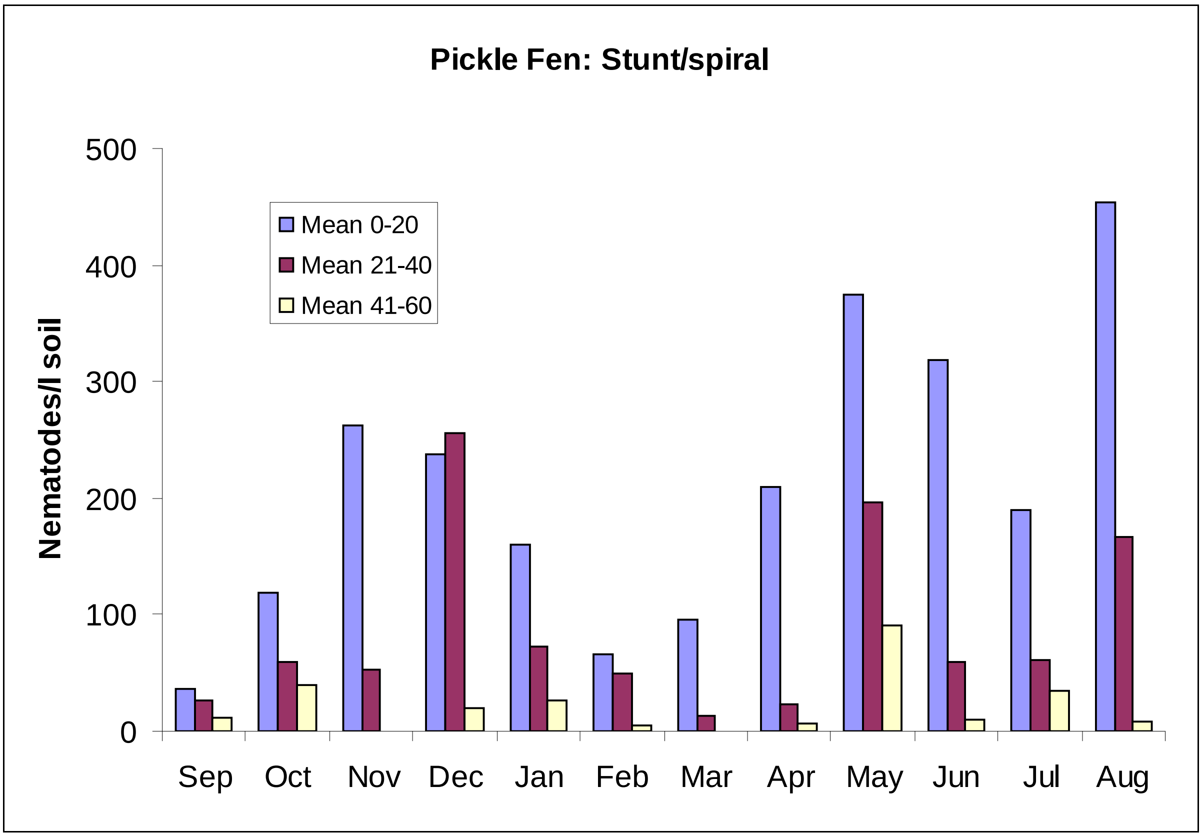

Figure 9. Mean numbers of nematodes at 0-20, 21-40 and 41-60 cm deep at three monitoring sites over 12 months between October 2010 and September 2011.

Table 12. Results of regression analyses to investigate the relationship between soil temperature (ºC) and soil moisture (% soil moisture by volume) and nematode numbers at three soil depths (0-20, 21-40, 41-60 cm) between September 2010 and August 2011 (x = soil temperature or soil moisture, y = nematode numbers).

Site	Nematode group	Equation			Probability (P)	% variation accounted for
		0-20	21-40	41-60		
Soil temperature						
West Dereham	Root lesion	y = 4.8x + 70.6	y = 4.8x + 40.2	y = 4.8x – 18.6	<0.05	15.5
	Stubby root	y = 16.4x + 473.0	y = 16.4x + 244.3	y = 16.4x + 140.4	<0.01	29.5
	Stunt/spiral	y = 20.0x + 270	y = 20.0x - 98	y = 20.0x - 198	<0.001	40.0
Christchurch	Root lesion	y = 146.7x - 559	y = 146.7x + 227	y = 146.7x - 891	<0.01	24.3
	Stunt/spiral	y = 161.3x + 758	y = 161.3x - 481	y = 161.3x - 1384	<0.001	43.9
Chatteris	Cyst juveniles	y = 95.8x + 14	y = 95.8x - 430	y = 95.8x - 916	<0.001	38.4
	Root lesion	y = -15.3x + 119	y = -15.3x + 1063	y = -15.3x + 479	<0.001	39.1
	Stunt/spiral	y = 3.1x + 176.6	y = 3.1x + 53.8	y = 3.1x – 11.1	<0.001	43.3
Soil moisture						
West Dereham	Root lesion	y = -5.4x + 259	y = -5.4x + 224	y = -5.4x + 170	0.056	13.3
	Stubby root	y = -16.7x + 1071	y = -16.7x + 828.7	y = -16.7x + 739.1	<0.01	25.6
	Stunt/spiral	y = -30.3x + 1251	y = -30.3x + 856	y = -30.3x + 782	<0.001	42.1
Christchurch	Root lesion	y = -60x + 2502	y = -471x + 13753	y = -532x + 11588	<0.001	41.5
	Stunt/spiral	y = -319.4x + 10347	y = -319.4x + 9344	y = -319.4x + 6873	<0.001	49.1
Chatteris	Cyst juveniles	y = -33.2x + 1758	y = -33.2x + 1471	y = -33.2x + 871	<0.05	17.1

Table 12. (cont'd)

Site	Nematode group	Equation			Probability (P)	% variation accounted for
		0-20	21-40	41-60		
	Root lesion	y = 33.1x + 255	y = 33.1x + 19	y = 33.1x - 460	<0.001	40.3
	Stunt/spiral	y = -1.9x + 250	y = -1.9x + 136.9	y = -1.9x + 65.7	<0.001	42.2

The effect of cultivation on nematode numbers at a range of soil depths

The numbers of nematodes pre- and post-cultivation recovered at three soil depths at five sites are summarised in Table 13. Data are only presented for root lesion and stunt/spiral nematodes as these were the most numerous at all monitored sites.

Table 13. Effect of cultivation practices on numbers of nematodes pre- and post-cultivation at five sites and three soil depths in March 2011.

Cultivation	Soil depth (cm)			Mean
	0-20	21-40	41-60	
Saffham Prior 1, Root lesion nematodes				
Pre-cultivation	123	218	48	130
Post cultivation	323	140	87	183
Mean	223	179	67	
SED (27DF) Cultivation	44.4			
SED (18DF) Soil depth	56.8			
SED (27DF) Interaction	77.0			
F probability cultivation	0.242			
F probability soil depth	<0.05			
F probability interaction	0.053			
Swaffham Prior 1, Stunt/spiral nematodes				
Pre-cultivation	3590	2010	565	2055
Post cultivation	2021	1240	1157	1473
Mean	2806	1625	861	
SED (27DF) Cultivation	394.9			
SED (18DF) Soil depth	312.5			
SED (27DF) Interaction	684.0			
F probability cultivation	0.152			
F probability soil depth	<0.001			
F probability interaction	0.097			
Swaffham Prior 2, Root lesion nematodes				
Pre-cultivation	3590	2010	565	2055
Post cultivation	2021	1240	1157	1473
Mean	2806	1625	861	
SED (27DF) Cultivation	394.9			
SED (18DF) Soil depth	312.5			

Table 13. (cont'd)

Cultivation	Soil depth (cm)			Mean
	0-20	21-40	41-60	
SED (27DF) Interaction	221.7			
F probability cultivation	0.571			
F probability soil depth	0.127			
F probability interaction	0.279			
Swaffham Prior 2, Stunt/spiral nematodes				
Pre-cultivation	3590	2010	565	2055
Post cultivation	2021	1240	1157	1473
Mean	2806	1625	861	
SED (27DF) Cultivation	394.9			
SED (18DF) Soil depth	312.5			
SED (27DF) Interaction	337.7			
F probability cultivation	0.204			
F probability soil depth	<0.001			
F probability interaction	0.607			
Chatteris, Root lesion nematodes				
Pre-cultivation	125	15	131	90
Post cultivation	33	60	125	73
Mean	79	38	128	
SED (27DF) Cultivation	44.4			
SED (18DF) Soil depth	56.8			
SED (27DF) Interaction	51.0			
F probability cultivation	0.588			
F probability soil depth	0.275			
F probability interaction	0.179			
Chatteris, Stunt/spiral nematodes				
Pre-cultivation	37	5	64	35
Post cultivation	220	57	0	92
Mean	128	31	32	
SED (27DF) Cultivation	22.7			
SED (18DF) Soil depth	25.0			
SED (27DF) Interaction	39.4			

Table 13. (cont'd)

Cultivation	Soil depth (cm)			Mean
	0-20	21-40	41-60	
F probability cultivation	<0.05			
F probability soil depth	<0.001			
F probability interaction	<0.001			
Upwell J, Root lesion nematodes				
Pre-cultivation	110	232	127	157
Post cultivation	347	182	165	231
Mean	228	207	146	
SED (27DF) Cultivation	49.4			
SED (18DF) Soil depth	54.9			
SED (27DF) Interaction	85.6			
F probability cultivation	0.142			
F probability soil depth	0.319			
F probability interaction	0.069			
Upwell J, Stunt/spiral nematodes				
Pre-cultivation	21	13	40	25
Post cultivation	31	0	12	14
Mean	26	7	26	
SED (27DF) Cultivation	15.8			
SED (18DF) Soil depth	11.9			
SED (27DF) Interaction	27.3			
F probability cultivation	0.502			
F probability soil depth	0.202			
F probability interaction	0.621			
Littleport, Root lesion nematodes				
Pre-cultivation	593	601	221	471
Post cultivation	975	1524	1538	1346
Mean	784	1062	880	
SED (27DF) Cultivation	274.5			
SED (18DF) Soil depth	207.9			
SED (27DF) Interaction	475.5			
F probability cultivation	<0.01			

Table 13. (cont'd)

Cultivation	Soil depth (cm)			Mean
	0-20	21-40	41-60	
F probability soil depth	0.415			
F probability interaction	0.390			
Littleport, Stunt/spiral nematodes				
Pre-cultivation	61	14	23	33
Post cultivation	40	72	160	91
Mean	50	43	92	
SED (27DF) Cultivation	34.3			
SED (18DF) Soil depth	36.3			
SED (27DF) Interaction	59.3			
F probability cultivation	0.101			
F probability soil depth	0.372			
F probability interaction	0.191			

In general, there was little effect of cultivation on nematode numbers. Only at Littleport was there a highly significant difference (P<0.001) in numbers pre- and post-cultivation for root lesion nematodes. Surprisingly numbers were almost three times higher post-cultivation than pre-cultivation. Over all sites there was no consistent trend to find greater numbers of nematodes pre- or post-cultivation. There was a significant difference in numbers of stunt/spiral nematodes (P<0.001 in each case) between soil depths at both Swaffham Prior sites and in root lesion nematode numbers (P<0.05) at Swaffham Prior 1. The trend was for nematode numbers to decrease with increasing soil depth. At Chatteris there was a highly significant interaction (P<0.001) between stunt/spiral nematode numbers at the three soil depths and numbers pre and post cultivation. This was due to numbers being highest post cultivation at 0-20 and 20-40 cm whereas at 40-60 cm the opposite was true.

Objective 4: Pot experiment to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

The effect of the treatments on nematode numbers are summarised in Table 14. An ANOVA was done on each individual nematode group and total free-living nematodes (all nematodes excluding stem nematode). Numbers of root lesion nematodes and total free-living nematodes differed significantly between treatments (P<0.05 in each case). Root lesion nematode numbers were lowest where Biofence meal was applied followed by Biofence granules and HDCI 037. The highest nematode numbers were where Vydate was used at 4000 g ai/ha. Although the most effective nematicides/biopesticides did not have significantly lower counts than the untreated control, Biofence meal reduced nematode numbers by 86% and Biofence granules and HDCI 037 by 72%. Nematorin 10G was the most effective standard nematicide and this treatment had 57% less root lesion nematodes than the untreated control. The lack of statistical significance between treatments may in part be due to variability in nematode numbers between samples.

Although numbers of stubby root and stem nematodes did not differ significantly between treatments there was a trend to find lowest counts where HDCI 036 or Biofence meal was used. Numbers of cyst juveniles were lowest where Vydate 10G was used but this treatment was not significantly better than any other. HDCI 038 reduced numbers of stem nematode by 43% compared with the untreated control but this difference was not statistically significant.

The effect of the nematicides on the time to 50% seedling emergence and AUC differed significantly between treatments ($P<0.001$, in each case, Table 15). This was primarily due to emergence in the Biofence treatments taking at least twice as long as in all the other treatments. As a result the AUC for these treatments was much lower than for all other treatments.

The dry weight of onion foliage differed significantly between treatments ($P<0.01$, Table 15). The lowest yield of foliage was recorded with Vydate 10G at 200g ai/ha and the highest was with Biofence meal. The dry weight yield of roots and the total plant dry weight did not differ significantly between treatments although there was a trend to find highest total dry weight where Biofence was used and the lowest where Vydate 10G was used.

Table 14. Mean numbers of nematodes (number/L soil) following treatment with a range of nematicides and biopesticides.

Treatment	Rate	Nematode numbers/l soil					
		Cyst juveniles	Root lesion	Stem nematode	Stubby root	Stunt/spiral	Total nematodes
Untreated control	-	17	29	819	17	333	396
Vydate 10G	2000 g ai/ha	8	33	147	25	250	317
Vydate 10G	4000 g ai/ha	4	63	198	108	379	554
HDCI 036	30 kg/ha	13	13	37	4	108	138
HDCI 037	1 kg/5L water	33	8	197	21	262	325
HDCI 038	2000 g ai/ha	33	21	468	67	221	342
Sesamin EC	10 L/ha	21	33	568	42	400	496
Sesamin EC	20 L/ha	13	45	677	42	312	412
Biofence meal	300 g/m^2	38	4	58	8	254	304
Biofence granules	300 g/m^2	17	8	324	67	304	396
SED (45DF)		13.8	16.2	298.8	32.1	98.3	109.2
F probabilty		0.229	<0.05	0.132	0.054	0.192	<0.05

Table 15. Mean time to 50% seedling emergence (days), area under the seedling emergence curve (AUC) and onion dry matter yield (g) following treatment with a range of nematicides and biopesticides.

Treatment	Rate	Time to 50% seedling emergence	AUC	Onion dry matter yield		
				Tops	Roots	Total plant
Untreated control	-	17.1	731.8	0.62	1.21	1.82
Vydate 10G	2000 g ai/ha	16.2	738.8	0.37	0.90	1.27
Vydate 10G	4000 g ai/ha	18.0	719.9	0.40	0.82	1.23
HDCI 036	30 kg/ha	17.9	712.7	0.42	1.19	1.61
HDCI 037	1 kg/5 l water	16.6	664.6	0.62	1.00	1.62
HDCI 038	2000g ai/ha	15.5	682.3	0.53	0.98	1.51
Sesamin EC	10 l/ha	14.4	721.4	0.51	1.40	1.91
Sesamin EC	20 l/ha	14.5	759.1	0.50	0.95	1.45
Biofence meal	300 g/m^2	52.3	187.3	0.77	1.57	2.34
Biofence granules	300 g/m^2	41.0	259.2	0.76	1.38	2.13
SED + (DF)		2.04 (45)	30.35 (45)	0.111 (44)	0.322 (44)	0.406 (44)
F probability		<0.001	<0.001	<0.01	0.331	0.152

Discussion

Objective 1: Pot experiments to establish the most damaging nematode species to onions

As in year one of this project, soil dilution was a very effective way of creating a range of populations of needle nematodes. Needle nematode numbers at the start of the experiment were approximately two thirds of the target population and there was a wide range of counts over which the impact on onion growth could be measured. The maximum needle nematode count was about 20 times greater than the guideline threshold (50 needle nematodes/L soil). At the end of the experiment needle nematode numbers had declined but the range of actual numbers was still sufficiently wide to provide a good test of their impact on onion growth.

Needle nematodes did not have any significant effect on onion growth and the same was true of root lesion nematodes, stubby root nematodes or stunt/spiral nematodes in year one of the project. Needle nematodes are one of the larger free-living species so might have been expected to have had some impact on crop growth particularly as the created populations were up to 20 times greater than the guideline threshold. 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 threshold is too conservative and well below the number of nematodes which can be tolerated by the crop. In general, results of pot experiments in both year 1 and 2 of this project suggest that the impact of needle, root lesion, stubby root and stunt/spiral nematodes on onion growth are much less than previously thought. These results were supported by additional studies with root lesion and stunt/spiral nematodes reported in Appendix 1. In addition, observations from the field show little if any crop damage where soil analysis has shown FLN numbers in excess of current thresholds. If the seedling stage of growth is not susceptible to nematode damage then it is highly unlikely that later growth stages are at risk from these pests. Field experiments to investigate the effect of high populations of nematodes on onion growth would be useful to confirm these conclusions. It is also possible that current onion cultivars are less susceptible to nematodes than some older cultivars, although there is no evidence of this. The cultivar vision used in this work is a currently commonly grown variety.

If onions are more tolerant of nematodes than previously thought this will have a significant impact on nematicide use and potentially increase the profitability of the crop. However, it should be borne in mind that stem nematode (*Ditylenchus dipsaci*) can have a significant impact on the yield of onion crops at relatively low levels so there will still be a need for soil analysis to assess the risk of crop damage. In addition, no experiments were done with northern root knot nematode (*Meloidogyne hapla*) which is thought to be a pest of onions. Although this nematode is relatively uncommon (recorded in less than 1% of samples submitted to ADAS Pest Evaluation Services) when it is present, numbers of several thousand per litre soil have been recorded so it would be prudent to determine whether it poses a risk to the onion crop. It is also a species which could become more common if temperatures continue to rise as a result of climate change. Its current multiplication is insufficient to produce enough eggs to assure their persistence between crops but an increase in soil temperature may allow it to overcome this constraint and become an economic crop pest in the UK.

Objective 3: Monitoring vertical distribution of free-living nematodes

Soil temperature followed a relatively predictable pattern across all three monitored sites. Overall temperature ranged between 1 and 16ºC and was generally lowest in December/January and highest in August/September. There was little difference in the range of temperatures recorded at all three soil depths. Soil moisture showed more variation between soil depths and overall ranged between 15 and 35% moisture by volume. There was a maximum difference of about 10-15% moisture by volume between soil depths. At the two mineral soil sites (West Dereham and Christchurch) soil moisture remained relatively constant within soil depths from September until March and then declined. At the organic site (Chatteris) soil moisture remained relatively constant within soil depths throughout the year with the exception of a slight increase during March.

Numbers of nematodes tended to decrease with increasing soil depth and there was some evidence that some species were most common at different soil depths. For example, stunt/spiral nematodes tended to be recorded most at 0-20 cm whereas in contrast root lesion nematodes were as common at 20-40 cm as at 0-20 cm.

Regression analyses showed that there was a trend for nematode numbers to increase with increasing soil temperature and decrease with increasing soil moisture. However, the amount of variation accounted for never exceeded 50% so the equations generated were unreliable as a means of predicting nematode numbers at a specific soil temperature or level of soil moisture. Nematodes are poikilothermic (cold blooded) and therefore their body temperature will reflect that of the surrounding soil. Consequently it would be expected that as soil temperatures decline nematodes would become less active. Soil extraction methods rely on nematodes being active so might be expected to recover least nematodes from samples taken when soil temperatures are at their lowest. There was a trend for highest nematode number in August/September when soil temperatures were at their highest. Nematodes are likely to be most active under these conditions and consequently would be expected to reproduce. The results suggest that there would be no disadvantage to soil sampling in the summer months although the numbers of nematodes present just before sowing are likely to give the best indication of potential crop damage. Also as data were only collected over one season it is possible that different trends would be observed in different years when both soil moisture and temperature were more variable. That nematode numbers decreased with increasing soil moisture is perhaps surprising as nematodes require moisture in order to move around in soil. However, nematodes are best able to move around when soil moisture levels allow the creation of water films around soil particles. As the soil becomes wetter and soil pores become full of water, nematodes will become less active. This might explain why numbers decline as soil moisture increases. The longer they remain inactive the more their food reserves will be depleted and the greater the chance that they will die. This will also affect the numbers recovered by soil extraction.

Soil cultivation appeared to have limited if any effect of nematode numbers. In some instances nematode numbers were higher post cultivation than pre-cultivation. This is surprising, soil cultivation is generally considered an effective cultural control option for soil pests. Perhaps the small size of nematodes means that they are less influenced by soil disturbance than larger pest species.

Some conclusions can be drawn with regard to how soil temperature, soil moisture and cultivation affect advice on when to take samples for free-living nematodes. It is important to stress that this study has measured the impact of these factors on the numbers of nematodes recovered by soil extraction. This should not be interpreted as a direct indication of how these factors affect the populations of nematodes in soil. In most instances soil extraction counts can be related to changes in nematode numbers in the soil but this is not always the case as extraction is dependent on active nematodes as has already been discussed. However, taking this into account it is possible to offer some advice on soil sampling.

Current advice is to sample to a depth of 15 cm and results suggest that in general this will give a good estimate of numbers present in soil. In the majority of cases (75%) most nematodes were recovered at 0-20 cm. It would appear best to avoid the coldest months which in 201/11 were January and February. To an extent this is common sense as nobody is keen to sample during particularly cold weather when it is possible that the soil will be frozen and it will be difficult to push a soil corer into the ground. Nematode counts from soil extraction are also likely to be low when the soil is waterlogged so it is better to allow conditions to improve before taking samples. Data from this study suggest that sampling pre- or post-cultivation is likely to have limited impact on the results of soil extraction.

It is important to bear in mind that neither stem nematode or root knot nematode were present at any of the monitored sites. Stem nematode is the most important nematode pest of onions and root knot nematode is also recorded as damaging the crop. Stem nematode can devastate crops even at low levels of soil infestation so it is important that soil sampling can be relied upon to detect this pest. Soil sampling strategies and extraction methods for stem nematode were the subject of a previous HDC project (FV 327 Onions: improving risk assessment for stem nematode). In the absence of specific data on stem nematode this project suggests that soil sampling for this pest should follow the same guidelines as suggested for other less damaging free-living species. The same approach should be adopted for root knot nematode until it is established whether it poses a threat to the onion crop.

Objective 4: Pot experiment to test the effectiveness of a selection of nematicides and biopesticides on the control of free living nematodes

In terms of efficacy of nematode control Biofence was the most promising of the novel products investigated. Biofence meal also tended to be more effective than the granular formulation. Statistically significant differences between treatments were only recorded for root lesion nematodes and the total free-living nematode counts (this could be a reflection of root lesion nematode data) and differences tended to be between the most effective treatments and Vydate which was least good at reducing nematode numbers. Despite the apparent lack of efficacy of Vydate it is possible that this treatment had an effect on nematode mobility such that the pests were disorientated and unable to locate the host crop. For example, the nematicide aldicarb was thought to have a nematistatic effect on nematodes such that movement and feeding were reduced (Evans, 1973). However, this effect may not be sufficient to kill nematodes some of which can withstand long periods of starvation (Evans and Perry, 1976). Therefore it is possible that Vydate has an initial nematistatic effect on nematodes during seedlings are able to grow away and establish. However, if nematodes are able to recover from this then results of soil extraction might show little change in pest numbers.

Biofence meal and granules reduced numbers of root lesion nematodes and total free-living nematodes by 82% and 76% respectively in comparison with the untreated control. HDCI 036 was the most effective standard treatment when compared with Vydate 10G.

Of the other novel products HDCI 037 reduced numbers of root lesion nematodes and stem nematode in comparison with the untreated control by 72% and 76% respectively. HDCI 038 reduced numbers of stem nematode by 43% compared with the untreated control. None of these differences were statistically significant.

The effectiveness of the Biofence treatments in reducing numbers of some free-living nematodes must be balanced against its effect on seedling emergence. Where Biofence was used seedling emergence took approximately twice as long as in all other treatments. Previous work with this product has shown a phytotoxic effect on weeds (W Parker, pers. comm.) However, despite late emergence the Biofence treatments produced the highest onion dry matter yield. This result is not surprising as the product is marketed as a 100% vegetable organic fertiliser.

Overall results suggest that Biofence is worthy of further investigation particularly if it is possible to overcome its effects on seedling emergence. There was also limited evidence that HDCI 037 and HDCI 038 may warrant further evaluation if alternative formulations are available.

Conclusions

Threshold levels

- Pot experiments suggest that populations of needle nematodes well above the current guideline threshold have no effect on onion growth.
- Results from year 1 and 2 of this project suggest that guideline thresholds for needle, root lesion, stubby root and stunt/spiral nematodes are far too conservative and that significant savings can be made on nematicide use.

Factors influencing nematode numbers

- There was a trend for nematode numbers to increase with increasing soil temperature and decrease with increasing soil moisture but regression equations were not sufficiently reliable to be able to predict nematode numbers at a specific soil temperature or level of soil moisture.
- Numbers of nematodes recovered by soil extraction tended to decrease with increasing soil depth. Most nematodes were usually found at 0-20 cm in comparison with 20-40 cm and 40-60 cm.
- There appeared to be no consistent effect of soil cultivation on the numbers of the different nematode groups recovered at the monitoring sites.

Soil sampling

- Soil sampling advice can be fine-tuned as a result of monitoring nematode numbers and soil moisture and temperature over one year at three soil depths. Most reliable counts are likely to be achieved by sampling at 0-15 cm and avoiding the coldest months and waterlogged soil. Samples can be taken pre or post cultivation
- Soil sampling for stem nematode should follow the above guidelines for the time being but further work may be necessary to determine how this pest behaves in soil as it was not recorded at any of the monitoring sites.

Control

- Of the current standard nematicides tested Nematorin gave the best reduction in total nematode numbers
- Biofence, particularly the meal formulation shows promise as a means of reducing nematode levels in soil but this must be balanced against the fact that it can double the time for seedling emergence. Further work is required to determine how to make best use of this product.
- There was also limited evidence that HDCI 037 and HDCI 038 may warrant further evaluation.

Nematode damage to onions

- Stem nematode remains the main nematode threat to the onion crop.
- No experiments were done with northern root knot nematode (*Meloidogyne hapla*) which is thought to be a pest of onions. Although this pest is recorded in less than 1% of samples it has potential to become more common if temperatures continue to rise as a result of climate change.

Knowledge and Technology Transfer

An article was published in HDC News (Risks re-assessed, HDC News Issue 184 p22-23) in June 2012. The results were also presented to the British Leek Growers association on 27 March 2012 and will be presented at The BOPA Biannual Onion Conference 16th November 2012.

Glossary

ANOVA – Acronym for analysis of variance. A statistical technique used to compare the sources of variability in an experiment.

Differential GPS – a means of accurately locating sampling points using the Global Positioning System satellite network linked to known reference points on the ground to obtain very high (sub-metre) accuracy.

Flegg modified Cobb method (Flegg, 1967) – A method of extracting free-living nematodes from soil which is specifically recommended for large species (eg, dagger and needle nematodes).

Seinhorst 2-flask method (Seinhorst, 1955) – A method of extracting free-living nematodes from soil, which is specifically recommended for small to medium sized species (e.g. stubby root nematode, root lesion nematodes, stunt/spiral nematodes).

References

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Evans, A A F, (1973). Mode of action of nematicides. *Annals of Applied Biology* **75,** 469-473.

Evans A A F and Perry R N. (1976). Survival strategies in nematodes. The organisation of Nematodes. Academic Press, New York, N A Croll (ed.) 383-424

Flegg, J J M (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology* **60**: 429-437.

Seinhorst, J W (1955). Een eenvoudige methode voor het afschieden van aaltjes uit grond.*Tijdschr. PIZiekt* **58**: 103-108.

Appendix 1. Additional experiments to evaluate nematode thresholds

Additional experiments were done by a visiting French student (from University of Lyon) to provide additional data on the relationship between onion growth and numbers of free-living nematodes. Results are presented here for experiments with root lesion (*Pratylenchus* spp.) and stunt/spiral nematodes (*Tylenchorynchus/Helicotylenchus/Rotylenchus* spp.).

Materials and methods

ADAS Pest Evaluation Services extract soil samples from farmers and growers to advise on the numbers of free-living nematodes and how they might affect crop growth. Once the extractions have been completed the soil is retained for one month in a cold store at 5ºC in case there is any need for a re-analysis. After the month has elapsed these soils are disposed of. These additional experiments used these retained soils and selected a number to provide a range of populations of either root lesion or stunt/spiral nematodes with limited numbers of other free-living species. This method was investigated as an alternative to soil dilution for creating a range of populations of different free-living nematode species.

As the soils came from a number of different sites and soil types they were also likely to vary in their nutrient content. This potentially could have influenced onion growth and confound any impact of nematode feeding. To combat the possibility of varying nutrient levels between pots it was decided to provide each with sufficient fertiliser to satisfy the requirements of the onion seedlings. Fertiliser applications equivalent to 100 kg N/ha, 75 kg P_2O_5/ha and 100 kg K_2O were measured out as pot doses (based on recommendations from an ADAS soil scientist). These were split and one half applied to the seedbed and the other half after germination.

The experiments used 10 cm diameter pots. Sufficient soil was measured out to fill the pot to the inner rim. This was then tipped into a polythene bag and the first fertiliser split added and incorporated by gently turning the bag through 360 degrees. The soil was then tipped back into the pot and 15 onion seeds (cv. Vision) evenly sown over the soil surface. About one centimetre of soil was then added to cover the seeds. Seeds were subjected to a germination test before sowing to ensure that they were viable.

Each pot was labelled and arranged in a glasshouse maintained at 20ºC and 14 hours daylength to mimic spring conditions. Pots were watered as necessary and the number of seedlings that emerged was assessed daily until there was no change over a period of five days. Once seedling germination was complete the plants were thinned to four per pot and these grown on to monitor whether there was any further impact of nematodes on growth.

After approximately six months onion yield was assessed. Plants were harvested and the dry matter yield assessed for both the roots and tops (foliage + bulb) by oven drying at 80ºC for 16 hours. The pot soil was also extracted using the Seinhorst two-flask technique to compare the initial and final needle nematode population.

Results

Germination test

The germination test of the onion seed (cv. Vision) showed it to be 88% viable. This was considered a sufficiently healthy seed lot with which to conduct the pot experiments.

Comparison of actual and target nematode numbers

Regression analysis was used to compare the target population of each nematode group to the actual population achieved by soil dilution. The actual population was measured once at the end of the experiment and compared with the nematode count from the initial extraction of the sample by ADAS Pest Evaluation Services. This initial count was considered to be the target population. The equation of the regression line and the percentage variation accounted for is given in Table 1. If 100% of variation is accounted for this represents a perfect fit between target and actual nematode populations. These data are also presented in Figures 1 and 2.

Table 1. Results of regression analyses to compare target and actual nematode populations (y = actual population, x = target population)

Nematode group	Regression line equation	Probability	% variation accounted for
Root lesion	y = 0.9138x	<0.001	71.7
Stunt/spiral	y = 0.5758x	<0.001	85.7

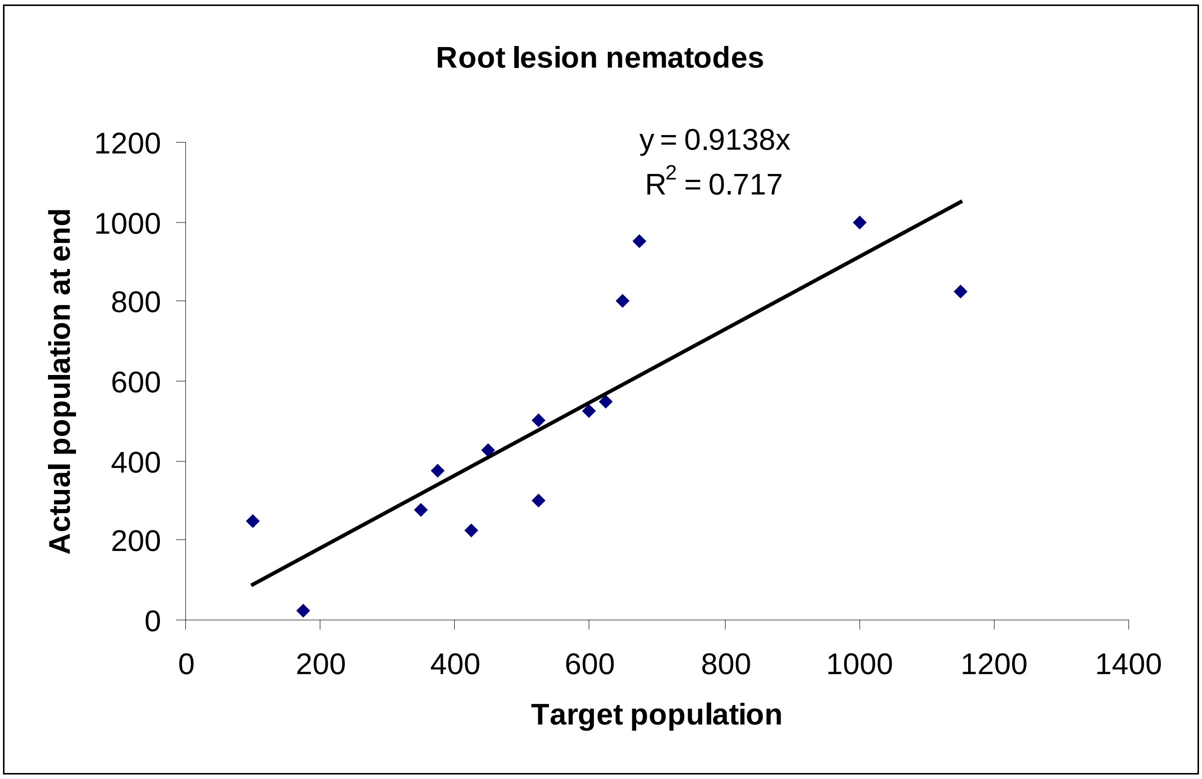

Figure 1. Actual root lesion nematode populations at the end of the experiment against target populations (number/litre soil)

Actual numbers of root lesion nematodes at the end of the experiment were about 70% of the target population but still provided a good range of counts over which to measure their impact on onion growth. There was a good spread of counts although the maximum of 1000/L soil was less than the current threshold of 2,500/L soil. The data for stunt/spiral nematodes was heavily influenced by a very high population of 14,000 nematodes in one sample. Therefore the counts were not spread evenly between the minimum and maximum limits. However, the influence of such a high count of nematodes on onion growth was of interest as it was significantly above the current threshold of 10,000 stunt/spiral nematodes/L soil.

Impact of nematodes on onion growth

Regression analyses were undertaken on three datasets to assess the impact of the actual nematode populations at the start of the experiment on onion growth as listed below:

1. 50% onion seed emergence - The time taken for 50% of onion seedlings to emerge in each pot was determined and the relationship with actual nematode number investigated. If seedling emergence was inhibited by increasing nematode number then the time to 75% emergence might be expected to increase.

2. Dry matter onion yield – The relationship between mean onion yield per plant and actual nematode number was investigated.

The results of analyses done using data on the time taken to 50% emergence of onion seedlings and its relationship with actual nematode numbers at the start of the experiment are summarised in Table 2 and Figures 3 and 4.

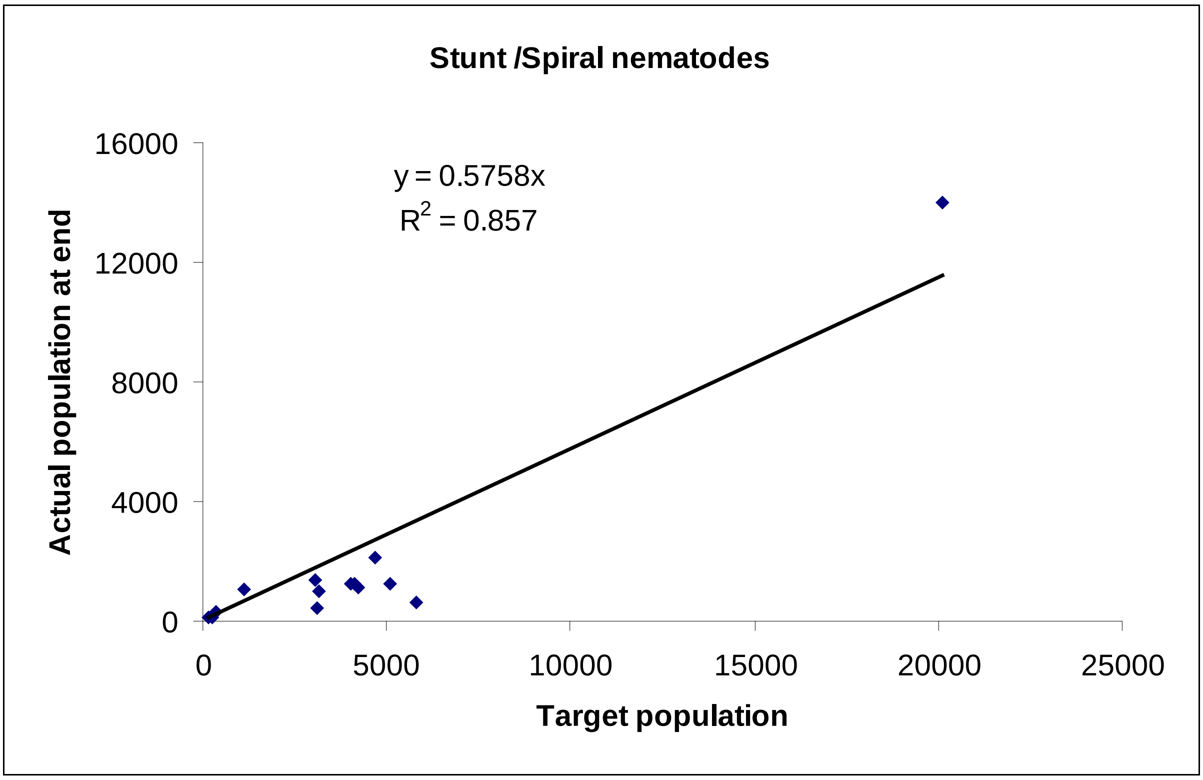

Figure 2. Actual stunt/spiral nematode populations at the end of the experiment against target populations (number/litre soil)

Table 2. Results of regression analyses to investigate the relationship between 50% onion seed emergence and actual nematode populations at the start of the experiment (x = actual population, y = time to 50% emergence)

Nematode group	Regression line equation	Probability	% variation accounted for
Root lesion	y = -0.00305 + 21.51	0.321	0.5
Stunt/spiral	y = -0.000267 + 25.54	0.530	0

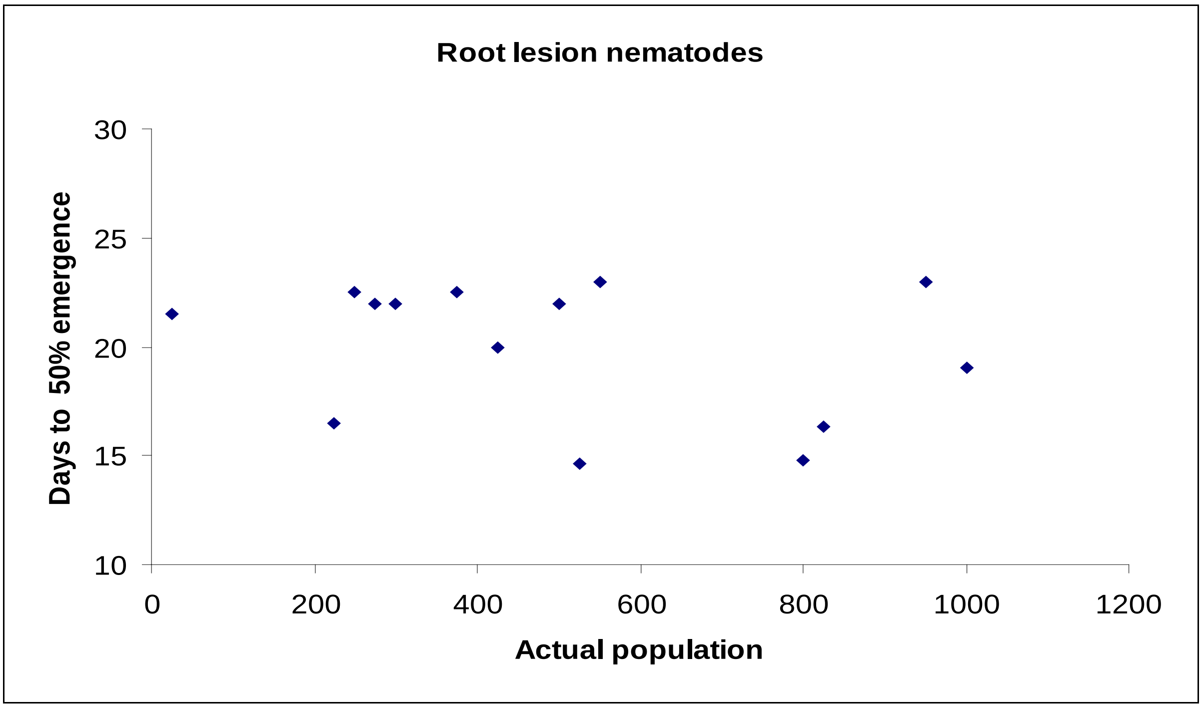

Figure 3. Relationship between time to 50% emergence and actual root lesion nematode population (number/litre soil) at the end of the experiment.

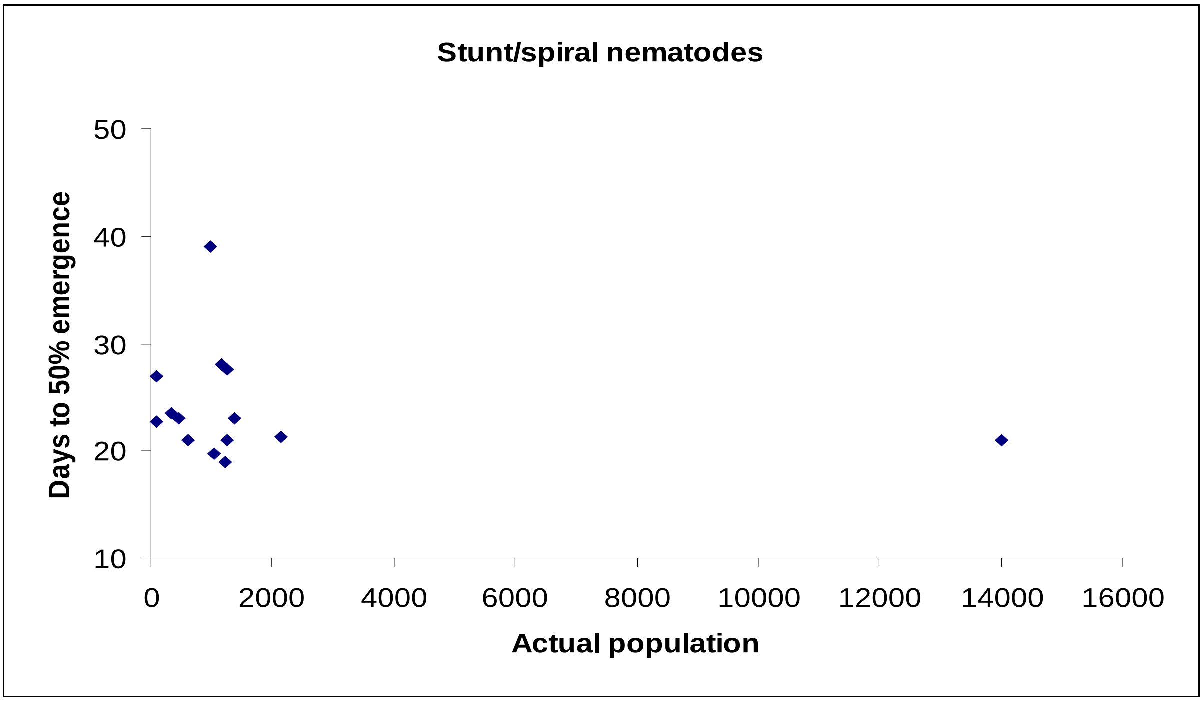

Figure 4. Relationship between time to 50% emergence and actual stunt/spiral nematode population (number/litre soil) at the end of the experiment.

The results of an analysis done using data on mean onion dry matter yield/plant and its relationship with actual nematode numbers at the end of the experiment is summarised in Table 3 and in Figures 5 and 6.

Table 3. Results of regression analyses to investigate the relationship between onion dry matter yield and actual nematode populations at the end of the experiment (x = actual population, y = seedling dry weight)

Nematode group	Regression line equation	Probability	% variation accounted for
Root lesion	y = 0.000193x + 0.1926	0.203	5.9
Stunt spiral	y = -0.0000162 + 0.4137	0.175	7.7

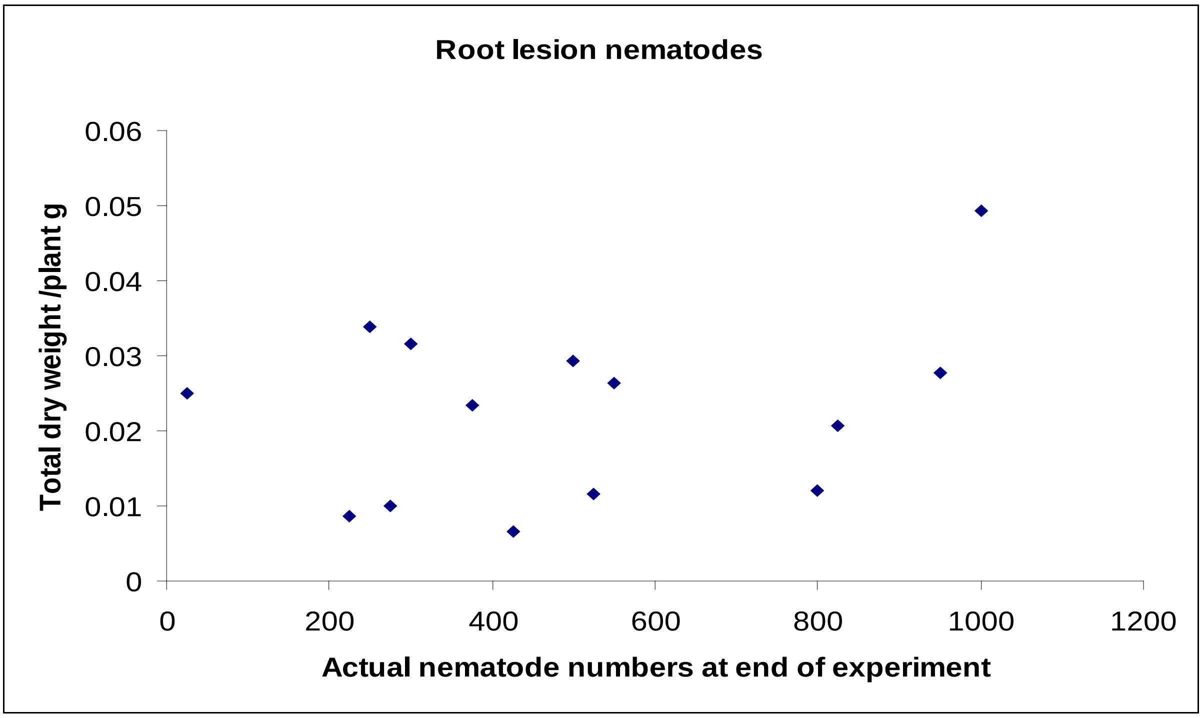

Figure 5. Relationship between onion dry weight and actual root lesion nematode populations (number/litre soil) at the end of the experiment.

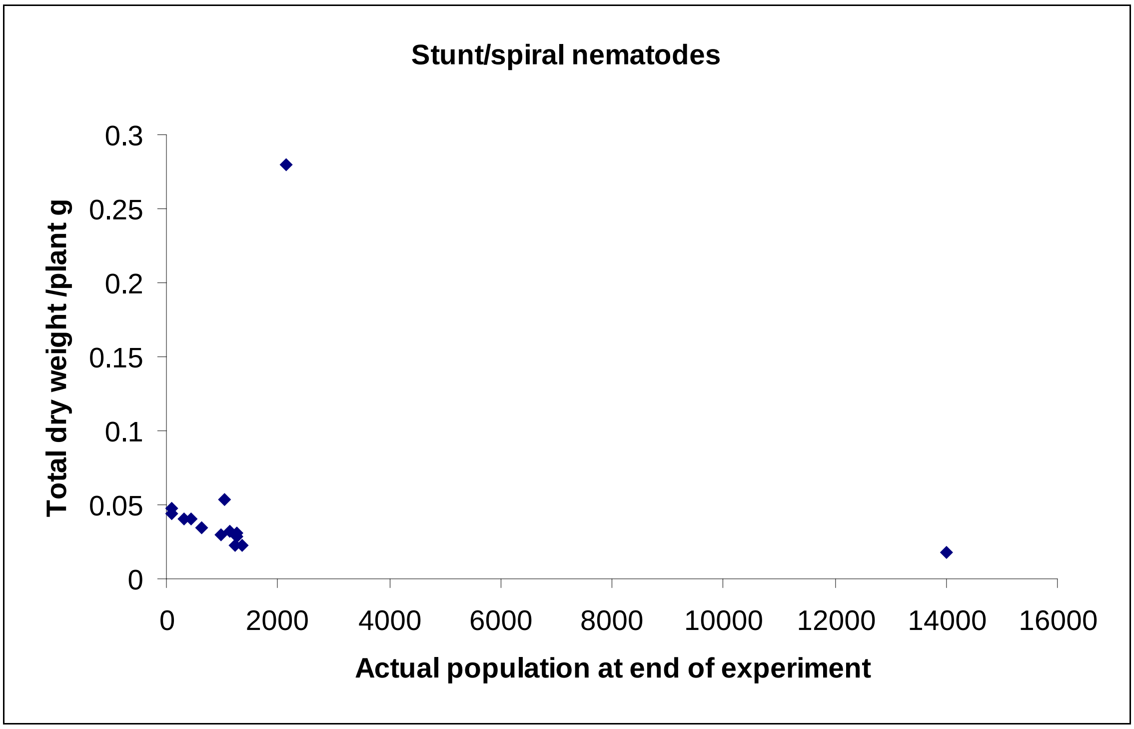

Figure 6. Relationship between onion dry weight and actual stunt/spiral nematode populations (number/litre soil) at the end of the experiment.

In general there was a very poor relationship between onion dry matter and numbers of both root lesion and stunt/spiral nematodes.

Discussion

Use of soil samples submitted to ADAS Pest Evaluation Services is an alternative to soil dilution as a means of producing a range of populations of different free-living nematode species. However, the randomness of nematode counts from these samples means that it is difficult to achieve an even spread of populations between the minimum and maximum counts. This was particularly evident with stunt spiral nematodes in this experiment. The majority of counts ranged between 100 and 2000 nematodes/L soil but one sample had 14,000 nematodes/L soil. The spread of populations for root lesion nematodes was much more even although the maximum count was less than the current threshold of 2,500/L soil.

In general, data supported results from the soil dilution studies with no clear relationship between nematode numbers and seedling emergence or onion dry weight. There was no evidence to suggest that increasing numbers of either root lesion or stunt/spiral nematodes within the ranges studies in these experiments had any deleterious effect on crop growth. In summary, this work is further indication that current 'anecdotal' thresholds for free-living nematodes in onions are too conservative and that there is scope for savings on nematicide use.