Project title:	Onions: improving risk assessment for free-living nematodes
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Project leader:	Steve Ellis, ADAS
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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

This project is re-defining the threshold levels to apply nematicides for free-living nematodes which could reduce the need to use them and ultimately save approximately £100/ha.

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 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 be the evaluation of potential alternative products for control of free-living nematodes. Alternative chemical control options such as Nemathorin are available and potential biopesticides are also worthy of investigation.

The specific objectives of this project are listed below:

1. To measure the effect of different populations of stubby root, needle, stunt/spiral and root lesion 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 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 1-3 have been started. Objective 4 will not begin until year 2

Summary of the project and main conclusions

- Pot experiments suggest that populations of stubby root, root lesion and stunt/spiral nematodes well above current guideline thresholds have no effect on onion growth.

- Results suggest that significant savings can be made on nematicide use.

- Stunt/spiral nematodes are most commonly recovered from soil samples followed by root lesion nematodes, stubby root nematodes, needle nematodes, cyst juveniles, dagger nematodes, stem nematodes and root-knot nematodes.

- Stem nematodes and root-knot nematodes are potentially important pests of onions but were recovered on average in less than 1% of soil samples.

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

A range of populations of root lesion, stubby root and stunt/spiral nematodes were created by soil dilution. This involved mixing soil infested with nematodes with the same soil which had been sterilized by autoclaving at 121ºC for 45 minutes. For example, to achieve a target nematode population of 1000 stubby root nematodes/l of soil, 1 l of soil containing 2000 stubby root nematodes/l soil was mixed with 1 l of sterile soil. A total of 30 target populations was created for each nematode group (Table 1).

Table 1

Nematode group	Provisional threshold (no./litre soil)	Target population range (no./litre soil)
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 (cv Vision). Pots were maintained in a polythene tunnel and watered as necessary.

Nematode numbers were also 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.

Objective 2: Analysis of historic sampling data

A total of 11,733 records for free-living nematode samples were extracted from the PES database over 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 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.

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 £100/ha and reduced environmental impact in terms of effects on non-target species and residue levels in soil.

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. Neither stem nematode (a semi-endoparasite) nor root-knot nematode (endoparasite) are true free-living nematodes. Free-living nematodes do not invade plants.

- 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 and reduce the environmental impact of pesticide use.

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, oxamyl (SOLA 20061890 Vydate 10G 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 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 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. 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 was 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 was 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 fosthiazate (Nemathorin) are available and potential biopesticides were also 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 aimed to improve the precision with which growers and agronomists sample land for free-living nematodes and to improve their confidence in the interpretation of results in order to decide on the suitability of the land for cropping with onions and the need for nematicide treatment. Additional data will also be generated in year two of the project 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, needle, stunt/spiral and root lesion 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 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

Materials and methods

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

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

Approximately 75kg of field soil was collected from sites known to be infested with stubby root nematodes, stunt/spiral nematodes and root lesion nematodes. It was also intended to collect soil from a site infested with needle nematodes. However, it was not possible to locate such a site and this work will be delayed until year 2. The soil was collected using spades, sampling to a depth of approximately 15 cm at a range of points across the field and was contained in plastic dustbins. The bins were returned to the laboratory and sampled using a 15 cm long x 2 cm diameter cheese-corer auger. A total of 20 cores was taken from each bin and each sample extracted twice, once using the Seinhorst two-flask technique (Seinhorst, 1955) and once using the Flegg-modified Cobb technique (Flegg, 1967). Soil samples were also sent to Fera to determine the predominant nematode species at each site.

After the original nematode population had been assessed, a range of populations was created by taking a known volume of the 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 5 kg batches at 121ºC for 45 minutes in cotton bags. After autoclaving, the soil was allowed to cool for 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. As an 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 soils were 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 the nematodes present later extracted using the Seinhorst two-flask technique to check the population. The target population ranges for each nematode group are given in Table 2.

Table 2. Target population ranges for root lesion, stunt/spiral and stubby root nematodes to be achieved by soil dilution.

Nematode group	Provisional threshold (no./litre soil)	Target population range (no./litre soil)
Root lesion	2500	0 – 3,350
Stubby root	200	0 – 6,902
Stunt/spiral	10,000	0 – 11,600

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.

Pots were maintained in a polythene tunnel and watered as necessary. A Tiny Talk data logger was located in one pot to monitor soil temperature throughout the experiment. 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 4 per pot and these were grown on to monitor whether there was any 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 80^{o}C for 16 hours. The pot soil was also extracted using the Seinhorst two-flask technique to compare the initial and final nematode populations.

Objective 2: Analysis of historic sampling data

A total of 11,733 records of free-living nematode samples processed in the period 31 October 2000 to 23 September 2010 was extracted from the PES database. 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.

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 will continue to be taken in year two of the project until 12 months of data have been collected. 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 species present. This 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 was used to measure soil temperature and soil moisture 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. 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 10m length of cord was attached. This was used to describe a semi-circle of radius 10m around which the 10 sample points were defined. At each point a single core was taken to a depth of 60cm and this sub-divided into cores from 0-20cm, 20-40cm and 40-60cm. Cores were taken with a 60mm radius corer which was hammered into the ground. Each of these sub-divisions was considered to be a replicate and was bagged and labelled separately. Therefore around each datalogger on each sampling occasion a total of 30 samples was taken (3 depths x 10 sampling points).

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. Early (February/March) and late (May/June) cultivation were compared and soil samples taken pre- and post-cultivation 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. A total of 10 cores was taken from the defined area at both pre- and post-cultivation sampling.

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.

Nematode species

The predominant root lesion nematode was *Pratylenchus thornei* (89%) followed by *Pratylenchus neglectus* (11%), the predominant stubby root nematode was *Paratrichodorus anemones* (76%), followed by *Trichodorus primitivus* (24%) and the predominant stunt/spiral nematode was *Helicotylechus vulgaris* (78%) followed by *H. canadensis* (15%) and Bitylenchus dubius (7%)

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 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 3. If 100% of variation is accounted for this represents a perfect fit between target and actual nematode populations.

Table 3. Results of regression analyses to compare target and actual nematode populations (x = actual population, y = target population)

Nematode group	**Regression line equation**		**Probability**		**% variation accounted for**	
	At start	At end	At start	At end	At start	At end
Root lesion	x = 2.43y	x = 0.89y	<0.001	<0.001	86.2	62.0
Stubby root	x= 0.78y	x = 0.56y	<0.001	<0.001	87.8	72.7
Stunt/spiral	$x= 0.001y^2 + 0.0104y$	x = 1.09y	<0.001	<0.001	90.8	82.0

Regression analyses showed a very highly significant fit between actual and target nematode population for each nematode group (P<0.001). For root lesion and stunt/spiral nematodes, at the end of the experiment this was not significantly different from a 1:1 relationship where the actual population was approximately equivalent to the target population. At the start of the experiment the relationship between actual and target nematode numbers for stunt/spiral nematodes was best described by a quadratic relationship. This was because at low target population's nematode numbers were slightly lower than the target whereas at high target populations nematode numbers were slightly higher than target populations. Numbers of root lesion nematodes at the start of the experiment were approximately 2.5 times higher than the target population and this is difficult to explain. It is possible that this was due to nematode reproduction. However, by the end of the experiment actual nematode numbers had returned to levels that were very similar to those in the target populations. For stubby root nematodes, actual nematode numbers were slightly less than target populations at both the start and end of the experiment. However, the range of actual nematode numbers was still sufficiently wide to provide a good test of their impact on onion growth.

Graphs of actual against target populations for each nematode group are given in Figure 1.

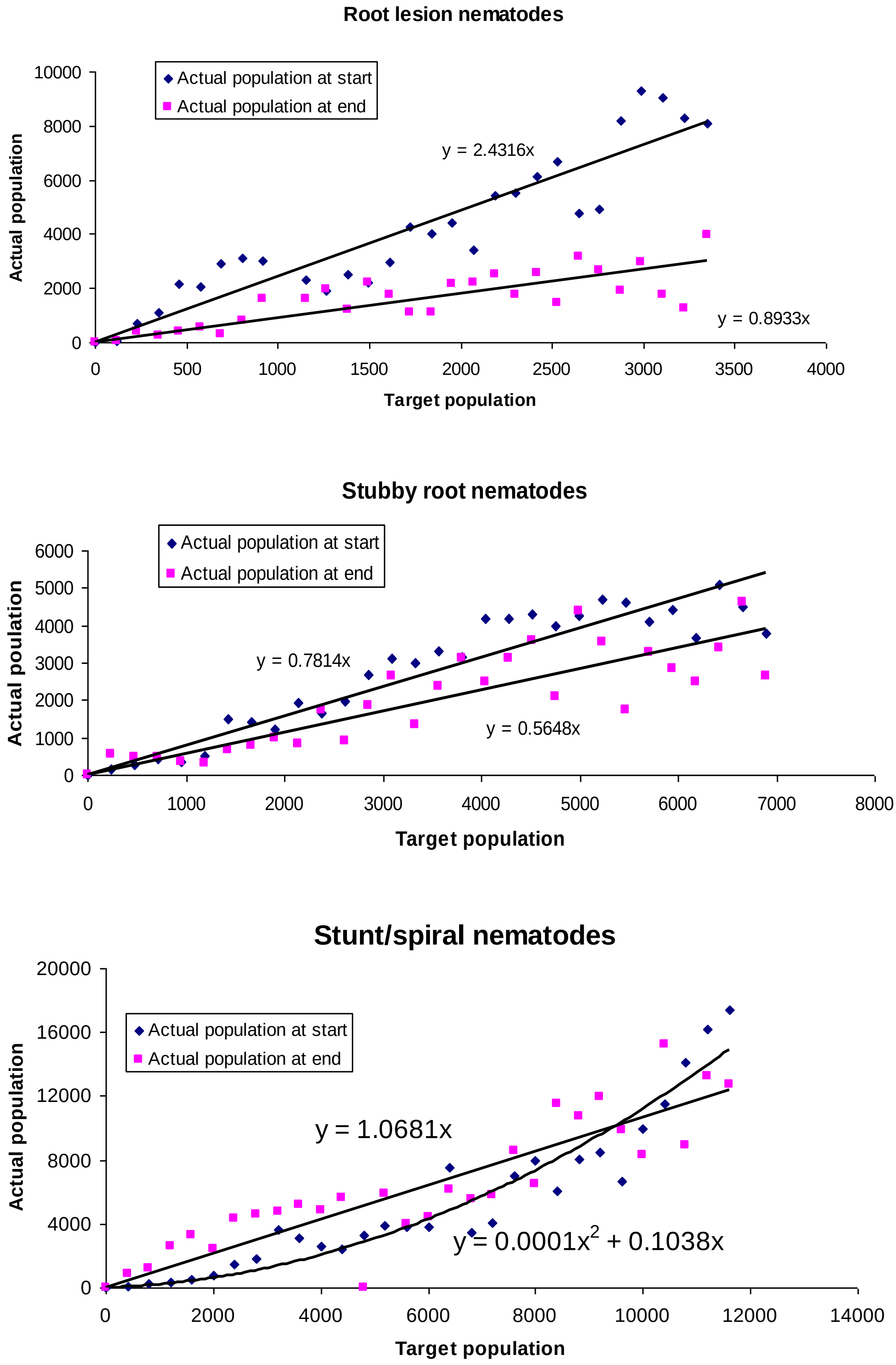

Figure 1. Actual nematode populations against target nematode populations (number/litre soil

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

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 50% 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 of data on the relationship between time taken to 50% emergence of onion seedlings and nematode numbers present at the start of the experiment are summarised in Table 4.

Table 4. 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 = -0.0003x + 16.595	0.388	0
Stubby root*	N/A	N/A	N/A
Stunt/spiral	y = -0.0003x + 18.454	0.027	13.8

*It was not possible to measure time to 50% emergence in the stubby root nematode experiment as very rapid emergence meant that all pots were beyond 50% emergence at the first assessment date

Overall there was no clear relationship between the time to 50% seedling emergence and actual nematode population.

The results of analyses of data on the relationship between area under the curve of seedling emergence against time and nematode numbers present at the start of the experiment are summarised in Table 5.

Table 5. 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**
Root lesion	y = 0.021x + 238.700	0.020	15.4
Stubby root	y = 0.007x + 82.570	<0.001	42.5
Stunt/spiral	y = 0.008x + 178.600	<0.001	39.9

Overall there was no clear relationship between the area under the seedling emergence against time curve (AUC) and actual nematode population. These data are shown as graphs in Figure 2.

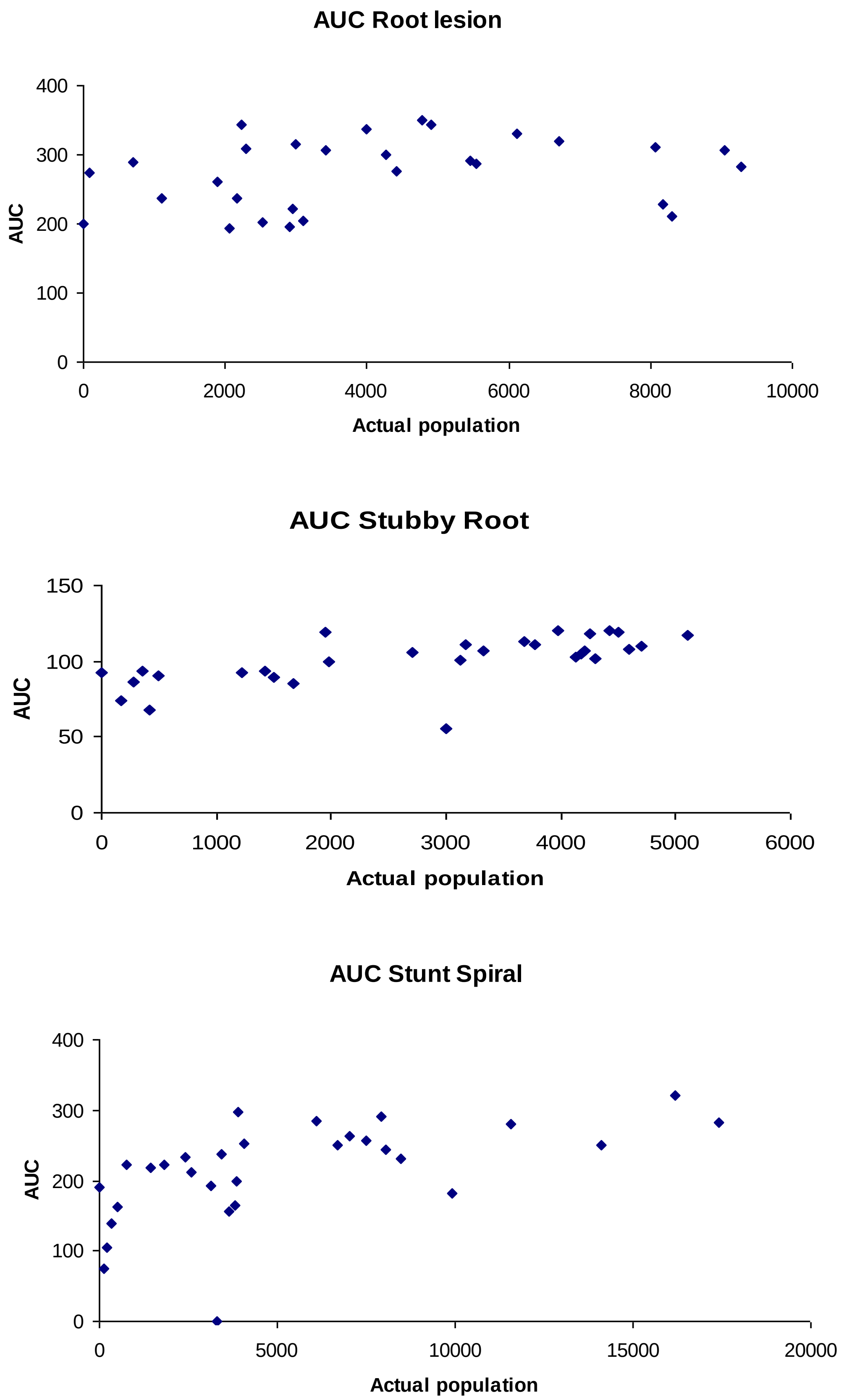

Figure 2. Relationship between area under the seedling emergence against time curve (AUC) and actual nematode population (number/litre soil) at the start of the experiment.

The results of analyses of data on the relationship between mean onion dry matter yield/plant and nematode numbers present at the start of the experiment are summarised in Table 6.

Table 6. 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**
Root lesion	y = 0.00004x + 0.2405	0.183	3.0
Stubby root	y = 0.000009x + 0.1032	0.095	6.4
Stunt/spiral	y = -0.000005x + 0.5641	0.648	0

Overall there was no clear relationship between onion dry matter yield and actual nematode population.

Objective 2: Analysis of historic sampling data

Over the period October 2000 until December 2010 a total of 11,733 soil samples were received by ADAS Pest Evaluation Services (PES) for extraction of free-living nematodes. All samples were subjected to two extraction methods, the Seinhorst two-flask technique (Seinhorst, 1955) for small- to medium-sized nematodes and the Flegg-modified Cobb technique (Flegg, 1967) for large nematode species. The combination of these two methods is considered to give the best estimate of nematode numbers in a soil sample. In each sample eight main nematode groups were identified and counted and numbers presented as numbers/litre soil. These nematode counts were used to advise farmers/growers and agronomists of the likelihood of the need for control (usually a nematicide) based on knowledge of the following crop and whether guideline thresholds were exceeded. Details of the nematode groups identified and counted and the guideline thresholds is given in Table 7.

Table 7. Nematode groups identified and counted by PES, typical species and guideline thresholds (number/litre soil)

Nematode group	Typical species	Guideline threshold
Cyst juveniles*	*Heterodera* spp *Globodera* spp	-
Dagger nematodes	*Xiphinema* spp	50
Needle nematodes	*Longidorus* spp	50
Root-knot nematodes	*Meloidogyne* spp	Presence
Root lesion nematodes	*Pratylenchus* spp	2500
Stem nematode	*Ditylenchus dipsaci*	Presence
Stubby root nematodes	*Trichodorus* spp *Paratrichodorus* spp	200
Stunt/spiral nematodes	*Helicotylenchus* spp *Rotylenchus* spp	10,000

* Cyst juveniles cannot be identified easily in the free-living state and previous cropping history is used as a potential indicator of the species present. For example, in a field previously cropped with potatoes the cyst juveniles could be potato cyst nematodes which would be potentially damaging to future potato crops.

All nematode counts from October 2000 to December 2010 were analysed to determine any trends or patterns in nematode incidence over the 10-year period. This included the relative abundance of each nematode group, the range of numbers encountered, the proportion of samples in which the guideline threshold was exceeded and any possible trends in nematode counts. The results of these analyses are discussed in this section.

Incidence of nematode groups by year

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 (Table 8). The proportion of samples in which these nematodes were recovered was stunt/spiral nematodes 98.6%, root lesion nematodes 94%, stubby root nematodes 72.2%, needle nematodes 38%, cyst juveniles 31.9%, dagger nematodes 0.7%, stem nematode 0.5% and root-knot nematodes 0.1%.

Overall there was relatively little variation in the proportion of samples infested with each nematode group between years. The greatest variability was for needle nematodes which showed a range of 25.2% between the highest and lowest incidence years. Stubby root nematodes showed a 23.8% difference between the highest and lowest incidence years and for cyst juveniles the equivalent range was 14.3%. In all other nematode groups the range between highest and lowest incidence years was less than 5%. In general, there were no clear trends between years to suggest that the incidence of free-living nematodes in soil samples was either increasing or decreasing.

For each free-living nematode group (except stem nematode and root-knot nematode) the proportion of times that group was recovered from soil samples in between 2001 and 2010 were ranked for each year. For example, if needle nematodes were recovered least often in 2007 that year was given a score of 1 and where they were recovered most often the year was given a score of 10. Other years were given scores between 1 and 10 depending upon nematode incidence. Stem nematode and root-knot nematode were excluded from this analysis as they spend most of their life cycle within the plant and so are less directly affected by soil conditions. The year 2000 was also omitted as sample numbers were much lower than for other years.

In general, cyst juveniles, dagger, needle, root lesion and stunt/spiral nematodes were recorded least often in 2007 and most often in 2004. There was also a trend for these nematodes to be recovered most often between 2001 and 2004 and least often between 2005 and 2010. Stubby root nematodes were the main exception to these trends as they were least common in 2006 and most common in 2003. Also these nematodes were relatively less frequently extracted between 2001 and 2004 than most other groups.

Table 8. Relative ranking of nematode group incidence between 2001 and 2010. (1 = year in which nematode group was least frequently recovered, 10 = year in which nematode group was most frequently recovered.

Year	**Nematode group**						
	Cyst juveniles	**Dagger**	**Needle**	**Root lesion**	**Stubby root**	**Stunt/spiral**	**Total**
2001	9	9	8	6	3	4	39
2002	10	10	7	8	2	8	45
2003	3	6	9	7	6	10	41
2004	8	8	10	10	10	7	53
2005	2	3	3	4	9	3	24
2006	7	5	6	3	5	1	27
2007	1	1	1	1	8	2	14
2008	4	6	5	1	7	5	28
2009	5	4	3	5	4	5	26
2010	6	2	2	8	1	9	28

Table 9. Free-living nematodes 2000-2010: Number and proportion of samples in which presence detected

Year	Total samples	Samples in which presence detected	
		Number	Proportion (%)
Cyst juveniles			
2000	91	32	35.2
2001	894	343	38.4
2002	901	354	39.3
2003	889	270	30.4
2004	947	363	38.3
2005	1334	362	27.1
2006	1281	446	34.8
2007	2426	607	25.0
2008	991	319	32.2
2009	1367	443	32.4
2010	612	209	34.2
Total	11733	3748	31.9
Dagger			
2000	91	1	1.1
2001	894	15	1.7
2002	901	16	1.8
2003	889	9	1.0
2004	947	12	1.3
2005	1334	4	0.3
2006	1281	6	0.5
2007	2426	3	0.1
2008	991	10	1.0
2009	1367	5	0.4
2010	612	1	0.2
Total	11733	82	0.7
Needle			
2000	91	49	53.8
2001	894	405	45.3
2002	901	388	43.1
2003	889	457	51.4
2004	947	498	52.6
2005	1334	454	34.0
2006	1281	490	38.3
2007	2426	696	28.7
2008	991	378	38.1
2009	1367	465	34.0
2010	612	177	28.9
Total	11733	4457	38.0
Root-knot			
2000	91	0	0
2001	894	1	0.1
2002	901	0	0
2003	889	0	0
2004	947	0	0
2005	1334	23	1.7
2006	1281	8	0.6
2007	2426	70	2.9
2008	991	3	0.3
2009	1367	49	3.6
2010	612	10	0.2
Total	11733	164	0.1

Table 9 (cont'd)

Root lesion			
2000	91	13	14.3
2001	894	41	4.6
2002	901	47	5.2
2003	889	43	4.8
2004	947	51	5.3
2005	1334	52	3.9
2006	1281	30	2.3
2007	2426	35	1.4
2008	991	14	1.4
2009	1367	58	4.2
2010	612	32	5.2
Total	11733	416	3.5
Stem nematode			
2000	91	0	0
2001	894	0	0
2002	901	0	0
2003	889	0	0
2004	947	0	0
2005	1334	2	0.1
2006	1281	11	0.9
2007	2426	8	0.3
2008	991	4	0.4
2009	1367	24	1.8
2010	612	4	0.7
Total	11733	53	0.5
Stubby root			
2000	91	72	79.1
2001	894	614	68.7
2002	901	573	63.6
2003	889	626	70.4
2004	947	794	83.8
2005	1334	1052	78.9
2006	1281	891	69.6
2007	2426	1805	74.4
2008	991	724	73.1
2009	1367	944	69.1
2010	612	372	60.8
Total	11733	8467	72.2
Stunt/spiral			
2000	91	91	100.0
2001	894	884	98.9
2002	901	898	99.7
2003	889	888	99.9
2004	947	940	99.3
2005	1334	1305	97.8
2006	1281	1245	97.2
2007	2426	2369	97.7
2008	991	983	99.2
2009	1367	1356	99.2
2010	612	611	99.8
Total	11733	11570	98.6

In summary, stunt/spiral and root lesions nematodes were the most commonly recovered nematode species, found in at least 94% of samples. In contrast, dagger, root-knot and stem nematodes were rarely found and were present in less than 1% of samples.

Maximum and minimum counts of nematode groups and range

The minimum count for all nematode groups was zero indicating that each group was absent from at least one sample between 2000 and 2010 (Table 10). Only 1.4% of all samples did not contain any stunt/spiral nematodes and root lesion nematodes were not present in 5.8% of samples. Stubby root nematodes were not present in 27.8% of samples. These were the most commonly recovered nematodes as previously discussed. 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). However, counts of this magnitude for these groups were very rare and in at least 90% of samples none was found. The maximum and minimum count together with the numbers of nematodes within 90% of the maximum to minimum range give a good indication of how many nematodes of each group are likely to be found in most soil samples. These data confirm the relative rarity of dagger nematodes, root-knot nematodes and stem nematodes even though very high counts of some species were recorded.

Table 10. Free-living nematodes 2000-2010: Numbers detected and range

Nematode group	**Number/litre**		**Proportion of samples with '0'**	**Numbers of nematodes comprising 90% of max-min range**
	Min	**Max**		
Cyst juveniles	0	8,500	69.8	125
Dagger nematodes	0	1,625	99.3	0
Needle nematodes	0	7,725	61.9	75
Root-knot nematodes	0	15,750	98.6	0
Root lesion nematodes	0	12,125	5.8	1500
Stem nematode	0	20,325	99.5	0
Stubby root nematodes	0	8,975	27.8	925
Stunt/spiral nematodes	0	33,975	1.4	4888

Proportion of nematode counts above threshold

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. These data are presented in Figures 3-7 for dagger nematodes, needle nematodes, root lesion nematodes, stubby root nematodes and stunt/spiral nematodes respectively. 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 is also shown in Figure 7.

In all these figures data for 2000 should be treated with caution as only 91 samples were analysed in this year. This could skew analyses of the proportion of sites over threshold for each nematode group. Consequently, 2000 has been omitted from subsequent discussion of results and is only included for completeness.

In view of the relative scarcity of dagger nematodes in soil samples it is not surprising that numbers rarely exceed threshold. Between 2000 and 2010 only 0.6% of samples or less had threshold counts of this species (Figure 3).

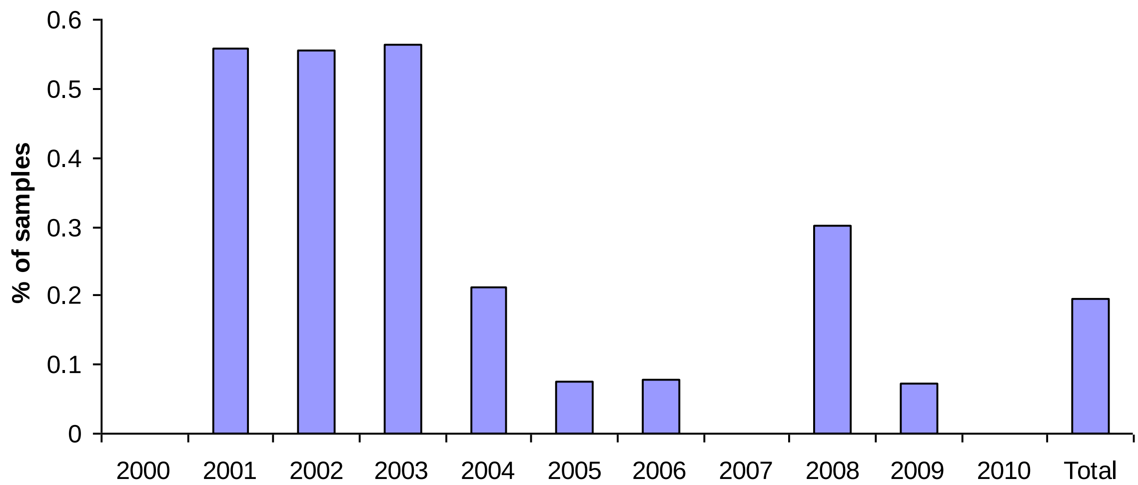

Figure 3. Proportion of samples (%) in which dagger nematode numbers exceed the 50/l soil threshold, 2000-2010 (Total = all sites over threshold 2000-2010)

Threshold counts of needle nematodes are much more common than for dagger nematodes (Figure 4) despite the threshold for both species being 50 nematodes/l soil. This reflects the relatively higher incidence of needle nematodes than dagger nematodes in soil samples. The proportion of samples over threshold ranged between 7.3% in 2007 and 23.7% in 2003. Over all years 14.1% of soil samples had counts of needle nematodes above the 50/l soil threshold.

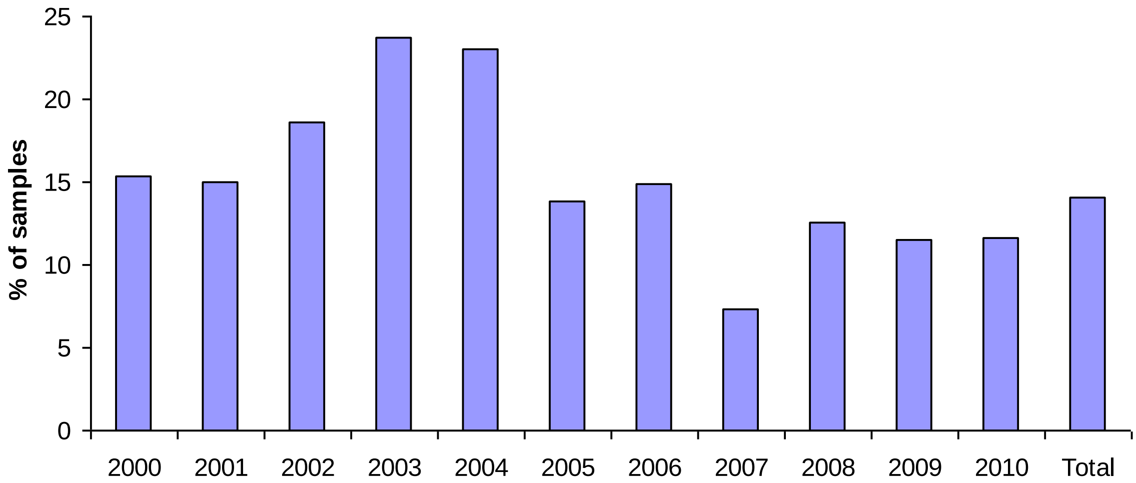

Figure 4. Proportion of samples in which needle nematode numbers exceed the 50/l soil threshold, 2000-2010 (Total = all sites over threshold 2000-2010)

The threshold for root lesion nematodes is 2500 nematodes/l soil. This was exceeded in only 1.4% of samples in 2007 and 2008 but 14.3% of samples in 2000 (Figure 5), and 5.2% in 2002 and 2010. Over all years, 3.5% of soil samples had counts of root lesion nematodes in excess of the threshold.

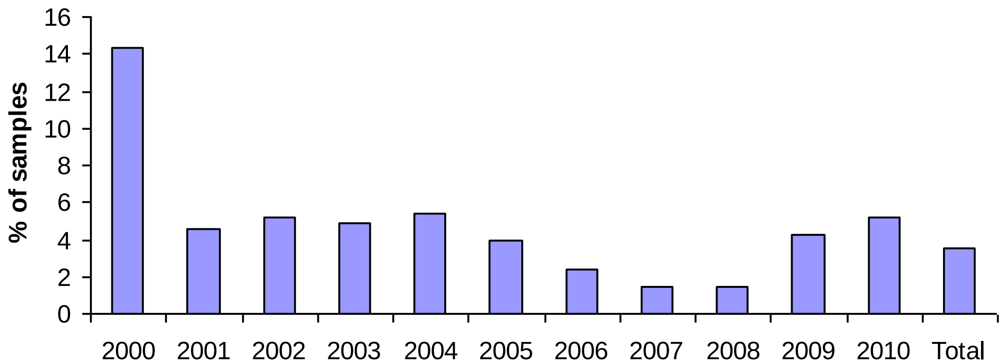

Figure 5. Proportion of samples in which root lesion nematode numbers exceed the 2,500/l soil threshold, 2000-2010 (Total = all sites over threshold 2000-2010)

The proportion of soil samples which exceeded the threshold for stubby root nematodes is given in Figure 6. Data are presented for both the 250 and 1000 nematodes/l soil thresholds as previously discussed.

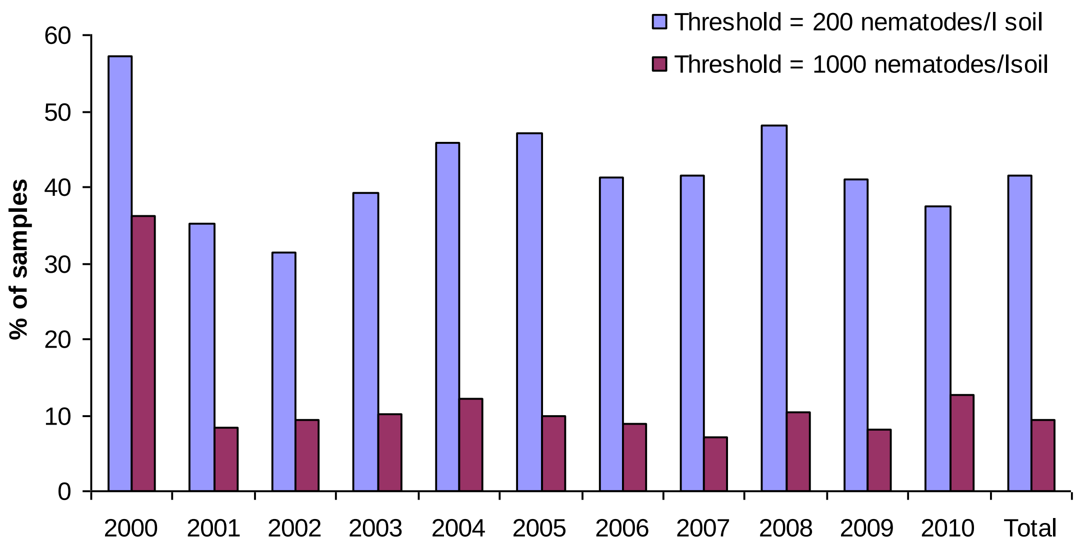

Figure 6. Proportion of samples (%) in which stubby root nematode numbers exceed threshold, 2000-2010 (Total = all sites over threshold 2000-2010)

The proportion of sites over the 200 nematodes/l threshold ranged between 31.1% in 2002 and 48.0% in 2008. Over all years, the proportion of sites over threshold was 41.5%. If the threshold is increased to 1000 nematodes/l soil then the proportion of samples above this is greatly reduced and ranges between 7.0% in 2007 and 12.6% in 2010. Over all years, the proportion over threshold is 9.4% which represents an approximate 75% decrease in sites over threshold compared with data for the 200 nematodes/l threshold. This would have a potentially significant impact on nematicide use.

The guideline threshold for stunt/spiral nematodes is 10,000/l soil. Therefore it is not surprising that threshold counts of this nematode are rarely recorded (Figure 7). The proportion of sites over threshold range from 0.9% in 2005 to 4.1% in 2004. In most years less than 1% of sites had threshold counts of stunt/spiral nematodes.

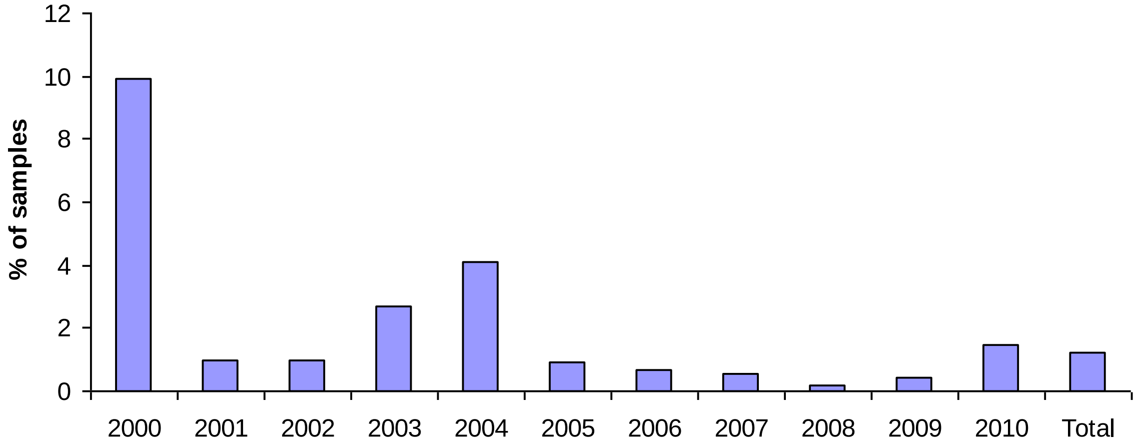

Figure 7. Proportion of samples (%) in which stunt/spiral nematode numbers exceed the 10,000/l soil threshold, 2000-2010 (Total = all sites over threshold 2000-2010)

Objective 3: Monitoring vertical distribution of free-living nematodes

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 11.

Stubby root nematodes were only recovered from Barshalls 1. Both stunt/spiral and root lesion nematodes were found at all sites and cyst juveniles at Euximoor 4 and Pickle Fen 1. Overall the combination of sites provides a good range of the most commonly recorded nematode groups in UK soil.

Table 11. 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

Figure 8. Data logger in situ at Christchurch, Cambridgeshire

The dataloggers were located at each site in August 2010 and the first monthly readings and soil samples started in September 2010. Once a years-worth of data has been collected this will be analysed in relation to soil moisture and temperature and the results reported in the final report for this project

Discussion

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

Soil dilution proved to be a very effective way of creating a range of populations of different nematode groups. For both root lesion and stunt/spiral nematodes the actual population was very close to the target population. Actual numbers of root lesion nematodes at the start of the experiment were approximately 2.5 times as high as the target population and this is very difficult to explain. It is possible that the increased numbers were due to nematode reproduction but by the end of the experiment the actual population was similar to that of the target population.

It is very interesting that none of root lesion nematodes, stubby root nematodes or stunt/spiral nematodes had any significant effect on onion growth. It would be expected that onions would be most susceptible to the effect of nematode feeding at the seedling stage as the root system is beginning to develop. However, there was no effect of increasing nematode numbers on seedling emergence or on the total area under the graph of seedling emergence against time.

There are no recognized thresholds for free-living nematodes in onions but as a guide about 2500 root lesion nematodes, 200 stubby root nematodes and 10,000 stunt/spiral nematodes per litre soil are thought to be potentially damaging. Numbers of nematodes in the created populations were well in excess of these thresholds at both the start and end of the experiment. This suggests that the current thresholds are too conservative and well below the number of nematodes which can be tolerated by the crop. These results were unexpected and if there is sufficient resource, repeat studies will be undertaken in year two of the project to confirm the results from year one.

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.

Objective 2: Analysis of historic sampling data

Over the period 2000-2010 the most frequently extracted free-living nematode groups were stunt/spiral nematodes, followed by root lesion nematodes, stubby root nematodes, needle nematodes, cyst juveniles, dagger nematodes, stem nematodes and root-knot nematodes. Stem nematodes (*D. dipsaci*) are potentially most damaging to an onion crop and these were only recovered in 0.5% of samples. As stem nematode spends most of its life within the plant it can be difficult to recover from soil. Recent work (HDC project FV 327 Onions: improving risk assessment for stem nematode) indicated that the Seinhorst two-flask technique is the most effective extraction method for this pest in soil. Soil analysis cannot prove that the pest is absent from a field but should identify fields at high risk from attack. Therefore it can be concluded that fields at high risk from stem nematode are relatively rare.

Equally, root-knot nematode is also a potential threat to onions but is also relatively rare in the UK.

Of the true free-living nematode species, stubby root nematodes and needle nematodes are potentially the most damaging. These nematode groups are much more commonly found than either stem nematode or root-knot nematode. Stubby root nematodes were found in approximately three quarters of samples and needle nematodes in just over a third of samples.

In general, there was relatively little variation in the proportion of nematode samples infested with each nematode group between 2000 and 2010.

Nematode recovery from soils might be expected to show greatest variation with annual rainfall as these pests require sufficient soil moisture to remain mobile. Most nematodes were recorded least often in 2007 but rainfall in this year was above average. Rainfall in England between 2000 and 2010 was only below average in 2003, 2005 and 2010. Therefore it seems likely that other factors apart from soil moisture were having a greater impact on nematode recovery. In general, trends in nematode incidence did not suggest that the risk posed by these pests is either increasing or decreasing.

The highest nematode count recorded between 2000 and 2010 was 33,975/l soil for stunt/spiral nematodes. Overall, these nematodes were the most commonly extracted from soil samples and also the most numerous. Despite stem nematodes and root-knot nematodes being rarely recovered from soil samples, when present these pests can occur in significant numbers. After stunt/spiral nematodes, stem nematode and root-knot nematode were recovered in greatest numbers. The occurrence of such high counts of these nematodes is probably dependent upon whether soil sampling encounters a dense aggregation in the field.

Data for the proportion of sites above current guideline thresholds for each nematode group shows that stubby root nematodes are most likely to exceed these 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. The nematode numbers at which the threshold is set will clearly have a significant influence on the area of crop treated with nematicide. If the stubby root nematode threshold is increased to 1000/l soil, as suggested by previous work (Ellis, unpublished data), 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.

Conclusions

- Pot experiments suggest that populations of stubby root, root lesion and stunt/spiral nematodes well above current guideline thresholds have no effect on onion growth.

- Results suggest that significant savings can be made on nematicide use.

- Stunt/spiral nematodes are most commonly recovered from soil samples followed by root lesion nematodes, stubby root nematodes, needle nematodes, cyst juveniles, dagger nematodes, stem nematodes and root-knot nematodes.

- Stem nematodes and root-knot nematodes are potentially important pests of onions but were recovered on average in less than 1% of soil samples.

- There was no evidence from historic sampling records that free-living nematode numbers are increasing or decreasing.

- The highest count of free-living nematodes was for stunt/spiral nematodes. There were also high individual counts of stem and root-knot nematodes even though these nematodes were only recovered from 0.5% of samples.

- Stubby root nematodes were most likely to exceed the current guideline threshold but results suggest that this is set at too conservative a level. Based on pot experiments, all but exceptionally high nematode numbers would require nematicide treatment.

- Stem nematode and root-knot nematode remain the main nematode threat to the onion crop.

Knowledge and Technology Transfer

To date there has been limited scope for technology transfer as the experimental work has been ongoing. A potential article for HDC News will be discussed on completion of this annual report.

Glossary

AUC – Area under the curve of seedling emergence against time used to compare onion seedling emergence in pots infested with different numbers of free-living nematodes.

Data logger – A piece of equipment that collects data (in this case soil moisture and temperature) remotely in the field

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.

Endoparasite – A nematode that spends virtually all its time within the plant such as stem nematode.

Flegg modified-Cobb technique – A method of extracting free-living nematodes from soil. Specifically designed for large species such as needle and dagger nematodes.

Free-living nematode – A nematode which spends its life in the soil and does not invade plant roots e.g. stubby root, root lesion and stunt/spiral nematodes

Regression analyses – A statistical technique designed to determine whether there is a relationship between two variables that can be described by a straight line or a curve

Seinhorst two-flask technique – A method of extracting free-living nematodes from soil specifically designed for small to medium sized species such as stubby root and root lesion nematodes.

Semi-endoparasite – A nematode which spends part of its life within the plant such as root-knot nematode

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