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| **Project title:** | Leeks: improving risk assessment for free-living nematodes |
| **Project number:** | FV 377a |
| **Project leader:** | Steve Ellis, ADAS |
| **Report:** | Final report, September, 2012 |
| **Previous report:** | N/A |
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| **Location of project:** | ADAS, High Mowthorpe |
| **Industry Representative:** | Philip Lilley of Hammond Produce Ltd |
| **Date project commenced:** | 1 April 2011 |
| **Date project completed (or expected completion date):** | 30 September 2012 |

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# 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.

Steve Ellis

Entomologist

ADAS

Signature ............................................................ Date ............................................

**Report authorised by:**

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Horticulture Research Manager

ADAS

Signature ............................................................ Date ............................................

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# CONTENTS



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# GROWER SUMMARY

## Headline

- Pot experiments indicate that threshold levels to control nematodes in leeks are higher than the guidelines so potentially, if the results reflect the field situation, the management of nematodes in leeks can be altered.

## Background

Free-living nematodes (FLN) are important pests of leeks as they can reduce crop vigour and growth. Currently there is no approved chemical control option for these pests. Vydate 10G has a SOLA (EAMU) for use on bulb onions and shallots (SOLA 20061890) and there was an EAMU for use on leeks which has now lapsed. While it may be possible to pursue a SOLA for leeks again, 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 leeks can be grown in the absence of nematicide without the significant risk of nematode damage.

The risk from free-living nematodes is currently assessed by considering field history, previous cropping and representative soil testing for the pests. There is however little information available on the species that are most damaging to leeks and at what level they pose a risk. With pressure from retailers to reduce nematicide use in general, the ability to predict those fields at risk from FLN using the results of reliable soil testing will become increasingly important.

The first and most fundamental component of risk assessment is to understand the nematode infestation level that justifies treatment. Currently, guideline thresholds for leeks 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 numbers per volume or weight of soil. This work was done in tandem with a similar study in onions FV 377 Onions: improving risk assessment for free-living nematodes an approach which allowed much more cost-effective use of research funding and sharing of resource between experiments.

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 nematode species.

This project aims to provide thresholds for FLN in leeks to help with interpreting the results of soil analysis and predicting where the crop can be grown with minimal risk of FLN

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damage. 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 objective of this project is as below:

1. To measure the effect of different populations of stubby root, needle, stunt/spiral and root lesion nematodes on the growth of leeks, in order to determine which species are potentially most damaging.

## Summary

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

A range of populations of needle, 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 sterilised by heating to 60ºC for 30 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.** Target and actual population ranges for root lesion, stunt/spiral and stubby root nematodes to be achieved by soil dilution – 2012

| Nematode group | Provisional threshold (no/litre soil) | Target population range (no/litre soil) | Actual population range (no/litre soil) |
|---|---|---|---|
| Needle | 50 | 0 – 810 | 0 - 1035 |
| Root lesion | 2,500 | 0 – 6,960 | 0 - 4197 |
| Stubby root | 200 | 0 – 1,900 | 0 - 1175 |
| Stunt/spiral | 10,000 | 0 – 2,700 | 0 - 7225 |

The target populations were made up in 1.5 l pots and sown with 20 leek seeds (cv. Belton or Phuston). Pots were maintained in a polythene tunnel and watered as necessary. Numbers of stunt/spiral nematodes were lower than the provisional threshold. A count of 2,700/l soil is, however, higher than has been recorded in 73% of samples over the last 10 years and therefore represents a higher than average population of the pest. The highest population created of stunt spiral nematode was 7225/l, which is higher than 96% of

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samples analysed over the last 10 years. Actual population of stunt spiral nematode can therefore be considered a significant infestation.

Nematode numbers were also assessed to determine how the actual populations compared with the target populations.

For needle nematodes the target population was very close to the actual population. For root lesion and stubby root nematodes the actual population was just over half of the target population and for stunt/spiral nematodes the actual population was almost twice that of the target population.

To assess the impact of nematode populations on leek growth seedling emergence was monitored daily and leek dry matter yield measured. There was no obvious effect of populations of needle, root lesion, stubby root or stunt/spiral nematodes on leek growth or yield. These results suggest that current guideline thresholds for free-living nematodes are far too conservative and that the crop can tolerate much higher populations of these pests. If these results reflect the field situation, potentially nematicide use in leeks could be reduced, which could greatly improve crop profitability.

## 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 nematodes. This could potentially realise a saving 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. These nematodes are only rarely recovered but can have a significant impact on the crop if present. With the exception of stem nematode, the majority of other free-living species appear to have limited effect on leek 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.

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## SCIENCE SECTION

### Introduction

Free living nematodes (FLN) are important pests of leeks as they can reduce crop vigour and growth. Currently there is no approved chemical control option for these pests. Vydate 10G has a SOLA (EAMU) for use on bulb onions and shallots (SOLA 20061890) and there was a SOLA for use on leeks which has now lapsed. While it may be possible to pursue an EAMU for leeks again, 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 leeks can be grown in the absence of nematicide without the significant risk of nematode damage.

The risk from free-living nematodes is currently assessed by considering field history, previous cropping and representative soil testing for FLN. However, there is little information available on those free-living nematodes that are most damaging to leeks and at what level they pose a risk. With pressure from retailers to reduce nematicide use in general, the ability to predict those fields at risk from FLN using the results of reliable soil testing will become increasingly important.

This project will investigate the most fundamental component of risk assessment which is to understand the nematode infestation level that justifies treatment. Currently, guideline thresholds for leeks 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.

### Rationale for study

The Assured Produce protocol for leeks 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.

This project aims to improve the confidence of growers and agronomists when interpreting the results of soil sampling for free-living nematodes in order to decide on the suitability of the land for cropping with leeks and the need for nematicide treatment. In future, protecting crops from free-living nematode damage will become increasingly reliant on integrated

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strategies that combine cultural and chemical control. Robust risk assessment will be fundamental to the success of such IPM programmes.

The specific objective of this project is as below:

1. To measure the effect of different populations of needle, root lesion, stubby root and stunt/spiral nematodes on the growth of leeks, in order to determine which species are potentially most damaging.

## Materials and methods

### *Objective 1: Pot experiments to establish the most damaging nematode species to leeks*

Approximately 75 kg of field soil was collected from sites known to be infested with needle nematodes, root lesion nematodes, stubby root nematodes and stunt/spiral nematodes. These sites were selected from the ADAS Pest Evaluation Services database which contains 10 years of data on soil analysis for free-living nematodes from sites in England and Wales. The soil was collected using spades to sample to a depth of approximately 15 cm at a range of points across the field and placed in plastic dustbins. The bins were returned to the laboratory and sampled to determine the level of infestation by the target nematodes. This was done using a 15 cm deep x 2 cm diameter cheese corer to take a total of 20 cores from each bin. Each sample was extracted twice, once using the Seinhorst two flask technique (Seinhorst, 1955) for small medium sized nematodes (e.g. stubby root, root lesion and stunt/spiral nematodes) and once using Flegg modified Cobb technique (Flegg, 1967) for large nematodes (e.g. needle and dagger nematodes).

A range of 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 heating in an oven at 60ºC for 30 minutes. This was done in cotton bags in 5 kg batches. After heating, 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. 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 soil was mixed on a sheet of polythene. This was folded carefully from one side to

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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 the Seinhorst two flask and/or the Flegg modified Cobb technique to check the nematode 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) |
|---|---|---|
| Needle | 50 | 0 – 810 |
| Root lesion | 2,500 | 0 – 6,960 |
| Stubby root | 200 | 0 – 1,900 |
| Stunt/spiral | 10,000 | 0 – 2,700 |

* The provisional threshold is anecdotal and there is no supporting evidence in the literature

Once all the pots had been filled with soil they were labelled and sown with 20 leek seeds (cv. Belton or Phuston) and covered to a depth of 1 cm with spare soil from the original mixture. Prior to sowing the leeks seed from both varieties was subjected to a germination test to ensure it was viable. This involved testing two batches each of 100 seeds. These were placed on four filter papers within a Petri dish. Sufficient water was added to moisten the filter paper. The lid was placed on the Petri dish and this was stored in a dark cupboard at approximately 20 $^{o}$C. Each dish was examined daily and the number of germinated seeds counted and removed.

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 in the needle nematode and root lesion nematode experiments were thinned to 4 per pot and these grown-on to monitor whether there was any further impact of nematodes on growth. This was not done for the stubby root and stunt/spiral nematode experiments as germination and establishment were slow. Leek yield was assessed up to six months after sowing. Plants were harvested and the dry matter yield assessed for both the roots and tops by oven drying at 80ºC for 16

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hours. The pot soil for the root lesion, stubby root and stunt/spiral nematode experiments was extracted using the Seinhorst two flask technique to compare the initial and final nematode population. For the needle nematode experiment the Flegg modified Cobb extraction method was used.

## Results

*Germination test*

The germination test of the onion seed, cv. Belton, showed it to be 97.5% viable the cv. Phuston seed was 92% viable. These were considered sufficiently healthy seed lots 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 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 (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 = 1.1x -88.9 | y = 0.045x + 6.5 | <0.001 | <0.01 | 67.9 | 26.5 |
| Root lesion | y = 0.6x + 217.8 | y = 0.02x + 52.9 | <0.001 | <0.001 | 83.6 | 73.5 |
| Stubby root | y = 0.6x – 21.3 | No sensible fit | <0.001 | N/A | 88.7 | N/A |
| Stunt/spiral | y = 2.0x + 85.6 | y = 0.268x + 114.2 | <0.001 | <0.001 | 55.6 | 51.8 |

Regression analyses showed a highly significant fit between actual and target nematode population for each nematode group at the start of the experiment (P<0.001). For needle nematodes the target population was close to the actual population (Table 4). For root lesion and stubby root nematodes the actual population was just over half of the target

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population and for stunt/spiral nematodes the actual population was almost three times that of the target population (Table 4). It is difficult to explain why numbers of stunt/spiral nematodes were higher than anticipated. It is possible that it was due to nematode reproduction. It is also possible that soil samples taken to assess nematode numbers in the bulked sample were not representative of the whole sample. Bulked samples were stored in dustbins and it is difficult to take samples from the total depth of soil.

The created populations of needle, stubby root and root lesion nematodes were both below and above their current guideline thresholds and so provided a good range over which to test the impact of nematode feeding on leek growth. The guideline threshold for stunt/spiral nematodes is estimated at 10,000/l soil. The target populations did not reach this level with a maximum count of 7225 stunt/spiral nematodes/l soil. This level is still higher than 96% of samples analysed by ADAS Pest evaluation Services over the last 10 years so can still be considered a significant infestation of stunt/spiral nematodes.

**Table 4.** Target and actual nematode populations at the start and end of the experiments

| Nematode group | Start of experiment | | End of experiment | Time of assessment (days after sowing) |
|---|---|---|---|---|
| | Target | Actual | Actual | |
| Needle | 0 - 810 | 0 - 1035 | 0 - 95 | 139 |
| Root lesion | 0 - 6,960 | 0 - 4197 | 0 - 1975 | 131 |
| Stubby root | 0 - 1,900 | 0 - 1175 | 0 - 250 | 225 |
| Stunt/spiral | 0 - 2,700 | 0 - 7225 | 0 - 1150 | 211 |

*Change in nematode numbers during the experiment*

Numbers of needle nematodes showed a big reduction over the duration of the study (Fig 1a). When pots were harvested the maximum count was 95/l soil. As this assessment was made almost five months after the experiment was established, some reduction in nematode numbers would have been expected. It is likely that nematode numbers were still well above the guideline threshold of 50/l soil for the period over which leeks were emerging when they would be most susceptible to attack. Also a count of 95 needle nematodes/l soil is still almost twice the current threshold.

Numbers of root lesion nematodes also declined over the life of the experiment (Fig 1b) although this was not as marked as with needle nematodes. The final maximum count was

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1975/l soil which is higher than 94% of counts recorded in samples extracted by Pest Evaluation Services over the last 10 years (Ellis, 2012). This is still a significant count of root lesion nematodes. As with needle nematodes it is likely that nematode numbers had not declined significantly during the period of leek emergence.

Stubby root and stunt/spiral nematode numbers also declined over the duration of the experiment. This would be expected as the final assessments were done approximately seven months after the pots were initially set up. This was because germination of seedlings (cv. Belton) was poor when seeds were sown in May 2012 and in some pots none emerged. As a result the pots were re-sown with fresh seed (cv. Phuston) in August 2012 and pots were then harvested in November 2012 when the plants had been given sufficient time to grow. It is difficult to estimate nematode populations in pots at the time of the second sowing of leek seed. It is possible that in some pots numbers would have declined as there would have been limited root material available on which nematodes could feed due to the poor growth of seedlings from the first sowing. To estimate numbers would have required destructive sampling of the pots and it was decided that the preferred option was to re-sow pots with leek seed. It is interesting that despite a significant reduction in stubby root nematode numbers the highest population recorded was still above the current threshold for this nematode group.

Graphs of actual against target populations for each nematode group are given in Figures 1b-d.

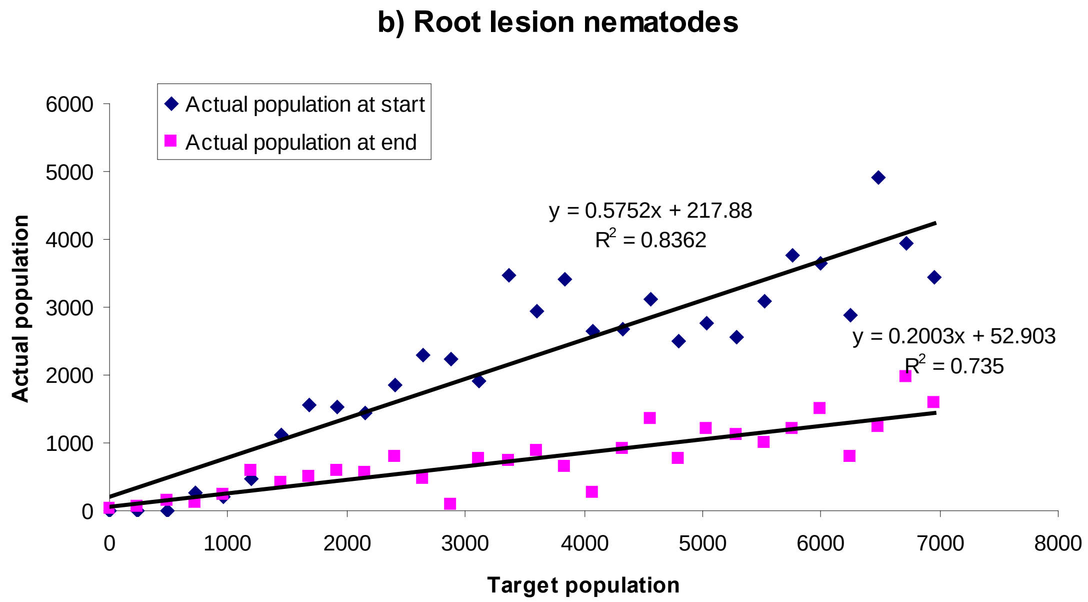


**Figure 1b.** Actual nematode populations against target nematode populations (number/litre soil (n = 30)

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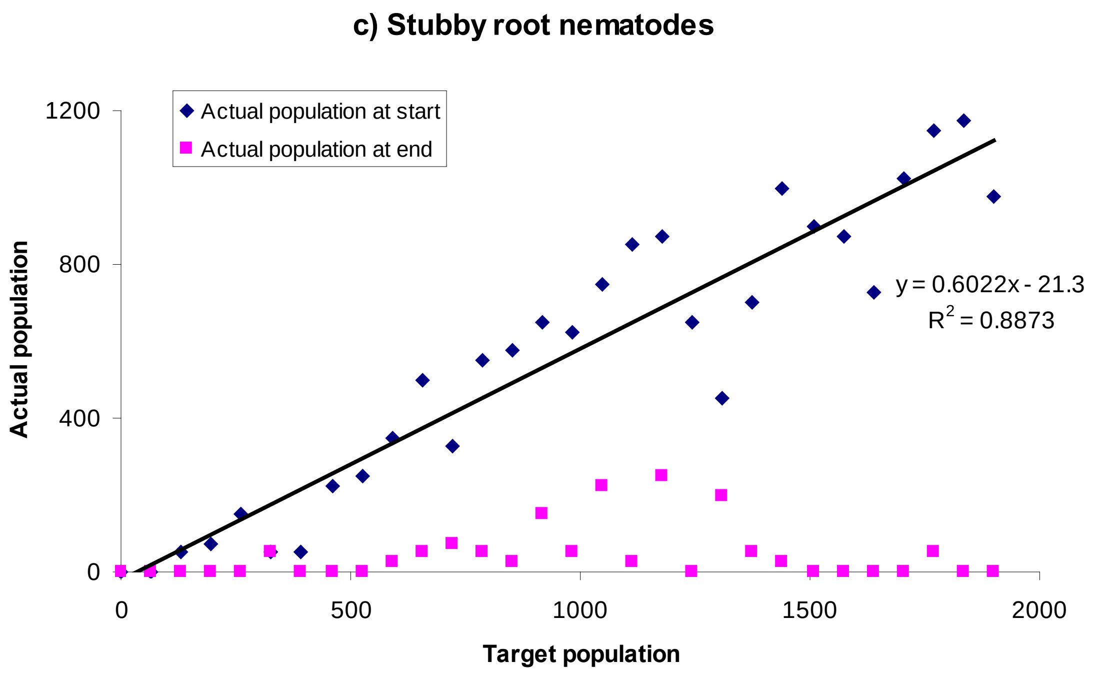


**Figure 1c.** Actual nematode populations against target nematode populations (number/litre soil (n = 30)

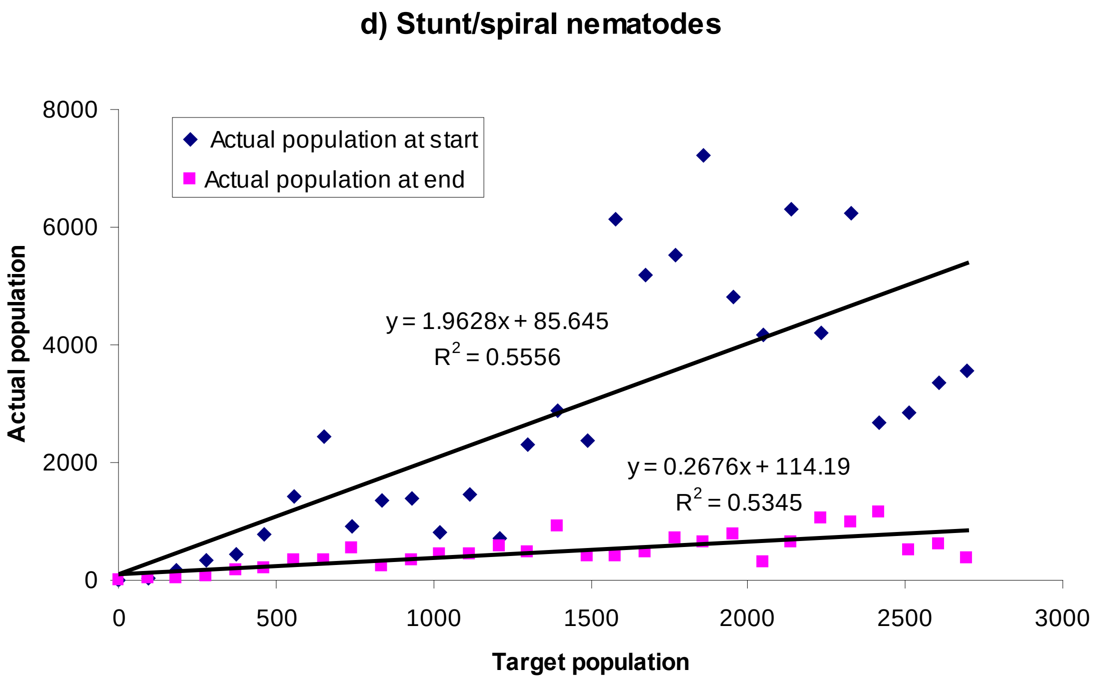


**Figure 1d.** Actual nematode populations against target nematode populations (number/litre soil (n = 30)

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

*Impact of nematodes on leek growth*

Regression analyses were undertaken on datasets for needle and root lesion nematodes to assess the impact of the actual nematode populations at the start of the experiment on leek growth. The variables examined are listed below:

1. 75 % leek seed emergence in the treated pots - The time taken for 75% of leek 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 leek yield - The relationship between mean leek yield per plant and actual nematode number was investigated.

These variables could not be assessed for the first sowing of leeks (cv. Belton) in the presence of stubby root and stunt/spiral as leek growth was poor as previously discussed. The data shown for these two nematode groups below relate to the second sowing of leeks (cv. Phuston)

*75% leek seed emergence*

Overall there was no clear relationship between the time to 75% seedling emergence and actual nematode population for all nematode groups. These data are shown as graphs in Figure 2. It was possible to plot a straight line through the data for stubby root nematodes but this only accounted for about 30% of the variation between data points. Also time to 75% emergence decreased with increasing nematode number.

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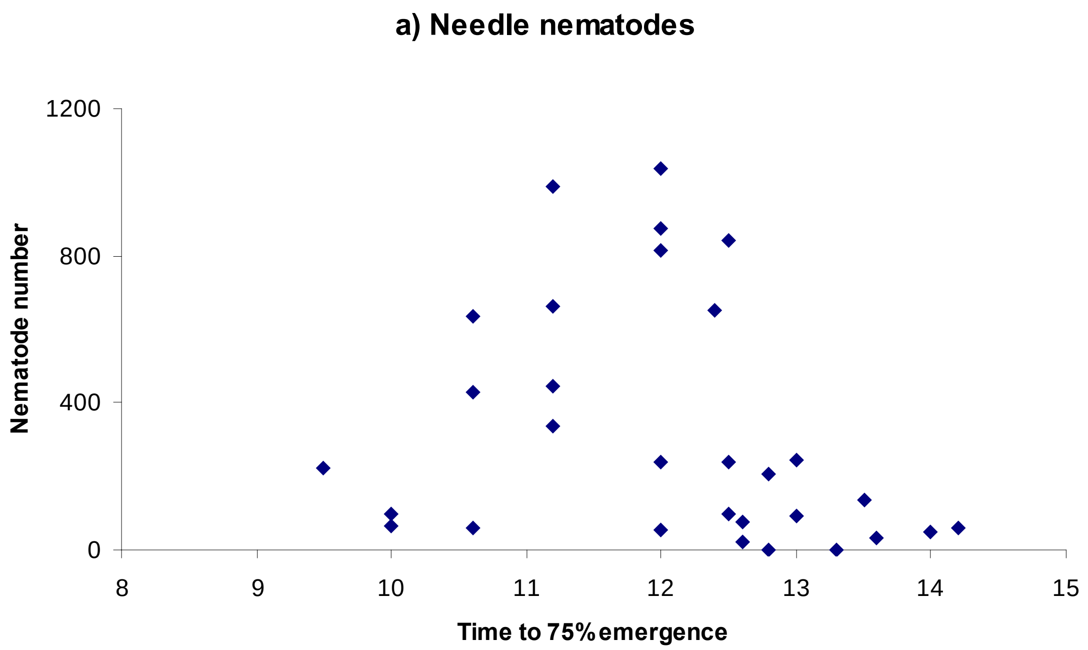


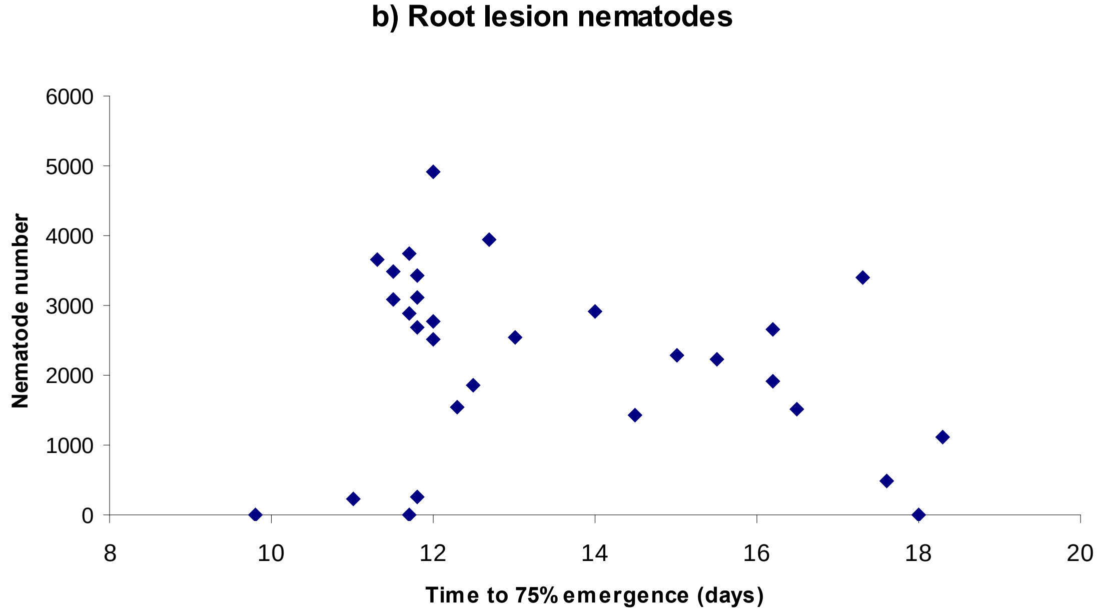


**Figure 2a-b.** Relationship between time to 75% seedling emergence and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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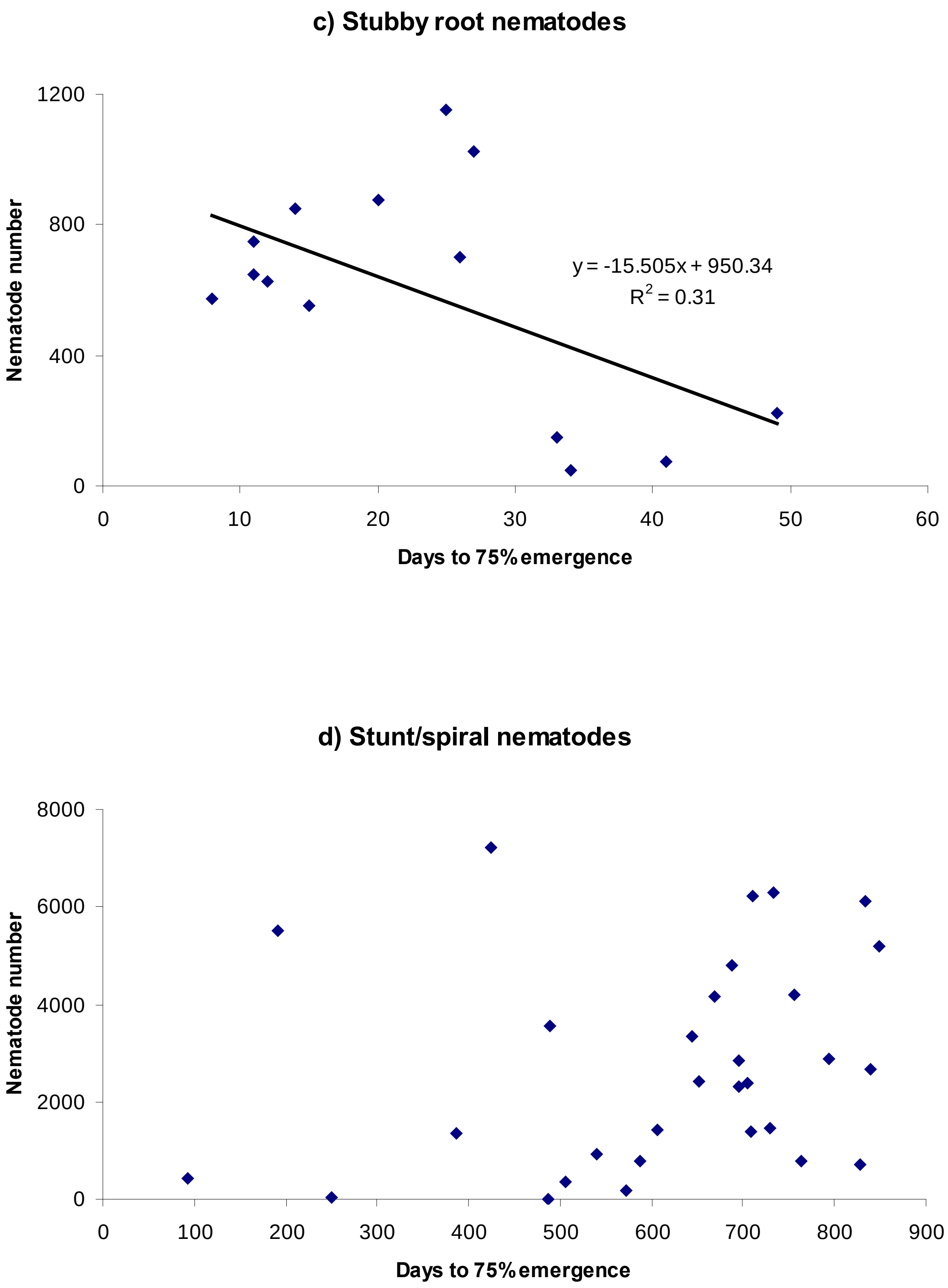


**Figure 2c-d**. Relationship between time to 75% seedling emergence and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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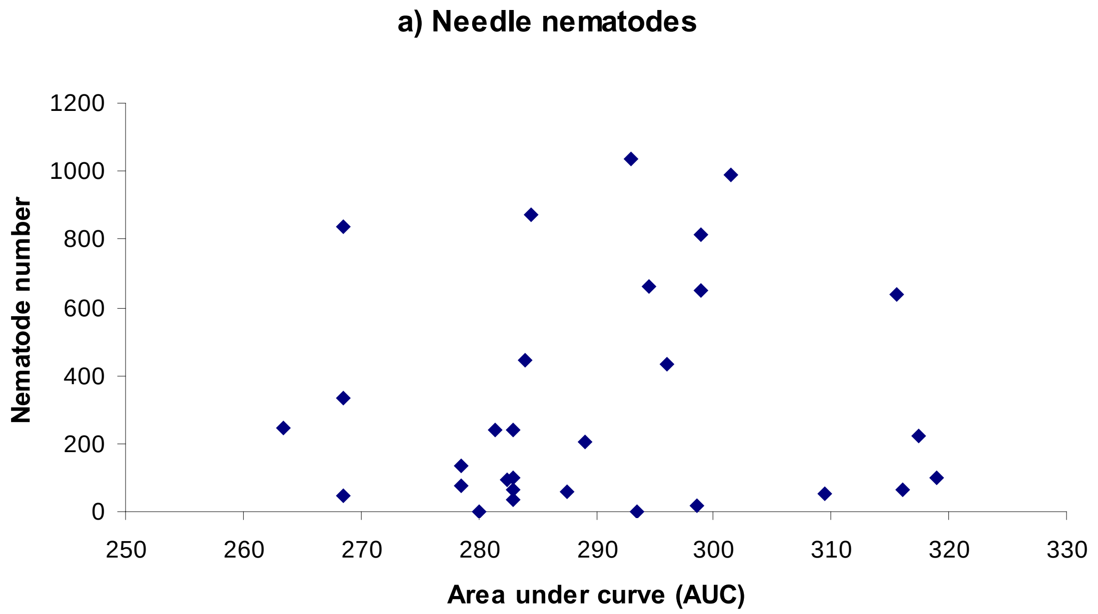
*Area under the seedling emergence curve (AUC)*

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 3.



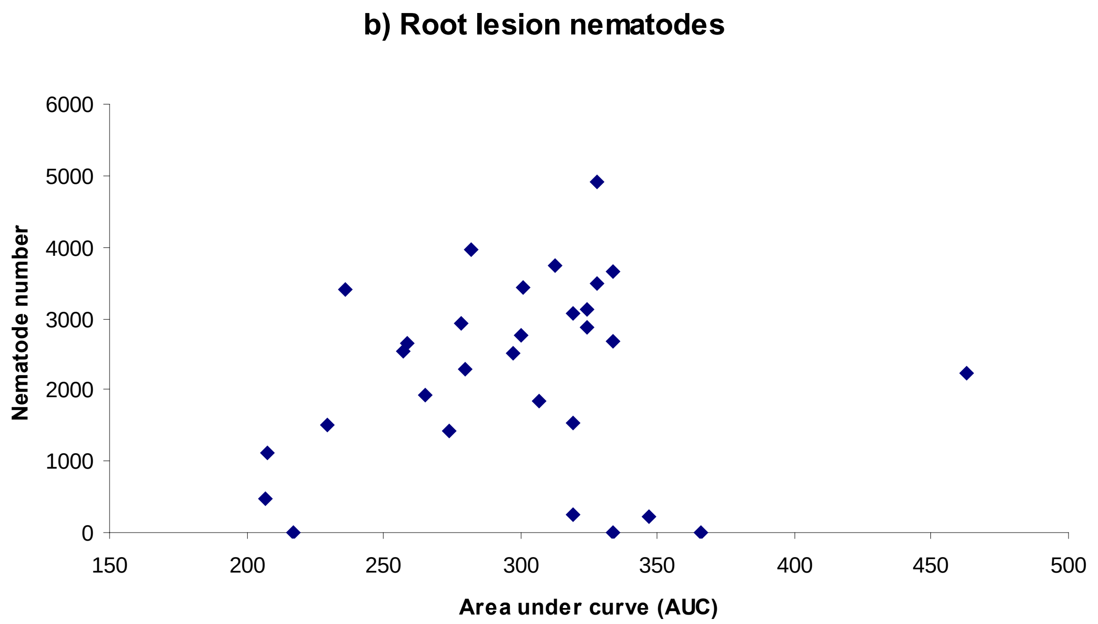




**Figure 3a-b.** Relationship between area under the seedling emergence against time curve (AUC) and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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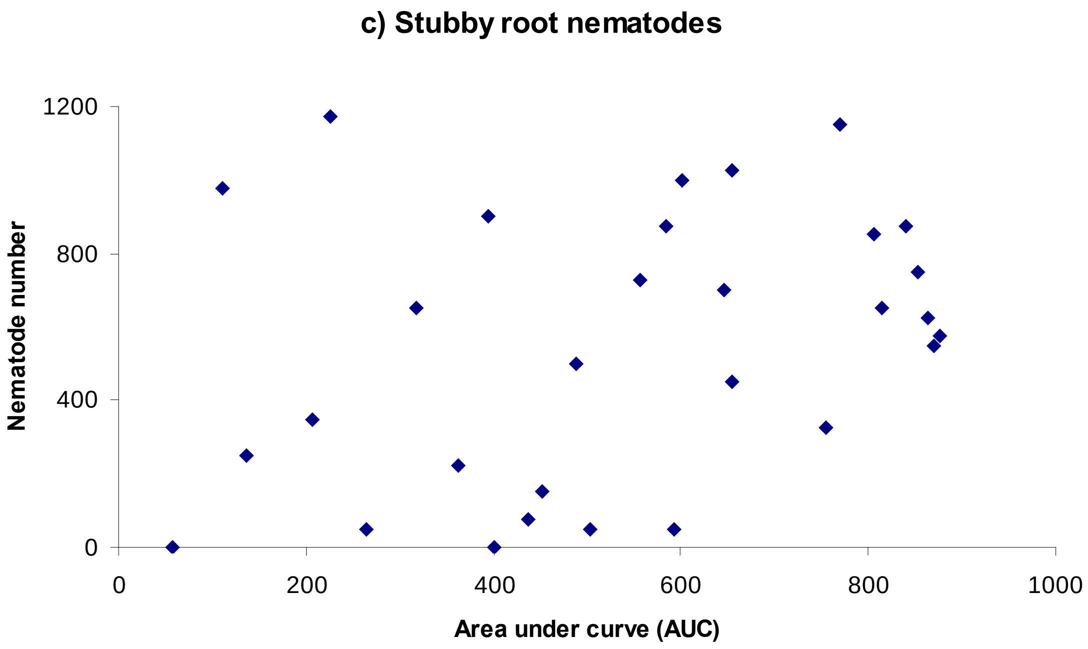


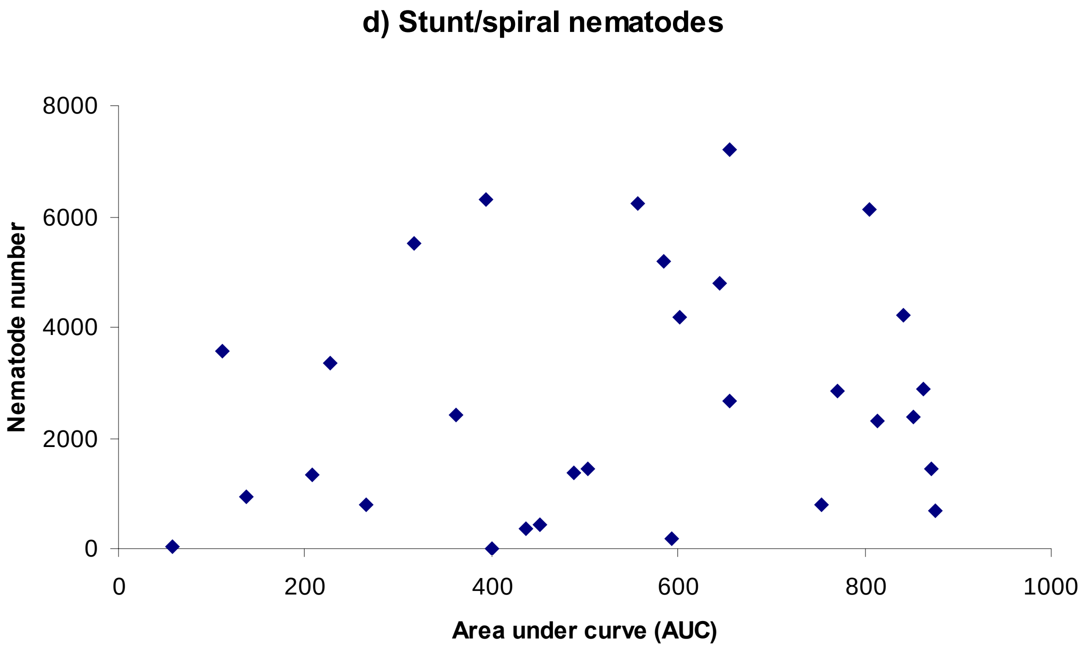


**Figure 3c-d.** Relationship between area under the seedling emergence against time curve (AUC) and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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*Dry matter leek yield*

The results of analyses done using data on mean leek dry matter yield/plant and its relationship with actual numbers of each nematode group at the start of the experiment are summarised in Table 5.

**Table 5**. Results of regression analyses to investigate the relationship between leek dry weight and actual nematode populations at the start of the experiment (x = actual population, y = seedling dry weight)

| Nematode group | Regression line equation | Probability | % variation accounted for |
|---|---|---|---|
| Needle | y = 0.000018x + 0.2394 | 0.616 | 0 |
| Root lesion | y = 0.000014x + 0.2015 | 0.022 | 14.4 |
| Stubby root | y = 0.000047x + 0.0470 | 0.004 | 22.9 |
| Stunt/spiral | No sensible fit | N/A | N/A |

Overall there was no clear relationship between leek dry matter yield and actual nematode population. These data are represented graphically in Figure 4.

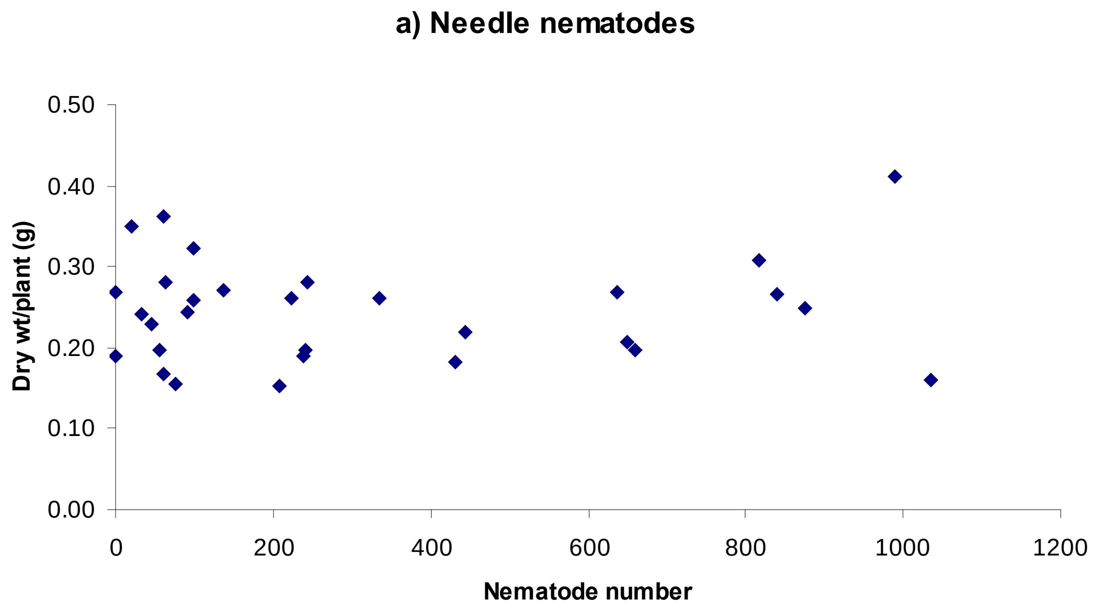


**Figure 4a.** Relationship between leek dry weight and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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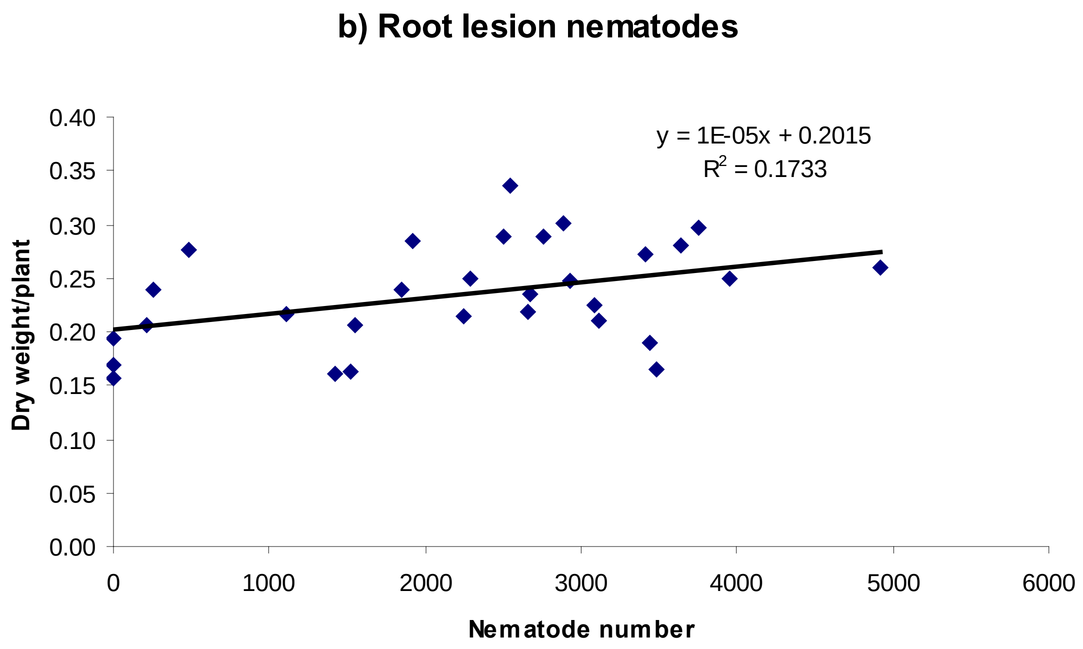


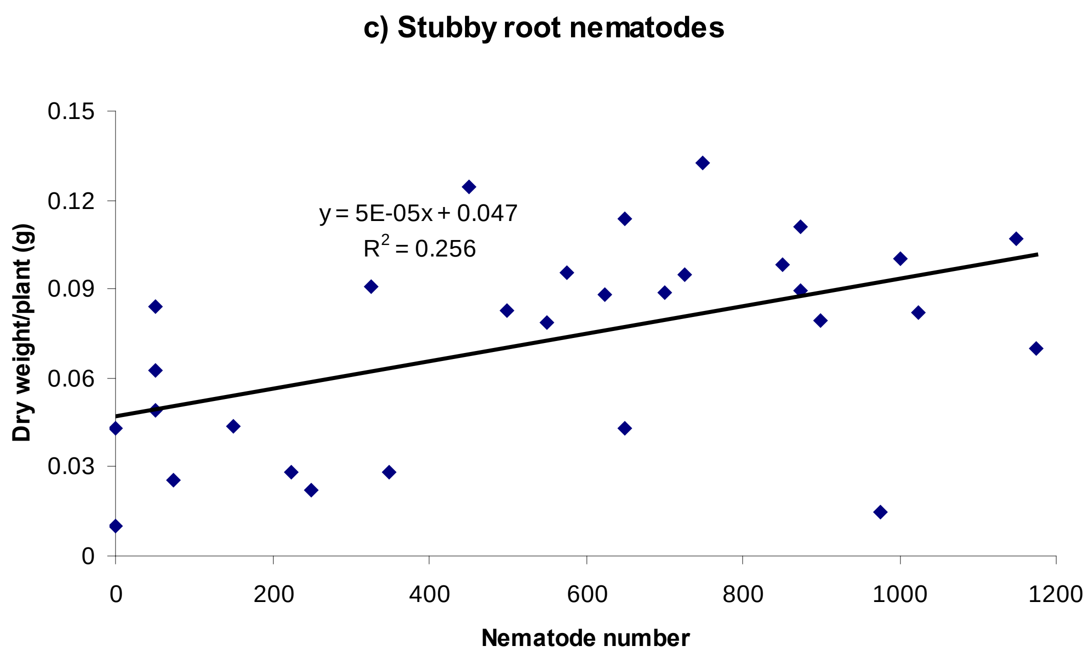


**Figure 4b-c.** Relationship between leek dry weight and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

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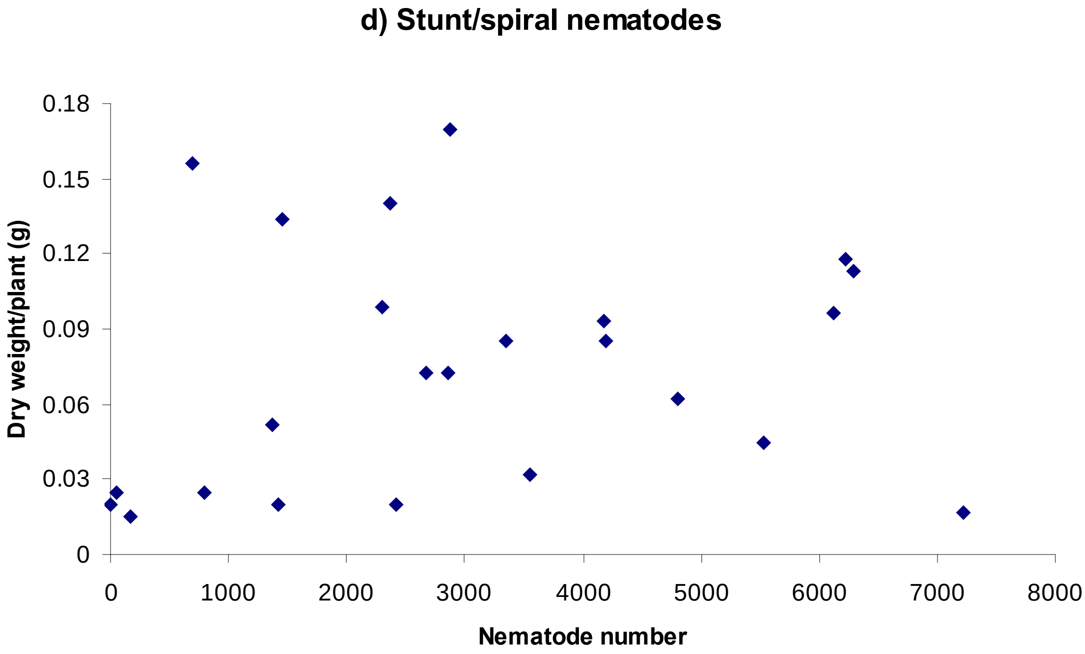


**Figure 4d.** Relationship between leek dry weight and actual nematode population (number/litre soil) at the start of the experiment (n = 30).

Although not analysed statistically, data for dry matter yield for the first sowing of leeks in the presence of stubby root and stunt/spiral nematodes supported the data for the second sowing of leeks. There was no indication that dry matter yield had been influenced by stubby root or stunt/spiral nematodes. For example a stubby root nematode population of 50/l soil (well below the guideline threshold of 200/l soil) produced a dry matter yield of 0.13 g/plant and a population of 1769/l soil (well above threshold) gave a dry matter yield of 0.17 g/plant. Similarly a stunt/spiral population of 50/l soil (below the guideline threshold of 10,000/l soil) produced a dry matter yield of 0.03 g/plant whereas a population of 3350/l soil produced a yield of 0.09 g/plant.

## Discussion

Soil dilution proved to be a very effective way to create a range of populations of different nematode groups. This technique had previously been shown to be effective in HDC project FV 377 Onions: Improving risk assessment for free-living nematodes. For needle nematodes the target population was very close to the actual population. For root lesion and stubby root nematodes the actual population was just over half of the target population and for stunt/spiral nematodes the actual population was almost twice that of the target population. This is difficult to explain but may be due to nematode reproduction or

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alternatively difficulties in taking a representative sample from the bulk of soil collected from the field. It could also be due to inefficient extraction of nematodes from soil samples but this is unlikely as scientific staff have many years of experience of extracting nematodes from soil. It is also unlikely that the same error would have been repeated in each sample of a batch of 30.

The created populations of needle, stubby root and root lesion nematodes were both below and above their current guideline thresholds and so provided a good range over which to test the impact of nematode feeding on leek growth. The guideline threshold for stunt/spiral nematodes is estimated at 10,000/l soil. The target populations did not reach this level with a maximum count of 7225 stunt/spiral nematodes/l soil. This level is still higher than 96% of samples analysed by ADAS Pest evaluation Services over the last 10 years so can still be considered a significant infestation of stunt/spiral nematodes.

Numbers of all nematodes declined over the duration over the duration of the study. As this assessment was made almost five to seven months after the experiment was established, some reduction in nematode numbers would be expected. In retrospect it would have been better to create spare pots of nematodes to extract at regular intervals after creating the target populations. This would give a better indication of how the population changed over the life of the study. It is likely that for the period over which leeks were emerging, numbers of all nematodes were close to the levels assessed at the start of the study .

Numbers of needle nematodes showed a big reduction over the duration of the study. When pots were harvested the maximum count was 95/l soil compared with 810/l soil at the start of the study. A count of 95 needle nematodes/l soil, as recorded at the end of the experiment, is still almost twice the current threshold.

Numbers of root lesion nematodes also declined over the life of the experiment although this was not as marked as with needle nematodes. The final maximum count was 1975/l soil which is higher than 94% of counts recorded in samples extracted by Pest Evaluation Services over the last 10 years. This is still a significant count of root lesion nematodes. As with needle nematodes, it is likely that root lesion nematode numbers had not declined significantly during the period of leek emergence.

Stubby root and stunt/spiral nematode numbers were assessed about seven months after the pots were set up due the poor growth of leeks (cv. Belton). It is therefore, perhaps, not surprising that numbers of both these nematode groups declined during the experiment. It is possible that in some pots there would have been limited root material available on which nematodes could feed due to the poor growth of seedlings from the first sowing. It is

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interesting that despite a significant reduction in stubby root nematode numbers the highest population recorded was still above the current threshold for this nematode group.

Results suggest that leek growth was not significantly affected by needle, stubby rot, stunt/spiral or root lesion nematodes. One would expect that leeks would be most susceptible to the effect of nematode feeding at the seedling stage as the root system is beginning to develop and very limited in extent. There was however no effect of increasing nematode numbers on either seedling emergence or on the total area under the graph of seedling emergence against time for all nematode groups. Needle nematodes are one of the largest free-living species found in soil and consequently would potentially be expected to pose the greatest threat to the crop. That this was not evident in this experiment suggests that smaller species are also unlikely to be damaging.

The results from the current project are supported by those from FV 377 Onions: Improving risk assessment for free-living nematodes. In this study there was no obvious effect of needle nematodes (up to 1305/l soil), root lesion nematodes (up to 3350/l soil), stubby root nematodes (up to 6902/l soil) or stunt/spiral nematodes (up to 11,600/l soil) on onion growth and yield. Similarly, unpublished data provided by Julia Droin (see Appendix 1) also showed that root lesion nematodes (up to 1,000/l soil) and stunt/spiral nematodes (up to 14,000/l soil) had little if any effect on onion growth.

There are no recognised thresholds for free-living nematodes in leeks but as a guide about 50 needle nematodes, 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 needle,root lesion and stubby root nematodes were well in excess of these thresholds at the start of the experiment and also at the end for needle and stubby root nematodes. Although highest created population of stunt/spiral nematodes (7225/l soil) was below the current threshold this is still a significant number and higher than numbers recorded in 96% of samples extracted by ADAS Pest Evaluation Services over the last 10 years. This suggests that the current thresholds are too conservative and well below the number of nematodes which can be tolerated by the crop. If leeks are more tolerant of free-living nematodes than previously thought this could potentially have a significant impact on nematicide use and the profitability of the crop. It should however be recognised that stem nematode (*Ditylenchus dipsaci*) can have a significant impact on the yield of leeks crops at relatively low levels so there will still be a need for soil analysis to assess the risk of crop damage from this species. The results from this work suggest that stem nematode remains the major nematode pest of leeks and that other free-living species have a limited impact on the crop.

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Root knot nematode (*Meloidogyne* spp) is also recorded as a potential pest of onions and may therefore be expected to attack leeks. As there is little, if any, information on root knot nematode infestations of leeks in the UK, stem nematode should be considered the main pest of this crop.

## Conclusions

- Pot experiments suggest that populations of needle, root lesion, stubby root and stunt/spiral nematodes well above current guideline thresholds have no effect on leek growth.
- Results suggest that significant savings can be made on nematicide use.
- Stem nematode remains the main nematode threat to the leek crop.

## Knowledge and Technology Transfer

The results of this study were presented to the leek growers association in November 2011 and April 2012.

## Glossary

**Dagger nematodes** – Free-living nematodes belonging to the genus *Xiphinema.*

**Free-living nematodes** – Nematodes that spend all or part of their life in the soil feeding on plant roots and do not form cysts (cf cyst nematodes)

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

**Needle nematodes** – Free-living nematodes belonging to the genus *Longidorus*

**Root lesion nematodes** – Free-living nematodes belonging to the genus *Pratylenchus*

**Seinhorst two-flask technique** – A method of extracting small to medium sized free-living nematode species from soil such as root lesion, stubby root and stunt/spiral nematodes.

**Stubby root nematodes** – Free-living nematodes belonging to the genus *Trichodorus* or *Paratrichodorus.*

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**Stunt/spiral nematodes** – Free-living nematodes belonging to the genus *Tylenchorynchus, Helicotylenchus or Rotylenchus*

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# APPENDIX 1. Additional experiments to evaluate nematode thresholds

These experiments were done by a visiting French student (Julia Droin from University of Lyon), who investigated the relationship between onion growth and numbers of free-living nematodes. Results are presented here to support the work undertaken on leeks. Data are provided 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 14 soil samples 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 day length 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

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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 |

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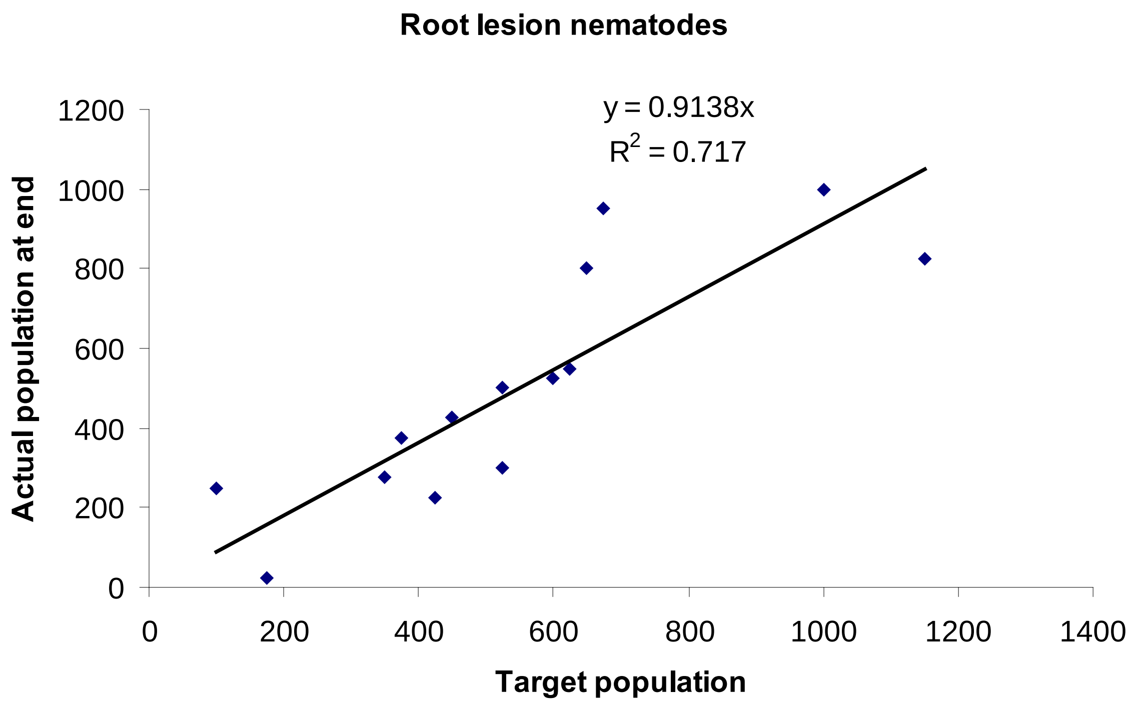


**Figure 1.** Actual root lesion nematode populations at the end of the experiment against target populations (number/litre soil)

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 datasets for three nematode species 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

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investigated. If seedling emergence was inhibited by increasing nematode number then the time to 50% 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.

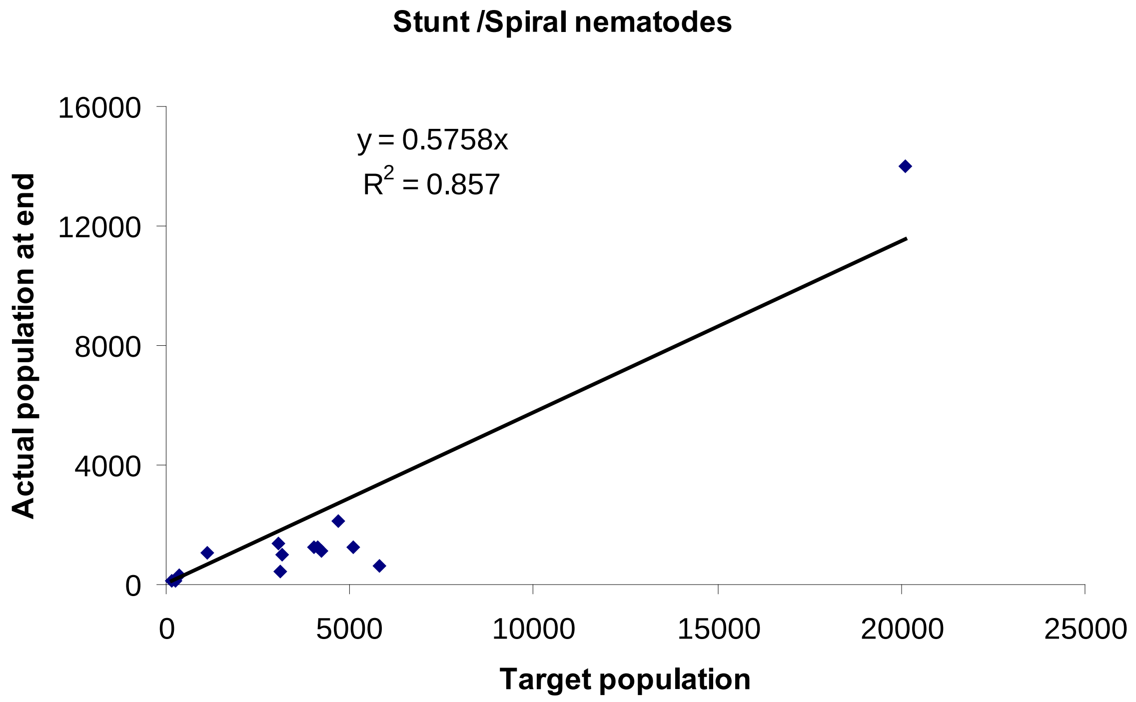


**Figure 2.** Actual stunt/spiral nematode populations at the end of the experiment against target populations (number/litre soil)

**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 |

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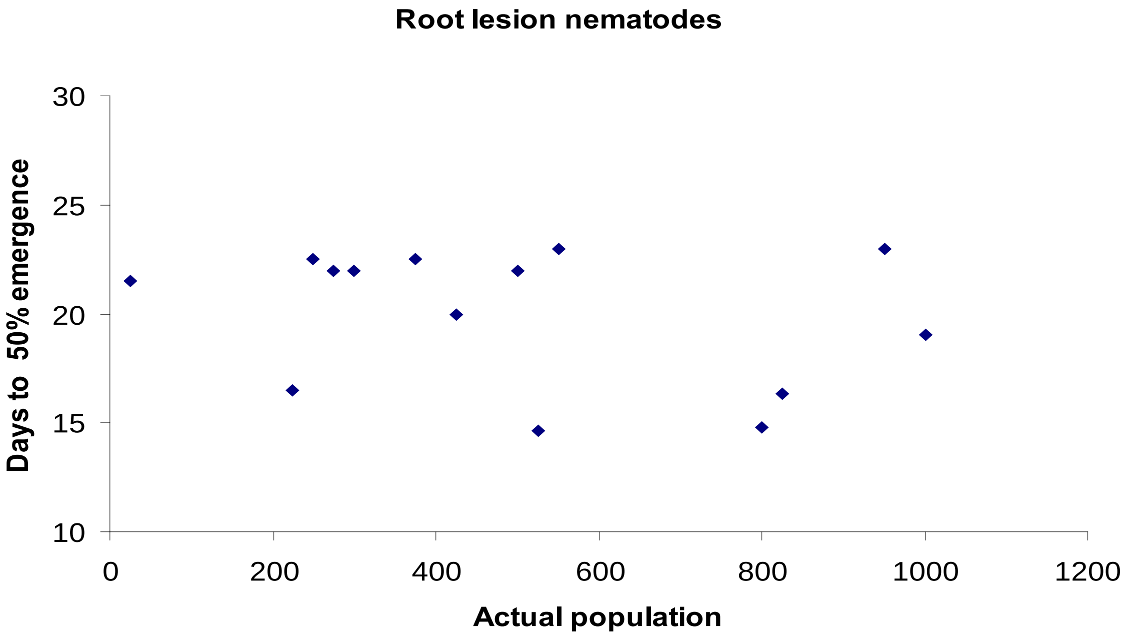


**Figure 3.** Relationship between time to 50% emergence and actual root lesion nematode population (number/litre soil) at the end of the experiment.

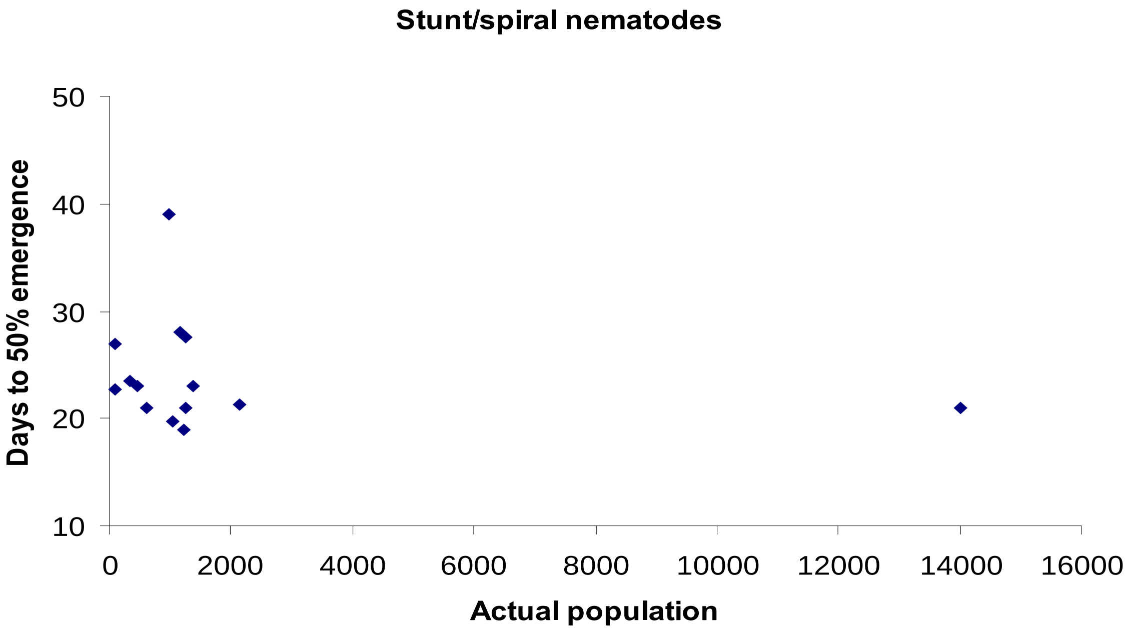


**Figure 4.** Relationship between time to 50% emergence and actual stunt/spiral nematode population (number/litre soil) at the end of the experiment.

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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 |

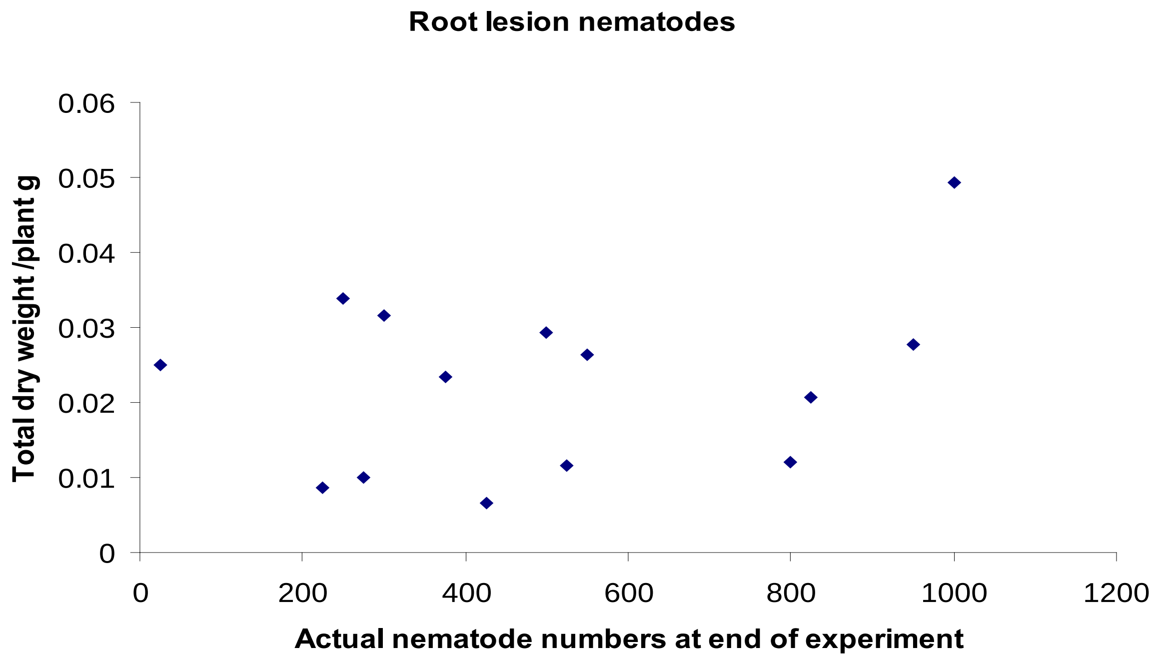


**Figure 5.** Relationship between onion dry weight and actual root lesion nematode populations (number/litre soil) at the end of the experiment.

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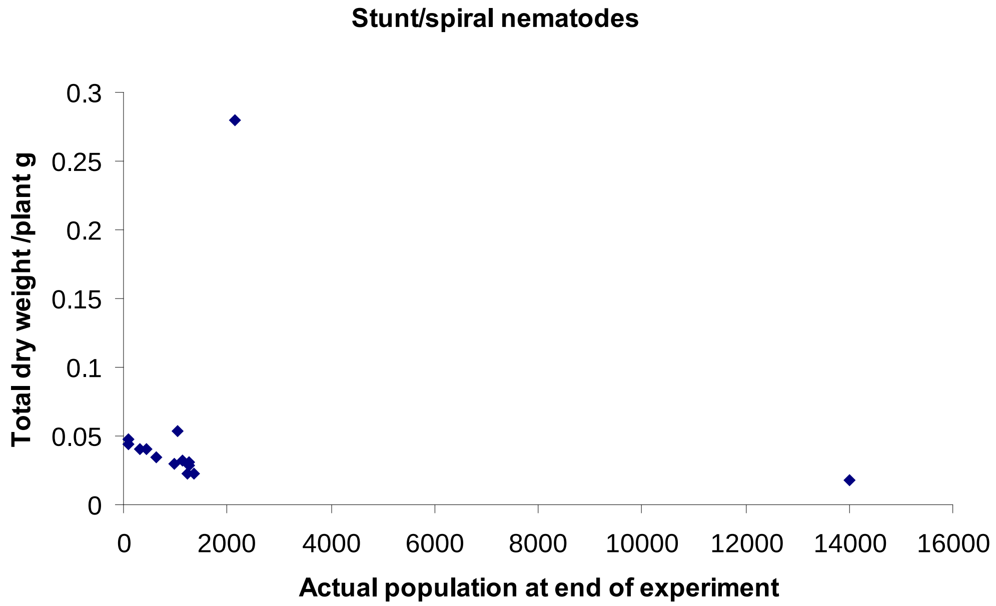


**Figure 6.** Relationship between onion dry weight and actual stunt/spiral nematode populations (number/litre soil) at the end of the experiment.

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.

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## APPENDIX 2. Comparison of actual and target nematode populations

a) Needle nematodes

| Pot number | Start of experiment | | End of experiment |
|---|---|---|---|
| | Target | Actual | Actual |
| 1. | 0 | 0 | 0 |
| 2. | 23 | 0 | 0 |
| 3. | 45 | 20 | 5 |
| 4. | 68 | 55 | 10 |
| 5. | 90 | 34 | 25 |
| 6. | 113 | 46 | 5 |
| 7. | 135 | 62 | 25 |
| 8. | 158 | 100 | 15 |
| 9. | 180 | 100 | 5 |
| 10. | 203 | 91 | 15 |
| 11. | 225 | 136 | 35 |
| 12. | 248 | 238 | 5 |
| 13. | 270 | 240 | 35 |
| 14. | 293 | 243 | 30 |
| 15. | 315 | 208 | 15 |
| 16. | 360 | 224 | 20 |
| 17. | 405 | 64 | 10 |
| 18. | 428 | 60 | 25 |
| 19. | 450 | 336 | 30 |
| 20. | 473 | 443 | 45 |
| 21. | 495 | 430 | 10 |
| 22. | 518 | 650 | 65 |
| 23. | 540 | 636 | 0 |
| 24. | 563 | 660 | 30 |
| 25. | 585 | 840 | 40 |
| 26. | 630 | 816 | 35 |
| 27. | 675 | 1035 | 10 |
| 28. | 720 | 75 | 10 |
| 29. | 765 | 990 | 30 |
| 30. | 810 | 875 | 95 |
| Range | 0 - 810 | 0 - 1035 | 0 - 95 |

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b) Root lesion nematode

| Pot number | Start of experiment | | End of experiment |
|---|---|---|---|
| | **Target** | **Actual** | **Actual** |
| 1. | 0 | 0 | 25 |
| 2. | 240 | 0 | 50 |
| 3. | 480 | 0 | 150 |
| 4. | 720 | 255 | 125 |
| 5. | 960 | 214 | 250 |
| 6. | 1200 | 482 | 575 |
| 7. | 1440 | 1107 | 425 |
| 8. | 1680 | 1545 | 500 |
| 9. | 1920 | 1520 | 600 |
| 10. | 2160 | 1429 | 550 |
| 11. | 2400 | 1846 | 800 |
| 12. | 2640 | 2292 | 475 |
| 13. | 2880 | 2240 | 75 |
| 14. | 3120 | 1920 | 750 |
| 15. | 3360 | 3478 | 725 |
| 16. | 3600 | 2929 | 875 |
| 17. | 3840 | 3409 | 650 |
| 18. | 4080 | 2654 | 275 |
| 19. | 4320 | 2680 | 900 |
| 20. | 4560 | 3115 | 1350 |
| 21. | 4800 | 2500 | 750 |
| 22. | 5040 | 2762 | 1200 |
| 23. | 5280 | 2545 | 1125 |
| 24. | 5520 | 3083 | 1000 |
| 25. | 5760 | 3750 | 1200 |
| 26. | 6000 | 3643 | 1500 |
| 27. | 6240 | 2880 | 800 |
| 28. | 6480 | 4917 | 1225 |
| 29. | 6720 | 3952 | 1975 |
| 30. | 6960 | 3440 | 1600 |
| Range | 0 - 6960 | 0 - 4917 | 0 - 1975 |

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c) Stubby root nematode

| Pot number | Start of experiment | | End of experiment |
|---|---|---|---|
| | Target | Actual | Actual |
| 1. | 0 | 0 | 0 |
| 2. | 66 | 0 | 0 |
| 3. | 131 | 50 | 0 |
| 4. | 197 | 75 | 0 |
| 5. | 262 | 150 | 0 |
| 6. | 328 | 50 | 50 |
| 7. | 393 | 50 | 0 |
| 8. | 459 | 225 | 0 |
| 9. | 524 | 250 | 0 |
| 10. | 590 | 350 | 25 |
| 11. | 655 | 500 | 50 |
| 12. | 721 | 325 | 75 |
| 13. | 786 | 550 | 50 |
| 14. | 852 | 575 | 25 |
| 15. | 917 | 650 | 150 |
| 16. | 983 | 625 | 50 |
| 17. | 1048 | 750 | 225 |
| 18. | 1114 | 850 | 25 |
| 19. | 1179 | 875 | 250 |
| 20. | 1245 | 650 | 0 |
| 21. | 1310 | 450 | 200 |
| 22. | 1376 | 700 | 50 |
| 23. | 1441 | 1000 | 25 |
| 24. | 1507 | 900 | 0 |
| 25. | 1572 | 875 | 0 |
| 26. | 1638 | 725 | 0 |
| 27. | 1703 | 1025 | 0 |
| 28. | 1769 | 1150 | 50 |
| 29. | 1834 | 1175 | 0 |
| 30. | 1900 | 975 | 0 |
| Range | 0 - 1900 | 0 - 1175 | 0 - 250 |

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d) Stunt/spiral nematode

| Pot number | Start of experiment | | End of experiment |
|---|---|---|---|
| | Target | Actual | Actual |
| 1. | 0 | 0 | 0 |
| 2. | 93 | 50 | 25 |
| 3. | 186 | 175 | 50 |
| 4. | 279 | 350 | 75 |
| 5. | 372 | 425 | 175 |
| 6. | 465 | 775 | 200 |
| 7. | 558 | 1425 | 325 |
| 8. | 651 | 2425 | 350 |
| 9. | 744 | 925 | 550 |
| 10. | 837 | 1350 | 225 |
| 11. | 930 | 1375 | 350 |
| 12. | 1023 | 800 | 450 |
| 13. | 1116 | 1450 | 450 |
| 14. | 1209 | 700 | 575 |
| 15. | 1302 | 2300 | 475 |
| 16. | 1395 | 2875 | 925 |
| 17. | 1488 | 2375 | 400 |
| 18. | 1581 | 6125 | 400 |
| 19. | 1674 | 5200 | 475 |
| 20. | 1767 | 5525 | 700 |
| 21. | 1860 | 7225 | 650 |
| 22. | 1953 | 4800 | 775 |
| 23. | 2046 | 4175 | 300 |
| 24. | 2139 | 6300 | 650 |
| 25. | 2232 | 4200 | 1050 |
| 26. | 2325 | 6225 | 1000 |
| 27. | 2418 | 2675 | 1150 |
| 28. | 2511 | 2850 | 525 |
| 29. | 2604 | 3350 | 600 |
| 30. | 2697 | 3550 | 375 |
| Range | 0 - 2697 | 0 - 7225 | |

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