PhD Summary Report No. 13

April 2009

Project No. RD-2005-3190

New cyst nematode threats to cereals in the UK

by

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January 2006- December 2008

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ABSTRACT

For the past 35 years cereal cyst nematodes (CCN) have been a minor problem in intensive cereal production in the UK and other areas of northern Europe, despite the susceptibility of most cereal cultivars to these widespread pests. Historically, the principal cereal cyst nematode, *Heterodera avenae* has been controlled by parasitic fungi that destroy the females and eggs of the nematode. This is an example of natural pest control in an intensive agriculture system. In nematode surveys conducted at Rothamsted Research the cereal cyst nematode, *Heterodera filipjevi* was found in fields in Essex and in Wales. This pest had not previously been recorded in the UK. The research project described aimed to assess the risk of *H. filipjevi* to intensive cereal production in the UK.

CCN populations were more diverse than expected. In a survey of cereal fields in the UK 65% were infested with CCNs and *H. filipjevi* was widespread. *Heterodera pratensis* was also present at one site but studies confirmed that most fields contained mixed populations of *H. avenae* and *H. filipjevi*. All CCN populations were suppressed by parasitic fungi that destroyed nematode females and eggs. Cyst nematode populations containing *H.* filipjevi only produced a single generation in a growing season and their ability to multiply on wheat was similar to that of *H. avenae*, which also has a single generation. Nematode females and eggs in all populations were parasitised by fungi including *Catenaria auxiliaris* and *Pochonia chlamydosporia* (also known to infect *H. avenae*). Results indicated that these fungi were major factors in limiting nematode reproduction on susceptible crops. The principal CCN pests had different responses to some plant defence compounds. DIBOA a hydroxamic acid produced by wheat and rye as a plant defence compound reduced the hatch and mobility of juveniles of *H.avenae* and increased their mortality more than of those of *H. filipjevi* that recovered from exposure to such compounds.

These findings suggest that *H. filipjevi* is not a significant new threat to intensive cereal production in the UK and it is effectively controlled by fungi that have successfully controlled *H. avenae* for many years. The unexpected widespread distribution of *H. filipjevi* in the UK may have resulted from the current predominance of autumn-sown cereals but this would need further testing.

INTRODUCTION

Temperate cereal crops are attacked by a number of different cyst nematodes worldwide. Nematodes are microscopic worms that move in the pore spaces in soil and must feed on plant roots to support their development. Cyst nematodes are characterised by the developing females swelling to form resistant cysts that each contain several hundred eggs, which may remain dormant in soil for several years. The infective juvenile nematodes hatch from these eggs, migrate in soil and penetrate roots just behind the root tip (Figure 1). If large numbers of juveniles invade, root growth is slowed and root systems are stunted making less efficient use of water and nutrients in soil and thereby reducing yields.

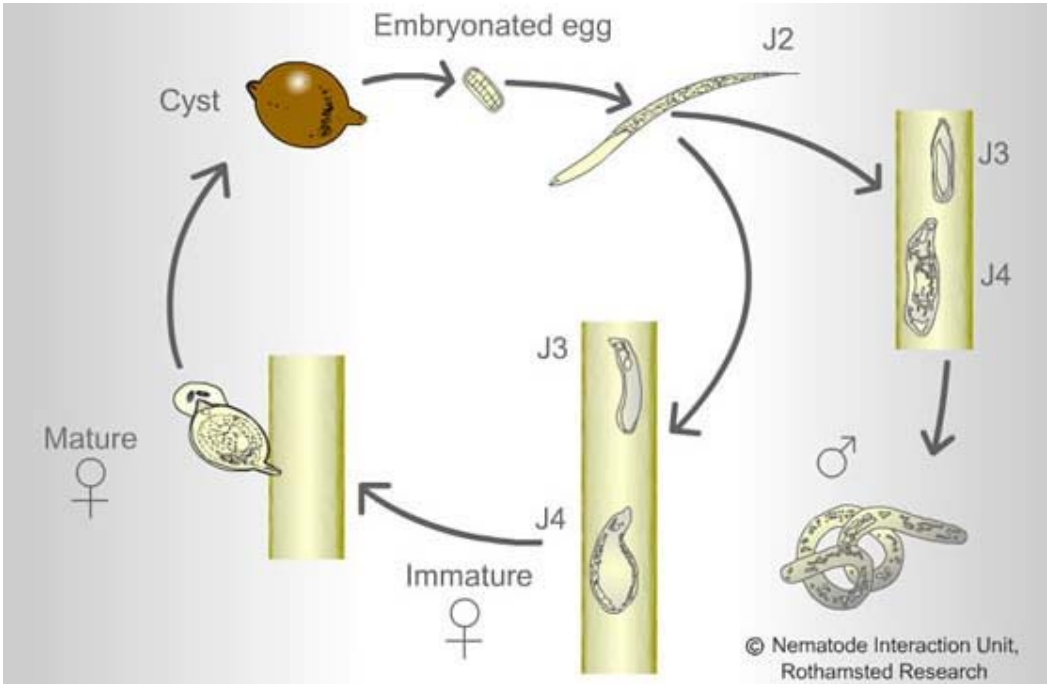

Figure 1 The life cycle of cereal cyst nematodes illustrating the infective second-stage juveniles (J2) that infects roots and the juveniles and adult females that depend on feeding cells within roots for their development.

The dominant cereal cyst nematode in the UK is the native pest, *Heterodera avenae*, which is a parasite of grasses and of temperate cereals. It is the most widespread cyst nematode in Britain. Crop susceptibility is in the order oats> wheat>barley and, in general autumn-sown cereals are more tolerant of nematode attack than those sown in the spring. These differences are because most nematodes hatch in the spring when soil temperatures are rising. Hence,

spring cereals with smaller root systems at that time than those sown in autumn, contain larger densities of infective nematodes. Nematode populations of <5 eggs per gram of soil at sowing time are not damaging whereas infestations >20 eggs per gram soil may cause 20% yield reductions in wheat.

In the UK, since the intensification of cereal production in the 1940's, when grassland was ploughed and cultivated to meet the demands for increased food production, *H. avenae* became a major constraint to cereal productivity. However, despite monocultures of susceptible cereals and much initial damage caused by the nematode, populations declined because of the build up of naturally occurring fungal pathogens of the nematode (*Pochonia chlamydosporia* and *Nematophthora gynophila*) that infect and kill the eggs and females of *H. avenae*. These pathogens have kept levels of this pest below the economic damage threshold and became the first example of the biological control of a nematode pest in the field. Such suppressive soils have become widespread in the UK and northern Europe and this major pest has declined in importance in UK cereal production over the past 30 years.

Recent reports of crop damage associated with *Heterodera spp*. In Scandinavia were thought to indicate a breakdown of these suppressive soils in controlling cereal cyst nematodes. However, detailed studies indicated that the dominant nematode in these soils causing significanrt crop damage was another species of cereal cyst nematode, *Heterodera filipjevi* and not *H. avenae*. In the UK, a site at Ceredigion, Wales, and a site in Essex have both suffered crop damage attributed to the presence of cereal cyst nematodes, which may have been *H. filipjevi*. Thus, it is important to establish whether *Heterodera spp.* are, again, becoming a problem pest in UK cereal fields, as is the case in Scandinavia and to determine if this is due to a shift in the nematode species complex and a reduction in the efficacy of natural soil suppression.

Nematicides and biological control products are too expensive for use on cereal crops and the main methods of control employed in those areas where suppressive soils provide insufficient levels of control, are crop rotations of host and non-host plants and the use of resistant cultivars.

In this project the species composition of populations of cereal cyst nematodes was determined in a limited survey of different fields in southern Britain in which cereals had been intensively grown. Populations were characterised using morphological and morphometrical analyses combined with biochemical and molecular studies and a host range test to determine if populations are currently more diverse than previously recorded. The effectiveness of natural soil suppressiveness against the species/pathotypes of cereal cyst nematodes found was also measured to determine whether those fungal agents that controlled *H. avenae* were still present and active in cereal fields. Finally, inherent defence mechanisms of cereal crops, such as the production of hydroxamic acid was investigated to determine if such biocides were active against UK populations of cereal cyst nematodes and may be manipulated for control in the future, if such a new threat to UK cereal production is found.

Aim and objectives of Study

To identify and characterise cyst nematodes that occur in soils intensively cropped with cereals in southern Britain and investigate their biology and ecology to assess their potential risk to intensive cereal production.

Objectives:

1. *To characterise species and populations on cereal host crops using morphological and molecular methods.*
2. *To study and compare the host range and population dynamics of* H. filipjevi *and other cereal cyst nematode populations, with those known for* H. avenae*.*
3. *To test the susceptibility of* H. filipjevi*, and any other cyst nematodes in the soils sampled, to the causal agents (fungal parasites* Nematophthora gynophila *and* Pochonia chlamydosporia*) of* H. avenae *population decline.*
4. *To study the impact of hydroxamic acids on nematode development and their importance in host plant resistance to* H. avenae *and* H. filipjevi*.*

EXPERIMENTAL APPROACHES AND RESULTS

Characterisation of cereal cyst nematode species

A limited field survey was conducted in June in order to establish the presence and biodiversity of cereal cyst nematode infestations by identifying species in soil

samples taken from cereal fields in southern Britain. The survey aimed to confirm whether *Heterodera filipjevi*, and other cereal cyst nematodes were present in any of the sites selected.

Five samples (2-3 Kg soil) were taken at random from within cereal rows from each of 25 fields from 14 farms. Nematode females, cysts (Figure 2) and eggs from a 200ml subsample of the soil from each site were extracted using routine methods and the numbers counted to measure the size of the infestation.

Figure 2 Cereal cyst nematode females (white) and cysts (brown) in soil extracts. Each cyst is formed from a dead female and may contain several hundred eggs, which survive several years in soil.

Morphological characterisation of species and populations was conducted using a reference slide collection held at Rothamsted. Key morphological features of the cysts that differentiate between the species include: the fenestration (areas of thin cuticle on the vulval cone of the cyst through which the infective juveniles emerge), the length of the vulval slit, the presence of bullae and the presence and length of the underbridge (Figure 3). Morphometric analysis was conducted on the following populations: Abbots Ripton, Cottenham, Clifton Reynes F1, Clifton Reynes F2, Cae Banadl, Symonds hyde, Tewes, Woburn Horsepool, Woburn Stackyard D1 and Woburn Stackyard D2. At x100 magnification, the following measurements were made: 1). length of stylet, 2). stylet knob width, 3). head tip to median bulb, 4). head tip to excretory pore, 5). body width at excretory pore, 6). anus to tail tip, 7). body width at anus, 8). hyaline tail to tail tip, 9). total body length (Figure 4)

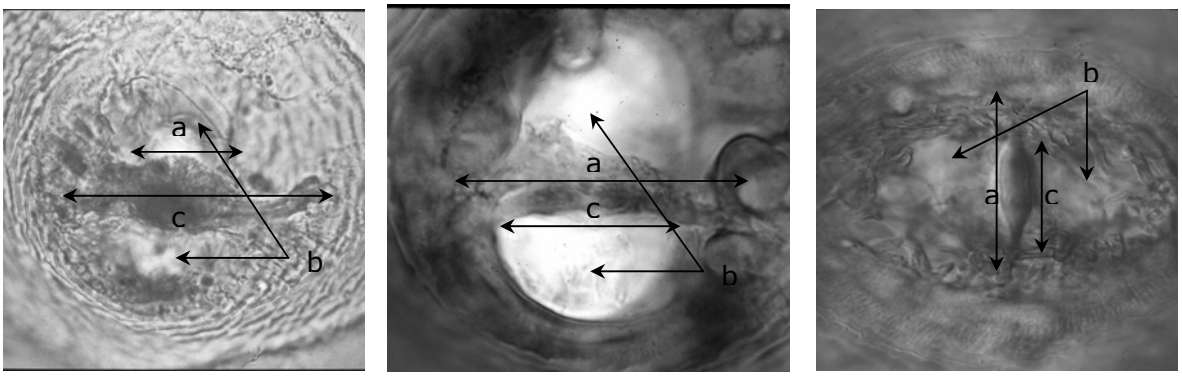

Figure 3 The diagnostic features of the vulval cone. Left to right, *H. hordecalis*, *H. mani* and *H. filipjevi* **a** length of underbridge, **b** fenestration, **c** width of nestra.

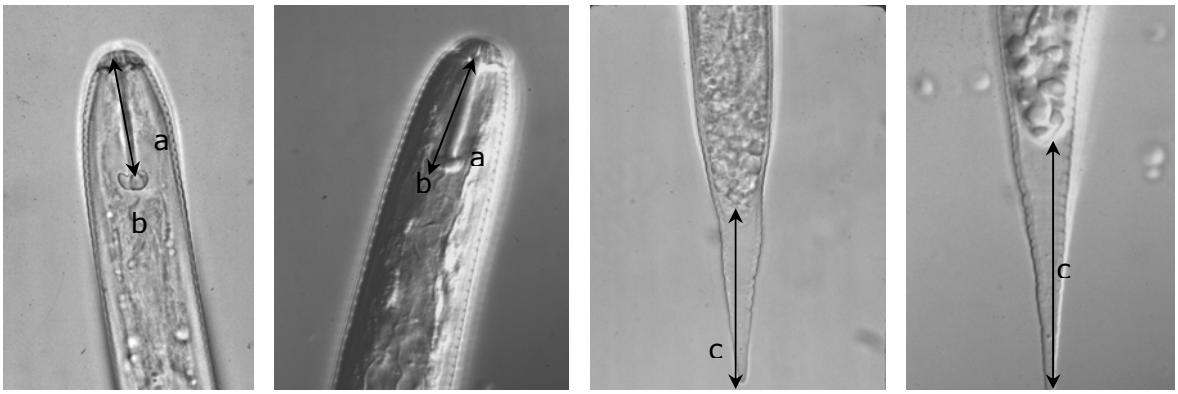

Figure 4 Some key characteristics used to identify cereal cyst nematode second-stage juveniles: Left to right: Head of *H.mani* juvenile, head of *H. filipjevi* juvenile, 10. tail of *H. filipjevi* juvenile and 11. tail of *H. avenae* juvenile, showing **a** stylet length, **b** stylet knob width and **c** hyaline tail length.

The field populations were analysed biochemically with the use of isoelectric focusing to compare protein profiles between populations and also compare them against known standard species: the *H. avenae* 'Ask' population and the *H. filipjevi* 'Etelhem' population, both from Sweden. These two populations are well characterised in terms of morphology, biochemical and molecular studies.

Isoelectric focusing (IEF), which uses protein separation for identification of nematode species was conducted using populations of cysts previously extracted and stored at -80°C. The Pharmacia Phastsystem was used to separate proteins based on their electrical charge on gels, which were analyzed using the Totalab

2.0 computer software package, which uses the presence and absence of the bands produced to allow comparisons between samples.

Another method of characterisation is the use of an International Test Assortment (or biotest) of oat and barley cultivars with different resistance genes which enable the separation field populations on a species and pathotype basis. Twenty pre-germinated seeds of each of the following cultivars of spring barley: Varde, Emir, Ortolan, Morroco and KVL 191 and oats: Nidar, Selma and Hedvig with different resistance genes were planted into pots containing soil from five sites: Cae Banadl, Cottenham, Clifton F1, Clifton F2 and Abbots Ripton (four replicates pots per field soil x cultivar treatment; 160 pots in total). The pots were placed in a glasshouse at a constant 15 ºC and watered as and when required. Eighteen weeks after planting the pots were removed and stored at 4 ºC for later examination. The roots and the soil was washed using the Fluidising Column to extract cysts and females which were counted and twenty-five cysts per treatment were frozen for later molecular (RFLP) analysis (see below).

Molecular characterisation of the populations was conducted on the cereal cyst nematodes that had reproduced on the oat and barley cultivars in the International Test Assortment (see above). The DNA from ten cysts from each individual sample was extracted using standard procedures. The DNA was amplified using AB28 and TW81 primers and digested using restriction enzymes BSuR1, HindIII and Msp1 to generate DNA fragments of different sizes that separated on a gel to create characteristic banding patterns called restriction fragment length polymorphisms (RFLPs), which act as fingerprints for the different species. The standard species used in the molecular analysis are population *Heterodera filipjev*i populations 'Etelhem' and '184' and *Heterodera avenae* population 'Ask'. *Heterodera filipjevi* population Etelhem is also a well characterised population from Sweden. The RFLP gels produced were analyzed using the Totalab 2.0 computer software package, which uses the presence and absence and intensity of the digestion bands produced to allow comparisons between samples. Thirty-two DNA samples were analysed from the presence and absence of bands and the band intensity data, which enabled the populations to be grouped and compared with the standards.

Key results

- Despite sampling fields mostly in intensive cereal production, 63% of the 92 soil samples contained cereal cyst nematodes but only half contained viable populations and all infestations were < 4 eggs g^{-1} soil, which is below the damage threshold for cereals in the UK (Table 1). In general, very few healthy females were found on the roots and in the soil collected around the roots for most field sites.
- The results of the morphological and morphometric measurements were complex involving overlapping ranges of characteristics. The data were subject to a range of statistical analytical techniques, including principal component analysis. In summary, almost all of the cysts cones examined were assigned to *Heterodera avenae* and *Heterodera filipjevi* (Figure 3).
- Measurements of one hundred juveniles from eight UK field populations confirmed that most populations (Abbots Ripton, Clifton Reynes Field 1, Cottenham, Tewes and Woburn Stackyard D Field 2) contained *H. avenae* and *H. filipjevi*. Only juveniles of *H. avenae* were found at Clifton Reynes Field 2 and juveniles of *H. filipjevi* were found at Woburn Stackyard D. Juveniles matching the measurements of H*eterodera pratensis* were found in samples collected at Cottenham and Woburn Stackyard D.
- The Abbots Ripton population and those from Clifton Reynes F1, F2 and Cottenham have a very similar protein profile to that for *H. avenae*. Abbots Ripton, Cae Banadl, Clifton Reynes F1 andF2, Cottenham also have a band at pI 4.4, revealing that these populations contain both *H. avenae* and *H. filipjevi*. Cae Banadl and Abbots Ripton samples also appeared to contain *H. pratensis* population (Figure 4).
- Differences in morphology of infective juveniles revealed greatest diversity in CCN populations compared to IEF separation of proteins that were not as sensitive when used on field populations containing mixed species of nematodes
- The Test Assortment grouped the nematode populations from Clifton Reynes F1 and F2, Cottenham and Abbots Ripton populations whereas the Cae Banadl population had a different response to the differential cultivars used and was the only population tested that was avirulent to the barley cultivar Ortolan, as was the reference Ask population (pathotype Ha11).

All populations appear to be avirulent on Hedvig, the only cultivar that is resistant to both *H. avenae* and *H. filipjevi* pathotypes.

- RFLP analysis distinguished *H. avenae* and *H. filipjevi* on the different soil and plant lines and confirmed earlier findings (Figures 5 & 6).

Table 1. The occurrence of cereal cyst nematodes in soil samples collected from 13 farms and 23 fields under intensive cereal production.

Site	Cropping History	CCN present
Tewes, Little Sampford, Essex	>30 years continuous wheat	Ha, Hf
Thaxted, Essex	Continuous winter wheat	
Abbots Ripton	Continuous wheat (32 years)	Ha* Hf^{+}
Cottenham, Cambridgeshire	Wheat/break rotation (oats, OSR or)	Ha, Hf, Hp^{-}
Clifton Reynes, Bedfordshire	Continuous wheat	Ha, Hf
	Continuous wheat	Ha, Hf
Woburn, Bedfordshire	Pasture	
	Continuous cereals	
	Continuous cereals	
	Continuous cereals	Ha, Hf
Cae Banadl, Wales	Continuous cereals	Hf
Bapton, Wiltshire	Spring barley	Not detected
Keysley, Wiltshire	Winter wheat	Not detected
Sonning, Berkshire	Winter oats	Not detected
Rothamsted, Hertfordshire	Winter oats	Not detected
	Bioenergy grasses	Not detected
	Continuous spring barley	Not detected
	Continuous winter wheat	Not detected
Morley, Norfolk	Spring barley	
Symonds Hyde	Continuous winter wheat	

**Heterodera avenae*; $^{+}$*H. filipjevi*; $^{-}$*H. pratensis*.

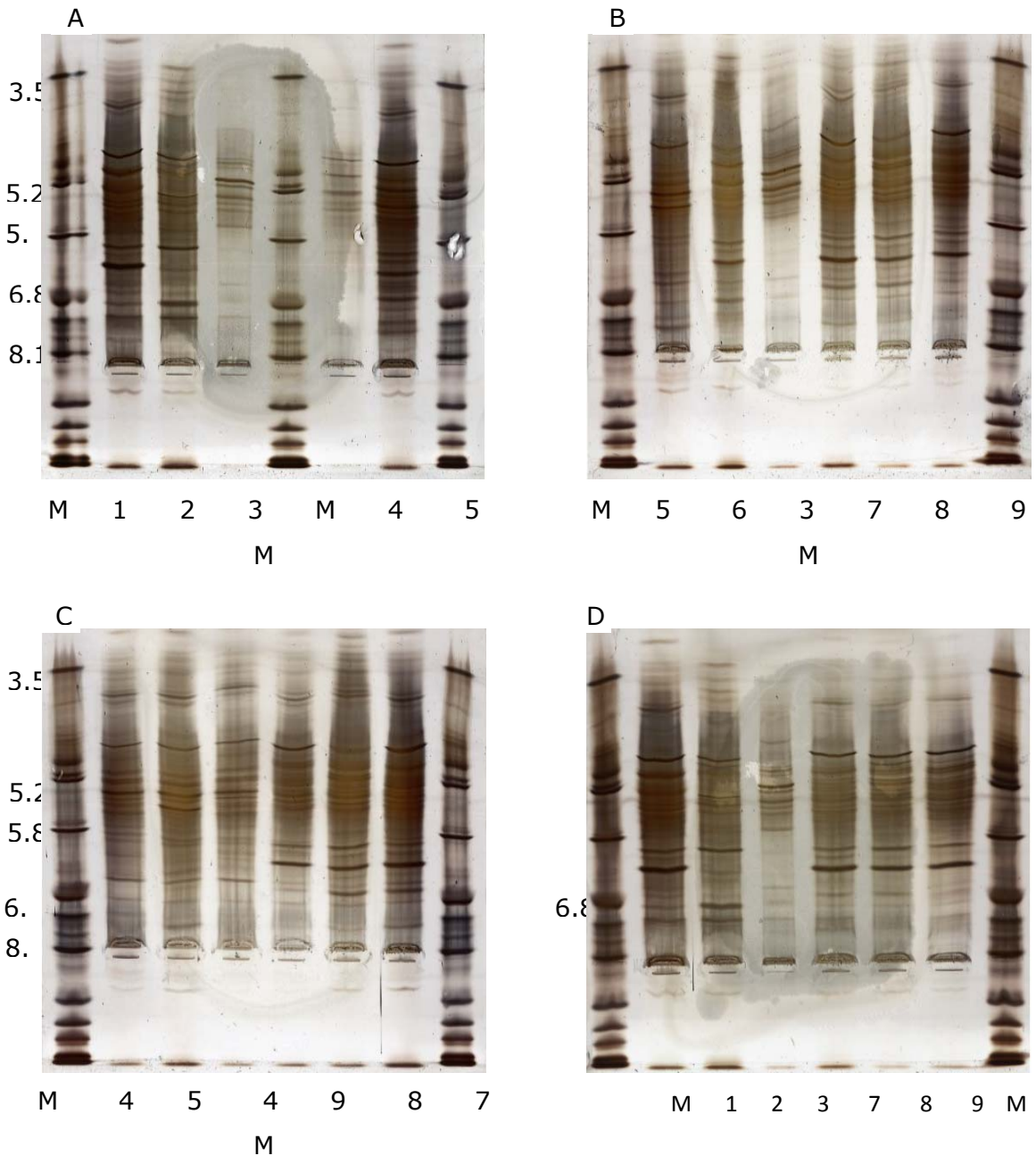

Figure 4 Gel A, lanes: M: marker (pI 3.5-9.3), 1: H. avenae standard (Ask, Ha 11), 2: H. filipjevi standard (Etelhem), 3: H. pratensis standard (Tømmerås), 4: Cae Banadl, 5: Abbots Ripton, 6: H. avenae + H. filipjevi (mix), 7: Cottenham, 8:Clifton Reynes Field 2, 9: Clifton Reynes Field 1.

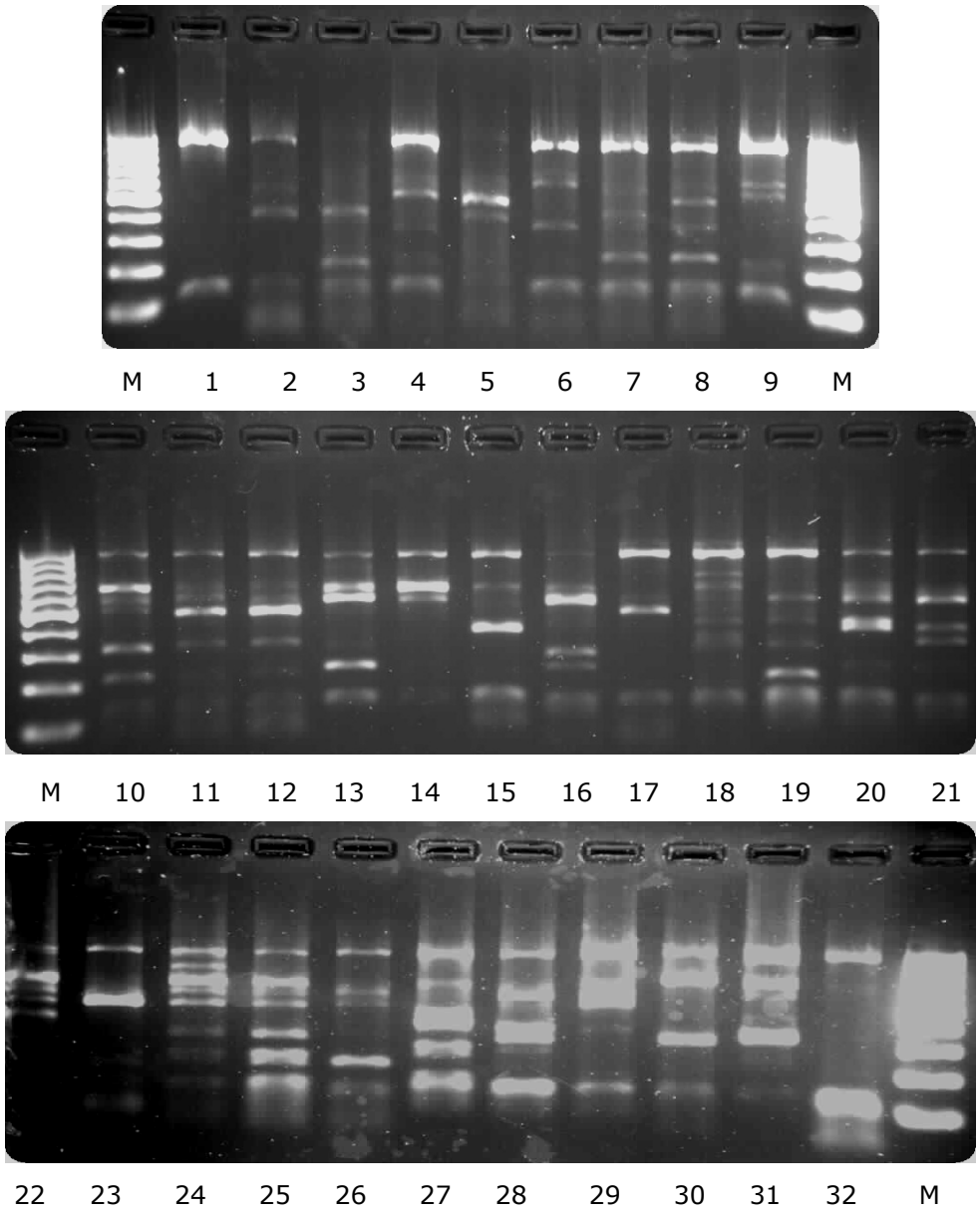

Figure 5 RFLP profiles of UK field populations generated by restriction enzyme Msp1. Lanes: M: 100bp marker, 1:H. avenae, 2:H. filipjevi, 3:Cottenham type B / KVL 191, 4:Clifton F2 / KVL 191, 5:Abbots R. / KVL 191, 6:Cottenham / Morocco, 7:Cottenham type B, 8:Cottenham type A, 9:Cae Banadl type A, 10:Cottenham type A / Emir, 11:Clifton F1 type B / Emir, 12:Clifton F1 type A / Emir, 13:Clifton F2 type B / Emir, 14:Clifton F2 type A / Emir, 15:C. Banadl / Selma, 16:Cottenham type A / Selma, 17:Clifton F1 / Selma, 18:Clifton F2 / Selma, 19:Abbots R. / Selma, 20:C. Banadl type A / Varde, 21:C. Banadl type B / Varde, 22:Cottenham type A / Varde, 23:Clifton F1 type A / Varde, 24:Clifton F1 type B / Varde, 25:Clifton F2 type A / Varde, 26:Abbots R. type B / Varde, 27:C. Banadl / Nidar, 28:Cottenham / Nidar, 29:Clifton F1 / Nidar, 30:Clifton F2 / Nidar, 31:Abbot R. / Nidar, 32:Clifton F2 / Hedvig.

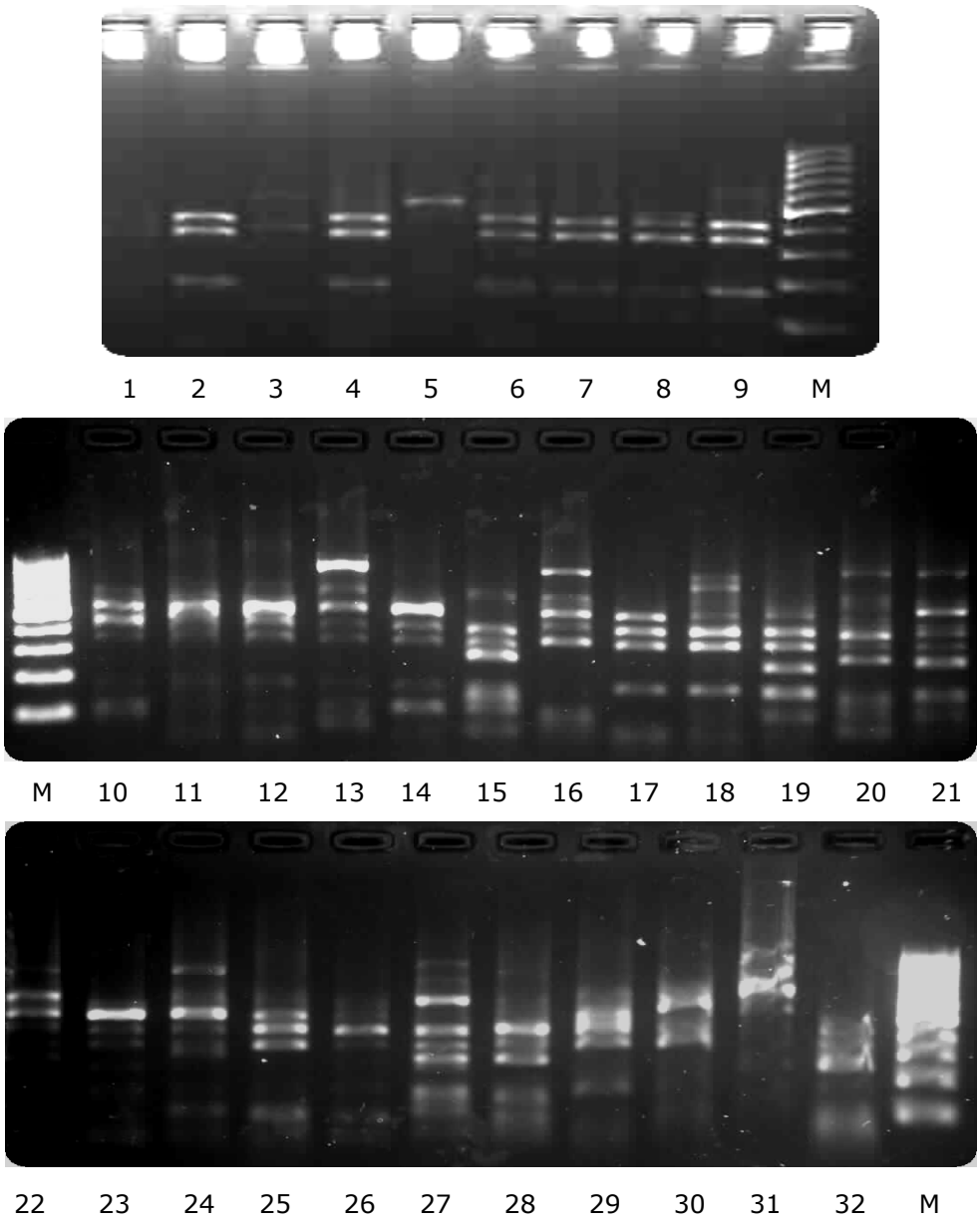

Figure 6 RFLP profiles of UK field populations generated by restriction enzyme BsuR1. Lanes: M: 100bp marker, 1:H. avenae, 2:H. filipjevi, 3:Cottenham type B/KVL 191, 4:Clifton F2/KVL 191, 5:Abbots R./KVL 191, 6:Cottenham/Morocco, 7:Cottenham type B, 8:Cottenham type A, 9:Cae Banadl type A, 10:Cottenham type A/Emir, 11:Clifton F1 type B/Emir, 12:Clifton F1 type A/Emir, 13:Clifton F2 type B/Emir, 14:Clifton F2 type A/Emir, 15:C. Banadl/Selma, 16:Cottenham type A/Selma, 17:Clifton F1/Selma, 18:Clifton F2/Selma, 19:Abbots R./Selma, 20:C. Banadl type A/Varde, 21:C. Banadl type B/Varde, 22:Cottenham type A/Varde, 23:Clifton F1 type A/Varde, 24:Clifton F1 type B/Varde, 25:Clifton F2 type A/Varde, 26:Abbots R. type B/Varde, 27:C. Banadl/Nidar, 28:Cottenham/Nidar, 29:Clifton F1/Nidar, 30:Clifton F2/Nidar, 31:Abbot R./Nidar, 32:Clifton F2/Hedvig.

The population dynamics of CCN populations and their susceptibility to fungal pathogens

The small infestations of cereal cyst nematode populations found in the fields sampled despite intensive cereal cropping could be either because they are recent introductions in the UK or because they are established but have become suppressed. The main aim of this section is to investigate whether there are any differences in the life cycle of the species of cereal cyst populations found in the survey and to understand the regulatory processes underlying any such differences, in particular, the impact of nematophagous fungi that are known to regulate *Heterodera avenae* populations.

The number of generations in a growing season for four field populations of cereal cyst nematodes out-of-doors was investigated. *Heterodera avenae* has only one generation per growing season across all of the geographic localities in which this pest has been found. A species or population able to complete more than one generation in a growing season is likely to become more abundant, especially if it is able to compete more effectively with natural enemies in soil. The timing of different stages in the life cycle and cyst production was monitored in four field populations.

In November, pre-germinated winter wheat (cv. Consort) and winter rye (cv. Carotop) seeds were transplanted (three per pot) into pots filled with soil from Cottenham, Cae Banadl, Abbots Ripton and Clifton Reynes (Field 1). The pots were then plunged in outdoor sand-beds in a randomised block design. Two months after planting, twenty-four pots of 3 replicates of each cereal-soil combination were sampled. The soil was carefully mixed and two sub-samples of 200g were taken for juvenile (J2) and female/cyst extraction and roots were stained to assess the development of juveniles within them. This was repeated on five sampling occasions. Females from each treatment were examined for fungal parasites and the percentage of infected eggs recorded.

Key Results

- All the field populations studied had only one generation in a growing season eventhough the Cottenham population, which appeared to contain

H. avenae, *H. filipjevi* and *H. pratensis* produced females over a longer period than other sites, with females observed as early as January.

- The overall trend in the numbers of cysts and females produced over time is similar for each population in (Figure 7).
- Except in soil from Cottenham, more ($P<0.01$) cysts and females were produced on wheat than on rye. Both nematodes reproduce on wheat but only *H. filipjevi* is able to reproduce on rye. The Clifton Reynes F1 population failed to reproduce on rye.

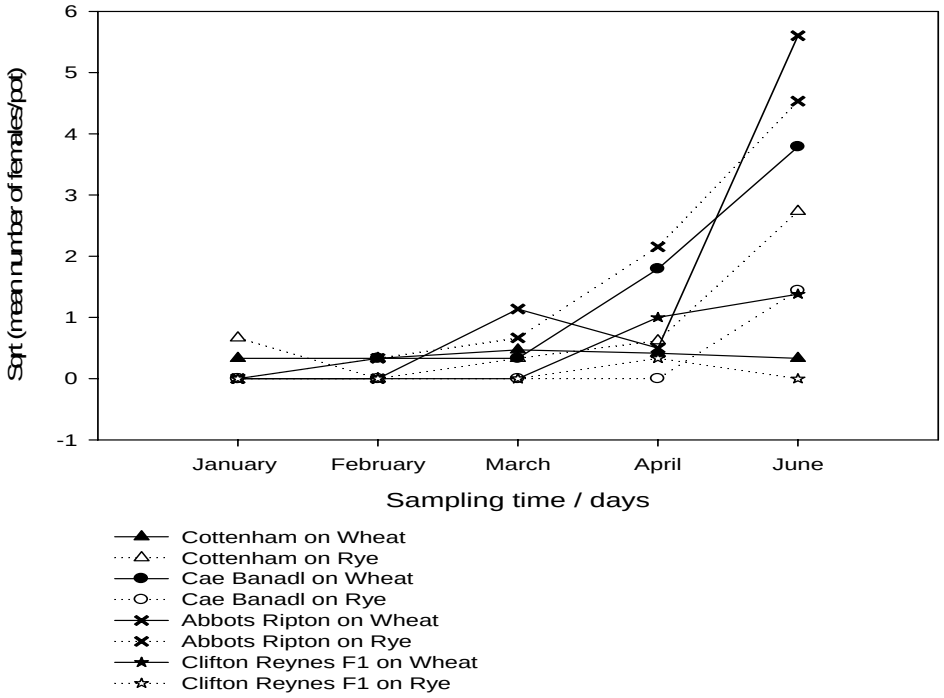

Figure 7 Mean number (square root) of females produced from four nematode populations on wheat and rye.

- J2 numbers in soil differed ($P<0.001$) according to initial population levels present in each field soil (Figure 8). These data support those for female production and for juvenile development in roots and for cyst populations (data not shown), which showed similar trends and demonstrate that all populations of the nematodes completed only one generation in a growing season.

- A small percentage of egg infection by parasitic fungi was found across all treatments and replicates.

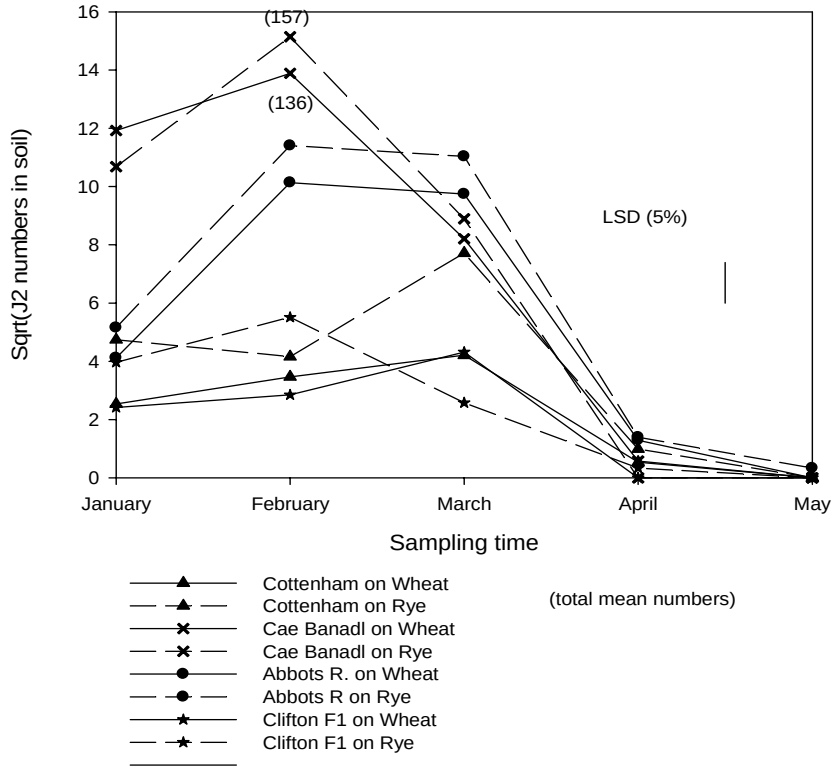

Figure 8 The mean number (square root) of J2 present on wheat and rye in four CCN-infested soils.

The effect of formalin application on field populations

Heterodera avenae populations are suppressed by key fungal agents such as *Pochonia chlamydosporia* and *Nematophthora gynophila* and formalin kills these fungi but not encysted nematode eggs and is a useful tool to estimate the impact of fungal pathogens (Kerry & Crump, 1998). The discovery that *H. filipjevi* is widespread in UK soils required investigation to determine if all CCN species can be controlled by such natural biocontrol agents. The equivalent to the field rate (3000l/ha) of formalin was applied to the soil from each site, which was held at field capacity. After three months all traces of formalin had disappeared from the treated pots before cereals were planted. Winter wheat seedlings (cv Consort) were planted in each pot placed in a glasshouse at 15 °C in a randomised block design and watered daily. At ten, 14 and 18 weeks, four

replicate pots per soil treatment were randomly selected and the females extracted and counted. The numbers of juveniles in roots and the fungal infection in nematode eggs was also measured. The abundance of two egg parasitic fungi Po*chonia chlamydosporia* and *Paecilomyces lilacinus* was estimated in soil treated with formalin and non-treated soil.

Key Results

- Unlike previous results with *H. avenae*, the application of formalin had a significant ($P<0.01$) nematicidal effect in all soils. Fewer females developed in treated soil and it was impossible to use this treatment to estimate the effect of the fungi. Presumably, juveniles of *H. filipjevi* were active in the autumn at the time of treatment and were killed.
- Typically, the decline in the number of female nematodes was not matched by an increase in cyst numbers (Figure 9) indicating that fungi may be active in all soils.
- The proportion of nematode eggs infected with fungi was significantly ($P<0.001$) greater in untreated soil for most populations tested (Figure 10) with the exception of the Cottenham population and the proportion of eggs increased with time in all soils.
- After soil plating for *P. chlamydosporia*, *P. lilacinus* and general fungi, significantly more colony forming units (CFU) were found in the non-treated compared to the formalin-treated soils. The differences in fungal abndance in the different treatments were correlated with the abundance of these fungi.

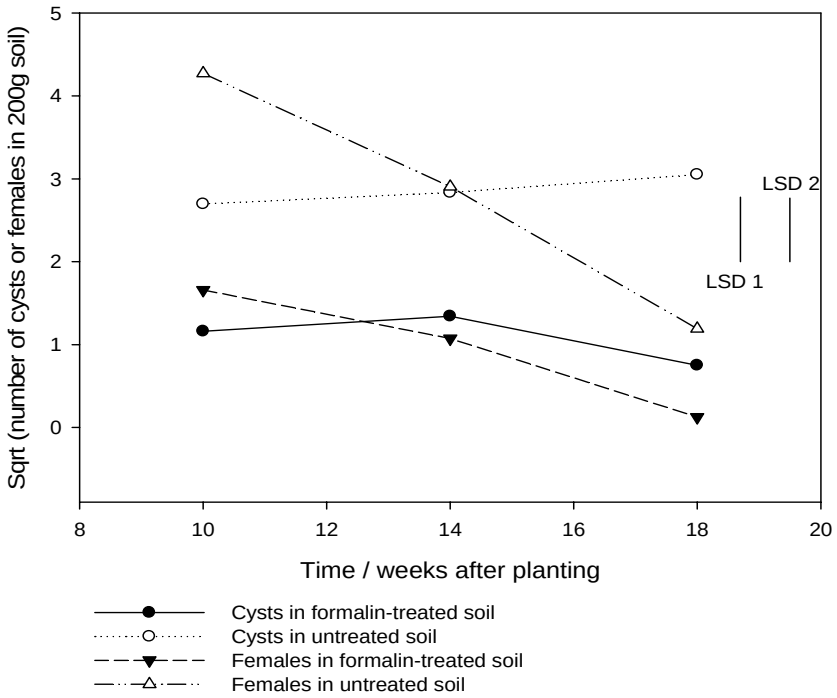

Figure 9 The effect of formalin on the production of females and cysts (Means of 4 replicates)

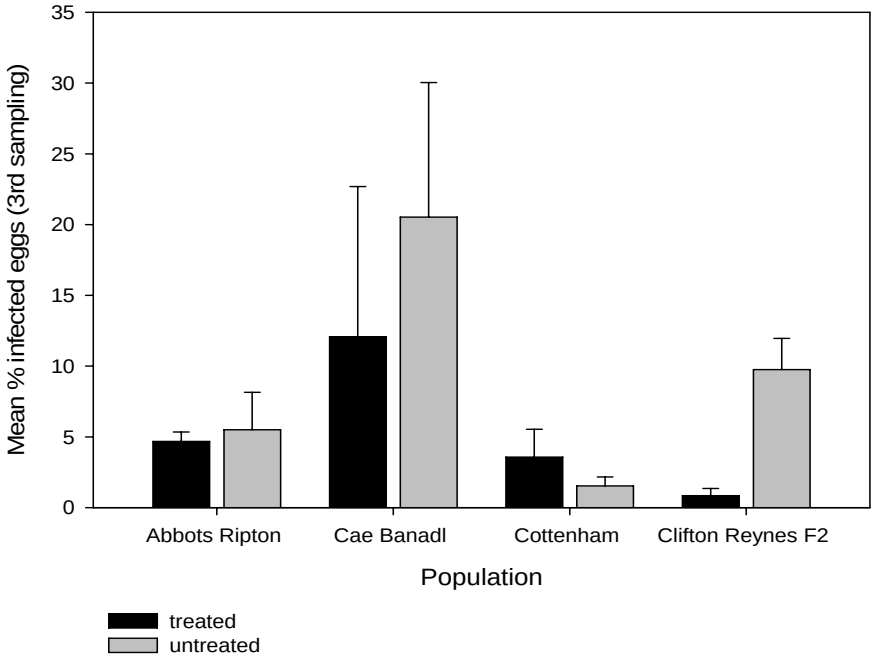

Figure 10 Mean percentage egg infection for formalin-treated and untreated field soils for the third sampling time.

The development of individual females on the roots of wheat and rye in five CCN- infested soils was monitored in transparent containers. The proportion of females that became infected by fungi and their rate of destruction were measured, and the causal agents identified. Soil was planted with wheat or rye in each of ten containers per soil. After six weeks, the chambers were checked twice weekly for the presence of females. The position of each female was marked on the outside of the container and the development of individuals noted on each occasion. This was repeated until all of the females had either reached maturity (i.e. tanned to become cysts) or disintegrated due to fungal infection.

Key results

- In the observation chambers, the time from initial female emergence to disintegration due to fungal infection ranged from 3 to >29 days on both wheat and rye roots.
- In the soils from Cottenham, Clifton Reynes F1 and F2, and Cae Banadl, the females were destroyed by the zoosporic fungus, *Catenaria auxiliaries* (Figure 11).
- In the conditions within the transparent chambers, of the females observed 48% (Abbots Ripton), 90% (Cottenham) and 84% (Clifton Reynes) were parasitised by *C. auxiliaris*, which took between 15-30 days to infect the nematode females and destroy them.

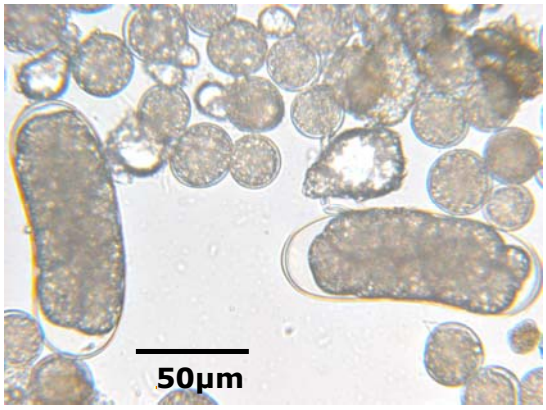

Figure 11 The contents of a nematode female infected with the globose spores of *Catenaria auxiliaris* that can be seen the between eggs.

The destruction of cyst nematode eggs with fungal agents *Pochonia chlamydosporia* and *Paecilomyces lilacinus* will be examined in an *in vitro* experiment in controlled conditions. The experiment will allow the rate of egg

destruction to be measured and will provide an indication of any differences in susceptibility between nematode species. Despite much study, it remains unclear how quickly some isolates of nematophagous fungi destroy the egg contents. Such information is important in which the impact of fungi on nematode survival are measured in repeated sampling. It is necessary to determine if eggs infected at one sampling survive to the next or whether all parasitized eggs result from new infection.

A method for the rapid fungal infection of nematode eggs recently developed at Rothamsted was used to study the rate of destruction of the egg contents. Three nematophagous fungal isolates (CCN Cottenham eggs [PcCC], isolate Pc280, originally isolated from potato cyst nematode [PCN] eggs, and *Paecilomyces* [Pl] also isolated from CCN were added ($5.5x10^4$ conidia ml^{-1}) to 0.0125% Yeast Extract Medium (YEM) and grown at 10°C, 15°C and 20°C. Into each 100 ml conical flask, 18ml of egg suspension (112 eggs ml^{-1}) and 2ml of spore suspension was placed giving the following treatment combinations: *H. filipjevi* eggs + isolate Pc CC, *H. filipjevi* eggs + Pl, PCN eggs + isolate Pc280, PCN eggs + Pl, Control: *H. filipjevi* eggs + 2ml YEM and Control: PCN eggs + 2ml YEM.

The egg/fungus suspensions were shaken using an Orbital Incubator (Jencons,UK), at 100 rev/min for 12 hours at 25 ºC to enable the onset of infection to occur. Suspensions were then washed and placed into a counting dish with four replicates per treatment per temperature. After 0, 8, 24, 36, and 45 days the dishes were examined and scored according to the following key stages: I = No infection; II = Hyphal attachment; III = Hyphae visible inside the egg; IV = Hyphae covering egg; V = Egg consumed.

Key Results

- The optimal temperature for growth of both *Pochonia chlamydosporia* and *Paecilomyces* strains is 25ºC, but this study has shown that the preferred temperature for optimal egg infection is at 15ºC and 20ºC for strains of *Pochonia chlamydosporia* and *Paecilomyces* used.

- The mean total percentage of infected eggs is not significantly different over time for *P. chlamydosporia* and *Paecilomyces* when means are taken across the temperatures and nematode species.
- The rate of infection between days 0 to 8 was higher for *H. filipjevi* but a larger proportion of eggs for PCN were infected compared to that for eggs of *H. filipjevi*.
- The size of egg may affect the rate of destruction. PCN eggs were notably larger than those of *H. filipjevi* and it would theoretically take less time to consume an *H. filipjevi* egg compared to that for PCN. However, the proportion of eggs consumed was similar for the treatments across all of the temperatures tested (Figure 12) .
- The overall mean rate of infection is greater in the *H. filipjevi* eggs than in the PCN eggs, with a rate of 0.598% day^{-1} for PCN and a rate of 0.912% day^{-1} for *H. filipjevi*.

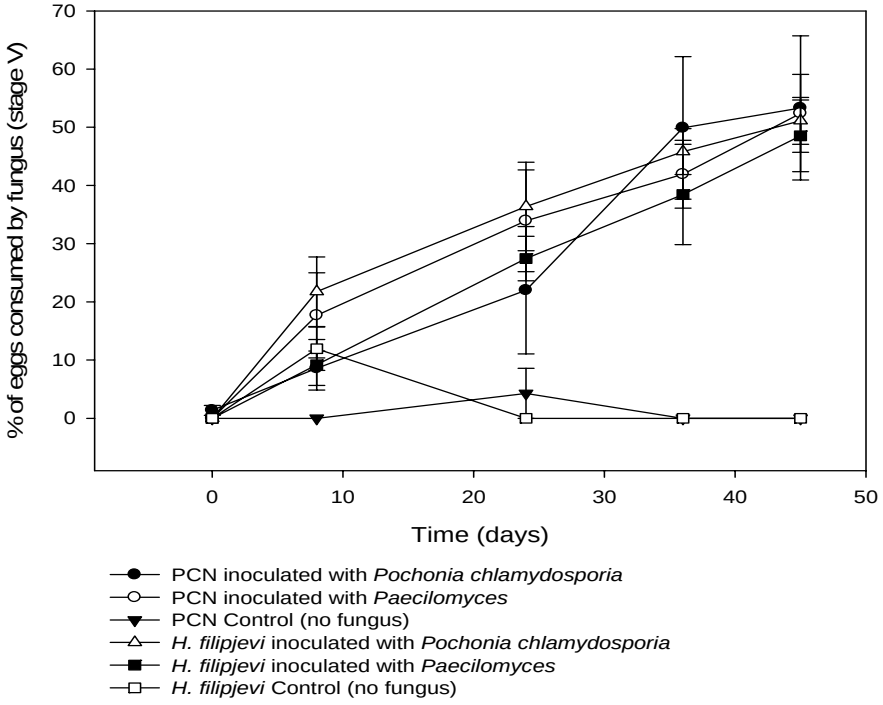

Figure 12 The percentage of eggs consumed over time for PCN and *Heterodera filipjevi* at 15 ºC.

Effects of DIBOA on *H. avenae* and *H. filipjevi* juvenile mortality.

Hydroxamic acids (Hx) are secondary metabolites that are involved in plant defence systems and are found in rye, wheat and maize, but not in oats and barley. The effect of hydroxamic acids on cereal cyst nematodes has not yet been studied. This experiment was designed to study the effect of a range of concentrations of DIBOA on the hatch and activity of the infective J2 of *H.avenae* and *H. filipjevi*.

The effect of DIBOA on the hatch rate of *Heterodera avenae* and *Heterodera filipjevi* was investigated. Dilutions of DIBOA at concentrations of 0, 12.5, 25, 50 and 100µg ml^{-1} were used after initial testing for their activity against laboratory standard cultures of root-knot nematodes and potato cyst nematodes that were available in much larger numbers than CCN. Four *Heterodera avenae* cysts from the 'Ask' population were gently placed into wells containing 1ml of each of the DIBOA solutions or the distilled water control. Each treatment was replicated five times in a 25-well plate. The dishes were then sealed with Parafilm and placed in an incubator at 17ºC. The cumulative number of active and inactive juveniles was counted twice weekly until no further hatching of juveniles was observed in any of the wells. This method was repeated for *H. filipjevi* from the Cottenham population.

Key Results

- Few juveniles of *H. avenae* hatched in the control compared to the treatments in which the cysts were exposed to the DIBOA solutions. Therefore DIBOA does not act to inhibit juvenile hatching process of *H.avenae* (Figure 13).
- However, there is a concentration gradient effect on the activity of the hatched juveniles and with time more become inactive with increased DIBOA concentrations (Figure 14).
- The total cumulative hatch of *H. filipjevi* was smaller than that for *H. avenae* but again exposure to most concentrations of DIBOA stimulated hatch (Figure 15); only exposure to 100µg ml^{-1} caused a slight but consistent reduction in the hatch.

- As with *H. avenae*, there is a concentration gradient effect on the activity of the hatched *H. filipjevi* juveniles and with time more become inactive with increased DIBOA concentrations (Figure 16).

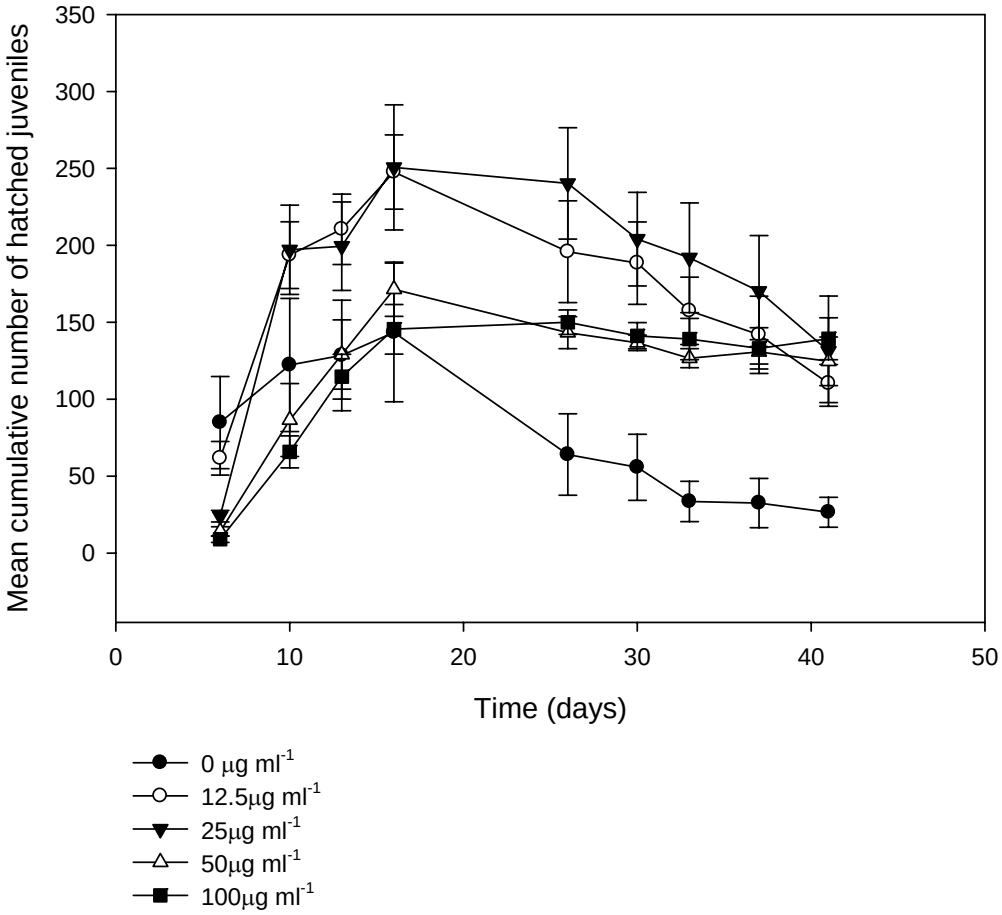

Figure 13 Cumulative hatch of juveniles of *H. avenae* exposed to different concentrations of DIBOA.

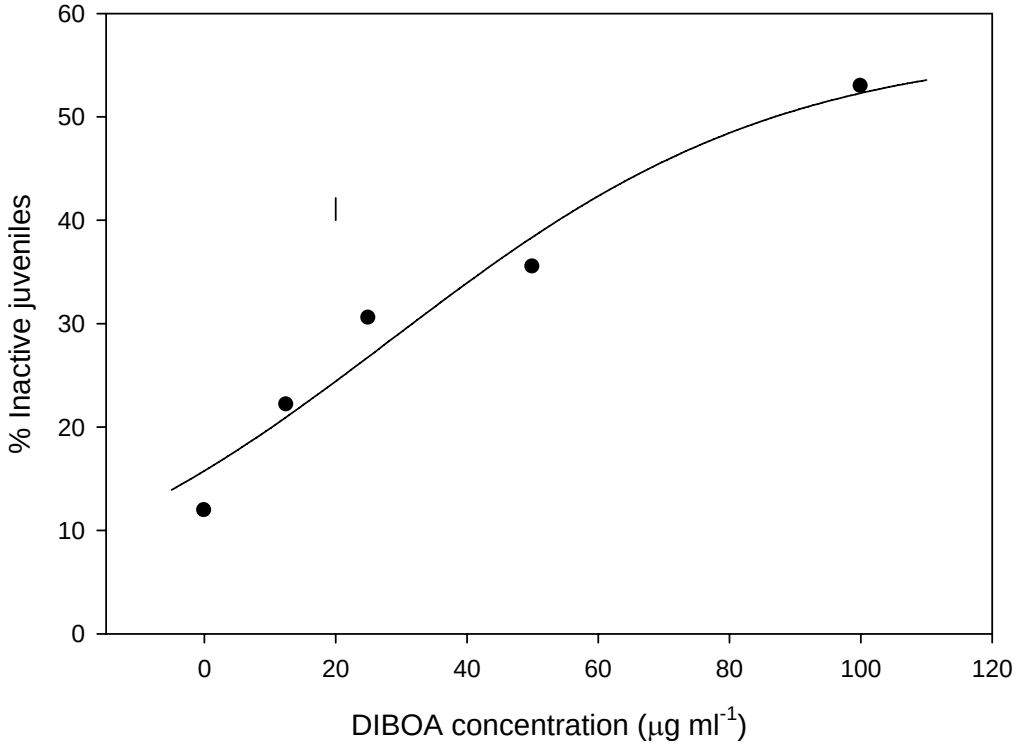

Figure 14 Mean proportion (%) of inactive *H. avenae* juveniles to different concentrations of DIBOA (means across days 13-33).

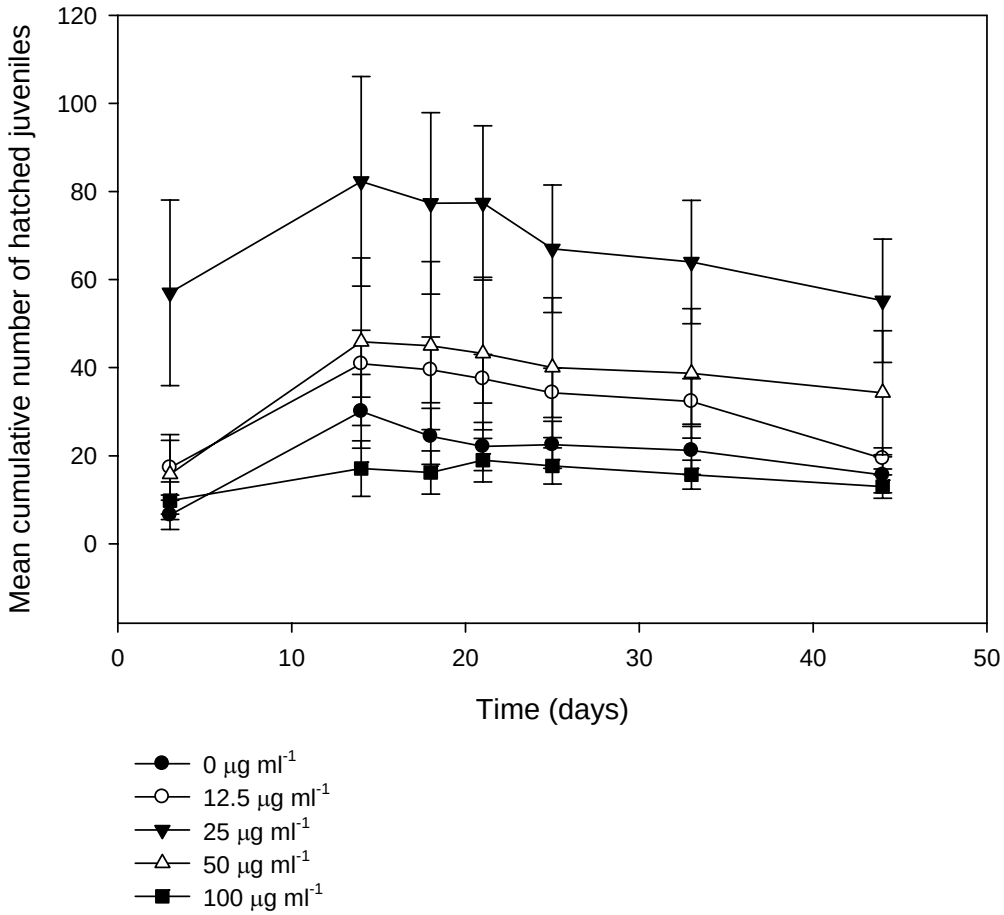

Figure 15 Cumulative hatch of juveniles of *H. filipjevi* exposed to different concentrations of DIBOA.

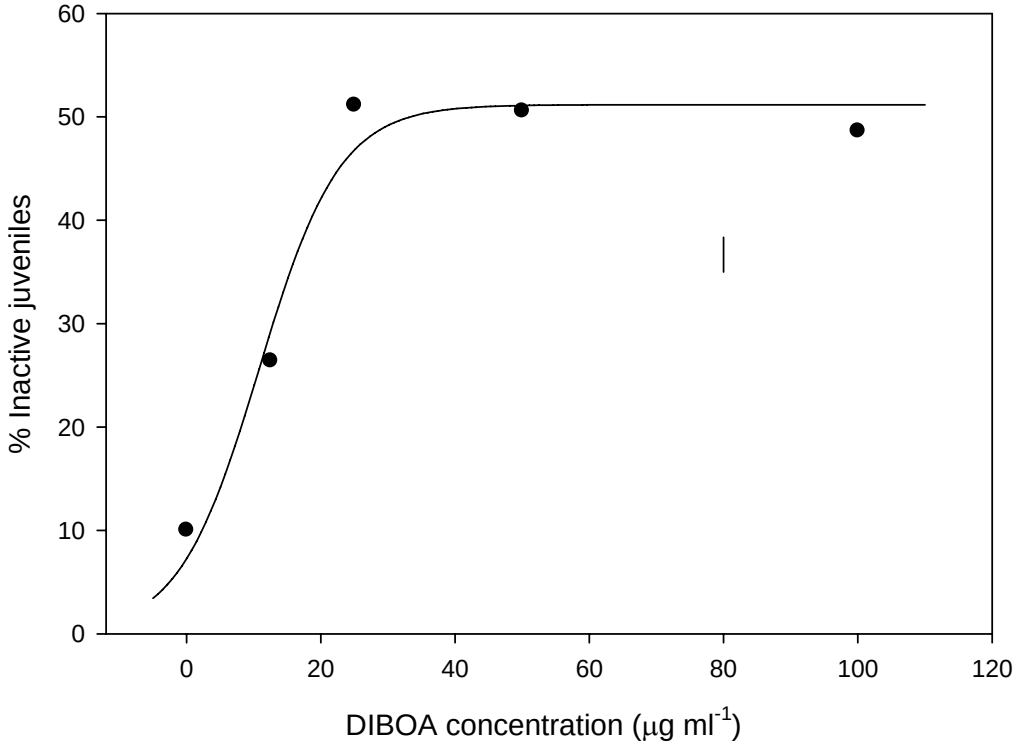

Figure 16 Mean proportion (%) of inactive *H. filipjevi* juveniles to different concentrations of DIBOA (means across days 14-33).

The Effect of DIBOA on *H. avenae* and *H. filipjevi* Juvenile Mortality was investigated. *Heterodera avenae* juveniles from the Cottenham population and *H. filipjevi* juveniles from the Cottenham, Clifton Reynes F2 and Abbots Ripton populations were hatched in tap water. The hatched juveniles were removed and stored at 4°C until used in the sensitivity assay. The juvenile suspension was adjusted to give approximately 30 juveniles in 100µl of water. A single aliquot of 30 juveniles was placed into each well, including the controls, with a total of 25 wells, with 5 replicates per concentration of DIBOA. To the controls, 900µl of sterilised distilled water was added to give a total of 1ml per well. To the other wells, 900µl of the DIBOA solution was added, with 5 replicates per concentration with the following concentrations to make total concentrations of 11.25, 22.5, 45 and 90 µg ml^{-1}. An initial count of active and inactive juveniles was taken before exposure and then the dishes were sealed with Parafilm and placed in an incubator at 17°C for 48 hours. After 48 hours a second count of active and inactive juveniles was taken. To determine if the effect could be

reversed, the juveniles were rinsed and transferred to sterilised distilled water (sd H_2O). The dishes were re-sealed and placed back in the incubator at 17°C for a further 24 hour period, after which a further count of active and inactive juveniles was conducted.

Key Results

- After 48 hours exposure of the juveniles to DIBOA, *H filipjevi* appeared significantly less sensitive to changes in DIBOA concentration than *H. avenae*. As in the hatching test *H. avenae* and *H. filipjevi* juveniles appeared to react differently to DIBOA (Figure 17). Above concentrations of 11.25µg ml^{-1} DIBOA, the mortality (inactivity %) of *H. avenae* juveniles and of those of *H. filipjevi* reaches a maximum, which was similar for both species (Figure 18).
- However, after transferral of juveniles into distilled water, the effect of DIBOA on cereal cyst nematode juveniles was partly reversible with significantly more juveniles of *H. filipjevi* becoming active compared to those of *H. avenae*, which remain inactive at concentrations >11.25µg ml^{-1}.

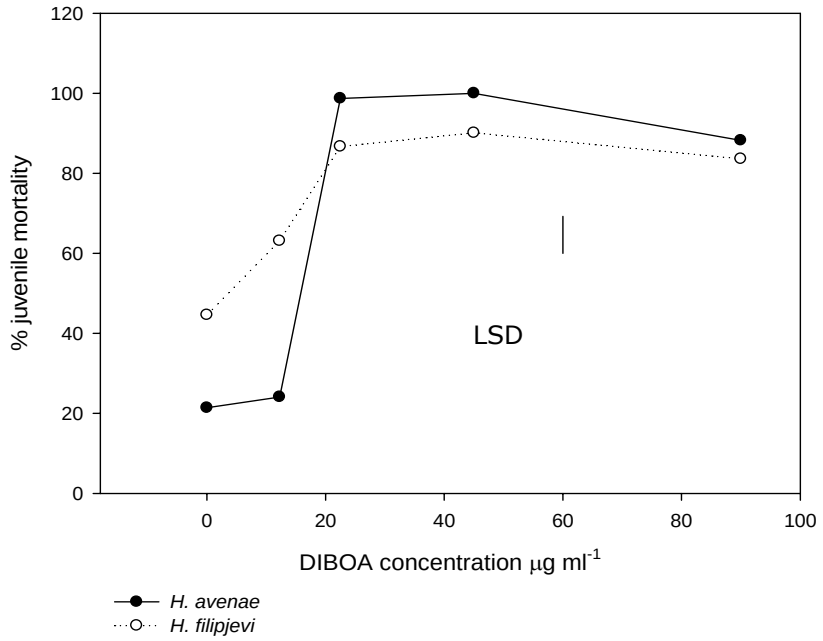

Figure 17 The effect of DIBOA concentration on % juvenile mortality for *H. avenae* and *H. filipjevi* after 48 hours exposure.

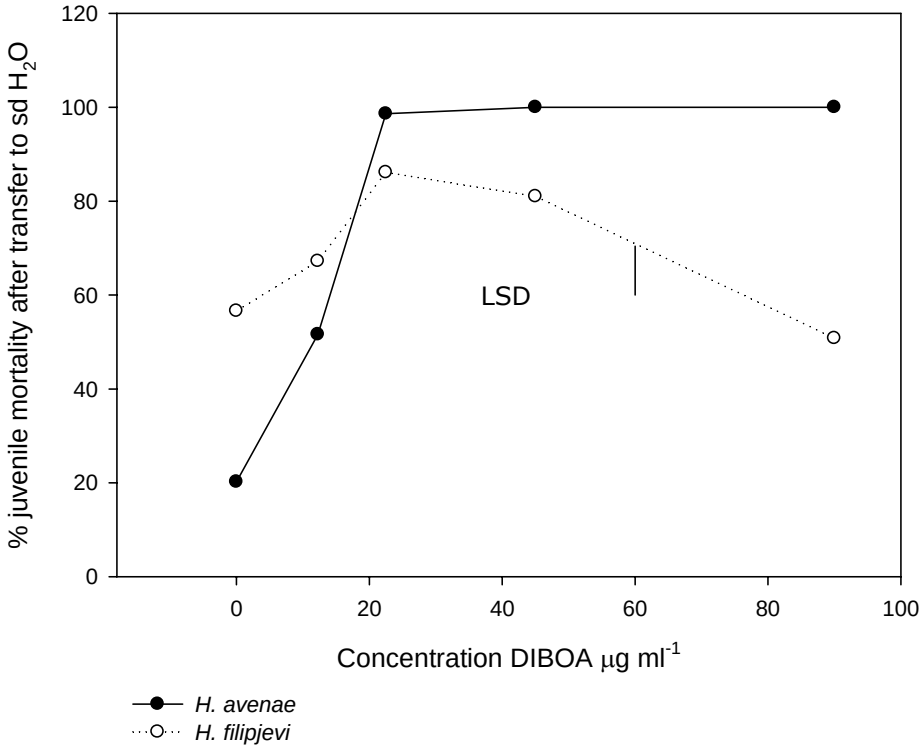

Figure 18 The effect of DIBOA concentration on % juvenile mortality for *H. avenae* and *H. filipjevi*, after transferal of juveniles to distilled water (sdH_2O).

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