Final Report

Managing potato cyst nematode through maximising natural decline and population suppression

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EXECUTIVE SUMMARY BY OBJECTIVES

The project aimed to identify the key factors that affect PCN-decline and to explore the manipulation of fungal parasites to increase egg mortality as an alternative to the application of chemical nematicides. The project was divided into eight overall scientific objectives:

Identify key factors that affect PCN population suppression and decline rates during and between potato crops.

- Soil sterilization increased the number of bacteria but this effect was not apparent after 3 years and there was no relationship with the rate of PCN decline.
- An inverse relationship was observed between the number of eggs per cyst and the level of egg infection by fungi in some soils but not others and the amount of infection on a single occasion was not predictive of PCN decline rates.
- Soil chemical properties could not be used to predict PCN decline rates in the limited range of soils tested in controlled experiments.

Develop new protocols for measuring decline rates in field soils, based initially on cyst content, and to relate population suppression and decline rates to differences in microbial populations using DNA and PLFA profiling techniques.

- The Phospholipid Fatty Acid (PLFA) analysis showed that the soil microbial communities differed between sites but the method was not sufficiently discriminating to identify populations of microorganisms that might be involved in the decline of PCN populations.
- PCR enabled discrimination of the nematode egg-parasitic fungi, *Pochonia chlamydosporia* and *Paecilomyces lilacinus*.
- Four new isolates of fungi were identified with possible potential to be used in the development of new management strategies.
- Although not originally written into the project a monoclonal antibody was successful in discriminating between viable and non-viable eggs and could be developed into an assay for the quantification of viable nematode populations in field soils.

Investigate the influence of edaphic factors and cropping regimes on decline rates of PCN.

- Weak interactions between soil type and cropping regime that affected PCN decline were not sufficiently large to be used as a method to manage PCN infestations.
- Although the majority of break crops had no effect, *Solanum sisymbriifolium* significantly increased PCN hatch in laboratory tests and could therefore be used in PCN management regimes.

Assess the effects of different soil applied fungicides and residual herbicides on PCN decline rates and the associated microbial communities estimated using the above methods

- *P. chlamydosporia* and *P. lilacinus* showed different levels of sensitivity to fungicides but were tolerant to herbicides. (Objective 4)

Investigate and establish the biotic and abiotic factors that influence growth and development of the three fungi, *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Plectosphaerella cucumerina*.

- The presence but not the type of decomposing roots had a significant, negative effect on egg parasitism by *P. chlamydosporia*.
- The spread of *P. chlamydosporia* from the site of application near the tuber into the soil increased with time, and could be found at both 10 cm and 15 cm distant from the plant.
- Populations of the fungus reached a maximum 10 cm from the tuber but they continued to increase after 12 weeks, at 15 cm distance from the plant. It is possible that this increase was caused by secondary production of conidia (secondary growth).

Investigate potential formulation and delivery of fungal agents, including commercially available product including any potential phytotoxicity implications.

- The type of formulation did not have significant effect on the control of PCN and when the fungi were applied at recommended rates these were not phytotoxic.

Investigate carbon and nitrogen acquisition and potential translocation by the three fungi. These important studies will greatly advance our understanding of nematode parasitic fungi and their development in bulk soils and rhizosphere with access to alternative nutrient sources.

- *Paecilomyces lilacinus, Monographella cucumerina* and *P. chlamydosporia* were limited and there was little proliferation above the initial inocula added to acid washed sand; all fungi were much more abundant in the presence of potato roots.
- *P. lilacinus* was the most abundant fungus in soil and *M. cucumerina* appeared to be able to grow only in the presence of potato roots.
- The presence of the plant had a different effect on the two isolates of P. *chlamydosporia* tested; isolate Pc392 being able to exploit the nutrients provided much more readily than isolate Pc280.
- In a second experiment on different crop plants, the presence of roots had no effect on fungal abundance but there were significant interactions between the fungi and the plant species that affected the numbers of Colony Forming Units (CFU) on roots.

Assess the importance of fungal parasites in the patch dynamics of PCN.

- There was a weak inverse relationship between the age of a PCN patch and the presence of nematophagous fungi involved in decline with the central area of the patch, the oldest and most established PCN area, having an increased fungal population than the edge of the patch where the youngest PCN population was present.

Assess the effects of different granular nematicides and a fumigant on PCN decline rates in soils of different textures and types with especial reference to the populations of three known PCN parasitic fungi and the general microbial community measured by a range of techniques including baiting, Molecular diagnosis, PFLA and HPLC.

- Granular nematicides were successful in controlling PCN populations at different sites and in several trials biological control fungi were also able to control PCN multiplication rates however, the biological control agent produced much less reliable effects and there were large differences in their ability to control PCN across different sites. **It is likely that the initial PCN population density in soil is critical in the ability of biological control fungi to succeed.**

1. EXPERIMENTAL SECTION

1.0 Introduction

Potato cyst nematodes (PCN) are the major pest constraint to potato production in the UK. About 64 % of potato land in England and Wales is infested with PCN and the nematode is increasing in abundance. Nematicides are a necessity to maintain the economic viability of some potato growing areas and in 2002, 27% (*c*. 30,000 ha) of the area of the potato crop was treated at an approximate cost of £10M. Consumer concern over the use of pesticides has prompted some supermarket chains to limit the amounts of nematicide that may be used by individual growers. If necessary, produce will be sourced outside the UK and this is particularly relevant to organic potato production, which is increasingly difficult, mainly due to there being few cultivars available that have partial resistance to *G. pallida*, the dominant species of PCN in the UK. The use of a partial resistors leads to the subsequent selection of a PCN population virulent to that cultivar.

For PCN, their narrow host range amongst solanaceous crops has meant that they may be effectively managed by crop rotations as long as volunteer potatoes are effectively controlled. However, the effectiveness of crop rotations is countered by the longevity of the nematode in soil and the spontaneous hatch of PCN eggs, which in the absence of a host crop is typically only c. 20-40 % per annum for *G. rostochiensis* and 10-30 % per annum for *G. pallida*. Hence, if soils become heavily infested, it can take many years for populations to decline to non-damaging levels (Whitehead, 1995).

As decline rates in different populations vary from 10 – 50 %, it is apparent that being able to predict or manipulate the occurrence of rapid decline rates could have important management consequences. Therefore, it is essential to understand the factors that affect decline rates between potato crops. Typically, on a mineral soil heavily infested (100 eggs g^{-1} soil) with *G. pallida*, it may take 13 years for the population to decline without the use of nematicides or resistant cultivars, to a level that is safe (< 5 eggs g^{-1} soil) to grow a susceptible potato crop. However, much of the early data on decline rates are difficult to interpret because population estimates were based on cysts and not eggs and the age and species structure of the populations was often not defined.

On average, *G. rostochiensis* has a 40% greater spontaneous hatch than *G. pallida* and is more readily controlled by crop rotation (Evans and Haydock, 2000), but differences between nematode populations and soil conditions greatly affect this hatch. Stone *et al*. (1973) demonstrated that non- host cropping with cereals, grasses and a range of horticultural crops had no effect on the rates of decline of *G. pallida* that varied between 15 and 24% per annum over a 7 year period. Regular cultivation of infested soil increased decline rates, which might be expected to be slower under grass (Turner, 1996).

Fungal parasites of females and eggs have long been recognised as the causal agents for the decline of populations of cereal cyst nematodes in monocultures of susceptible crops. Soils suppressive to potato cyst nematodes have also been recognised but are much less widespread. However, similar types of fungi have been found attacking the eggs of both pests and three species, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Monographella*

(syn. *Plectosphaerella) cucumerina*, have been identified as pathogens of PCN. There is a need to assess the importance of these fungi in the population dynamics of the nematode. In general, applications of such biological control agents require integration with other control measures to provide practical levels of nematode control and they have been successfully used with granular nematicides, crop rotation and partially resistant cultivars.

In general, most research on PCN has concentrated on the management of the pests in the potato crop and little effort has been placed on affecting the survival of the nematode between crops, although this is known to differ significantly in different soils. The Defra commissioned review (2003) of research priorities for PCN Management recognised the need for more information on the factors affecting decline rates and on the opportunities for their manipulation. The project set out to:

- to understand the factors that affect decline rates; and
- to manipulate the fungal parasites of potato cyst nematodes (PCN) in order to:
  (a) significantly increase nematode egg mortality between potato crops,
  (b) reduce the occurrence of nematode "hot spots" in fields, and
  (c) decrease the need for long crop rotations, beyond those required for other diseases.

The hypotheses tested were:

- It is possible to manage field soils in such a way as to enhance the decline rate of PCN without the addition of a chemical or biological agent, using certain cropping regimes and cultivation techniques with the potential for organic production of potatoes.

- The addition of a commercially produced fungal agent developed for the control of PCN will instigate or promote population suppression to PCN in UK soils.

- Fungal parasites of PCN significantly reduce the survival of eggs of the nematode in soil and establish an equilibrium population, which would allow reductions in the long rotations especially needed to manage *G. pallida*.

- The presence of crop rhizospheres or fresh organic matter may enable the fungus to proliferate but prevent it from switching to the parasitic phase and the consequent large scale parasitism of PCN eggs.

- Neither the addition of a commercially available fungal agent or those developed at Rothamsted will have a detrimental effect on beneficial non-target organisms.

- Some soil-applied pesticides will have a greater detrimental effect on the three PCN parasitic fungi, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Plectosphaerella cucumerina* that influence the suppression and decline rate of PCN, than others.

- The three PCN parasitic fungi, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Monographella cucumerina* have a preference for PCN as a nutrient source and will translocate nutrients across the mycelial web as far as efficiency allows so that low levels of PCN will keep the fungi in parasitic rather than saprophytic phase.

- Using a combination of techniques such as cyst assessment, DNA markers and PFLA profiling, it is possible to ascertain the potential level of suppression and decline rate of PCN in a field soil.

The specific scientific objectives were:

1. To identify the key factors that affect PCN population suppression and decline rates during and between potato crops.

2. To develop new protocols for measuring decline rates in field soils, based initially on cyst content assessment, and to relate population suppression and decline rates to differences in microbial populations using DNA and PFLA profiling techniques.

3. To investigate the influence of edaphic factors and cropping regimes on decline rates of PCN

4. Assess the effects of different soil applied fungicides and residual herbicides on PCN decline rates and the associated microbial communities estimated using the above methods

5. Investigate and establish the biotic and abiotic factors that influence growth and development of the three fungi, *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Monographella cucumerina.*

6. Investigate potential formulation and delivery of fungal agents, including the commercially available product and measure any potential phytotoxicity .

7. Use radiolabelling in laboratory and glasshouse studies, to investigate carbon and nitrogen acquisition and potential translocation by the three fungi. These important studies will greatly advance our understanding of nematode parasitic fungi and their development in bulk soils and rhizosphere with access to alternative nutrient sources

8. Assess the importance of fungal parasites in the patch dynamics of PCN.

9. To assess the effects of different granular nematicides and a fumigant on PCN decline rates in soils of different textures and types with especial reference to the populations of three known PCN parasitic fungi and the general microbial community measured by a range of techniques including baiting, Molecular diagnosis, PFLA and HPLC.

2. MATERIALS AND METHODS

2.0 General Methods

Extraction of cysts from soil and the estimation of potato cyst nematode populations
Standard procedures were used for the extraction and quantification of cysts and eggs *of Globodera* spp, as outlined by Shepherd (1986).

Methods for working with nematophagous fungi
Standard procedures were used for working with nematophagous fungi were followed and can be found in *A manual for research on* Verticillium chlamydosporium*, a potential biological control agent for root-knot nematodes* (Eds Kerry and Bourne, 2002*).* Methods used but not incorporated within this manual are detailed below under the relevant objectives.

Egg viability and infection by nematophagous fungi
Release of encysted eggs: Cysts were put in small excavated glass blocks and were rinsed twice with sterilised distilled water (SDW). The cysts were then carefully put in a drop of sterilised distilled water on a sterilised channelled aluminium slide (7.5 × 2.5cm, the channel is 15 mm wide, 0.05mm deep) and were squashed with a glass slide to release the eggs. 250 µl of SDW were added to suspend the eggs and the egg suspension was transferred into a 2 ml tube. The slide was washed another three times with 250 µl SDW to make 1 ml egg suspension. The egg suspension was mixed and 0.5 ml was transferred to another 2 ml tube. One tube (0.5 ml) was used to assess the egg infection, the other tube (0.5 ml) was be used to assess the egg viability.

Egg infection: 0.5 ml of egg suspension was centrifuged at 13000 rpm for 4 min, the supernatant was discarded and 1 ml SDW was added to re-suspend the eggs. The suspension was again centrifuged and the supernatant was discarded .The eggs were then washed with 1ml SDW, and then re-suspended in 0.6 ml SDW. 0.2 ml of the egg suspension was spread onto a plate containing water agar with antibiotics. Each sample had three replicated plates (each with 0.2 ml egg suspension. Plates were incubated at 25°C for two days. After incubation, the numbers of infected and uninfected eggs were counted using a dissecting microscope and the percentage of egg parasitism was calculated.

Egg viability: 0.5 ml of egg suspension was centrifuged at 13000 rpm for 4 min, the supernatant was discarded and the eggs were re-suspended in 0.5 ml 0.05% (W/V) aqueous solution of Meldola Blue. Samples were incubated at 20°C in darkness for five days. After this time, the stain was removed and the samples were centrifuged at 13000 rpm for 4 min, the supernatant was discarded, then 1 ml distilled water was added to the tube to wash the eggs. The tubes were mixed well and were centrifuged and the supernatant discarded. One ml of distilled water was added, the eggs were re suspended and were left incubating at 20 C, for one day. After this time, 0.1 ml egg suspension was transferred to a counting tray and the number of viable eggs (not stained) , dead eggs (stained dark blue), empty eggs, active and dead juveniles were counted using a dissecting microscope. Three replicated counts (i.e. counting three 0.1 ml egg suspensions) were made on each sample.

Soil Analysis: Total nitrogen and carbon analysis was undertaken by the Soil Analytical Group at Rothamsted using a LECO CNS 2000 Combustion Analyser (LECO Corporation, St

Joseph, Michigan, USA). Briefly soil samples were air dried and weighed into 'boats' and placed in the auto-loader. In the combustion chamber, the furnace and flow of oxygen caused the soil sample to combust and any elemental C, S and N were converted to CO_2, SO_2, N_2, and NOx. Water was then removed and the gas left to equilibrate before the nitrogen and carbon were quantified. The soil pH was also measured using standard techniques.

2.1 Methods objective 1

To identify the key factors that affect PCN population suppression and decline rates during and between potato crops.

Soils: Twelve soils (Table 2.1) that had been identified as having substantially different rates of decline and cropping histories were collected and selected to further assess PCN decline rates over three seasons.

Experimental details: The 12 soil samples were thoroughly mixed and a 200 g sub sample removed before being divided into two sets and between 8-9 kg of each soil placed in heavy duty plastic bags, and stored at 4^{o}C until used. One set of bags of each of the 12 soils was irradiated with a minimum absorbed radiation dose of 50 kGy at Isotron plc, Daventry, UK, and then returned to the cold store. The soil from both the irradiated and non-irradiated field samples was put into large plant pots (26.5 cm diameter) and placed in the sand plunge at Rothamsted Research. Cysts that had been extracted from Waddington Stone Pit soil (a soil known to have a large population of PCN) were placed in 5 small nylon mesh bags (each containing 25 cysts) and were buried in the pots containing the different soils. The pots were kept free of weeds throughout the experiment. The experiment was set up using randomised blocks and each treatment had three replicates. At each sampling time point (November 06, February 07, November 07, March 08 and October 08), one bag of cysts was taken and the number of eggs/cyst, infected eggs and egg viability (%) was measureded. Data were analysed using Analysis of Variance (ANOVA) and correlation analysis using GenStat 11th Edition®.

Soil analysis: The percentage of carbon (%C), nitrogen (%N), pH, and phosphate (PO_4-POlsen) was determined for each of the soils tested. Using this information, the soils were grouped according to their similarities (Euclidean distance) using principal coordinate analysis, in GenStat 11th Edition®.

TABLE 2.1 THE TWELVE SOIL SAMPLES ANALYSED IN THE EXPERIMENT: NUMBERS OF EGGS PER G OF SOIL, RATE OF PCN DECLINE AND CROPPING HISTORY

Soil/ Field name	Eggs/g soil	PCN Decline rate	Crop history
Cranberry 21	13	Fast	Brassica crops in rotation; no fumigants, organic manure (composted).
Cranberry 22	13	Fast	
Mill Dam Close	23	Not known	Had potatoes as crop in 2005
Lansome	1	Not known	Had potatoes as a crop in 2000
Lees	34	Slow	Organic soil, Had potatoes as crop in 2005
Severals	32	Slow	
Plantation	29	Slow	
Field 45	1	Moderate	
Matthews 9	6	Fast	Organic soil, no compost, no manure added
Parish A	4	Fast	
Parish B	8	Fast	
Wincups	45	Fast	

2.2 Methods objective 2

Develop novel methods for predicting rates of decline

a) *The relationship between cyst size, numbers of eggs per cyst and cyst age: Use of the Atkinson algorithm*: It has been found that there is a high correlation between the size of a newly formed cyst and its egg content. An algorithm (Atkinson, 2001) has been developed that relates cyst size and the time from the last potato crop to predict the rate of egg loss for PCN infested field soils in Bolivia. In order to investigate whether similar relationships occur with UK fields preliminary investigations were conducted to assess whether cyst size was related to egg content. The width and length of 32 cysts was measured and the relationships between cyst volume, cyst area, percentage of egg hatch and number of eggs per cyst was assessed. The Analysis of Variance (ANOVA) and regression analysis were applied to the results using GenStat 10th Edition®.

b) *Use of PLFA analysis*: Samples of soil (25 g) from each of the 12 field sites were collected and placed at -80^{o}C and then freeze dried using a Heto PowerDry™ (Thermo Electron Corp) and a Genovac™ pump. PLFAs were extracted from replicated 5g samples of soil from each site using the method of Bardgett *et al*. (1996). Fatty acid nomenclature was as follows: fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, cis18:1g7 and cy19:0 represented current known bacterial PLFAs (Frostegard *et al*., 1993; Federle, 1986; Tunlid *et al*., 1989), 10Me18:0 was indicative of actinomycetes and 18:2g6 was used as an indicator of fungal biomass (Federle, 1986).

c) *Use of DNA markers to identify and quantify nematophagous fungi*. Techniques for the identification of the nematophagous fungi *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Monographella cucumerina* had been developed previously (Hirsch *et al*., 2000; Mauchline *et al*., 2002; Atkins *et al*., 2003 and 2005). These were used to confirm identification from selective media and also tested for the quantification of fungi from soil samples.
Direct extraction of fungal DNA from soil: Field soil (Severals) and clean sand were inoculated with spores of *P. chlamydosporia* at different rates (500000, 5000, 500, 50 and 0

chlamydospores per g of soil/sand). 0.25 g sub samples were taken from each treatment and DNA was extracted using PowerSoil™ DNA Extraction kit. A second kit (ZRSoil®) was tested on the soil samples from Severals. After extraction, the quantity of DNA was measured using spectrophotometer and ITS PCR was performed using REDE Extract-N-Amp Tissue PCR kit (SIGMA).

Combined technique (selective media followed by conventional PCR): soil samples were mixed thoroughly; 1g sub-samples were taken and suspended in 9 ml of 0.05 % water agar. The soil suspensions were mixed for 20 seconds using a vortex mixer and a second dilution was made, transferring 1 ml of the soil suspension into 9 ml of 0.05 % water agar. 200 µl of the soil suspension (10^{-2} dilution) was plated onto different selective media (SM) according to the method described by Kerry and Bourne (2002). The SM was specific for each fungus under study. For PCR, colonies were picked from the SM after 14 days of growth, and were transferred onto corn meal agar or potato dextrose agar plates, in order to have enough mycelium for the extraction of DNA. After 1 week, the mycelium was collected and the DNA was extracted and amplified using REDE Extract-N-Amp Tissue PCR kit (SIGMA). The kit contains all the reagents needed to rapidly extract and amplify genomic DNA from animal tissues, and it is very efficient in extracting DNA from fungal material. Specific PCR primers and cycling parameters were used following the protocols developed by Hirsch *et al*., 2000; Mauchline *et al*., 2002; Atkins *et al*., 2003 and 2005.

d) *Use of trehalose to predict PCN decline*

Trehalose is the main fluid surrounding unhatched juveniles in eggs of PCN (Clarke and Hennessy, 1976; Perry *et al*., 1983). Eggs of *G. rostochiensis* contain 0.34 M trehalose surrounding the perivitelline space, as opposite to the juvenile, which does not contain this sugar in its composition (Clark & Hennessy, 1976). Exposure to potato root diffusate (PRD) is thought to induce changes in the permeability of the eggshell, especially the inner lipoprotein layer membrane (Jones *et al*., 1998), causing trehalose to leak out of the eggs, allowing the juvenile to hydrate and start the behavioural sequence which will lead to eclosion. The length of exposure, type of PRD (such as age of the host plant) and concentration of PRD are thought to affect the rate of hatching. However, spontaneous hatch of nematodes has been reported under field conditions (Perry *et al*., 1983), without apparent stimuli from the plant.

The experiment was set up using cysts from one population of PCN (Barnes). Eggs were extracted from cysts and separated in batches of 100 eggs. The eggs were placed in excavated glass blocks (100 eggs per glass block, 5 replicates per treatment) and the following treatments were applied: exposure of eggs to PRD for 30 minutes followed by incubation in soil leachate (weekly PRD pulse), exposure to PRD and incubation in PRD (positive control), exposure and incubation in soil leachate (negative control). The excavated glass blocks were covered with glass lids to avoid evaporation and were incubated at 20ºC. Every week for 8 weeks, the numbers of juveniles that hatched was recorded, the juveniles were removed and new PRD or soil leachate was added to each treatment. A second experiment was set up using similar treatments and replicates as the above experiment but this time the treatments were left at 5ºC for 2 weeks, prior to incubation at 20 ºC. A General Linear Model (GLM) was used to study the effects of PRD on hatching after 8 weeks, for each temperature scenario. The numbers of juveniles that were hatched in each week (1-8 weeks, 8 observations per treatment) was modeled in order to access the distribution of time to hatch for each of the treatments. For each replicate, a cumulative distribution of counts of juveniles on log (time) was fitted, to estimate the mean, the standard deviation and the proportion of juveniles that hatched in time, given the total number of eggs. The method used the differences in cumulative count for each replicate and applied the Cumdistribuition procedure, in GenStat® (2007) (Brain and Butler, 1988).

e) *Use of* ELISA *to predict PCN decline*: Antibodies are available that can distinguish *Globodera pallida* from *G. rostochiensis* and there is some evidence that these antibodies may recognise a 34 kDa antigen that is related to viability (Robinson *et al*., 1993; Davies *et al*., 1996). Therefore, although not originally written into the project the consortium decided that it would be helpful if these antibodies could be used to identify and quantify viable eggs within cysts. Experiments were set up to test the hypothesis that the presence of the 34 kDa protein is related to egg viability and can be developed as a diagnostic to identify and quantify viable PCN eggs. Briefly, known numbers of eggs and juveniles, both live and dead, were homogenised in a small volume of homogenising solution and then diluted in coating buffer and incubated overnight in wells on and ELISA plate (Nunc, Maxisorb™). An immunoabsorbant assay was undertaken using standard procedures (Davies and Carter, 1995).

2.3 Methods objective 3

Determine rates of decline in soils under different break crops and in commercial cropping systems treated with selected fungal agents.

*Assess the rate of PCN decline under different break crops***:** Soil samples from an organic soil (Severals) and a mineral soil (Woburn) were obtained and stored at 4°C until use. Soil (500g) was then placed in a plant pot (12 cm diameter) to which were added 2 week old seedlings of wheat, pea, oil seed rape and one series of pots was left fallow. A small nylon bag containing 25 PCN cysts (Waddington or Plantation population) was also placed in the soil with the seedlings. The pots were then placed in the plunge and arranged in a randomised block with four replicates, and were watered as necessary. The experiment was harvested in August 2007. To assess the effects of the type of crop, soil (Severals or Woburn) and PCN population (Waddington or Plantation), the numbers of eggs per cyst and the proportions of eggs that were viable and fungus infected were analysed using ANOVA (3-way interaction), in GenStat 11th Edition®.

Assess the rate of PCN decline in field trials using selected nematophagous fungi under commercial cropping.

Field sites 2007: Five field sites were identified (Whaplode, Chatteris, Holme Farm, Holbeach Hurn, Red Houses and Aerodrome, Dr A. Barker, Branston, personal communication) and three one metre square plots, incorporating two potato ridges, were randomly placed in a 25 metre square area within each field.

Fungal inoculum e: A selected isolate of *Paecilomyces lilacinus* was bulked up and formulated as an application-ready wettable spore powder by BCP (Biological Control Products SA). The name of the product as supplied to Rothamsted was PL LINK. The spore concentration was 4 x 10^9 spores /gram. The recommended application rate was 1000 gram per ha (4 x 10^8 spores m^2) which equates to 0.1 gram inoculum per m^2. The product was applied together with a spore activator at a ratio 1:1 w/v following the manufacturer's instructions. Briefly, the spore activator was diluted with twice the volume of water and the product was applied shortly after planting of the potato crop but before crop emergence to ensure that the product is present when nematode hatch will occur. A second application of the product was applied 3-4 weeks after the first application.

Sampling: Soil samples were taken from the treatment blocks before the first treatment and before the second treatment and at the end of the season. The amount of PCN present was determined together with the level of *Paecilomyces* present in the soil by means of dilution plating onto the selective medium. At the end of the field season the yield was determined comparing the treated blocks with the yield of an equal surface area used a as control.

Field sites 2008: Further field trials were undertaken in 2008 as a consequence of the results obtained in 2007. Trials were undertaken in Suffolk (Sutton/Shottisham) and Norfolk (Castle Rising). PCN counts (Pis) were done prior to planting and in the planning of the trial treatments were blocked in accordance with the egg counts at the end of the experiments Pf/Pi were calculated to see if the fungi were having any effect on the multiplication of the nematodes.

Fungal inocula: Selected isolates of *Paecilomyces lilacinus* (PL Link) and *P. chlamydosporia* (Pc 280) were bulked up and formulated either as a liquid, wettable powder or paste by BCP (Biological Control Products SA). The fungi were then applied once according to BCPs recommended instructions as described above after planting but prior to plant emergence.

Sampling: Soil samples were taken just prior to harvest as described above.

2.4 Methods objective 4

Impacts of herbicides and fungicides on the activity of biological control fungi

The sensitivity of the nematophagous fungi *Paecilomyces lilacinus* (PL link) and *P. chlamydosporia* (Pc 280) to fungicides and herbicides which are commonly applied to the potato crop was studied using the method adapted from Pijls *et al.* (1994). The following pesticides were tested: 1) Fungicides – Tatoo (mancozeb + propamocarb hydrochloride), Monceren IM (imazail + pencycuron) and Amistar (azoxystrobin); 2) Herbicides – Basagran (bentazone), Stomp (pendimethalin) and Sencorex (metribuzin). Fungal spore suspensions were made by growing each isolate on PDA (potato dextrose agar), at 25°C, for 10 days. Spores were harvested in water, counted within a haemocytometer and adjusted to a concentration of 2.5 x 10^5 spores/ml. A sample (100 µl) of the spore solution was added to 100 µl double strength Czapek Dox liquid medium with 2% yeast extract in a 96 well microtitre plate amended with 12 final fungicide concentrations (each half diluted from 100 µg/ml to 0.098 µg/ml and a 0 µg/ml control). Plates were incubated in the dark at 25°C for 72 hours, after which optical densities at 630nm were taken with a Bio-Tek plate reader. Replicate readings were used to fit dose response regression curves and the EC_{50} value (fungicide concentration at which 50% of growth is inhibited) of each isolate, was calculated using the program GenStat 11th Edition®.

2.5 Methods objective 5

Investigate and establish the biotic and abiotic factors that influence growth and development of the three fungi *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Plectosphaerella cucumerina*.

Field soil and treatments: The aim of these experiments was to determine if the infection of *G. pallida* eggs by the nematophagous fungus *Pochonia chlamydosporia* (Pc 280) in soil

could be affected by the presence/decomposition of a plant crop. It was hypothesized that the growth/decomposition of certain types of plant crops can provide nutrients for the fungus and therefore can affect the switch of *P. chlamydosporia* from saprotrophic to parasitic growth The experiment was set up in pots containing 1 kg of commercial compost and sand (3:1) inoculated with three doses of *P. chlamydosporia* (500, 1000 and 5000 chlamydospores g/soil). One week after inoculation, seedlings of wheat, corn, cabbage, mustard and kale were planted. There was also an unplanted fallow and each treatment consisted of 4 replicates. The pots were arranged in the plunge in a randomised block designed experiment. After 6 weeks, plant tops were cut and *G. pallida* cysts were baited in the soil (10 cysts/pot), following the protocol described by Atkins *et al.* (2003). Plants shoot length, fresh shoot weight and shoot dry weight was recorded in each treatment. One and two months after decomposition of the roots in the soil, cysts were removed and the percentage of egg infection was measured. At the end of the experiment the number of CFU/ g dry soil was obtained for the pots inoculated with 5000 chlamydospores/g soil. The percentage of carbon and nitrogen in soil was also measured in all treatments.

Monitoring the growth and spread of nematophagous fungi from the rhizosphere into the bulk soil: A selected isolate of *Pochonia chlamydosporia* (Pc 280) was applied to ridged potatoes in small field plots (4 ridges, 4 plants in each ridge). The soil was a sandy loam and it was free of potato cyst nematodes and *P. chlamydosporia*. The four plants in the middle of the plot were inoculated with *P. chlamydosporia* at a concentration of 5×10^6 chlamydospores per plant. The spread of the fungus was measured in the ridge, 10 and 15 cm away from the potato plant, at different time intervals during the growing season. In order to be precise in the method of soil sampling, metal grids were inserted in the soil, at planting. Four grids were buried around each inoculated plant, 2 grids were placed 10 cm away from the plant, and 2 grids were buried 15 cm away from the plant. Each metal grid was composedof 16 cubes, each cube = 3 X 3 X 3; the size of the grid = 15 cm. The soil samples were taken from 8 cubeses in the grid, at 10 and 15 cm distance, one week after planting and monthly for 3 months. At each time, soil samples were mixed thoroughly; 1g sub samples were removed and suspended in 9 ml of 0.05 % water agar solution. The soil suspensions were whirly-mixed for 20 seconds and a second dilution was made, transferring 1 ml of the soil suspension into 9 ml of 0.05 % water agar. 200 µl of the soil suspension (10^{-2} dilution) was plated into selective medium (three plates per sample), following the protocol described by Kerry and Bourne (2002). After 14 days of incubation at 25^{o} C, the number of colony forming units (CFU) was counted. The results were expressed in numbers of CFU g/ dry soil. The CFU/ soil data were analysed using ANOVA to compare differences between time and distance of fungal spread. As multiple observations (repeated measures) from each plant were taken, GLM analysis was used to analyse counts of observed presence/absence of CFU out of three technical replicates across the total grid cells.

The effect of root exudates from sugar beet, Solanum sisymbriifolium*, wheat, oil seed rape and pea on PCN hatch*: Many nematodes require host plant root diffusates to induce or increase hatch (Jones *et al*., 1998). Therefore seedlings of sugar beet, oil seed rape, *S. sisymbriifolium,* pea and wheat were placed in standard potting compost and root diffusate was collected using standard techniques at two, four, six and eight weeks (Southey,1986). Hatching tests were performed and a standard potato root diffusate was used as a positive control.

Use of cryo-scanning electron microscopy to monitor egg/cyst colonisation: The use of the scanning electron microscope to document the development of fungal bio-control agents in the emerging female / newly formed cyst was achieved using a Leica CM 1850 cryo ultra

microtome and a Jeol 6700 Feg Scanning Electron Microscope. Briefly, transparent pots (8 cm) with three small holes in the bottom were filled with sterilised sand inoculated with *Pochonia chlamydosporia*, *Monographella cucumerina* (10^7 chlamydospores per pot) and *Paecilomyces lilacinus* (10^9) into which were placed small chitted potatoes (Maris Piper). The potato chits were allowed to establish and were then inoculated with potato cyst juveniles (population Severals, 2000 per pot). Infection of the developing females by the fungi was monitored between 6 – 12 weeks after nematode addition by washing away the sand and dissecting females from the cleaned roots. Females or cysts recovered from each treatment were prepared for examination by planing away 10-micron thick sections of the nematode body until sufficient internal cavity was revealed to expose the eggs and the fungus. For each treatment a single female/cyst was mounted in a drop of OTEC tissue mounting medium onto the cryostat chuck. The sample was plunged into liquid nitrogen and cryo fixed for 2 minutes, mounted in the pre cooled cryostat at -30 ◦ C and cryo planed to a depth of about 50 microns, using a stainless steel blade. Thin sections were examined on glass slides to check that eggs were present before removing the specimen and transferring it to the Gattan Alto 2500. The sample was then sublimated for 2 minutes at -95 C and then coated for 90 seconds with gold palladium before viewing via the Jeol 6700 Scanning Electron Microscope.

2.6 Methods objective 6

Investigate the potential formulation and delivery of fungal agents, including commercially available products including any potential phytotoxicity implications

See methods objective 3.

2.7 Methods objective 7

Investigate the carbon nitrogen acquisition and potential translocation by three fungi

The specific objectives of this experiment were: a) to assess if nutrients in the rhizosphere of potato roots increase abundance of different fungal species/biotypes; b) to examine if break crops (wheat, oil seed rape, sugar beet and potatoes) differ in their ability to support selected fungal isolates in soil and their rhizospheres. The experiment was done in two phases (one per objective):

The abundance of two *Pochonia chlamydosporia* biotypes Pc280 (*P. c.* var. *chlamydosporia*), Pc 392 (*P.c.* var. *catenulata*), and single isolates of *Paecilomyces lilacinus* (PL link) and *Monographella cucumerina* (CABI 380408) were compared when the carbon and nitrogen source for fungal growth was only provided through the root system of potato plants. Differences in colony forming units counts (CFU) at the end of the experiment were used as a measure of the nutrition derived from the plant by each fungal isolate. Sterilised acid washed sand (170 g; pH 6) were weighed and placed in a re-sealable polythene bag and 5 ml of water was added to humidify the sand before adding the chlamydospores (5000 per g of sand) of *P. chlamydosporia*. Sand and chlamydospores were thoroughly mixed and transferred into boiling tubes. A similar procedure was followed for *P. lilacinus* and *M. cucumerina* but the inoculum was added as 5 ml of spore suspension. Potato chits (cv. Cara) were planted into

half the boiling tubes containing the inoculated sand, wrapped in aluminium foil and kept in a growth chamber at 20 °C, and 16 h of light (300 μmol.m^2.sec^{-1}) for four weeks. The boiling tubes containing only inoculated sand were sealed with parafilm® and watered regularly to keep them moist. Boiling tubes containing plants were watered daily. As soon as potato shoots had emerged, they were manually sprayed twice a day with a foliar fertilizer (Phostrogen) care being taken to avoid leakage to the sand. Four weeks later, fresh shoots and root systems were weighed and fungal populations from soil and roots were isolated in selective media and the CFU counted. CFU numbers were adjusted according to weight differences between dry and wet soil obtained from 1 g of soil taken per each boiling tube. The experimental design was a 2-way randomised design with 4 blocks. There was a total of 10 treatment combinations including fallow sand (i.e. fungus but no plant), and 4 replicates per treatment. The analysis of soil CFU data was made using ANOVA with a square root transformation to account for heterogeneity of variance across the treatment combinations using GenStat Release 8.2.

Based on results from the above experiment, *P. chlamydosporia* biotypes Pc 392 and 280 as well as *P. lilacinus* were selected to be used in this experiment. Methods were similar to those described before. Modifications included the use of sterilised acid washed sand-grit (1:1 w/w) instead of sand only. Potato (cv. Maris Piper) and spring cultivars of wheat (cv. Paragon), oilseed rape (cv. Heros), sugarbeet (cv. Dominica) were included as break crops. Seeds were surface sterilised and germinated at 25 °C into Petri dishes containing nutritive agar (Kerry *et al*., 1984). Seedlings (3 cm long) were taken from the Petri dishes and planted into boiling tubes previously filled with sand grit (170 g) inoculated with each fungus. After 4 weeks in a growth chamber, plants were removed from the boiling tubes. Shoots and root systems were weighed and fungal abundance (CFU) in sand and rhizosphere were evaluated as in phase 1. The experimental design used was a randomised block with three blocks. Treatments included the 5 types of crops (including fallow) with 4 types of fungus (including control) and three replicates, giving a total of 60 boiling tubes One g of sand was taken at random from 20tubes, after sand-grit had been inoculated so as to assess the initial CFU counts. CFU data were recorded.

2.8 Methods objective 8

Assess the importance of the fungal pathogens of nematode eggs on the patch dynamics of PCN

As part of a Sappio-Link Project (No 112) several fields, Hanger, Steadmans and Bemrose (near Dawsmere Lincolnshire) containing PCN had been mapped in detail. Transects, 75, 100 and 100 m respectively, were set up across patches and 500 g soil samples taken every 5 m with an auger were obtained. Twenty observation points per transect were collected for Steadmans and Bemrose and fifteen observation points were collected for Hanger. The samples were returned to the laboratory and the cysts extracted, counted and the number of eggs infected with fungi from each sample was recorded using standard techniques. Data were analysed in GenStat 11th Edition using ANOVA, regression and correlation analysis.

2.9 Methods objective 9

Assess the effects of different granular nematicides and a fumigant on PCN decline rates in soils of different textures and types with reference to nematophagous fungi.

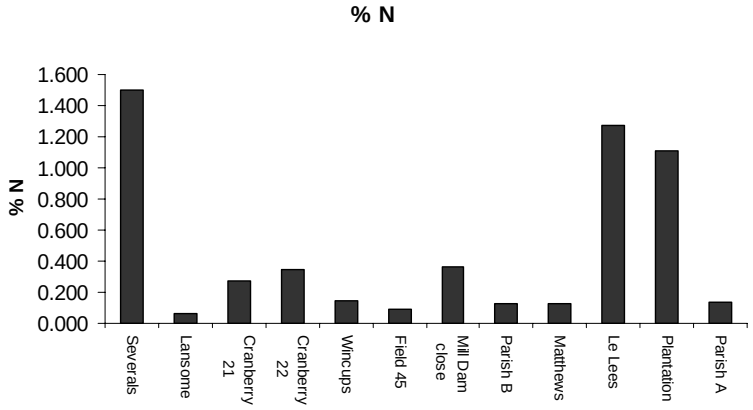
Effects of nematicides on soil colonization, egg viability and egg infection (2006 samples). Twelve soil samples (three treatments, four replicates) were sampled from five field experiments (in total 60 soil samples) in late September to early October 2006. Soil samples (in total 48) from Newton (Derwent), Ferrybidge (Yorkshire), Westhorpe (Lincolnshire) and Chennels were diluted and plated on three media: Martin's Rose Bengal (for general soil fungi), semi-selective media (for *P. chlamydosporia*) and *Paecilomyces* plates. Two dilutions of each soil sample were plated on four replicate plates of each media. Cysts were extracted from the 12 soil samples from Westhorpe, Lincolnshire, Chennels, Severals and Ferrybridge using the fluidising columnmethod (Trudgill *et al*., 1972). Egg suspensions were made for assessment of egg viability (by Meldola's blue staining) and egg infection (by egg plating).

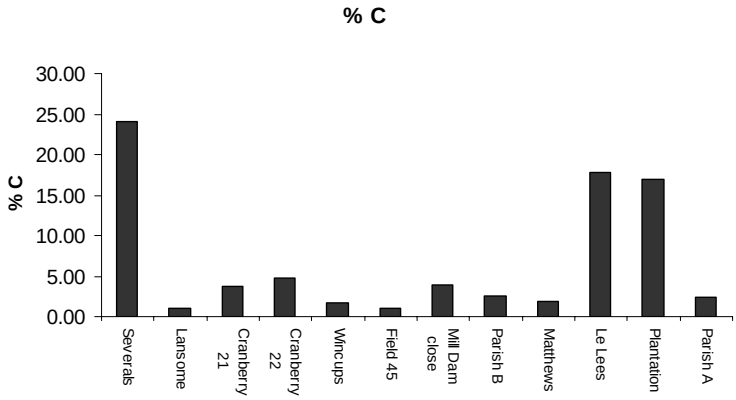
3. RESULTS AND DISCUSSION

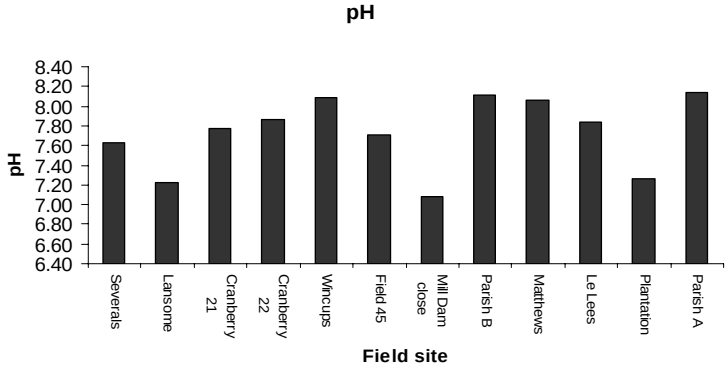
3.1 Objective 1

To identify the key factors that affect PCN population suppression and decline rates during and between potato crops.

Assess the rates of decline in a range of field soils of different textures and cropping:
Soil analysis: The soil analysis which was investigated further using principal coordinate analysis in GenStat® (Figure 3.1.1 & 2). The soils were grouped according to their similarities for percentage C and N, available PO_4 and pH. The analysis generated five distinct groups. Group 1 included soils from Matthews, Wincups, Parish A and Parish B, group 2 included Field 45, Cranberry 21 and Cranberry 22, group 3 included Mill Dam Close and Lansome, group 4 included Severals and Le Lees and finally, in the group 5 the field soil Plantation was included (Figure 3.1.2). The total carbon and nitrogen for the majority of soils were between 1.0 to 5.0% and 0.1 to 0.4% respectively, with the exception of Severals, Lees and Plantation which were between 17 and 25 % and 1.0 to 1.5%. These three soils were therefore highly organic. The pH of all the soils was slightly alkaline and ranged between 7.0 and 8.2. The quantity of available phosphate was variable across the different field sites, ranging from 39-45 ppm (Wincups and Field 45) and 144 ppm (Mill Dam Close). Interestingly, a trend was observed between soil chemical properties and rates of decline for PCN. Soils with similar PCN decline rates often shared similar chemical properties (Table 2.1). For example, the soils from group 1 (Matthews, Wincups, Parish A and Parish B) had all been observed to have fast decline rates for PCN.







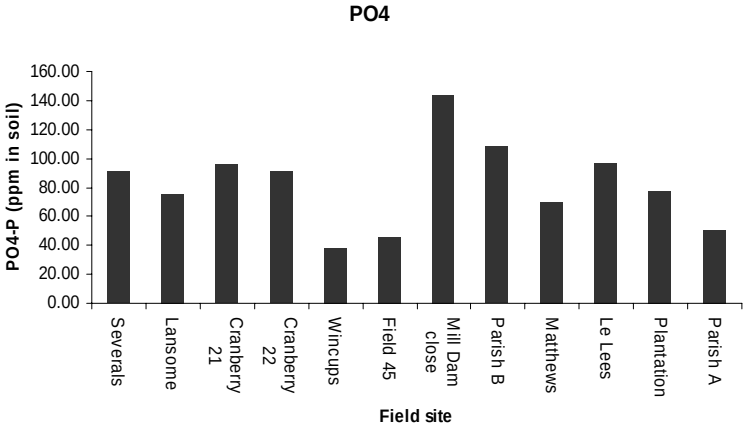


FIGURE 3.1.1. SOIL ANALYSIS USING LECO ANALYZER MEASURING NITROGEN, CARBON, PH AND PHOSPHATE.

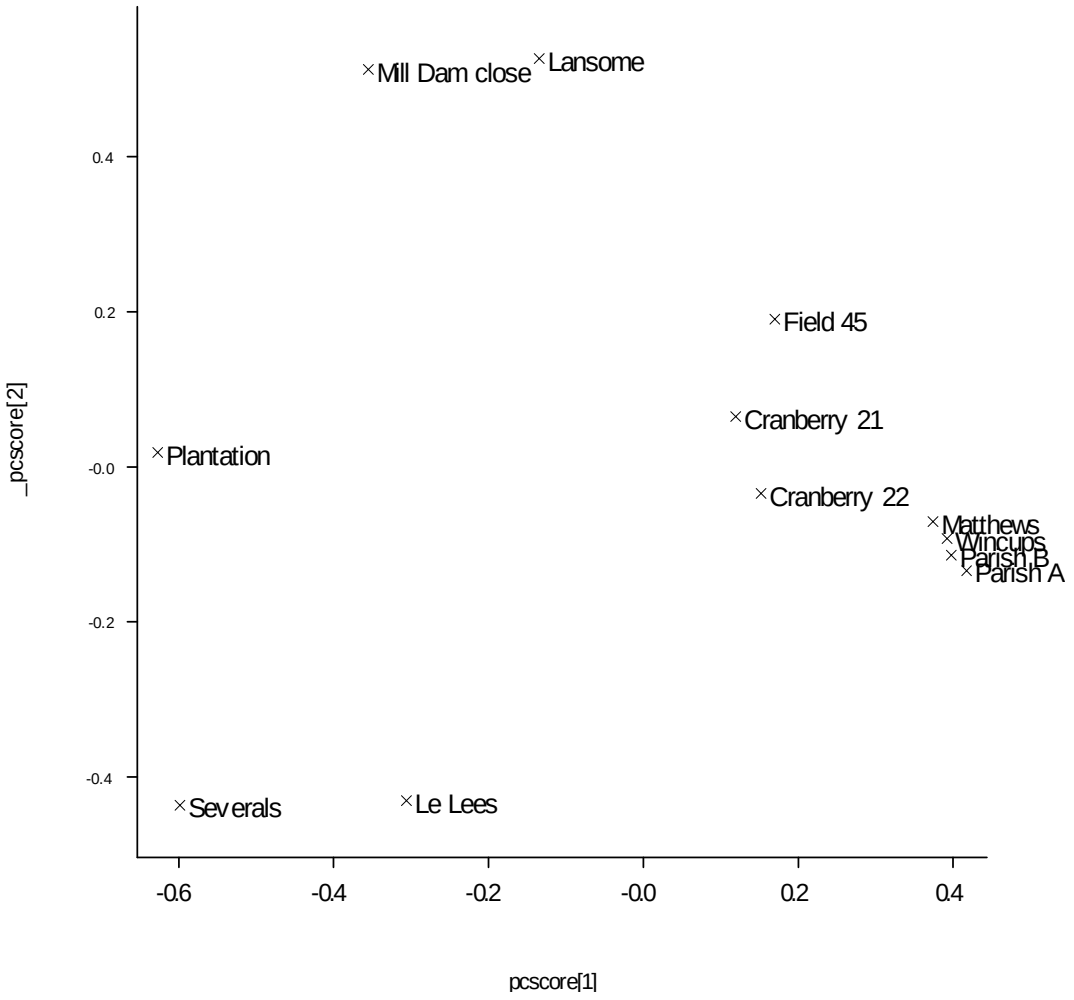


FIGURE 3.1.2 DISTRIBUTION OF TWELVE SOILS ACCORDING TO SIMILARITIES IN PERCENTAGE C. AND N, AVAILABLE PO4, AND PH USING PRINCIPAL COMPONENT ANALYSIS. Using this information five groups were formed: group 1- *Matthews*, *Wincups*, *Parish B* and *Parish A*; group 2 – *Field 45*, *Cranberry 21* and *Cranberry 22*; group 3 – *Mill Dam Close* and *Lansome*; group 4 – *Severals* and *Le Lees* and group 5 – *Plantation*.

Effect of soil sterilization on the numbers of eggs per cyst and proportion of infected eggs in 12 soils with different PCN decline rates – interactions with the different soil groups

The effects of soil sterilization on the numbers of eggs/cyst and proportion of infected eggs was accessed in soils from 12 field sites. At each sampling time (November 06, February 07, November 07, March 08 and October 08) one bag containing 25 cysts was taken and the number of eggs/cyst, infected eggs and percentage egg viability was quantified. Using ANOVA, the results showed no significant interactions between the numbers of eggs/cyst found in each soil and sterilisation ($p=0.141$) and the sterilisation of the soil was not found to affect the numbers of eggs which were subsequently found in cysts during the course of the experiment. However, a significant interaction was found between the number of eggs per cyst and time ($p<0.001$; LSD (5%) = 0.5726) and the number of eggs/cyst significantly

decreased during the course of the experiment (Table 3.1). Similarly, no significant interactions were found between the Pf/Pi numbers and the sterilization of soil (p=0.793; LSD (5%) = 0.130; Figure 3.1.3). The ANOVA on the numbers of infected eggs showed no significant interactions with soil sterilisation (p=0.145) but a significant interaction was found for time (Table 3.1). Greater rates of egg infection were found in the winter compared to spring. A significant overall negative correlation was found between percentage of infected eggs and number of eggs/ cyst (r= -0.2671; p<0.001). This correlation was stronger in the winter than in the spring, reinforcing the effect the effect of time in fungal infection.

TABLE 3.1 AVERAGE NUMBER OF EGGS PER CYST IN TWELVE SOILS WITH DIFFERENT DECLINE RATES FOR PCN, DURING A TWO-YEAR EXPERIMENT AND AVERAGE INFECTION (SQUARE ROOT OF PERCENTAGE EGG INFECTION) IN TWELVE SOILS WITH DIFFERENT DECLINE RATES FOR PCN, DURING A TWO-YEAR EXPERIMENT (LSD (5%) = 0.273)
.

Time	Nov-06	Feb-07	Nov-07	Mar-08	Oct-08
Eggs	87.8	75.0	73.2	77.6	20.1
Infection	1.6	1.38	1.85	1.46	1.82

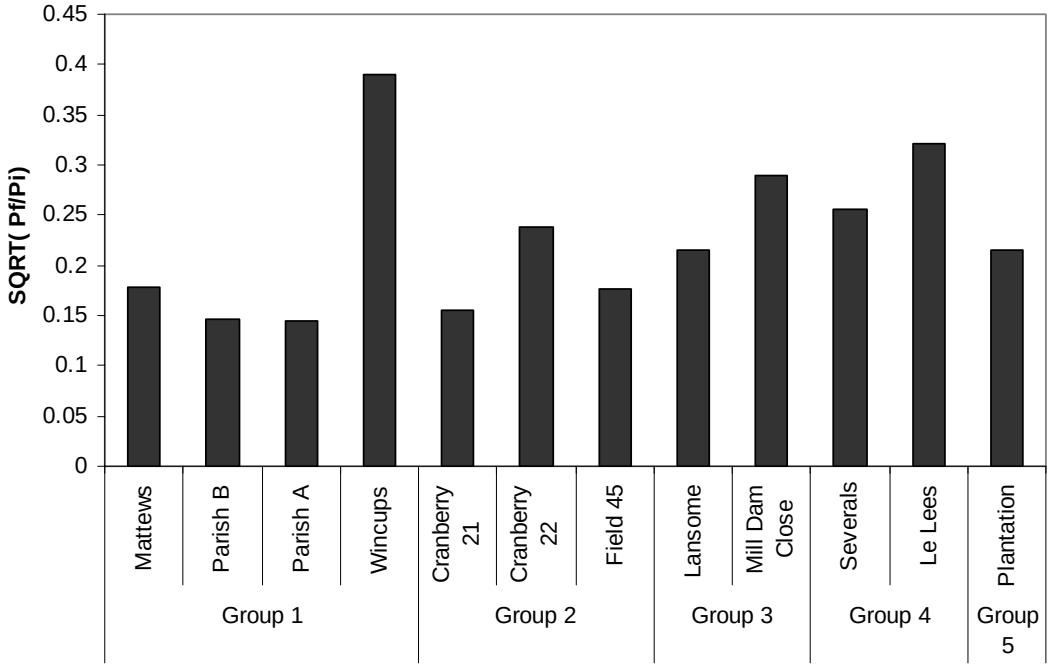


FIGURE 3.1.3. PF/PI'S ON THE NUMBER OF EGGS PRESENT IN SOILS WITH DIFFERENT DECLINE RATES FOR PCN. THE INTERACTION BETWEEN PF/PI'S AND TREATMENT (STERILIZATION OF SOIL) WAS NOT SIGNIFICANT (P=0.793; LSD (5%) = 0.130).

The effects of soil sterilisation on number of eggs per cyst and the proportion of eggs infected that occurred in the different soils over time can be seen in Figures 3.1.4 A to C. The soils have been grouped broadly into three groups according to their chemical characteristics. It was hypothesised that there would be a decline in egg numbers during the period of the experiment and that there would be an inverse relationship between the decline in egg number and the proportion of eggs infected and that sterilisation of the soil would remove such a relationship. This type of relationship in the non-sterile soils can broadly be seen in the soils Lees, Plantation, Cranberry 22, Parish A and B and Field 45 but sterilisation of the soil does not seem to affect this broadly inverse relationship. However, it should be noted that

throughout the whole experiment the percentage of egg infection was never recorded on any one occasion to be above 15 % and in the majority of cases it never rose above 8 % (Figures 3.1.4 A to C). Infected eggs may have their contents destroyed within a few weeks at summer soil temperatures (15 C) and it is difficult to predict the total amounts of parasitism from samples collected on a single occasion. The marked decline in nematode populations in all soils during the last year of the experiment may have been caused by the extremely wet summer in that year.

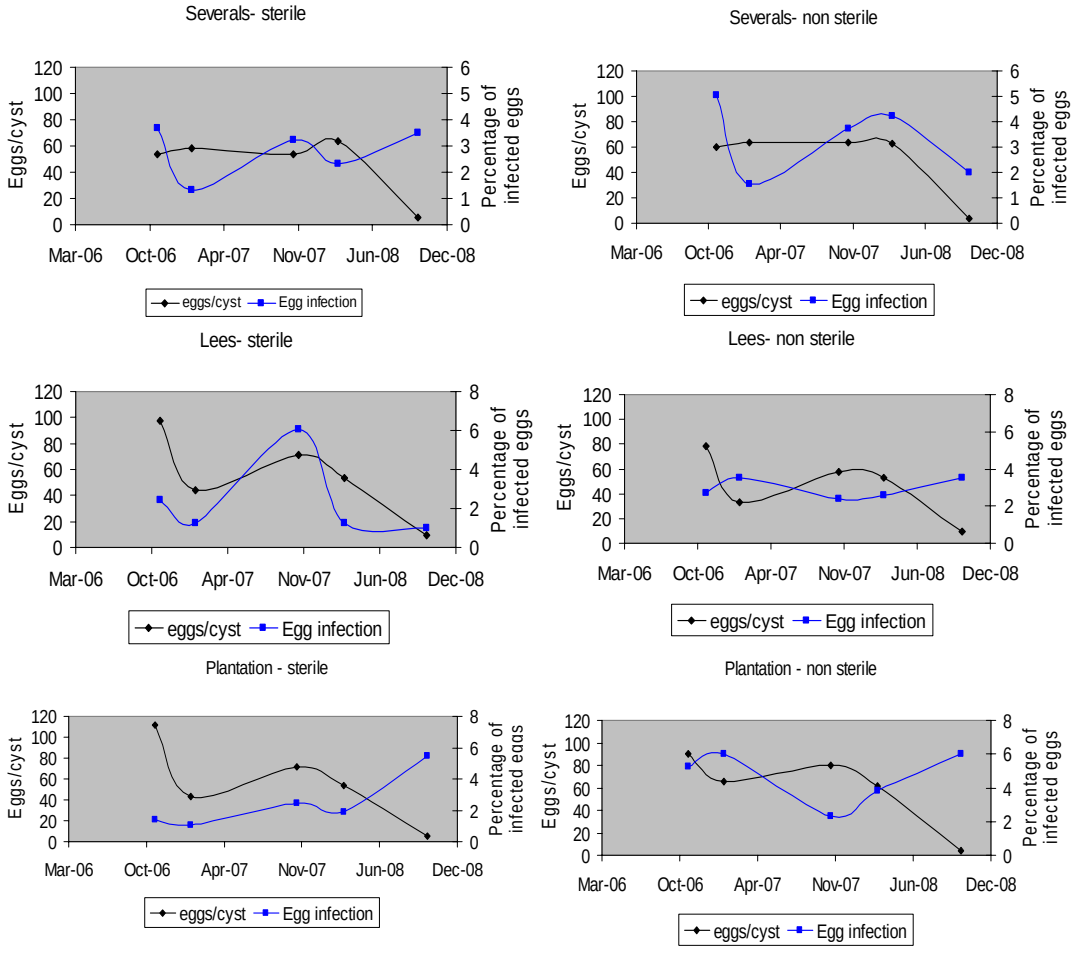


FIGURE 3.1.4 A PERCENTAGE FUNGAL EGG INFECTION OF POTATO CYST NEMATODE EGGS EXTRACTED FROM THREE SOILS (SEVERALS, LEES AND PLANTATION), STERILISED AND NON-STERILISED, WITH A HIGH ORGANIC CONTENT WHERE THE PERCENTAGE NITROGEN AND PERCENTAGE CARBON WERE GREATER THAN 1.0 AND 15.0 RESPECTIVELY AND MAINTAINED AS FALLOW IN A SAND PLUNGE FROM MARCH 2006 TO NOVEMBER 2008 WITH PERIODIC SAMPLING (BARS = SE)

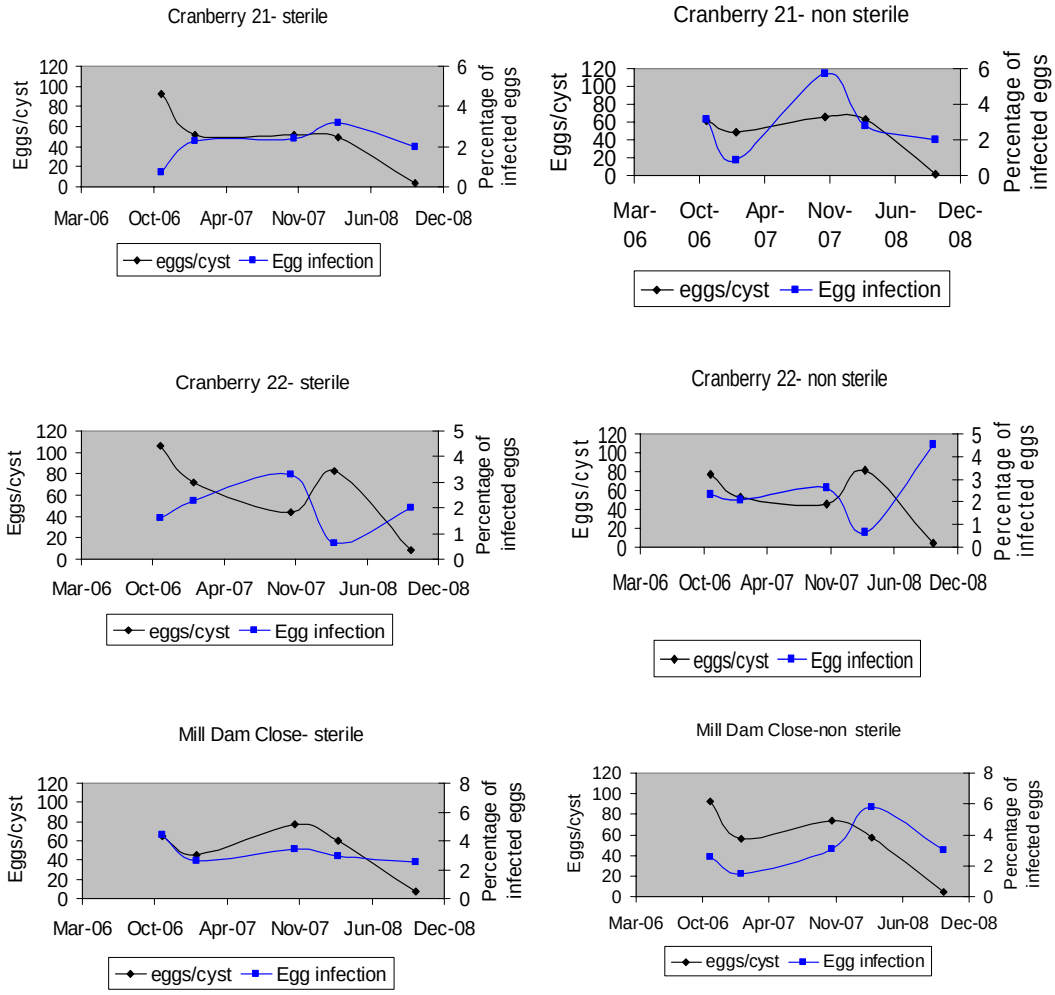


FIGURE 3.1.4 B PERCENTAGE FUNGAL EGG INFECTION OF POTATO CYST NEMATODE EGGS EXTRACTED FROM THREE SOILS (CRANBERRY 21, CRANBERRY 22, MILL DAM CLOSE), STERILISED AND NON-STERILISED, WITH A MODERATE ORGANIC CONTENT WHERE THE PERCENTAGE NITROGEN AND PERCENTAGE CARBON WAS BETWEEN 0.2 AND 1.0 AND 0.25 AND 15.0 RESPECTIVELY AND MAINTAINED AS FALLOW IN A SAND PLUNGE FROM MARCH 2006 TO NOVEMBER 2008 WITH PERIODIC SAMPLING (BARS = SE).

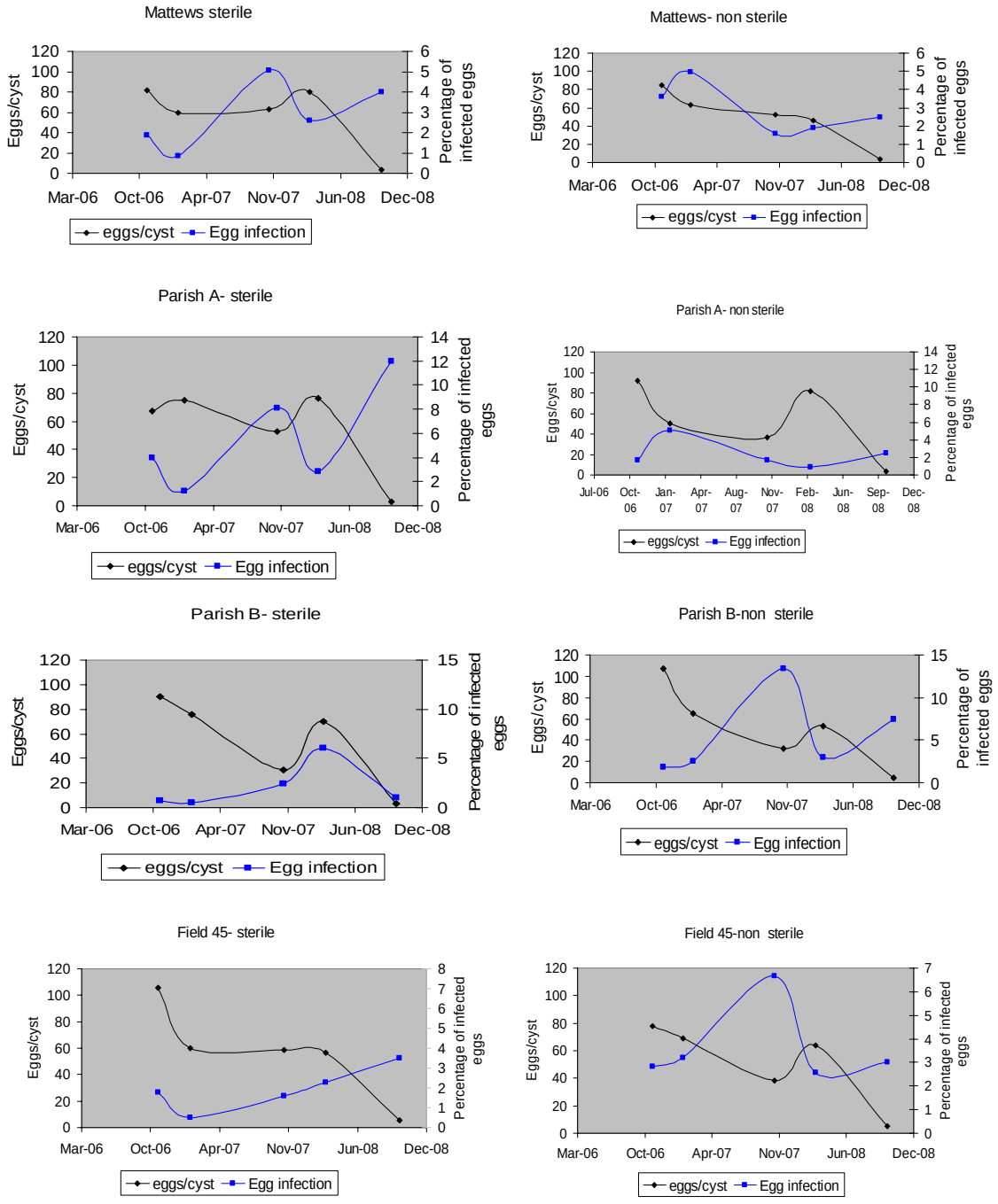


FIGURE 3.1.4 C PERCENTAGE FUNGAL EGG INFECTION OF POTATO CYST NEMATODE EGGS EXTRACTED FROM THREE SOILS (MATTHEWS, PARISH A, PARISH B, FIELD 45,), STERILISED AND NON-STERILISED, WITH A MODERATE ORGANIC CONTENT WHERE THE PERCENTAGE NITROGEN AND PERCENTAGE CARBON WAS BETWEEN 0.2 AND 1.0 AND 0.25 AND 15.0 RESPECTIVELY AND MAINTAINED AS FALLOW IN A SAND PLUNGE FROM MARCH 2006 TO NOVEMBER 2008 WITH PERIODIC SAMPLING (BARS = SE).

General Soil Microbial Community

Using dilution plates the number of colony forming units of bacteria, fluorescent *Pseudomonads* and fungi were determined by dilution plating onto potato dextrose agar, Kings B medium and corn meal agar, respectively and the results can be seen in Figure 3.1.5. The number of bacteria ranged between 5 to 70 x 10^6, of which the number of *Pseudomonads* was <20 % of the overall total bacterial community (Figure 3.1.5 A). This compares with a number of between 0 and 15 x 10^4 colony forming units of fungi (Figure 3.1.5 B).

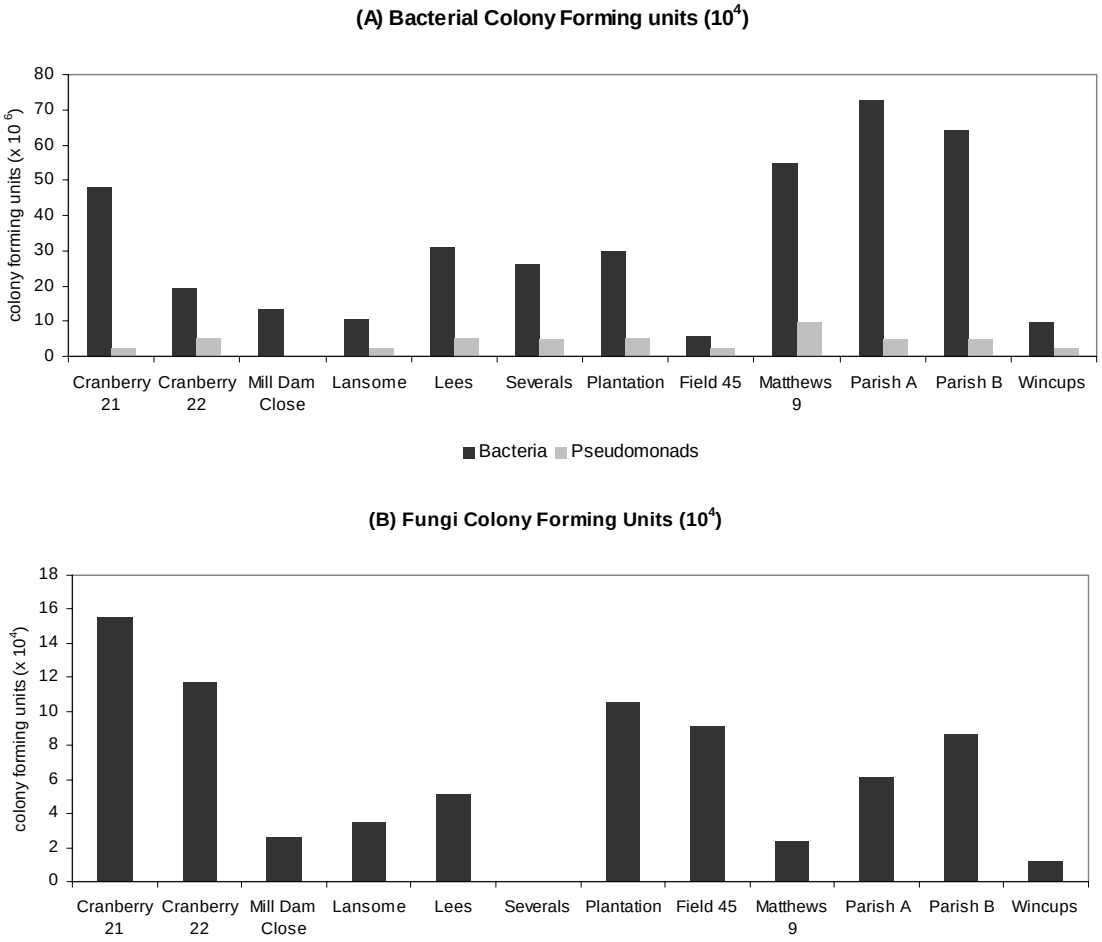


FIGURE 3.1.5. A) THE NUMBER OF COLONY FORMING UNITS PER GRAM OF SOIL ASCERTAINED BY DILUTION PLATING ONTO POTATO DEXTROSE AGAR AND KING'S B MEDIUM FOR EACH OF THE TWELVE SOILS PLACE IN THE SAND PLUNGE B) THE NUMBER OF COLONY FORMING UNITS OF FUNGI ASCERTAINED BY DILUTION PLATING ONTO CORN MEAL AGAR.

The effects of time since the last potato crop on the microbial community and its effect on PCN decline:

Two PCN infested soils were collected: one (Mill Dam Close) had been cropped with potatoes in the previous season and the other (Lansome) had last had a potato crop 5 years previously. These two treatments were duplicated and the soil irradiated as described above. The soils were placed in large pots and placed in the sand plunge as described above. The pots remained fallow and weeded. Twenty five cysts in nylon bags were placed in the pots and removed on a regular basis to assess fungal activity as measured by egg infection. Each treatment consisted of three replicates. There was very little difference in the fungal populations of the bulk wet soil between Mill Dam Close and Lansome with 60.5 and 57.0

cfu/g wet soil respectively. The irradiated Lansome soil had 35.5 cfu/g wet soil which was not significantly different from the other two, however the irradiated Mill Dam Close soil had 187.5 cfu/g wet soil which was significantly larger (x3) than any of the others. This large difference had very little effect on egg infection that ranged from just below 2% of eggs infected to just over 4% of eggs infected (Figure 3.1.6).

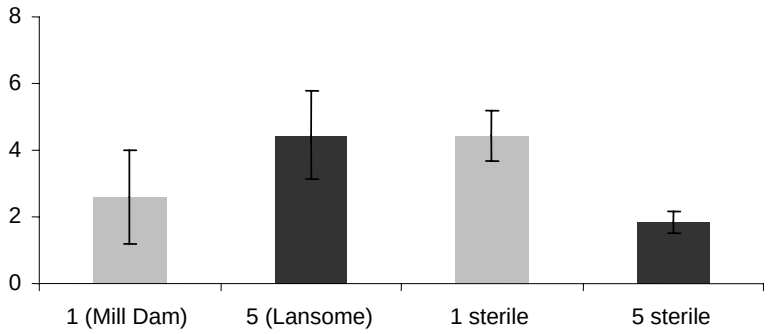
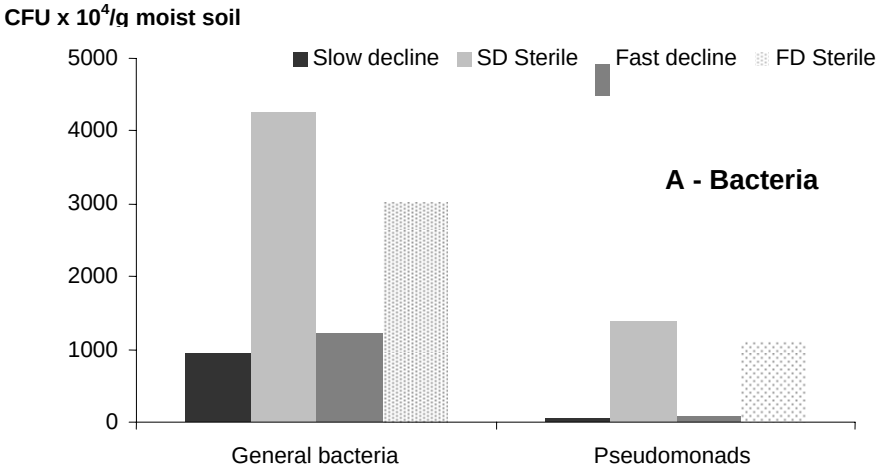


FIGURE 3.1.6. THE NUMBER OF INFECTED EGGS FROM POTATO CYST NEMATODES IN TWO SOILS; MILL DAM CLOSE (GREY) THAT HAVE HAD A POTATO CROP THE PREVIOUS SEASON AND LANSOME (BLACK) THAT HAD NOT BEEN CROPPED WITH POTATO FOR 5 YEARS HALF OF WHICH HAD BEEN IRRADIATED AND HALF OF WHICH HAD NOT BEEN IRRADIATED (SAMPLES TAKEN 1 MONTH AFTER RADIATION).

Comparison of the microbial community between a fast and a slow decline soil:

Using dilution plates the number of colony forming units of bacteria, fluorescent *Pseudomonads* and fungi were determined by dilution plating onto potato dextrose agar, Kings B medium and corn meal agar respectively and a comparison made between sterilised and non-sterilised soils (Figure 3.1.6). The results show that there is very little difference in either the overall total bacterial community or the fluorescent *Pseudomonas* community between the fast decline or the slow decline soils and the number of bacteria per unit weight of soil range from 10×10^6 to 40×10^6 (Figure 3.1.6 A). Sterilization of the soil led to an increase in the bacterial numbers per unit weight of soil compared with the non-sterilised soil. The sterilization of the soil reducesthe number of colony forming units of fungi present in the soil bringing the number down from between 70 to 90×10^3 colony forming units per gram soil to around 30×10^3 colony forming units per gram soil (Figure 3.1.6 B).



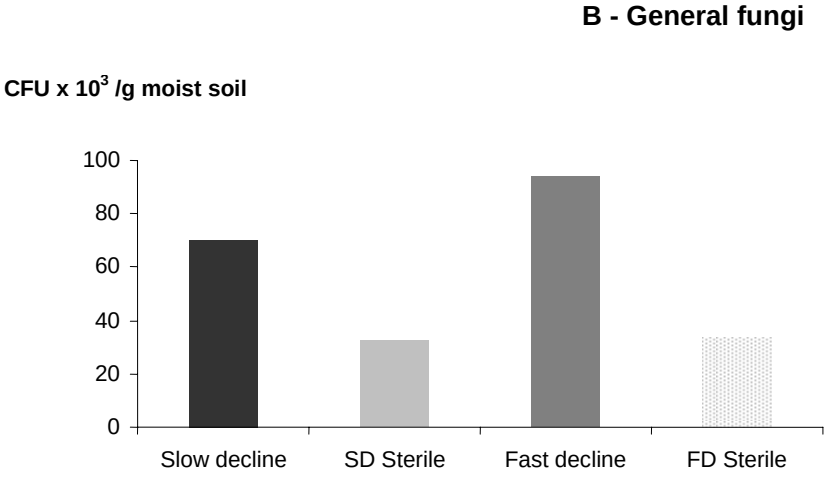


FIGURE 3.1.6. THE NUMBER OF COLONY FORMING UNITS PER GRAM SOIL OF GENERAL BACTERIA AND *PSEUDOMONADS* (A) AND GENERAL FUNGI (B) IN A FAST PCN DECLINE (WINCUPS) AND SLOW PCN DECLINE (SEVERALS) SOILS THAT WERE EITHER STERILISED () OR NON-STERILISED.

3.1 Concluding comments for objective 1

Although the soils could be grouped according to the chemical properties this was not predictive of any observable decline in the number of viable eggs. There appeared to be a correlation between the known decline rate observed by crop advisors in the field and the soil type but this could not be replicated in well-controlled experiments with standard nematode populations in pots and may have been an inherent feature of the nematode infestation in each field. The impact of soil factors on decline has been variable with high decline rates generally occurring in free-draining soils, and increased organic matter and clay contents have reduced rates in some tests (Marshall, 1998) and increased them in others (Brown, 1978). As soil sterilisation did not have a significant effect on changes in the numbers of eggs/cyst, the impact of the microbial community on decline rates would appear to be small in the 12 soils tested. Little decline in average nematode populations occurred until 2008 and in the conditions of this experiment there did not appear to be a steady decline rate, as predicted. In 2008, the summer was especially wet but it is not clear if this affected the rate of nematode eggs hatching. In general the abundance of fungi and bacteria was not easily related to the reduction in egg numbers or the rate of nematode population decline. Sterilization of the soil tended to increase the number of bacteria present at the beginning of the experiment but by the end after 3 years no effect could be observed on the rate of PCN decline. Interestingly, fungal infection of eggs was consistently low, usually below 10% and never above 15 %, throughout the whole experiment and although an inverse relationship could be observed between the number of eggs per cyst and the level of egg infection by fungi, this was only the case in a proportion of the soils used in the experiment and therefore not of any use in being predictive of decline rates.

Results objective 2

Develop novel methods for predicting rates of decline

The relationship between cyst size hatch and numbers of eggs per cyst was examined: Thirty PCN cysts were measured in width and length and the numbers of full eggs, empty eggs and juveniles contained in each cyst were counted, as well as the percentage of hatch. The relationships between the cyst volume, cyst area, percentage of egg hatch and the numbers of eggs/cyst was analysed using ANOVA and regression analysis in GenStat®. Models were fitted for each relationship studied. There was a significant relationship between the cyst volume and number of eggs per cyst (Figure 3.2.1). A similar significant relationship was found between cyst circular area and eggs/cyst (figure not shown). The model showed an asymptotic exponential significant curvature ($p=0.008$), suggesting that there is a limit in the number of eggs produced, as the cyst volume increases. For this population, the model estimated that the maximum number of eggs per cyst is 499.4. Weak or no relationships were found between percentage of egg hatch and cyst circular area or volume. Hence, it is possible to predict rates of decline from a knowledge of cyst size and the time of the last potato crop.

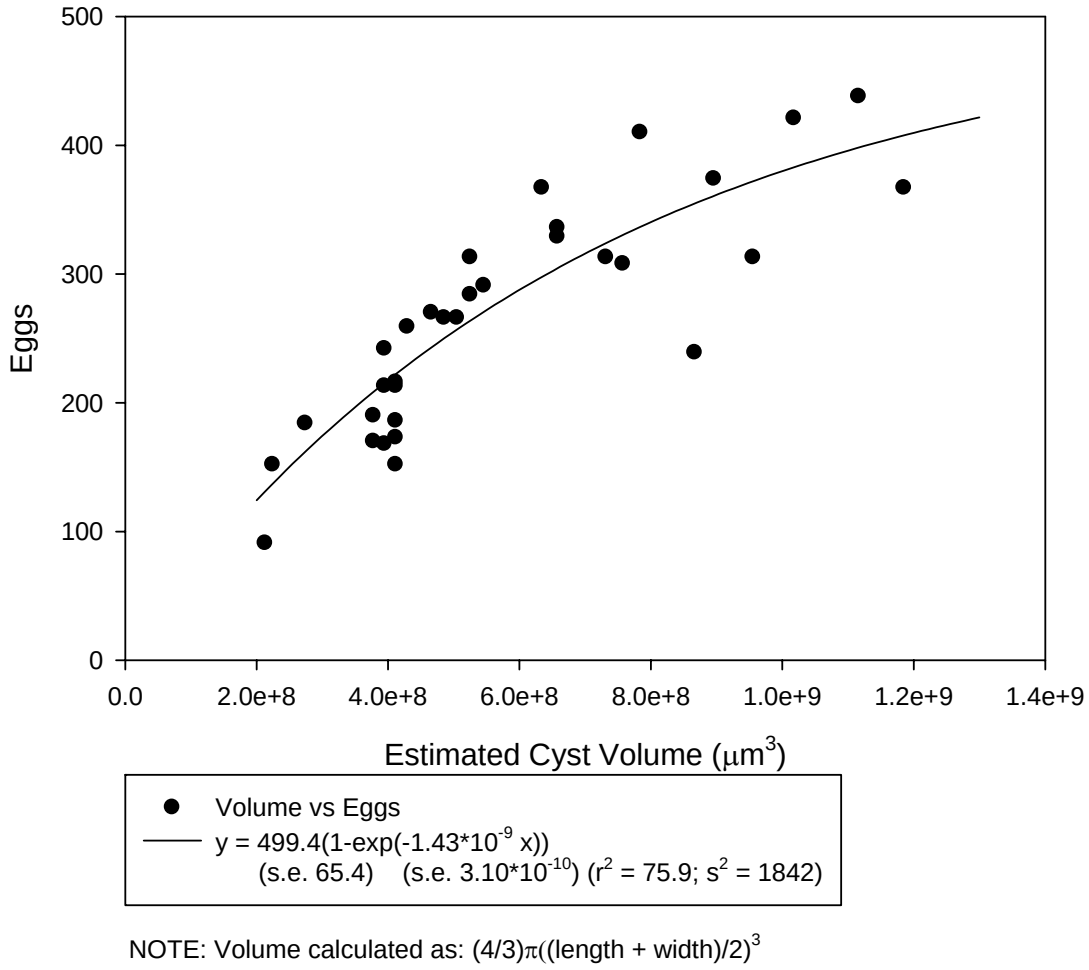


FIGURE 3.2.1 RELATIONSHIP BETWEEN CYST VOLUME AND NUMBER EGG PER CYST.

Investigate the use of PLFA techniques as possible indicators of microbial communities that may influence or determine PCN decline rates. The PLFAs showed that soil sterilization had a large effect on both Gram positive and Gram negative bacteria reducing their highest fatty acid concentrations in all soils to less than 0.2 µmol/g soil. While some of the non-sterilised soils seems to have very low concentrations of fatty acids that were comparable with the sterilised soil samples, for example Cranberry 21, Lansome and Parish A by far the majority of these soils had fatty acids indicative of these bacteria at concentrations greater than 0.2 µmol/g g soil. However, in general in the non-sterilised soils there was much variation between the soil samples (Fig. 3.2.2).

The fungal microflora in both the sterilised and non-sterilised soil samples was broadly similar and again significant levels of variation could be seen between the various soil samples (Fig 3.2.2 A and B). However, the actinomycete populations in the sterilised soil samples were again reduced but in Cranberry 21 and 22, Lansome and Parish B fatty acid concentrations were similar to those in the non-sterilised soil.

It is of interest that the sterilisation of the soil had a large effect on the number of colony forming units as measured by dilution plating (Fig 3.1.6 B) compared with the fatty acid

analysis where very few differences could be seen between the sterilised and the non-sterilised soil samples (Fig 3.2.2).

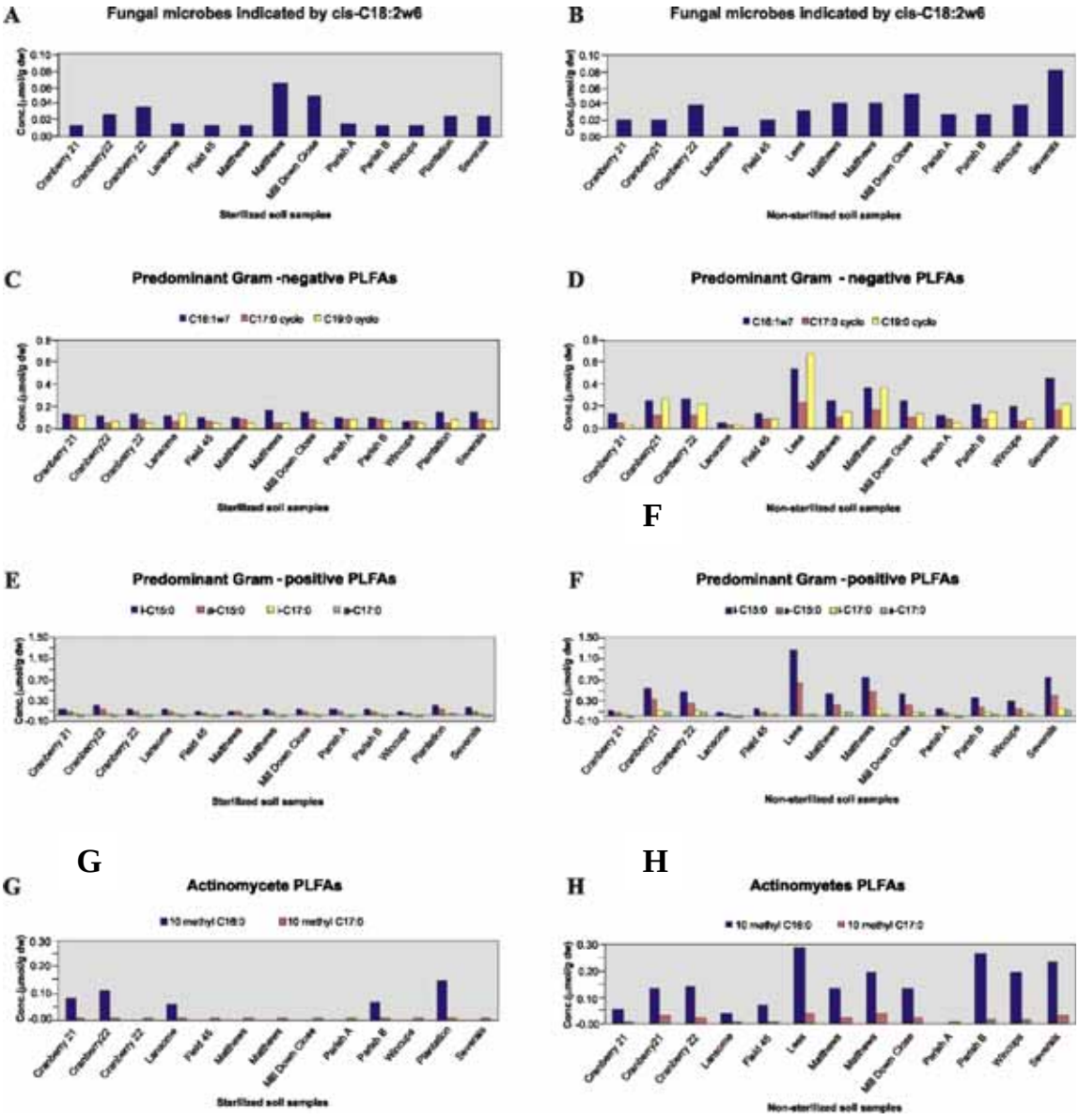


FIGURE 3.2.2 PLFA ANALYSIS PROFILES OF THE DIFFERENT SOIL SHOWING FUNGI AS MEASURED BY CIS-C18:2W6 FOLLOWING SOIL STERILISATION (A) AND IN NON-STERILISED (B) SOIL; GRAM-NEGATIVE BACTERIA AS MEASURED BY C16:1W7, C17:0 AND C19:0 FOLLOWING SOIL STERILISATION (C) AND IN NON-STERILISED (D) SOIL; GRAM-POSITIVE BACTERIA AS MEASURED BY I-C15:0, A-C15:0, I-C17:0 AND A-C17:0 FOLLOWING SOIL STERILISATION (E) AND IN NON-STERILISED (F) SOIL; ACTINOMYCETES AS MEASURED BY 10 METHYL C16:0, 10 METHYL C17:0 FOLLOWING SOIL STERILISATION (G) AND IN NON-STERILISED (H) SOIL

Use of DNA markers to identify and quantify nematophagous fungi

PCR with specific primers enabled the identity and presence of *P. chlamydosporia* (Fig 3.2.3) and *P. lilacinus* (Fig 3.2.4) to be confirmed from colonies grown on selective media. Using these it has been possible to obtain unambiguous identifications of the fungi, which can be very difficult using solely morphology and colony growth form on selective media. However, the use of PCR for the identification of *P. cucumerina* was unsuccessful, due to problems in the specificity of the primers developed previously by Atkins *et al* (2003). It would appear

from the results that the use of morphological techniques to unambiguously identify *P. chlamydosporia* is much more difficult than for *P. lilacinus*. Only one of the colonies tested out of a total of 17 (6 %) was confirmed as *P. chlamydosporia* by PCR (Table 3.1), whereas 39 of the colonies tested out of a total of 50 (78%) was confirmed as *P. lilacinus* by PCR (Table 3.2). However, attempts to exploit the use of PCR to identify and quantify nematophagous fungi directly from soil have been unsuccessful. The direct extraction of fungal DNA from field soil failed in a soil where the content of organic matter was high (*Severals*), using 2 DNA extraction kits, PowerSoil™ and ZRSoil® (Fig 3.2.5). Although fungal DNA could be extracted from sand using the PowerSoil™ kit, at high rates of fungal inoculation (Fig 3.2.5), the method showed limitations and the identification of the fungi from soil was further done combining plating and PCR techniques. Selective plating and PCR gave more accurate interpretation of the fungal dynamics in soil than using either method alone. Similar results had been found by Mauchline *et al* (2002). Using dilution plating and PCR techniques, several new isolates of *P. chlamydosporia* were identified. Two isolates were obtained from a field site at Whaplode and three from sites at Sutton, Chatteris and Wickham Market all of which were isolated from PCN eggs (Table 3.4). These isolates are securely maintained in a collection of nematophagous fungi held at Rothamsted Research.

Pochonia chlamydosporia

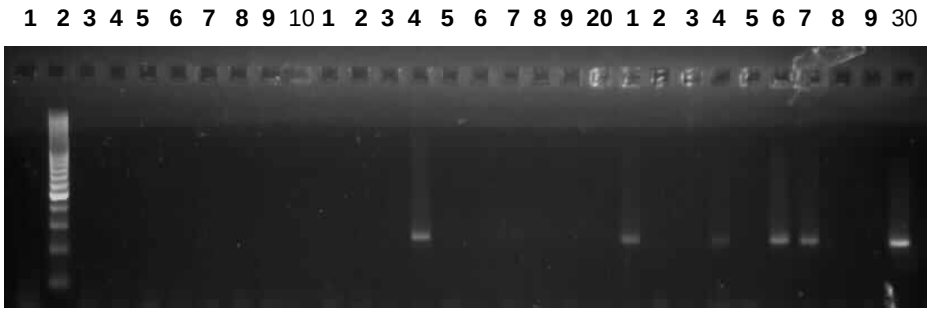


FIG 3.2.3 DETECTION OF *POCHONIA CHLAMYDOSPORIA* USING SPECIFIC PRIMERS BASED ON THE B-TUBULIN GENE. LANE 1- NEGATIVE CONTROL, LANE 2 – MARKER; LANES 14 – CHATTERIS, 21- WHAPLODE, 24- CHATTERIS, 26-407 (SUFFOLK) ARE POSITIVE. LANES 27 AND 30 ARE POSITIVE CONTROLS.

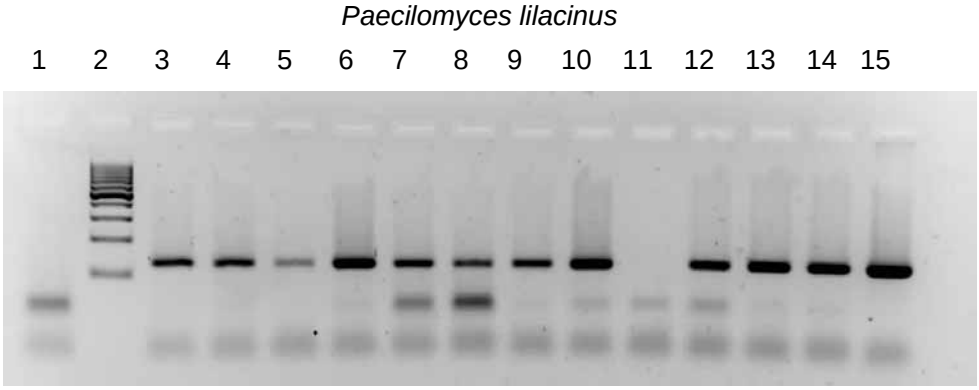


FIG 3.2.4 DETECTION OF *PAECILOMYCES LILACINUS* USING SPECIFIC PRIMERS. LANE 1-CONTROL, LANE 2-MARKER, LANE 3-H2, 4-H2, 5-H2, 6-H3 C+, 7- H3 C+, 8- CHATTERIS 2, 9- CHATTERIS3, 10- UTC 106 (NORFOLK), 11- 204 (YORKSHIRE), LANES 12-15- POSITIVE CONTROLS.

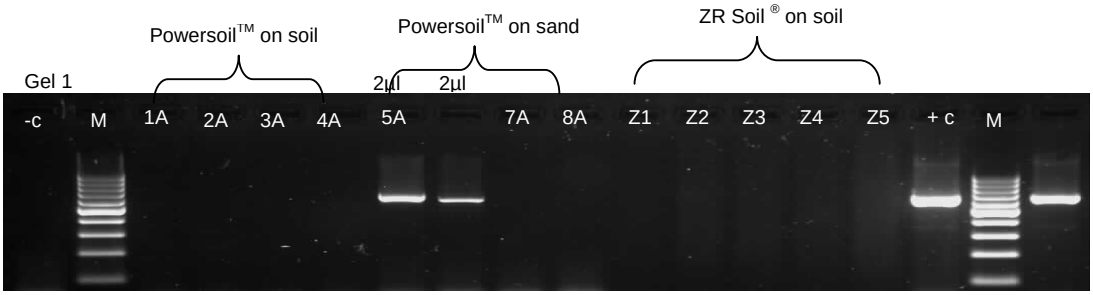


FIGURE 3.2.5 GEL OF PCR PRODUCTS OF DNA EXTRACTED DIRECTLY FROM SOIL (1A TO 4A; Z1 TO Z5) AND SAND (Z1 TO Z5) USING 2 DIFFERENT EXTRACTION KITS, POWERSOIL™DNA EXTRACTION KIT AND ZRSOIL® ,SHOWING INABILITY TO DETECT FUNGI FROM SOIL.DNA WAS DETECTED IN SAND INOCULATED WITH 500000 AND 5000 CHLAMYDOSPORES OF *POCHONIA CHLAMYDOSPORIA* (SAMPLES 5A AND 6A, RESPECTIVELY) USING POWERSOIL™ DNA EXTRACTION KIT.

TABLE 3.1 DNA APPROACHES TO STUDY *POCHONIA CHLAMYDOSPORIA*.

Site	CFU/0.2µl soil (X100)	PCR confirmed
ROTHAMSTED FIELD TRIALS AFTER 1ST APPLICATION (MAY 2007)		
Holme Farm (H)	0	0
Holme Farm (H) Control	1	0
Redhouse (RH)	4	0
Redhouse (RH) Control	1	0
Holbeach Hurn (HH)	0	0
Holbeach Hurn (HH) Control	0	0
Whaplode (W)	1	0
Whaplode (W) Control	2	0
Chatteris (CH)	0	0
Chatteris (CH) Control	0	0
SUFFOLK (MAY 2007)		
Mocap	1	1
PL Link formulation	0	0
Untreated control	0	0
NORFOLK		
Mocap	3	0
Untreated control	4	0

TABLE 3.2 DNA APPROACHES TO STUDY *PAECILOMYCES LILACINUS*.

Site	CFU/0.2µl soil (X100)	PCR confirmed
ROTHAMSTED FIELD TRIALS AFTER 1ST APPLICATION (MAY 2007)		
Holme Farm (H)	11	9
Holme Farm (H) Control	5	5
Redhouse (RH)	2	2
Redhouse (RH) Control	1	1
Holbeach Hurn (HH)	0	0
Holbeach Hurn (HH) Control	2	0
Whaplode (W)	0	0
Whaplode (W) Control	0	0
Chatteris (CH)	7	7
Chatteris (CH) Control	4	3
SUFFOLK (MAY 2007)		
Mocap	2	2
PL Link formulation	5	2
Untreated control	2	2
NORFOLK		
Mocap	5	4
Untreated control	4	2

TABLE 3.4: NEW ISOLATES OF *POCHONIA CHLAMYDOSPORIA* FOUND DURING THE COURSE OF THE PROJECT (CONFIRMED USING PCR –ß TUBULIN PRIMERS)

Name	Site	Origin	Date of isolation
407	Bayer trial, Sutton, Suffolk	Soil	June 07
W3 E1 W3 E2	Whaplode	PCN eggs	May 07
C3E1	Chatteris	PCN eggs	May/07
104	Bayer trial, "Paul Goddard", Wickham Market Suffolk	Soil	Jun/08

Use of trehalose to predict PCN decline

Exposure of PCN eggs to potato root diffusate (PRD) induces changes in the permeability of the eggshell, especially the inner lipoprotein layer membrane (Jones *et al*., 1998), causing trehalose to leak out of the eggs and for the juveniles to hatch. In experiments in which eggs were maintained at 20 ºC, the time for juveniles to hatch was longer in soil leachate than in PRD. The rate of hatch following a PRD pulse treatment in which following exposure to PRD, eggs were maintained at 5 ºC for two weeks and then brought back to 20 ºC, was intermediate and not statistically significantly different either other treatments (Table 3.3).

Using GLM analysis, the predicted probabilities of a juvenile to hatch after 8 weeks at 20 ºC, for each of the three treatments, was calculated. As may be expected, the probability of the PRD pulse treatment lies between the probability of the negative and positive control; all treatments were significantly different ($p<0.05$) (Table 3.4). The Cumdistribution analysis found that there was no lag in log (time) before a response to the treatment applied ($p>0.05$) (Fig. 3.2.6). At 5ºC, the results were less conclusive as the response from the PRD (positive control) was not different from the soil leachate treatment (negative control) (Figure 3.2.7). The predicted probabilities of a juvenile hatching after 8 weeks at 5ºC were lower than at 20ºC (Table 3.5).

Comparison between the two temperatures: the probabilities of juveniles to hatch after a PRD pulse and the PRD treatment (positive control) decreased at 5 ºC, when compared to 20 ºC, showing that the cold temperature was reducing the rate of the hatch. However, this experiment would need to be repeated in order to determine the effect of the low temperatures with prior exposure to PRD and spontaneous hatch. In all tests, application of PRD whether as a continuous or pulsed exposure resulted in the release of trehalose, which was only detected in untreated eggs.

TABLE 3.3 MEAN TIME TO JUVENILE HATCH (LOG (TIME SCALE)) AT 20ºC, CONSIDERING THE ANALYSIS OF PARAMETERS FROM THE FITTED DISTRIBUTION TO CONSIDER OVERALL DIFFERENCES ACROSS TREATMENTS.

Treatment	Soil leach (negative control)	PRD pulse	PRD (positive control
	1.047	0.945	0.778

LSD (5%) = 0.2198, SED = 0.101

TABLE 3.4 PREDICTED PROBABILITY OF A JUVENILE HATCHING FOR EACH TREATMENT, AFTER 8 WEEKS, USING GENERALISED LINEAR MODEL (GLM) ANALYSIS (20 ºC). THE DIFFERENCES BETWEEN NUMBERS OF JUVENILES WHICH WERE HATCHED IN EACH TREATMENT WAS SIGNIFICANTLY DIFFERENT (P<0.05)

Treatment	Hatch (%)	Standard error (SE)
Soil Leach (negative control)	8.85	1.33
PRD pulse	23.88	2.01
PRD (positive control)	53.67	2.44

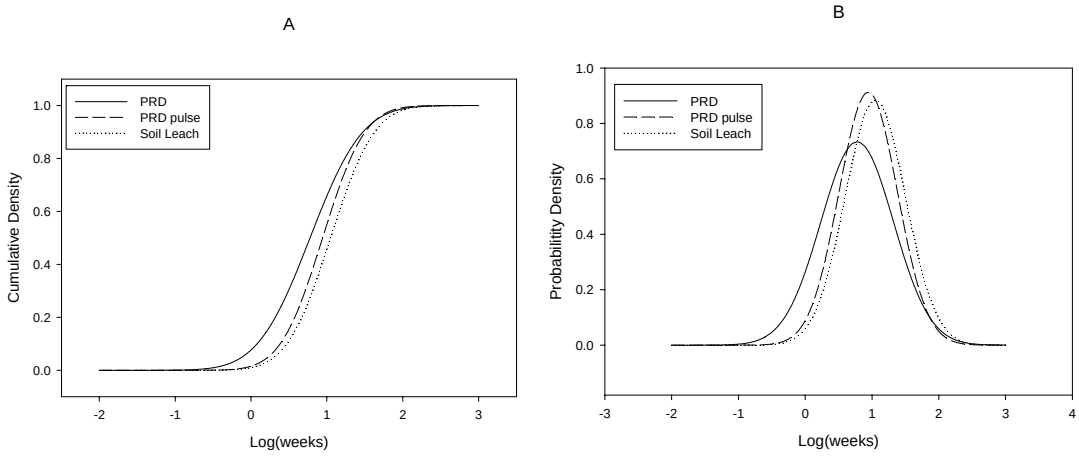


FIG 3.2.6 CUMULATIVE (A) AND PROBABILITY DISTRIBUTION (B) OF TIME TO HATCH, AT 20 ºC

TABLE 3.5 PREDICTED PROBABILITY OF A JUVENILE HATCHING FOR EACH TREATMENT, AFTER 8 WEEKS, USING GENERALISED LINEAR MODEL (GLM) ANALYSIS (5 ºC).

Treatment	Hatch (%)	Standard error (SE)
Soil Leachate (negative control)	10.69	2.85
PRD pulse	18.29	3.61
PRD (positive control)	30.41	3.85

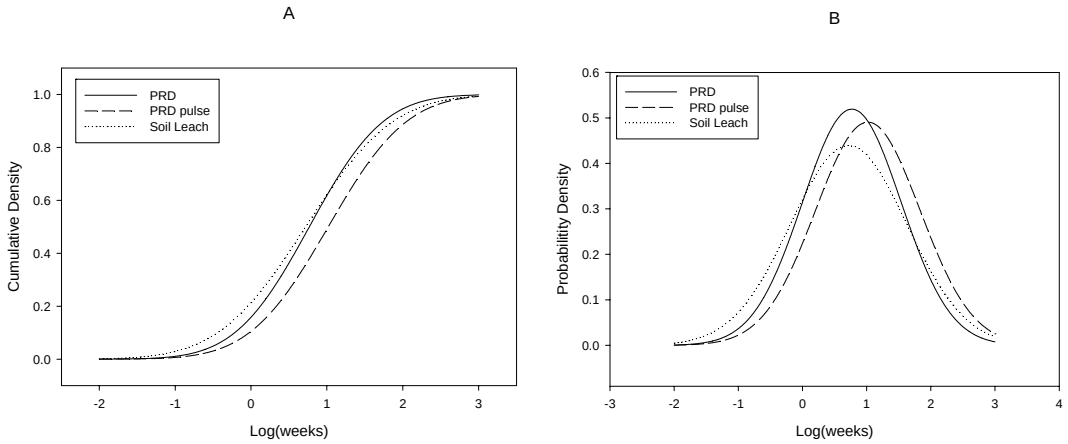


FIG 3.2.7 CUMULATIVE (A) AND PROBABILITY DISTRIBUTION (B) OF TIME TO HATCH, AT 5 °C.

Use of ELISA to predict PCN decline

A monoclonal antibody (MAC 356) was successfully resurrected after > 15 years storage in liquid nitrogen and was tested to check the viability of *G. pallida* and *G. rostochiensis* eggs. The results showed that MAC 356 was able to distinguish between dead and live eggs of *G. pallida*. The antibody was tested against other populations of *G. pallida* and showed similar results (not shown). MAC 356 was active against live *G. pallida* eggs and was not reactive to juveniles or dead eggs (Figure 3.2.8). Although some reactivity was shown when MAC 356 was tested on live eggs of *G. rostochiensis*, the reactivity was lower than on *G. pallida* eggs, and the antibody reacted to live juveniles of *G. rostochiensis* (Figure 3.2.9). To conclude, MAC 356 can be used to assess the viability of eggs of *G. pallida* but is not discriminative when tested against eggs of *G. rostochiensis.*

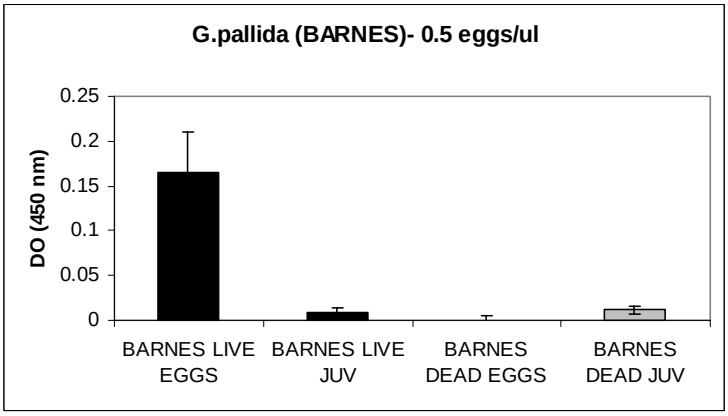


FIGURE 3.2.8 USE OF THE MONOCLONAL ANTIBODY MAC 356 AGAINST EGGS OF *GLOBODERA PALLIDA* (BARNES POPULATION). THE ANTIBODY SHOWED REACTIVITY AGAINST LIVE EGGS OF *G. PALLIDA* BUT NOT TO LIVE JUVENILES OR DEAD EGGS.

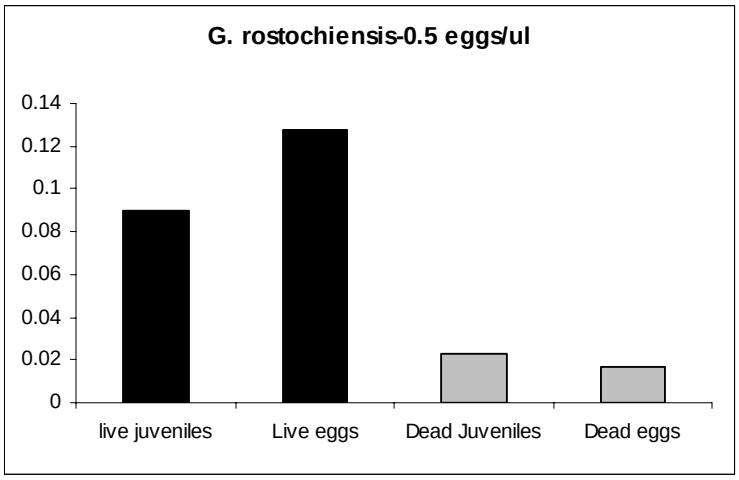


FIGURE 3.2.9 USE OF THE MONOCLONAL ANTIBODY MAC 356 AGAINST EGGS OF *GLOBODERA ROSTOCHIENSIS*. THE ANTIBODY SHOWED REACTIVITY AGAINST LIVE EGGS AND JUVENILES OF *G. ROSTOCHIENSIS*.

3.2 Concluding comments for objective 2

The PLFA analysis of the soils showed that the microbial communities between the different field soils were different but the method was not sufficiently discriminating to identify populations of microorganisms that might be involved in the decline of PCN populations. This was especially true for the fungal community and PFLA analysis did not reveal the differences observed with selective media for specific nematophagous fungi. The use of PCR was discriminating and could be successfully used in identifying both *P. chlamydosporia* and *P. lilicinus*. However attempts to use PCR to identify these fungi directly from soil was unsuccessful and the use of the semi-selective media was a necessary key step in the identification process. Using such techniques four new *P. chlamydosporium* isolates were identified, which should be characterised for their potential as new biological control agents. Although not originally written into the research of the project a monoclonal antibody was successful in discriminating between viable and non-viable eggs and could be developed into an assay for the quantification of nematodes in field soils.

3.3 Results objective 3

Determine rates of decline in soils under different break crops and in commercial cropping systems treated with selected fungal agents.

Is there an interaction between cropping regime, soil type and nematode population on the rate of PCN decline? Due to the non-normal distribution of the percentage egg infection data, a logit transformation with an adjustment (0.1) was used to account for variance heterogeneity. There was a significant weak interaction between soil, crop and nematode population ($p= 0.030$). However, a significant difference in the percentage of egg infection was found between Severals and Woburn soil but only for the PCN population from Waddington (Table 3.6). The percentage of PCN egg infection from Woburn soil and Severals soil under different break crops ranged from 0 to around 14 percent (Figure 3.3.1)

TABLE 3.6 LOGIT (EGG INFECTION) OF THE TWO POPULATIONS OF PCN STUDIED (PLANTATION AND WADDINGTON), IN TWO SOILS (SEVERALS AND WOBURN). THE VALUES ARE MEAN VALUES. SEVERALS SOIL: SED= 0.449 ON 43 DF; LSD (5%) = 0.906

Soil	Population	Plantation	Waddington
Severals		-3.37	-3.99
Woburn		-3.57	-2.76

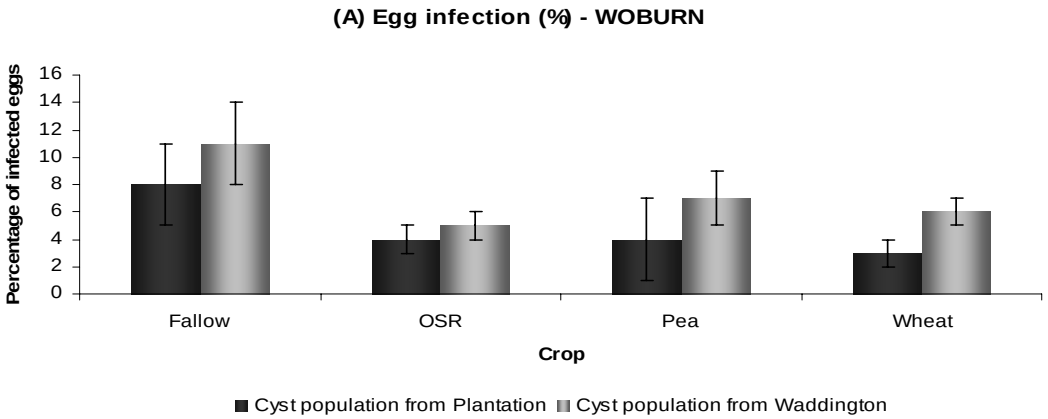


FIGURE 3.3.1 EFFECTS OF DIFFERENT BREAK CROPS (OSR, PEAS, WHEAT) AND A FALLOW ON THE AVERAGE PERCENTAGE EGG INFECTION, IN TWO SOILS (A)- WOBURN AND (B)-SEVERALS

Similarly as above due to the natural variation associated with the estimated numbers of viable eggs/cyst, calculated as: Percentage of egg viability * numbers of eggs/ cyst, a log transformation was used to account the variance of heterogeneity. No major relationships were found but a significant difference was found between pea and OSR, for the population of Waddington. Results showed a weak interaction between crop and nematode population (p= 0.074). All the correlations between egg infection, egg viability and cyst content counts were not significant (p> 0.238) (Table 3.7)

TABLE 3.7. EFFECTS OF DIFFERENT BREAK CROPS (OSR, PEA, WHEAT) AND A FALLOW CROP ON THE AVERAGE NUMBER OF VIABLE EGGS/CYST, COMPARING TWO POPULATIONS OF PCN (PLANTATION AND WADDINGTON). SED= 0.3862 ON 44 DF; LSD= 0.7784

Crop	Population	Plantation	Waddington
Fallow		7.951	7.764
OSR		8.080	7.522
Pea		7.611	8.331
Wheat		8.310	7.731

Assess the rate of PCN decline in field trials using selected nematophagous fungi under commercial cropping

Field sites 2007: The results to the 2007 field trials can be seen in Figures 3.3.3.and 3.3.4. Interestingly, there was an increase in both tuber yield and number of tubers at all sites in the treatment plots although this increase was not statistically significant. At Whaplode, the crop was severely damaged due to potato blight and had to be abandoned. Due to the yield results of the 2007 data it was decided to repeat the experiments.

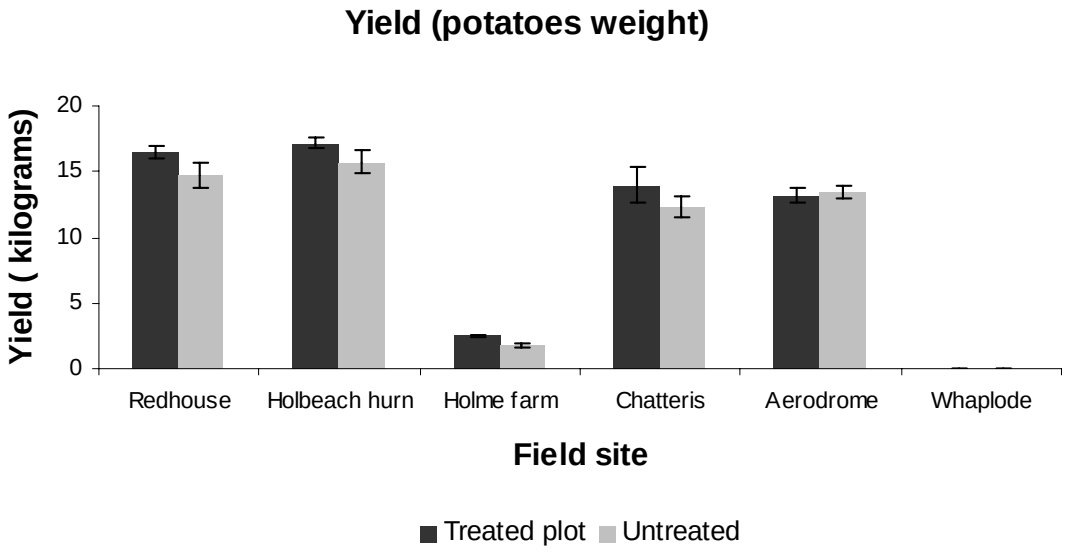


FIG 3.3.3 YIELD OF TUBERS PER PLOT FOR COMMERCIAL POTATOES TREATED WITH *PAECILOMYCES LILACINUS* AT SIX SITES (ANOVA BETWEEN TREATED AND UNTREATED PLOTS $P > 0.05$).

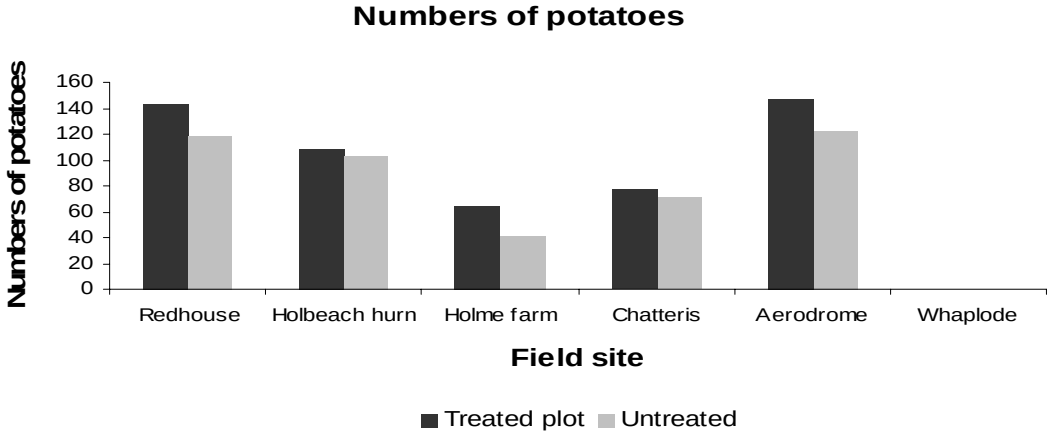


FIG 3.3.4 NUMBER OF TUBERS PER PLOT FOR COMMERCIAL POTATOES TREATED WITH *PAECILOMYCES LILACINUS* AT SIX SITES (ANOVA BETWEEN TREATED AND UNTREATED PLOTS $P > 0.05$)

Field sites 2008: Because the earlier field trials had appeared promising it was decided to undertake two more carefully control experiments following the 2007 trials. There was no

statistically significant difference in either the tuber yield or the number of tubers produced at either of the sites (Figs 3.3.5 and 3.3.6). Indeed, there was a slight reduction in overall tuber weight across all treatments in comparison with the control. There was also no statistically significant difference between the various formulations although the *Pochonia* paste appeared to reduce both number of tubers and tuber weight to the largest extent compared to the other treatments. Interestingly, although none of the treatments had an effect on either tuber weight or tuber number there was a statistically significant effect on the ratio of Pf/Pi (Fig 3.3.7). At the Suffolk site all biological control treatments reduced the Pf/Pi ratio from around 19 to less than 10, showing a 50 percent reduction in nematode multiplication rate. However, this result was not repeated at the Norfolk site where the Pf/Pi ratio was around 14 for the untreated control; although at this site the *Pochonia* paste reduced the Pf/Pi ratio this was not the case with any of the other treatments and both the powder and liquid formulated *Paecilomyces* treatments increased the Pf/Pi ratio.

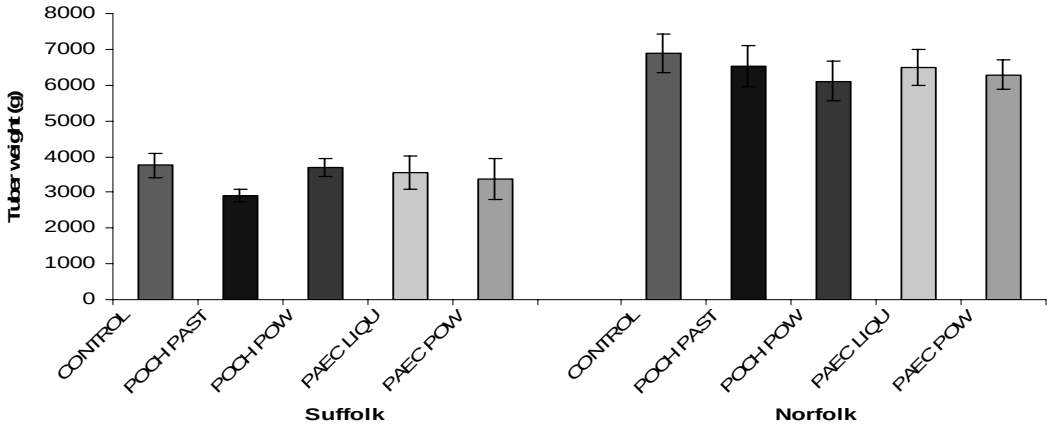


FIG 3.3.5 YIELD OF TUBERS PER PLOT AT TWO SITES, SUFFOLK AND NORFOLK, COMPARING AND UNTREATED CONTROL WITH THE BIOLOGICAL CONTROL AGENTS *PAECILOMYCES LILACINUS* (PAEC), AS A LIQUID (LIQU) OR A WETTABLE POWDER (POW) FORMULATION, AND *POCHONIA CHLAMYDOSPORIA* (POCH) AS A PASTE (PAST) OF WETTABLE POWDER (POW), (ANOVA BETWEEN TREATMENTS $P > 0.05$).

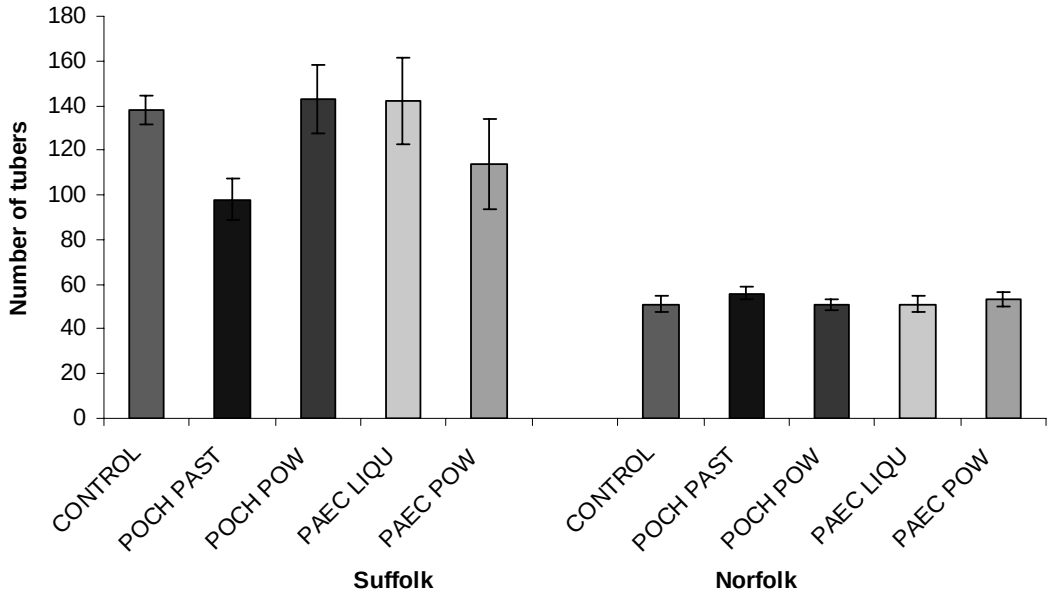
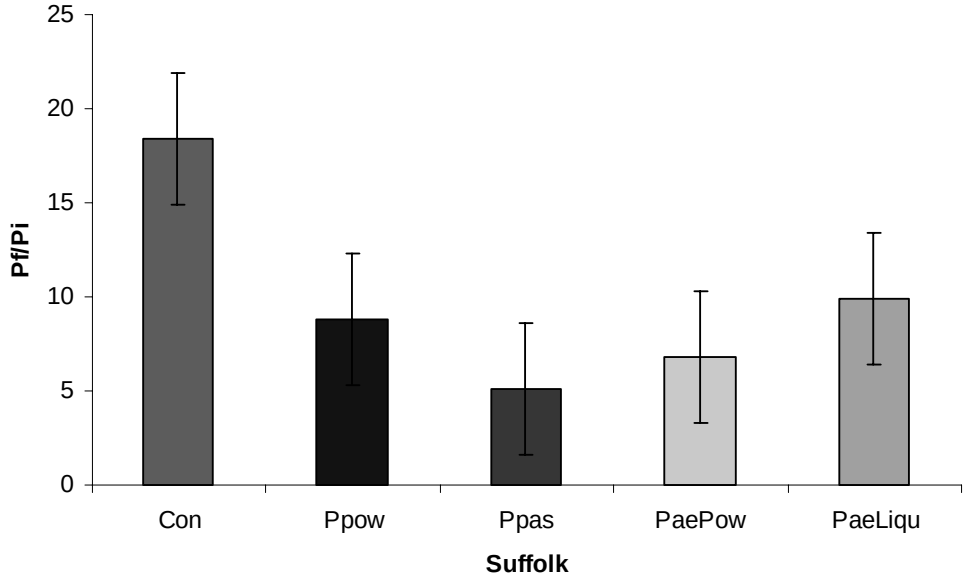


FIG 3.3.6 NUMBER OF TUBERS PER PLOT AT TWO SITES, SUFFOLK AND NORFOLK, COMPARING AND UNTREATED CONTROL WITH THE BIOLOGICAL CONTROL AGENTS *PAECILOMYCES LILACINUS* (PAEC), AS A LIQUID (LIQU) OR A WETTABLE POWDER (POW) FORMULATION, AND *POCHONIA CHLAMYDOSPORIA* (POCH) AS A PASTE (PAST) OF WETTABLE POWDER (POW), (ANOVA BETWEEN TREATMENTS $P > 0.05$).



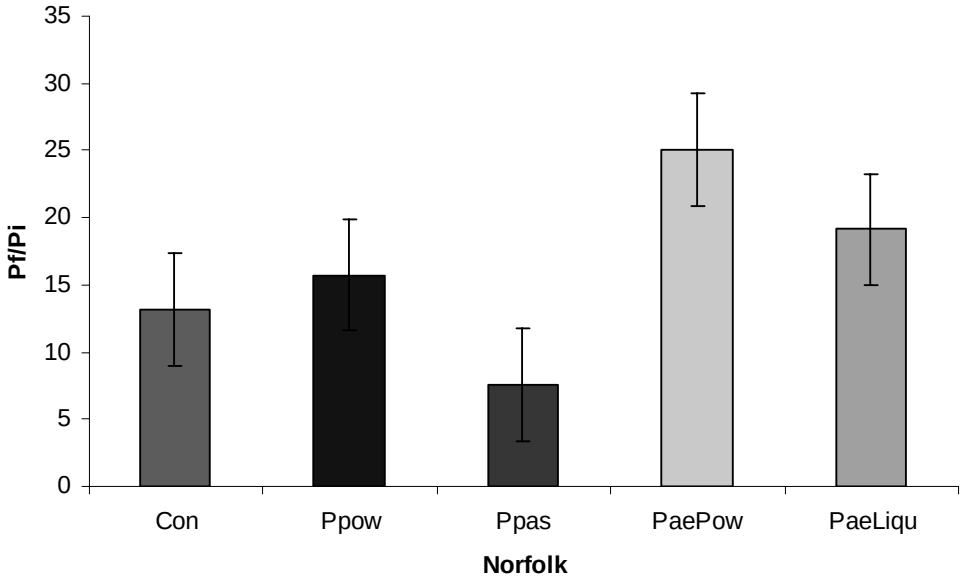


FIG 3.3.7 PF/PI RATIO AT TWO SITES, SUFFOLK AND NORFOLK, COMPARING AND UNTREATED CONTROL WITH THE BIOLOGICAL CONTROL AGENTS *PAECILOMYCES LILACINUS* (PAEC), AS A LIQUID (LIQU) OR A WETTABLE POWDER (POW) FORMULATION, AND *POCHONIA CHLAMYDOSPORIA* (POCH) AS A PASTE (PAST) OF WETTABLE POWDER (POW), (ANOVA BETWEEN TREATMENTS $P < 0.05$).

3.3 Concluding comments for objective 3

Although there were weak interactions between soil type and cropping regime that affected PCN decline these were not regarded as sufficiently large to be used as a method to manage PCN infestations. Although the majority of break crops had no effect it should be noted that *Solanum sysimbrifolium* (Objective 5) did have a significant effect on PCN hatch in laboratory tests and could therefore be used in PCN management regimes. The trend for there being an increase in both tuber yield and the number of tubers in the 2007 trials was not maintained during the 2008 season and this requires some explanation. There are two major factors that could have played a role here: 1) during the 2007 season a double application was undertaken to give the fungi a better prospect of establishment whereas in the 2008 growing season only one application was made (in line with the manufacturers recommendations) but also with the thought that farmers would want to reduce the number of application rates to the minimum; therefore application rate and timing appear to be having an effect. 2) Differences in the soil microflora may also have had a profound effect, during the 2007 season it was apparent that soft rot of the tubers was a problem at several sites and at Holme Farm in particular, soft rot was being controlled in the fungal treated plots. Therefore, in 2007 there could have been an indirect effect of the control of soft rots which was not at all observed during the 2008 season. This is potentially an important finding for the exploitation of the biological control agent and requires further investigation. Tobin et al. (2008b) demonstrated significant control of PCN multiplication by *P. chlamydosporia* at two field sites and the fungus was compatible with the nematicide fosthiazate and combined applications reduced nematode infestations by up to 84%. However, the application rates of the fungus inoculum were equivalent to 3-5 t. ha^{-1}, which are impractical on a field scale and there is a need to improve formulations and production methods.

Results objective 4

Impacts of herbicides and fungicides on the activity of biological control fungi

Impact of three herbicides (Sencorex, Basagran and Stomp) on the activity of the nematophagous fungi Pochonia chlamydosporia *and* Paecilomyces lilacinus: Data were analysed using ANOVA in GenStat® but showed no significant relationships between the different herbicides and the two fungi tested (p=0.760, df =2). In both fungi, the concentration of herbicides had no effects on fungal growth (p=0.436, df =22) (Figure 3.4.1). There was no overall differences between the three herbicides tested (Table 3.8).

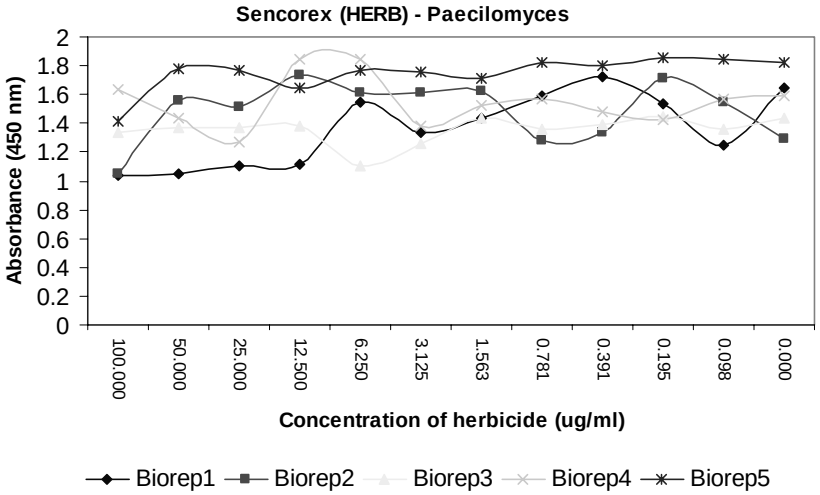


FIGURE 3.4.1. THE EFFECT OF HERBICIDES SENCOREX ON THE GROWTH OF *PAECILOMYCES LILACINUS*. THE DIFFERENT CONCENTRATIONS OF HERBICIDE PRODUCED NO SIGNIFICANT EFFECTS ON THE GROWTH OF THE FUNGUS. SIMILAR RESULTS WERE FOUND FOR BASAGRAN AND STOMP, IN BOTH *POCHONIA CHLAMYDOSPORIA* AND *PAECILOMYCES LILACINUS*.

TABLE 3.8 COMPARISON BETWEEN THE OVERALL EFFECTS OF HERBICIDES ON THE GROWTH OF THE TWO FUNGI TESTED (MEAN LOG (ABSORBANCE-BLANKS).

Herbicide	Basagran	Sencorex	Stomp
LSD (5%) = 0.1574 Sed = 0.0755	-0.365	-0.354	-0.393

Impact of three fungicides (Monceren, Amistar and Tatoo) on the activity of the nematophagous fungi Pochonia chlamydosporia *and* Paecilomyces lilacinus*:* Both of the fungi responded differently compared to the herbicides; the fungicides caused significant reductions ($p<0.001$, df = 1) on nematophagus fungal growth and differences could be found between the different fungicides and their effects on the growth of both *P. chlamydosporia* and *P. lilacinus* ($p<0.001$, df = 2). A strong effect of the concentration of fungicide on the activity of both fungi was found ($p<0.001$, df = 11). The two fungi showed different levels of sensitivity to fungicides (Figure 3.4.2).

Non-linear regression analysis was applied to the data (means of the three technical replicates) to describe the relationship between absorbance and concentration for the three fungicides applied to the two fungi. The general form of the equation for the model, which was found to be appropriate, was:

$$y = a + c/(1 + \exp(-k \times \log(Conc) - m))$$

where y represents the absorbance data, a is the asymptotic response for increasing concentration, $a + c$ is the estimated control response (at 0 concentration), k the exponential rate of decrease in absorbance as concentration increases and m is the $\log_e$ of the concentration providing 50% reduction in absorbance from the control response (LC50). Note that this model provided a better fit to the data than fitting a simple exponential decay model on the raw concentration scale [$y = a + b\exp(-k(Conc))$], the present model having one extra parameter.

A parallel curves analysis was used to find out which parameters a, b, k and m required to be estimated separately for fungi (Poch. and Paec.) and/or fungicides (Amistar, Tatoo and Monceren) using a forward selection approach. The addition of extra (separate) parameters in the model was assessed using the F-test on the respective degrees of freedom (which are the number of extra parameters fitted and the corresponding residual degrees of freedom. As the fungi should have the same absorbance in the control condition regardless of fungicide, separate estimates of the control response for the fungicide were not fitted.
The best model was (model below):

$$y = a_i + c_l/(1 + \exp(-k_i \times \log(Conc) - m_j))$$

where $i = 1,\ldots, 6$ for the 2 fungi by 3 fungicides, and $j = 1,\ldots, 3$ for the three fungicides and $l = 1, 2$ for the 2 fungi. Hence, separate c parameters were required for the fungi, separate a and k parameters were required for fungi and fungicides, but separate m parameters were only required for the fungicides. This means that the action of each fungicide on both fungi was similar in this respect, although the exponential rates of decrease were different.

A summary of the modelling is given below, indicating how the best model was derived.

Model		**RSS**	**d.f.**	
Common *a*, *b* and *k*		42.40	355	
Fungicides				
Separate *k*		37.66	353	
Separate *c*		37.71	353	
Separate *a*		39.59	353	
Separate *m*		**35.79**	**353**	
Fungi				
Separate *k*		22.46	354	
Separate *c*	**(1)**	**18.87**	**354**	**P < 0.001 (Fitted first)**
Separate *a*		29.36	354	
Separate *m*		19.34	354	
Fungicides and Fungi				

Fungicides	*Fungi*			
Separate *m*	**Separate *c***	**(2) 7.516**	**352**	**P < 0.001 vs. (1)**
Separate *m, a*	**Separate *c***	**(3) 6.908**	**350**	**P < 0.001 vs. (2)**
Separate *m, a, k*	**Separate *c***	**(4) 6.386**	**348**	**P < 0.001 vs. (3)**
Separate *m, a, k*	**Separate *c, a***	**(5) 6.061**	**345**	**P = 0.004 vs. (4)**
Separate *m, a, k*	**Separate *c, a, k***	**(6) 5.868**	**342**	**P = 0.01 vs. (5)**
Separate *m*, *a*, *k*	Separate *c*, *a*, *k*, *m*	(7) 5.850	339	P = 0.789 vs. (6)

No further separations of parameters provided a significant ($P < 0.05$) improvement in the fit following model **(6)**. Hence, separate estimated responses at the control condition were not imposed for the fungicides, and the LC50s were significantly different for the fungicides, but were the same for the two fungi they were exposed ($P = 0.789$). The residual mean square (s^2) from the model was 0.017 on 342 degrees of freedom and the percentage variance accounted for was 91.6%. The LC50s for the three fungicides are given in Table 3.9 with standard errors (SE). The estimated LC50 for Amistar was the highest, due to the effect of this fungicide being so dramatic.

TABLE 3.9 LC50S FOR THE THREE FUNGICIDES TESTED. THE LC50S WERE SIGNIFICANTLY DIFFERENT FOR THE FUNGICIDES, BUT WERE THE SAME FOR THE TWO FUNGI THEY WERE APPLIED TO ($P = 0.789$).

	LC50s (µg ml^{-1})	**SE**
Amistar	0.068	0.006
Tatoo	1.910	0.257
Monceren	0.716	0.133

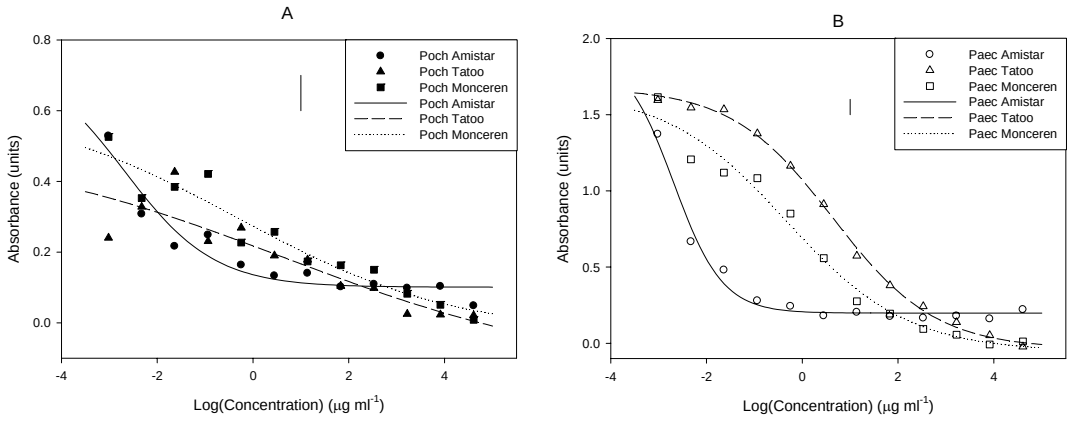


FIGURE 3.4.2 DOSE RESPONSE MODEL CURVES FOR THE FUNGICIDES AMISTAR, TATOO AND MONCEREN ON THE GROWTH OF *POCHONIA CHLAMYDOSPORIA* (A) AND *PAECILOMYCES LILACINUS* (B)

3.4 Concluding comments for objective 4

The method used in which the nematophagous fungi were grown in liquid cultures treated to the pesticide treatments proved sensitive for assessing effects on fungal growth. The two fungi *P. chlamydosporia* and *P. lilacinus* showed different levels of sensitivity to fungicides but were tolerant to herbicides. *P. chlamydosporia* was more tolerant to high concentrations of fungicides than *P. lilacinus*.

Results objective 5

Investigate and establish the biotic and abiotic factors that influence growth and development of the three fungi *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Plectosphaerella cucumerina*

Infection of G. pallida *eggs in soil by the nematophagous fungus* Pochonia chlamydosporia *(Pc 280) in the presence of a plant crop and in fallow soil:* The presence of decomposing roots had a negative effect on egg parasitism by *P. chlamydosporia*. Although the infection levels were low in all treatments (<10 %), significant differences were found between the control plots (fallow) and plots where plant roots had been left to decompose ($p<0.05$; Figure 3.5.1). There were no significant differences between the type of plant, rate of spore inoculation or time for decomposition. Two months after the decomposition of roots, the fungus could be recovered from the soil, in both fallow and plant treatments (roots of cabbage and wheat) but was below the inoculation level (5000 chlamydospores/g soil) (Figure 3.5.2). Significant differences in the percentage of carbon and nitrogen in soil could not be detected between the treatments (Figure 3.5.3).

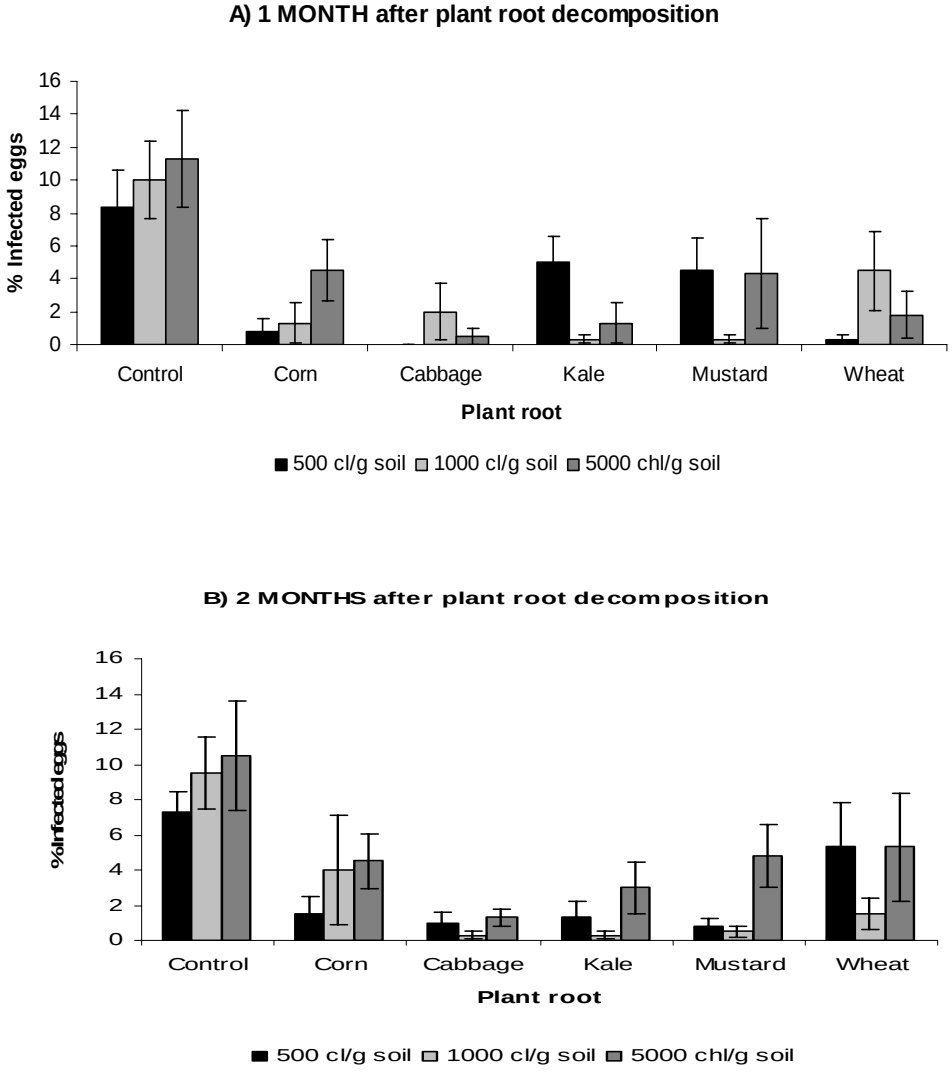


FIGURE 3.5.1 PERCENTAGE OF *GLOBODERA PALLIDA* EGGS INFECTED WITH *POCHONIA CHLAMYDOSPORIA* IN SOIL INOCULATED WITH DIFFERENT RATES OF SPORES (CHLAMYDOSPORES), ONE MONTH (A) AND TWO MONTHS AFTER ROOT DECOMPOSITION (B) , OR IN FALLOW SOIL (CONTROL).

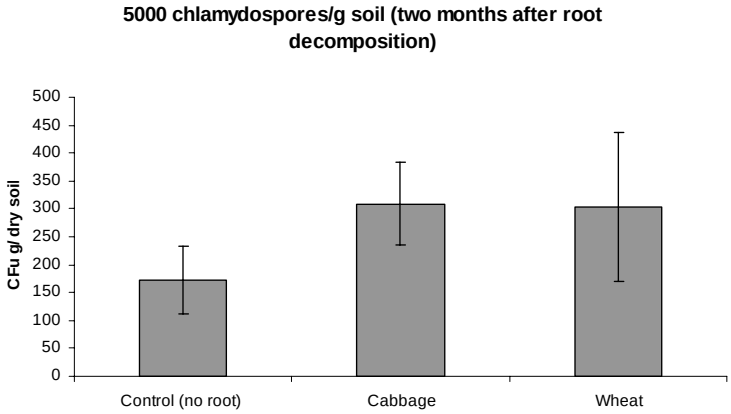


FIGURE 3.5.2 NUMBER OF CFU BELONGING TO *POCHONIA CHLAMYDOSPORIA* FOUND IN THE SOIL 2 MONTHS AFTER ROOT DECOMPOSITION, SHOWING THAT THE FUNGUS WAS ALIVE AND PRESENT IN FALLOW AND PLANTED SOILS

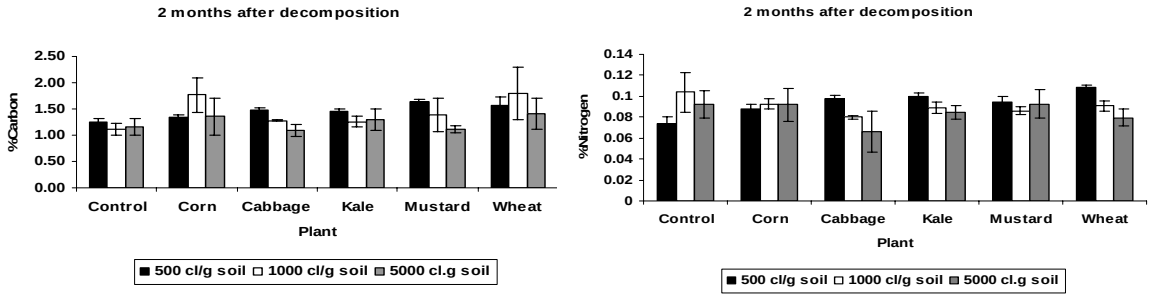


FIGURE 3.5.3 PERCENTAGE OF CARBON AND NITROGEN PRESENT IN THE SOIL, TWO MONTHS AFTER ROOT DECOMPOSITION.

Monitoring the growth and spread of nematophagous fungi from the rhizosphere into the bulk soil: The abundance and spread of the fungus was examined in small plots of potatoes grown out-of-doors. Firstly, a model was fitted to the binary data (presence/absence of CFUs), in order to test whether the depth at which samples were taken had an effect on the probability of occurrence. As there was no significant ($p>0.05$) effect of position of sampling on the grid, the data was fitted using probabilities calculated as one per each grid (values out of 8) to enable the analysis of distance and time effects using a binomial distribution ($n=8$). By applying this model, the ANOVA showed a significant main effect of time ($p=0.013$) for the CFU/ g soil data (Figure 3.5.4). An increase on the numbers of CFUs with time was found, reaching a plateau after 8 weeks. When data was analysed using GLM analysis, the results of approximate F-tests found a strong effect of distance (10 and 15 cm), time and interaction between these two factors (Figure 3.5.5). The probability of observing the presence of the fungus in the soil increased with time, for both distances but appears to be reaching a plateau for 10 cm over time. However, the probability of spread is still increasing at 12 weeks, for 15

cm distance. It is possible that this increase is caused by secondary production of conidia (secondary growth).

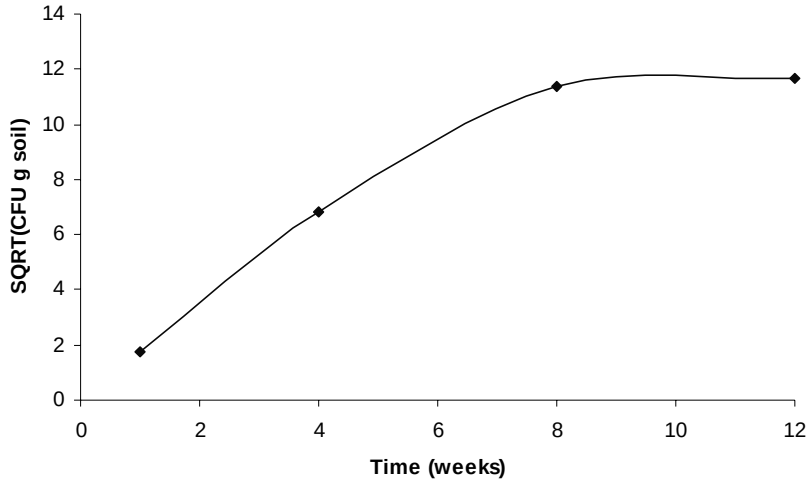


FIGURE 3.5.4 NUMBERS OF CFUS (SQRT CFU) FOUND IN SOIL AND RELATIONSHIP WITH TIME. SIGNIFICANT DIFFERENCES BETWEEN TIME AND FUNGAL ABUNDANCE WERE FOUND (SED= 2.492; LSD (5%) = 5.747), USING ANOVA.

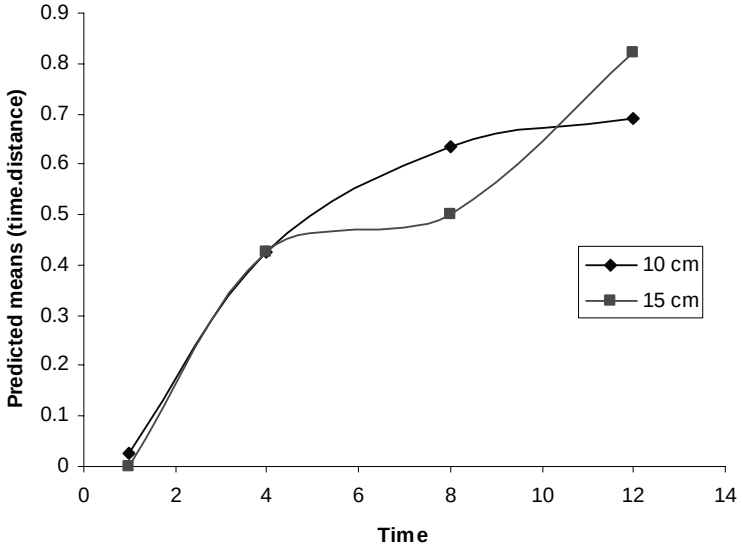


FIGURE 3.5.5 PREDICTED MEANS FOR THE INTERACTION TIME X DISTANCE OF FUNGAL SPREAD ON SOIL CFU DATA, TAKEN AT 10 AND 15 CM DISTANCE FROM AN INOCULATED PLANT. THE PROBABILITY OF OBSERVING THE PRESENCE OF THE FUNGUS IN THE SOIL INCREASED WITH TIME, FOR BOTH DISTANCES.

The effect of root exudates from sugar beet, Solanum sysimbriifolium*, wheat, oil seed rape and pea on PCN hatch:* The greatest hatch can be seen from potato in which around 4500

juveniles have hatched from the 20 cysts, the next highest hatch is with *S. sisymbrifolium* with a hatch of around 500 (Fig 3.5.6). However, the root diffusate collected from *S. sisymbrifolium* plants at two weeks increases this hatch to over 1400 juveniles which is much higher but still less than 50% of the potato root diffusate hatch (Fig 3.5.7).

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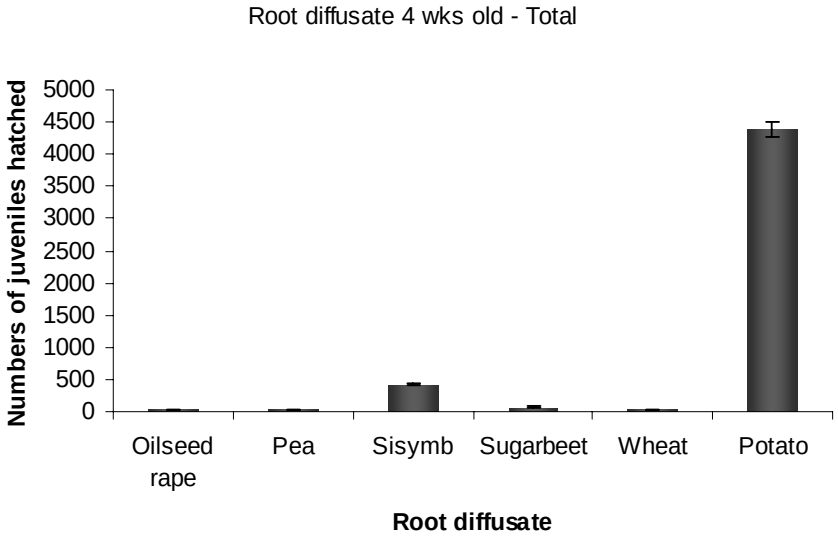

FIG 3.5.6 COMPARISON OF HATCH OF POTATO CYST NEMATODES PLACED IN STANDARDISED SOLUTIONS OF ROOT DIFFUSATE COLLECTED FROM OILSEED RAPE, PEA, *S. SISYMBRIFOLIUM*, SUGARBEET, WHEAT AND POTATO (BARS = SE)

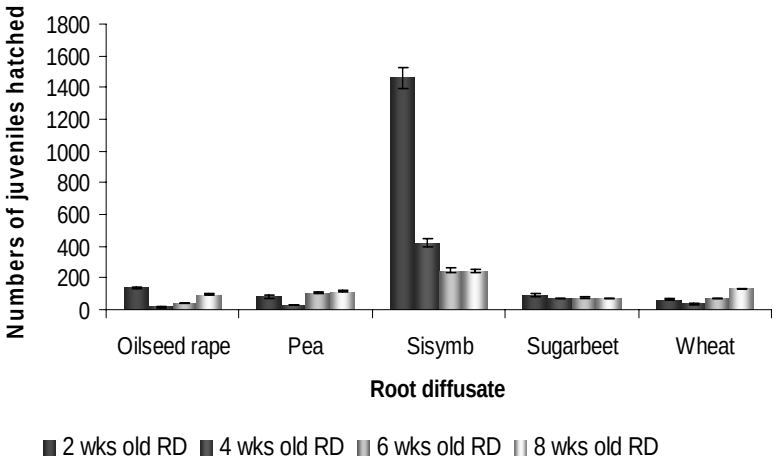

FIG 3.5.7 COMPARISON OF HATCH OF POTATO CYST NEMATODES PLACED IN STANDARDISED SOLUTIONS OF ROOT DIFFUSATES COLLECTED AT 2, 4, 6, 8 WEEK OLD PLANTS OF OILSEED RAPE, PEA, *S. SISYMBRIFOLIUM*, SUGARBEET, AND WHEAT (BARS = SE)

3.5 Concluding comments for objective 5

The presence of decomposing roots had negative effect on egg parasitism by *P. chlamydosporia*. Significant differences were found between the control plots (fallow) and plots where plant roots were decomposing. There were no significant differences between the type of plant, rate of spore inoculation or time for decomposition. Two months after the decomposition of roots, the fungus could be recovered from the soil, in both fallow and plant treatments (roots of cabbage and wheat) but was below the inoculation level. Presumably, the roots provide an alternative food source that maintains *P. chlamydosporia* in its saprotrophic phase and prevents the switch to parasitic activity.

The spread of *P. chlamydosporia* from the site of application near the tuber into the soil was significant, increased with time, and could be found at both 10 cm and 15 cm distant from the plant. It appears to have reached a maximum at 10 cm. However, the spread continued to increase at 12 weeks, at 15 cm distance from the plant. On agar, colonies of the fungus grow by about 1.5mm/day and so the dispersal observed in soil in field conditions is close to this rate and it is possible that the spread observed was caused by the production and dispersal of conidia (secondary spread).

The use of *S. sisymbriifolium* in combination with nematophagous fungi could be useful in the management of PCN, especially in organic systems.

Use of cryo-scanning electron microscopy to monitor egg/cyst colonisation: Initial tests showed that cryo-scanning microscopy can be used to monitor egg infection in nematode cysts. An experiment was set up in which potatoes were grown in sand with PCN and nematophagous fungi. Initial tests involved the application of fungi at very high rates but this proved to be phytotoxic in that the tuber chits did not establish. A subsequent experiment was done and the fungi applied at a lower rate. Cysts have been collected throughout the growing season and examined using cryo-scanning electron microscopy to observe egg infection (Fig 3.5.8). Cryo-scanning electron microscopy has shown that all the three fungi *P. chlamydosporia*, *P. lilacinus* and *M. cucumeria* have the ability to degrade the internal reproductive system of the female to such an extent that no eggs could be observed and were therefore clearly not viable (Fig 3.5.8).

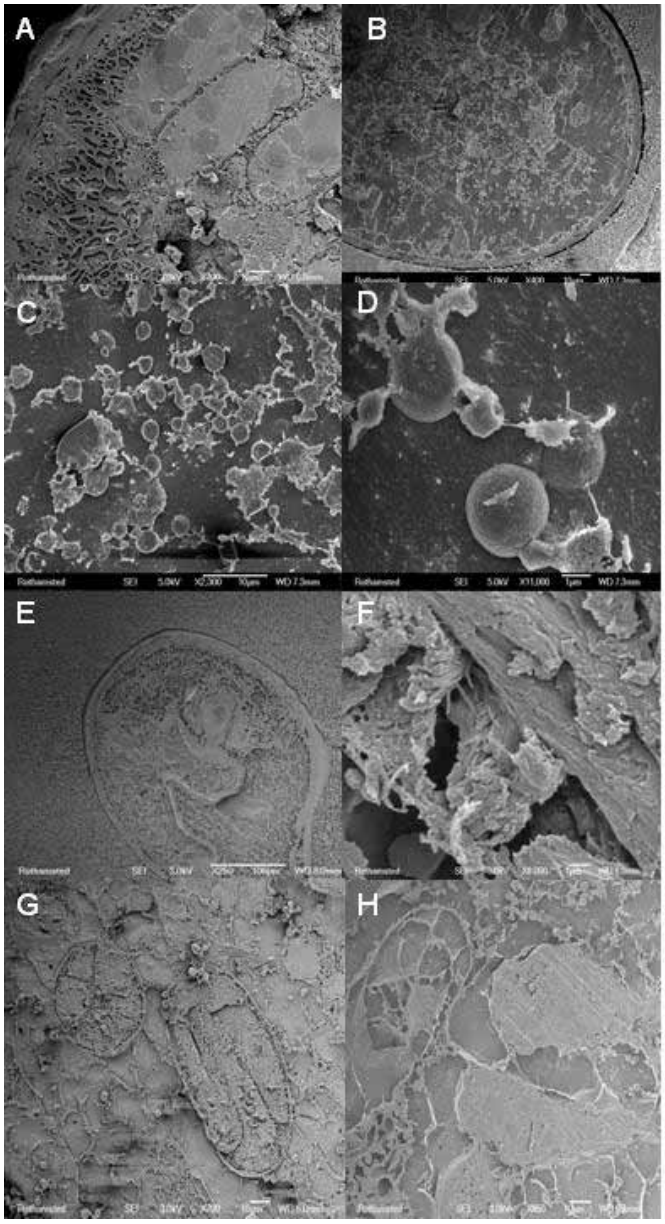

FIGURE 3.5.8 UNINFECTED POTATO CYST NEMATODE FEMALE WITH EGGS AT 46 DAYS (A), INFECTED POTATO CYST NEMATODE FEMALE INFECTED WITH *POCHONIA CHLAMYDOSPORIA* AT 84 DAYS SHOWING NEMATODE AND FUNGAL SPORES AT LOW (B), MEDIUM (C) AND HIGH MAGNIFICATION (D), INFECTED POTATO CYST NEMATODE FEMALE WITH *PAECILOMYCES LILACINUS* AT 46 DAYS (E), INFECTED POTATO CYST NEMATODE FEMALE WITH *PAECILOMYCES LILACINUS* AT 46 DAYS, CLOSE UP SHOWING HYPHAL MYCELIUM (F), UNINFECTED AND INFECTED POTATO CYST NEMATODE FEMALE WITH *MONOGRAPELLA CUCUMERIA* AT 56 DAYS, CLOSE UP SHOWING HYPHAL MYCELIUM (G AND H RESPECTIVELY).

Results objective 6

Investigate the potential formulation and delivery of fungal agents, including commercially available products including any potential phytotoxicity implications

Throughout all the experiments undertaken during the project, both in field studies and in pot experiments, all plants were observed for any evidence of phytotoxic effects at normal application rates. At no point during these studies were phytotoxic effects observed at any of the application rates that were administered in field or pot tests. However, during the cryo-scanning studies (see Objective 5 above) in order to ensure infection fungi were initially applied at three times the recommended application rate and the chitted tubers failed to develop and establish.

Results objective 7

Investigate the carbon nitrogen acquisition and potential translocation by three fungi

Assess if nutrients in the rhizosphere of potato roots increase abundance of different fungal species/biotypes.

CFU differences between fallow sand, sand and rhizosphere from the tubes containing potatoes were used as a measure of the nutrition derived from the plant by each fungal isolate. There were significant main effects and interactions between absence/presence of a plant and type of fungus. The fungi reacted differently to the presence of the plant, *M. cucumerina* and *P.c.* var. *catenulata* (Pc392) being the most different. There was significant difference ($p<0.05$) between treatments (potato vs no potato) for isolate Pc392 (Table 3.10; Figure 3.7.1). Similar results were obtained from CFU obtained from the rhizosphere. *Paecilomyces lilacinus*, Pc280 and Pc392 were significant different from *M. cucumerina* but were not significant different amongst them (Table 3.11; Figures 3.7.2 and 3.7.3).

TABLE 3.10 ANOVA (SQUARE ROOT TRANSFORMATION) OF CFU PER G OF ACID WASHED SAND.

Fungus	None	*Monographella cucumerina*	*Paecilomyces lilacinus*	Pc280	Pc392
No potato	0.0	0.0	193.4	67.5	103.2
Potato	0.0	15.0	185.2	67.9	202.8

$P<0.05$

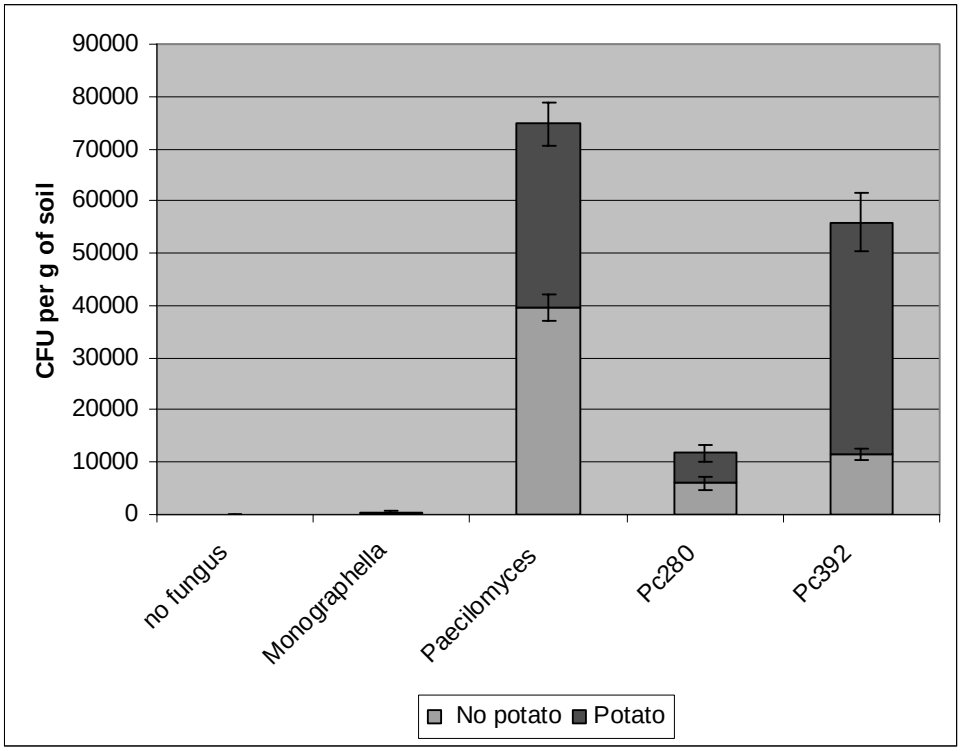

FIGURE 3.7.1 SOIL CFU NUMBERS (MEANS OF RAW DATA). (I) –STANDARD ERROR OF MEAN BARS

TABLE 3.11. ANOVA (LOG (CFUROOTS+1)) OF CFU FROM POTATO RHIZOSPHERE.

Fungus	None	*Monographella cucumerina*	*Paecilomyces lilacinus*	Pc280	Pc392
Potato	0.0	3.181	13.469	11.455	14.377

P<0.05

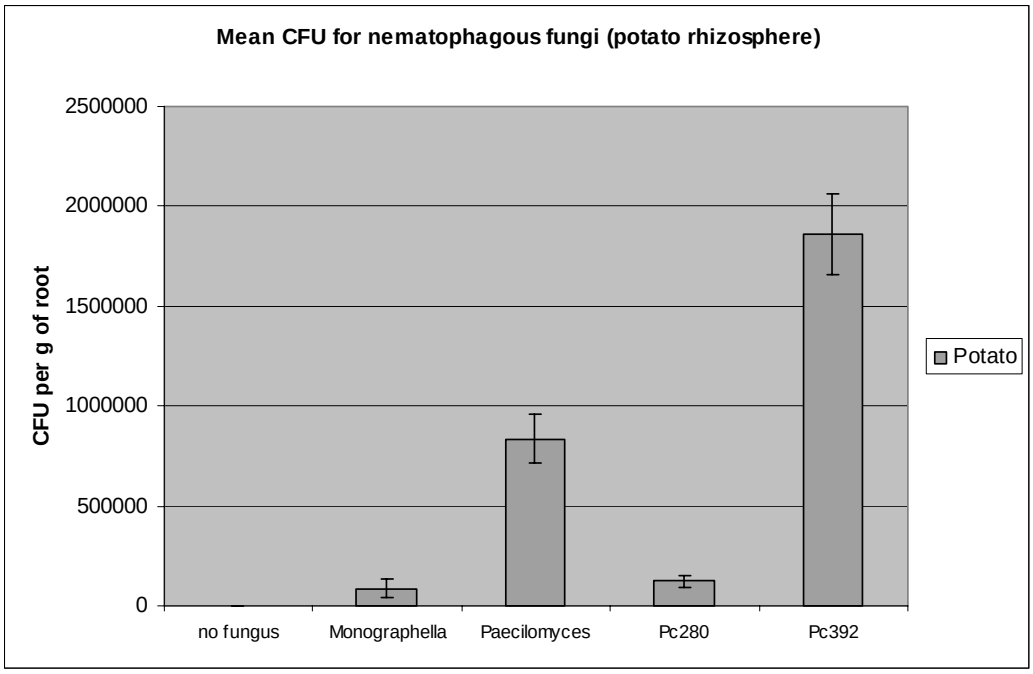

FIGURE 3.7.2 NEMATOPHAGOUS FUNGI CFU COUNTS (POTATO RHIZOSPHERE)

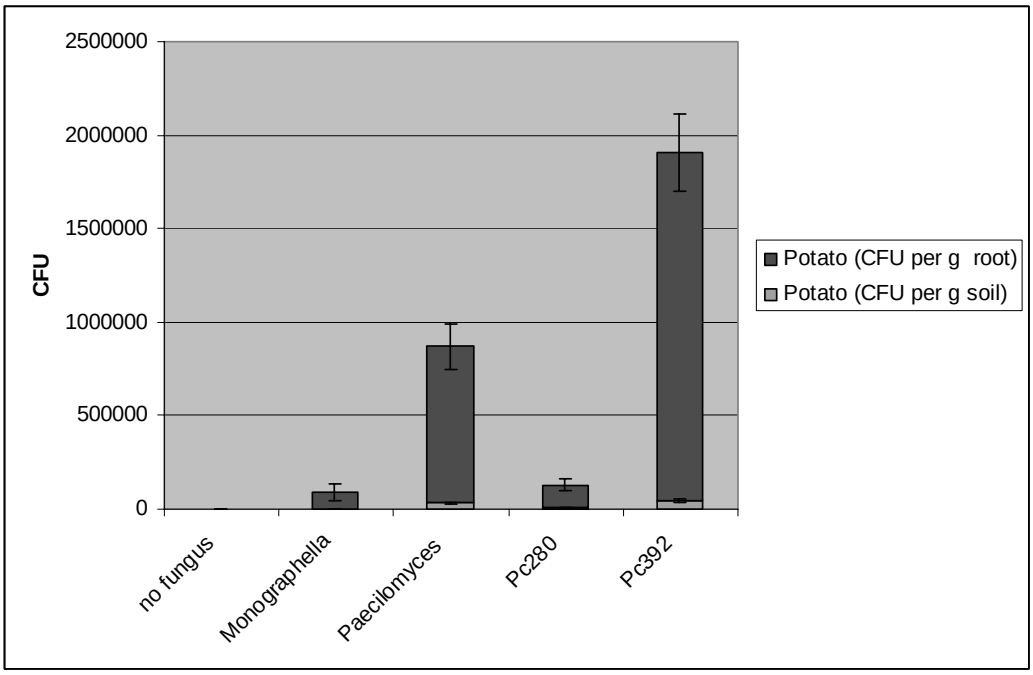

FIGURE 3.7.3 NEMATOPHAGOUS FUNGI CFU COUNTS (POTATO SOIL AND RHIZOSPHERE).

In the second experiment, similar techniques were used to measure the effects of the root exudates from different plants used as break crops in potato rotations, on the abundance of the different fungal isolates. There were significant differences ($P<0.001$) in the abundance of the three isolates in the sand but no significant differences due to crops ($P=0.988$) or to any interaction between crops and fungus ($P=0.180$)

The fungal populations in roots were also measured (Table 3.12). There was a significant interaction between crops and fungi (P=0.003) with a strong main effect of fungus (P<0.001). There was no significant main effect of crop (P=0.460). *P. lilacinus* was most abundant on the roots of oilseed rape and on those of sugar beet whereas *P. chlamydosporia* isolate 392 was more abundant on the roots of all crops than isolate 280, except on potato roots.

TABLE 3.12 MEANS (SQUARE ROOT TRANSFORMATION) OF CFU PER G OF ACID WASHED SAND-GRIT OF THREE ISOLATES OF NEMATOPAGOUS FUNGI AND THE NUMBERS PER G OF ROOT IN DIFFERENT CROPS.

Fungus	Fallow		OSR		Potato		SB		Wheat	
	Sand-grit	Root	Sand-grit	Root	Sand-grit	Root	Sand-grit	Root	Sand-grit	Root
Control	0.0	0.0	0.0	0.0	6.1	0.0	0.0	0	0.0	0.0
Paecilomyces lilacinus	382.7	0.0	410.9	2152	441.2	669	386.4	1916	416.5	957
Pc280	152.7	0.0	179.5	372	155.7	570	162.5	261	165.3	292
Pc392	488.7	0.0	435.1	574	420.2	573	438.4	NA	380.2	1488

3.7 Concluding comments for objective 7

In acid washed sand in which the available nutrients were minimal, growth of all three fungi was limited and there was little proliferation above the initial inocula added to soil; all fungi were much more abundant on the surface of roots than in the sand. *Paecilomyces lilacinus* was the most abundant fungus in soil. *Monographella cucumerina* appeared to be able to grow only in the presence of plant roots and the presence of the plant had a different effect on the two isolates of *P. chlamydosporia* tested; isolate Pc392 being able to exploit the nutrients provided much more readily than isolate Pc280. It is clear from the data collected that these fungi are able to exploit nutrients diffusing from potato roots to increase their abundance and support saprotrophic development. In the second experiment, the populations of the fungi in the fallow treatment were larger than in the previous experiment and the presence of roots had no effect on fungal abundance but there were significant interactions between the fungi and the plant species that affected the numbers of cfu on roots.

Results objective 8

Assess the importance of the fungal pathogens of nematode eggs on the patch dynamics of PCN

The data on cyst and egg numbers and fungal egg parasitism collected along each transect, at 3 field sites (Hanger, Steadmans Bemrose) was compared to assess differences between fields, spatial relationships between nematode abundance and egg parasitism along the transect. Although no significant differences in the percentage of eggs parasitised were found

between the 3 field sites (data analysed on the $\log_{10}$ scale, p=0.327), there was a suggestion that eggs at the field site in Hanger had greater parasitism than in Steadmans, in which the level pf egg infection was lower (Figure 3.8.1). Due to much variability in the results, clear conclusions could not be made in this respect. In terms of spatial distribution, the 3 fields showed differences in the distribution of parasitised eggs along the transect. At Bemrose, a significant autocorrelation was found at 5 metres (between pairs of sampling points 5 metres apart) ($p<0.05$), suggesting that fungal parasitism has a spatial dimension (Table 3.12). A similar, strong indication of autocorrelation was also found in the field site located in Hanger. There was not enough data collected at Steadmans to calculate autocorrelation of distance and parasitism. Similar autocorrelations were found when the numbers of cysts were compared with distance. At Hanger and Bemrose, significant autocorrelation was found between cyst numbers 5 metres apart (Table 3.13). The correlation dropped at 10 m apart, suggesting that patches are 5-10 metres diameter.

Relationship between eggs per g soil with number of cysts: A parallel lines regression analysis was used to explain the numbers of eggs per gram of soil in terms of numbers of cysts at the three sites. No differences were found between the relationships for Steadmans and Bemrose (slopes p= 0.830, intercepts 0.573) (Fig.3.8.2). However, significant differences were found for thetransect sampled in Hanger compared to Steadmans and Bemrose. The rate of increase in eggs was 10-fold more for Hanger than for the other two sites.

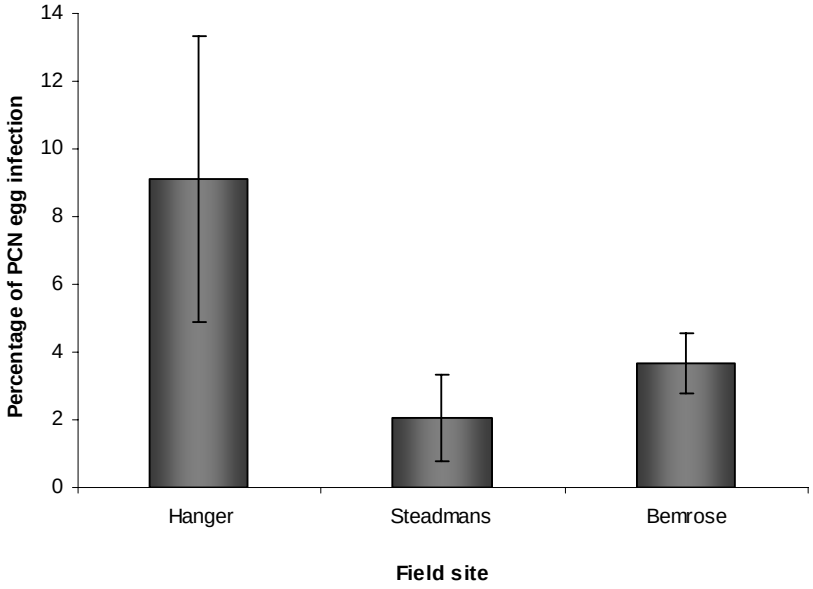

FIGURE 3.8.1 NO SIGNIFICANT DIFFERENCES WERE FOUND BETWEEN THE 3 FIELD SITES ON THE PERCENTAGE OF PARASITISED EGGS. HOWEVER, THERE WAS A SUGGESTION THAT HANGER HAD GREATER NUMBERS OF EGGS PARASITISED.

TABLE 3.13 AUTOCORRELATION OF DISTANCE IN THE TRANSECT OF EGG PARASITISM FOUND IN THE FIELD SITE OF BEMROSE A SIGNIFICANT AUTOCORRELATION (P<0.05) WAS FOUND AT 5 METERS. WITH 20 OBSERVATIONS, VALUES OF CORRELATION OUTSIDE +/- 2/SQRT(20) = 0.447 ARE SIGNIFICANT AT 5 % LEVEL FOR BEMROSE.

Distance (meters)	Autocorrelation (egg parasitism)
0	1.000
5	**-0.665**
10	0.108
15	0.201
20	-0.006
25	-0.158
30	0.115
35	0.011
40	-0.148
45	0.154
50	-0.070
55	-0.052
60	0.021
65	0.000
70	0.021
75	-0.014
80	-0.043
85	0
90	0
95	0

TABLE 3.14 AUTOCORRELATION OF CYST NUMBERS FOUND IN THE FIELD SITES OF HANGER AND BEMROSE. A SIGNIFICANT AUTOCORRELATION (P<0.05) WAS FOUND AT 5 METRES. WITH 15 OBSERVATIONS, VALUES OF CORRELATION OUTSIDE +/- 2/SQRT (15) = 0.516 ARE SIGNIFICANT AT 5 % LEVEL FOR HANGER. WITH 20 OBSERVATIONS, VALUES OF CORRELATION OUTSIDE +/- 2/SQRT (20) = 0.447 ARE SIGNIFICANT AT 5 % LEVEL FOR BEMROSE (--- REPRESENTING MISSING VALUES).

Distance (meters)	Autocorrelation (Hanger)	Autocorrelation (Bemrose)
0	1	1
5	**-0.557**	**-0.451**
10	0.038	0.213
15	-0.015	-0.048
20	0.173	-0.243
25	-0.236	0.092
30	0.031	-0.005
35	0.164	-0.025
40	-0.064	0.121
45	-0.159	-0.287
50	0.231	0.136
55	-0.167	-0.062
60	0.073	0.064
65	-0.011	0.167
70	---	-0.103
75		-0.023
80		0.013
85		-0.182
90		0.124
95		---

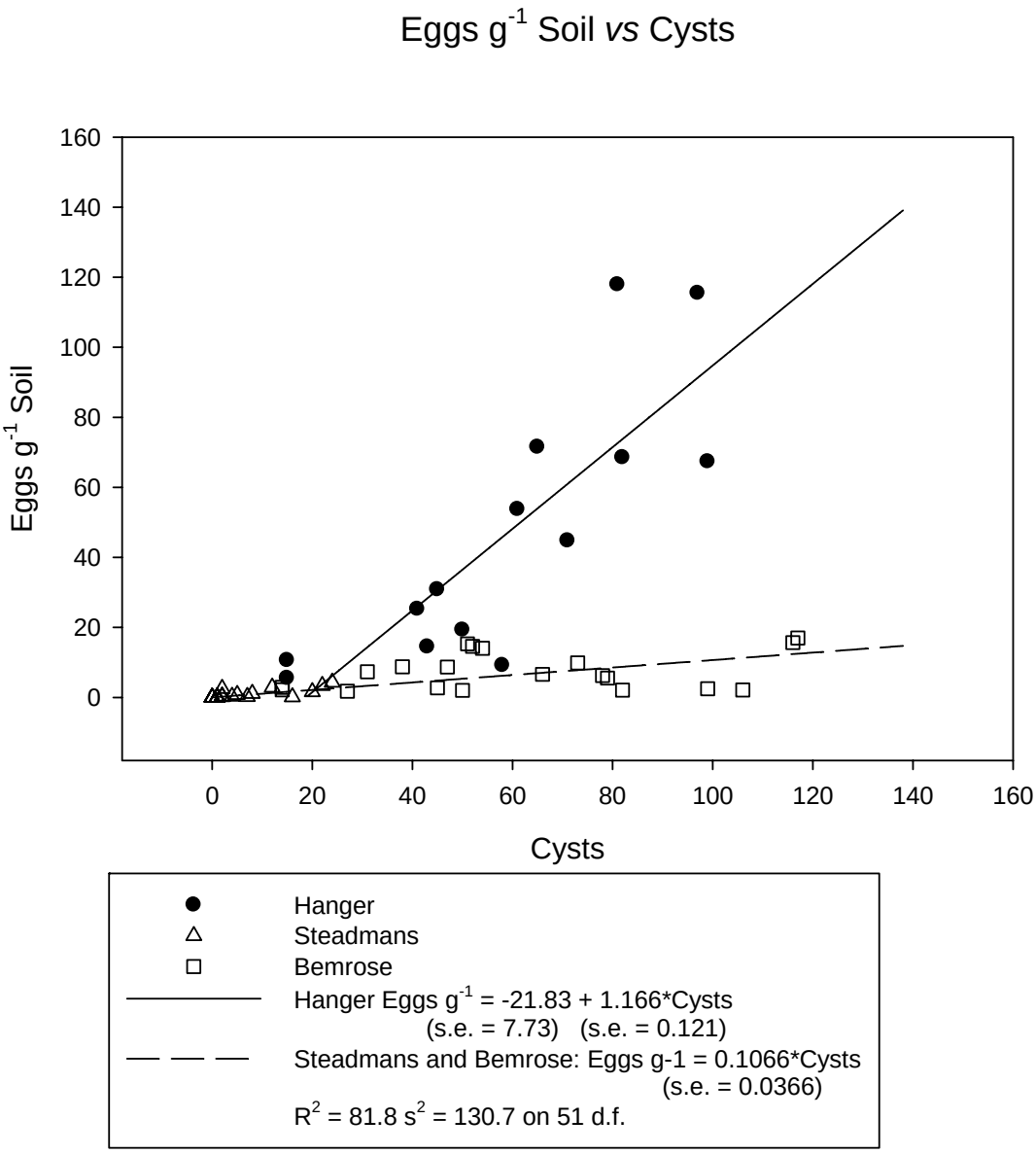

FIGURE 3.8.2 RELATIONSHIPS OF NUMBERS OF EGGS PER GRAM OF SOIL WITH NUMBERS OF CYSTS IN THREE FIELD SOILS (HANGER, STEADMANS AND BEMROSE).

3.8 Concluding comments for objective 8

There was an interesting trend for an inverse relationship to exist between the age of a PCN patch and the presence of nematophagous fungi involved in decline. The central area of the patch, the oldest and most established PCN area, had an increased presence of these fungi than the edge of the patch where the youngest PCN population was present. Although this might be expected from knowledge of the relatively slow development of suppressive soils,

this trend was not statistically significant due to general variability and there is a need for more detailed sampling.

Results objective 9

Assess the effects of different granular nematicides and a fumigant on PCN decline rates in soils of different textures and types with reference to nematophagous fungi.

One of the problems in identifying field sites in 2006 and 2007 was that farmers that had moderate to high PCN initial counts did not plant the fields to potatoes and therefore locating useful field sites was difficult. The PCN counts in fields that were planted with potatoes therefore tended to be low and were probably not the best sites to undertake the testing of biological control agents, whose effects are generally density dependent with greatest kills at high pest densities. It is difficult to draw any robust conclusions from the multiplication data of these early field trials due to the highly variable PCN multiplication (Fig 3.9.1).

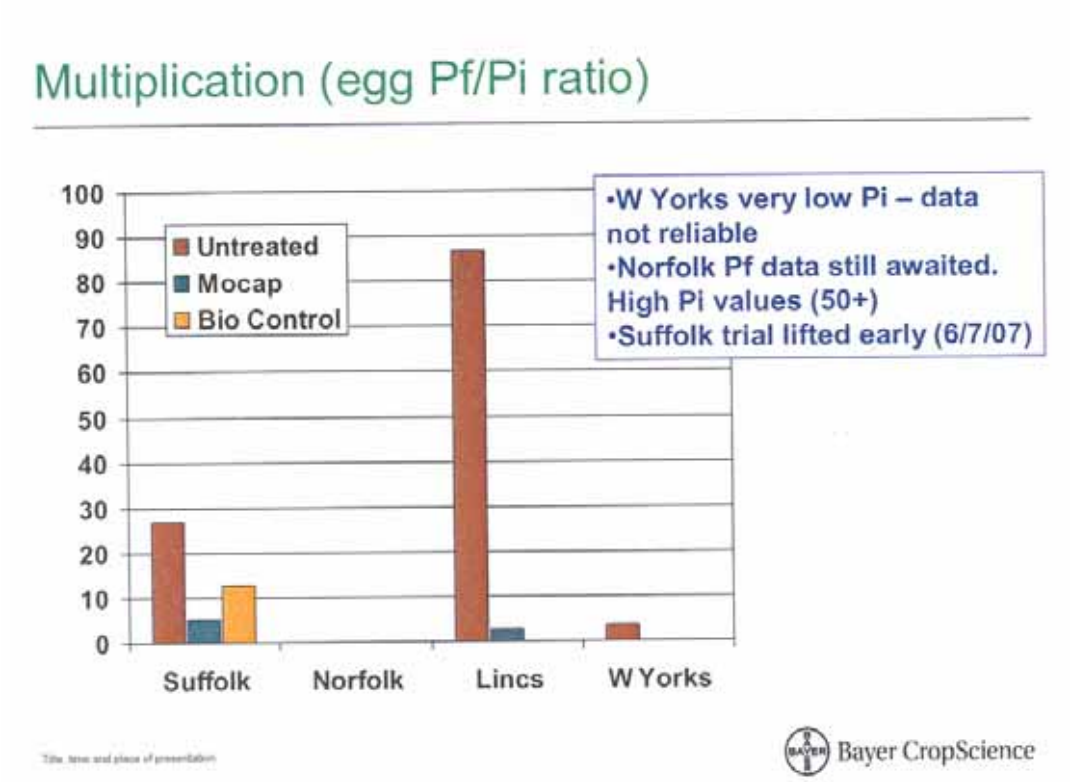

FIGURE 3.9.1 MULTIPLICATION RATES OF PCN FROM THREE SITES IN WHICH PLOTS WERE TREATED WITH EITHER A STANDARD APPLICATION OF MOCAP, *PAECILOMYCES LILACINUS* (BIOLOGICAL CONTROL AGENT) AND AN UNTREATED CONTROL.

Although nematode multiplication rates were difficult to assess it was possible to monitor fungal abundance, egg numbers and their levels of infection (Figs 3.9.2 to 3.9.4). Monitoring these trials it can be seen that the number of colony forming units present in the soil was approximately 600 cfu per gram of dry soil. However, the variation between replicates was very high (± 400 cfu per gram dry soil) and no differences could be observed between treatments (Fig 3.9.2). Interestingly, there were significant reductions in the number of viable

eggs and the number of eggs per cyst in the fungal treated plots when compared to the Mocap treated plots (Fig 3.9.3).

BAYER field trials testing the biocontrol product 'PL Link' (*Paecilomyces lilacinus*)

1. Bayer trials 2007

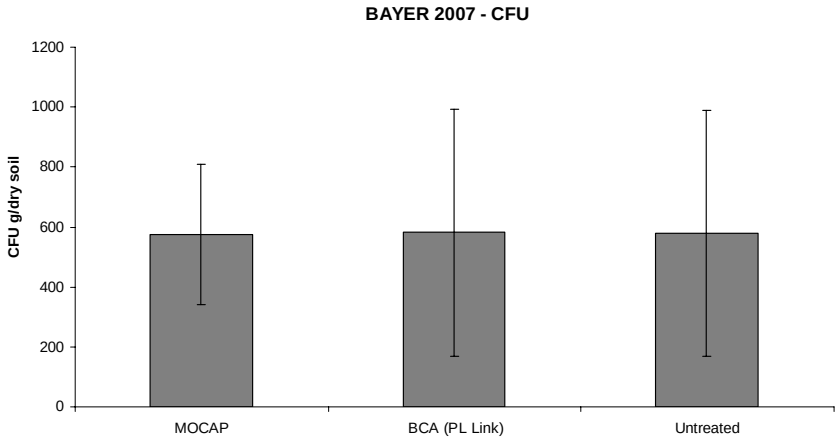

FIGURE 3.9.2 ABUNDANCE OF *PAECILOMYCES LILACINUS* IN THE DIFFERENT TREATMENTS MOCAP, *P. LILACINUS* AND AN UNTREATED CONTROL (NUMBER OF COLONY FORMING UNITS PER GRAM OF DRY SOIL) ON POTATOES (VARIETY MARFONA) IN A FIELD INFESTED WITH *G. ROSTOCHIENSIS* GROWN AT SUTTON, SUFFOLK. NO DIFFERENCES BETWEEN THE TREATMENTS WERE FOUND ($P>0.05$)

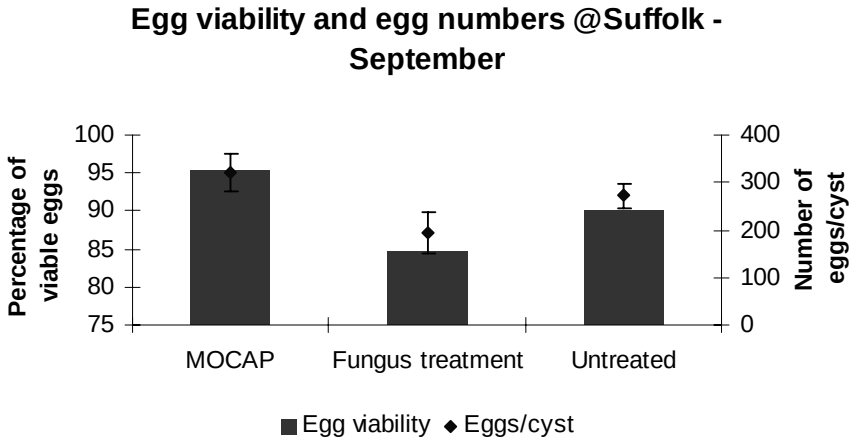

FIGURE 3.9.3 COMPARISON BETWEEN THE PERCENTAGE OF EGG VIABILITY AND NUMBERS OF EGGS PER CYST IN THE DIFFERENT TREATMENTS MOCAP, *P. LILACINUS* AND AN UNTREATED CONTROL ON POTATOES (VARIETY MARFONA) IN A FIELD INFESTED WITH *G. ROSTOCHIENSIS* GROWN AT SUTTON, SUFFOLK. ($P < 0.05$).

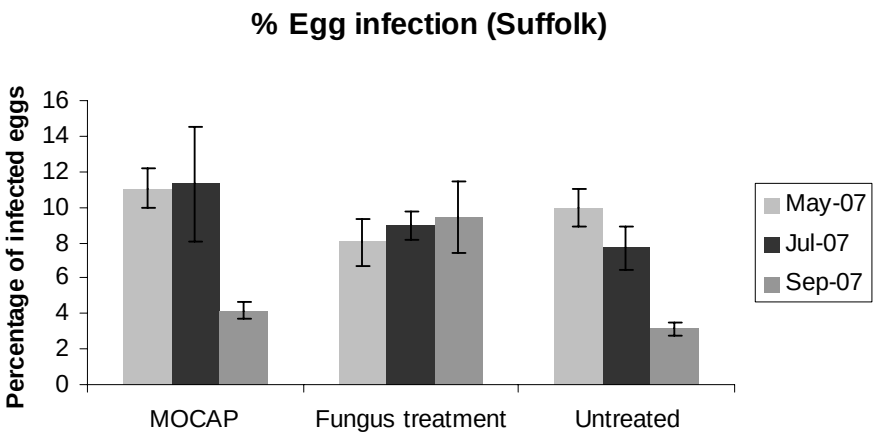

FIGURE. 3.9.4 PERCENTAGE OF INFECTED EGGS RECOVERED FROM CYSTS IN THE SOIL WITH THE DIFFERENT TREATMENTS MOCAP, *P. LILACINUS* AND AN UNTREATED CONTROL ON POTATOES (VARIETY MARFONA) IN A FIELD INFESTED WITH *G. ROSTOCHIENSIS* GROWN AT SUTTON, SUFFOLK.

The percentage egg infection in the 2007 field trials ranged from between 4 % and 14 %. Lowest levels of infection were seen in the Mocap treated and untreated plots in September 2007, and there were significant differences between these infection levels and the levels of infection produced in the fungal treated plots where infection remained relatively high at around 10 % ($p < 0.05$) (Fig 3.9.4).

2. Bayer trials 2008

A) Ivy House Farm, Wainfleet, Lincolnshire

The multiplication rates of the nematodes can be seen in Table 3.15 and ranged from 1.4 to 1.8 but no significant difference could be observed between the different treatments and the untreated control, although both the Mocap and the *P. lilacinus* treatments were slightly lower. All treatments had the effect of increasing tuber yield from between 109 % to 161 % ($p < 0.05$). The fungal treated plots gave a yield of 31.2 t/ha compared to 28.6 t/ha for the untreated controls. Yields of the nematicide treated plots were 46.2, 45.9 and 33.3 for Mocap, Nemathorin and Vydate respectively (Table 3.16). *Paecilomyces lilacinus* was only detected in the fungal treated plots at planting however by July it was detected in all plots, but was of significantly greater abundance in the fungal treated plot followed by the untreated plot($p < 0.05$). In July the fungal abundance in the Mocap treated plots was around 100 cfu per gram dry soil compared to 400 and 500 cfu per gram dry soil for the untreated and fungal treated plots respectively (Fig. 3.9.5). Interestingly by harvest the fungal abundance had increased further to 700 cfu per gram dry soil in the fungal treated plots but had fallen to 100 cfu per gram dry soil in the other two treatments.

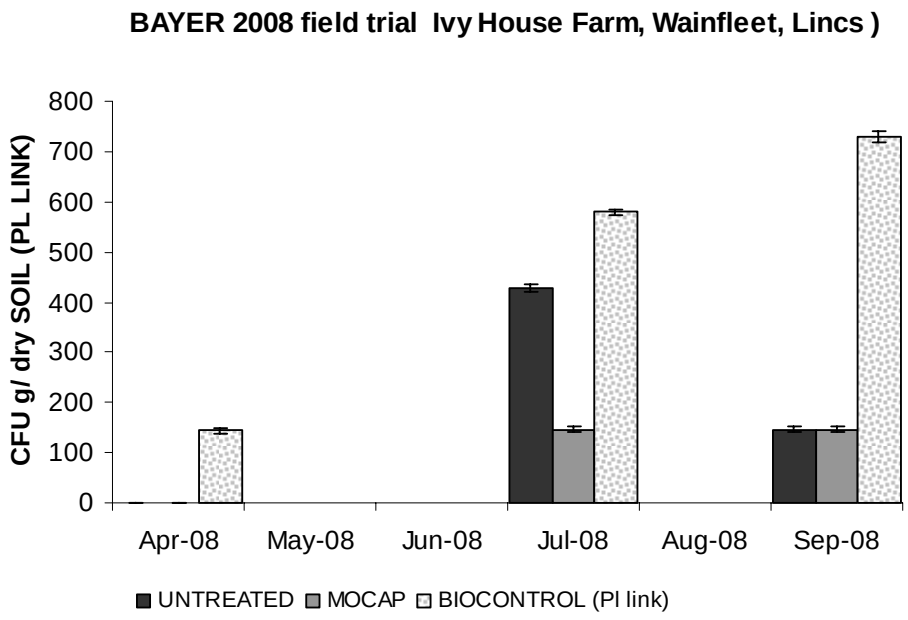

FIG 3.9.5 ABUNDANCE OF *PAECILOMYCES LILACINUS* IN SOIL, IN EACH TREATMENT (NUMBER OF COLONY FORMING UNITS PER GRAM OF DRY SOIL) IN POTATO (VARIETY MARIS PIPER) PLOTS INFESTED WITH *G. PALLIDA* IN DIFFERENT SAMPLING TIMES (APRIL, JULY AND SEPTEMBER 2008) GROWN AT IVY HOUSE FARM, WAINFLEET, LINCOLNSHIRE.

TABLE 3.15 BAYER RESULTS. PI AND PFS

Part Rated Ass. Class Rating Data Typ Rating Unit Scale Transformation ARM Action Code Untreated				CYST COUNT NUMBER Pi		CYST COUNT NUMBER Pf		CYST QTIENT NUMBER Pf/Pi @DIV[2,1]	
Entry No.	Entry/Trt. Description	Dosage	Dosage Unit / Appl. Code	1		2		3	
1	UNTREATED			80.0	a	148.5	a	1.8	a
2	MOCAP	10	KG AI/HA A	93.5	a	130.0	a	1.4	a
6	TEST COMPOUND	1 0.33	L/HA BC	101.5	a	169.3	a	1.7	a
LSD (P=.05)				33.46		114.53		1.07	
Standard Deviation				19.34		66.19		0.62	
CV				21.09		44.35		37.21	
Grand Mean				91.67		149.25		1.66	
Replicate F				1.548		0.333		0.877	
Replicate Prob(F)				0.2964		0.8027		0.5036	
Treatment F				1.263		0.352		0.615	
Treatment Prob(F)				0.3484		0.7169		0.5713	

Means followed by same letter do not significantly differ (P=.05,
Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

TABLE 3.16 YIELD DATA

ID08GBRP10RB01						
Richard Dawson, Ivy House Farm, Wainfleet, Lincs						
	YIELD (t/ha)					
	Rep 1	Rep 2	Rep 3	Rep 4		
					MEAN	% rel
UTC	23.63	29.00	29.55	32.35	**28.63**	100.0
66.7 kg/ha Mocap 15G	50.45	52.75	39.30	42.08	**46.15**	161.2
100kg/ha Mocap 10G	42.88	42.50	42.23	52.80	**45.10**	157.5
30kg/ha Nemathorin	37.95	43.25	57.88	44.88	**45.99**	160.6
55kg/ha Vydate	36.50	29.53	32.10	34.88	**33.25**	116.1
Bio-control agent	34.50	23.25	33.83	33.20	**31.20**	109.0

B) Park Farm, Loudham, Wickham Market, Suffolk

The multiplication rates of the nematodes can be seen in Table 3.17 and ranged from 0.6 to 0.8 but no significant difference could be observed between the different treatments and the untreated control, although both the Mocap and the *P. lilacinus* treatments were slightly lower. *Paecilomyces lilacinus* was only detected in the fungal treated plots at planting however by July it was detected in all plots, but was not significantly greater in the fungal treated plot compared to the untreated control (Fig 3.9.6). Fungal abundance was highest at harvest.

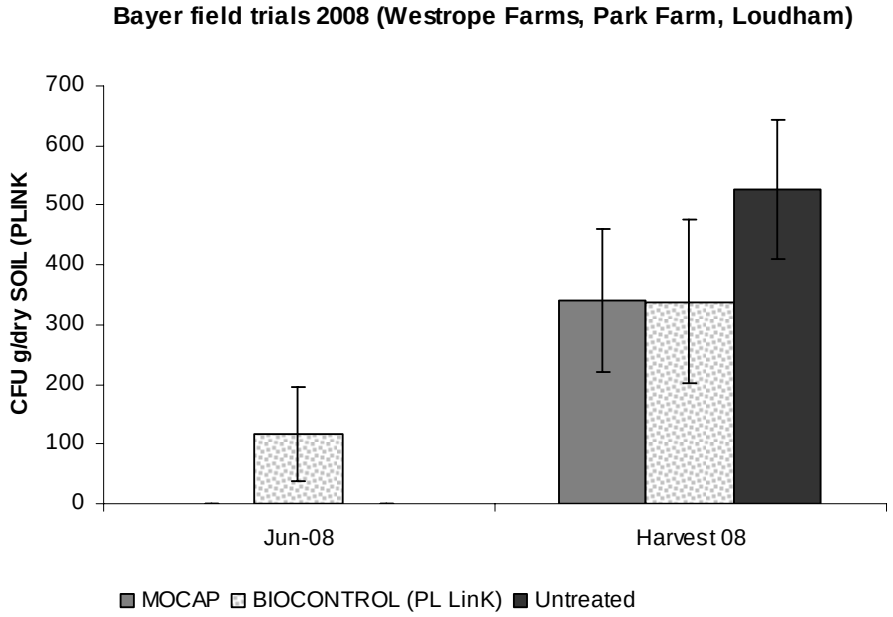

FIG. 3.9.6 ABUNDANCE OF *PAECILOMYCES LILACINUS* IN SOIL, IN EACH TREATMENT (NUMBER OF COLONY FORMING UNITS PER GRAM OF DRY SOIL) IN POTATO (VARIETY MARIS PIPER) PLOTS INFESTED WITH *G. PALLIDA* IN DIFFERENT SAMPLING TIMES (JUNE AND SEPTEMBER 2008) GROWN AT PARK FARM, LOUDHAM, WICKHAM MARKET, SUFFOLK.

TABLE 3.17 MULTIPLICATION OF PCN AT PARK FARM USING DIFFERENT TREATMENTS.

Part Rated Rating Data Typ Rating Unit Scale Transformation ARM Action Code Untreated					CYST COUNT NUMBER Pi		*CYST* *COUNT* *NUMBER* *Pf*		CYST QTIENT *NUMBER* Pf/Pi @DIV[2,1]	
Entry No.	Entry/Trt. Description	Dosage	Dosage Unit	Appl. Code	1		*2*		3	
1	UNTREATED				41	a	*30*	*a*	0.8	a
2	MOCAP	10	KG AI /HA	A	51	a	*28*	*a*	0.6	a
6	TEST	0.33	L/HA	BC	41	a	*32*	*a*	0.8	a
LSD (P=.05)					54		*38.5*		0.21	
Standard Deviation					31.2		*22.3*		0.12	
CV					70.38		*75.05*		17.63	
Grand Mean					44.33		*29.67*		0.7	
Replicate F					0.882		*0.254*		1.285	
Replicate Prob(F)					0.5015		*0.8559*		0.362	
Treatment F					0.137		*0.036*		4.126	
Treatment Prob(F)					0.8744		*0.9644*		0.0746	

3.9 Concluding comments for objective 9

Granular nematicides were successful in controlling PCN populations at different sites and in several trials biological control fungi were also able to control PCN multiplication rates however, the biological control agent produced much less reliable effects and there were large differences in there ability to control PCN across different sites. It is likely that the initial PCN population density in soil is critical in the ability of biological control fungi to succeed.

4. FUTURE DIRECTONS

- Microplot experiments established with PCN infested soil to develop methods to manage the nematode throughout the crop rotation, which includes a trap crop and the application of a selected isolate of *P. chlamydosporia*:

    - Continue the studies on the spread of the *P. chlamydosporia* in soil using irrigation, to access distribution of the fungus after application. (Follow up from objective 5).
    - The effects of rotation on the decline of PCN (cereal–oilseed rape-potato, or sisymbriifolium-potato or other break crops).
    - Test the new isolates o*f P.chlamydosporia* obtained in the project and assess their potential as biological control agents.

- Development of a potential quantitative diagnostic kit for PCN using antibodies (follow up from objective 2)

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