Final Report

Sustainable Long Term Management of Wireworms on Potato

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1. SUMMARY

The increasing incidence of wireworm damage in all-arable rotations and risk of total crop failure has led some growers to consider wireworm as a more important pest than potato cyst nematode. Reasons for the apparent increase in the importance of wireworm populations (principally *Agriotes obscurus*, *A. sputator* and *A. lineatus*) over the past 10-15 years were thought to be a combination of agronomic, cultural and ecological factors associated with changes in cropping practice. Results from the 'Rotational Experiment' completed as part of the current project have confirmed the influence of cultivation on wireworm population size. Wireworm populations in winter wheat plots cultivated conventionally declined significantly throughout the experiment. However, in winter wheat plots cultivated with minimum tillage, wireworm populations did not decline significantly and were comparable in size to populations in grass plots.

Wireworm risk assessment has recently advanced throughout Europe with the development of pheromone trapping systems for *Agriotes* spp. The sensitivity of this pheromone monitoring tool was confirmed here. However, it was also apparent that traps may be placed for as little as 3 hours in each field in order to provide a reliable early warning of the presence of click beetles and to inform the need for more time-consuming soil sampling for wireworms.

Two significant scientific obstacles have hindered the development of sustainable control options. Firstly, our inability to identify wireworms accurately to species limits our ability to interpret population processes at a fine scale, and secondly, at a landscape level there is a lack of understanding of adult dispersal potential (highlighted by issues surrounding the interpretation of pheromone trap catches) and hence the importance of population refuges in non-farmed land has been unclear. This project has gone some way to removing these obstacles, by successfully delivering a T-RFLP technique to identify the main UK pest species and by demonstrating that movement of adult beetles across farm landscapes is greater than previously understood, with field margins being potentially important refuges.

The ability of the potato industry to manage wireworm populations has arguably not significantly progressed for the last 50 years due to the fact that no new suitable chemistry has become available. Biofumigants and biocontrols investigated in the project achieved at best inconsistent wireworm control, but although the incorporation into the soil of mustard grown in rotation with potato does not appear to provide effective wireworm control on its own it may prove to be a useful soil conditioner while also suppressing weeds.

The project has confirmed the importance of mechanical cultivation and rotational factors.

1.1. Producer information summary

1.1.1. Background and commercial objective

The incidence of wireworm (click beetle) damage on potato in the UK has been on an upward trend in recent years. This trend has been seen both in traditional high-risk situations following long-term grass and where crops are grown in fields in all-arable (no grass) rotations (a situation known as 'arable wireworm'). This is particularly serious for major UK potato growers, packers and processors as wireworms can cause a severe loss of tuber quality, even at low populations. This can make the difference between a crop worth >£100/tonne, or one ploughed in at a significant loss. Because of this risk of total crop failure, some potato growers now regard wireworms as a more serious issue than potato cyst nematode. Wireworms are polyphagous, and in the UK carrot, sugar beet and leek growers have also been affected, although damage levels in cereals (which are less susceptible than many root crops) are not currently causing concern. The reasons for the apparent increase in the importance of wireworm populations (principally *Agriotes obscurus*, *A. sputator* and *A. lineatus*) are likely to include a combination of agronomic, cultural and ecological factors associated with changes in cropping practice in farming that have occurred over the last 10 to 15 years (Parker & Howard, 2001).

Although wireworm risk assessment has recently advanced throughout Europe with the development of pheromone trapping systems (Furlan *et al*., 2002), the ability of the potato industry in particular to manage wireworm populations has arguably not significantly progressed for the last 50 years. This is partly due to the fact that no new, suitable chemistry which combines the required soil persistency with an acceptable environmental profile has been forthcoming, but the biological and technical knowledge base required to implement an effective IPM strategy for wireworms, encompassing both rotational (wireworms take four years to complete their development in the soil) and short-term (in-crop) management, is also poor. The rotational element in particular has been largely hitherto ignored, but is a critical issue for those growers with a persistent 'arable wireworm' problem – a situation that is becoming more common. On a biological level, two significant scientific obstacles hinder the development of sustainable control options. These are, firstly, our inability to identify wireworms accurately to species, which limits our ability to interpret population processes at a fine scale and, secondly, at a landscape level, there is a lack of understanding of adult dispersal potential (highlighted by issues surrounding the interpretation of pheromone trap catches) and hence the importance of population refuges in non-farmed land. Sustainable wireworm management can only be achieved if there is a concerted research effort aimed at improving the biological knowledge base and expanding the range of potential control options.

The aim of this project was to improve substantially the ability of the UK potato industry to manage wireworm populations both strategically (around the rotation) and tactically (in the growing crop) by developing a better understanding of population dynamics at a landscape scale and developing novel control techniques that can be used independently or integrated with insecticide use at different points in the rotation.

The specific objectives of the project were:

- To provide a robust method for identifying *Agriotes* wireworms.

- To assess the impact of cultural and rotational factors on the survival of wireworms in all-arable rotations ('Rotational Experiment').
- To investigate the magnitude and timescale of dispersal of click beetles on a landscape scale.
- To investigate behavioural responses of click beetles to pheromones.
- To assess the impact of novel control strategies on reducing wireworm populations rotationally and in the potato crop ('Biofumigant Experiments').

1.1.2. Commercial and environmental sustainability benefits

The project aimed to substantially improve the ability of the UK potato industry to manage wireworm populations both strategically (around the rotation) and tactically (in the growing crop). Improved management of this pest would reduce crop wastage/rejection and sub-optimum quality damage through better prevention of tuber quality losses. These improvements would impact positively on the efficiency and economic operation of both farmers and packers. Reduced reliance on soil-applied pesticides for wireworm control would help reduce costs and lead to enhanced compliance with UK and European (EUREP-GAP) crop assurance protocols.

Specific benefits to emerge from this project include:
Development of a robust 'terminal restriction fragment length polymorphism' (or T-RFLP)-based method to identify larvae of *Agriotes*.
Improved understanding of cultivation factors influencing the survival and population dynamics of wireworm which potentially explains the emergence of 'arable wireworm' in recent years.
Identification of field margins as a source of new populations and proof that click beetles disperse widely across farmland.
Confirmation of species-specific behavioural response differences to pheromone traps.
The information that short-period pheromone trapping (approx. 3 hours trap exposure) can provide a sensitive early warning of the presence of the pest, which can in turn determine the need for more time-consuming soil sampling for wireworms.

1.1.3. Summary of results and conclusions

Objective 1: A 'terminal restriction fragment length polymorphism' (or T-RFLP) based method was developed for the identification of species of wireworm including *Agriotes sputator*, *A. obscurus* and *A. lineatus*. Development of this identification method has overcome one of the significant scientific obstacles that has until now hindered the development of sustainable control options. Our ability now to identify wireworms accurately to species will allow interpretation of population processes at a fine scale.

Objective 2: Results from the 'Rotational Experiment' have provided evidence for the recent emergence of 'arable wireworm' in crops grown in fields in all-arable (no grass) rotations. Starting at similar populations densities in 2006, plots cultivated conventionally for winter wheat production in 2007 and 2008 were found to have significantly lower numbers of wireworms than plots left to grass or in wheat that was cultivated using minimum tillage. Very short-period pheromone trapping (approx. 3 h) of click beetles was effective for the detection of the pest and may be used to inform the need to complete more time consuming soil sampling for wireworm.

Objective 3: We have shown that there are interspecific differences in click beetle movement in fields and have identified possible differences between the behaviour of males and females. Our results also demonstrate that invasion of fields from refuges can be expected, with field margins potentially playing an important role. Taken with results from previous LINK projects, this suggests that click beetles disperse widely across agricultural landscapes and that new wireworm colonisation of suitable (uncultivated) habitat should be expected. These results also inform the developments in short-duration pheromone trapping proposed under Objective 2.

Objective 4: Inter-specific differences in walking speeds which are consistent with estimated attraction ranges of pheromone traps have been identified. Behavioural response of click beetles to pheromones was investigated and it was found that exposure impeded movement of *A. sputator* under the experimental conditions used.

Objective 5: Assessment of the impact of novel control strategies on reducing wireworm populations did not identify candidates that may improve tactical (in the growing crop) control of this pest. The most effective product tested only achieved 30% wireworm mortality in pot trials. Similarly, incorporating 'biofumigant' mustard into the rotation did not consistently improve strategically (around the rotation) control of wireworm. However, incorporating mustard may have other benefits including acting as a soil conditioner and weed suppressant.

2. THE RESEARCH PROGRAMME

2.1. Introduction

Wireworms, the larvae of click beetles (Coleoptera: Elateridae), are recognised worldwide as pests of potato. Up to 39 species from 12 genera have been recorded as attacking potato, although the number of important species in any one global region is constant and relatively low (Jansson & Seal, 1994). The species that most commonly attack potato in the UK are *Agriotes lineatus*, *A. sputator* and *A. obscurus*. Other species (e.g. *Athous haemorrhoidalis*) have also been recorded as attacking potato, but these are generally much less common in agricultural land and are usually found in mixed populations with *Agriotes* species. Potato crops are particularly susceptible to attack as wireworm damage to tubers reduces crop quality rather than yield. Even low populations can cause an economic level of damage. Typical crop losses in North America range from 5 to 25% (Jansson & Seal, 1994), and are comparable to damage levels seen in the United Kingdom when insecticides are used on potato for wireworm control (e.g. Parker *et al.* 1990).

In the United Kingdom, high wireworm populations have traditionally been associated with fields in long-term grassland (Miles, 1942; Anon, 1948) as this undisturbed habitat is generally favourable for wireworm survival. However, in recent years, wireworm damage has become an increasing problem for UK potato growers. Factors contributing to this increase probably include increasingly stringent quality demands from retailers, an increase in the use of old pasture as 'clean' potato land free of soil-borne skin finish diseases, and an apparent increase in wireworm damage in fields in all-arable rotations (Parker & Howard, 2001). This increase in so-called 'arable wireworm' problems has occurred in all the main potato growing areas in the UK.

The general increase in the perception of wireworms as a serious problem for UK potato growers has highlighted a number of shortcomings in both the knowledge base and in the techniques required to manage wireworms in a sustainable and effective way. These include a poor knowledge of factors relating to the maintenance of wireworm populations in fields in all-arable rotations, and the need to develop alternatives to the currently limited options for conventional control with insecticides. These could include the use of biofumigants, which have been shown to have potential against wireworms (Furlan *et al.,* 2004). In addition, although the introduction of pheromone traps for click beetles has improved the ability to detect low wireworm populations (Furlan *et al.,* 2002), the interpretation of pheromone catches requires care (e.g. Blackshaw & Vernon, 2006), as well as an understanding of the way in which different species in the *Agriotes* complex (principally *A. sputator*, *A. lineatus* and *A. obscurus*) react to traps. Separating out the ecological differences between these three species is hampered by the lack of a method of distinguishing between the larvae (wireworms) on physical features alone.

The aim of this project is to improve substantially the ability of the UK potato industry to manage wireworm populations both strategically (around the rotation) and tactically (in the growing crop) by developing a better understanding of population dynamics at a landscape scale and developing novel control techniques that can be used independently or integrated with insecticide use at different points in the rotation.

2.2. Objective 1: Methodology for identifying wireworms

2.2.1. Introduction

Adult beetles originating from the UK, Canada and Austria were successfully sequenced at the 16S gene, and restriction sites were identified in the sequences (specific restriction enzymes recognise and cut DNA at these specific sequences). The presence of restriction sites for particular enzymes varies consistently and reliably for the target species *Agriotes sputator*, *A. obscurus* and *A. lineatus*. These differences were used to develop a 'terminal restriction fragment length polymorphism' (or T-RFLP) technique that identifies wireworms.

For completeness, a summary of the development work and results is given below. For a full version of the described work see Ellis et al., (2009).

2.2.2. Methods

2.2.2.1. Sampling

Specimens used in this study were part of a more extensive collection of biological material recovered in 2004 from organic farmland in the South Hams region of Devon, UK. Adult males captured in the summer in Yatlor traps using individual sex pheromones, were identified to species and stored at -20 °C within 12 hours of sampling. Larvae came from soil cores (10 cm diameter x 10 cm deep) collected from February to April. Additional samples of both adults and larvae from Canada were kindly contributed by Dr. Robert Vernon of the Pacific Agri-Food Research Centre and samples of adult *A. obscurus* from Austria by Dr. Michael Traugott, University of

Innsbruck. Further UK samples of larvae from Hertfordshire were also donated by Michael Tait (Syngenta).

2.2.2.2. DNA extraction, PCR and sequencing

DNA was extracted from both adult beetles and larvae using a standard salt/chloroform protocol (Rico *et al.*, 1992). Polymerase chain reaction (PCR) primers were developed for the 16S region of mitochondrial DNA. Conserved regions of 16S were identified by alignment with sequence data from other Elateroids. Primers based in these regions were chosen after checking for self-annealing, formation of hairpins, GC content, etc. Six to nine adults of each species (23 in total) were initially sequenced at the 16S region from as wide a geographic range as possible (*A. lineatus* - 5 UK samples and 4 Canadian; *A. obscurus* - 5 UK, 1 Canadian and 2 Austrian samples; *A. sputator* - 6 UK samples).

2.2.2.3. Development of T-RFLP probe and larval identifications

Initially, adults of known identity were amplified at 16S by PCR, except the reverse primer was fluorescently labelled with FAM (*A. lineatus* - 1 UK sample and 2 Canadian; *A. obscurus* - 1 UK and 3 Austrian samples; *A. sputator* - 4 UK samples). DNA concentration was then quantified on a Nano-Drop 1000 spectrophotometer (LabTech). Sample DNA (~1µg) was then simultaneously digested with 4 units of Hpy188I, 1 unit of HpyAV, and 2.5 units of AseI in 25µl reaction volumes containing 0.25µl 100X BSA and 2.5µl 10X NEB 2 buffer made up with DNA-grade H_2O. All three enzymes do not perform optimally in the same buffer: the number of units of each was adjusted as above to optimise collective performance. Digests were performed at 37°C for 2 hours followed by inactivation at 65°C for 15 minutes. Following digestion, fluorescently labelled fragments were sized against an internal size standard (LIZ-500) on an Applied Biosystems 3130 genetic analyzer and visualised in Genemapper v. 4. Following development using known adults, larvae originating from the UK (12) and Canada (24) were then identified to species using T-RFLP. Larval samples were always run against positive controls (adults). Following T-RFLP analysis, genetic identifications of a sub-set of the larvae (n=15) were confirmed by direct sequencing.

2.2.2.4. Sequence analysis

Sequences were edited and aligned using BioEdit 7.0.5.3 utilizing Clustal W. Initial sequences were aligned against other Elateroid sequences: Cardiophorinae sp., *Stenagostus rhombeus*, *Athous haemorrhoidalis, Ampedus balteatus,* and *Agrypnus murinus* (GenBank accession numbers AJ862749, AJ862750, AJ 862748, AJ862747, AJ862746, respectively). Following T-RFLP identifications, summary data for all individuals sequenced (adults and larvae) were generated in DnaSP (GC content) and MEGA (distance calculations). Distances between species and geographic groups of *A. obscurus* were estimated. Standard errors were calculated by a bootstrapping procedure with 10,000 replications.

2.2.3. Results

2.2.3.1. Sequence data

One haplotype was observed in *A. lineatus* (GenBank accession number EU285481), two in *A. sputator* (GenBank accession numbers EU285480, EU285485) and three in *A. obscurus* (GenBank accession numbers EU285482, EU285483, EU285484). Sequences were AT rich, as often observed in insect mtDNA. Sequence divergence between species ranged from 0.029 ± 0.009 to 0.06 ± 0.013. Distances between UK, Canadian and Austrian *A. obscurus* samples indicate closer alliance of Canadian to Austrian samples than either to the UK, but this is highly speculative since sample sizes are very limited (the only two sequences of Austrian *A. obscurus* included in the analysis were both of the same haplotype, yet three haplotypes were found in total).

Generated sequences were found to vary consistently and reliably for the presence of restrictions sites of Hpy188I (recognition site TCNGA (cuts between N and G) present in *A. sputator* and *A. obscurus*), HpyAV (recognition site N_6GAAGG (cuts before N_6) present in *A. obscurus* and *A. lineatus*) and AseI (recognition site ATTAAT (cuts between TT) present in *A. lineatus* only), hence these were chosen for probe development. Intraspecific nucleotide substitutions were not found to occur at any restriction site. Finally, PCR amplification of adult *A. obscurus* can be problematic, but the addition of BSA enhances the reaction.

2.2.3.2. T-RFLP probe and larval identification

T-RFLP digests of adult sequences utilising Hpy188I, HpyAV and AseI all yielded the predicted pattern of peaks, though fragments consistently differed from the exact anticipated size (7bp difference maximum). This is perhaps most likely explained by the fluorescent dye affecting migration of the product. Although partial digestion may occur, leading to the presence of additional peaks, banding patterns remain diagnostic and entirely consistent and assignments can be made with confidence. For *A. sputator* a 379 base pair (bp) fragment is produced, with potential for a peak at 401bp in the case of partial digestion; for *A. obscurus* a 271bp peak is produced with potential for an additional peak at 379bp and for *A. lineatus* a 171bp peak is produced with potential for an additional peak at 271bp. Although there is the potential to falsely assign individuals in the case of complete failure of a particular enzymatic digestion this is easily avoided through use of positive controls.

Of the initial sample of 36 larvae (24 originating from Canada and 12 from the UK), 30 were found to be *A. obscurus*, 5 were found to be *A. sputator* and none was *A. lineatus.* One individual did not ever amplify. Identities of a sub-set of 15 of the 35 *Agriotes* larvae identified by T-RFLP were confirmed by direct sequencing. The sub-set included 4 *A. sputator* (UK samples) and 11 *A. obscurus* (both UK and Canadian samples). Sequencing confirmed that the T-RFLP identities of these individuals were all correct.

It also proved possible to differentiate larvae of the European species *A. sordidus* using this method, with a peak at 144bp.

2.2.4. Discussion

The results clearly demonstrate the simplicity and reliability of use of a T-RFLP based method of identifying larvae of *Agriotes.* Although adult PCR amplifications of *A. obscurus* can be problematic, this matters little for larval identification. Indeed, it is not necessary to use adult material for analysis with the T-RFLP probe as long as some larvae are of known identity for use as positive controls. Use of a T-RFLP approach is time efficient as it avoids post-PCR steps such as excision and clean-up of DNA bands from agarose gels, cycle sequencing and DNA precipitation; following DNA extraction it is possible to have the results of T-RFLP identifications in as little as one day, depending on the number of individuals to be genotyped.

Of the 36 wireworms identified so far utilizing the probe, it is interesting to note that none was *A. lineatus.* Although a possible explanation of this is inhibition of restriction enzyme activity in larvae of this species, this seems unlikely. Firstly adults of this species amplify well by PCR, and digestion with the probe produces fragments as expected. Secondly, none of the larvae sequenced showed a probe pattern of *A. lineatus* but all showed either the sequence of *A. sputator* or that of *A. obscurus*. Alternative explanations are that this is a result of the sampling, e.g. that larvae of this species respond differently to trapping and collection methods in the field, or simply that *A. lineatus* larvae are less prevalent in the specific parts of the agricultural areas that were sampled. Previous studies in British Columbia (Canada) that have attempted to relate adult pheromone trap catches of *A. lineatus* and *A. obscurus* with total wireworm bait trap counts have found evidence of strong correlations between *A. obscurus* and total wireworm catch (at one site), but no evidence for any correlation between *A. lineatus* and total wireworm catch (Blackshaw & Vernon, in press). Twenty-four of the 38 larvae identified by T-RFLP in this study were donated from the same region though not the same site. Furthermore recent work has suggested a possible relationship between *A. obscurus* and wireworms in agricultural land in the UK, but not between wireworms and *A. sputator* or *A. lineatus* (Hicks & Blackshaw, 2008). These observations are suggestive of a stronger prevalence of *A. obscurus* larvae than *A. lineatus* in agricultural fields at least at some localities.

2.3. Objective 2: Rotational factors affecting wireworm populations

2.3.1. Introduction

The aim of this long-term experiment was to investigate the interaction between cultivation and insecticidal seed treatment on the maintenance of wireworm populations in an arable rotation. Wheat was grown with/without clothianidin (Deter) seed treatment, using either a plough-based cultivation regime or a non-plough regime for two successive years (harvest years 2007 and 2008); potatoes were gown in 2009, overlaid with different in-crop treatments for wireworm including a defatted mustard meal (BioFence), which was also assessed as part of Objective 5 of the project. Permanent grass followed by potatoes was used as a control.

2.3.2. Methods

2.3.2.1. Experimental site

The experimental site was in an all-arable rotation at Babraham Farms, Babraham, Cambridge (OS grid reference TL 511519). Soil sampling on 2 May 2006 in four separate blocks (*ca*. 1 ha each) showed the field population of wireworms (mixed *Agriotes* and *Athous* spp.) to be *ca*. 120,000/ha overall, with local variation from 0 to 270,000/ha. This is typical of a low-level population that would not cause significant damage to cereals or grass, but is capable of causing economic damage to potato. The entire field was ploughed (August 2006) prior to the start of the experiment.

2.3.2.2. Experimental design

A factorial plus control experimental design was used, where the main factor in the wheat plots was cultivation type, with seed treatment/no seed treatment sub-plots. Grass plots were sown as controls. Each of the three main treatments (Table 1) was replicated six times (18 plots in total). Main plot size was 20 m x 30 m and sub-plots 10 m x 30 m. All winter wheat plots (C2 grade, cv. Einstein) were drilled at 172 kg/ha. Deter-treated sub-plots used seed treated at 2 l/tonne (equivalent to 50g a.i./100kg seed). Grass plots (a medium term ley mix of perennial ryegrass, timothy and white clover) were drilled at 40 kg seed/ha on 8 September 2006 using a Maschio/Sulky drill combination. All 'normal cultivation' wheat plots received two passes of the Maschio and one pass from a Cambridge roller prior to drilling.

Treatment Code	Crop	Main plot: tillage	Sub-plot: clothianidin ST
A	Grass	Normal	n/a
B1	Wheat	Normal	No
B2	Wheat	Normal	Yes
C1	Wheat	Minimal	No
C2	Wheat	Minimal	Yes

TABLE 1. SUMMARY OF TREATMENTS APPLIED IN THE 'ROTATIONAL EXPERIMENT'

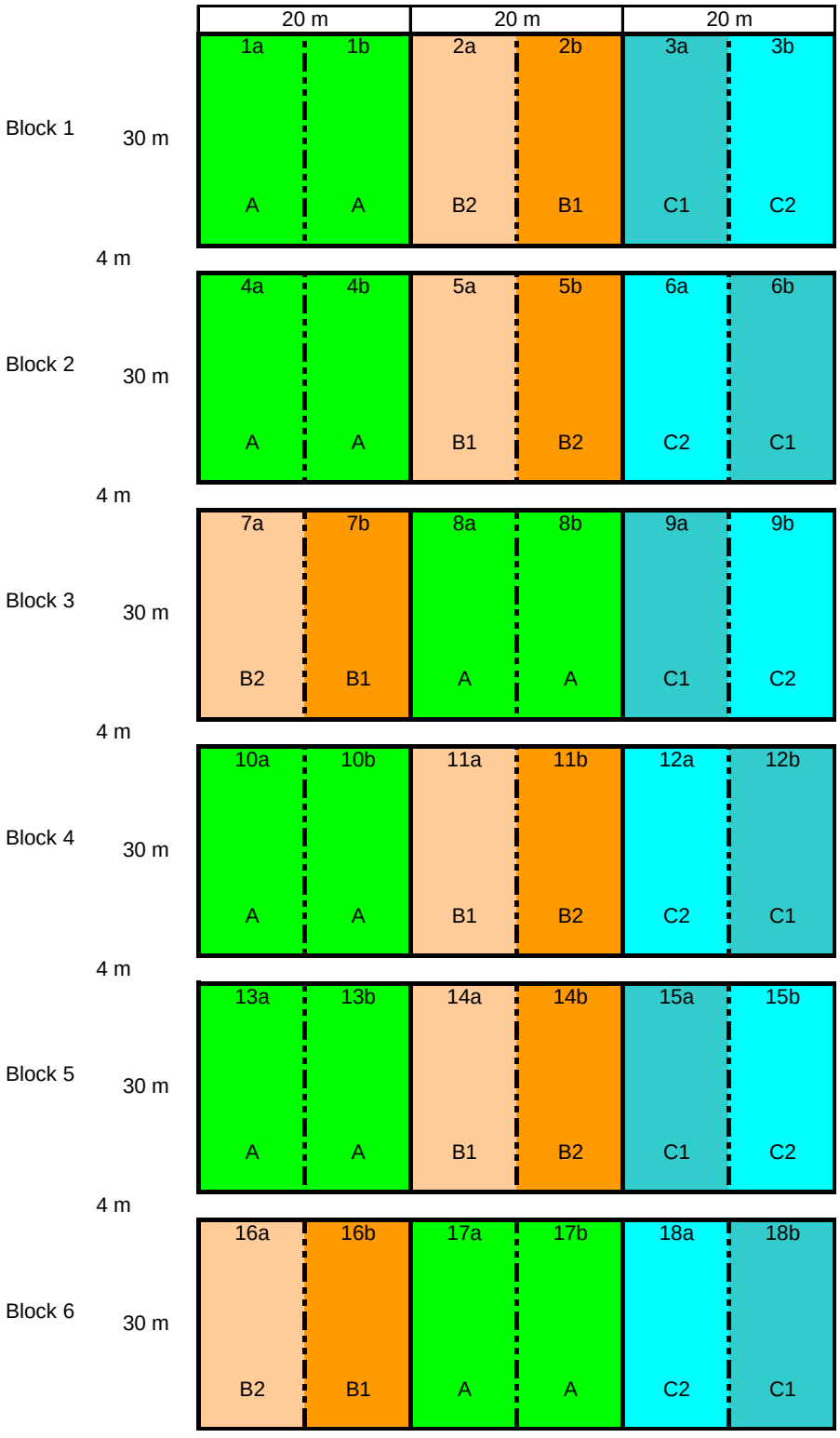


FIGURE 1. FACTORIAL PLUS CONTROL EXPERIMENTAL DESIGN USED IN 2006/07 AND 2007/08 FIELD SEASONS.

2.3.2.3. Assessments

Within the rotational experiment a number of specific assessments were completed:

1. Autumn cereal plant count
2. Spring & summer/autumn soil sampling for wireworm
3. Potato tuber assessment
4. Short period pheromone trapping
5. Very short period pheromone trapping

2.3.2.3.1. Autumn cereal plant count

Plant counts were done in the wheat sub-plots on 18 December 2007 (wheat at growth stage 12) by counting the plants in five x 0.1 m^2 quadrats per plot.

2.3.2.3.2. Spring & summer/autumn soil sampling for wireworm

Soil samples (10 x 10 cm diameter cores per main plot, 5 x 10 cm cores per wheat sub-plot) were taken on 17 April 2007, 22 August 2007, 11 April 2008 and 16 October 2008. Fifteen cores per main plot were taken in autumn 2006 and 5 cores per main plot on 31 March 2009 and 22 October 2009. Each soil core was placed into a separate bag and labelled before being taken to the laboratory in order to extract wireworms. Wireworms were initially identified to genus using conventional taxonomic keys before confirming the identification using the molecular tools developed in objective 1.

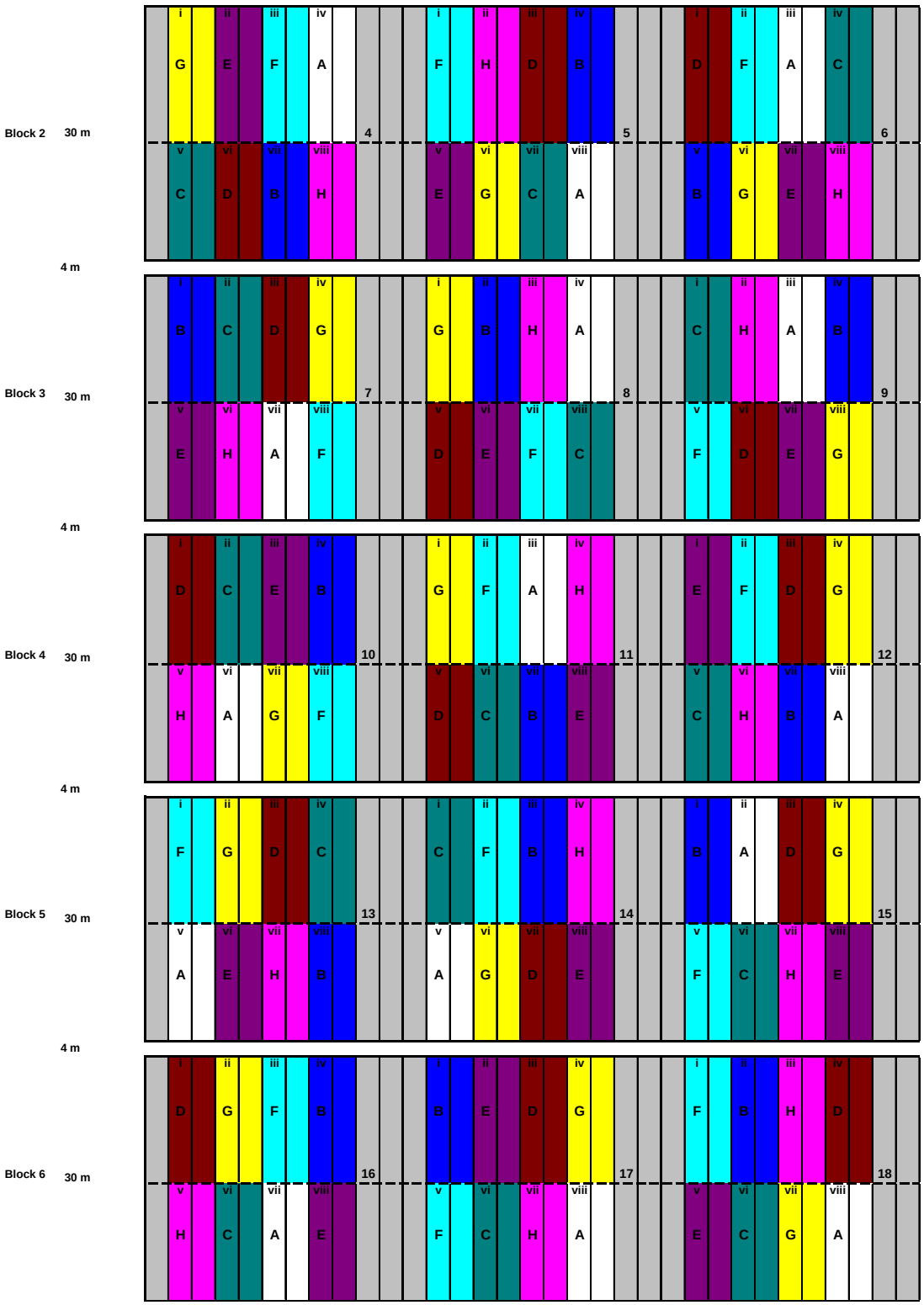
2.3.2.3.3. Potato tuber assessment

The entire experimental field site was cultivated and prepared for potato planting in April 2009. On 21 April after ridging the main experimental plots were marked out again, but due to the enlarged headland that had to be established for machinery manoeuvring it was not possible to re-establish Block 1 (see Figure 1.). Each main plot then consisted of 11 x 30 m beds. Main plots were subdivided into eight sub-plots so that each sub-plot consisted of 2 x 15 m beds. The beds at the edge of each main plot became guard rows. For each main plot the following eight treatments were applied randomly to the eight sub-plots:

Code	Treatment	Rate
A	Untreated	-
B	Untreated	-
C	BioFence	3 t/ha
D	BioFence	6 t/ha
E	Mocap	60 kg/ha
F	Nemathorin	15 kg/ha
G	Mocap + Biofence	60 kg/ha + 3 t/ha
H	Nemathorin + Biofence	15 kg/ha + 3 t/ha

TABLE 2. SUMMARY OF TREATMENTS APPLIED IN THE 'ROTATIONAL EXPERIMENT'

The completed experimental design was as follows:



(See key overleaf)

Treatment	Crop	Soil Treatment
A	Desiree	Untreated
B	Desiree	Untreated
C	Desiree	BioFence @ 3 t/ha (Italian field rate)
D	Desiree	BioFence @ 6 t/ha (double rate)
E	Desiree	Mocap @ 60 kg/ha (broadcast)
F	Desiree	Nemathorin @ 15 kg/ha (broadcast)
G	Desiree	Mocap @ 60 kg/ha (broadcast) + BioFence @ 3 t/ha
H	Desiree	Nemathorin @ 15 kg/ha (broadcast) + BioFence @ 3 t/ha
Guard Rows	Desiree	Nemathorin @ 15 kg/ha

FIGURE 2. EXPERIMENTAL DESIGN USED IN 2009 FIELD SEASONS.

Due to the small area of each sub-plot, treatments were applied manually. Guard rows were treated using conventional mechanical application of Nemathorin at 15 kg/ha. The treated experimental area was planted with potatoes (cv. Desiree) on 22 April 2009.

Assessments: visual assessment of crop establishment was completed on 3 June 2009 in order to record any phytotoxic properties of the soil-applied treatments. Finally, the potato crop was harvested on 16 October 2009. A sample of 100 tubers from each sub-plot was taken back to the laboratory for assessment of wireworm damage. Tuber damage data was analysed using a logistic model which transforms the data within GLIM using a logistic transformation. Data for the number of wireworm holes was analysed by first log transforming the data within GLIM.

2.3.2.3.4. Short-period pheromone trapping

Because of the short range of attraction of the traps and likely rate of movement of the beetles, traps were only run for short periods (3-4 days maximum for each trapping period) to reduce the risk of confounding data by drawing in beetles from neighbouring plots. To prevent pheromones for different species interfering, trap/pheromone combinations for only one species at a time (either *Agriotes sputator, A. lineatus* or *A. obscurus*) were used.

Pheromone trapping was also done concurrently for all three species in 2007, 2008 and 2009 on the far side of a neighbouring field (*ca*. 400 m away) to give a general indication of the timing and level of activity of click beetles in the field during the experimental trapping period.

Trap set-up and handling*:* Yatlor funnel traps and pheromones (supplied by Csalomon, Hungary) were used. Pheromones were inserted into the lower vane assembly of the trap immediately prior to use. Traps were placed on the ground, ensuring that the bottom edge of the funnel was in contact with the soil all the way round (beetles enter the trap by walking).

Experimental area*:* there were three trapping rounds (3-4 days duration for each) for each species in 2007 and 2008 (see Table 3. for actual trapping schedules).

		2007		2008	
Period	**Species**	**Date out**	**Date assessed**	**Date out**	**Date assessed**
f	*Lineatus*	10/05/2007	14/05/2007	09/05/2008	13/05/2008
1	*Sputator*	14/05/2007	17/05/2007	02/05/2008	06/05/2008
1	*Obscurus*	17/05/2007	21/05/2007	06/05/2008	09/05/2008
2	*Lineatus*	21/05/2007	25/05/2007	20/05/2008	23/05/2008
2	*Sputator*	25/05/2007	29/05/2007	13/05/2008	16/05/2008
2	*Obscurus*	29/05/2007	01/06/2007	16/05/2008	20/05/2008
3	*Lineatus*	01/06/2007	05/06/2007	27/05/2008	30/05/2008
3	*Sputator*	05/06/2007	08/062007	30/05/2008	02/06/2008
3	*Obscurus*	08/06/2007	12/06/2007	23/05/2008	27/05/2008

TABLE 3. TRAPPING SCHEDULES USED IN EXPERIMENTAL AREA IN 2007 AND 2008.

On each trapping round, one trap for the relevant species was placed in the centre of each of the 18 main plots. The wheat/grass was cut down immediately around the trap to ensure air-flow around the trap. A new trapping round for the next species was started on the same day that a trapping period was completed for the previous species.

Commercial field: six additional traps (2 each for each species) were run in 2007 and 2008 and 12 additional traps (4 each for each species) in the field neighbouring the experiment. These traps were approximately 400 m from the experiment; individual traps were set away from the headland and 40 m apart. Traps were checked every 3-10 days throughout May into early June.

Assessments: beetles were collected from each trap by opening the trap base and emptying the trap catch into a plastic bag. These were returned to the laboratory for identification, initially using conventional taxonomic keys. Subsequently each beetle was identified using the molecular tools developed in objective 1.

2.3.2.3.5. Very short-period pheromone trapping

Following the trapping schedule completed on 29 May in 2008, a trap fitted with the *A. sputator* pheromone was placed in the centre of each of the 18 main plots at 10.30 am on 30 May 2008. Each trap was then emptied after approximately three hours. Similarly, in 2009, a trap fitted with the *A. sputator* pheromone was placed in the centre of 15 of the main plots (blocks 2-6) at approximately 10.00 am on 7 May 2009. Each trap was then emptied after 3, 6, 24, 48 and 72 hours.

Assessments: as above.

2.3.3. Results

2.3.3.1. Autumn cereal plant count

The results of the plant counts made on 18 December 2007 are summarised in Figure 3. The overall mean plant population across all wheat plots was 297.7 plants/ m^2, within the normal range expected for winter wheat crops in late autumn. Severe rabbit grazing occurred in the minimum tillage area of the trial, reducing the plant populations in these plots.

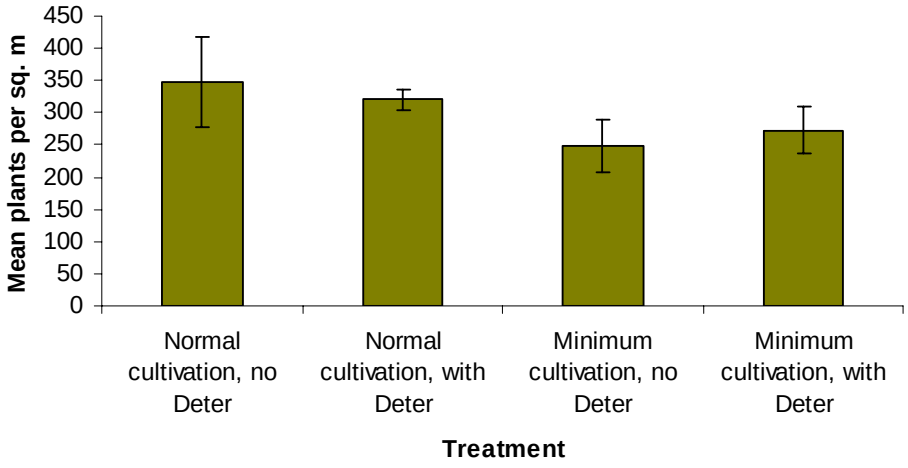


FIGURE 3. MEAN NUMBER OF PLANTS/M^2 FOR SUB-PLOT TREATMENTS IN THE WINTER WHEAT ON 18 DECEMBER 2007. ERROR BARS ARE 95% CONFIDENCE LIMITS

Low levels of wireworm damage were observed in some of the wheat plots (generally less than 5 plants/m^2 attacked) and no significant differences between treatments were recorded (Figure 4.).

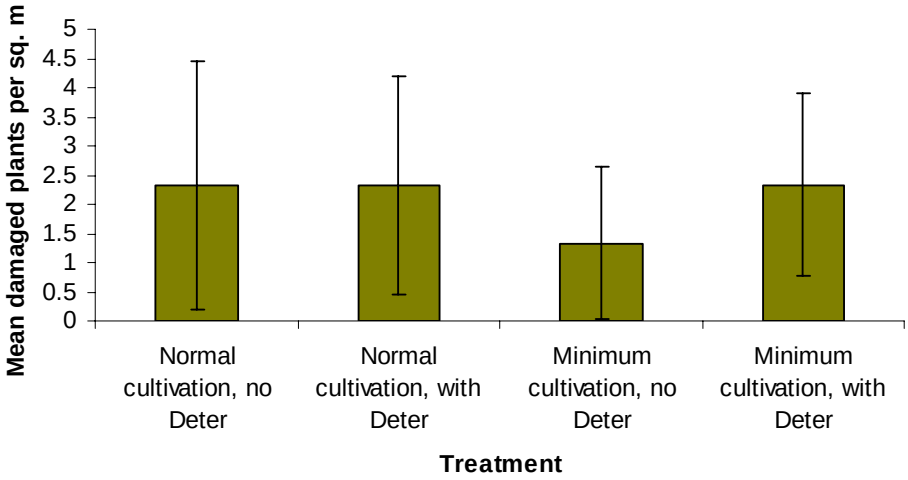


FIGURE 4. MEAN NUMBER OF PLANTS/M^2 ATTACKED BY WIREWORMS FOR SUB-PLOT TREATMENTS IN THE WINTER WHEAT ON 18 DECEMBER 2007. ERROR BARS ARE 95% CONFIDENCE LIMITS.

2.3.3.2. Spring & summer/autumn soil sampling for wireworms

Low numbers of wireworms were found throughout the experimental period, with a maximum of 17 collected at any one sampling point. The following tables summarise the results of the sampling throughout the experimental period for *Agriotes* spp. (Table 4.), *Athous* spp. (Table 5.) and total wireworms (Table 6.).

Treatment	Aut-06	Apr-07	Aug-07	Apr-08	Oct-08	Mar-09	Oct-09
A – grass	13,889	62,500	0	41,667	20,833	83,333	100,00
B – winter wheat (normal cultivation)	41,667	0	0	20,833	20,833	0	0
C- winter wheat (minimum cultivation)	13,889	83,333	0	0	104,167	83,333	0
$F_{2,10}$ (ANOVA)	0.53	0.84	-	1.67	2.50	1.43	1.00
P	0.606	0.458	-	0.237	0.132	0.285	0.410

TABLE 4. *AGRIOTES* SPP. HA-1 IN MAIN PLOT TREATMENTS AT BABRAHAM FARMS (POPULATION CONVERSION BASED ON 15 CORES PER MAIN PLOT IN AUTUMN 2006, 10 CORES PER MAIN PLOT IN 2007 & 2008 AND 5 CORES PER MAIN PLOT IN 2009).

Treatment	Aut-06	Apr-07	Aug-07	Apr-08	Oct-08	Mar-09	Oct-09
A – grass	83,333	20,833	0	125,00	104,167	83,333	0
B – winter wheat (normal cultivation)	55,556	20,833	0	41,667	0	0	0
C- winter wheat (minimum cultivation)	27,778	83,333	0	125,00	104,167	83,333	50,000
$F_{2,10}$ (ANOVA)	0.29	2.14	-	1.00	1.92	1.00	1.00
P	0.757	0.168	-	0.402	0.196	0.402	0.410

TABLE 5. *ATHOUS* SPP. HA-1 IN MAIN PLOT TREATMENTS AT BABRAHAM FARMS (POPULATION CONVERSION BASED ON 15 CORES PER MAIN PLOT IN AUTUMN 2006, 10 CORES PER MAIN PLOT IN 2007 & 2008 AND 5 CORES PER MAIN PLOT IN 2009).

Treatment	Aut-06	Apr-07	Aug-07	Apr-08	Oct-08	Mar-09	Oct-09
A – grass	97,222	83,333	0	166,667	125,000	166,667	100,000
B – winter wheat (normal cultivation)	97,222	20,833	0	62,500	20,833	0	0
C- winter wheat (minimum cultivation)	41,667	166,667	0	125,00	208,333	166,667	50,000
$F_{2,10}$ (ANOVA)	0.35	2.40	-	0.84	**4.30**	1.60	0.55
P	0.714	0.141	-	0.460	**0.045**	0.250	0.600

TABLE 6. TOTAL WIREWORMS HA-1 IN MAIN PLOT TREATMENTS AT BABRAHAM FARMS (POPULATION CONVERSION BASED ON 15 CORES PER MAIN PLOT IN AUTUMN 2006, 10 CORES PER MAIN PLOT IN 2007 & 2008 AND 5 CORES PER MAIN PLOT IN 2009).

A trend for increased numbers of *Agriotes* spp. and *Athous* spp. in grass or minimum cultivation winter wheat plots compared with wireworm numbers in normal cultivation winter wheat plots was apparent from April 2007. Despite this, a significant treatment effect was only found in October 2008 for total estimated wireworm populations, which coincided with the largest number of wireworms collected. A second approach was taken to analyse total wireworm populations across the entire sampling period. Here instead of extrapolating estimated wireworm populations from soil samples, the proportion of soil cores in which wireworm were found was taken and analysed as a split plot design, with date as the split plot factor. Taking this approach, a significant treatment effect was recorded ($F_{2,75} = 12.16$, $P = 0.002$). Using least significant difference to compare the different treatments the trend apparent from the above table is confirmed i.e. wireworm populations in grass and minimum cultivation winter wheat plots were significantly higher than in normal cultivation winter wheat plots.

In 2008 care was taken to keep separate soil samples from each sub-plot within each main plot in order to allow the effect of clothianidin (Deter) seed treatments on estimated wireworm populations to be recorded. These results are summarised in Table 7 and show that Deter seed treatment had no significant effect on wireworm population size. Similarly there was no significant interaction between cultivation and seed treatment.

Cultivation	Seed Treatment	*Agriotes spp.*		*Athous spp.*		Total	
		Apr-08	Oct-08	Apr-08	Oct-08	Apr-08	Oct-08
B – winter wheat (normal cultivation)	Deter	0	0	41,667	0	41,667	0
C – winter wheat (minimum cultivation)	Deter	0	83,333	125,000	83,333	125,000	166.667
B - winter wheat (normal cultivation)	None	41,667	41,667	41,667	0	83,333	41,667
C – winter wheat (minimum cultivation)	None	0	125,00	83,333	125,000	83,333	250,000
$F_{2,10}$ (ANOVA)		1.00	0	0.27	0.15	0.79	0.08
P		0.333	1.000	0.609	0.703	0.388	0.776

TABLE 7. WIREWORM HA-1 IN SUB PLOT TREATMENTS AT BABRAHAM FARMS (POPULATION CONVERSION BASED ON 5 CORES PER SUB PLOT IN 2008).

The size class of recovered wireworms is summarised in the following graph (Figure 5.).

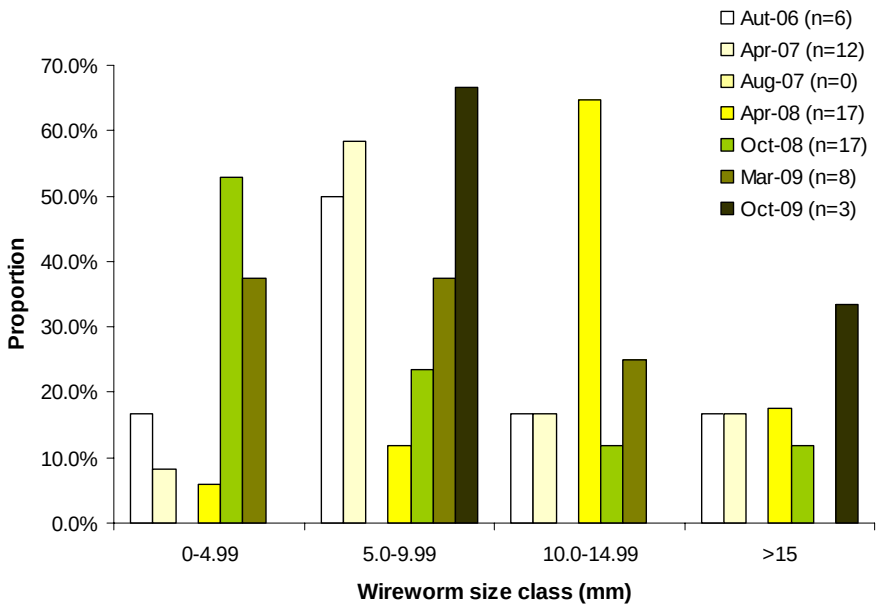


FIGURE 5. RELATIVE PROPORTIONS OF WIREWORMS (ALL SPECIES) IN DIFFERENT SIZE CLASSES FOUND AT DIFFERENT SAMPLING DATES.

2.3.3.3. Potato tuber assessment

2.3.3.3.1. Phytotoxic effects of soil applied treatments

Visual assessment of crop establishment on 3 June 2009 identified clear phytotoxic effects associated with the BioFence treatments (Figure 6.). Reduced crop establishment was most obvious in plots treated with BioFence at 6 t/ha but was also seen in plots treated with BioFence at 3 t/ha on its own or in combination with Mocap or Nemathorin.

FIGURE 6. PHYTOTOXIC EFFECTS OF BIOFENCE TREATMENTS REDUCING CROP ESTABLISHMENT AS SEEN ON 3 JUNE 2009.

2.3.3.3.2. Tuber damage assessments

Low levels of wireworm damage were recorded with an average of 1.88% of tubers damaged by wireworm feeding across all treatments. GLM analysis found no significant effect of previous cultivation on either percent of damaged tubers or mean number of wireworm holes (Table 8.).

Treatment	% Damaged Tubers	Mean No. Wireworm Holes/Tuber
Grass	1.68	0.03
Wheat: normal	2.20	0.04
Wheat: min till	1.75	0.04
Deviance Ratio $_{2,119}$	0.70	1.13
Approx. F prob.	0.498	0.326

TABLE 8. EFFECT OF PREVIOUS CULTIVATION ON PERCENT DAMAGED TUBERS AND MEAN NUMBER OF WIREWORM HOLES.

Similarly, soil treatments did not significantly affect either percent damaged tubers or mean number of wireworm holes (Table 9.). However, it is interesting to note that the untreated controls had on average 40% more damaged tubers and 42% more wireworm holes than treated plots.

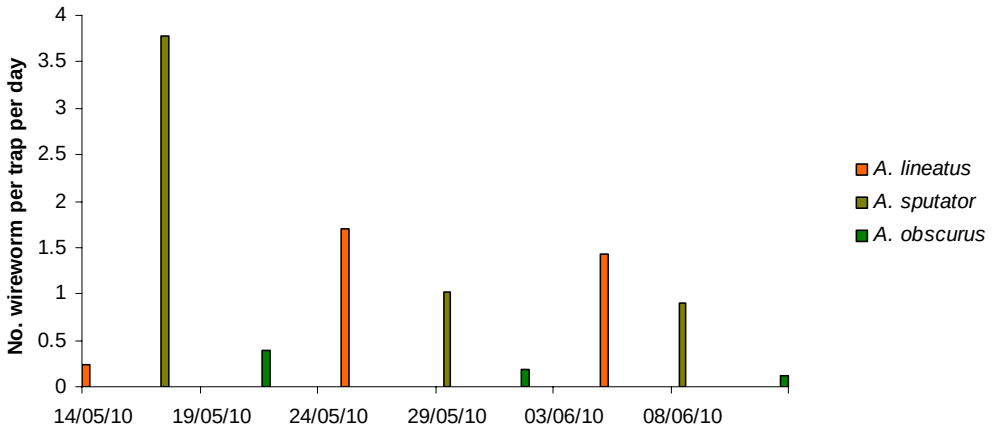
Code	Treatment	Rate	% Damaged Tubers	Mean No. Wireworm Holes/Tuber
A+B	Untreated	-	2.70	0.05
C	BioFence	3 t/ha	1.80	0.03
D	BioFence	6 t/ha	1.53	0.03
E	Mocap	60 kg/ha	1.27	0.02
F	Nemathorin	15 kg/ha	1.73	0.03
G	Mocap + Biofence	60 kg/ha + 3 t/ha	1.33	0.02
H	Nemathorin + Biofence	15 kg/ha + 3 t/ha	1.93	0.03
		Deviance Ratio $_{6,119}$	1.17	1.19
		Approx. *F* prob.	0.330	0.316

TABLE 9. EFFECT OF PREVIOUS CULTIVATION ON PERCENT DAMAGED TUBERS AND MEAN NUMBER OF WIREWORM HOLES.

2.3.3.4. Short-period pheromone trapping

Patterns of activity: the overall patterns of activity in the experimental area and in the neighbouring field were comparable (see Figure 7. and 8.). The relative proportions of the different species caught between the two areas are given in Table 10.

a) 2007



b) 2008

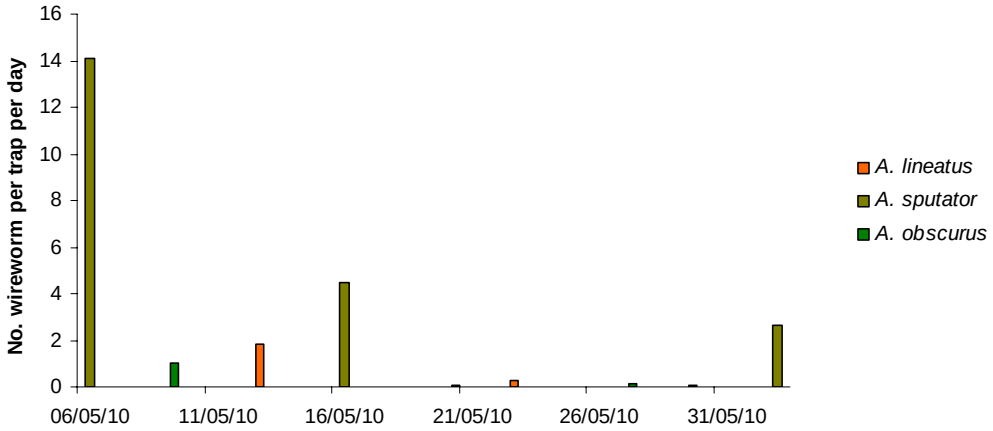
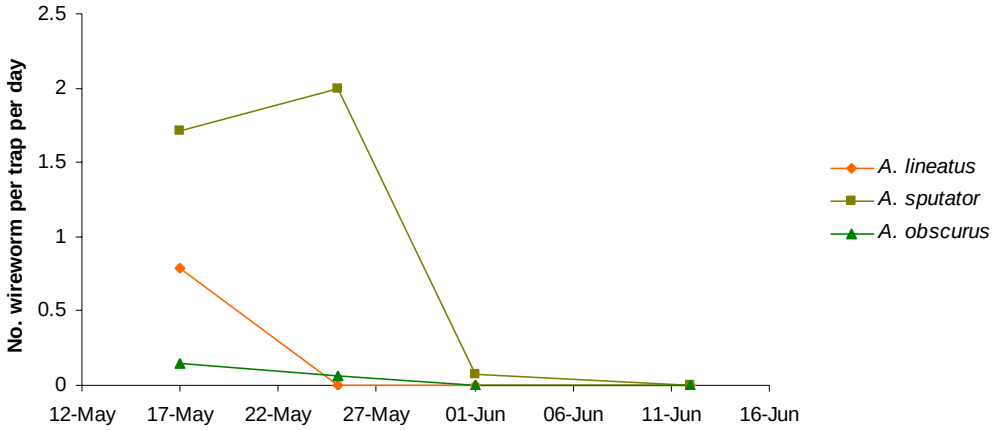
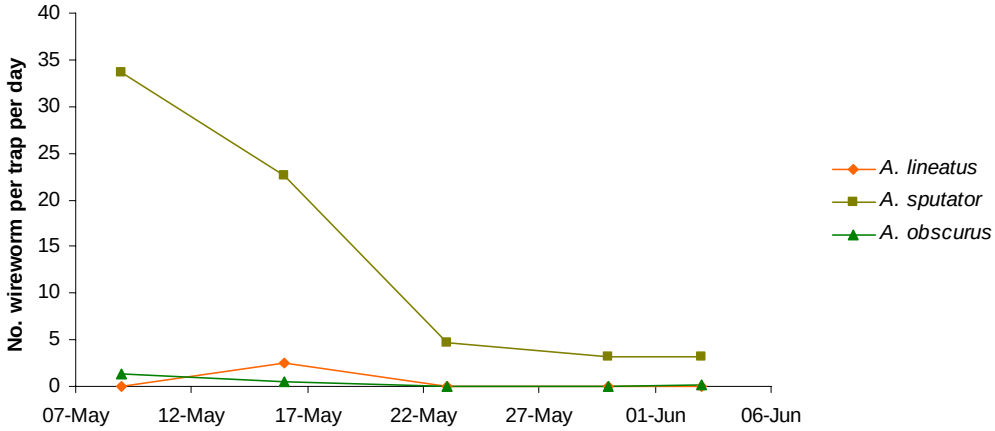


FIGURE 7. OVERALL TRAP CATCHES BY *AGRIOTES* SPECIES IN THE EXPERIMENTAL AREA IN 2007 AND 2008

a) 2007



b) 2008



c) 2009

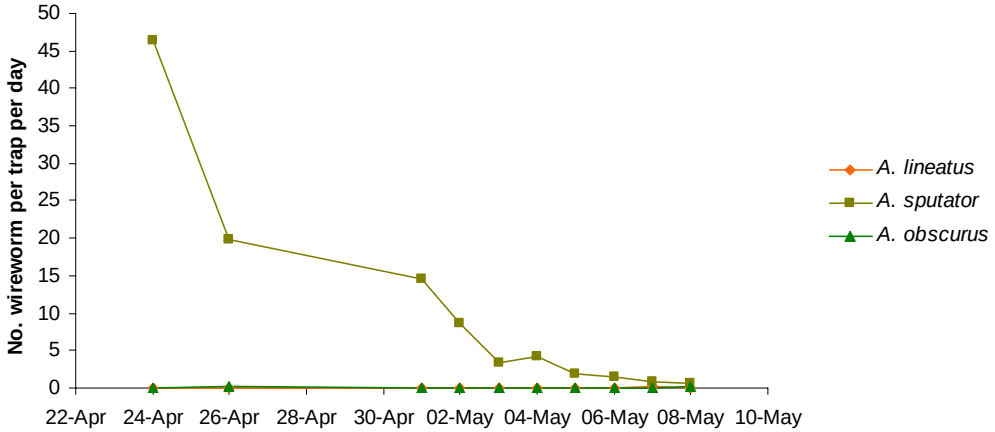


FIGURE.8. OVERALL TRAP CATCHES BY *AGRIOTES* SPECIES IN THE NEIGHBOURING FIELD IN 2007, 2008 AND 2009.

Year	Site	Total caught	Relative proportions (%)		
			A. lineatus	*A. sputator*	*A. obscurus*
2007	Experimental site	616	39	53	8
	Neighbouring field	71	16	80	4
2008	Experimental site	1615	9	87	4
	Neighbouring field	988	4	93	3
2009	Neighbouring field	1414	<1	99	1

TABLE 10. RELATIVE PROPORTIONS (%) OF DIFFERENT *AGRIOTES* SPECIES CAUGHT IN THE EXPERIMENTAL AREA AND NEIGHBOURING FIELD

2.3.3.4.1. Effect of main plot treatment on trap catches

Results are summarised in Table 11 for 2007 and Table 12 for 2008. In 2007 significantly higher numbers of beetles were trapped in grass plots compared to either of the two wheat treatments, while in 2008 significantly higher numbers were trapped in wheat minimum tillage plots. In addition, while numbers of *A. lineatus* were significantly affected by main plot treatments in 2007 no significant effect was found in 2008. By contrast, numbers of *A. sputator* and *A. obscurus* trapped were not affected by main plot treatments in 2007 but significant differences for both species were recorded in 2008.

Treatment	*A. lineatus* Mean	*A. sputator* Mean	*A. obscurus* Mean	Total Mean
Grass	21.00	22.50	4.17	44.67
Wheat: normal	8.67	12.50	0.83	22.00
Wheat: min till	10.67	19.50	2.83	33.00
$F_{2,17}$ (ANOVA)	33.10	2.66	3.88	18.17
P	**<0.001**	0.119	0.057	**<0.001**

TABLE 11. EFFECT OF MAIN PLOT TREATMENT ON THE MEAN NUMBER OF *AGRIOTES* SPECIES CAUGHT IN PHEROMONE TRAPS IN 2007

Treatment	*A. lineatus* Mean	*A. sputator* Mean	*A. obscurus* Mean	Total Mean
Grass	10.83	46.70	2.00	59.53
Wheat: normal	8.33	65.80	2.50	76.63
Wheat: min till	6.00	111.50	6.67	124.17
$F_{2,17}$ (ANOVA)	2.02	5.95	8.11	5.95
P	0.183	**0.020**	**0.008**	**0.020**

TABLE 12. EFFECT OF MAIN PLOT TREATMENT ON THE MEAN NUMBER OF *AGRIOTES* SPECIES CAUGHT IN PHEROMONE TRAPS IN 2008

2.3.3.4.2. Spatial analyses

In principle, if there is a real trend towards different numbers of beetles in plots with different treatments, it may be possible to detect these in terms of differences in the spatial pattern of beetle distribution in the experiment. As the pheromone trap locations can be assigned an x,y coordinate within the experiment, initial spatial analyses were done. These were:

Calculation of an index of aggregation (I_a) using SADIE (Spatial Analysis by DistancE Indices). Index values significantly greater than 1 indicate that counts exhibit a degree of aggregation. I_a was calculated for each trap period for each species (Table 13.).

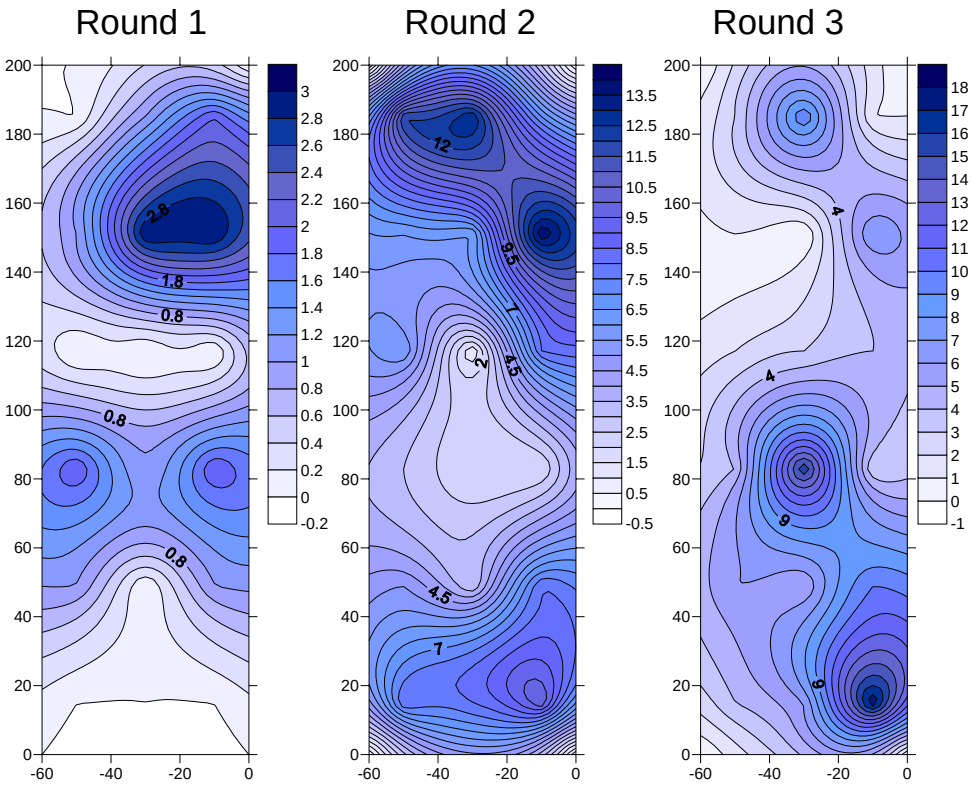
Production of interpolated maps using kriged data (Figures 9. & 10.)
Production of 'red-blue' plots (Perry *et al.*, 1999) which, in simple terms, calculate an index of clustering for each data point, which can then be interpolated to produce a contour map with patches (red) where the cluster index is >1.5 and gaps (blue) where the index is <1.5 (Figure 11.).

	A. sputator		***A. lineatus***		***A. obscurus***	
Trap round	**I_a**	**P_a**	**I_a**	**P_a**	**I_a**	**P_a**
1	1.310	0.103	1.395	0.103	1.965	0.013
2	0.795	0.782	1.453	0.077	1.545	0.026
3	1.056	0.333	1.454	0.090	0.731	0.846
Total	1.220	0.180	0.987	0.397	1.770	0.013

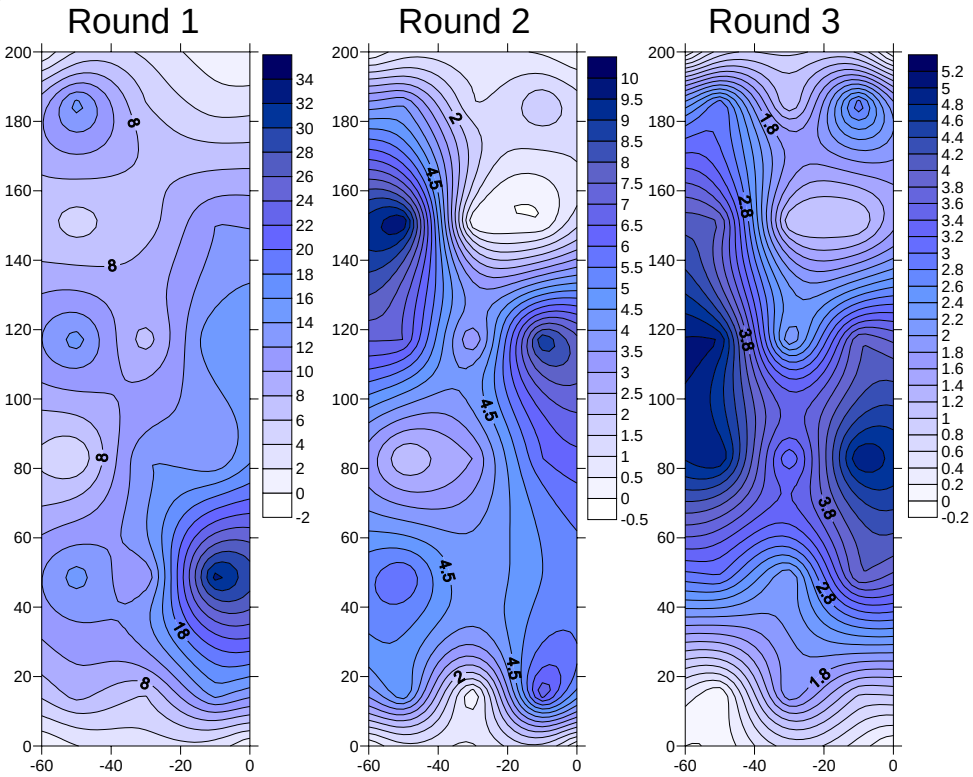
TABLE 13. VALUES OF I_a AND ASSOCIATED PROBABILITY (P_a) FOR EACH *AGRIOTES* SPECIES IN EACH TRAPPING ROUND.

These results indicate that the degree of aggregation in the pheromone trap catch data for each species is no more than would be expected in a random permutation of the counts in every instance for *A. sputator* and *A. lineatus*. However, significant values of I_a were found for *A. obscurus* in the first and second trapping rounds and for the total trap catches, indicating some aggregation in the distribution of the pheromone trap catches for this species.

a) *A. lineatus*



b) *A. sputator*



c) *A. obscurus*

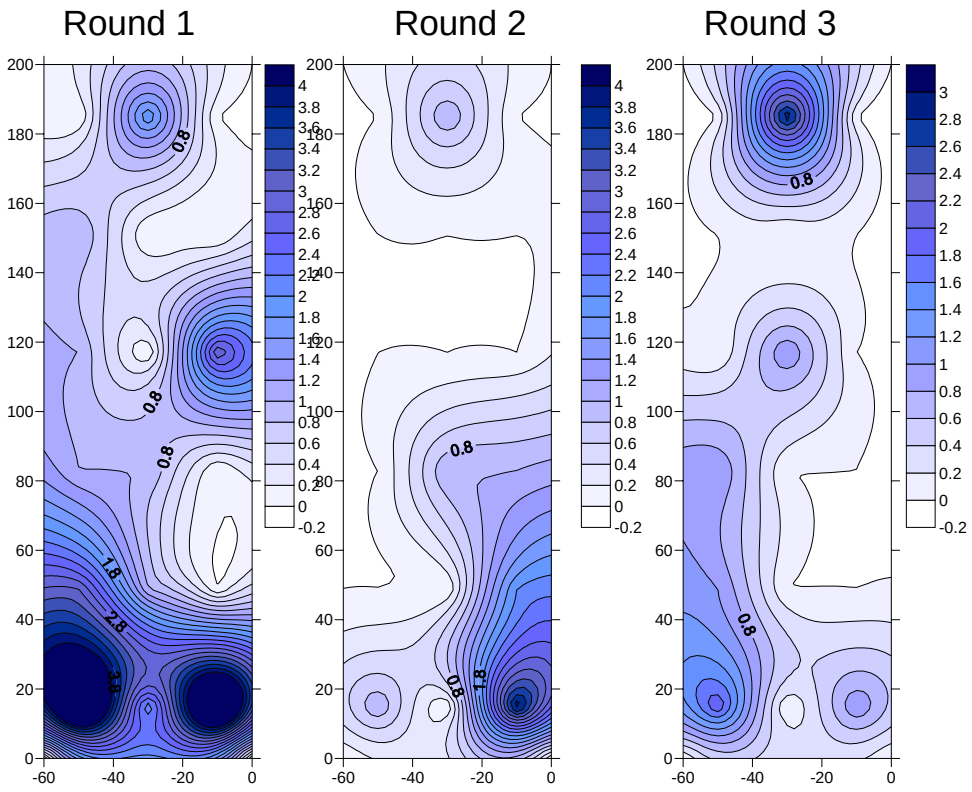


FIGURE 9. INTERPOLATED MAPS OF CLICK BEETLE DISTRIBUTION IN THE EXPERIMENTAL AREA BASED ON PHEROMONE TRAP CATCHES FOR EACH *AGRIOTES* SPECIES IN EACH TRAPPING ROUND.

When interpolated maps based on the aggregated data (from all three trapping rounds) for each species were produced (Figure 10.), there was no evidence of any association between main plot treatment and beetle distribution for *A. obscurus* and *A. sputator*. However, for *A. lineatus*, there was an apparent association between 'peaks' of beetle distribution on the map and the location of the grass plots.

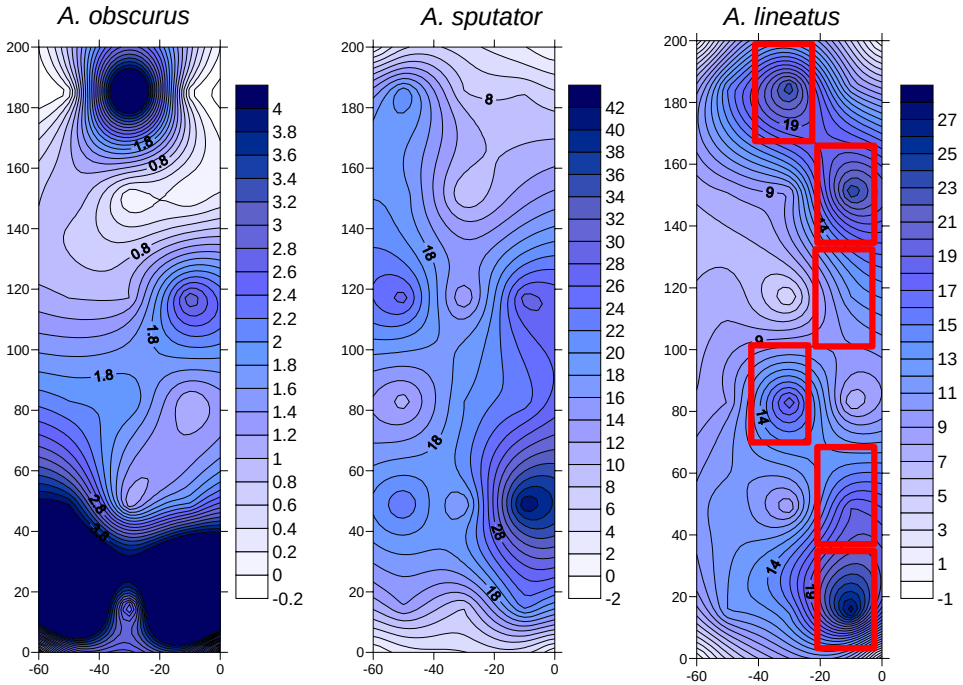


FIGURE 10. INTERPOLATED MAPS OF CLICK BEETLE DISTRIBUTION IN THE EXPERIMENTAL AREA BASED ON AGGREGATED PHEROMONE TRAP CATCHES IN MAY/JUNE 2007 FOR EACH SPECIES. RED BLOCKS IN *A. LINEATUS* MAP INDICATE LOCATION OF GRASS PLOTS.

The red-blue plots based on the aggregated trap catch results for all three species are given in Figure 11. These show some limited evidence of patchiness but patches and gaps are not closely related to the location of particular treatment plots.

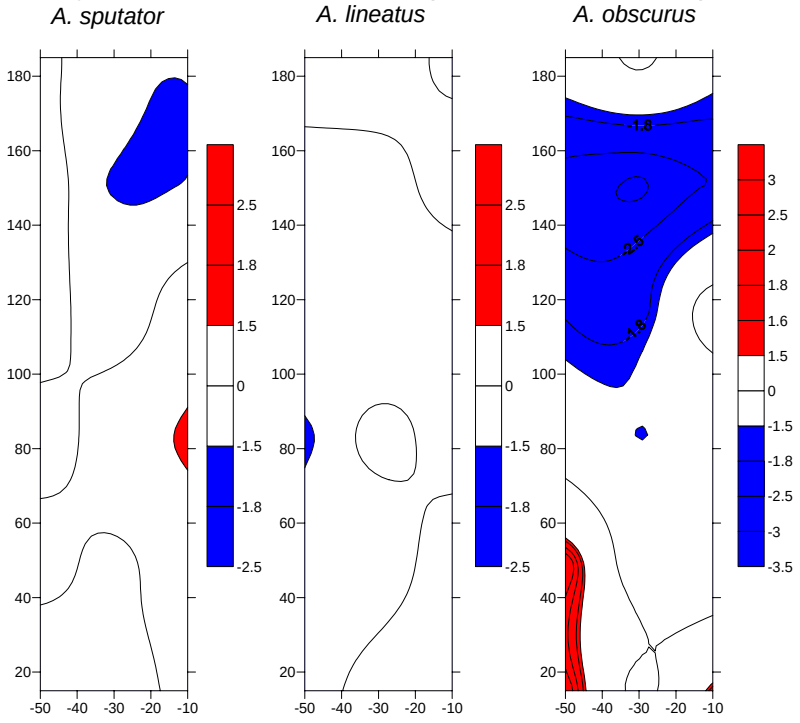


FIGURE 11. RED-BLUE PLOTS OF THE EXPERIMENTAL AREA BASED ON AGGREGATED PHEROMONE TRAP CATCHES IN FOR EACH SPECIES. RED = 'PATCH', BLUE = 'GAP'.

2.3.3.5. Very short-period pheromone trapping

The following table (Table 14.) summarises catches of *A. sputator* during a three hour period within each of the 18 experimental plots in 2008. No significant difference between the main plot treatments was found, although reasonable numbers of beetles were trapped despite the short duration of the experiment. By comparison, the three rounds of 3/4 day trapping completed immediately before this experiment did find a significant difference between numbers of *A. sputator* trapped in main plot treatments.

	***A. sputator* – 3 hour trapping**
Treatment	**Mean**
Grass	2.00
Wheat: normal	4.83
Wheat: min till	2.00
$F_{2,17}$ (ANOVA)	1.47
P	0.276

TABLE 14. EFFECT OF MAIN PLOT TREATMENT ON THE MEAN NUMBER OF *AGRIOTES SPUTATOR* CAUGHT DURING THREE HOUR PHEROMONE TRAPPING PERIOD ON 30 DECEMBER 2008.

The following figure (Figure 12.) summarises catches of *A. sputator* emptied after 3, 6, 24, 48 and 72 hours when placed within each of the 15 experimental plots in 2009. The fitted regression line accounts for 97.6% of the variance.

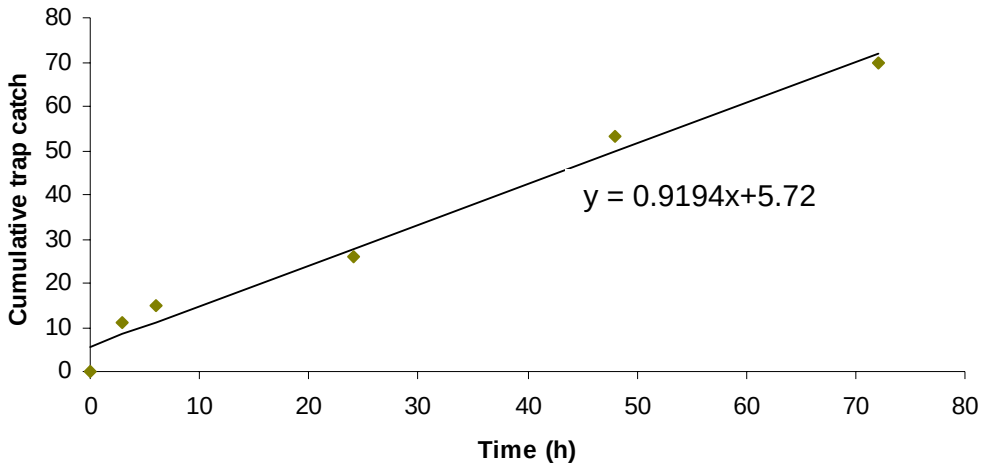


FIGURE 12. CUMULATIVE CATCHES OF *A. SPUTATOR* CAUGHT BETWEEN 7 AND 10 MAY 2009. THE TREND LINE ILLUSTRATES THE INITIALLY FASTER RATE OF CATCH AND SUBSEQUENT SLOWER RATE OF CATCH THROUGHOUT THE REMAINDER OF THE EXPERIMENTAL PERIOD.

2.3.4. Discussion

Overall wireworm populations were low throughout the experiment at the Babraham site and on average were only slightly above the limit of detection for soil core sampling (Parker, 1996). In addition, as wireworm population estimates based on soil core sampling use a multiplication factor of 62,500, estimated populations fluctuated widely between plots. These fluctuations made statistical analysis of the wireworm population estimates difficult and a significant treatment effect was found for only one (October 2008) of the seven samples taken. The sample taken in October 2008 recorded the highest number of wireworms (17 across all plots) with significantly more wireworm in minimum cultivation plots than normal cultivation plots. Subsequent analysis of the number of wireworms per soil core taken for all sample dates confirmed that throughout the course of the experiment wireworm numbers remained at the same level in grass and minimum cultivation plots but declined in conventionally-cultivated plots so that numbers were significantly lower. Therefore it would appear that cultivation is an important factor in determining wireworm population size and that minimum cultivation may be an important factor in explaining the emergence of so-called 'arable' wireworm.

Despite the significant effect of cultivation on estimated wireworm population size this did not result in significant differences in potato tuber damage. Tuber damage attributed to wireworm was low across all treatments (mean of 1.88%). This low level of damage probably explains the lack of a significant treatment effect recorded for both of the cultivation treatments and for the pesticide treatments applied immediately before planting. However, notwithstanding these inconclusive results, the use of BioFence appears to be both impractical, due to phytotoxicity to sprouting tubers, and uneconomic, currently costing £4,400 to treat at 3 t/ha, for the control of wireworms.

In contrast to soil sampling, which lacked the sensitivity required to accurately determine the wireworm populations present at the Babraham site throughout the course of the experiment, pheromone trapping caught large numbers of click beetles in each year of the project. Interestingly, the proportion of each species of *Agriotes* trapped switched from approximately equal numbers of *A. lineatus* and *A. sputator* in 2007 to almost entirely *A. sputator* in 2009. It not clear why this switch occurred, although the interpolated maps point to the relatively high mobility of *A. sputator* and *A. lineatus,* with little similarity in numbers caught in the different trapping rounds. By contrast, there was some evidence of patches persisting in certain locations for *A. obscurus.* Behavioural responses of click beetles to pheromones are further described in Objective 4 of the project.

Because of the short range of attraction of the traps and likely rate of movement of the beetles, pheromone traps were only run for short periods (3-4 days maximum for each trapping period) to reduce the risk of confounding data by drawing in beetles from neighbouring plots. However, results from 2008 for all three species and from 2009 for *A. sputator* showed that the trapping period may be further reduced to just three hours. Therefore, traps may be simply set in the morning and collected in the afternoon for monitoring purposes. This approach would have the additional benefit that the same pheromone lures may be used in several separate fields as each lure remains effective for a number of weeks.

2.4. Objective 3: Magnitude and timescale of click beetle dispersal

2.4.1. Introduction

Until recently it has been assumed that click beetle dispersal is relatively constrained and this has been the basis for advice on the use of pheromone traps as a monitoring tool. Studies of click beetle distributions at landscape (Blackshaw & Vernon 2006) and field (Blackshaw & Vernon 2008) scales have suggested that they are more mobile than previously thought. In particular, a regional-scale survey in an earlier LINK project (Hicks 2009) showed that whilst adult males of all three UK species of interest (*A. lineatus*, *A. obscurus* and *A. sputator*) were recovered from pheromone traps in nearly all of the 95 fields sampled, wireworms were only found in 19 of these. Most significantly, no *A. lineatus* larvae were recovered even though this was the most numerous species of adult trapped.

These data indicate that dispersal is a hitherto underestimated component of click beetle/wireworm ecology and that it may be key to understanding how new populations become established in a field and the timescale over which this can occur. Two research questions arise from this; where do click beetles come from to invade fields, and how far do they move?

2.4.2. Methods

2.4.2.1. Mark-Release Recapture Studies

Two areas, 72m x 72m were marked out in April 2007. One was sown with wheat and the other left fallow. In the previous year, they had been treated with insecticide to kill any wireworms present and preliminary sampling confirmed this. In each site, six 1m linear gutter traps, leading to a pit-fall trap, were placed at regular intervals parallel to and 1m in from the boundaries. Within the zone created by these traps 36 normal pit-fall traps were systematically positioned to be equidistant. In addition, 25 cross traps made from four half metre lengths of gutter trap with a pitfall at the centre were positioned in a randomised latin square. The pitfall traps were deemed to be the location of the trap and the distance to, and direction from, the centre of the plot was determined.

Most male click-beetles were captured using sex pheromone traps and females (and some males) in forage traps. Beetles were grouped for release and given a distinctive marking on the thorax using acrylic paint. Batches were held in captivity and maintained on slices of apple until there were sufficient available (min. 200) for release.

In the first study, beetles were released from a single point at the centre of each field. Dates of release are shown in Table 15.

		Wheat		Fallow
8 May	*A. obscurus*	Male		
		Female		
10 May	*A. obscurus*	Male	*A. obscurus*	Male
25 May	*A. lineatus*	Male	*A. lineatus*	Male
	A. obscurus	Male	*A. obscurus*	Male
30 May	*A. obscurus*	Male	*A. obscurus*	Male
		Female		Female

TABLE 15. RELEASE DATES FOR BATCHES OF MARKED *AGRIOTES* CLICK-BEETLES IN WHEAT AND FALLOW FIELDS.

Traps were examined periodically starting one hour after release up to a maximum of 561 hr which was the longest duration between release and recapture for any individuals.

Distance travelled to capture – as a straight line – and angle to trap from the release point were used as response variables and analysed separately. For each capture, explanatory variables were recorded: field, species-gender group, time since release (hrs), and period of recapture (early May, late May or June).

Counts for directions travelled were non-normally distributed and so were square-root transformed prior to analysis. A Generalised Linear Model (GLM) was applied.

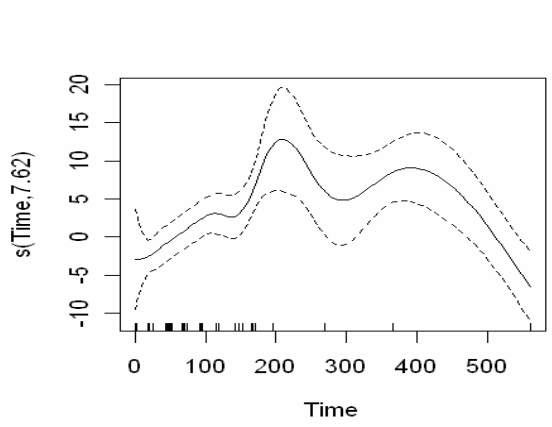


FIGURE 13. LOESS SMOOTHING FUNCTION APPLIED TO THE TIME SINCE RELEASE VARIABLE FOR THE GAM ANALYSIS OF DISTANCE TRAVELLED.

Preliminary analyses of the distance data showed that data collected 561 hr after release were outliers and so subsequently omitted. The relationship between time and distance was non-linear and so a Generalised Additive Model (GAM) was fitted with a Loess smoothing function (Figure 13) applied to the time since release variable. The preliminary GAM analysis violated the assumption of homogeneity so the eventual fitted model was a GAM with Poisson distribution and a log-link function.

In a second study male *A. obscurus* click beetles were released in batches of 40 at five randomised locations on each side of the wheat field on May 10 2007. Marked and unmarked beetles (male and female) were recovered from pitfall traps. Count data from each trap were used to generate kriged contour plots using SURFER©.

2.4.2.2. Genetic mixing of populations

The original intention had been to develop a microsatellite technique to investigate the genetic relatedness between click beetle populations at different geographic scales. Resource problems were encountered with this approach and it was decided to opt for the use of Amplified Fragment Length Polymorphisms (AFLP) as a more pragmatic option.

This approach has several advantages over other molecular markers, since data is highly reproducible, no *a priori* sequence data is required, and there is a high level of resolution. The first step involves restriction-ligation, in which DNA is digested with two restriction enzymes, and adaptors are ligated onto the ends of the fragments produced. A pre-selective PCR is carried out, where these restriction fragments are amplified with two PCR primers that have corresponding adaptor and restriction site-specific sequences. A selective PCR, using primers that amplify only a selection of the fragments, results in a manageable number of fragments for analysis when samples are processed using a genetic analyser. AFLPs are scored using software based on shared peaks between individuals and analyses can be performed to estimate genetic relatedness at different scales, and the relationship between geographic and genetic distance can be used to assess the extent of dispersal for each species.

2.4.3. Results

2.4.3.1. Mark-Release Recapture Studies

Overall recovery of released beetles was of the order 15-16% which is relatively efficient for this kind of study and provided sufficient data for analysis.

The optimal GLM for distance travelled was based on the date of recapture variable and the field/gender-species group interaction terms but only explained 9.8% of the total deviance (Table 16).

	Df	Deviance	Residual Df	Residual Dev.	F	P(>F)
Null			587	112367		
Date	2	1124	585	111243	6.55	0.04*
Field:Group	4	9929	580	101314	11.37	$1.7e^{-10}$***

TABLE 16. ANALYSIS OF DEVIANCE FOR GLM MODEL DIRECTION ~ DATE + FIELD:GROUP + E_i, 9.8% OF DEVIANCE IS EXPLAINED.

The directions travelled during the second period of recapture (late May) differed from the other two periods and the Field:Group interaction was attributable to the *A. lineatus* male release in the wheat field which was the only response that differed statistically.

The optimal model from the GAM analysis was Distance ~ Date + Field + Group + s(Time) with all variables significant at $P<2e^{-16}$ and 22.1% of the deviance explained. Boxplots showed that less distance was moved by female *A. obscurus*, in the wheat field and in the last recovery period (early June).

Recovery of marked beetles released at the edges (Figure 14) showed that dispersal into the field was rapid. The first beetles were recovered one hour after release at a distance of one metre from the field edge. By 19 hr after release beetles were in the middle of the field, having moved some 35-40m.

These results were consistent with what was observed from captures of wild male (Figure 15) and female (Figure 16) beetles.

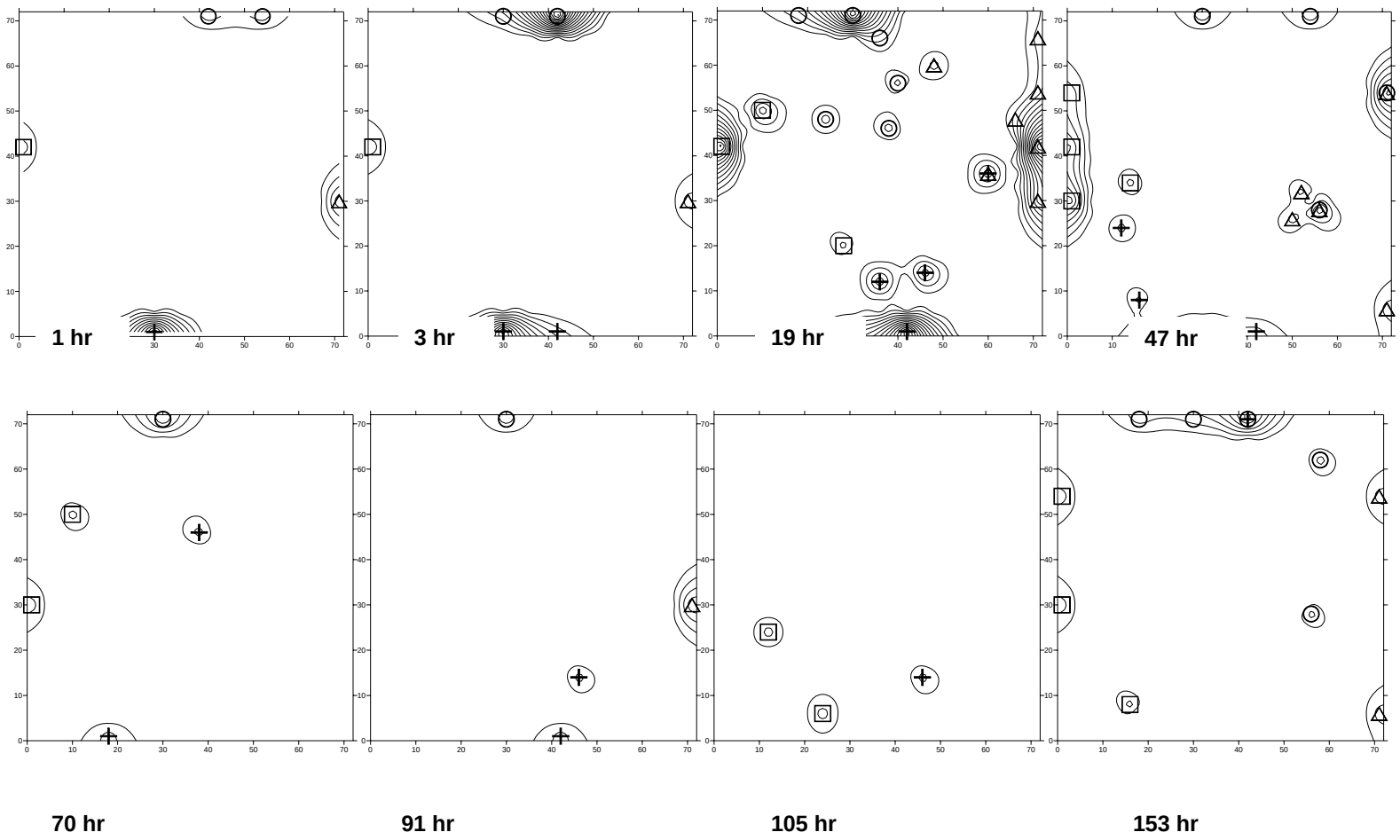


FIGURE 14. RECAPTURE OF MARKED BEETLES AT TIME INTERVALS IN PITFALL TRAPS FOLLOWING RELEASE. SYMBOLS SHOW THE FIELD EDGE AT WHICH THE BEETLES WERE RELEASED; NORTH (CIRCLE), SOUTH (CROSS), EAST (SQUARE) AND WEST (TRIANGLE).

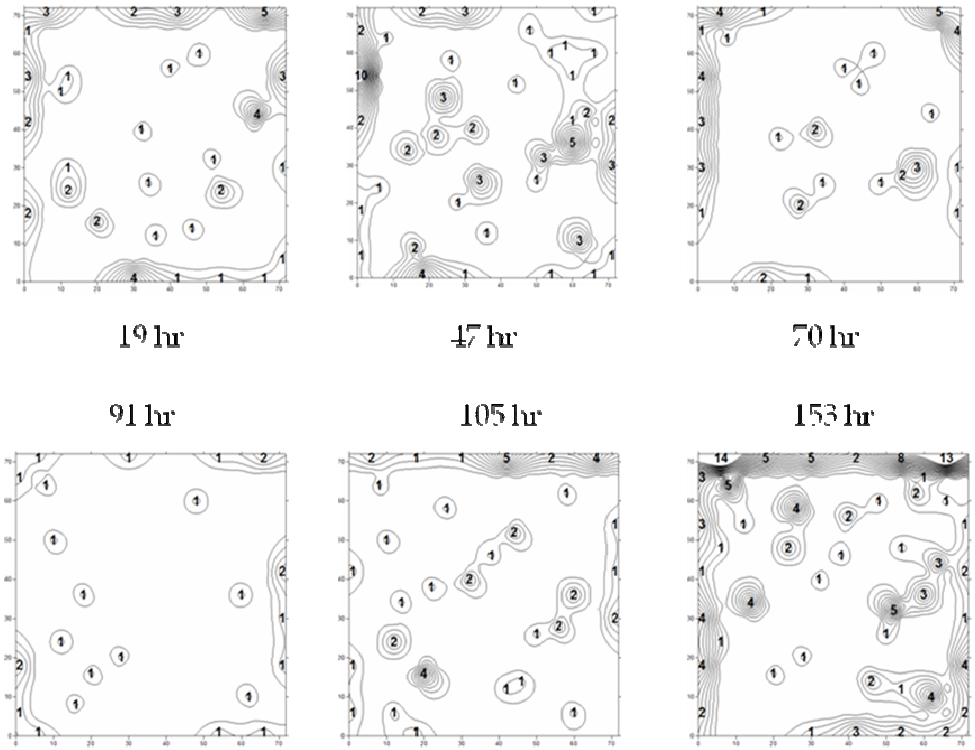


FIGURE 15 SPATIAL DISTRIBUTION OF CAPTURED WILD *A. OBSCURUS* MALES AT DIFFERENT TIMES AFTER RELEASE FROM THE FIELD EDGE. NUMBERS ARE ACTUAL COUNTS IN PITFALL TRAPS.

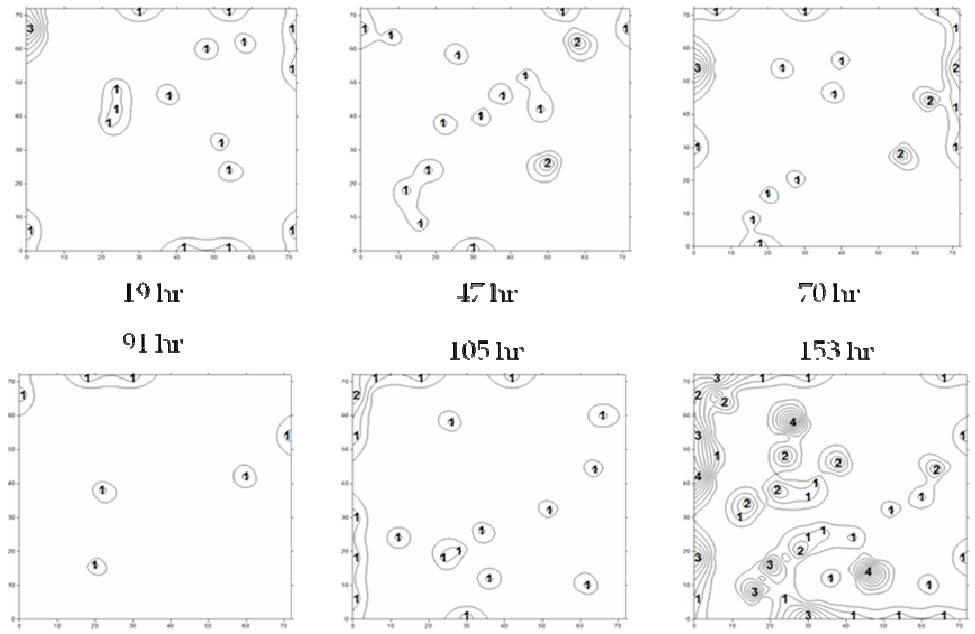


FIGURE 16 SPATIAL DISTRIBUTION OF CAPTURED WILD *A. OBSCURUS* FEMALES AT DIFFERENT TIMES AFTER RELEASE FROM THE FIELD EDGE. NUMBERS ARE ACTUAL COUNTS IN PITFALL TRAPS.

2.4.3.2. Genetic Mixing of Populations

Selective primer combinations are currently being chosen based on the number of polymorphic peaks and variability and reliability between different samples (within the same species). Once the optimal primer combination(s) are chosen they will be applied to click beetle and wireworm samples of each species from Devon (field and farm scales), Somerset, Cambridge and Scotland. These results will be presented later as an addendum to this report.

2.4.4. Discussion

The differences in direction travelled revealed by the analysis are relatively minor with only 9.8% of deviance explained. The fact that *A. lineatus* males caused the significance of the field:species-gender group effect may also be linked into the observed recovery date effect since this species-gender group was only released during the second (late May) recapture period. At this stage it is difficult to draw conclusions but the data do suggest that there are not large biological differences in play, and that any effect is principally environmental.

The situation with distance travelled is different, with substantially more deviation (22.1%) being explained by the GAM. Furthermore, all explanatory variables were involved in the optimal model suggesting that there may be biological as well as environmental differences. The evidence suggests that female *A. obscurus* may not travel as far as males. This should not be assumed to be because they move at a slower speed since the time to recapture says nothing about the trajectory of travel between the release point and pitfall trap. Indeed, this point is apparent from the smoothed time function (Figure 13) where the first 100 hr or so shows a roughly linear relationship which becomes more variable subsequently. In other words, the beetles captured after 100 hr are not walking in a straight line even if they are up until that point. Possible reasons for females moving less distance than males in the same time period could also include a more tortuous path and maybe a shorter lifespan. This latter may also explain why distances moved by beetles recaptured in the late June period were significantly lower; this is towards the end of the adult activity period and death may have intervened. Alternatively, the presence of more mature plants in both fields might have impeded progress.

The difference between the wheat and fallow in distance moved seems counterintuitive since the density of plants - and potential for disruption of walking trajectories - will be greater in the cereal. At the start of the study the fallow field was clear of weeds but a large number had germinated and grown by the end. It is possible that the row structure of the wheat crop facilitated more rapid movement away from the release site though this is difficult to reconcile with the analysis of the directional data.

It is clear from the study of edge-released *A. obscurus* that they will move from field margins into bare soil areas. The wild beetle captures also show that both males and females are active in this respect. At this stage there is nothing to suggest that this dispersal is anything but random with the implication that click beetles are much more spatially active than previously thought. The rate of movement into the field could be as high as an estimated 2m hr-1 as indicated by Figure 15. This needs to be compared with the *ca*. 42m attraction range of a pheromone trap reported by Hicks

and Blackshaw (2008) or the 80m dispersal estimated by Schallhart *et al*. (2009) using stable isotopes.

We have shown that dispersal of *Agriotes* beetles across arable fields is a real phenomenon. We have also started to reveal possible differences in dispersal behaviour between species and between genders. This reinforces the view (Blackshaw & Vernon 2008; Hicks & Blackshaw 2008) that we should not treat *Agriotes* wireworms as a single pest complex.

2.5. Objective 4: Behavioural response of click beetles to pheromones

2.5.1. Introduction

The first recommendations for the use of pheromone traps to monitor wireworms treated each trap-species complex as identical. This view was challenged by Blackshaw & Vernon (2008) who reported differences between *A. lineatus* and *A. obscurus* spatial patterns within fields that were most likely to have been caused by differential responses to their respective pheromone traps. The existence of such differences was confirmed by Hicks and Blackshaw (2008) in a mark-recapture study. These results negated the recommended use of pheromone traps for monitoring.

Given that wireworm is actually a pest complex of several species and that pheromone traps remain a potentially useful tool in locating hotspots it is desirable to understand the dynamics of the trapping process better. One component of this is the responsiveness of the different species to their synthetic pheromone lures. It is also of interest to see if the differential attraction ranges of pheromone traps, as reported by Hicks and Blackshaw (2008), are linked to beetle walking speeds.

2.5.2. Methods

In order to investigate adult click beetle walking behaviour and response to pheromones a locomotion compensator was used to track beetle movements over 5 minutes, measuring average walking speed, track length, straightness (how straight or tortuous the track is), upward length (net upward displacement, or movement towards stimulus) and straightness (how straight the insect is moving towards the stimulus) among other parameters.

Adult male click beetles of each of the three species *A. lineatus*, *A. obscurus* and *A. sputator* were collected using pheromone traps from June to July. To get an idea of whether/how often the three species fly, the pheromone traps were modified by placing a circle of sticky trap paper inside the top of the trap.

The beetles were tracked in still air, but some *A. sputator* beetles were tested with air flow with pheromones to see if this altered their walking behaviour.

2.5.3. Results

Only two flying beetles were caught – one *A. obscurus* and one *A. sputator*. A click beetle was also observed flying away from the lid of an *A. obscurus* trap, but the species was unknown. In accordance with other observations and studies of click

beetle movement, this suggests flight does occur but not on a large scale under UK conditions.

In total 28 *A. sputator*, 20 *A. lineatus* and 14 *A. obscurus* were tested on the locomotion compensator. *A. lineatus* was the fastest, followed by *A. obscurus* and *A. sputator* (Figure 17).

Five of the *A. sputator* beetles were tested both in still air and in air flow containing pheromones. Adult male click beetle speed and straightness (ranging from 0 to 1) may be expected to increase in response to pheromones, but speed in still air is higher than in air flow with pheromones, as is straightness (the average value is closer to 1 and so the tracks are straighter; Figs 2.5.2 and 2.5.3). Upward straightness, which can range from -1 to +1, was -0.05 when pheromones were applied, and upward length was 12.1, indicating that there was little if any movement towards the stimulus.

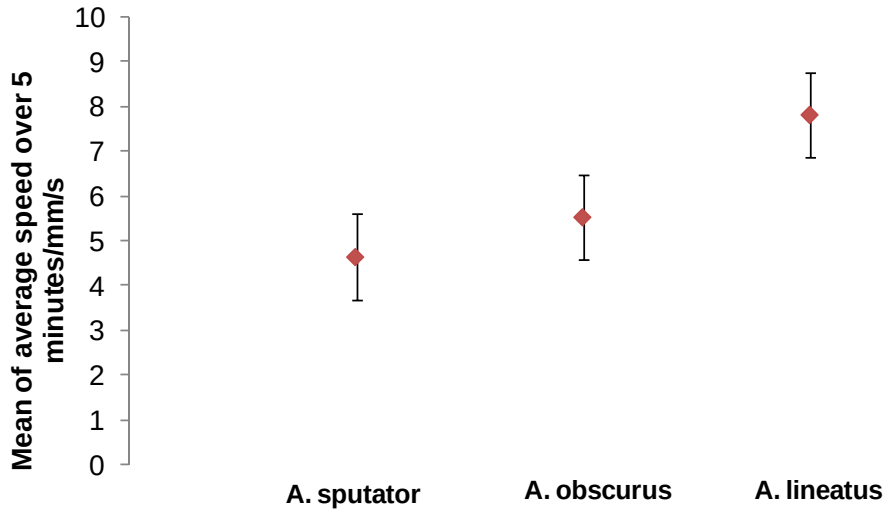


FIGURE 17. MEAN OF THE AVERAGE SPEED OVER 5 MINUTES FOR *A. SPUTATOR*, *A. OBSCURUS* AND *A. LINEATUS* ADULT CLICK BEETLES (WITH STANDARD ERROR BARS.)

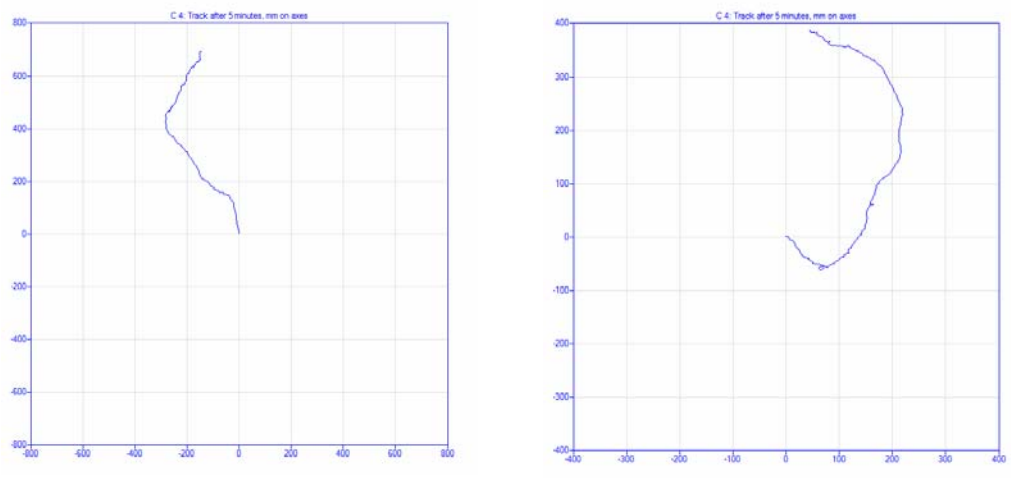


FIGURE 18. EXAMPLE OF TRACKS PRODUCED BY *A. SPUTATOR* ON THE LOCOMOTION COMPENSATOR WITH PHEROMONES (LEFT) AND WITHOUT PHEROMONES (RIGHT). THE BEETLE STARTS WALKING FROM THE CENTRE OF THE GRAPH.

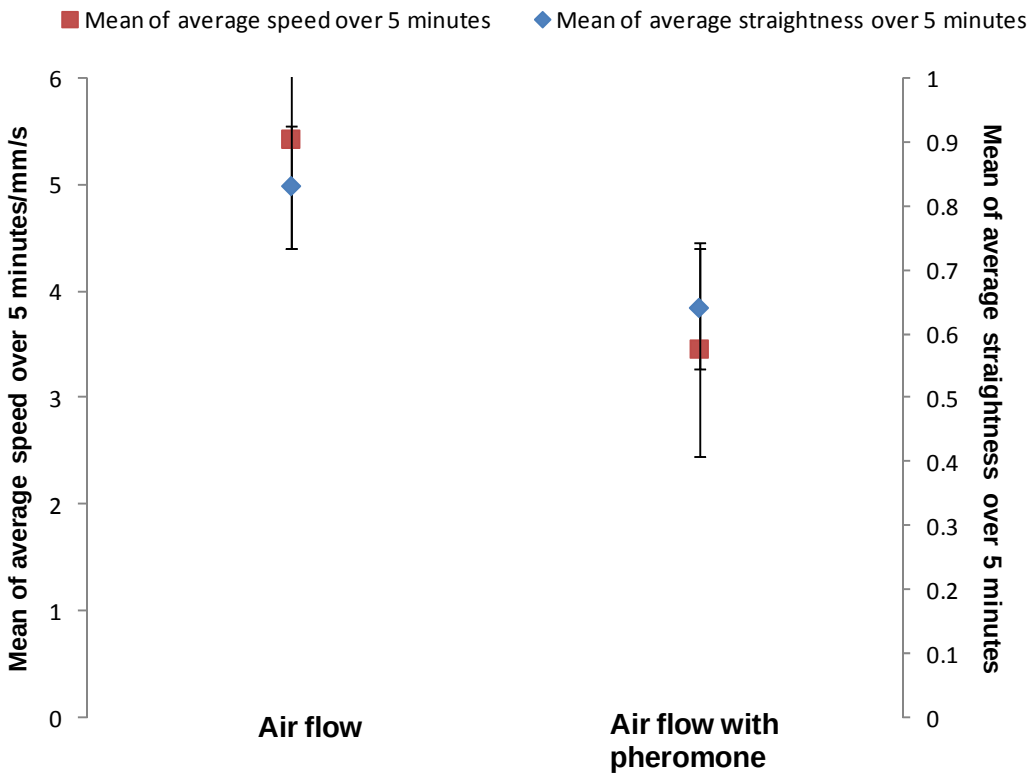


FIGURE 19. CHANGES IN MOVEMENT OF A. SPUTATOR WITH AND WITHOUT PHEROMONES IN AN AIR FLOW.

2.5.4. Discussion

Walking speeds in this study (Figure 17) were consistent with the differences in trap attraction range reported by Hicks and Blackshaw (2008). Thus it would appear that walking speed will be an important component for the normalisation of trap counts for each species.

The effect of exposure to a pheromone source was also clear cut, albeit unexpected. For *A. sputator* at least, pheromones appear to have reduced walking speed and straightness (Figure 18). We postulate that responses are dose-sensitive and that when there is too much exposure decision-making is impaired and movement is reduced.

Studies into the locomotory behaviour of the click beetles will continue beyond the end of the project and will be presented as an addendum to this report.

2.6. Objective 5: Impact of novel biocontrol agents

2.6.1. Introduction

The aim of work done under this objective was to evaluate alternatives to conventional insecticides for wireworm control. These alternative wireworm controls may not necessarily replace current insecticides, but instead may enhance the effectiveness of insecticides by using them in conjunction with, for example, novel biocontrol agents. Two approaches were taken.

Firstly, initial laboratory bioassays were conducted on the efficacy of commercial formulations of the entomopathogenic fungus *Metarhizium anisopliae* (BioCane and ChaferGuard) and the entomopathogenic nematodes *Heterorhabditis megidis* (Nemasys H), *Steinernema carpocapsae* and *Steinernema feltiae*.

The second approach was to investigate the field use of Caliente brand mustards, grown as green manures, and a proprietary de-fatted mustard seed meal (BioFence). Previous laboratory (Furlan *et al.,* 2004) and field (Furlan, personal communication) studies have shown that mustards bred specifically for high glucosinolate content can be toxic to wireworms when the green material is chopped and incorporated into the soil when there is sufficient soil moisture present to allow myrosinase enzymes to convert glucosinolates released from ruptured cells into isothiocyanates (ITC). The mustards shown to be toxic to wireworms in this way are *Brassica juncea* (Caliente Brand 99) and *Eruca sativa* (Nemat) (both from Plant Solutions Ltd, UK). A de-fatted mustard meal produced from *Brassica carinata* (BioFence) was also shown to be effective against wireworms in laboratory bioassays (Furlan *et al.,* 2004). In addition to the field trials laboratory experiments were conducted to examine the possible interaction between the rate of biofumigant applied (de-fatted mustard meal - BioFence), and soil moisture content on wireworm survival (see Objective 2).

2.6.2. Methods

2.6.2.1. Assessment of novel biocontrol agents and biofumigants

The first part of this objective was to complete *in vitro* testing of candidate non-chemical controls for *Agriotes* spp. wireworms. The objectives of this work were to:

- Establish the optimum product rate and soil water content for maximum effect when using de-fatted mustard meals (*Brassica carinata* meal – BioFence) as biofumigation agents.

- Test the efficacy of a range of non-chemical agents for the control of wireworms in a soil-based medium. The candidate materials included isolates of the fungal pathogen *Metarhizium anisopliae* (BioCane and ChaferGuard), soil-active insect-pathogenic nematodes (*Heterorhabditis megidis, Steinernema feltiae, Steinernema carpocapsae*), and de-fatted *Brassica carinata* meal (BioFence).

This work was done in pots of soil in semi-field conditions.

The second part of this objective was to test under field conditions the efficacy of Caliente brand mustards and Nemat, grown as green manures but which are known to have biofumigant properties, as well as the BioFence.

2.6.2.2. Effect of product rate and soil moisture on the efficacy of BioFence:

Treatments are given in the following table (Table 17.) and in each case the treatment was replicated four times, with each pot being a ‘plot’, giving a total of 36 pots.

Treatments	BioFence Application rate (t/ha)	Water volume (% soil capacity)
1A	0.00	80
1B	0.00	40
1C	0.00	20
1D	2.00	80
1E	2.00	40
1F	2.00	20
1G	4.00	80
1H	4.00	40
1I	4.00	20

TABLE 17. PRODUCT RATE AND SOIL MOISTURE CONDITIONS USED TO TEST EFFICACY OF BIOFENCE.

The treatments described in Table 16 were established by first determining the water-holding capacity of a sample of John Innes No. 2 potting compost. This was done by oven-drying a two litre sub-sample of the compost before adding measured quantities of water (e.g. 20 to 50 ml increments) to the cooled, dry soil until water could no longer be absorbed. The quantity of water added up to this point represented the maximum water-holding capacity of the sample of compost and this could be expressed as a percentage of the dry weight of the compost. It was then possible to calculate the amount of water required to wet compost in a 1.5 litre pot to 80%, 40% and 20% of compost capacity.
BioFence was prepared by first weighing out 250 g of the extruded granules and grinding these to a coarse powder with a pestle and mortar. Sufficient compost to fill 36 x 1.5 litre pots (approx. 54 litres) was dried, split into three equal sub-samples and assigned to the following three treatments:

Twelve pots prepared with dried compost. Four pots re-wetted to 80% capacity (treatment 1A), four pots to 40% capacity (treatment 1B) and four pots to 20% capacity (treatment 1C).

Twelve pots prepared with dried compost. To each pot 3.53g of BioFence powder was added and mixed thoroughly. As above, four pots each re-wetted to 80% (treatment 1D), 40% (treatment 1E) and 20% (treatment 1F) capacity.

Twelve pots prepared with dried compost. To each pot 7.06g of BioFence powder was added and mixed thoroughly. As above, four pots each re-wetted to 80% (treatment 1G), 40% (treatment 1H) and 20% (treatment 1I) capacity.

For each treatment, drainage holes in the bottom of pots were covered with fine wireworm-proof mesh or muslin prior to filling with compost, to prevent wireworms escaping. Three untreated wheat seeds were placed in each pot to provide a food source for the wireworms. Ten late-instar *Agriotes* wireworms were placed into a 5 cm deep hole in the centre of each pot immediately after each treatment was prepared. These holes were then gently covered with compost. Pots were placed in plant-pot saucers and placed in a shaded environment at 18-20°C for 10 days. Pots were initially not watered but after 2 days were watered daily with a standard amount per pot. After 10 days, the contents of the pots were examined for the presence of wireworms, recording wireworms found as either (i) live with unimpaired mobility (ii) live with impaired mobility (iii) dead (iv) missing. Any found in the pot saucers were also recorded.

An additional pot trial was completed using a similar method to that previously described, but including a range of *Agriotes* wireworm species. Species tested were: *A. brevis*, *A. sordidus*, *A. litigiosus*, *A. ustulatus* and *A. obscurus* (which was thought to be the species used in the previous pot trial). For each species tested, five wireworms were placed into a pot. Pots were either left untreated or treated with 1.54 g of BioFence. Each treatment combination was replicated five times and wireworm survival was assessed after 15 days.

2.6.2.3. Efficacy of novel control agents:

The novel treatments are listed in the following table. In each case the treatment was replicated ten times, with each pot being a 'plot', giving a total of 60 pots.

Treatments	Product	Rate
2A	Untreated	-
2B	*Heterorhabditis megidis* (Nemasys H)	1 million/m^2
2C	*Steinernema carpocapsae*	1 million/m^2
2D	*Steinernema feltiae*	1 million/m^2
2E	BioCane (*Metarhizium*)	99 kg/ha
2F	Chafer Guard (*Metarhizium*)	33 kg/ha

TABLE 18. NOVEL TREATMENTS FOR CONTROL OF WIREWORM.

The above treatments were established by first autoclaving and then oven drying sufficient compost to fill 60 x 100 ml containers (approx. 6 litres). For untreated (2A) containers compost was re-wetted as previously described to produce a soil moisture content of 15% ± 2%. Three wheat seeds were placed into each container together with one late instar *Agriotes* wireworm, which was carefully placed into a small hole in the centre of each container and then gently covered. Finally 2 ml of water was added to each container. Each nematode treatment (2B, 2C & 2D) was prepared as described for treatment 1A with the 2 ml of water replaced by 2 ml of a nematode suspension. Each nematode suspension was prepared by diluting a nematode pack (50 million nematodes/pack) in 5 litres of water. After mixing thoroughly, 1 litre of this suspension was added to 9 litres of clean water in order to produce a suspension of approx. 1000 nematodes per millilitre (confirmed by microscope counts). Two millilitres of this suspension could then be applied to each container to achieve the target rate, equivalent to 1 million/m2. The BioCane and Chafer Guard treatments (2E & 2F) were also prepared as described for treatment 1A. Each product was added at a rate of 1 million conidia/g of air-dried soil, thoroughly mixing the treated compost before adding the wheat seeds and wireworm. The lid of each container was pierced to allow gaseous diffusion but not escape of wireworms, and was screwed on. The containers were then placed in a darkened area at a temperature of 18°C for 60 days (allowing for maximum mortality due to *Metarhizium* infection). Assessments were completed after 60 days, recording wireworms as either (i) live and mobile (ii) writhing only (iii) dead (iv) missing.

2.6.2.4. Efficacy of Caliente brand mustards and BioFence under field conditions

Field experiment: in 2006, a long-term field experiment was set up at North Cadbury, Somerset (courtesy of Mr Archie Montgomery) to evaluate the effect of either one or two preceding mustard crops on subsequent wireworm damage to potato.

Experiment site: the field (OS grid reference ST 612257) was initially sampled on 4 April 2006 while still in permanent grass, and was found to have an established wireworm population of *c*. 580,000/ha. The site was ploughed in April 2006 and *c*. 1 ha left fallow (the rest of the field was planted with maize) until the start of the experiment in July 2006.

Experiment design: the 1 ha experiment site was divided into two roughly equal plots (Half A and Half B). For the biofumigant plot experiment a randomised complete block design with four treatments (Table 19.) replicated six times (24 plots in total) was used (Figure 20.). Plot size was 12 m x 12 m in 2006 and 12 m x 10 m in 2007 with 2 m guards (left fallow) between each plot.

Code	Treatment
A	*Eruca sativa* (Nemat) sown at 10.3 kg/ha; incorporated after c. 10 weeks
B	*Brassica juncea* (Caliente 99) sown at 10.0 kg/ha; incorporated c. 10 weeks
C	*Brassica carinata* de-fatted meal (BioFence); incorporated at 2.5 t/ha
D	Untreated

TABLE 19. TREATMENTS USED IN THE BIOFUMIGANT FIELD EXPERIMENT.

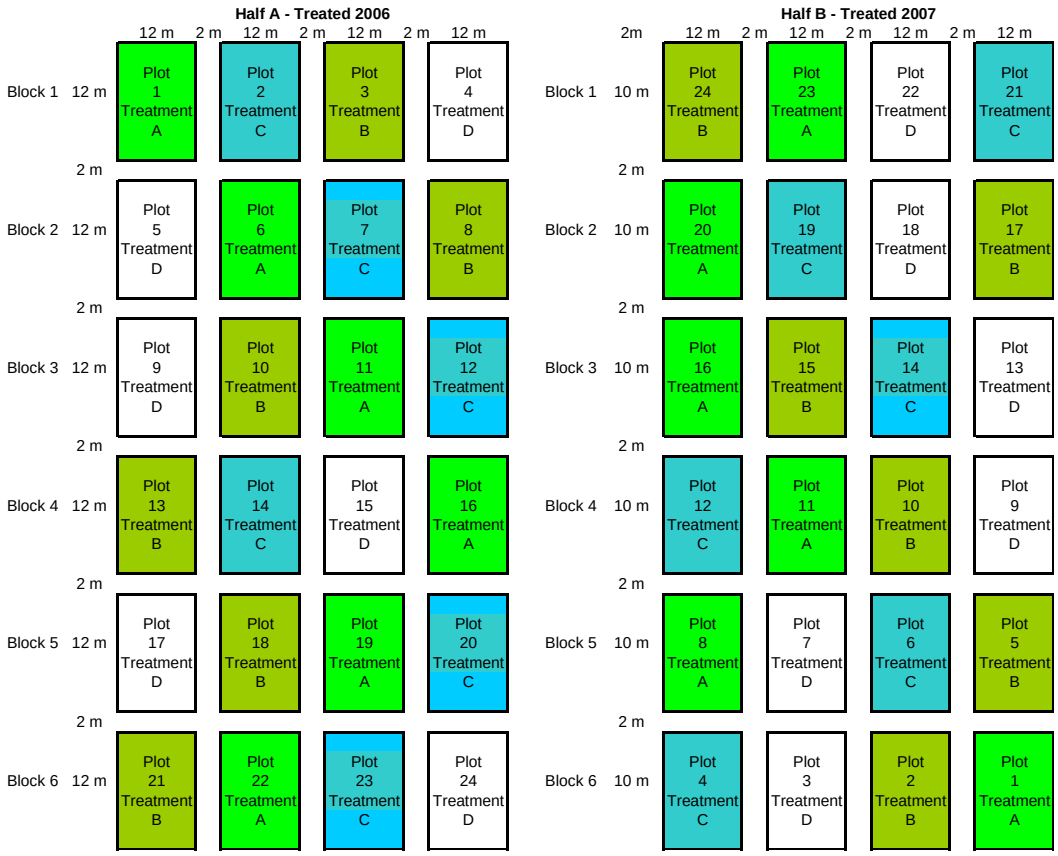


FIGURE 20. BIOFUMIGANT EXPERIMENT PLOT DESIGN USED ON HALF A IN 2006 AND HALF B IN 2007.

2.6.2.4.1. Methods

In 2006 the entire experiment site was ploughed, pressed and marked out on 26 July. All mustard plots were precision drilled in Half A on the same day. On 28 July 2006, Half B (*c*. 0.5 ha) was drilled with Nemat as per Treatment A (Table 2.6.3.). Untreated and BioFence plots remained fallow. On 4 October 2006, the BioFence pellets were spread on the soil surface in the appropriate plots. The mustard treatments were then chopped and incorporated the same day using a front-mounted flail mower and a rear-mounted power harrow working to *c*. 10 to 15 cm depth. The BioFence treatments were incorporated in the same pass. All untreated plots also received a pass with the power harrow.

In 2007 the entire experiment site was ploughed, pressed and marked out on 19 July. Mustard plots were precision-drilled in Half B on the same day. As Half B had been cropped with Nemat in 2006, this allowed comparisons to be made between areas receiving one or two crops of mustard or BioFence treatment. Half A was left fallow in 2007. Both mustards germinated quickly and evenly in 2007 and received a top-dressing of 100 kg ha^{-1} of nitrogen and 20 kg ha^{-1} of sulphur (applied by hand) *c*. 1 week after emergence. On 25 September 2007, the BioFence pellets were spread on the soil surface. The mustard treatments were then chopped and incorporated the same day using the method previously described. As in 2006 the BioFence treatments were incorporated in the same pass and all untreated plots also received a pass with the power harrow.

In 2008 the whole experiment site (Half A and B) was planted with potatoes (Fambo) on 24/05/08 with half of each plot being additionally treated with Mocap (ethoprophos) at 60 kg/ha. Potatoes were harvested on 27/08/08 and assessed for tuber damage (Figure 21.).

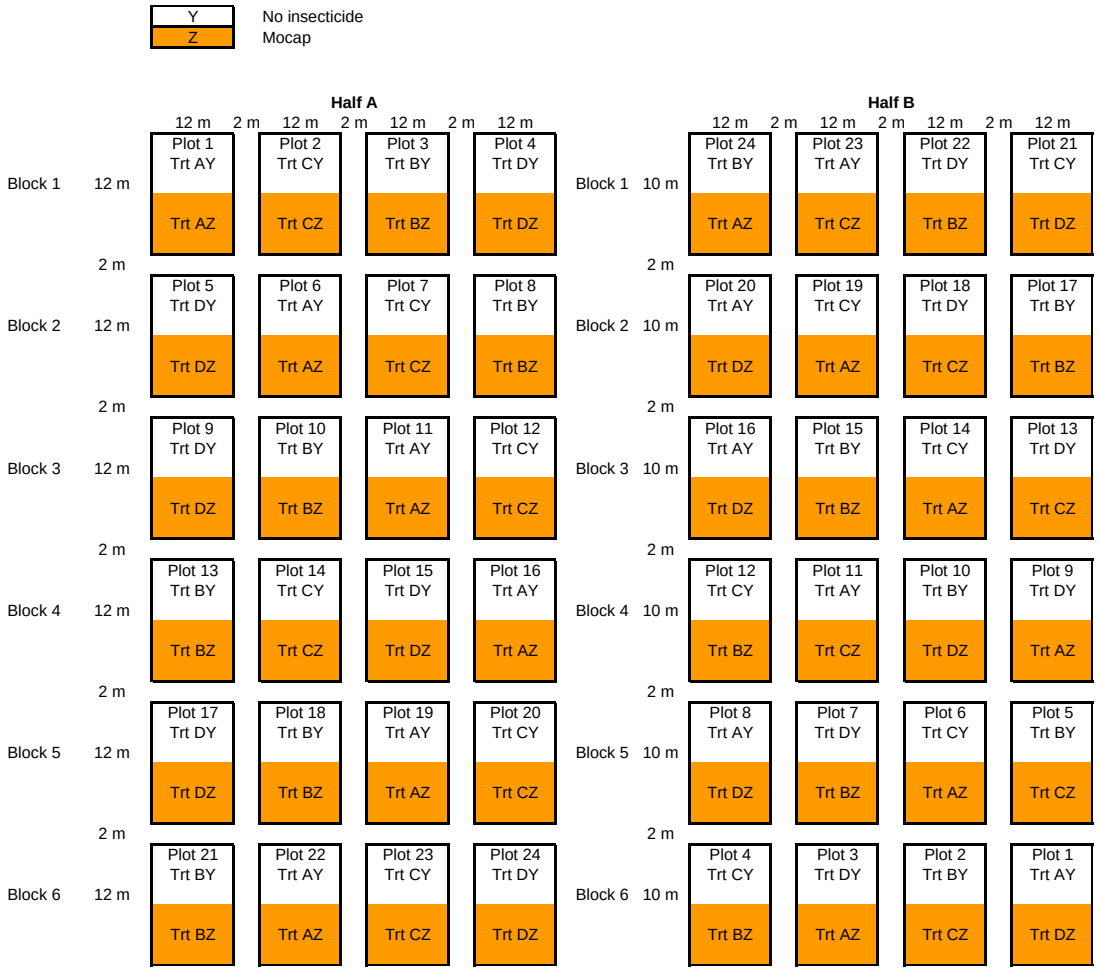


FIGURE 21. POTATO PLANTING AND MOCAP APPLICATIONS IN 2008 OVERLAYING BIOFUMIGANT EXPERIMENT PLOT DESIGNS USED IN 2006 AND 2007.

Assessments: in 2006 and 2007, to sample for wireworms, four 15 cm diameter soil cores were taken from each plot pre-drilling in July, immediately pre-incorporation in September and 21 days after incorporation. All samples were processed through a large soil washer and wireworms extracted by flotation. All wireworms found were measured to determine their size class. The effect of isothiocyanates generated by the incorporated mustards and meal is thought to be short-lived (24-36 hours maximum), so 21 days was considered sufficient for any biological effect on wireworms to have occurred.

Immediately prior to incorporation, 0.5 m^2 areas of both Nemat and Caliente 99 were cut and weighed to determine the amount of fresh material likely to be incorporated. In 2006, the fresh weight of Caliente 99 incorporated was estimated at 22 t ha^{-1} and for Nemat 35 t ha^{-1}. In 2007 the foliage was very wet when samples were taken, but the likely fresh weight of both mustards was in excess of 60 t ha^{-1}.

Tuber damage assessments completed in 2008 were based on visual assessment of 100 tubers selected at random from each plot. The presence of wireworm damage and number of wireworm holes found were recorded for each tuber.

2.6.3. Results

2.6.3.1. Effect of product rate and soil moisture on the efficacy of BioFence:

The effects of product rate and soil moisture content on the efficacy of BioFence are given in Figure 22.

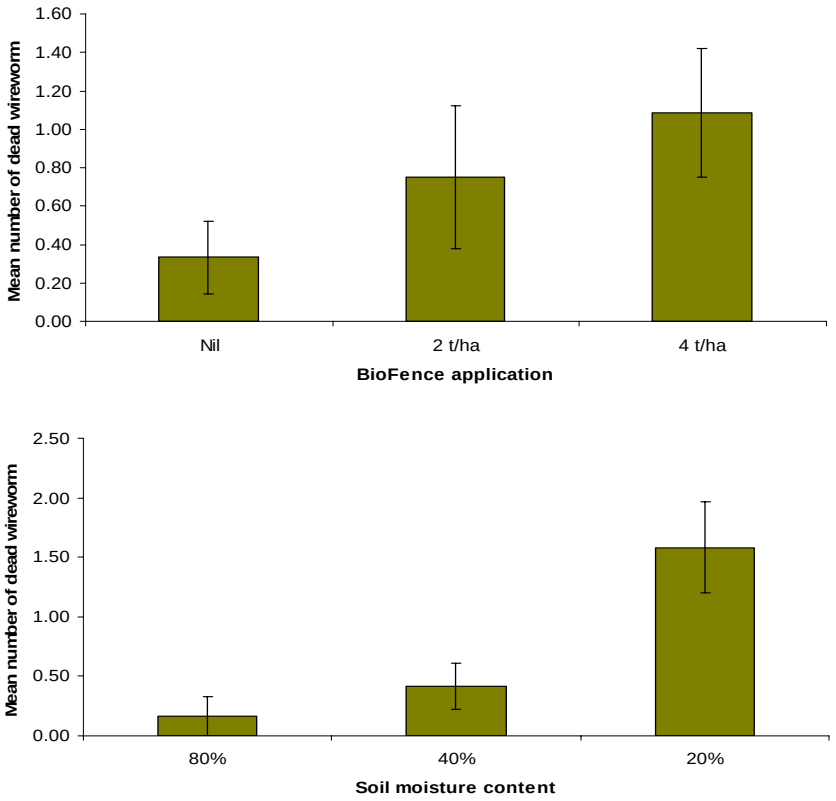


FIGURE 22. EFFECTS OF RATE OF BIOFENCE DE-FATTED MUSTARD MEAL AND SOIL MOISTURE CONTENT ON WIREWORM MORTALITY

Although increasing the rate of BioFence application increased wireworm mortality this increase was not significant ($F_{2,24}$ = 1.77, *P* = n.s.). By contrast, soil moisture significantly affected wireworm mortality (F2,24 = 7.18, *P* = 0.004), with increased mortality in soils with lower moisture content. Analysis of the BioFence pellets used in the experiment confirmed the presence of the glucosinolate sinigrin (2-propenyl glucosinolate) at a rate of 122.8 µmol/g of dry matter.

Results from the experiment investigating efficacy of BioFence against different *Agriotes* species are summarised in Table 20.

***Agriotes* species**	**% control mortality (no BioFence)**	**% treatment mortality (BioFence applied at 1.38 g/l)**
Agriotes brevis	5.56	18.18
Agriotes sordidus	12.50	20.00
Agriotes litigiosus	0.00	35.29
Agriotes ustulatus	0.00	15.79
Agriotes obscurus	0.00	27.78

TABLE 20. MORTALITY OF DIFFERENT *AGRIOTES* SPECIES IN THE PRESENCE OR ABSENCE OF BIOFENCE.

Where BioFence was applied, mortality in all species of *Agriotes* tested was broadly similar in the second pot trial to the first, although there was a wider range of wireworm mortality in the first trial (4-38%). The rate of BioFence applied in the second trial was lower than either of the two rates tested in the first (equivalent to approx. 1 t/ha).

2.6.3.2. Efficacy of novel control agents:

The effect of the novel control agents on the mortality of individually treated wireworms is summarised in Figure 23.

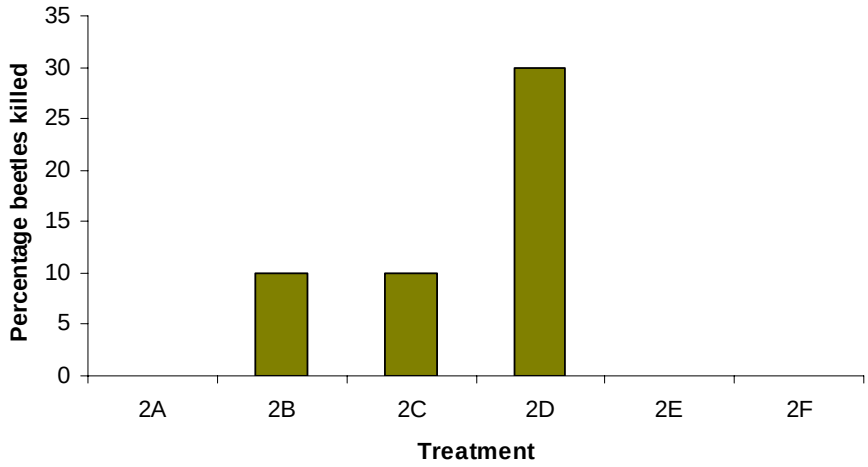


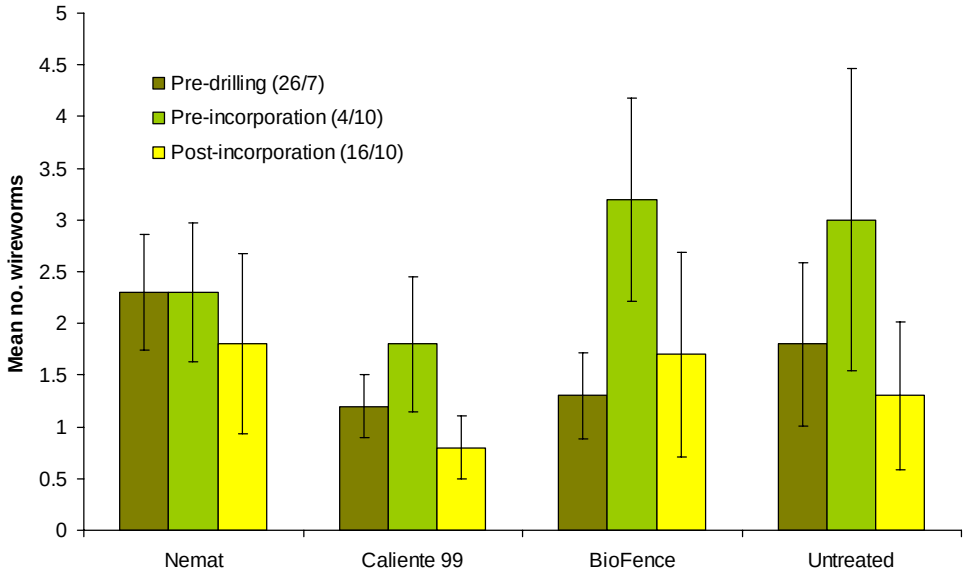
FIGURE 23. EFFICACY OF NOVEL CONTROL AGENTS; 2A = UNTREATED; 2B = *HETERORHABDITIS MEGIDIS*; 2C = *STEINERNEMA CARPOCAPSAE*; 2D = *STEINERNEMA FELTIAE*; 2E = BIOCANE (*METARHIZIUM)*; 2F = CHAFER GUARD (*METARHIZIUM*).

Across all treatments a total of just five individuals (8%) were killed. However, although dead individuals were recorded from three different treatments, three of the five dead wireworms were recorded from pots treated with *Steinernema feltiae* (2D) representing a 30% kill. It is perhaps also worth noting that for this treatment one individual was recorded as writhing and a further wireworm was missing.

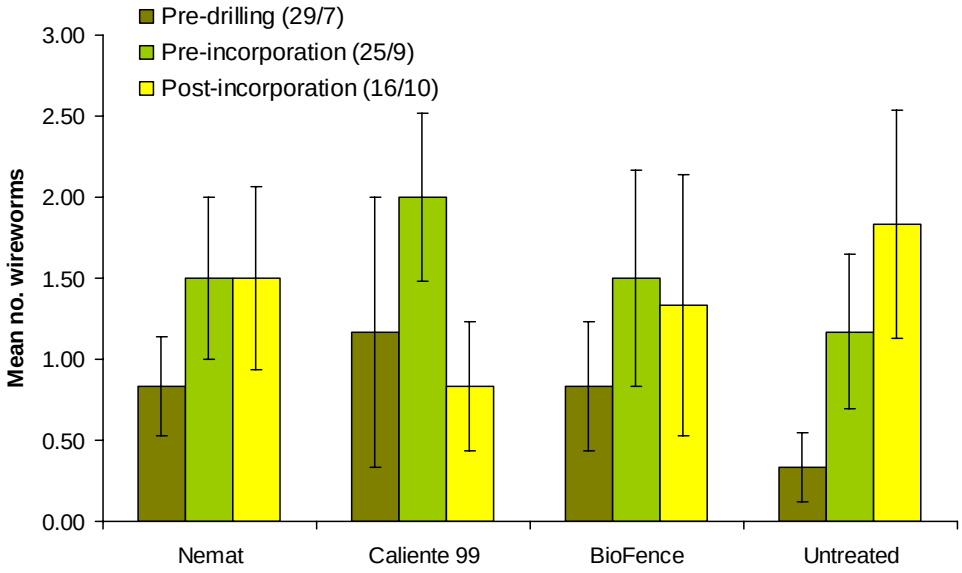
2.6.3.2.1. Efficacy of Caliente brand mustards and BioFence under field conditions:

The application of fertiliser ensured a highly vigorous crop and the size of the plants and hence the biomass production was significantly higher in 2007 than in 2006. The Caliente 99 was infested with turnip sawfly (*Athalia rosae*) in both 2006 and 2007, although only suffered significant damage in 2006. Soil moisture assessments in 2006 indicated that the water content of the soil was only 27% at the time of incorporation. In addition it was not possible to effectively seal the soil. In 2007 no soil moisture assessments were done on the day of incorporation as it was raining most of the day and the mustard foliage was very wet. This should have ensured sufficient moisture to activate the myrosinase enzyme system which releases isothiocyanates. Evidence that some biological effect had been obtained was seen on 16 October (21 days post-incorporation). Weed re-growth (principally docks and thistles) was clearly suppressed in the plots that had grown mustard plants, although a similar effect was not seen in the BioFence plots.

The results of the wireworm counts completed in 2006 and 2007 are summarised in Figure 24. As may be expected, there were no significant differences in wireworm numbers between treatments at the pre-drilling or pre-incorporation sampling occasions in either year. However, there were also no significant differences between treatments at the post-incorporation assessment in 2006 ($F_{3,15}$ = 3.767, *P* = 0.818) or in 2007 (F3,15 = 0.530, *P* = 0.670), although fewest wireworms were found in the Caliente 99 plots in both years.



a)



b)

FIGURE 24. MEAN NUMBER OF WIREWORMS PER PLOT FOUND PRE-DRILLING, PRE-INCORPORATION OF BIOFUMIGANT TREATMENTS AND 21 DAYS POST-INCORPORATION IN 2006 (A) AND 2007 (B). ERROR BARS ARE STANDARD ERROR OF THE MEAN.

The results of the potato tuber damage assessments completed in 2008 for plots in which mustard was grown/BioFence was applied in 2006 only, are summarised in Figure 25. Similarly, potato tuber damage assessments completed in 2008 for plots in which Nemat was grown in 2006 and mustard was again grown/BioFence applied in 2007 are summarised in Figure 26.

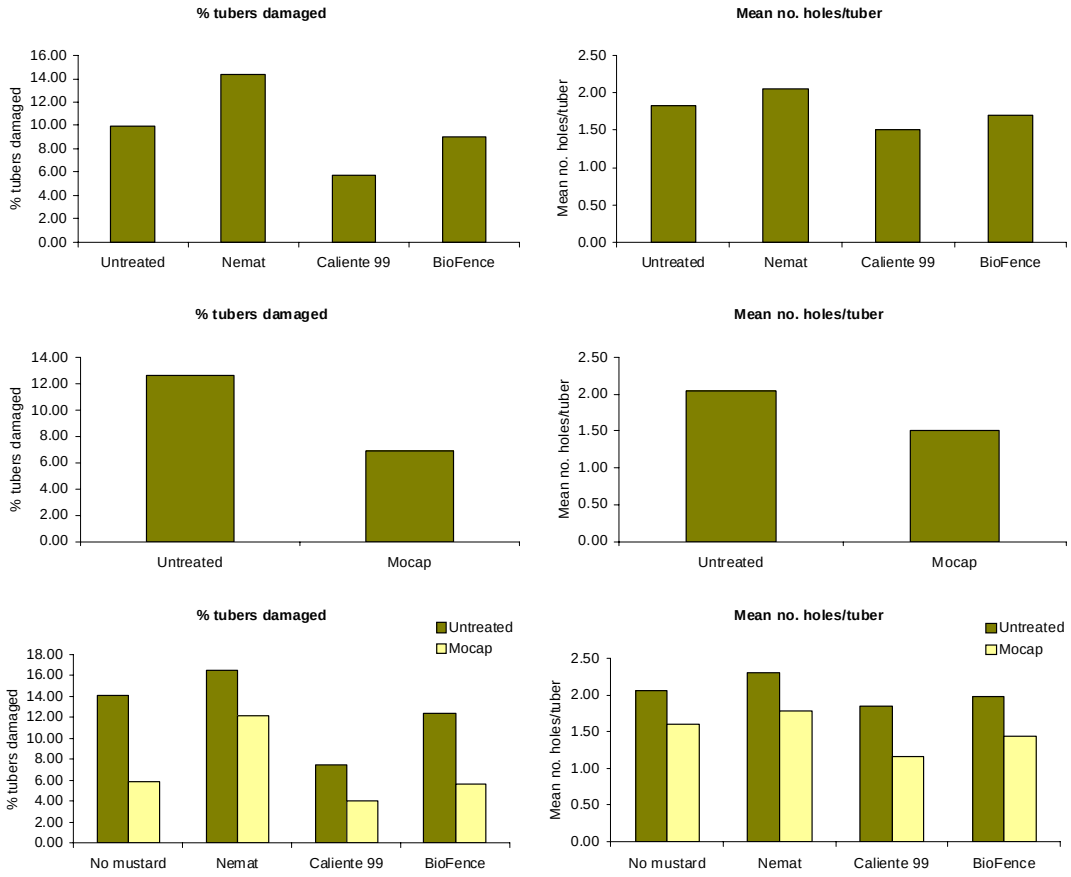


FIGURE 25. SUMMARY OF WIREWORM DAMAGE TO POTATO TUBERS GROWN IN PLOTS IN WHICH MUSTARD WAS GROWN/BIOFENCE APPLIED IN 2006 ONLY.

Analysis of the data summarised in Figure 25 showed that the percent of tubers with wireworm damage was significantly affected by the mustard treatment ($F_{3,35} = 3.86$, $P = 0.017$). The most effective of the mustard treatments was Caliente 99 and the least effective was Nemat. However, there was no significant difference in the number of wireworm holes per tuber. The efficacy of Mocap was confirmed with both a significant reduction in the percent of damaged tubers (F1,35 = 10.00, $P = 0.003$) as well as the number of wireworm holes per tuber (F1,35 = 12.97, $P = 0.001$). There was no interaction between mustard treatment and Mocap.

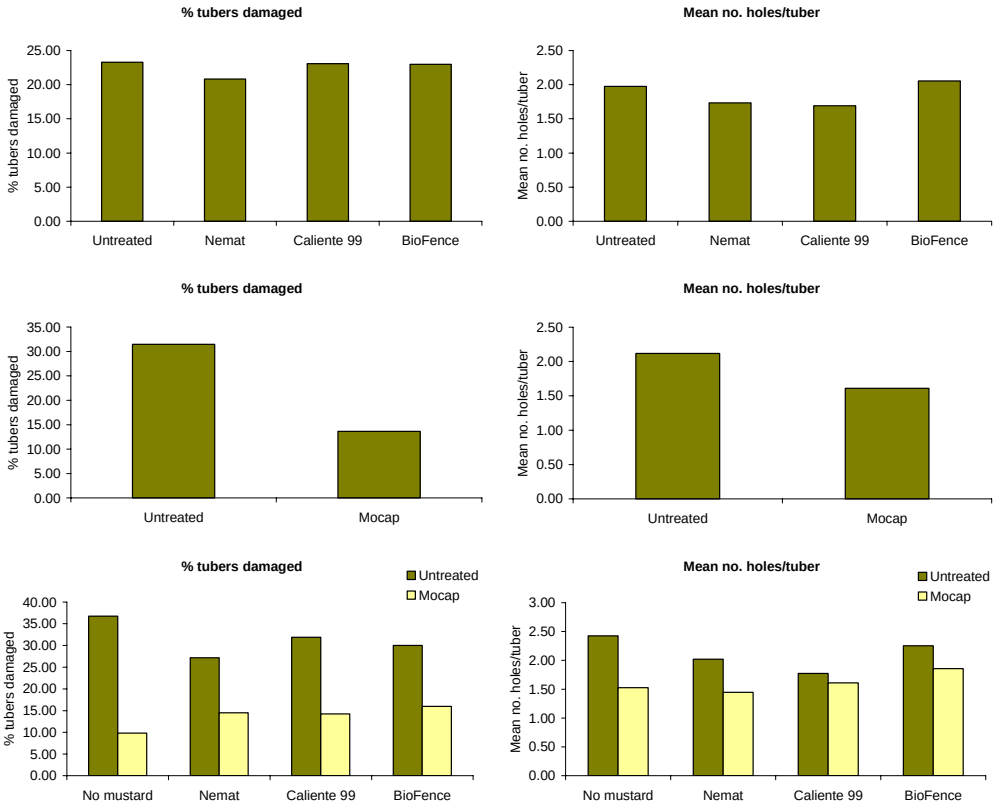


FIGURE 26. SUMMARY OF WIREWORM DAMAGE TO POTATO TUBERS GROWN IN PLOTS IN WHICH MUSTARD WAS GROWN/BIOFENCE APPLIED IN 2006 AND 2007.

Analysis of the data summarised in Figure 26 showed that, unlike in the previous set of data, the percent of tubers with wireworm damage was not significantly affected by the mustard treatment. However, application of Mocap was again found to significantly reduce the percent of damaged tubers ($F_{1,35} = 27.16$, $P < 0.001$) as well as the number of wireworm holes per tuber (F1,35 = 18.73, $P < 0.001$). Again there was no interaction between mustard treatment and Mocap.

2.6.4. Discussion

Results presented here contrast with those of Furlan *et al.,* (2004) where fresh mustard plant material and defatted mustard meal were found to be effective in killing range of *Agriotes* species *in vitro*. However, in the present study, soil moisture significantly affected wireworm mortality while rate of defatted mustard meal (BioFence) did not significantly affect mortality in pot trials completed. In addition, there was no evidence that the *Agriotes* species used in the study (thought to be *A. obscurus*) was less susceptible than those used by Furlan *et al.* (2004).

Similarly field trials using *Brassica juncea* (Caliente 99) and *Eruca sativa* (Nemat) incorporated as fresh plant material, and de-fatted mustard meal incorporated as BioFence pellets, were not shown to significantly reduce wireworm populations. The results of potato tuber damage assessments were also inconsistent, with a significant reduction in damage apparent when soils were treated with biofumigant once but not when treated twice, prior to growing potatoes.

However, incorporating fresh mustard plant material did reduce the presence of weeds while nutritional benefits to the soil and following potato crop should not be overlooked. Therefore, intercropping with mustard plants such as Caliente 99 or Nemat may be useful agronomically, although the beneficial effects do not appear to include significant control of wireworms.

None of the biocontrol agents tested in pot trials produced encouraging results with only low levels (<30%) of wireworm mortality recorded for all products tested. In contrast, Ansari *et al.* (2009) recorded 90-100% mortality of *A. lineatus* post inoculation when treated with a specific strain of *Metarhizium anisopliae*. While the importance of the entomopathogenic strain cannot be excluded, the two experiments also differed in the extent to which wireworm were exposed to the biological control agent. In the present study biocontrol agents were added to compost while Ansari *et al*. dipped wireworms into fungal suspensions, so that the wireworms were very likely to have been exposed to far more spores in the latter experiment.

2.7. Conclusions

A T-RFLP technique for a DNA-based method for identifying wireworms was developed and proof-tested. The method is reliable and time efficient and will be of value to those wishing to address problems in understanding wireworm ecology, control and risk assessment, in particular assessing spatiotemporal distributions in the agricultural landscape and associations between adult and larval distribution.

Data from the long-term rotational experiment at Babraham indicated that wireworm populations remained at pre-treatment levels in grass and minimum cultivation wheat plots but declined significantly in conventional cultivation wheat plots. Cultivation therefore appears to be important in determining wireworm population size and the wider adoption of minimum cultivation appears to be a potential explanation for the emergence of ‘arable wireworm’.

Wireworm populations were low throughout the experiment period at Babraham and at the limits of sensitivity for soil testing. In contrast, pheromone trapping was effective throughout the experiment period, catching large numbers of click beetles. Sensitivity of pheromone trapping was such that trapping duration may be reduced to just 3 hours. *Agriotes sputator* and *A. lineatus* were trapped in similar numbers at the start of the experiment, however, by 2009 *A. sputator* was the dominant species by number caught in pheromone traps. The mobility of adult *A. sputator* and *A. lineatus* populations was also apparent from interpolated maps produced in 2007.

Field work on biofumigants has not demonstrated a measurable effect on wireworm populations. However, the biofumigants did have a very noticeable impact on weed germination and re-growth in the plots where mustard was grown in 2007 and 2008. Mustard may then be a useful addition to the rotation, although not significantly affecting wireworm population size or damage to tubers.

There was no evidence from pot trials that defatted mustard meal (BioFence) or entomopathogenic nematodes or fungi may provide useful biological control of wireworms.

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4. APPENDICES

4.1. Scientific publications

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Parker, W., Lole, M. and Blackshaw, R. (in prep). Evaluation of biofumigants for the control of wireworms.

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