Research Report

Development of drip irrigation as a delivery system for the improved targeting and control of nematode pests in potatoes

Work undertaken between April 2004 and March 2007

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1. Summary

The purpose of this study was to examine whether drip irrigation techniques could be used to deliver doses of nematicide to achieve a more effective control of potato cyst nematode (PCN), compared to the use of granular nematicides by targeting the timing of applications to coincide with peaks in PCN hatch.

Samples of PCN collected from field sites were studied to understand their hatching profiles. There was a large variation between the total number of juveniles hatched from cysts from different sites. This can be explained by the different ages of the cysts and different periods since the last potato crop. Despite the large variation in the time when the peak hatch was reached, up to 50 % of juveniles generally hatched between 7-23 days after being exposed to Potato Root Diffusate. It is difficult to extrapolate findings from these controlled laboratory experiments to a field situation, as under laboratory conditions cysts are constantly exposed to fresh root exudates whereas under field conditions there will be more variation in the exposure to hatching stimuli depending on the development of the root system and the growth rate of the potato roots. Therefore, timing the application of liquid nematicide according to crop development may be a more reliable practice to maximise the efficacy of the applied nematicide rather than attempting to tailor the timing of application according to lab-based estimates of the hatching profiles of the different PCN populations present in a particular field.

During 2004, 2005 and 2006 a total of 8 replicated field trials were conducted to assess the efficacy of different application regimes of liquid oxamyl (Vydate® 10L, Du Pont) through a drip-irrigation system compared to the normal application of granular Vydate at planting.

The level of control obtained by application of Vydate® 10L by means of drip-irrigation was equivalent to that provided by an application of Vydate® 10 G (granule) as evidenced by Pf/ Pi and root invasion data. Results of field trials in 2005 and 2006 suggest that there is potential to decrease the dosage of Vydate® 10L to 75% compared to the normal dose of 5.5 l a.i. /ha and to still provide the same level of control as an application of Vydate® 10 G. Yields were not negatively affected by application of Vydate through a drip irrigation system. There were no additional benefits with regards to control of non-target pests (aphids, free living nematodes). This technology, if adopted, could lead to significant environmental benefits as there is no exposure of granules to either non-target organisms or the contractor applying the treatment.

2. Experimental Section

Introduction

Strategies for control of PCN (*Globodera pallida* and *Globodera rostochiensis*) mainly include the use of fumigant and non fumigant nematicides and crop rotation. Applications of nematicides are made before the crop is sown or at planting and large volumes of soil need to be treated requiring high use rates. The currently used fumigants are biocidal. Efficacy of the treatment can be highly variable and is reliant on a good surface seal to the soil. Due to the ability for PCN to multiply rapidly a fumigant treatment is usually followed by an application of a granular nematicide to control surviving viable cysts. Thus, applications of crop protection products are significant and costly.

Application rates of granular products are generally high (because there are no options for applications post planting) and products are required to persist for sufficient periods to prevent pest attack. In order to protect the root zone, products need to be effectively incorporated into the soil at planting. Product degradation has often begun before the crop begins to grow significantly so product use rate is less targeted at susceptible crop stages and may not persist for sufficient time to protect the vulnerable stages of the crop. Populations of *G. pallida* have become dominant at the expense of *G. rostochiensis* (due in part to the availability of *G. rostochiensis* resistance that is available in key varieties such as Maris Piper). *G. rostochiensis* populations are generally easier to control with granular nematicides as they tend to hatch earlier than *G. pallida,* so persistence of product is less of an issue. *G. pallida* populations have a greater temporal range of hatching and some populations may continue to hatch up to 10 weeks after sowing of the crop. At this point the degradation of the granular nematicide applied at planting is almost complete.

Concerns about the sustainability of current practices to control damaging populations of nematodes require the development of new and novel solutions to the problem if GB growers are to remain competitive. Furthermore, the use of high rates of crop protection products is less favoured because of potential environmental risks and the potential impact of food products that could contain residues. The safe use of products is also a pre-requisite and the development of closed system transfer systems that limit potential exposure risks to users and the non target environment is now a goal.

Project Aims

The aim of the research is to evaluate a novel delivery system for nematicides allowing the better targeting of PCN, especially *G. pallida*, using the minimum input of nematicide. The nematicide is delivered in a liquid form through a drip irrigation system. This approach will remove the risk to operators through the use of a closed system of delivery of the product and will also prevent the accidental risk of ingestion of product by non-target organisms that could potentially occur with granular formulation of the product. The risks to non-target soil organisms would be no greater than with the current delivery method.

The complicated inter-relationship between levels of nematicide in the soil, plant development and the behaviour of the different nematode species is fundamental to the long-term sustainability of the technique. A better understanding of pest biology with regard to hatching patterns could lead to a more targeted delivery of the nematicide if significant differences in hatching patterns occurred in different PCN populations.

The work was carried out to identify minimum concentrations of nematicide required under field conditions to prevent damage to potato crops and to control the build up of pest levels in the field. This was studied through field trials over a range of typical potato growing areas.

Free-living nematodes (e.g., *Trichodorus* species*)* which can transmit viruses causing loss of quality could potentially be controlled using drip irrigation applied nematicides. As Vydate is taken up systemically by the crop another beneficial side effect could be the control of sap-feeding insects, most importantly, aphids. This potential effect was also evaluated in the study.

Material and methods

Field trials to study the efficacy of liquid oxamyl applied using drip irrigation.

Field trials to assess both the efficacy and optimise the application system were carried out during 2004-2006. An overview of the treatments used and the applications dates of the different treatments are given in Tables 1 & 2. Seven out of the eight trials were situated in commercial potato fields and the crop husbandry conformed to standard agronomic practice. The 2006 trial at Long Hoos was planted within a wheat field. The potato variety grown was cv. Maris Piper at the G's, Woburn and Long Hoos sites and cv. Saturna at the Chennels site. The field trials were a split plot design with 4 replicates for each treatment. In 2006 at the Chennels and G's sites each treatment block receiving an application of Vydate® was paired with an untreated control to counteract the spatial variability in PCN infestations.

The drip irrigation was set up as for normal commercial practice under guidance from one of the project industrial partners (Field GB). The application of both the granular and liquid oxamyl was carried out by Agrisearch. The granular oxamyl was applied to the soil prior to ridging and planting using a purpose made wheeled granule spreader. The liquid oxamyl (Vydate® 10L) was applied by means of the above mentioned drip irrigation system. The delivery system for the liquid nematicide was a proto–type supplied by Cypherco Ltd.

The application rates of the liquid nematicide were based on a previous laboratory study carried out at Rothamsted and sponsored by DuPont in 2002. In this study it was shown that the number of cysts was significantly smaller at the end of the 60 day trial in treatments with liquid oxamyl at an equivalent rate of 5.5 kg a.i. /ha. compared to the control treatment. It was also shown that split dose applications (equalling a total of 5.5.kg/ha a.i) at either 4, 7 or 14 day intervals led to a significant smaller number of cysts compared to the control treatment. In a field situation, split dose applications can be advantageous to synchronise the application of pesticide with expected peaks in juvenile hatching from cysts related to crop development.

TABLE 1: TREATMENTS USED IN FIELD TRIALS DURING 2004, 2005 AND 2006

	Chennels 2004	G's 2004	Chennels 2005	G's 2005	Woburn 2005	Chennels 2006	G's 2006	Long Hoos 2006
Control	√	√	√	√	√	√	√	√
Granular Vydate 55kg / ha at planting	√	√	√	√	√	√	√	√
Liquid Vydate 55 l/ha at planting	√	√						
Liquid Vydate 55l/ha targeted			√	√	√	√	√	√
6 applications @ 14 day interval 9.1 l/ha	√	√						
wk 1,4 and 7 after planting 13.3 l/ ha	√	√						
wk 1,4 and 7 after planting 18.3 l/ha	√	√	√	√	√			
3 times targeted 18.31l/ha			√	√	√	√	√	√
2 times targeted 35.8 l/ha and 19.3 l/ha			√	√	√			
2 times targeted 20.6 l/ha (75% dose)			√	√	√	√	√	

TABLE 2: TREATMENT DAYS EXPRESSED AS DAYS AFTER PLANTING FOR THE DIFFERENT TREATMENT REGIMES DURING THE 2004, 2005 AND 2006 FIELD TRIALS.

	Chennels 2004	G's 2004	Chennels 2005	G's 2005	Woburn 2005	Chennels 2006	G's 2006	Long Hoos 2006
Date planted	3 May	17 May	3 May	9 May	29 April	3 April	3 May	25 April
Treatment								
Granular Vydate 55kg / ha at planting	0	0	0	0	0	0	0	0
Liquid Vydate 55 l/ha at planting	0	0						
Liquid Vydate 55l/ha targeted			15	11	18	38	17	23
6 app. @ 14 day interval 9.1 l/ha	7, 21 38, 52 66, 90	7, 21 38, 52 66, 90						
wk 1,4 and 7 after planting 13.3 l/ ha	7, 28 49	7, 28 49						
wk 1,4 and 7 after planting 18.3 l/ha	7, 28 49	7, 28 49	7, 29 51	7, 28 49	7, 29 47			
3 times targeted 18.31l/ha			15,3670	11, 23 62	18, 39 69	38,59.80	17 37 48	23,44,65
2 times targeted 35.8 l/ha and 19.3 l/ha			15 44	11, 28	18, 47			
2 times targeted 20.6 l/ha (75% dose)			15 36	11, 23	18, 39	38,59	17 37	

Root invasion and life stage studies

During the growing season root samples were collected in order to study root invasion and development of the juveniles as influenced by the different treatments. The root samples were collected at the time of application of the liquid Vydate® (in 2004, 2005) or one week after application (2006). The roots were washed and stained in a 0.05 % acid fuchsin lactoglycerol solution (Southey, 1986). A 10 gram sub-sample of the stained roots was put in 100 ml of distilled water and homogenised using a blender. From each sample 3 replicate sub-samples of 10 ml were assessed for the presence of Globodera *spp*. The individuals that were found were assigned to the corresponding life-stages (J2, J3, female J4, male J4, mature female, mature male.

Pf/ Pi ratio

Prior to planting soil samples were collected to establish the initial cyst density (Pi) in each plot. The samples were taken from the two inner rows over a 3 meter length, approximately the same place was chosen for the Pi and Pf samples. The bulk sample consisted of around 30 subsamples taken with a cheese corer in a zigzag pattern. Cysts were extracted from 200 gram of air dried soil using the Fenwick can method (Fenwick, 1949). The number of eggs was counted from a 25 cyst random aliquot to establish the number of eggs per gram dry soil, a standard procedure for expressing the PCN density of any given field. The cysts were crushed to release the eggs and suspended in a set volume, an aliquot of this was used to count the eggs (Bijlo, 1954). At the end of the growing season soil samples were again collected and used to determine the final PCN density (Pf). Again the number of eggs per cyst was counted. This information was used to calculate the Pf/ Pi ratio for both the number of cysts and for the number of eggs per gram soil.

Yield

At the end of the growing season yields were assessed after the haulms had been killed of. The tubers were graded according to the following categories undersize (<45 mm), marketable (45-85 mm) and oversize (>85mm). A 1 metre 3 row section was harvested from each plot. Diseased, damaged and misshapen tubers were graded out. The yield is expressed as the mean in kg of the treatment blocks.

The fate of field applied oxamyl

In 2006, the fate of field applied Vydate® was monitored in the three field trial sites. The following treatments were sampled:

- 7 days after each application of liquid Vydate® (see Table 2 for dates);
- Vydate® 10G (5.5 kg a.i /ha);
- Vydate® 10L (5.5.l a.i./ha) applied once coinciding with crop emergence;
- and Vydate® 10 L (1.83 l a.i. /ha applied at crop emergence and at two 3 weekly intervals).

For each treatment samples were taken from 3 replicate plots. The Vydate® 10G treatment blocks were only sampled on the first date, the other treatment blocks were sampled on all three dates. On the first date the sample collected was split in a 0-15 cm and a 15-30 cm below soil level sample, on both other dates the samples were taken from the 0-30 cm section below soil level. Sampling was done by exposing a soil profile in the potato ridge and taking out a 15 x 5 cm area down to the required depth. The drip-irrigation tape was situated just below the soil surface. This soil was mixed and a 200 ml sample was taken. Samples were cooled immediately and taken to SAL (Scientific Analysis Laboratories, Bar Hill, Cambridge) for HPLC analysis of oxamyl residues.

Hatch profiles and development of a hatching assay

The novel application system of liquid nematicides through drip irrigation allows for timed applications. There is some anecdotal evidence that emergence patterns of juveniles from cysts differ between different field populations and drip irrigation applied nematicide could be applied when peaks in emergence occur if this is consistent in different seasons. In this study hatching profiles of the different field populations used in the 2004, 2005 and 2006 field trials were determined, this with a view to evaluate the validity of the above approach. Juveniles of PCN (*Globodera* spp.) hatch from cysts in the soil under the influence of root exudates from their host plant.

The hatching profiles were obtained by soaking cysts in potato root diffusate (PRD) and recording emergence of juveniles over time. Potato root diffusate was extracted from greenhouse grown potato plants cv. Maris Piper. For each population a total of 30 cysts (5 replicates) were used, after each observation the PRD was drawn off and replaced with fresh PRD. In between observations the samples were kept in a dark incubator at 18˚C.

The hatch profiles obtained by soaking in PRD are likely to be influenced by the variations inherent to a product extracted from growing plants, this is especially relevant when comparing hatching profiles from different years. There is also a considerable cost, both monetary and in labour, attached to obtaining PRD. To address these issues a reliable hatching agent to replace PRD would be useful. A preliminary test evaluated the use of picrolinic acid as an alternative agent for establishing hatching. However, as indicated in earlier work (Greet, 1974), picrolinic acid does elicit a strong hatch in *G. rostochiensis* but fails to do so in *G. pallida*. In previous studies both zinc chloride (Greet, 1974) and sodium metavanadate (Greet, 1974; Whitehead, 1992) have been shown to hatch cysts of *G. pallida*.

A study was conducted to assess whether hatch profiles obtained by sodium metavanadate and zinc chloride solutions are comparable to hatch profiles obtained by PRD.

Cysts of *G. palli*da from 3 populations obtained from fields used in the 2006 field trials (Severals, Chennels and Long Hoos) were used. The basic procedure was similar to the hatching assay described above using PRD with additional treatments, each treatment contained 25 cysts (3 replicates). The following solutions were used: tap water (control), PRD, sodium metavanadate (1 mM) and zinc chloride (3 mM).

The effect of drip irrigation applied oxamyl on non–target pests

To assess whether potential control of non-target pests can be achieved using drip irrigation applied oxamyl two experiments were carried out in the 2006 field season. One experiment looked at the effect of drip-irrigation applied oxamyl on aphids, which are a major pest problem primarily due to their role as virus vectors. The other experiment looked at the effect of drip-irrigation on plant parasitic nematodes other than *Globodera spp.*, nematodes in the family Trichodoridae are known vectors for Tobacco Rattle Virus the causal agent of spraing in potato tubers (Ploeg, 1992).

The effect of drip irrigation applied oxamyl on aphids

The study to look at the additional control of aphids by means of drip irrigation applied oxamyl as a nematicide was carried out in the 2006 field season. The replicated split plot trial at Long Hoos was used to conduct the study. The control treatment blocks (5,7 and 9) and the

treatment blocks that received 3 equal applications of drip-irrigation applied liquid oxamyl were used.
Two species of aphid *Myzus persicae* (peach-potato aphid) and *Macrosiphum euphorbiae* (potato aphid) were used in the experiment. The aphids were put on the potato leaves in clip cages.

In each treatment block 6 clip cages were put on individual plants (i.e. one clip cage per plant), each cage contained one mature female aphid. In each block there were 3 clip cages with *M. persicae* and 3 clip cages with *M. euphorbiae*. From each treatment block 3 leaves were collected from a plant without a clip cage to be analysed for the level of oxamyl in the leaf material. This analysis was carried out by SAL (Scientific Analysis Laboratories, Bar Hill, Cambridge). The clip cages were monitored every three days during a 9 day period and the state of the aphid (dead or alive) was recorded. The number of juvenile aphids was recorded if present and new progeny were removed after each assessment.

The effect of drip irrigation applied oxamyl on free living nematodes

To study the effect of drip irrigation applied oxamyl on nematodes other than *Globodera* spp. soil samples were taken from the three replicated field trials in 2006. The treatments sampled were Vydate® 10 G (granular) applied once at planting at a rate of 55 kg/ ha, Vydate® 10 L (liquid) applied once coinciding with crop emergence and the control treatment. A composite sample was taken from each treatment block, after mixing nematodes were extracted from 200 gram field soil using the Whitehead tray technique (Whitehead & Hemming, 1965). The total number of nematodes in each sample was counted and the number of plant parasitic nematodes was recorded at 400 x magnification.

Statistical analysis

Statistical analysis was carried out using ANOVA, $p \leq 0.05$ after checking for equality of variance.

Results

Field trials to study the efficacy of liquid oxamyl applied using drip irrigation

2004

The results from the field trials conducted during the 2004 field season are shown in Figures 1 to 8. In all figures, except Figure 4, the treatments are as follows:

1. Granular Vydate® (55 kg/ha) at planting by soil incorporation.
2. Liquid Vydate® (55 l/ha) at planting by drip irrigation.
3. Liquid Vydate®, 6 applications of 9.1 l/ha at 14 day intervals.
4. Liquid Vydate®, 3 applications of 18.3 l/ha at 1,4 and 7 weeks after planting
5. Liquid Vydate®, 3 applications of 13.3 l/ha at 1,4 and 7 weeks after planting
6. Control no application of nematicide.

The results showing Pf/Pi ratios for *Globodera* spp. under different treatments are shown in Figures 1 and 6 for the field sites Chennels and G's, respectively. The Pf/ Pi ratio expressed as eggs per gram is lowest for treatment 4 (3 applications of 18.3 l/ha) although there is no

significant difference between this treatment and the control or the other treatments (ANOVA, $p \leq 0.05$).

The total number of nematodes recovered from one gram root at 34 and 68 days after planting at field site Chennels is shown in Figures 2 and 3. There was no significant difference between the control and any of the treatments or between the treatments (ANOVA, $p \leq 0.05$).The number of nematodes recovered from one gram root samples and categorised according to the life stage for field site Chennels is shown in Figure 4. The number of nematodes recovered from one gram root samples and categorised according to the life stage for field site G's is shown in Figure 7. There was no significant difference between the treatments and the control treatment at the field site Chennels and the field site G's (ANOVA, $p \leq 0.05$). The yield data categorised in marketable and undersized are shown in Figures 5 and 8 for the field sites Chennels and G's, respectively. There was no significant difference in the yield between the control treatment and any of the treatments (ANOVA, $p \leq 0.05$).

FIGURE 1: THE MULTIPLICATION OF PCN (PF/PI RATIO) VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE CHENNELS IN 2004.

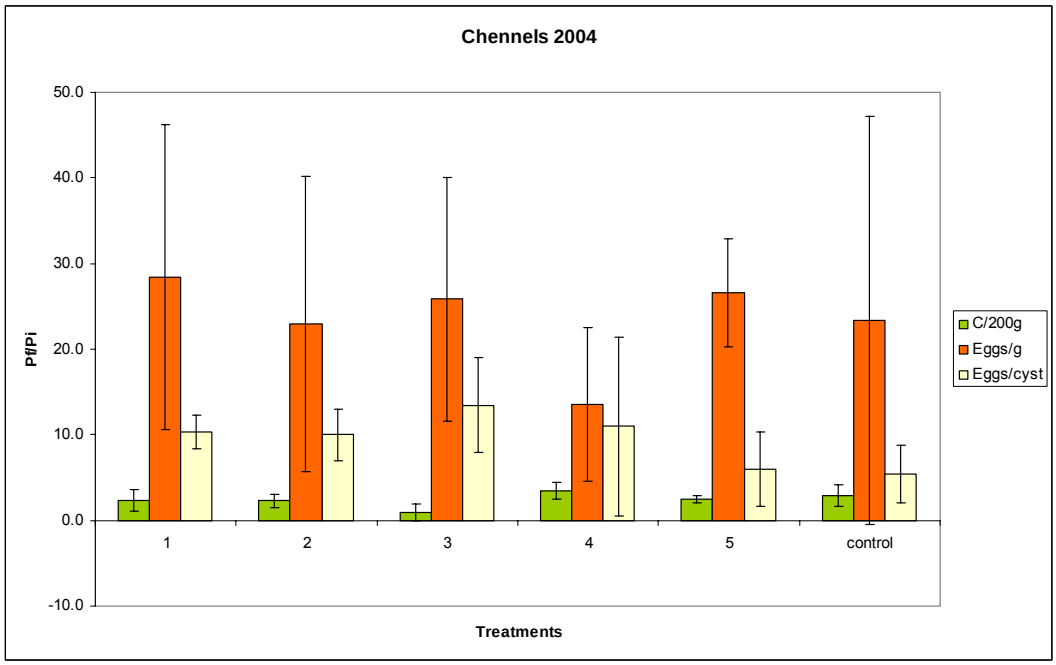


FIGURE 2: TOTAL NUMBER OF ALL LIFE STAGES OF PCN, FROM ROOTS SAMPLED 34 DAYS POST PLANTING AT FIELD SITE CHENNELS IN 2004 (ANOVA P≤0.05, LSD= 417.78).

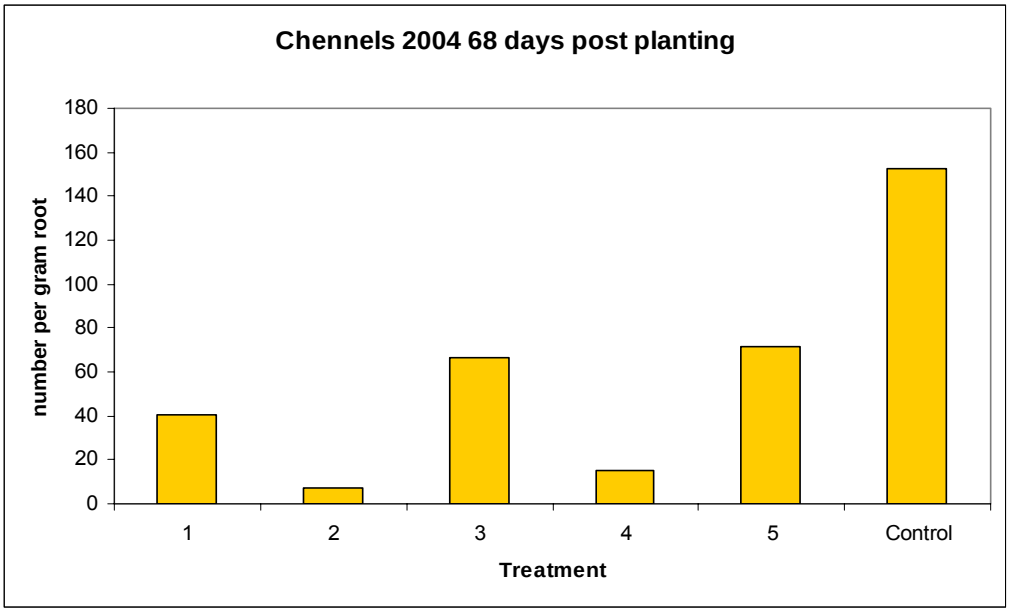


FIGURE 3: TOTAL NUMBER OF ALL LIFE STAGES OF PCN, FROM ROOTS SAMPLED 68 DAYS POST PLANTING AT FIELD SITE CHENNELS IN 2004 (ANOVA P≤0.05, LSD= 298.17).

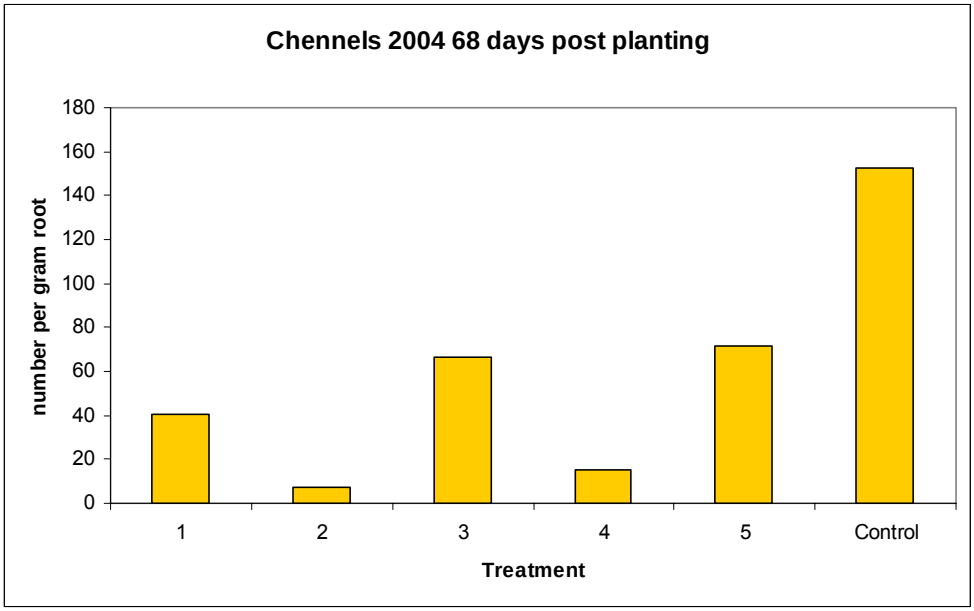


FIGURE 4: THE NUMBER OF NEMATODES PER LIFE STAGE PER GRAM OF ROOT AT THE SIX SAMPLE DATES AT CHENNELS, 2004. NO SIGNIFICANT DIFFERENCES WERE FOUND BETWEEN TREATMENTS. TREATMENT 1 IS THE CONTROL, TREATMENT 2-6 ARE THE SAME AS TREATMENT 1-5 ABOVE.

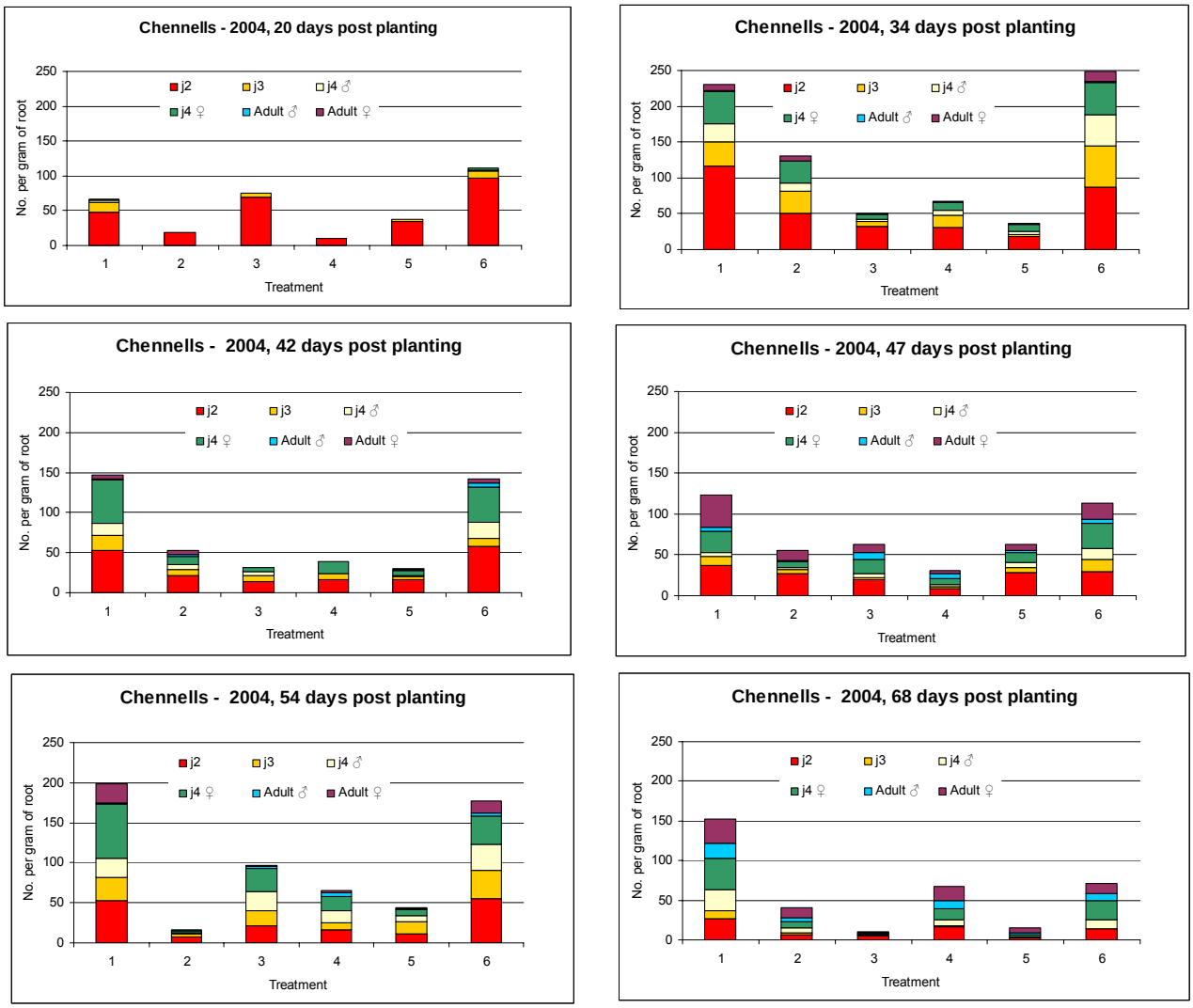


FIGURE 5: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE AND UNDERSIZED (< 55 MM) CATEGORIES (ANOVA P≤0.05, LSD 16.4).

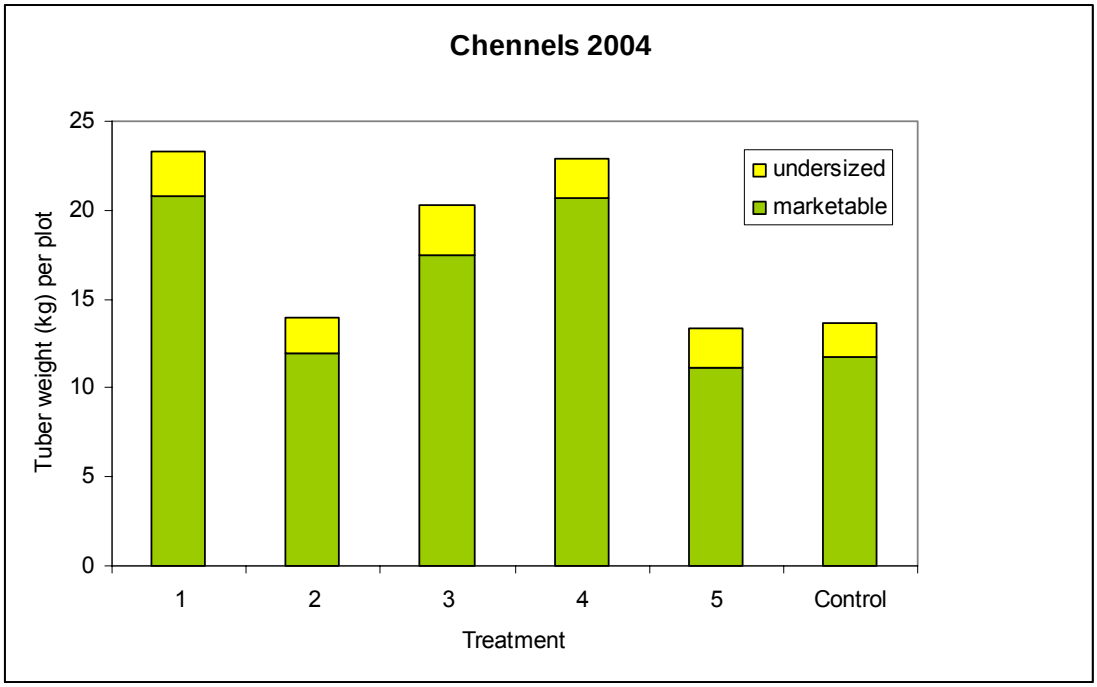


FIGURE 6: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER VOLUME OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE CHENNELS IN 2004.

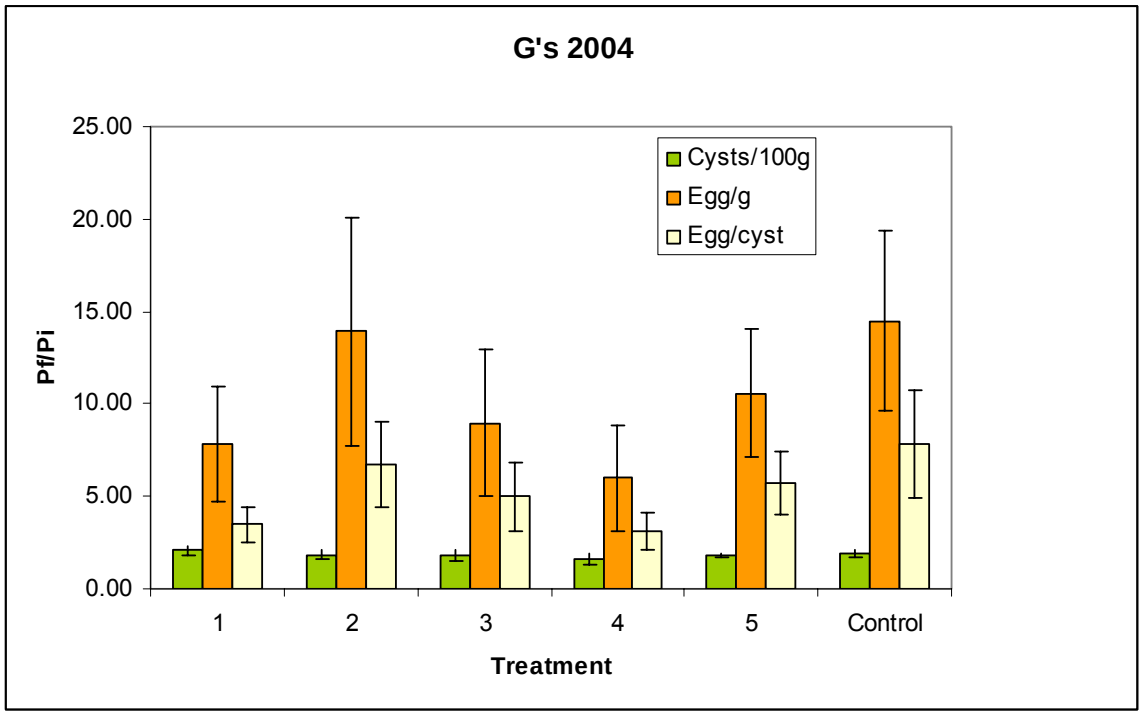
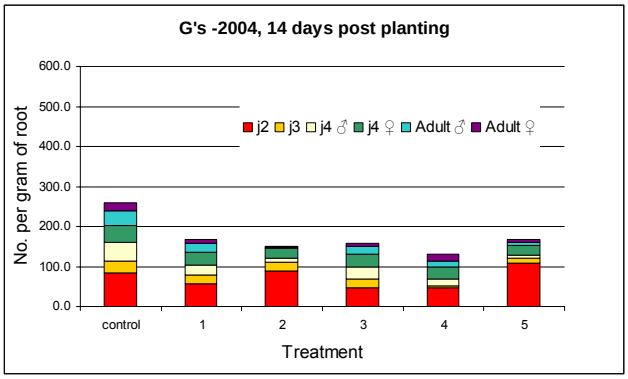
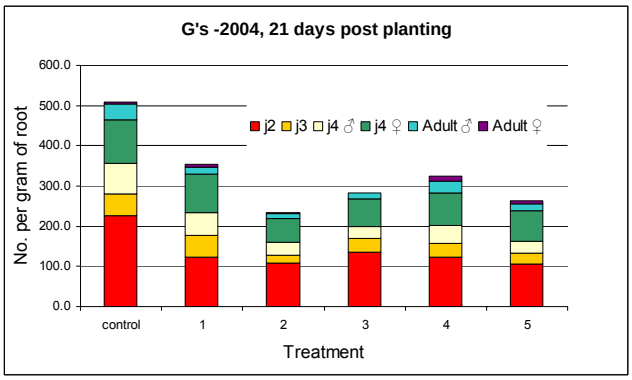
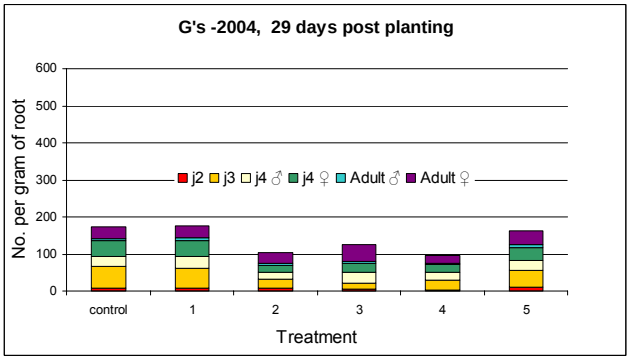
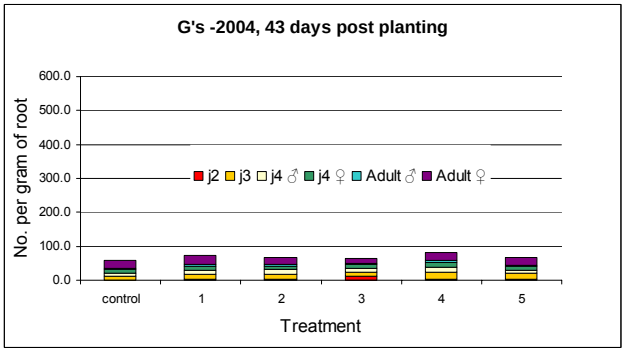


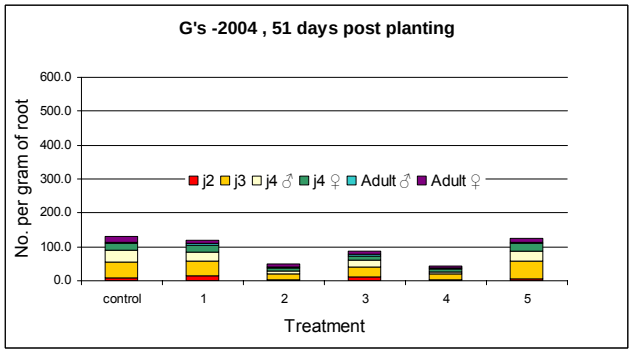
FIGURE 7: THE NUMBER OF NEMATODES PER LIFE STAGE PER GRAM OF ROOT AT THE SIX SAMPLES DATES AT G'S, 2004.











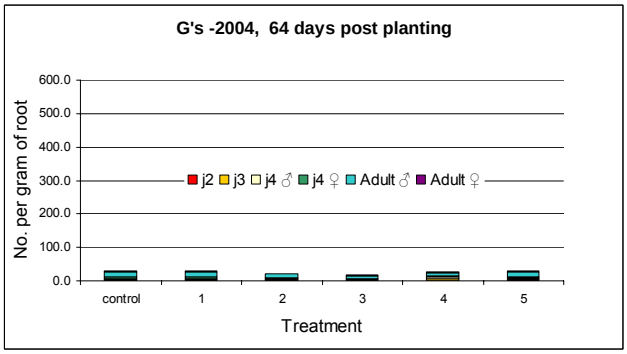
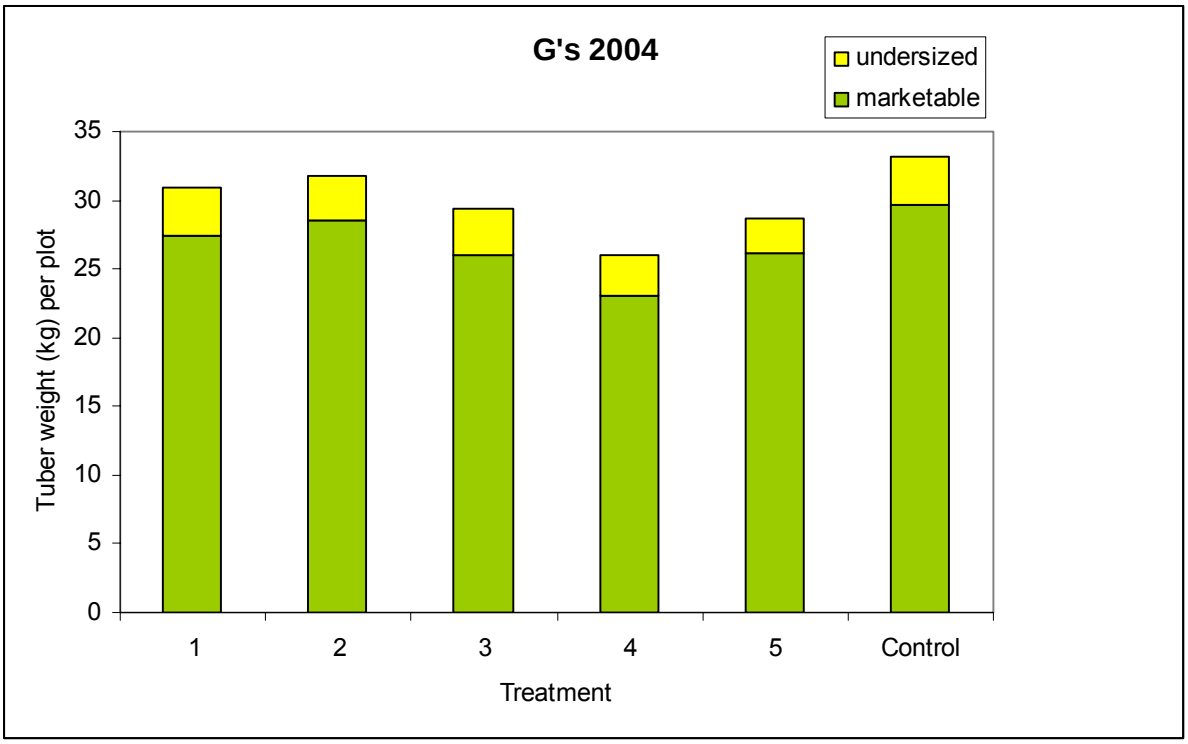


FIGURE 8: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE AND UNDERSIZED (< 55 MM) CATEGORIES (ANOVA P≤0.05, LSD 14.1).



2005

The results from the field trials conducted during the 2005 field season are shown in figures 9 to 17. Figures 9 to 11 show the results from the field trial at Chennels. Figures 12 to 14 show the results from the field trial at G's. Figures 15 to 17 show the results from the field trial at Woburn. In all figures the treatments are as follows:

1. Granular Vydate®, (55 kg/ha) at planting by soil incorporation.
2. Liquid Vydate®, (55 l/ha) timed with crop emergence.
3. Liquid Vydate®, 3 applications of 18.3 l/ha at 1, 4 and 7 weeks after planting.
4. Liquid Vydate®, 3 applications of 18.3 l/ha timed with crop emergence and subsequent crop development.
5. Liquid Vydate®, 2 applications of 35.8 l/ha and 19.3 l/ha timed with crop emergence and subsequent crop development.
6. Liquid Vydate®, 2 applications of 20.6 l/ha (75 % of commercially recommended dose) timed with crop emergence and subsequent crop development.
7. Control treatment

There was no significant difference in the Pf/Pi ratio between the control treatment and any of the other treatments at any of the three field sites (ANOVA, p≤0.05). There was no significant difference in the number of nematodes recovered between the control treatment and any of the other treatments at any of the three field sites (ANOVA, p≤0.05).

The yield data categorised in marketable, undersized and oversized are shown in Figures 11 and 14 for the field sites Chennels and Severals, respectively. In 2005 no grading of tubers took place at Woburn so yields are shown as total yield (Figure 17). There was no significant difference in yield between the control treatment and any of the other treatments at any of the field sites (ANOVA, p≤0.05).

FIGURE 9: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER 200 GRAM OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE CHENNELS IN 2005.

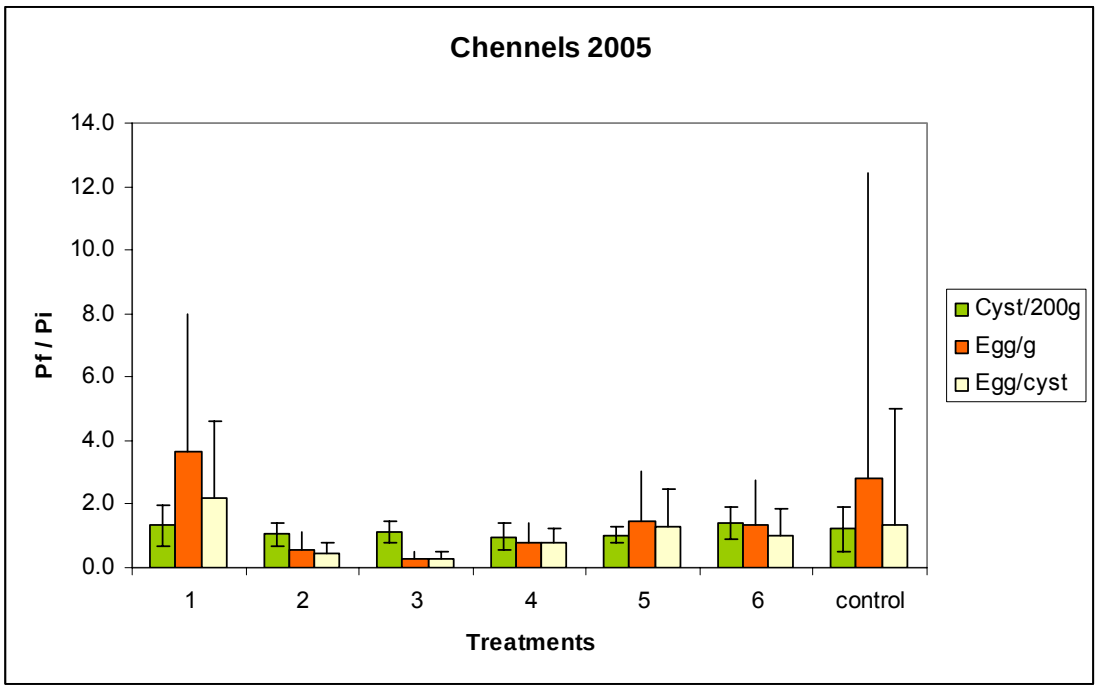


FIGURE 10: THE NUMBER OF NEMATODES PER LIFE STAGE PER GRAM OF ROOT AT FOUR SAMPLES DATES AT CHENNELS, 2005.

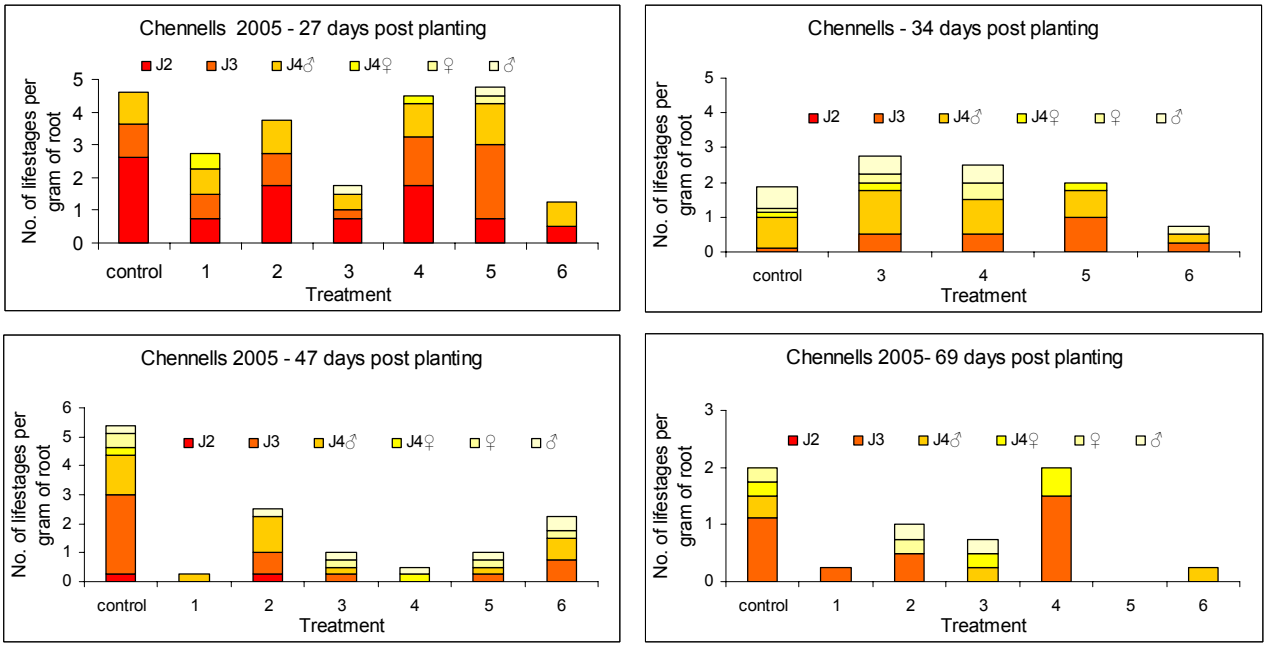


FIGURE 11: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE AND UNDERSIZED (< 55 MM) CATEGORIES (ANOVA P≤0.05, LSD 6.52) FOR FIELD TRIAL CHENNELS IN 2005.

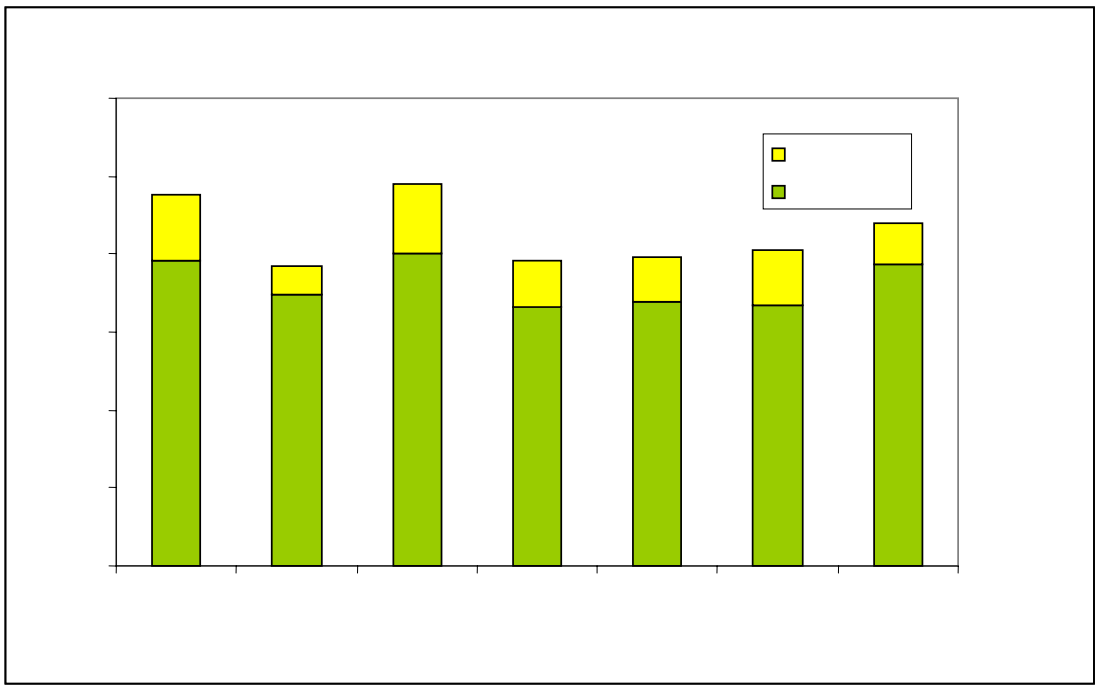


FIGURE 12: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER VOLUME OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE G'S IN 2005.

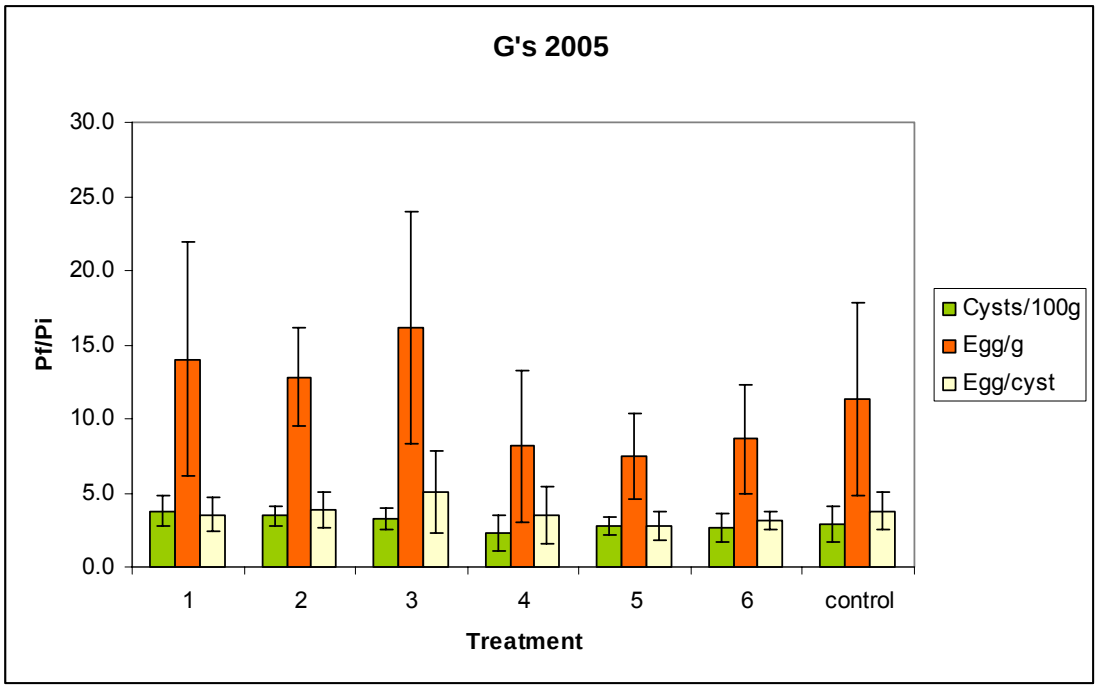


FIGURE 13: THE NUMBER OF NEMATODES PER LIFE STAGE PER GRAM OF ROOT AT THREE SAMPLE DATES AT G'S, 2005.

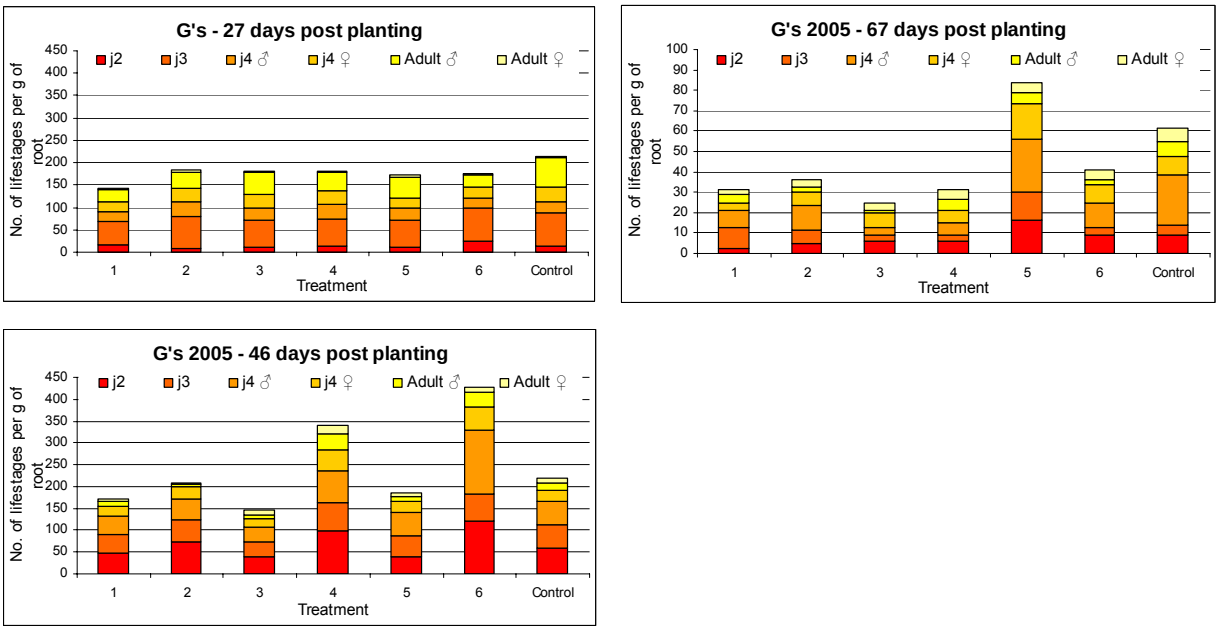


FIGURE 14: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE, UNDERSIZED (< 45 MM) AND OVERSIZED (> 85MM) CATEGORIES FOR THE FIELD TRIAL G'S IN 2005. (ANOVA P≤0.05, LSD 3.13).

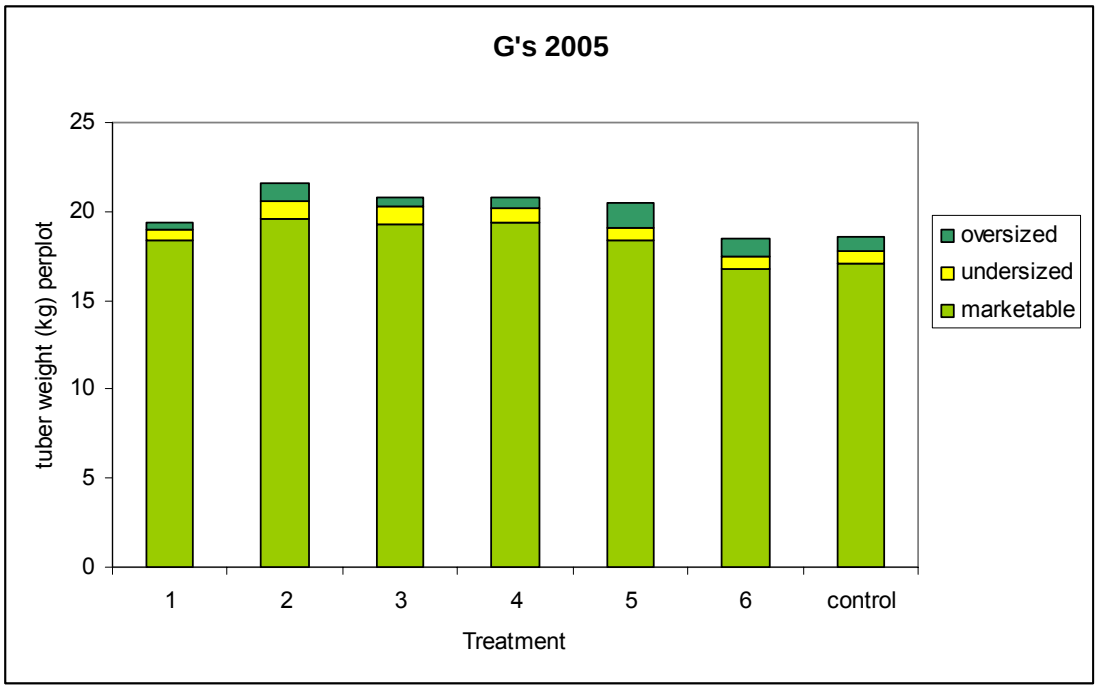


FIGURE 15: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER 200 GRAM OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE WOBURN IN 2005.

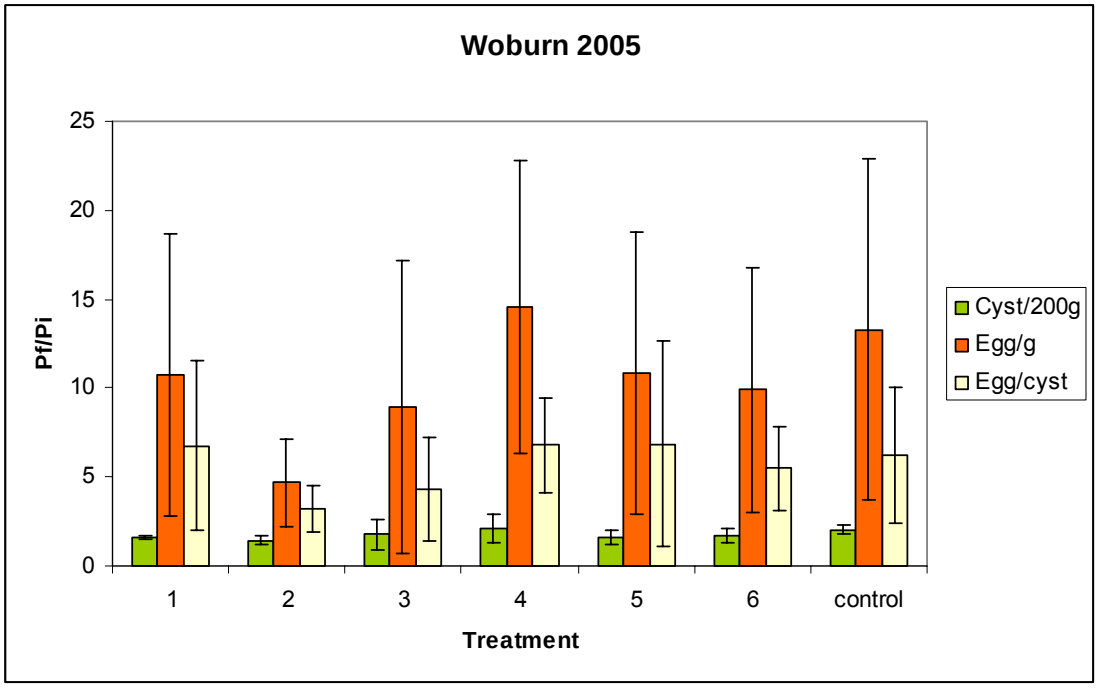
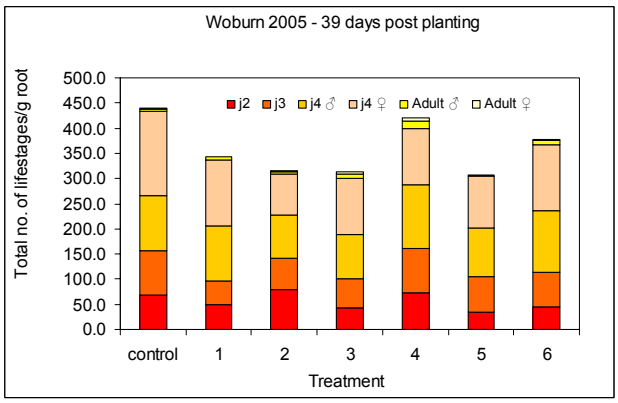
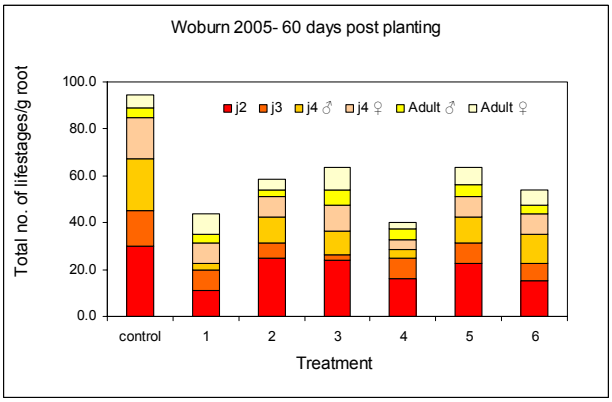


FIGURE 16: THE NUMBER OF NEMATODES PER LIFE STAGE PER GRAM OF ROOT AT THREE SAMPLE DATES AT WOBURN, 2005.





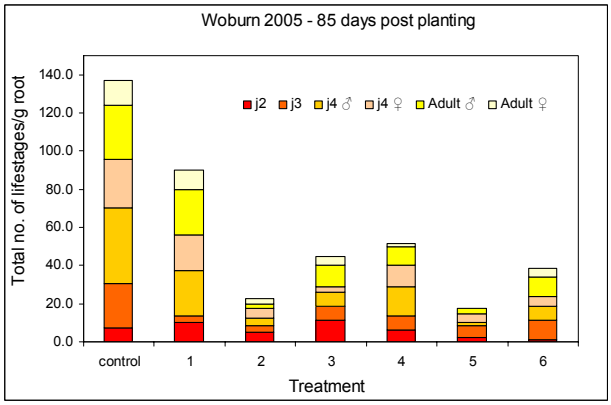
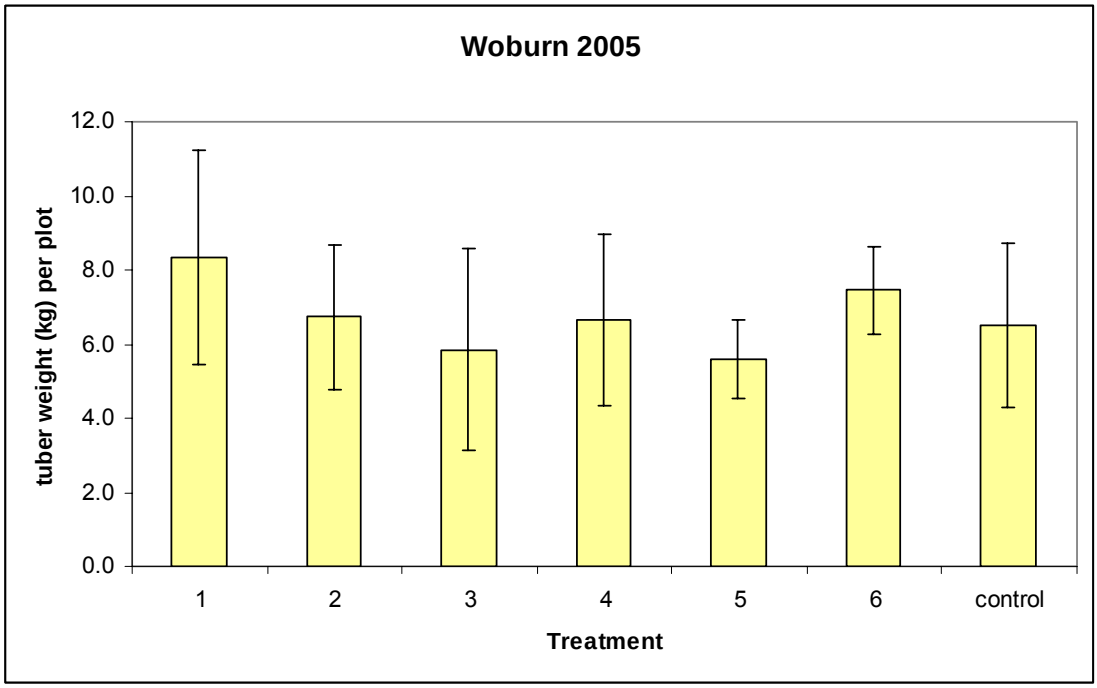


FIGURE 17: THE AVERAGE TOTAL YIELD PER TREATMENT PLOT. (ANOVA P≤0.05, LSD 13.52).



2006

The results from the field trials conducted during the 2006 field season are shown in figures 18 to 26. Figures 18 to 20 show the results from the field trial at Chennels , Figures 21 to 23 show the results from the field trial at G's and figures 24 to 26 show the results from the field trial at Long Hoos. In all figures the treatments are as follows:

1. Granular Vydate®, (55 kg/ha) at planting by soil incorporation.
2. Liquid Vydate®, (55 l/ha) timed with crop emergence.
3. Liquid Vydate®, 3 applications of 18.3 l/ha timed with crop emergence and subsequent crop development.
4. Liquid Vydate®, 2 applications of 20.6 l/ha (75 %of commercially recommended dose) timed with crop emergence and subsequent crop development.
5. Control treatment.

There was no significant difference in the Pf/Pi ratio between the control treatment and any of the other treatments at any of the three field sites (ANOVA, p≤0.05). There was no significant difference in the number of nematodes recovered between the control treatment and any of the other treatments at any of the three field sites (ANOVA, p≤0.05) except for the sample date 25 May at field site Long Hoos where the number of nematodes recovered from root samples was significantly lower in treatment one (Granular Vydate® (55 kg/ha) at planting by soil incorporation) compared to the control treatment.

The yield data categorised in marketable, undersized and oversized are shown in Figures 20, 23 and 26 for the field sites Chennels, Severals and Long Hoos, respectively. There was no significant difference in yield between the control treatment and any of the other treatments at the field sites Chennels and Severals (ANOVA, p≤0.05). The yield was significantly higher in the treatment 1 and 2 (Granular Vydate® (55 kg/ha) at planting by soil incorporation and an application of Liquid Vydate® (55 l/ha) timed with crop emergence) compared with the control treatment.

FIGURE 18: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER 200 GRAM OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE CHENNELS IN 2006.

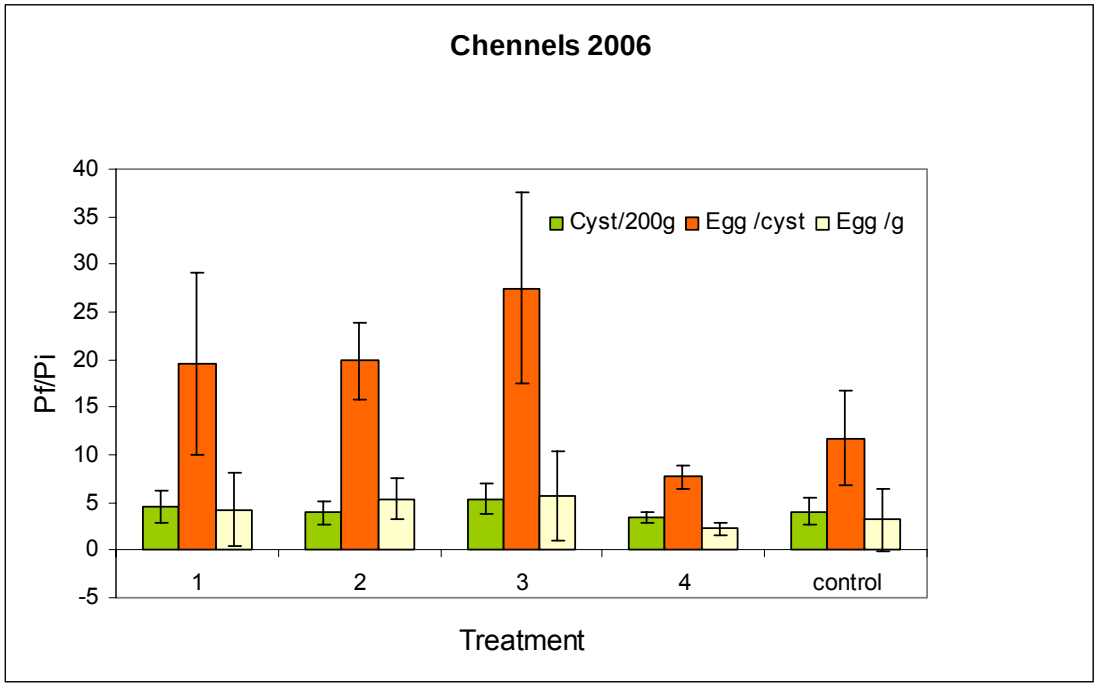


FIGURE 19: TOTAL NUMBER OF ALL LIFESTAGES OF PCN, FROM ROOTS SAMPLED ON 3 DATES AT FIELD SITE CHENNELS IN 2006.

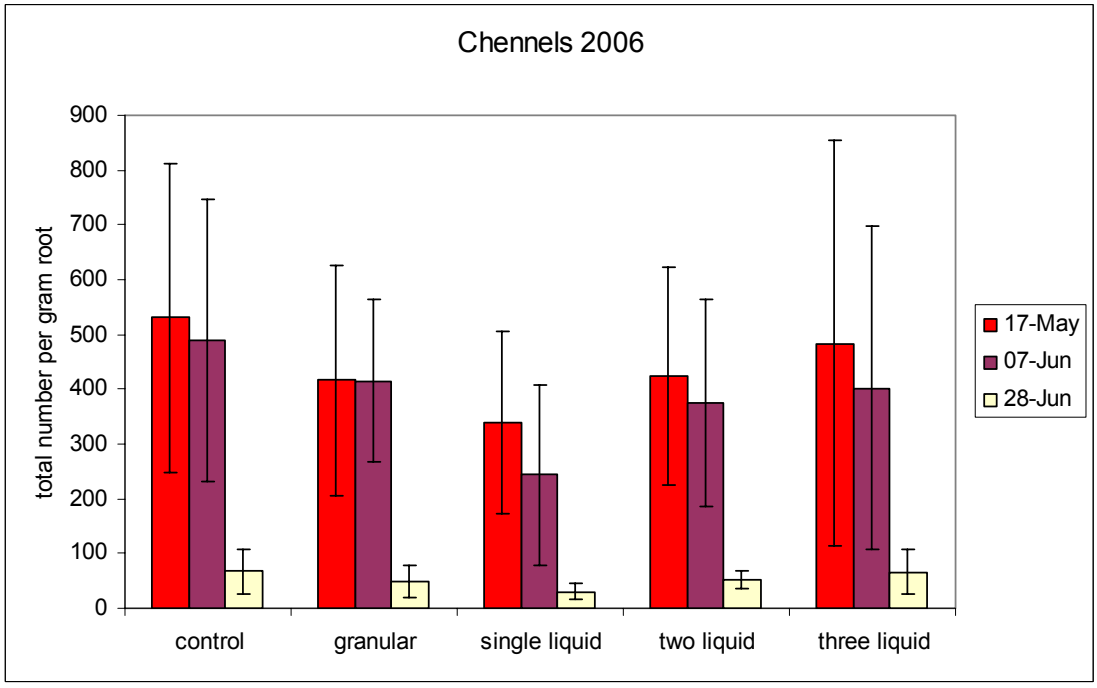


FIGURE 20: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE AND UNDERSIZED (< 55 MM) CATEGORIES (ANOVA P≤0.05, LSD 4.51).

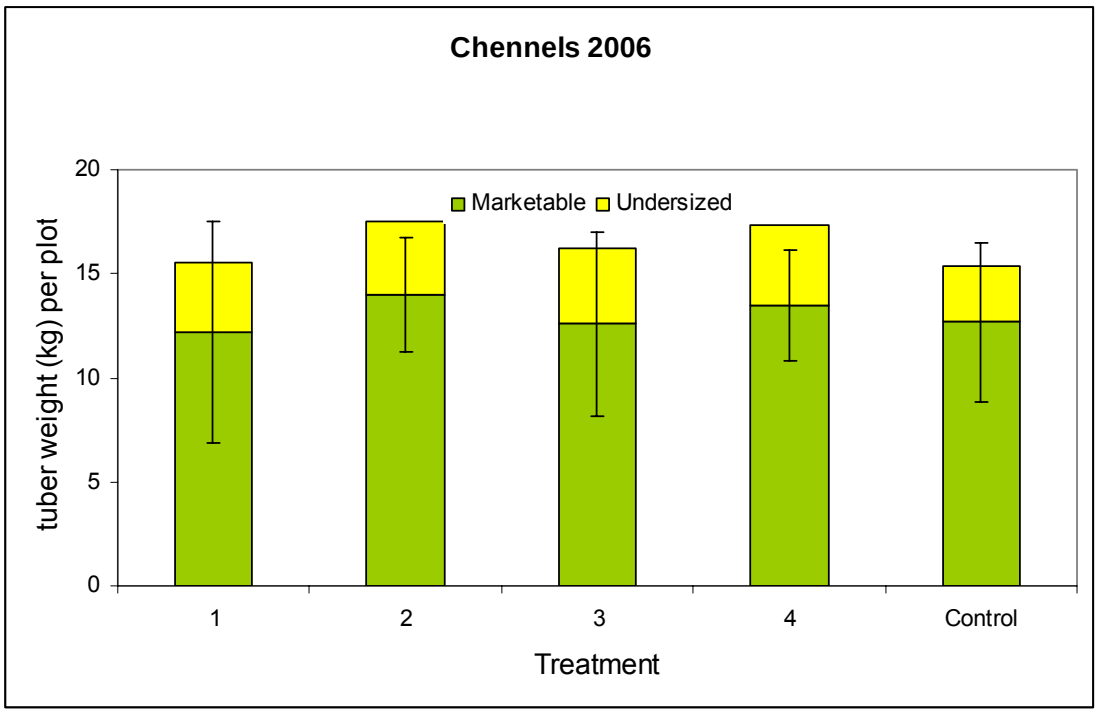


FIGURE 21: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER 100 GRAM OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE G'S IN 2006.

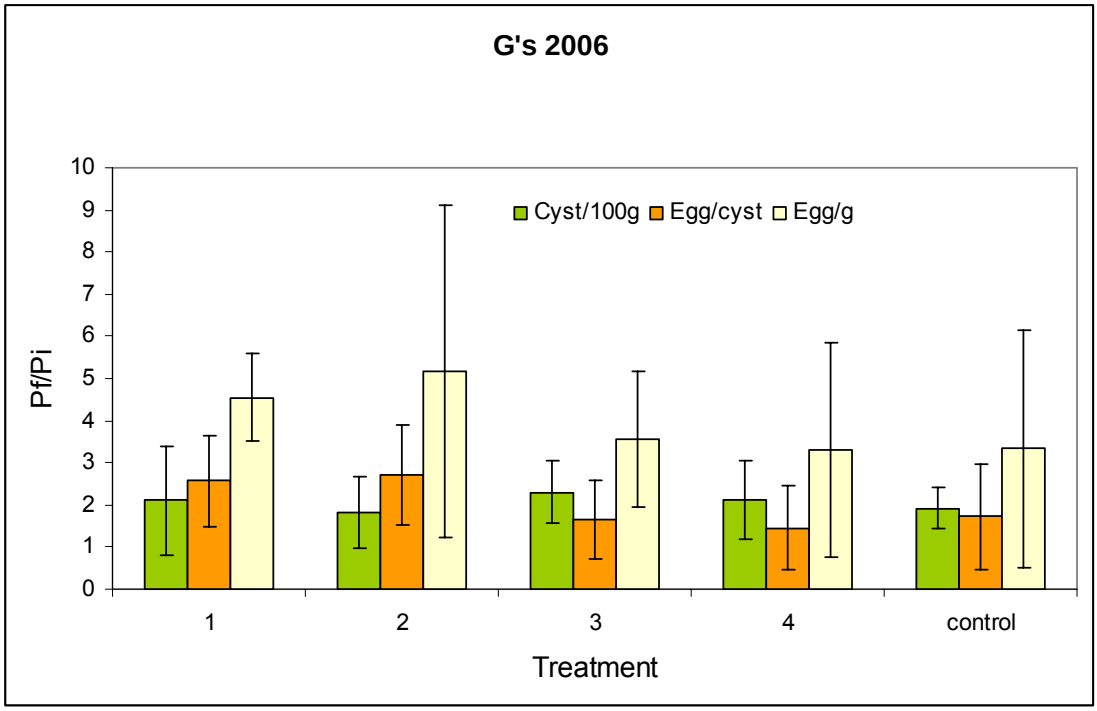


FIGURE 22: TOTAL NUMBER OF ALL LIFESTAGES OF PCN, FROM ROOTS SAMPLED ON 3 DATES AT FIELD SITE G'S IN 2006 (ANOVA P≤0.05, LSD= 417.78).

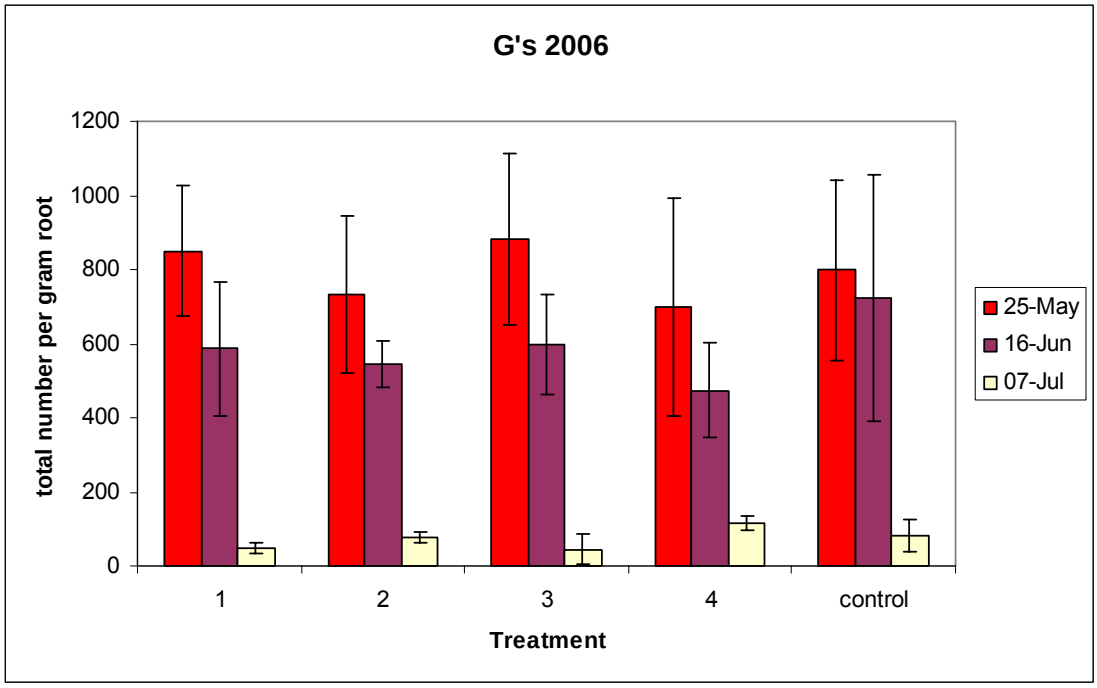


FIGURE 23: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE, UNDERSIZED (< 55 MM) AND OVERSIZED (>85MM) CATEGORIES (ANOVA P≤0.05, LSD 5.33).

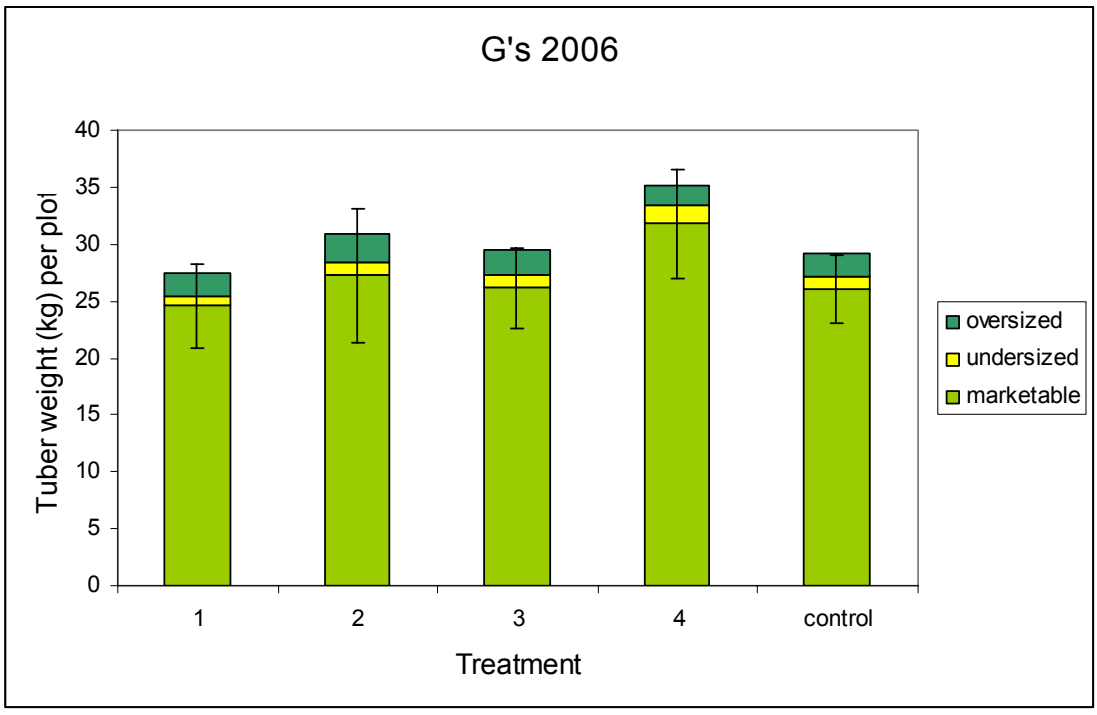


FIGURE 24: THE MULTIPLICATION OF PCN EXPRESSED AS THE PF/PI RATIO FOR CYSTS PER 200 GRAM OF SOIL, VIABLE EGGS PER GRAM OF SOIL AND VIABLE EGGS PER CYST AT FIELD SITE LONG HOOS IN 2006.

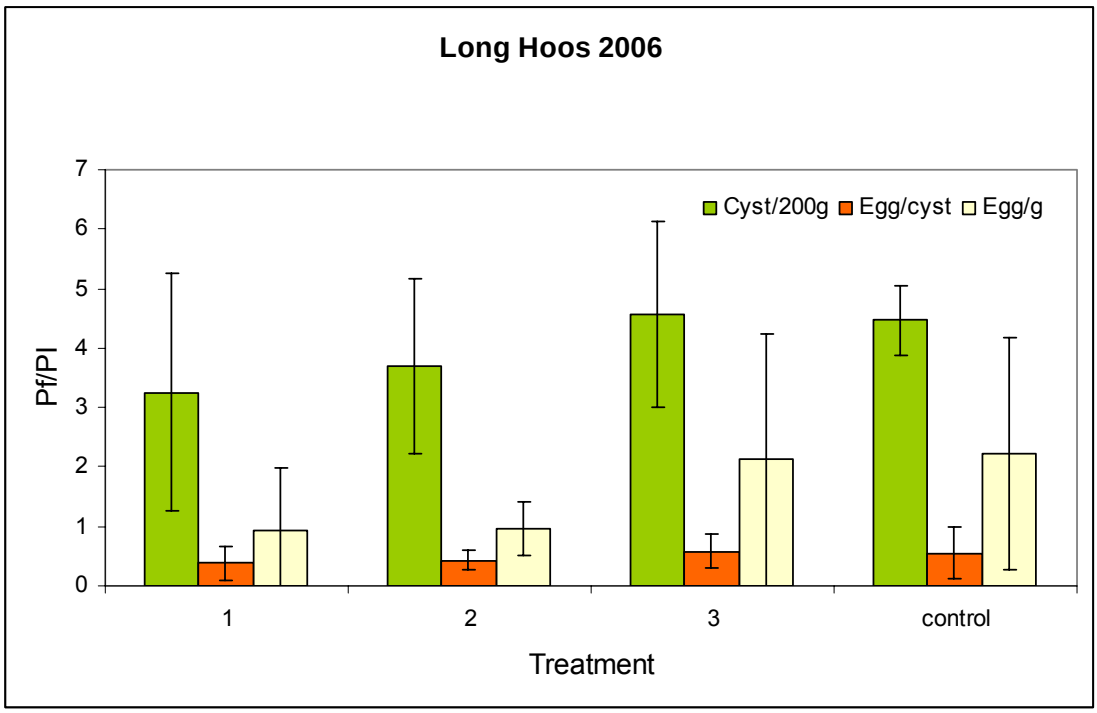


FIGURE 25: TOTAL NUMBER OF ALL LIFE STAGES OF PCN, FROM ROOTS SAMPLED AT 3 SAMPLE DATES AT FIELD SITE LONG HOOS IN 2006. DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS.

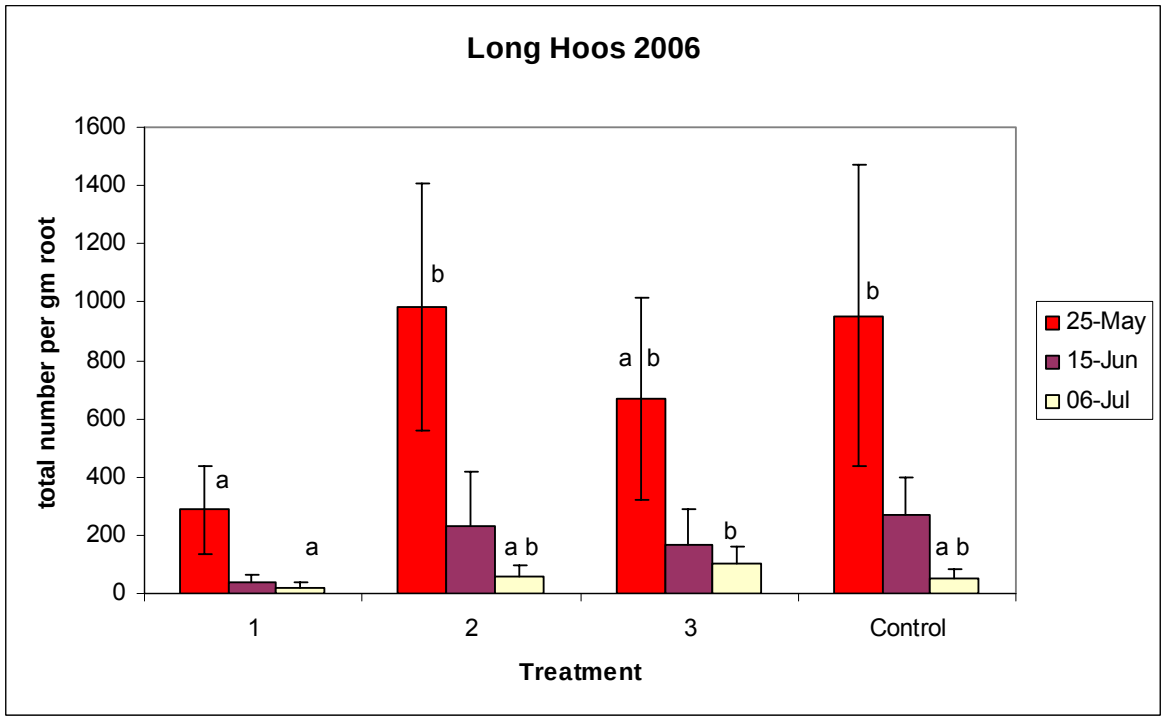
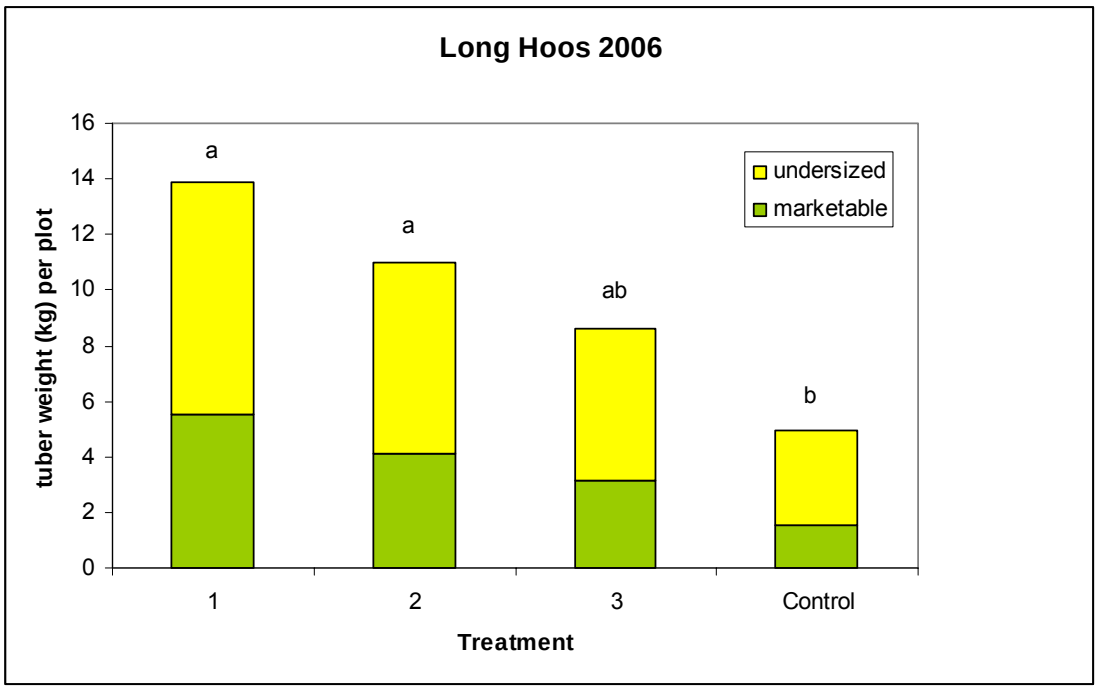


FIGURE 26: THE AVERAGE YIELD PER TREATMENT PLOT DIVIDED INTO MARKETABLE AND UNDERSIZED (< 55 MM) CATEGORIES (ANOVA P≤0.05, LSD 1.95).



The fate of field applied Vydate®

The results from the oxamyl residue analysis are shown in figures 27 to 29. The results shown are only those for the first out of 3 sampling dates. At the other sampling dates the residue levels were < 0.08 ppm (Chennels), < 0.06 ppm (Severals) and < 0.13 ppm at Long Hoos for all treatments analysed. The level of oxamyl residue at field site Severals was greatest in the single liquid (55 l/ha) treatment (treatment 2) at both soil depths sampled (Fig. 27). This treatment also showed up the highest level at field site Chennels, although the amount found was much higher (6 ppm) than that found at Severals (Fig. 28). At Long Hoos the residue level was highest in the 0-15 cm depth for the granular at planting treatment but not at the 15-30 cm depth for the same treatment (Fig. 29). The treatments were as follows:

1. Granular Vydate®, (55 kg/ha) at planting by soil incorporation.
2. Liquid Vydate®, (55 l/ha) timed with crop emergence.
3. Liquid Vydate®, 3 applications of 18.3 l/ha timed with crop emergence and subsequent crop development.
4. Liquid Vydate®, 2 applications of 20.6 l/ha (75 %of commercially recommended dose) timed with crop emergence and subsequent crop development.
5. Control treatment.

FIGURE 27: THE LEVEL OF OXAMYL (PPM) IN SOIL SAMPLED FROM 15-30 CM FROM SOIL SURFACE IN DIFFERENT TREATMENT BLOCKS AT FIELD SITE SEVERALS (G'S) IN 2006. SAMPLED 7 DAYS AFTER LAST APPLICATION, EXCEPT TREATMENT 1 (GRANULAR AT PLANTING) SAMPLED 24 DAYS AFTER APPLICATION.

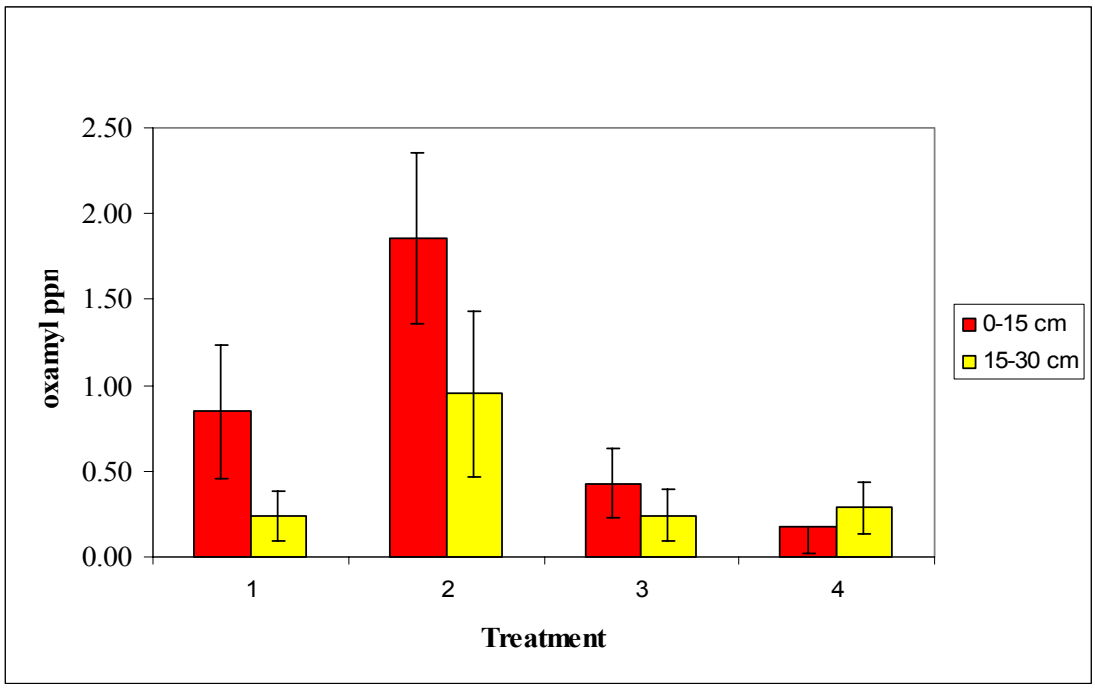


FIGURE 28: THE LEVEL OF OXAMYL (PPM) IN SOIL SAMPLED 0-15 CM/ 15-30 CM FROM SOIL SURFACE IN DIFFERENT TREATMENT BLOCKS AT FIELD SITE CHENNELS IN 2006. SAMPLED 7 DAYS AFTER LAST APPLICATION, EXCEPT TREATMENT 1 (GRANULAR AT PLANTING) SAMPLED 45 DAYS AFTER APPLICATION.

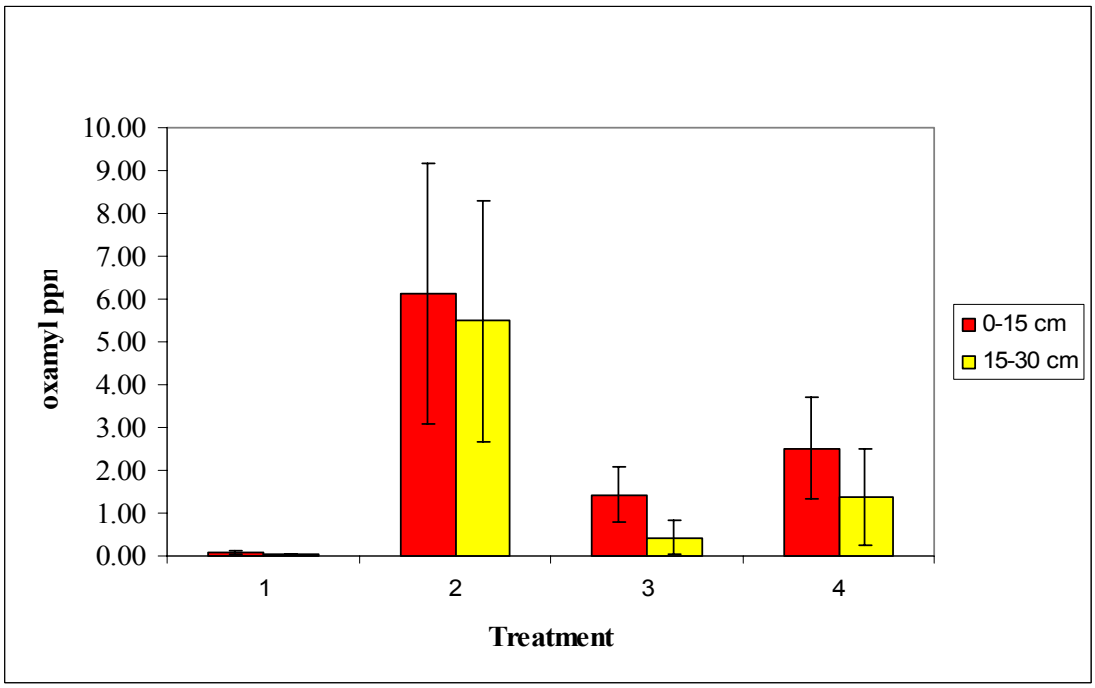
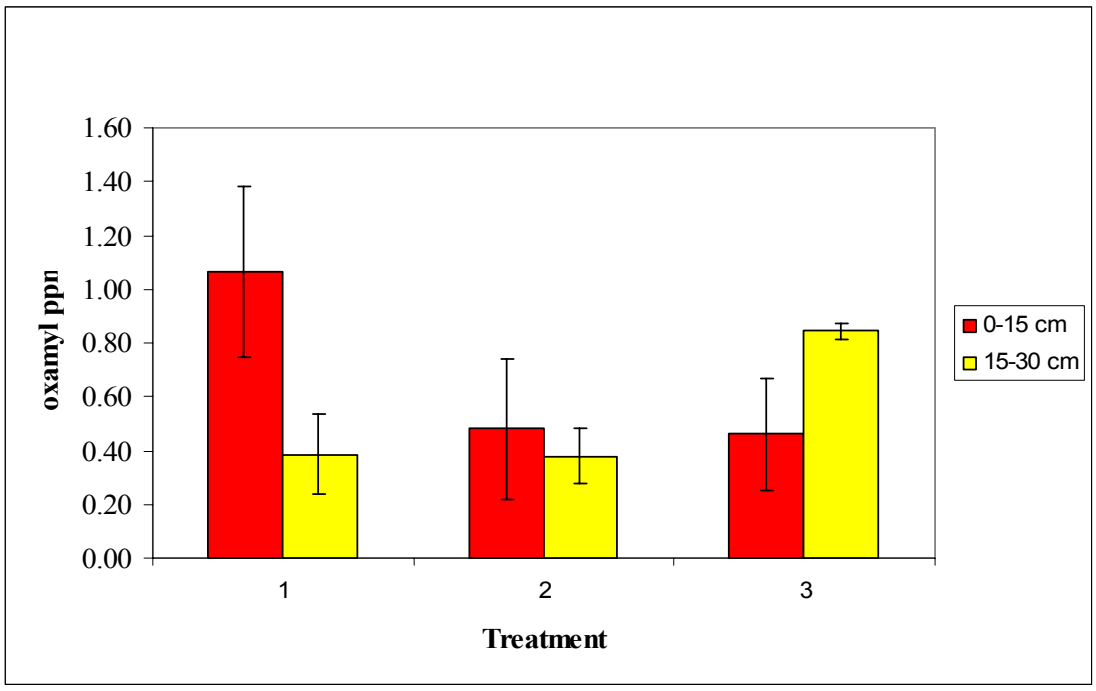


FIGURE 29: THE LEVEL OF OXAMYL (PPM) IN SOIL SAMPLED 0-15 CM/ 15-30 CM FROM SOIL SURFACE IN DIFFERENT TREATMENT BLOCKS AT FIELD SITE LONG HOOS IN 2006. SAMPLED 7 DAYS AFTER LAST APPLICATION, EXCEPT TREATMENT 1 (GRANULAR AT PLANTING) SAMPLED 30 DAYS AFTER APPLICATION.



Hatching profiles and hatch tests

The results from the hatch tests with Potato Root Diffusate (PRD) to characterise the field populations used in the field trials are shown in figures 30 to 35. The results show that there is a large variation in the number of juveniles hatched from each batch of cysts (see Figs. 30, 32 and 34). The hatch for each population as a percentage of the total hatch of each population is shown in Figures 31, 33 and 35.

FIGURE 30: NUMBER OF JUVENILES HATCHED FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS AND DEMONSTRATION PLOTS DURING 2004.

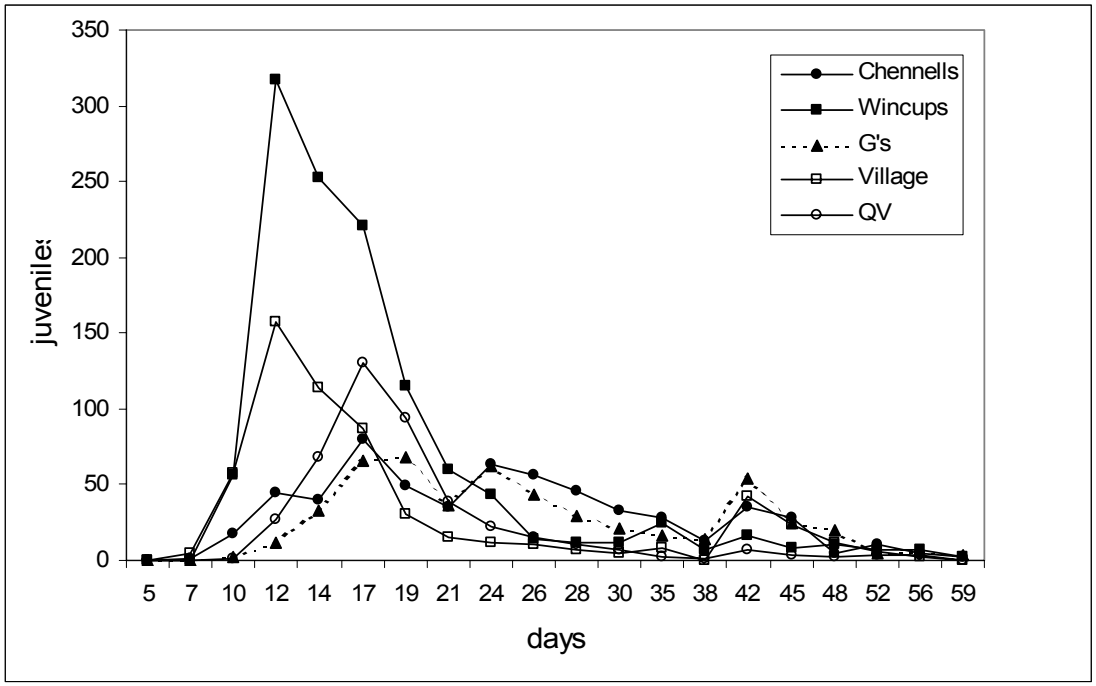


FIGURE 31: PERCENTAGE OF JUVENILES OF TOTAL JUVENILES HATCHED PER OBSERVATION DATE FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS AND DEMONSTRATION PLOTS DURING 2004.

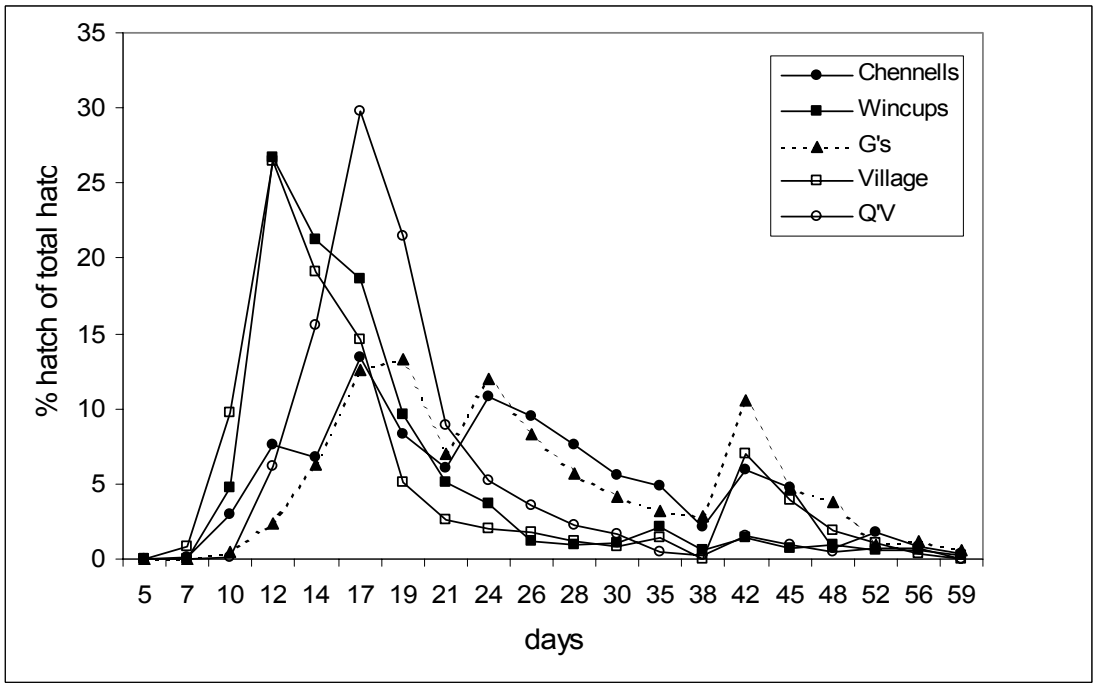


FIGURE 32: NUMBER OF JUVENILES HATCHED FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS IN 2005.

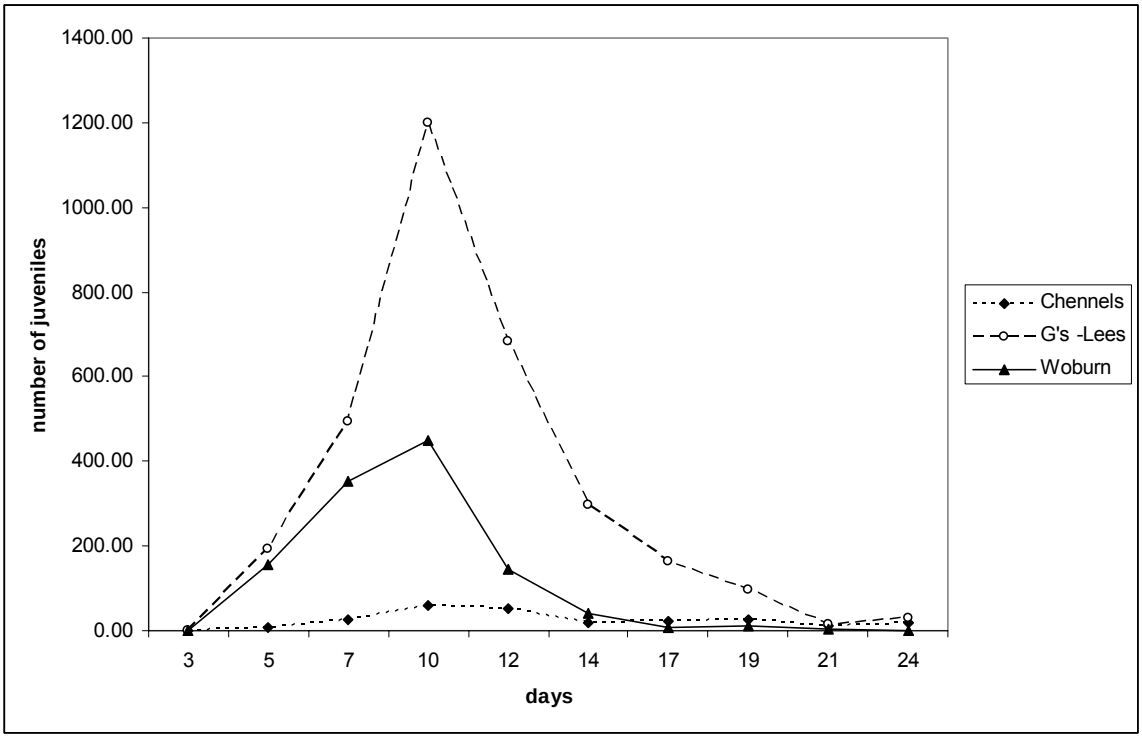


FIGURE 33: PERCENTAGE OF JUVENILES OF TOTAL JUVENILES HATCHED PER OBSERVATION DATE FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS IN 2005.

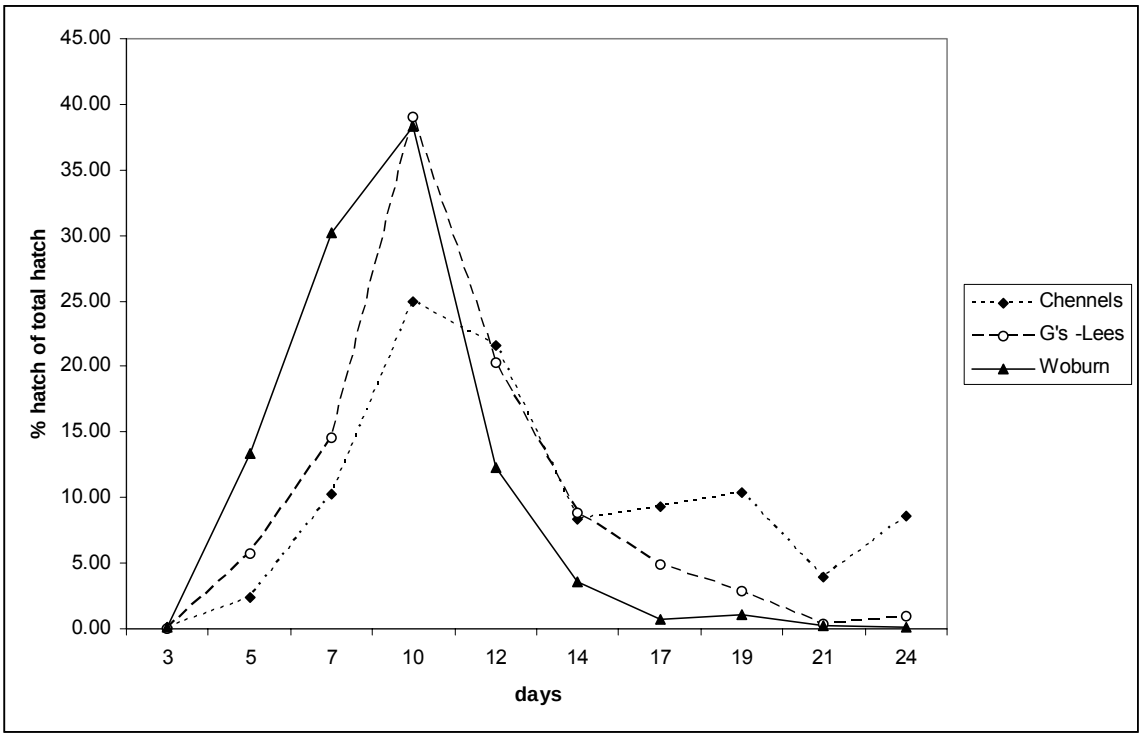


FIGURE 34: NUMBER OF JUVENILES HATCHED FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS IN 2006.

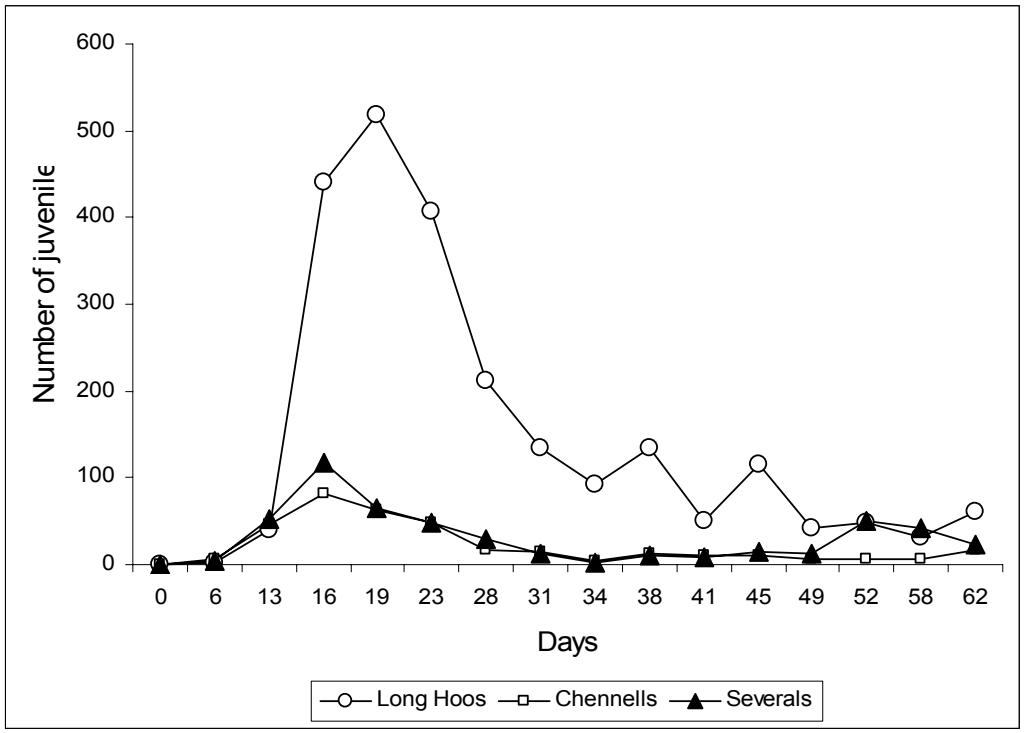
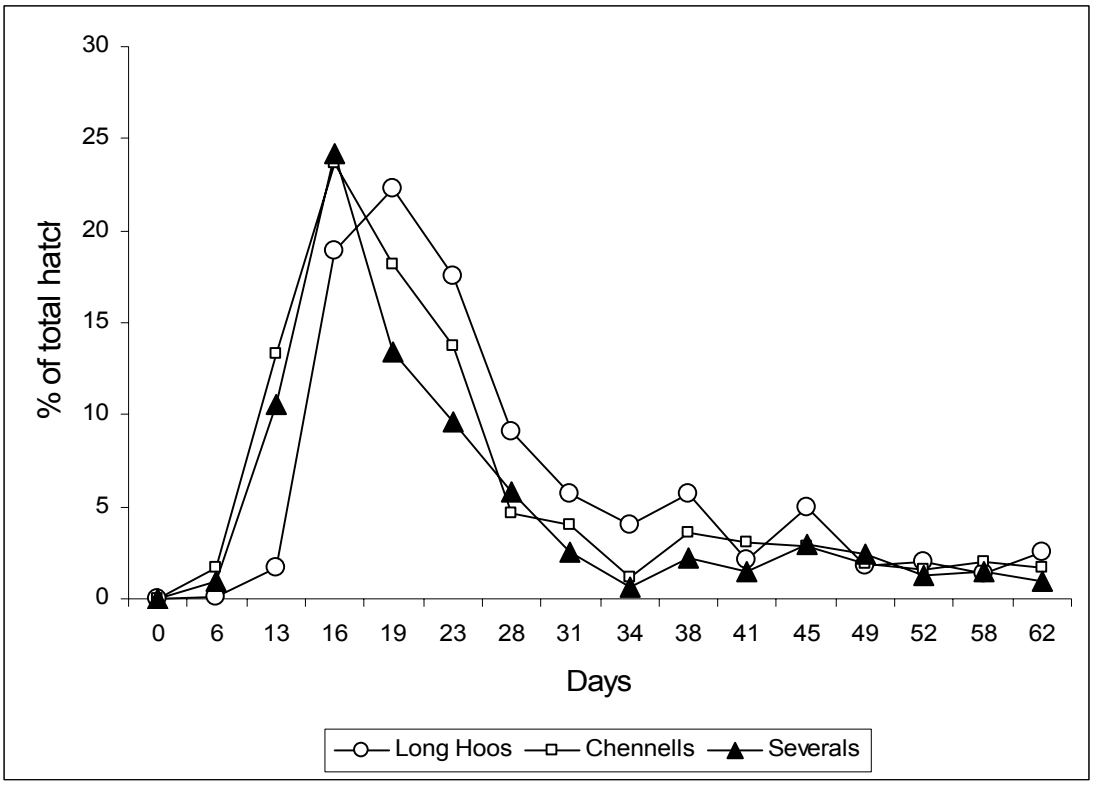


FIGURE 35: PERCENTAGE OF JUVENILES OF TOTAL JUVENILES HATCHED PER OBSERVATION DATE FROM CYSTS AFTER EXPOSURE TO POTATO ROOT DIFFUSATE. *GLOBODERA PALLIDA* POPULATIONS FROM FIELDS USED IN REPLICATED FIELD TRIALS IN 2006.



The results from the hatch assays comparing the hatch effected by PRD with the hatch effected by sodium metavanadate and zinc chloride are shown in figures 36 to 38. There is little effect on hatch for compounds other than PRD, except for the Long Hoos population were sodium metavanadate hatched a larger number of juveniles (Figure 38).

FIGURE 36: THE NUMBER OF JUVENILES HATCHED FROM 25 CYSTS (3 REPLICATES) AFTER EXPOSURE TO POTATO ROOT DIFFUSATE (PRD), SODIUM META VANADATE (SMV), ZINC CHLORIDE OR STERILE WATER. *G. PALLIDA* POPULATION CHENNELS.

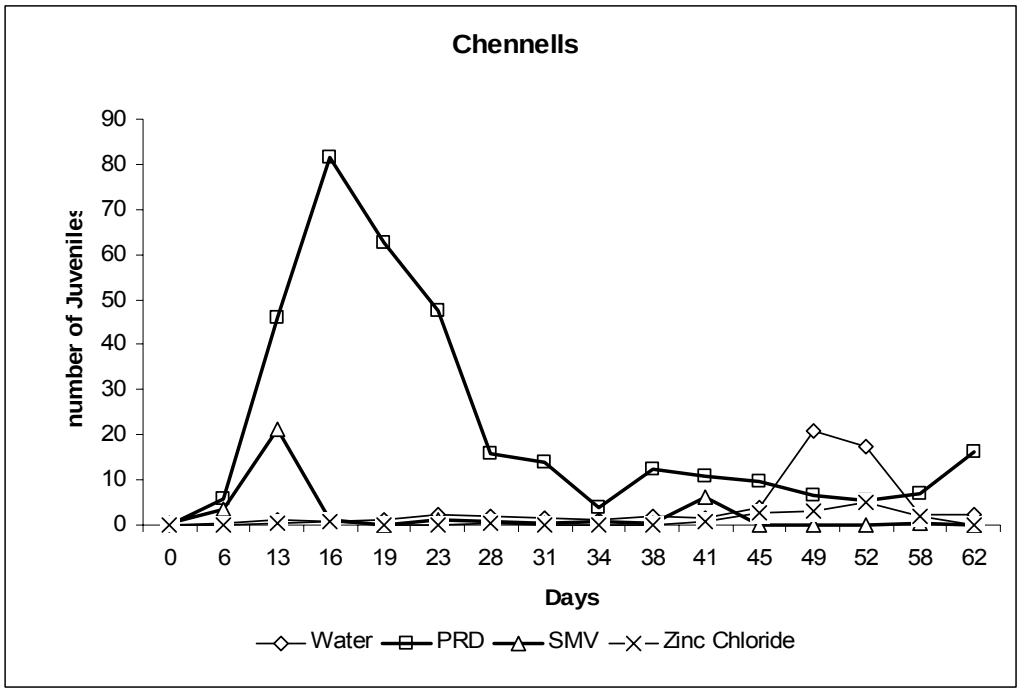


FIGURE 37: THE NUMBER OF JUVENILES HATCHED FROM 25 CYSTS (3 REPLICATES) AFTER EXPOSURE TO POTATO ROOT DIFFUSATE (PRD), SODIUM META VANADATE (SMV), ZINC CHLORIDE OR STERILE WATER. *G. PALLIDA* POPULATION SEVERALS.

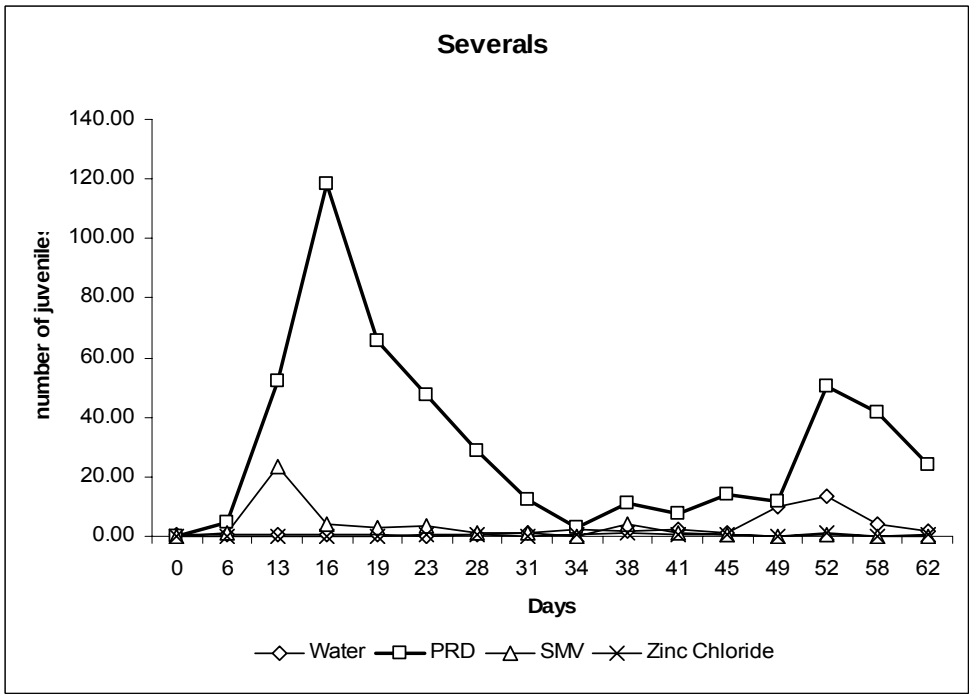
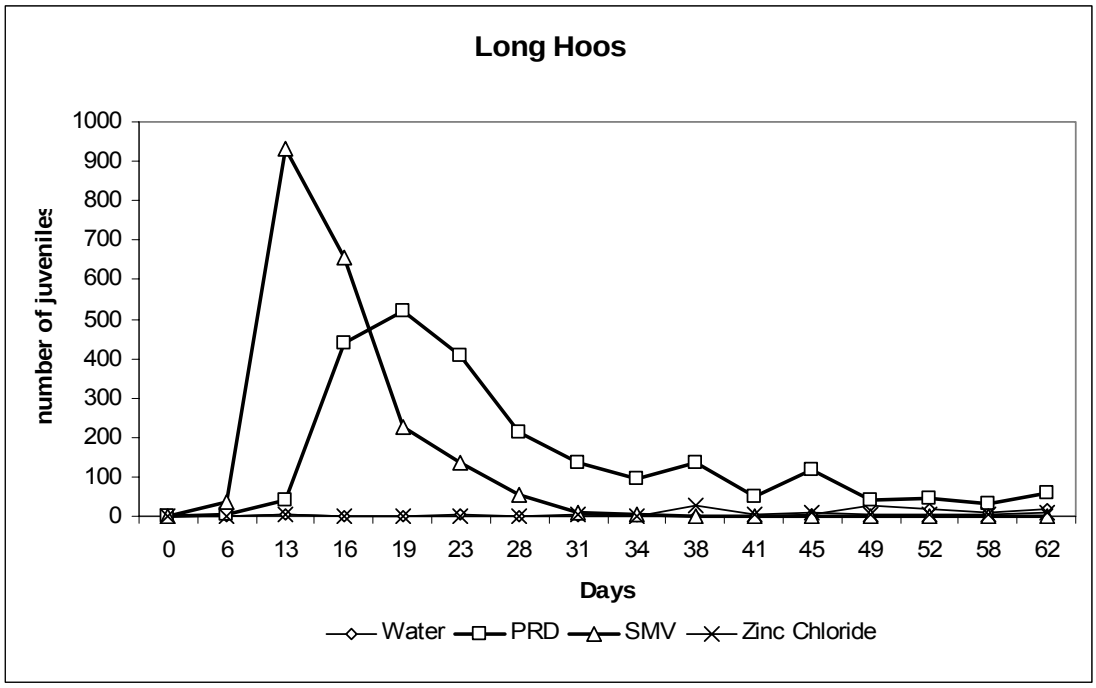


FIGURE 38: THE NUMBER OF JUVENILES HATCHED FROM 25 CYSTS (3 REPLICATES) AFTER EXPOSURE TO POTATO ROOT DIFFUSATE (PRD), SODIUM META VANADATE (SMV), ZINC CHLORIDE OR STERILE WATER. *G. PALLIDA* POPULATION LONG HOOS.



The effect of oxamyl on non –target pests

The results comparing the effect of granular or liquid applied Vydate at a rate of 55 l/ha (10 % a.i.) against a non-treated control on the total number of nematodes and the number of plant parasitic nematodes in soil samples taken from replicated treatment blocks are shown in figures 39 to 41. There was no significant difference between the treatments at the field sites Chennels and Severals for the total number of nematodes or the number of plant parasitic nematodes plant parasitic nematodes (Figs. 39 and 40). The total number of nematodes was significantly lower in the granular Vydate treatment compared to the non-treated and liquid Vydate treatment at field site Long Hoos (ANOVA, $p \leq 0.05$), there was no significant difference between the treatments for the number of plant parasitic nematodes (Figure 41) (ANOVA, $p \leq 0.05$).

FIGURE 39: THE TOTAL NUMBER OF NEMATODES AND THE NUMBER OF PLANT PARASITIC NEMATODES RECOVERED FROM A 200G SOIL SAMPLE FROM FIELD SITE CHENNELS.

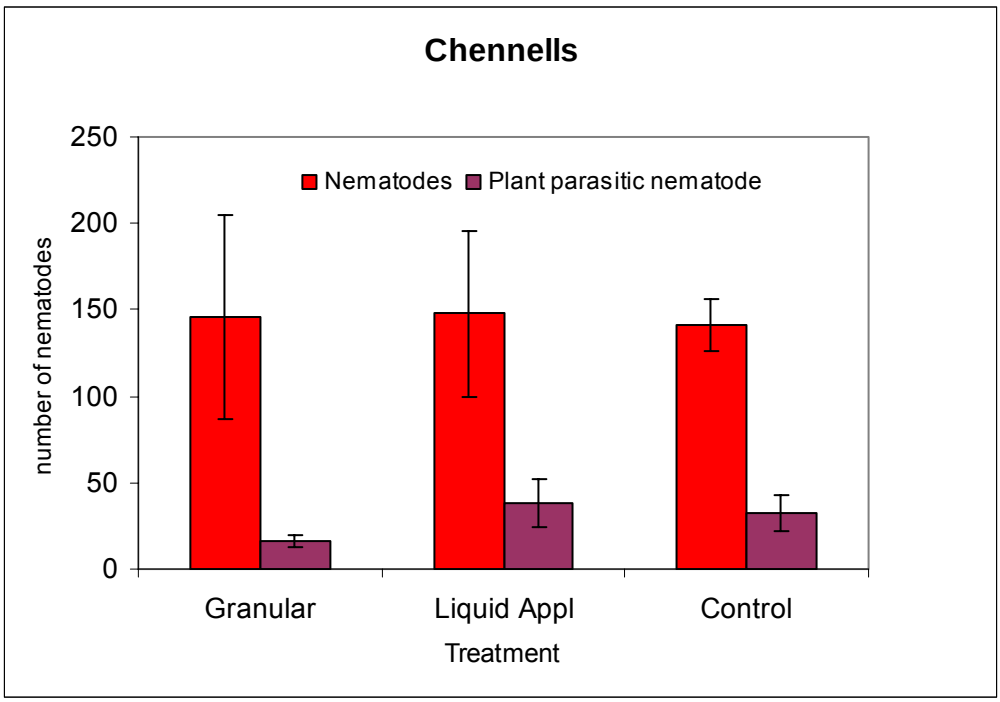


FIGURE 40: THE TOTAL NUMBER OF NEMATODES AND THE NUMBER OF PLANT PARASITIC NEMATODES RECOVERED FROM A 200G SOIL SAMPLE FROM FIELD SITE G'S (SEVERALS).

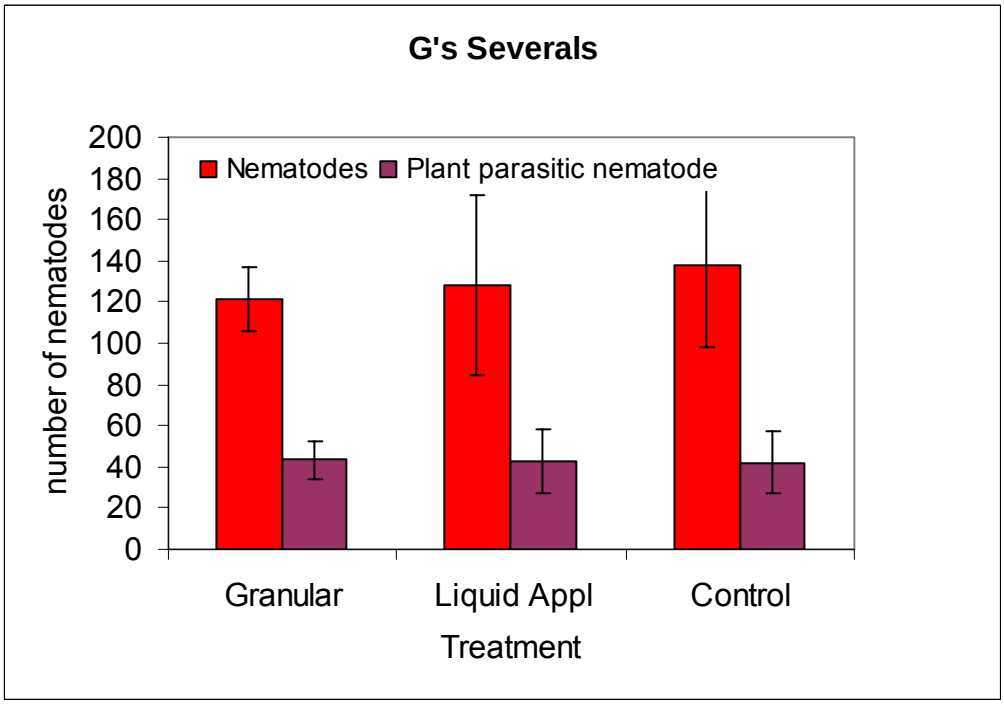
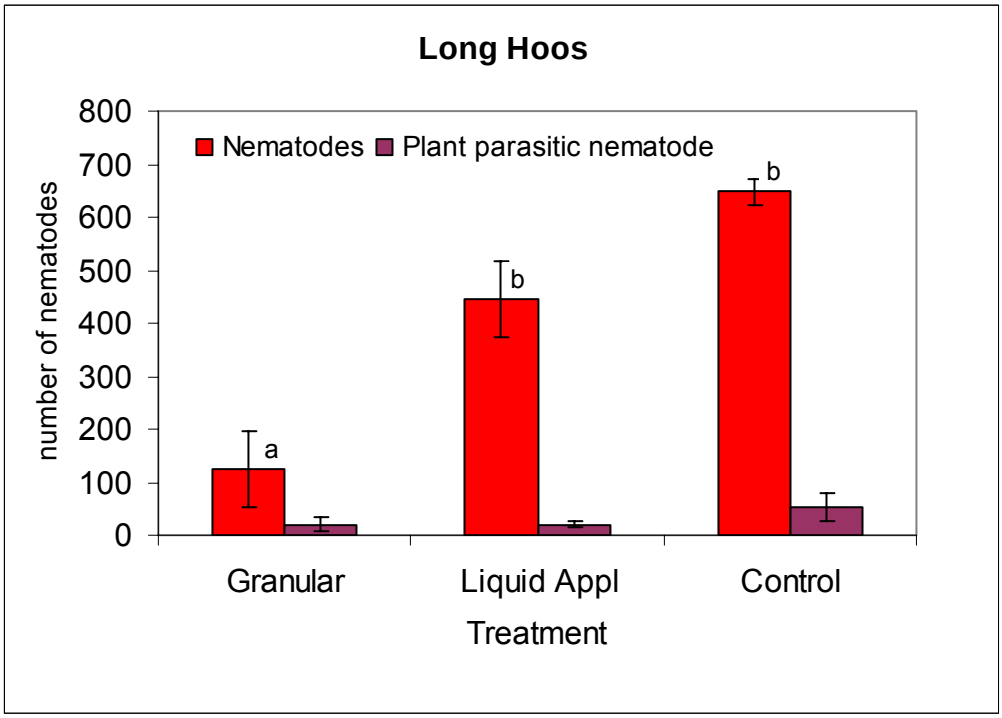


FIGURE 41: THE TOTAL NUMBER OF NEMATODES AND THE NUMBER OF PLANT PARASITIC NEMATODES RECOVERED FROM A 200G SOIL SAMPLE FROM FIELD SITE LONG HOOS. DIFFERENT LETTERS INDICATE A SIGNIFICANT DIFFERENCE (ANOVA, $p \leq 0.05$) BETWEEN TREATMENTS.



In the experiment concerning the possible non-target effect on two aphid species the only species recovered in high enough numbers was *M. persicae* and this was also the species for which fecundity was recorded (Figure 42). It was not possible to determine whether the missing individuals of M. *euphorbiae* had died or escaped although no dead individuals were found. The amount of oxamyl (in ppm) present in leaf material comparable to that on which the clip cages with aphids were placed is shown in Figure 43.

FIGURE 42: TOTAL NUMBER OF PROGENY COLLECTED FROM MATURE FEMALE (5 REPLICATES) OVER A SEVEN DAY PERIOD. (ANOVA $P \leq 0.05$, LSD 7.75)

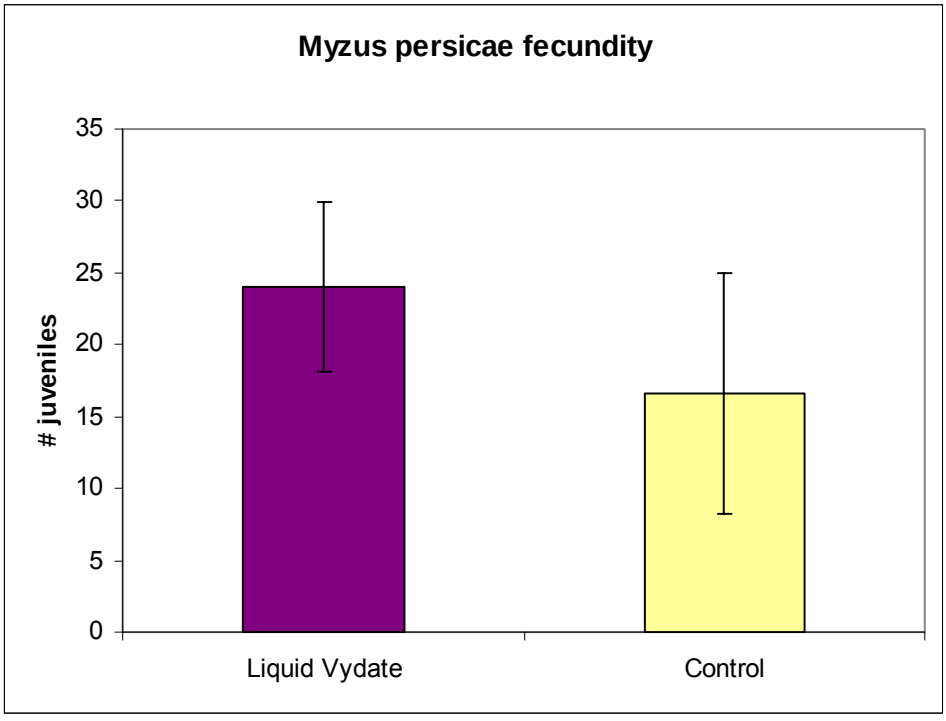
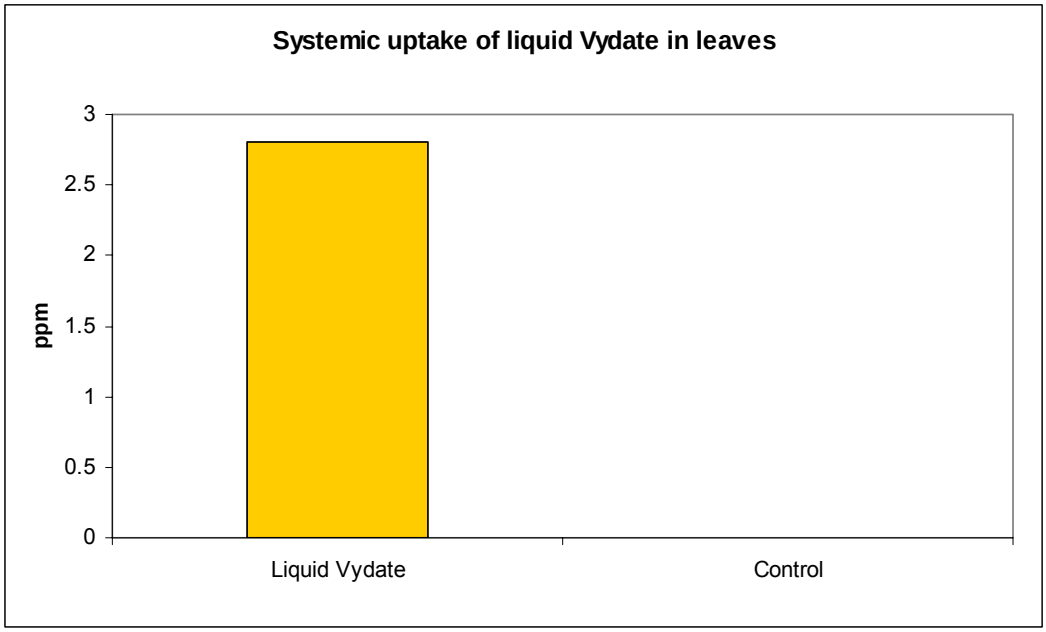


FIGURE 43: THE AMOUNT OF OXAMYL (PPM) IN POTATO LEAF MATERIAL 7 DAYS AFTER A DRIP IRRIGATION APPLICATION OF 18.3 L/HA OXAMYL (10% A.I.).



Discussion

Successful application of oxamyl through drip irrigation was carried out during three field trial seasons. The yield results for all seasons show that there was no detrimental effect on yield, due to phytotoxicity, for any of the liquid Vydate treatments compared to the control or the granular Vydate treatment. There were no significant differences between treatments for yield except for the field site Long Hoos in 2006. Together with the field site Woburn in 2005, this site was located on an experimental site run by Rothamsted Research. On both sites as the experimental potato crop was the only potato crop grown no overhead irrigation was available this partly explains the relatively low yield compared to the other sites that were all situated in commercial potato fields. In 2006 at Long Hoos, there was a significant difference in yield between the single dose granular, single dose liquid and the control treatment. This can be explained by the fact that the plants experienced drought stress and were less able to tolerate the stress due to PCN invasion.

Although the results from root invasion studies seem to indicate differences between treatments (i.e. Figures 3 and 4) the variation in number between the replicates was such that no significant difference was found. A significant difference in the total number of nematodes in the potato roots was found between the treatments at Long Hoos in 2006. However as the crop was very drought stressed, as evidenced by the comparatively low yield, these results are not thought to be representative. The lower number of nematodes invading the roots in both the granular and single liquid treatment did not lead to significant differences in the Pf/ Pi values (Figure 24).

It was expected that differences would be found between treatments for the Pf/ Pi values, however due to the large spatial variability in PCN density the large standard deviation of the means meant that no significant differences were found. In 2006 the trial layout was changed in order to try to deal with these huge spatial variations, unfortunately this approach did not overcome the problem.

In 2006 at the field site Chennels the early planted crop had a delayed emergence due to cold weather, this meant that the application of the liquid nematicide was 45 days later than the application of the granular nematicide. The HPLC results show that in the granular nematicide treatment virtually no active ingredient was present whereas in the single liquid treatment around 6 ppm where present. This is well above the amount required to effect nematode control and clearly showed the strength of being able to deliver the liquid nematicide at a time after planting as opposed to granular nematicides that have to be incorporated into the soil at time of planting. A low residual amount was also found for the granular treatment at Severals. The residual amounts shown for the split dose treatments (treatments 3 and 4) indicate that not enough active ingredient would be present one week after application to effect nematode control. Under laboratory conditions an exposure to 1 mg / litre (1 ppm) for 7 days caused a 50% suppression of hatch, with an almost complete inhibition at 4 mg/ litre (4 ppm) (Evans & Wright, 1982).

The results (Figures 27, 28 and 29) also show clear differences between the field sites likely to be caused by differences in soil type as the rate of breakdown can vary dramatically depending on soil moisture content, temperature and physical and chemical characteristics such as texture and pH (Leistra, Bromilow & Boesten, 1980).

The drip irrigation application of nematicides allows for the timing of application to coincide with expected peaks in nematode hatch. In order to understand variations in hatching and possible differences in hatching profiles between PCN populations, those from the field trials

were screened. There was a large variation between the total of number of juveniles hatched from cysts from different sites (Figures 30, 32 and 34), this can be explained by the different ages of the cysts and different periods since the last potato crop. There is also a variation between natural decline rates of eggs in cysts depending on soil type and the activity of antagonistic organisms. Although there was variation in the time when the peak hatch was reached in general up to 50 % of juveniles hatched between 7-23 days after being exposed to Potato Root Diffusate. It is difficult to extrapolate findings from the controlled laboratory experiments to a field situation as under laboratory conditions cysts are constantly exposed to fresh root exudates. Under field conditions there will be more variation in the exposure to hatching stimuli depending on the development of the root system and the growth rate of the potato roots. However it seems to be that there is a variation in the number of juveniles depending on cyst age and environmental factors as discussed previously but there appears to be less variation between the occurrence of peaks in hatching between field populations. In practice, this would mean that timing the application of liquid nematicide with crop development would be a reliable practice to maximise the efficacy of the applied nematicide but tailoring the timing of application to differences in hatching rates in different PCN populations is unlikely to be successful.

The results from the study looking at a possible artificial hatching agent to establish hatching profiles shows that both sodium metavanadate and zinc chloride failed to elicit a hatch response comparable to PRD in the cyst populations from both the Chennels and Severals field sites. Previously it was found that both sodium metavanadate and zinc chloride were able to hatch juveniles from cyst of *G. pallida* (Greet, 1974; Whitehead, 1992) however it is not known whether these studies were conducted with field collected aged cysts or cysts obtained from pot cultures. Sodium metavanadate elicited a strong hatching response in cysts from the Long Hoos site (Figure 38). A possible explanation for this is that the site at Long Hoos had a potato crop the previous year. The eggs in new cysts might behave differently to a hatch stimulus than aged cysts, however the hatch response elicited by sodium metavanadate was not comparable to the one elicited by PRD and therefore sodium metavanadate cannot be used as a reliable alternative hatching agent for PRD.

Although HPLC analysis of leaf material showed a high level of oxamyl indicating systemic uptake of drip-irrigation applied oxamyl (Figure 43), this seems not to have led to a reduction in the number of offspring produced by mature female aphids (Figure 42). It also did not lead to mortality of the adult females.

The study to look at the possible control of free living nematodes by means of drip-irrigation applied oxamyl showed no significant difference for the total number of nematodes and the number of plant parasitic nematodes at the field sites Chennels and Severals. The total number of nematodes includes bacteriovorous nematodes that have a very short generation time and can rapidly recolonise and area after nematicide application. Also the effect of oxamyl is reversible at low doses and nematodes are not necessarily killed (Pree *et al*., 1987). The significant effect of the granular application on the total number of nematodes at the field site Long Hoos can be explained by the very dry conditions meaning the oxamyl dissipates less rapidly from the granule.

Conclusions

The level of control obtained by application of Vydate® 10L by means of drip-irrigation is equivalent to that provided by an application of Vydate® 10 G (granule). There is a potential to decrease the dosage of Vydate® 10L compared to the normal dose of 5.5 l a.i. /ha and still achieve the same level of control as an application of Vydate® 10 G as evidenced by field trial results in 2005 and 2006. There were no additional benefits with regards to control of non-target pests (aphids, free living nematodes). This technology if adopted would lead to significant environmental benefits as there is no exposure of granules to either non-target organism or the contractor applying the treatment. The technique also allows a more efficient use of the nematicide as delivery of the product can be timed with crop development and subsequent nematode hatch. In laboratory assays no significant differences were found between the timing of peaks in hatching between different PCN populations. The delivery of the product can therefore be timed with crop emergence.

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