**DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS** **CSG 15**

Research and Development

# Final Project Report

(Not to be used for LINK projects)

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| Project title | Biological control of leatherjackets using insect pathogens. |
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## Executive summary (maximum 2 sides A4)


Leatherjackets, the larvae of craneflies, can be important pests of organically-grown crops. They can cause particular damage to field vegetables grown after a grass ley or on land converted to organic production from pasture. Unfortunately, there are as yet no effective alternatives to chemical insecticides that can be used by organic farmers. A reliable method of controlling leatherjackets would be of financial benefit to UK organic farmers who operate on narrow margins and face stiff competition from foreign imports.

The susceptibility of laboratory reared leatherjackets (*Tipula paludosa*) to 123 dipteran-active strains of insect pathogenic fungi, nematodes and the bacterium *Bacillus thuringiensis* (*Bt*) was measured using single-dose, laboratory bioassays. Seven strains of *Bt* and four strains of entomopathogenic nematodes exhibited high pathogenicity to leatherjackets and were selected for further examination in multiple-dose bioassays. Two strains of entomopathogenic fungi were weakly pathogenic to leatherjackets, and were not considered suitable for further study. The susceptibility of leatherjackets to *Bt* declined with increasing age of the host, and there was a 100-fold increase in $LC_{50}$ between first and third instars. There were strong indications that *Bt* acted as an antifeedant when applied to leaf material, which prevented third instar larvae from acquiring a lethal dose at all but the highest concentrations. However, incorporation of *Bt* into a bran bait significantly lowered the dose at which the bacterium was effective against third instars. Of the four strains of entomopathogenic nematodes examined in multiple-dose bioassays, *Heterorhabditis megidis* HRI-HUK and *Steinernema feltiae* HRI-NOR14 showed the highest pathogenicities with median lethal concentrations ($LC_{50}$) of 935 and 739 nematodes per insect respectively after six days. More nematodes were seen to be recycling in HRI-NOR14 than in HRI-HUK. Some tipulid larvae were able to survive nematode infestation by the encapsulation of invading juveniles.

Laboratory observations of the feeding behaviour of leatherjackets showed that most individuals resided in the top 5 cm of the soil. Although leatherjackets are generally thought of as root feeders, laboratory experiments showed that they also consume significant quantities of foliage, usually at night. There were opportunities, therefore, to treat leatherjackets in the field with *Bt* applied as a bran bait to the soil surface, or by spraying *Bt*

onto foliage. Entomopathogenic nematodes, which do not have to be ingested to infect their hosts, could be applied to the soil surface as a drench. The effectiveness of these strategies has been studied in glasshouse and field experiments.

Observations of field populations of leatherjackets confirmed their status as sporadic, usually localised pests. Insect pathogens could offer means of controlling leatherjackets that is compatible with the holistic management systems employed on organic farms, but remedial treatments are warranted only where crops have been grown after grass. Mycosed craneflies, most probably infected with species of *Entomophthora*, were regularly observed during field excursions. However, there was a low incidence of leatherjackets naturally infected with entomopathogens on organic farms and these micro-organisms are unlikely to contribute significantly to leatherjacket population dynamics, despite entomopathogenic fungi and nematodes being common in the soils studied. Whether natural control could be increased by augmentation or modifications of current cultural practice remains to be investigated.

# Scientific report (maximum 20 sides A4)

## 1. Introduction

The value of the UK organic food market is currently estimated at £200 million p.a., and has increased five fold in the last ten years (Anon (1), 1997). However, the UK still imports most of its organic produce, and there is a requirement to raise home production, particularly in field vegetables. Organic farming accounts for 0.3% of total British farm output with 880 organic farms covering 49,000ha. It is estimated that, by the year 2000, the UK organic output will be 1.5% of total agricultural production (Anon (2), 1997). Pest management in organic crops is based largely on prevention, and at present in the UK this approach appears to work well, possibly because the intensity of production on many organic farms is low. However, there are still occasions when remedial treatments of pests are required, although unfortunately there are few effective pest treatments available to organic growers. The demand for such products is set to increase significantly as the organic sector expands and becomes more competitive.

Leatherjackets, the soil-dwelling larvae of crane flies (Diptera; Tipulidae), can be serious pests of organically-grown crops. There are two species of tipulids (*Tipula oleracea* and *T. paludosa*) that damage crops in the UK. *Tipula paludosa* is the more common and when in abundance can cause considerable damage. The larvae are thought to feed just below the soil surface on the roots and underground stems of their host plants, but will also attack foliage. Seedlings and immature plants are particularly vulnerable. Craneflies oviposit preferentially in grassland, and hence leatherjackets can be a particular problem where pasture has been converted to organic production, or on crops planted after a grass ley. Field vegetables and young cereals are most at risk. In the absence of chemical insecticides, organic farms rely on good husbandry practices to encourage early root development and hence ameliorate yield losses due to leatherjacket activity. Such methods are not always successful, and there is a need for a remedial treatment to act as a second line of defence against leatherjackets.

Populations of leatherjackets are thought to be regulated naturally by the weather and predation (Coulson, 1962). They are also susceptible to diseases caused by insect pathogens (Sherlock, 1973) and some of these micro-organisms may have potential as biocontrol agents. Insect pathogens are host specific, do not harm the environment, and can be applied with conventional farm equipment.

## 2. Objectives

The aim of this project was to identify and evaluate insect pathogens (fungi, nematodes and the bacterium *Bacillus thuringiensis*) as biological control agents of leatherjackets. The objectives of the proposal were as follows:

1. To identify insect pathogens pathogenic to leatherjackets by laboratory bioassay.
2. To isolate insect pathogens from leatherjackets on organic farms.
3. To characterise pathogens virulent to leatherjackets.
4. To study the feeding behaviour of leatherjackets.
5. To evaluate selected pathogens on glasshouse experiments.
6. To evaluate pathogens in an organic crop production system in the field.

## 3. Results

### 3.1 Objective 1: To identify insect pathogens pathogenic to leatherjackets by laboratory bioassays

*3.1.1 Insect rearing*

All laboratory experiments were done with *T. paludosa*. Craneflies of *T. paludosa* were collected from three sites: i) organic pasture at the Henry Doubleday Research Association, Ryton upon Dunsmore, Warwicks: ii) an organic farm in Herefordshire, iii) pastureland used to monitor populations of *T. paludosa* at ADAS Drayton, Warwicks. Adults were paired, then mated females were decapitated, and wings and legs amputated.

Females were then placed on moist filter paper in a sterile 9cm diameter Petri dish to oviposit. Eggs were incubated at 20$^{O}$C ± 2$^{O}$C and hatched within ten days. Neonate larvae were transferred to moist compost in 13.5 cm diameter polyethylene pots and fed a mixture of bran and grassmeal (1:2). Using this method, approximately 70 adults could be reared over a period of 10 weeks from every female mated. Insect numbers were bulked up for the bioassay programme.

*3.1.2 The effect of Bacillus thuringiensis (Bt) on neonate leatherjackets*

A laboratory bioassay was developed to measure the susceptibility of neonate leatherjackets to *Bt*. The assay was developed in three stages. In stage one, the mortality of neonates in untreated controls was measured over time. Chinese cabbage (var. *Kasumi*) leaf discs (2 cm diameter) were placed onto 1.5% water agar in compartmentalised Petri dishes (20 compartments per dish). One neonate larvae was added to each well and the dishes maintained at 20$^{O}$C ± 2$^{O}$C. Mortality was assessed after 48 hours. In stage two, *B. thuringiensis* var. *israelensis* (*Bti*), which had been reported previously to kill leatherjackets, was characterised as a positive control. *Bti* was grown in Proflo B4 and harvested at 95% autolysis. The broth was diluted in 0.1% Triton-X100 at rates of 9.1 µl broth, 2.5 µl broth, 0.69 µl broth, 0.19 µl broth and 0.054 µl broth ml$^{-1}$ Triton-X100 respectively. Chinese cabbage (var. *Kasumi*) leaf discs (2 cm diameter) were suspended in aqueous suspensions of *Bti* and then placed onto 1.5% water agar in compartmentalised Petri dishes (20 compartments per dish). One neonate larvae was added to each well and the dishes maintained at 20$^{O}$C ± 2$^{O}$C. Each concentration was assayed against 25 larvae. Mortality was assessed as before. The median lethal concentration was calculated by regression analysis following the logistic transformation of dose-mortality data. This gave a mean $LC_{50}$ of 0.71 µl broth ml$^{-1}$ Triton-X100. In stage three, 93 strains of *Bt* from the HRI collection and with known dipteran activity, were cultured as *Bti* and each assayed against 25 neonate leatherjackets at a single dose of 0.71 µl broth ml$^{-1}$ Triton-X100. Seven strains exhibited a similar pathogenicity to that of the *Bti* positive control. The remaining 86 strains caused between 0-27% mortality although the majority (54) caused no deaths (Table 1).

**Table 1: Range of mortalities of *Bacillus thuringiensis* strains against neonate leatherjackets**

| % mortality | 0 | 1-10 | 11-20 | 21-30 | >30 |
|---|---|---|---|---|---|
| No. of *Bt* isolates | 54 | 16 | 13 | 3 | 7 |

Ninety three strains of *Bt* assayed against neonate leatherjackets at a single dose of 0.71 µl broth ml$^{-1}$. Mortality assessed 48 hours post treatment.

*3.1.3 The effect of entomopathogenic fungi on third instar leatherjackets*

A laboratory bioassay was developed to measure the pathogenicity of fungi to third instar leatherjackets. The apparatus used was modified from the leatherjacket rearing system (3.1.1). Pilot experiments were done to measure the mortalities of leatherjackets over time in untreated controls under different regimes of disturbance. Third instar leatherjackets were treated by direct immersion in an aqueous suspension of fungal conidia and maintained individually within 20 ml damp compost (John Innes, F2) in 30 ml, ventilated Universal bottles at 20$^{O}$C and 16L:8D photoperiod. Bran / grassmeal (1:2) was supplied *ad libitum*. Mortality was assessed every five days for four weeks. Using this method, 24 fungal strains from the HRI culture collection with known dipteran activity were each bioassayed against 25 third instar leatherjackets at a single dose ($10^8$ conidia ml$^{-1}$). Two strains of *Beauveria bassiana* showed 30% and 40% mortality within 30 days. It is unlikely that these isolates would be effective in the field and they were not studied further.

*3.1.4 The effect of entomopathogenic nematodes on third instar leatherjackets*

Using the laboratory bioassay developed in 3.1.3, ten strains of entomopathogenic nematodes from the HRI culture collection were each bioassayed against 20 third instar leatherjackets. A single dose of nematodes (500) was applied directly to 20 g compost (John Innes, F2) of 15 bioassay chambers (30 ml ventilated Universal bottles) immediately after the addition of one leatherjacket per chamber. Leatherjackets were maintained at 20$^{O}$C and a 16L:8D photoperiod and mortality assessed every seven days for four weeks. Four strains (*Heterorhabditis megidis* HRI-HUK, *Steinernema* sp. C1 HRI-MGL66, *S. feltiae* HRI-128 and *S. feltiae* HRI-NOR14) killed leatherjackets, causing 100% mortality by 14 days post application.

### 3.2 Objective 2: To isolate insect pathogens from leatherjackets on organic farms

The soil provides a good environment for entomopathogens as it acts as a buffer against extreme conditions and contains a relatively high number of insects (Keller & Zimmerman, 1989). Entomopathogens are commonly isolated from the soil (Vanninen, Husberg & Hokkanen, 1989), but their role in the natural regulation of insect populations is poorly understood. A better understanding of the interactions between soil insects and their pathogens could improve microbial control, for example by identifying methods to enhance their activity. As part of this study, natural populations of leatherjackets were assessed for diseases and the background level of soil-borne entomopathogenic fungi and nematodes measured using larvae of the greater wax moth *Galleria mellonella* as a living selective medium. *Galleria mellonella* larvae are particularly susceptible to entomopathogens and can be used to isolate entomopathogenic fungi and nematodes from the soil (Bedding & Akhurst, 1975; Zimmerman, 1986).

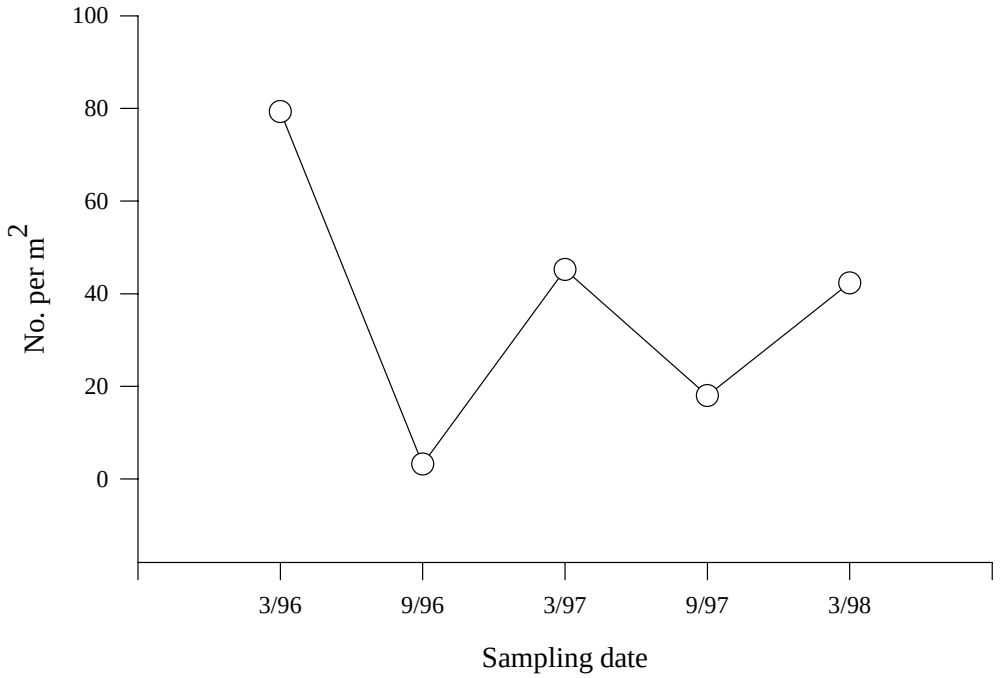
*3.2.1 Isolation of entomopathogens*

The frequencies of occurrence of leatherjackets, their associated pathogens and the background level of entomopathogenic fungi and nematodes was measured on 13 organic farms in the Southwest, West, and Midlands (including dairy farms, mixed farms, and stockless holdings) and on an area of (non-organic) permanent pastureland at ADAS Drayton. Approximately 2000 soil cores (c. 400 ml), taken at a depth of 5-10 cm along transects of the fields, were collected over a two year period from different stages of crop rotations and placed in individual polyethylene bags.

Leatherjackets were collected from samples by hand and placed in individual sterile Petri dishes to avoid cross infection. Half of each batch of leatherjackets was examined for disease within a few days of sampling. The other half was maintained in individual Petri dishes at 20$^{O}$C for six weeks (dependent on which larval stage was collected) prior to examination. Pathogens were identified according to the criteria of Carter (1976). At the same time, one fourth instar larva of *G. mellonella* was added to the soil sample. Soil bags were stoppered with a foam bung, sealed with a rubber band and maintained at 20$^{O}$C and examined every week for one month. Insect cadavers were removed and surface sterilised in 1% sodium hypochlorite solution for three minutes, washed three times in sterile distilled water and placed on damp filter paper within a sealed Petri dish. Cadavers were incubated at 20$^{O}$C until nematodes or fungi had emerged from or developed on the integument. Fungi were identified *in situ* or after isolation onto malt agar (2.5% malt extract, 1.0% agar). Nematodes were identified *in situ*.

Leatherjacket populations were small on all farms studied except one, which was undergoing conversion and where leatherjackets had caused considerable damage to field vegetables grown after pasture. The population of leatherjackets monitored over the three years of the project at ADAS Drayton varied widely from year to year (Figure 1). A low incidence of disease (1.7 %) was recorded from approximately 850 second and third instar leatherjackets collected from organic grassland and field vegetable crops. Pathogens isolated included the entomopathogenic fungus *Beauveria bassiana*, two unidentified fungal isolates, the nematode *Rhabditis tipulae*, and *Tipula* nuclear polyhedrosis virus (TNPV). Parasitism by the tachinid fly, *Siphona geniculata*, was also observed. However, none of the fungal isolates or the nematode recovered from leatherjackets were active in subsequent laboratory bioassays. Mycosed craneflies, most probably infected with species of *Entomophthora*, were regularly observed during field excursions.

**Figure 1: Leatherjacket population survey at ADAS Drayton**




Overall, 1191 isolates of entomopathogenic fungi or nematodes were obtained from 2100 samples of soil (56.7% occurrence). Entomopathogenic fungi were obtained at every site sampled and were isolated from 46.7% of samples, while entomopathogenic nematodes were isolated from 10% of the samples (Table 2). The diversity of species was low, only six species of entomopathogenic fungi and one species of nematode being isolated. Most habitats were dominated by *Beauveria bassiana* and /or *Metarhizium anisopliae,* accounting for 49.1% and 43.7% of the fungal strains found respectively. Despite the wide range of soils sampled, overall no correlation was observed between the frequency of fungal occurrence and a range of abiotic factors including soil type, pH, organic matter content and cultivation techniques. There were indications, however, that fungal occurrence was related to habitat type. It is likely that the distribution, abundance and persistence of entomopathogens is governed by a range of interacting variables and the relative importance of a single factor is difficult to measure. The widespread and frequent occurrence of entomopathogenic fungi and nematodes is strong evidence of an involvement in the population dynamics of some insect species. However, even though entomopathogens were common at all sites, the incidence of disease in leatherjackets was low. It seems unlikely, therefore, that leatherjacket control could be improved by manipulating natural populations of soil-dwelling entomopathogens.

**Table 2: The % frequency of occurrence of entomopathogenic fungi and nematodes isolated from soils in the UK**

| Species | Habitat | |
|---|---|---|
| | Grassland | Mixed Organic |
| *Beauveria bassiana* | 27.5 | 15.9 |
| *Metarhizium anisopliae* | 12.2 | 33.2 |
| *Paecilomyces farinosus* | 0.8 | 0.0 |
| *Paecilomyces fumosoroseus* | 3.2 | 0.8 |
| *Steinernema* spp. | 8.5 | 12.3 |

Soil samples were taken from 13 organic farms in the Southwest, West, and Midlands (including dairy farms, mixed farms and stockless holdings) and on an area of (non-organic) permanent pastureland at ADAS Drayton. Entomopathogenic fungi and nematodes were recovered using the "*Galleria* bait" method.

### 3.3 Objective 3: To characterise pathogens virulent to leatherjackets.

*3.3.1 Multiple dose bioassays of Bacillus thuringiensis strains against neonate leatherjackets*
Seven strains of *Bt* identified in Objective 1 were studied further in multiple dose bioassays against neonate leatherjackets, and compared against *Bti*, using the bioassay developed in 3.1.2. Each strain of *Bt* was grown concurrently in Proflo B4 and harvested at 95% autolysis. The broths were diluted in 0.1% Triton-X100 at rates of 9.1 μl broth, 2.5 μl broth, 0.69 μl broth, 0.19 μl broth and 0.054 μl broth $ml^{-1}$ Triton-X100. Leaf discs (2.0 cm) were suspended in the aqueous suspensions of *Bt* broth and placed onto 1.5% water agar in compartmentalised Petri dishes and maintained as before. Each concentration was assayed against 40 neonate larvae. The mortalities of duplicate populations of neonate leatherjackets were recorded 48 hours after treatment. Median lethal concentrations were calculated by regression analysis following the logistic transformation of dose-mortality data (Table 3). Of the seven *Bt* strains studied, two exhibited similar potencies to the *Bti* positive control, the remainder were less potent. There was significant variation between replicates attributed to changes in the susceptibility of different batches of insects and differences in food consumption.

**Table 3: Activities of strains of *Bacillus thuringiensis* against neonate leatherjackets**

| Code number | Broth number | mean $eLC_{50}$ μl broth $ml^{-1}$ Triton x100 |
|---|---|---|
| *Bti* | | 1.1 |
| M02008 | J01039 | 4.6 |
| M02766 | J01425 | 1.25 |
| M04058 | J02083 | 3.3 |
| M03398 | J02598 | 45.7 |
| B00298 | J01520 | 1.6 |
| M02082 | J01101 | 4.2 |
| M04028 | J02047 | 6.6 |

Eight strains of *Bt* assayed against neonate leatherjackets at concentrations from 9.1 - 0.054 μl broth.$ml^{-1}$ Triton X100. Mortality assessed 48 hours post treatment. $eLC_{50}$ refers to an estimate of the median lethal concentration (s.e.d = 4.9 at 7 degrees of freedom).

*3.3.2 Plasmid profiles of Bacillus thuringiensis strains that killed neonate leatherjackets*
The plasmid analysis of the seven strains of *Bt* with activity against neonate leatherjackets, studied in 3.3.1, was performed as described by Jarrett (1985). DNA preparations were analysed on vertical 0.6% agarose gels ( 14 x 14 x 0.3 cm). Sodium dodecyl sulphate was added to the electrophoresis buffer to a final concentration of 0.1% (wt/vol) and samples were electrophoresed for 1 h at 5 mA followed by 3.5 h at 25 mA. Plasmid size was determined by comparison with the previously characterised *Bti* IPS82. Five of the seven strains showed identical profiles to that of *Bti*. Two strains (J01425 and J01101) exhibited different plasmid profiles to *Bti* and each other.

*3.3.3. Multiple dose bioassays of Bacillus thuringiensis strains against third instar leatherjackets*
The susceptibility of third instar leatherjackets to two strains of *Bt* (*Bti* standard and J01425) was measured using the laboratory bioassays developed in 3.1.3. Third instar leatherjackets were maintained individually within 20 ml damp compost (John Innes, F2) in 30ml, ventilated Universal bottles. The two *Bt* strains were grown concurrently in Proflo B4 and harvested at 95% autolysis. The broths were made up to 10 ml at rates of 1 ml broth, and 0.1, 0.01, 0.001 ml broth $ml^{-1}$ Triton-X100. Leaf discs (2cm diameter) of Chinese cabbage (var *Kasumi*) were treated with the four doses of bacterial suspension and placed on the surface of the compost. The bioassay chambers were maintained at $20^{O}C$ and a 16L:8D photoperiod. Larval mortality (Table 4) and leaf disc damage were assessed at 48 h intervals for seven days. Leatherjacket mortality occurred only at the highest doses and was consistently higher with *Bti* treated leaf discs. This dose also inhibited average leaf consumption by 48%.

**Table 4: Mean % mortality of third instar leatherjackets fed *Bacillus thuringiensis* treated leaf discs**

| | Day 2 | Day 4 | Day 6 | Day 7 |
|---|---|---|---|---|
| *Bti* | 0 | 33.3 (6.67) | 60 (20.0) | 86.67 (6.67) |
| J01425 | 0 | 13.3 (6.67) | 53.3 (17.61) | 73.3 (6.67) |

Figures refer to treatment of leaf discs with undiluted *Bt* broth (Proflo B4, harvested at 95% autolysis). Figures in parenthesis refer to the standard error of the mean.

*3.3.4 The susceptibility of third instar leatherjackets to Bacillus thuringiensis var. israelensis formulated as a bait*

Laboratory bioassays (3.1.3) were done to measure the susceptibility of third instar leatherjackets to *Bti* formulated as a bait. Leatherjackets were housed in 20 ml of damp compost (John Innes, F2) within ventilated Universal bottles. *Bti* was grown in Proflo B4, harvested at 95% autolysis and incorporated into bran at three rates; 2ml broth.$g^{-1}$ bran, 1ml.$g^{-1}$ bran and 0.5ml.$g^{-1}$ bran. Aliquots (1g) of each *Bti*-bran mixture was applied to the surface of the compost in 15 bottles. Larvae were incubated at 20$^{O}$C and a 16L:8D photoperiod and mortality was measured at intervals of 48 hours for eight days (Table 5). The median lethal concentrations at eight days post treatment (calculated by the logistic regression of dose response) was 0.84 ml broth.$g^{-1}$ bran. Leatherjackets were more susceptible to *Bti* applied in bran than on leaf material (3.3.3).

**Table 5: Mean % mortality of third instar leatherjackets treated with *Bacillus thuringiensis* var *israelensis* incorporated in a bran bait**

| Concentration ml /$g^{-1}$ | 2 days | 4days | 6 days | 8 days |
|---|---|---|---|---|
| 2ml broth /g bran | 0 | 40 (4.47) | 66.7 (4.80) | 93.3 (1.06) |
| 1.5 ml broth /g bran | 0 | 0 | 41.6 (3.40) | 70.5 (2.52) |
| 1ml broth /g bran | 0 | 0 | 33.3 (5.52) | 73.3 (13.3) |
| 0.75 ml broth /g bran | 0 | 0 | 29.0 (2.15) | 29.0 (2.15) |
| 0.5 ml broth /g bran | 0 | 0 | 6.7 (3.40) | 26.7 (6.67) |

Aliquots, 1g of *Bti* bran mixture applied to the surface of compost (20 ml) in bioassay chamber, 1 leatherjacket per chamber. Figures in parenthesis refer to the standard error of the mean

*3.3.5 Multiple dose bioassays of entomopathogenic nematodes against third instar leatherjackets*

Four strains of leatherjacket-active nematodes *(H. megidis* HRI-HUK*, Steinernema* sp. C1, HRI-MGL66, *S. feltiae* HRI-128 and *S. feltiae* HRI-NOR14), identified in 4.1.4, were studied further in multiple dose bioassays against third instar leatherjackets. Bioassay chambers were treated with five nematode concentrations; $10^4$, 5 x $10^3$, $10^3$, 5 x $10^2$ and $10^2$ nematodes larvae$^{-1}$. Each nematode concentration was applied directly to 20 g of compost (John Innes, F2) in each of 15 bioassay chambers (30 ml ventilated Universal bottles) immediately after the addition of one leatherjacket per chamber. Leatherjackets were maintained at 20$^{O}$C and a 16L:8D photoperiod and mortality assessed every four days for four weeks. All strains killed third instar leatherjackets at the highest nematode concentration per larvae but only two strains (*H. megidis* HRI-HUK and *S. feltiae* HRI-NOR14) exhibited good control at lower nematode concentrations. Median lethal concentrations were calculated by regression analysis following the logistic transformation of dose-mortality data (Table 6). Of the four strains *H. megidis* HRI-HUK and *S. feltiae* HRI-NOR14 had significantly ($p < 0.05$) lower $LC_{50}$s at 935 nematodes larvae$^{-1}$ and 735 nematodes larvae$^{-1}$ respectively. Nematodes were observed recycling in more leatherjackets killed by HRI-NOR14 than HRI-HUK. It was observed that some tipulid larvae were able to survive nematode infestation by the encapsulation of invading juveniles, particularly at the lower doses applied.

**Table 6: Activities of strains of entomopathogenic nematodes against third instar leatherjackets**

| Nematode strain | Species | Mean $eLC_{50}$ nematodes /larvae |
|---|---|---|
| HRI-HUK | *Heterorhabditis megidis* | 935 |
| HRI-MGL66 | *Steinernema* sp. C1 | 1897 |
| HRI-128 | *Steinernema feltiae* | 1624 |
| HRI-NOR14 | *Steinernema feltiae* | 739 |

Four strains of entomopathogenic nematode assayed against third instar leatherjackets at concentrations of $10^4$, 5 x $10^3$, $10^3$, 5 x $10^2$ and $10^2$ nematodes. larvae$^{-1}$. Mortality assessed 6 days post treatment. $eLC_{50}$ refers to an estimate of the median lethal concentration. (s.e.d on square root transformed data = 1.1 at 8 degrees of freedom).

### 3.4 Objective 4: To study the feeding behaviour of leatherjackets

The feeding behaviour of leatherjackets was studied in the laboratory, to develop strategies for the application of microbial pesticides in the field. Many soil-dwelling pests, including leatherjackets, are difficult to control with conventional insecticides, because of poor soil penetration and enhanced microbial degradation. Application strategies for microbial pesticides of leatherjackets must be based on an understanding of the mode of infection of the pathogen. *Bacillus thuringiensis*, for example, infects its host per os and thus should be applied to plants or formulated as an ingestible bait. Entomopathogenic nematodes, on the other hand, actively seek out their hosts and thus can be applied as a soil drench.

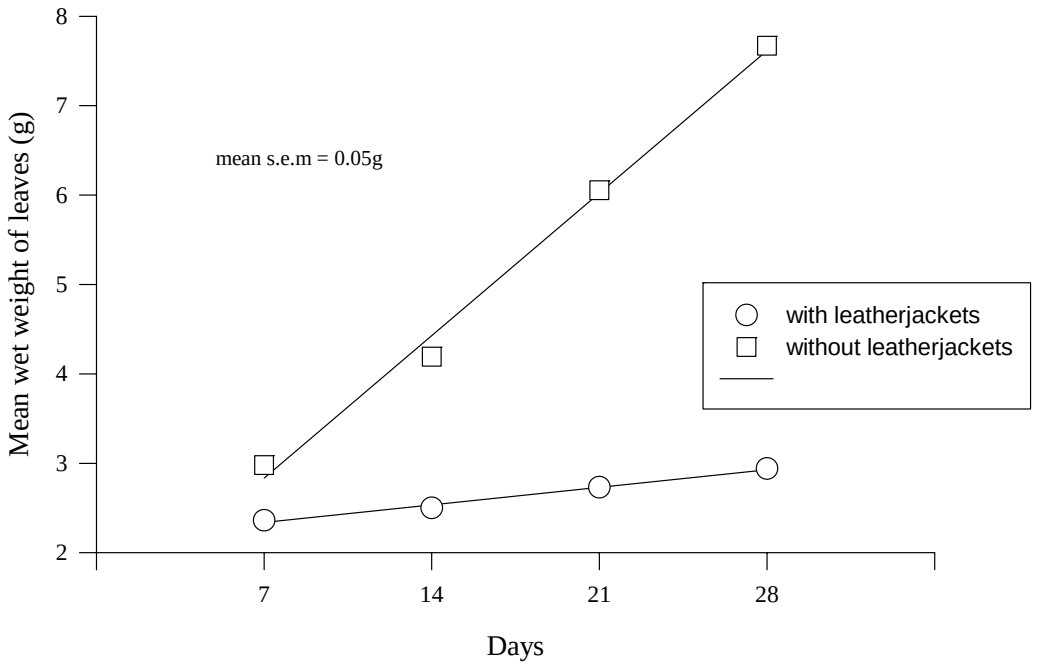
*3.4.1 Distribution of leatherjackets in relation to plant roots*

Leatherjackets are generally thought to be root feeders but field observations indicated that they can also feed on the soil surface. The movement of leatherjackets was observed within root chambers (10 x 7.5 x 1.5 cm) constructed from two glass plates separated by polyethylene foam spacers. Grass seedlings were grown for two weeks in compost (John Innes, F2) within root chambers at 20$^O$C and a 16L:8D photoperiod. Third instar leatherjackets were placed on the compost beside each plant stem, one leatherjacket per chamber, and the position of the larvae was noted at 8am (L) and 6pm (D) for seven days (three repetitions, 42 observations). Leatherjackets were observed within soil on 32 occasions (76%) and on the soil surface on 12 occasions (24%). Most soil surface activity (10 / 12 occasions) occurred during darkness.

*3.4.2 Determining the feeding location of third instar leatherjackets*

The preferred feeding location (roots or leaves) of third instar leatherjackets was determined in a choice experiment. Grass seedling were grown in compost (John Innes, F2) in 7.5 cm polyethylene pots (two seedlings per pot) in the glasshouse at c. 20$^O$C for two weeks. The compost of each pot was partitioned by a vertical nylon mesh, one seedling on each side. One seedling per pot was cut at soil level, the other half was covered at soil level with plaster of Paris. Leatherjackets were added to the compost surface of 20 pots, one insect per pot and maintained in the glasshouse at c. 20$^O$C for four weeks. Five pots were destructively sampled weekly for four weeks and leaf weights compared by a single factor ANOVA with those from pots with no leatherjackets. Leatherjackets were frequently observed on the soil surface and leaf damage was evident throughout the experiment. The wet weight of the above ground material (leaf and stem) of pots containing leatherjackets (mean weight = 2.5 g) was significantly different ($p < 0.05$) from pots containing no leatherjackets after 14 days (mean weight = 4.17g) (Figure 2). The rate of increase in leaf material was 0.196 g. day$^{-1}$ in pots with leatherjackets and 1.594 g. day$^{-1}$ in pots without leatherjackets.

**Figure 2: Mean wet weights of leaf material with and without leatherjackets**




Weight of leaf material in the presence or absence of leatherjackets (min. samples number, n = 5).

### 3.5 Objective 5: To evaluate selected pathogens in glasshouse trials

The effect of *Bti*, *H. megidis* HRI-HUK and *S. feltiae* HRI-NOR14, on third instar leatherjackets feeding on barley or cabbage, was measured in a glasshouse experiment. One week old seedlings of barley and cabbage were transplanted into 7.5 cm polyethylene pots containing damp compost (John Innes, F2) to which one third instar leatherjacket was added. *Bti* ( + 20 μl of etalfix) was applied directly to the leaves of plants at rates of 1 ml broth, 0.75 ml broth/ 0.25 ml Triton-X100, 0.5ml broth/ 0.5 ml Triton-X100 and 0.25ml broth/0.75 ml Triton-X100, in a volume of 10 ml using a hand held spray gun. *Bti* was also incorporated into a bran bait at rates of 2 ml broth.$g^{-1}$ bran, 1.5 ml broth.$g^{-1}$ bran, 1 ml broth.$g^{-1}$ bran and 0.5 ml broth.$g^{-1}$ bran, in a volume of 10 g and applied to the surface of the compost. Nematodes were applied directly to the compost at rates of 2 x $10^3$, 1.5 x $10^3$, $10^3$ and 5 x $10^2$ nematodes $larvae^{-1}$. Each treatment was applied to 10 larvae and the experiment was replicated three times. All treatments were fully randomised. Leaf damage was assessed every two days and leatherjacket mortality was assessed after 14 days. *Bti* incorporated into a bran bait and the nematode strain *S. feltiae* HRI-NOR14 exhibited better control of leatherjackets than the *Bti* leaf spray or the nematode strain *H. megidis* HRI-HUK (Table 7). A reduction in leaf damage compared with control plants was only observed at the highest doses of *Bti*-bran bait and *S. feltiae* HRI-NOR14 and corresponded to 9.1% and 13.8% increase in mass of leaf material respectively. There was no evidence of any effect of crop type. The *Bti* leaf spray only killed leatherjackets at the highest dose studied. The median lethal concentrations at fourteen days post treatment (calculated by the logistic regression of dose response) for *Bti*-bran bait and *S. feltiae* HRI-NOR14 was 1.66 ml broth.$g^{-1}$ bran and 1.382 x $10^3$ nematodes $larvae^{-1}$ respectively. In both cases the $LC_{50}$ was approximately twice that found in laboratory bioassays.

**Table 7: Activities of *Bacillus thuringiensis* and entomopathogenic nematodes against third instar leatherjackets under glasshouse conditions**

| Treatment | Mean $eLC_{50}$ | |
|---|---|---|
| | cabbage plants | barley plants |
| HRI-HUK | 2249 nematodes $larvae^{-1}$ | 2999 nematodes $larvae^{-1}$ |
| HRI-NOR 14 | 1354 nematodes $larvae^{-1}$ | 1409 nematodes $larvae^{-1}$ |
| *Bti* bran bait | 1.66 ml broth.g $bran^{-1}$ | 1.58 ml broth.g $bran^{-1}$ |
| *Bti* leaf spray* | 13.3 | 20.0 |

*Bti* and entomopathogenic nematodes applied to leatherjackets feeding on cabbage or barley seedlings maintained under glasshouse conditions. Mortality assessed 14 days post treatment.
* no dose response observed, figures refer to % mortality at highest dose (undiluted *Bt* broth).

### 3.6 Objective 6: To evaluate pathogens in an organic crop production system in the field

The effect of *Bti* and *H. megidis* HRI-HUK on third instar leatherjackets was measured in a split plot field experiment on the UKROFS field facility at HDRA Ryton. The design of the field experiment had to be adapted immediately prior to treatment due to damage by rabbits which eradicated some plots. Each plot covered 0.5 $m^2$, contained 18 cabbage plants (var *January King*) and was surrounded by a polyethylene barrier, sunk to a depth of 10cm, to prevent leatherjackets migrating between plots. Treatments consisted of; (i) a *Bti* leaf spray of pure *Bti* broth in a volume of 100 ml; (ii) a *Bti* bran bait formulation at a rate of 2ml broth.$g^{-1}$ bran in a mass of 200g; and (iii) *H. megidis* HRI-HUK applied as a drench at a rate of 5 x $10^5$ $m^2$; iv) untreated control. Each treatment was replicated four times in separate plots. *Bti* leaf spray was applied weekly using a hand held spray gun with an addition of 20 μl of etalfix (leaf sticker), *Bti* bran bait was also applied weekly by hand and the nematodes were applied only once in a volume of 100 ml using a watering can. To each plot 25 third instar leatherjackets were added. Crop damage was assessed twice weekly and crop yield measured four weeks post treatment. Numbers of leatherjackets were counted and assessed for sub lethal effects. Leatherjacket mortality was compared by single factor ANOVA following arcsine transformation. Further rabbit damage made it difficult to draw conclusions of the effect of the treatments on total yield of the plots. However, significantly more leatherjackets were recovered from control plots (52.5% recovery rate) than from treated plots (Table 8). The *Bti* bait treatment showed the greatest potential in controlling leatherjackets in the field (mean treatment mortality = 96.5%), closely followed by the *Bti* spray (mean treatment mortality = 91.75%) and the nematode treatment *H. megidis* HRI-HUK (mean treatment mortality = 86.75%).

**Table 8: Mean % mortality of third instar leatherjackets under field conditions**

| | Block 1 | Block 2 | Block 3 | Block 4 | Mean |
|---|---|---|---|---|---|
| HRI-NOR 14 | 100 | 87 | 87 | 73 | 86.75 (2.02) |
| *Bti* bran bait | 93 | 100 | 100 | 93 | 96.5 (3.09) |
| *Bti* spray | 87 | 93 | 87 | 100 | 91.75 (5.51) |
| Untreated | 60 | 60 | 50 | 40 | 47.5 (4.79) |

Mortality assessed 4 weeks post treatment. Figures in parenthesis refer to the standard error of the mean.

## 4. Conclusions and future research

Strains of *Bacillus thuringiensis* and entomopathogenic nematodes that kill leatherjackets were identified and characterised in laboratory and field experiments. The pathogens were applied in the field using strategies that exploited the mode of infection and host feeding behaviour. The strains studied in this project were obtained from a small fraction of the available gene pool, and there is scope to widen the bioassay programme to select pathogens with greater virulence to leatherjackets.

Leatherjackets are usually localised and sporadic pests. Biopesticides of leatherjackets would probably be most cost effective when used to control populations feeding on field vegetables planted after grassland. In general, the need for biological controls of pests in organic farming is likely to increase, as the intensity and area of production rises to meet consumer demand.

The finding that insect pathogenic fungi and nematodes are extremely common in agricultural habitats may well indicate an involvement in the population dynamics of some insect species. However, the incidence of disease in leatherjackets was low and there would appear to be few opportunities to enhance control by naturally occurring pathogens. Despite this, there is considerable opportunity to study further the ecology of soil-dwelling, insect pathogenic fungi and nematodes, to characterise their role as natural regulators of pest populations and to identify strategies to enhance their activity where they have been shown to interact with pest populations.

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