Project title:	Novel approaches for the management of cabbage root fly
Project number:	FV 364 (HDC PhD Studentship)
Project leaders:	Dr Andy Evans, Scottish Agricultural College Dr Nick Birch, The James Hutton Institute Dr Tom Shepherd, The James Hutton Institute
Report:	Annual report, May 2012
Previous report:	Annual report, May 2011
Key staff:	William Deasy (PhD student)
Location of project:	Scottish Agricultural College, Edinburgh The James Hutton Institute, Dundee
Industry Representative:	Mr Andrew Orr, East of Scotland Growers, Cupar
Date project commenced:	26th April 2010
Date project completed (or expected completion date):	30th April 2013

DISCLAIMER

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

William Deasy
PhD student
SAC Edinburgh/James Hutton Institute

Signature .... Date ....

Dr Andy Evans
Entomologist
SAC Edinburgh

Signature .... Date ....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature .... Date ....

[Name]
[Position]
[Organisation]

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

Research is ongoing to identify the chemicals that cabbage root fly larvae use to find calabrese roots. These chemicals will be evaluated as treatments that divert or prevent colonization of roots by cabbage root fly larvae. Other treatments are also being evaluated that can 'switch on' natural defences against cabbage root fly.

Background

Cabbage root fly is an economically important specialist insect pest of plants in the Brassicaceae family. Damage is caused by below-ground larvae feeding on plant roots. Plants can be attacked at any growth stage but the most serious damage is caused to young transplants soon after planting in the field.

Cabbage root fly control in the UK is currently reliant almost predominantly on pest forecasting (e.g. the HDC Pest Bulletin), pre-planting application of an organophosphorus insecticide (chlorpyrifos), use of crop covers (where applicable), and plant resistance. Current pesticide legislation is placing a greater emphasis on Integrated Pest Management (IPM) programmes. Under an IPM system, growers are encouraged to employ a combination of available chemical, cultural, and biological control methods in order to minimise the harmful side effects that can result from exclusive use of chemical insecticides. The ongoing review and withdrawal of several pesticides as a result of environmental, food safety and operator health concerns, means that growers are faced with fewer chemical control options to utilise while alternatives are being researched and developed.

The number of generations of cabbage root fly per year depends on prevailing climatic conditions. In the UK, there are normally two generations in the north and three in the south during a growing season. The life cycle involves an above ground adult stage and soil dwelling larval stage. Females oviposit at the base of the shoot or in the soil near the roots. Larvae that emerge from eggs move through the soil to locate host-plant roots to feed on in order to survive.

While only limited information exists about how cabbage root fly larvae detect and find roots, the consensus is that chemical cues released in Brassica plant root exudates, either as volatiles or in solution, play a key role in root location. Through a combination of techniques, including choice-test bioassays, metabolomic analysis, detailed behavioural observations, glasshouse and field trials, this project aims to identify compounds in root exudates that larvae exploit to locate roots to feed on. This will facilitate testing and development of potential control methods, utilising attractant and repellent compounds, to disrupt normal orientation behaviour for use as part of a sustainable IPM programme.

Plants protect themselves against insect attack using many defense strategies, such as secondary compounds that are toxic, repellent or anti-digestive, or morphological traits, which can negatively affect the performance of the herbivore. Elicitors are compounds that characterise attack and whose perception by the plant can induce a defensive response both locally in herbivore-attacked regions and systemically in undamaged parts.

Sugar sensing and signalling pathways interact with plant hormone signalling mechanisms to control metabolism, growth and stress responses. It has recently been hypothesised that extracellular sugars, occurring outside their normal compartment, indicate a disrupted or damaged plant cell, triggering hormone-mediated defense responses. The aims of this work are to investigate how sugar sensing affects Brassica plants' defense system and growth, and whether exogenous foliar and root applications of aqueous solutions of sugars can mimic and elicit inducible resistance against cabbage root fly.

Gucosinolate-containing plants in the Brassicaceae family, incorporated into soil as biofumigants, represent a potential source for pest, disease and weed control. Isothiocyanates, products of glucosinolate-myrosinase hydrolysis, are unpalatable and toxic to many generalist and specialist insects. Despite the fact that several specialist insects including *Delia* spp. have evolved mechanisms to cope with the toxicity of these compounds, beyond certain levels even these insects can be repelled and/or deterred. Using glasshouse pot tests and field trials, this work aims to evaluate the effect of an isothiocyanate-containing liquid biofumigant formulation ('Caliente' mustard), applied as a root drench, on cabbage root fly oviposition, egg survival, and larvae, along with resulting crop yields.

Summary of the results and main conclusions

This research aims to utilise the chemicals present in root and plant exudates that newly hatched cabbage root fly larvae use to locate roots to feed on, to disrupt their behaviour and reduce the larval colonisation of calabrese plants.

Sugar sensing in plants has recently been discovered to be involved in triggering inducible and systemic resistance to insects, nematodes and fungi. This project will determine whether the application of sugars and other ‘elicitors’ to foliage and/or seed can induce defence mechanisms in calabrese plants that can protect roots from cabbage root fly damage.

The most effective treatments will be utilised in a novel system of cabbage root fly pest management that disrupts host-plant location by the larvae. This will be evaluated in field trial. The potential delivery of these treatments will be in the form of incorporation into soil-applied slow-release granular formulations, seed coatings, foliar /soil sprays and/or treated plugs for transplants.

Promising results from laboratory, field (Figure 1) and glasshouse experiments to date, along with ongoing glasshouse studies investigating combinations of treatments, will form the basis for the 2012 field trial to further evaluate effective treatments for cabbage root fly management. Behavioural bioassays testing compounds identified from root volatiles experiments (Figure 2) will continue in efforts to elucidate the mechanisms underpinning larval host-plant location and avoidance of negative root signals (e.g. repellent compounds).

Figure 1. Field trial 2011 (Kelso, Scotland)

Figure 2. Root volatiles collection

Financial Benefits

At this stage in the project (end of Year 2 out of 3) we are not at a stage to be able to give an accurate estimate of financial benefits to growers. The financial benefits will become clearer once data from field trials in Years 2 & 3 have been obtained and fully analysed.

Action Points

At this point trials are underway to determine the optimal approaches for the application of these alternative treatments to reduce cabbage root fly damage, so it is too early to offer growers specific action points to achieve significant benefits for cabbage root fly management.

SCIENCE SECTION

Introduction

1. This project is investigating the hypothesis that cabbage root fly (*Delia radicum* L.) larvae recognize and orient towards *Brassica* root-derived semiochemical cues released in the soil to locate host-plant roots to feed on, and that other root volatiles can act as repellent signals to newly hatched larvae. To test this, we are assessing larval responses in behavioural bioassays to roots of host- and non-host plants along with chemical compounds identified from analyses of broccoli and other roots.
2. A field trial conducted in 2011 and ongoing glasshouse pot trials are studying plant inducible defence responses in broccoli against cabbage root fly, with the longer term aim of adding new control strategies to the IPM toolbox for this key pest of *Brassica* crops.

Materials and methods

Root volatiles collection

A novel sampling technique using solid phase micro extraction (Figure 1) has been developed to entrain volatiles in the immediate vicinity of growing roots (Figure 2). Compounds released by intact and damaged broccoli roots were analysed by gas chromatography mass spectrometry (Figure 3) and identified by comparing with pure standards.

Figure 1. Solid phase micro extraction (SPME)

Figure 2. Root volatiles collection using SPME

Figure 3. Gas chromatography mass spectrometry (GCMS)

Identification of attractants/repellents

Larval responses to host- and non-host roots and identified compounds are currently being evaluated in ongoing behavioural bioassays (partially developed during year 1 of the project) using the Noldus EthoVision video camera and software system.

Field trial 2011

Broccoli variety 'Parthenon' plants were used in the field experiment. Soil samples were collected for nutrient analysis (Analytical Services Department, SAC, UK) on day one of the trial (Table 1).

Table 1. Soil nutrient analysis results

Determination	Result	Units	Status
pH	6.7		
Extractable phosphorus	22.7	mg/l	High
Extractable potassium	473	mg/l	Very high
Extractable magnesium	279	mg/l	High

Trial plots were set up in a commercial crop located in Kelso, Scotland (Figure 4) using a randomized complete block design consisting of four blocks. Each block contained 10 plots corresponding to the treatments: untreated; Chlorpyrifos; selected plant defence elicitors (Sigma-Aldrich, UK); and Caliente (Plant Health Care, UK) applied to leaves or roots. A plot comprised 36 plants. The experiment commenced on the 16th June and assessment (root damage and marketable yield) was conducted immediately post-harvest on the 9th September. Oviposition by *Delia radicum* was monitored weekly for the duration of the trial using soil samples removed from a 5 cm diameter around plant stems. Eggs were separated from the soil by flotation on water, identified and counted. For damage and yield assessment, 10 plants were randomly selected from each treatment plot at harvest. Plant stems were cut at the soil surface to separate roots from aboveground parts. In the laboratory, a measurement of the fresh weight of each individual head and washed root was recorded along with visual assessment scores for larval feeding damage to roots (Table 2) and marketable yield (Tables 3). Pupae washed from soil surrounding the roots were collected by floating on water, identified and counted.

Table 2. Root damage assessment

Score	
0	Undamaged
1	<25%
2	25-50%
3	>50%
4	>75%

Table 3. Broccoli marketable yield assessment

Assessment parameter	Score
Head development	0= no head, 1= partially developed, 2= fully developed
Head shape	1= flat, 10= domed
Evenness of head shape	1= uneven, 10= even
Bud colour	1= pale, 10= dark
Bud size	1= small, 10= large
Evenness of bud size	1= uneven, 10= even
Pest damage	0= undamaged, 10= all damaged
Disease	0= absent, 1= present

Statistical analysis was carried out using GenStat 14th Edition. A general analysis of variance (ANOVA) was performed for each assessment parameter. Least significant differences (LSD) at 5% were calculated for the determination of significant differences between treatments, including controls, when the *F*-ratio of the ANOVA was significant.

Figure 4. Field trial 2011 (Kelso, Scotland)

Figure 5. Larval feeding damage to broccoli roots

Glasshouse pot trial

Plants

Broccoli variety ‘Parthenon’ plants were used in the glasshouse test (Figure 6). Seeds (Sakata Seed, UK) were germinated at 21°C, 16/8 h L: D photoperiod in modules containing Levington M2 compost. At the 2 - 3 true leaf stage seedlings were transplanted to individual four litre pots containing a 3:1 Sinclair compost: sand mix for growing on at 21°C/16°C day: night temperature, 16/8 h L: D photoperiod. Pots were watered daily.

Insects

D. radicum larvae used for plant infestation were obtained from our own culture at The James Hutton Institute. The populations originated from pupae and eggs collected from several commercial *Brassica* crops in East Lothian, Fife and Kelso, Scotland. The culture was maintained using similar methods described by Finch and Coaker (1969).

Treatments and infestation

At the 5-6 true leaf stage plants were treated with a single application of a selected treatment: 50 ml root drench of plant defence elicitors (Sigma-Aldrich, UK); and 0.5 grams per plant TDE5/CRF (coded product). For infestation, 10 eggs per plant were placed in the growing media within 1 cm of the stem using a fine soft brush 24 hours post application for the plant defence elicitors, and immediately after applying TDE5/CRF.

The experiment was a randomized complete block design with two blocks consisting of 16 treatment plots per block. Each plot consisted of 10 plants. Five plants were selected at random from each treatment plot for assessment: root damage (Table 2); root fresh weight; leaf fresh weight; and number of pupae (larval survival and development). Statistical analysis was carried out using GenStat 14th Edition. A general analysis of variance (ANOVA) was performed for each assessment parameter. LSD at 5% were calculated for the determination of significant differences between treatments including controls when the *F*-ratio of the ANOVA was significant.

Figure 6. Glasshouse pot trial

Results

Root volatiles collection

Solid phase micro extraction (SPME) and gas chromatography mass spectrometry (GCMS) has revealed a profile of compounds which show a consistent, reproducible pattern pre- (Figure 7) and post-damage (Figure 8).

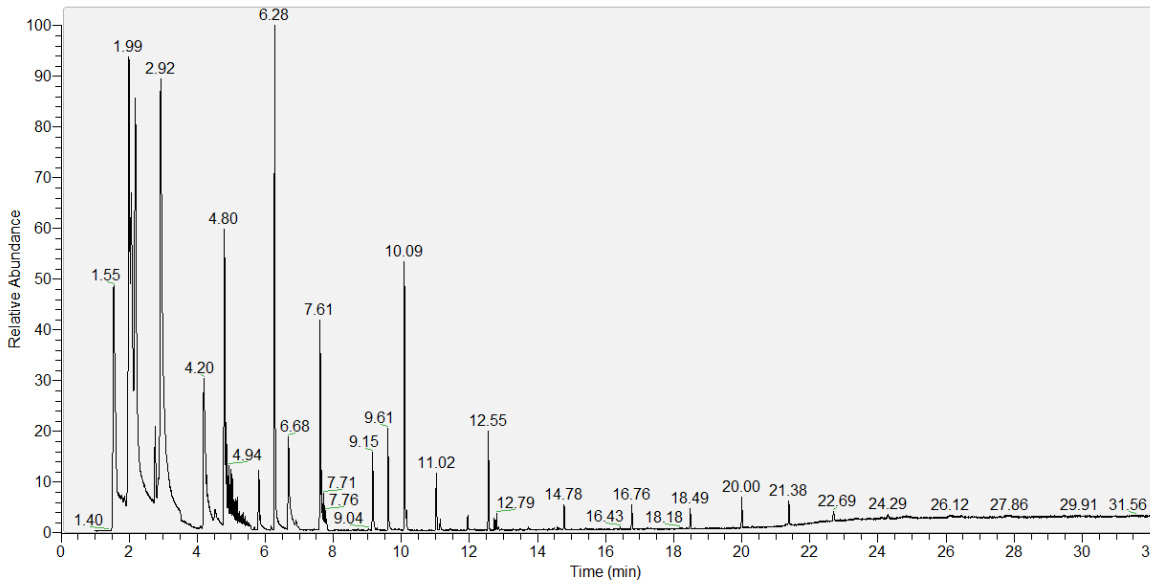

Figure 7. Typical chromatogram for intact broccoli roots

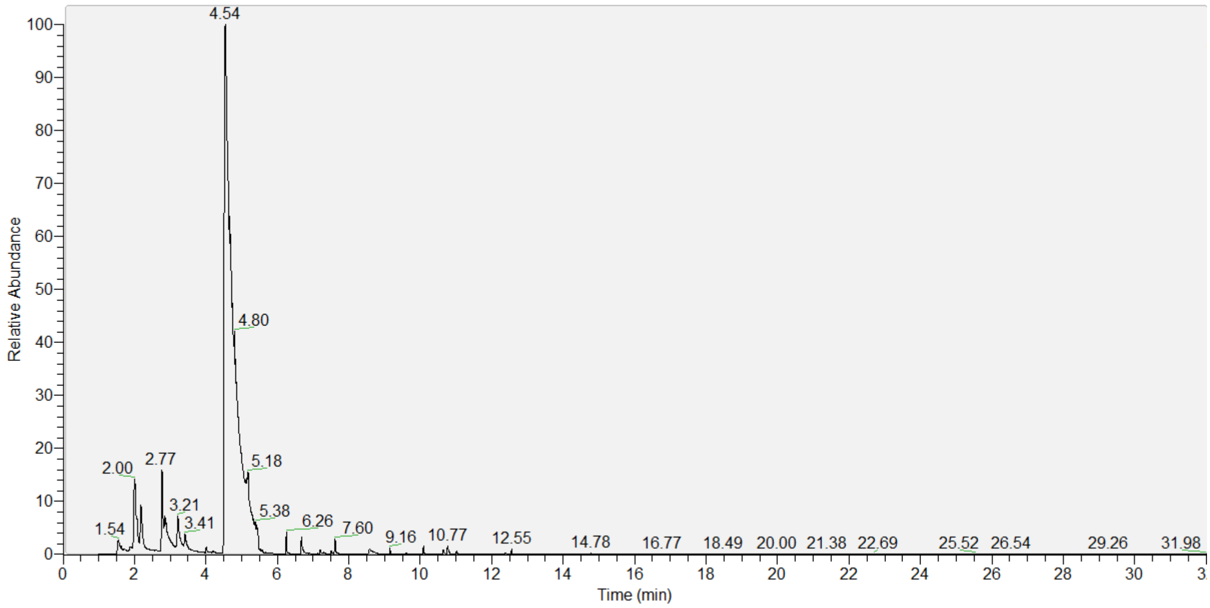

Figure 8. Typical chromatogram for damaged broccoli roots

Identification of attractants/repellents

Preliminary results using behavioural bioassays and EthoVision (Figure 9) suggest that *D. radicum* larvae can use root volatiles to locate host- and non-host plants. Larval responses to specific SPME-GCMS identified root-derived compounds are currently being tested in ongoing experiments.

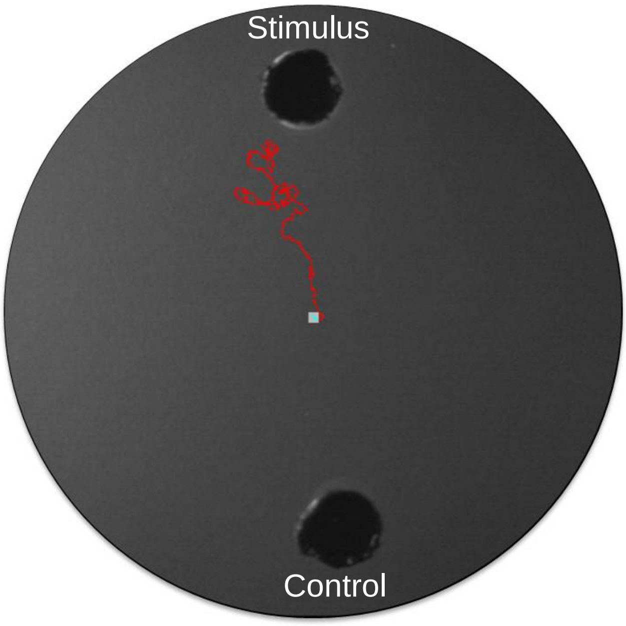

Figure 9. Larval tracks in response to a test stimulus recorded using EthoVision

Field trial 2011

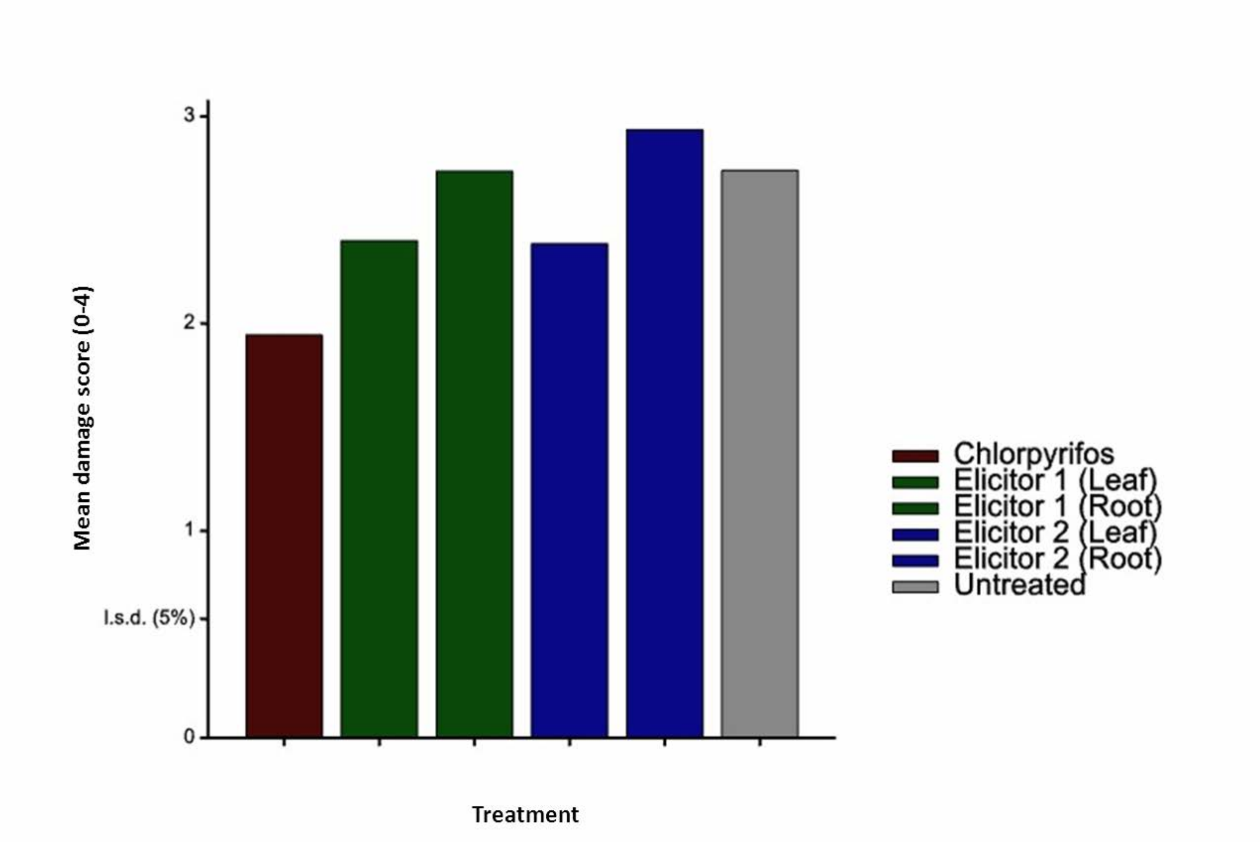

Figure 10. Mean root damage score (0-4) for *D. radicum* larval feeding on field grown broccoli plants (*n* = 40 per treatment). $LSD_{(p\ =\ 0.05)}$ between means is shown on the y-axis. (0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = > 50%, 4 = > 75%).

Table 4. Mean root damage score (0-4) for each treatment and LSD of means (5% level).

Treatment	Mean damage score (0-4)
Chlorpyrifos	1.944
Elicitor 1 (Leaf)	2.400
Elicitor 1 (Root)	2.735
Elicitor 2 (Leaf)	2.386
Elicitor 2 (Root)	2.936
Untreated	2.739
	$LSD_{(p\ =\ 0.05)}$ = 0.5738

In this field study, both plant defence elicitors did not differ significantly from untreated plants, though there was some evidence that larval feeding damage was reduced where treatments were applied to leaves (Table 4). Plants treated with Caliente (Dazitol), a biofumigant containing mustard and chilli pepper extracts, were not assessed due to the adverse phytotoxic effects on growth when used at the supplier's recommended rate.

Glasshouse pot trial

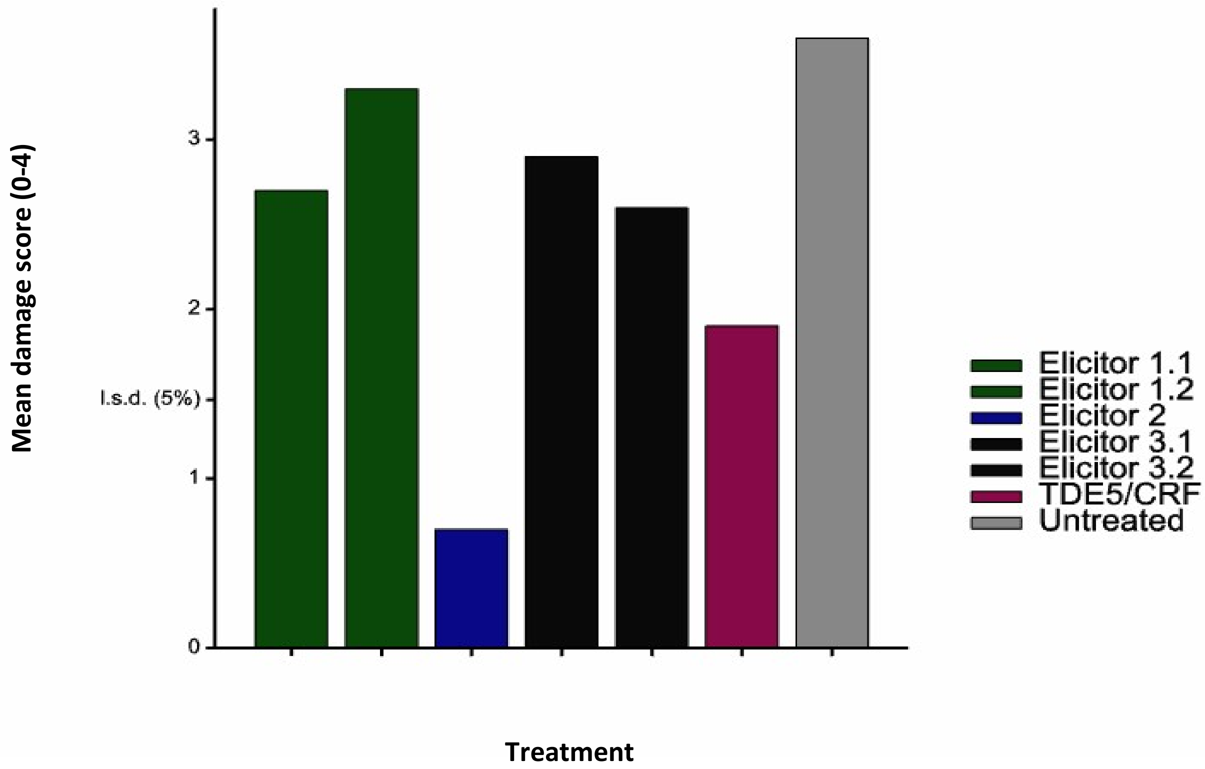

Figure 11. Mean root damage score (0-4) for *Delia radicum* larval feeding on glasshouse grown broccoli plants (n = 10 per treatment). $LSD_{(p\,=\,0.05)}$ between means is presented on the y-axis. (0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = > 50%, 4 = > 75%).

Table 5. Mean root damage score (0-4) for each treatment and LSD of means (5% level).

Treatment	Mean damage score (0-4)
Elicitor 1.1	2.70
Elicitor 1.2	3.30
Elicitor 2	0.70
Elicitor 3.1	2.90
Elicitor 3.2	2.60
TDE5/CRF	1.90
Untreated	3.60
	$LSD_{(p\,=\,0.05)}$ = 1.466

Under glasshouse conditions in this experiment, root damage was significantly reduced in plants treated with a plant defence elicitor as a soil drench and TDE5/CRF in comparison to untreated plants. All other treatments did not differ significantly from the control.

Discussion & Conclusions

- Promising results from laboratory, field and glasshouse experiments to date, along with ongoing glasshouse studies investigating combinations of treatments, will form the basis for the 2012 field trial.
- Behavioural bioassays testing compounds identified from root volatiles experiments will continue in efforts to elucidate the mechanisms underpinning larval host-plant location and avoidance of negative root signals (e.g. repellent volatiles).

Knowledge and Technology Transfer

- Oral presentation HDC protecting your field veg crop 2011.
- Oral presentation HDC Studentship Conference 2011.
- Poster session SAC Postgraduate Research Conference 2012.
- Oral presentation The James Hutton Institute Postgraduate Student Competition 2012.
- A4 poster (taken by Dr Nick Birch) 7th International IPM Symposium 2012.
- Proceedings paper submitted to IWSM July 2012 (collaboration with Dr Bruce Worton, University of Edinburgh).

References

Finch, S. and Coaker, T.H. (1969). A method for the continuous rearing of the cabbage root fly *Erioischia brassicae* (Bch.) and some observations on its biology. Bulletin of Entomological Research, 58: 619-627.

Kergunteuil, A. Dugravot, S. Mortreuil, A. Le Ralec, A. and Cortesero, A. M. (2012). Selecting volatiles to protect brassicaceous crops against the cabbage root fly, *Delia radicum*. *Entomologia Experimentalis et Applicata* 1-9, 14th International Symposium on Insect-Plant Interactions, DOI: 10.1111/j.1570-7458.2012.01257.x.

Koštál, V. (1992). Orientation behavior of newly hatched larvae of the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), to volatile plant metabolites. Journal of Insect Behavior, 5: 61-70.

Pierre, P. S. Dugravot, S. Cortesero, A. M. Poinsot, Denis. Raaijmakers, C. E. Hassan, H. M. and van Dam, N. M. (2011). Broccoli and turnip plants display contrasting responses to belowground induction by *Delia radicum* infestation and phytohormone applications. Phytochemistry, 73: 42-50.

Ross, K. T. A. and Anderson, M. (1992). Larval responses of three vegetable root fly pests of the genus *Delia* (Diptera: Anthomyiidae) to plant volatiles. Bulletin of Entomological Research, 82: 393-398.

Shepherd, T. Birch, A. N. E. Jorna, C. Mitchell, C. Cross, J. Hall, D. and Farman, D. (2009). Profiling of raspberry cane wound volatiles using a combination of SPME and GC-TOF-MS. Poster presented at: Metabomeeting 2009; 5-8th July 2009; Norwich BioSciences Institutes, UK.

