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| **Project title:** | Novel approaches for the management of cabbage root fly |
| **Project number:** | FV 364 |
| **Project leader:** | Andy Evans<br>SRUC |
| **Report:** | Final report, May 2014 |
| **Previous report:** | Annual report, May 2011<br>Annual report, May 2012 |
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| **Location of project:** | 2010-2011 SRUC<br>2011-2014 The James Hutton Institute |

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| **Industry Representative:** | Richard Haacker<br>Andrew Orr, East of Scotland Growers Ltd., Cupar, Fife, KY15 4RD |
| **Date project commenced:** | 01 May 2010 |
| **Date project completed** | 29 April 2014 |

The results and conclusions in this report are based on an investigation conducted over a four-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

## *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

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SRUC/The James Hutton Institute/University of Edinburgh

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*CONTENTS*

## *GROWER SUMMARY*

### Headline

- Candidate host plant location cues for cabbage root fly larvae were identified which may have potential application as attractants, repellents or biopesticides in future integrated pest management (IPM) strategies for this pest.
- Promising treatments from glasshouse and field trials require further research and development (e.g. formulation) before they can be considered for grower application.

### Background

Cabbage root fly (*Delia radicum*) is a key pest of *Brassica* crops. Female adult flies lay their eggs in the soil near *Brassica* host plants. Larvae that emerge must then quickly find the roots of their host plants to feed on to survive. The main pesticide used against cabbage root fly larvae chlorpyrifos (Dursban® WG) is currently under registration review. Consequently, there is a need for research into alternatives that comply with EC Directives and National Action Plans for sustainable use of pesticides and implementation of IPM in member states.

To gain new perspectives on cabbage root fly pest management, this project examined in detail how cabbage root fly larvae find the roots of *Brassica* plants in the soil. In addition, this project investigated the potential of 'switching on' inducible plant resistance against cabbage root fly in broccoli 'Parthenon' using plant defence elicitors. Induced resistance is that which appears only when a plant is attacked. Elicitors are compounds which activate one of several biosynthetic pathways involved in inducible resistance.

The elicitors used in this study were selected because previous studies have suggested that they have the potential to induce plant defence responses, but they have not previously been tested on Brassicas challenged by cabbage root fly.

### Summary

A combined bioassay test and video-tracking method was developed to record and analyse cabbage root fly larval behavioural responses to host and non-host plant root exudates and volatiles in the laboratory. Experiments showed that larvae follow specific plant chemical cues that are released by *Brassica* roots. A new soil probe method was developed to collect root volatiles *in situ* from glasshouse- and field-grown broccoli 'Parthenon' plants pre- and post-cabbage root fly infestation. Chemical analyses showed that sulfur compounds were the main volatiles released, particularly following root damage by cabbage root fly larval

 

feeding. In bioassay tests, a major sulfur volatile released by broccoli ‘Parthenon’ roots, was attractive to larvae, but toxic at the highest dose tested.

Glasshouse and field trials using broccoli ‘Parthenon’ were conducted to evaluate the efficacy of two experimental elicitors methyl jasmonate (MeJA) and D-Fructose, along with dimethyl disulfide (DMDS) and Caliente®/Dazitol against cabbage root fly compared to other crop protection products Dursban® WG, spinosad (Tracer®), entomopathogenic nematodes (Entonem) and garlic granules (ECOguard®). MeJA and garlic reduced larval performance under glasshouse conditions whereas D-Fructose and DMDS did not at the concentrations and modes of application tested. In field trials, MeJA combined with reduced rate Dursban® WG, Tracer®, and Entonem all showed partial efficacy for controlling cabbage root fly larvae, as did Caliente®/Dazitol. MeJA, DMDS and Caliente®/Dazitol were phytotoxic at several of the concentrations/volumes tested.

Overall, further research is needed into formulation, mode of application and timing to improve efficacy of promising treatments for potential use in future IPM strategies against cabbage root fly. Such research would ideally be in collaboration with a company having expertise in soil applied formulations. In addition, much research is still needed to characterise cabbage root fly larval responses to many of the compounds identified in this project before product development work could be undertaken.

## Financial Benefits

At this research and development stage it is too early to assess financial benefits.

## Action Points

New knowledge from this project can be used in future research and development of alternative semiochemical-based treatments for cabbage root fly compatible with IPM.

## *SCIENCE SECTION*

### Introduction

*Delia radicum* L. (Diptera: Anthomyiidae), the cabbage root fly, is a specialist root-feeding insect pest of *Brassica* crops. The ongoing registration review of chlorpyrifos, in line with current EU policy directed towards significantly reducing pesticide use and implementing integrated pest management (IPM) (Hillocks, 2012), opens new opportunities to research alternative pest management strategies for cabbage root fly.

Better understanding the host plant location of cabbage root fly larvae may provide valuable insights into how to manipulate their behaviour to reduce their chance of survival when searching in the soil for feeding sites on the roots of their host plant. Previous studies on cabbage root fly larval orientation behaviour have found that neonate larvae are attracted to odours from pieces of host plant roots in arena bioassays. Larvae showed attracted responses and the capacity to orientate by concentration gradients to a number of compounds that are general and specific volatile constituents of *Brassica* plants. Some of these compounds also elicited repulsion, particularly at high doses/concentrations (Finch and Skinner, 1974, Finch, 1977, Koštál, 1992, Ross and Anderson, 1992, den Ouden *et al*., 1996). These behavioural studies, along with physiological investigations on cabbage root fly larval sensory organs (Ryan and Behan, 1973, Ross and Anderson, 1991) demonstrate that larvae have specialised adaptive ability to detect and use *Brassica* host plant chemicals for orientation. However, the exact identity of the full profile of candidate cues specifically released from intact and/or induced growing roots of *Brassica* host plants still remains unclear.

One of the aims of this project was to identify the semiochemistry underpinning cabbage root fly larval host plant location.

 

Promotion of resistance in *Brassica* plants through the application of defence induction treatments (elicitors and phytohormones) and induced volatiles (semiochemicals) has the potential to influence cabbage root fly oviposition behaviour and larval growth and development. Previous studies have found that changes in non-volatile (e.g. glucosinolates) and volatile (e.g. sulfur compounds) *Brassica* plant chemistry induced by cabbage root fly larval feeding or phytohormone application to mimic root herbivory, can both positively and negatively affect cabbage root fly host plant selection and egg laying, reduce larval performance and numbers of emerging adults, and attract the main natural enemies of cabbage root fly (Baur *et al*., 1996a, Baur *et al*., 1996b, Hopkins *et al*., 1999, Neveu *et al*., 2002, Soler *et al*., 2005, van Dam and Raaijmakers, 2006, Ferry *et al*., 2007, Ferry *et al*., 2009, Kergunteuil *et al*., 2012, Pierre *et al*., 2012, Pierre *et al*., 2013).

This project further aimed to investigate whether plant defence induction treatments (MeJA, D-Fructose), an induced volatile (DMDS) and a biofumigant formulation (Caliente®) affect *D. radicum* larval performance and adult oviposition preference.

## Materials and methods

### Larval behavioural bioassays

Test stimuli (Table 1) were presented to a newly hatched individual cabbage root fly larva in choice-test bioassay arenas (Figure 1a). An EthoVision® video-tracking (Noldus; Tracksys Ltd., UK) technique (Figure 1b) was developed to record larval movement in response to the test stimuli. Bioassays were conducted using either a randomised complete block design or Latin square design. The data for results presented in this report were analysed using the Rayleigh test of uniformity on GenStat 16th Edition (VSN International Ltd., UK).

**Table 1:** Test stimuli presented to newly hatched cabbage root fly larvae in choice-test bioassays.

| Test stimuli |
|---|
| Allyl isothiocyanate (Sigma-Aldrich, UK) |
| 2-Chlorophenyl isothiocyanate (Sigma-Aldrich, UK) |
| Caliente®/Dazitol (Plant Health Care, UK) |
| Broccoli root exudates (*Brassica oleracea* L. convar. *botrytis* L. Alef. var. *cymosa* Duchesne 'Parthenon'; Sakata UK Ltd.) |
| Chinese cabbage root exudates (*Brassica pekinensis* L. 'Richi'; Sakata UK Ltd.) |
| Tomato root exudates (*Solanum lycopersicum* L. 'Shirley'; Enza Zaden UK Ltd.) |
| Volatiles from macerated broccoli 'Parthenon' roots |
| Volatiles from macerated Chinese cabbage 'Richi' roots |
| Volatiles from macerated tomato 'Shirley' roots |
| Volatiles from macerated *Brassica nigra* L. roots (Radboud University Nijmegen, The Netherlands) |
| Volatiles from macerated cauliflower roots (*Brassica oleracea* L. var. *botrytis* 'Atalaya'; Sakata UK Ltd.) |
| Sulfur volatile (Sigma-Aldrich, UK) |

 

**a** **b**

**Figure 1: a,** Choice-test bioassay arena consisting of a treatment (test stimulus) versus a control. **b,** EthoVision® video-tracking setup. A prepared arena was placed inside the enclosure before starting the test and recording larval movement.

## Root exudates and volatiles collection

Root exudates were collected from plants grown in nutrient solution (Murashige and Skoog and 3% sucrose/Ruakura) (Figure 2a). A new solid phase micro extraction (SPME; Supelco, UK)-based method was developed during this project for collecting root volatiles from glasshouse- and field-grown plants (Figure 2b and 2c, respectively).

**a** **b** **c**

**Figure 2: a,** Root exudates collection for bioassay tests and chemical analysis. **b,** Root volatiles collection in the glasshouse for chemical analysis. **c,** Root volatiles collection under field conditions for chemical analysis.

 

**Chemical analysis**

The non-volatile metabolite composition of root exudates was measured by gas chromatography mass spectrometry (GC-MS; Thermo Finnigan Trace DSQ GC-MS system). Root volatiles were analysed by SPME-GC-MS (Thermo Electron Corporation Trace DSQ™ II Series Quadrupole system) (Figure 3). Principal component analysis (GenStat) was applied to investigate the effect of *D. radicum* larval feeding damage on the composition of volatiles released by broccoli roots.

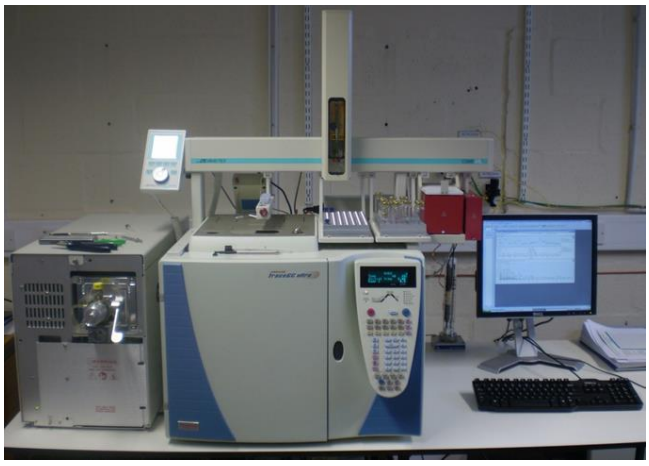

**Figure 3:** Gas chromatography mass spectrometry.

**Glasshouse trials**

Broccoli 'Parthenon' plants were used in the glasshouse tests (Figure 4a and 4b). Untreated seeds were germinated at 21°C, 16:8 hours (light:dark) photoperiod in modules containing Levington M2 compost. At the 2-3 true leaf stage plants were transplanted to individual 4 L containers with a 3:1 Sinclaire compost: sand mix for growing on at 21°C:16°C (day:night) temperature, 16:8 hours (light:dark) photoperiod. Natural day light was supplemented when required with artificial lighting (MASTER SON-T PIA Green Power; Philips, Guildford, UK) to maintain irradiance >200 W $m^{-2}$. Containers were watered to field capacity daily and allowed to drain freely. Plants were watered from the base following treatment applications. Cabbage root fly larvae used for plant infestation were obtained from our own culture at The James Hutton Institute.

 

**a** **b**

**Figure 4:** Glasshouse pot trials. **a,** Experiment 1. **b,** Experiment 2.

Table 2 displays a summary of the treatments used in glasshouse experiment 1. A single root drench application was applied to plants at the 5-6 true leaf stage using a 60 mL Plastipak syringe. The treatments were: 50 mL of distilled water applied to untreated control plants; 0.5 g/plant garlic granular formulation (Garlic), supplied by ECOspray Ltd., UK, placed on the growing substrate around plant stems (S. Silvester ECOspray Ltd, personal communication, 2012); 50 mL 1000 ppm MeJA aqueous solution (Sigma-Aldrich, UK) (Loivamäki *et al*., 2004); 50 mL 10 ppm D-Fructose aqueous solution (Sigma-Aldrich, UK) (Derridj *et al*., 2009); 50 mL 100 ppm D-Fructose aqueous solution; 50 mL 1 mM DMDS ethanol/distilled water solution (Sigma-Aldrich, UK); 50 mL 10 mM DMDS ethanol/distilled water solution; and 50 mL ethanol/distilled water solution (Sigma-Aldrich, UK). For infestation, 10 cabbage root fly 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 all experimental treatments except Garlic, which had eggs applied directly after applying the granules as recommended by ECOspray Ltd. Control plants for each treatment remained uninfested. The level of infestation was chosen as 10 larvae per plant as this is close to field infestation rates reported for cultivated *Brassica* species (Finch and Ackley, 1977, van Dam and Raaijmakers, 2006).

 

**Table 2:** Glasshouse experiment 1 treatments.

| Treatment | Application(s) |
|---|---|
| 50 mL of distilled water (control)/plant | 1 root drench |
| 0.5 g Garlic/plant | 1 root drench |
| 50 mL 1000 ppm MeJA/plant | 1 root drench |
| 50 mL 10 ppm D-Fructose/plant | 1 root drench |
| 50 mL 100 ppm D-Fructose/plant | 1 root drench |
| 50 mL 1 mM DMDS/plant | 1 root drench |
| 50 mL 10 mM DMDS/plant | 1 root drench |
| 50 mL ethanol and distilled water (control)/plant | 1 root drench |

Table 3 shows a summary of the treatments used in glasshouse experiment 2. At the 3-4 true leaf stage plants were treated with a single 50 mL root drench application using a 60 mL Plastipak syringe. The treatments were: distilled water for untreated control plants; 100 ppm MeJA aqueous solution; 1000 ppm MeJA aqueous solution; 100 ppm MeJA (25 mL) and 100 ppm D-Fructose (25 mL) aqueous solution; 100 ppm D-Fructose aqueous solution; 100 ppm MeJA (25 mL) and 10 mM DMDS (25 mL) ethanol/distilled water solution; and 100 mM DMDS ethanol/distilled water solution. For infestation, 10 cabbage root fly eggs per plant were placed in the growing media within 1 cm of the stem using a fine soft brush 24 hours post treatment. Control plants for each treatment remained uninfested.

**Table 3:** Glasshouse experiment 2 treatments.

| Treatment | Application(s) |
|---|---|
| 50 mL of distilled water (control)/plant | 1 root drench |
| 50 mL 100 ppm MeJA/plant | 1 root drench |
| 50 mL 1000 ppm MeJA/plant | 1 root drench |
| 25 mL 100 ppm MeJA and 25 mL 100 ppm D-Fructose/plant | 1 root drench |
| 50 mL 100 ppm D-Fructose/plant | 1 root drench |
| 25 mL 100 ppm MeJA and 25 mL 10 mM DMDS/plant | 1 root drench |
| 50 mL 100 mM DMDS/plant | 1 root drench |

 

To measure the leaf fresh weight, broccoli plant stems with leaves attached were cut at soil level using a secateurs (Felco®, UK) and weighed. Root (main and fine) were subsequently cleaned with water, dried with paper tissue and weighed. For external cabbage root fly damage assessment, washed roots were scored visually by placing into one of five categories outlined in Table 4 described by Hopkins (1994). Individual root damage scores were: 0, no visible damage present; 1, less than 25% root damage; 2, between 25 and 50% root damage; 3, over 50% root damage; and 4, more than 75% root damage with the tap root often girdled or severed. Cabbage root fly pupae retrieved by flotation on water and sieving during the cleaning procedure were counted.

**Table 4:** Cabbage root fly larval root damage assessment score.

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

Glasshouse experiment 1 was a randomised complete block design with two blocks consisting of 16 treatment plots per block. Each plot consisted of 10 broccoli plants. Five plants were selected at random from each treatment plot for assessment of leaf fresh weight, root fresh weight, cabbage root fly larval root damage, and number of pupae.

Glasshouse experiment 2 was a randomised complete block design with two blocks consisting of 18 treatment plots per block. Each plot consisted of six broccoli plants. 10 plants were selected at random from each treatment for assessment of leaf fresh weight, root fresh weight, cabbage root fly larval root damage, and number of pupae.

Statistical analysis was carried out using GenStat. A general parametric analysis of variance (ANOVA) was performed for the normally distributed variables assessed; broccoli leaf fresh weight, root fresh weight, and *D*. *radicum* root damage. A general linear model (GLM) approach was used to calculate ANOVA for the Poisson distributed variable; number of *D*. *radicum* pupae. 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.

 

## Field trials

### 2011 field trial (Experiment 1)

The field trial site was located in Kelso, Scotland (NT 76804 26878 UK Grid Reference) and was part of a commercial crop of broccoli (Figure 5a). Soil samples were collected for nutrient analysis (Analytical Services Department, SRUC, UK) on day one of the trial. Fertiliser was applied by the grower to the entire field, including the trial plots, immediately before transplanting with no further machine intervention to the plots beyond this. No insecticide or fungicide was used in the experimental plots. Beds containing the plots consisted of three rows of plants with a spacing distance of 33 cm between each plant.

**a** **b**

**Figure 5:** Field trial 2011. **a,** Site Kelso, Scotland. **b,** Cabbage root fly oviposition monitoring procedure.

Broccoli ‘Parthenon’ plants were used in the trial. Both treated and untreated module grown transplants at the 3-4 true leaf stage were supplied by East of Scotland Growers Ltd. Transplanting was undertaken manually. Table 5 shows a summary of the treatments used in field experiment 1. Chlorpyrifos (Dursban® WG) was the only application applied pre-transplanting by the plant producers (30 g in 5 L of water per 5, 000 plants). All other experimental treatments used on untreated plants were either applied as root drenches, using a 100 mL Plastipak syringe, or to leaves with Hozelock Plus Killaspray sprayers (B&Q, UK). The first applications of D-Fructose (Sigma-Aldrich®, UK), methyl jasmonate (MeJA) (Sigma-Aldrich®, UK) and Dazitol™ (Caliente®, Plant Health Care, UK) were immediately post-transplanting. D-Fructose and MeJA were applied on two additional consecutive dates over 10 day intervals. For treatment preparation and application, MeJA

 

1000 ppm aqueous solution with 0.01% Tween® 20 (Sigma-Aldrich®, UK) (Loivamäki *et al*., 2004) was made from 4.362 M concentration MeJA and applied at 50 mL per plant to roots and leaves on treatment date one, and to foliage only, until runoff, on subsequent dates as the number of leaves increased. 10 ppm aqueous solution of D-Fructose (Derridj *et al*., 2009) with 0.01% Tween® 20 was applied to roots and leaves at 50 mL per plant immediately after transplanting. Leaves only were sprayed until runoff for two further applications. Aqueous solution Dazitol™ treated plants received one post-transplanting root application only, at the label recommended rate (for in crop application to soil for biofumigation control of nematodes) of 20 L Dazitol™ and 15 mL of water/ha which was 0.5 mL Dazitol™ and 0.375 µL of water/plant.

**Table 5:** 2011 field trial (Experiment 1) treatments.

| Treatment | Application(s) |
|---|---|
| Untreated | |
| Chlorpyrifos (30 g in 5 L of water)/5, 000 plants | 1 root drench |
| 50 mL 1000 ppm MeJA/plant | 1 root drench |
| 50 mL 1000 ppm MeJA/plant | 3 leaf |
| 50 mL 10 ppm D-Fructose/plant | 1 root drench |
| 50 mL 10 ppm D-Fructose/plant | 3 leaf |
| 0.5 mL Dazitol™/plant | 1 root drench |

Cabbage root fly egg laying was monitored by removing soil samples from around the stem base. Plants for oviposition assessment were individually marked with a 20 cm length of wooden stake placed in the soil 15 cm away from the plant. Four plants were used in each treatment plot and monitored weekly for the duration of the field study from 16 June to 9 September 2011. The same plants were used throughout. Soil samples were removed from a 5 cm diameter around plant stems to a depth of 2 cm using a spoon (Figure 5b). The four samples from each treatment plot were combined in labelled plastic bags for laboratory counting of eggs. Removed soil was replaced with fresh soil to bring the site back to normal. *D*. *radicum* eggs were retrieved by washing the samples through a 2 mm aperture sieve (larger material discarded) and a second 750 µm aperture sieve, on which the eggs were retained. Collection was achieved by flotation on water in a filter paper-lined glass funnel and using a fine brush, before hatched and unhatched eggs were examined and counted. Plants in each plot were analysed for differences in the number of eggs between treatments.

 

The roots and surrounding soil of broccoli plants selected for assessment were hand-lifted from 12 to 13 September 2011. 10 plants were randomly selected from the centre of each treatment plot for assessment. Prior to lifting roots and soil, plant stems were cut at soil level and the heads retained for assessment. Roots and adjoining soil (25 cm deep, 25 cm diameter) were subsequently removed using a spade and placed in separate individually labelled plastic bags (Polybags Ltd., UK) for laboratory measurement of root fresh weight, visual scoring of cabbage root fly larval feeding damage and counting of pupae and larvae. To measure the fresh weight, roots (main and fine) were cleaned with water, dried with paper tissue and weighed. For external cabbage root fly damage assessment, washed roots were scored visually by placing into one of five categories outlined in Table 4. *D. radicum* pupae and larvae retrieved by flotation on water and sieving during the cleaning procedure were counted.

Experimental broccoli plant heads were manually harvested from assessment plants in accordance with industry standards from 9 to 10 September 2011 corresponding with the commercial crop harvest. The same 10 plants selected for yield assessment were used for root assessment. Harvested broccoli heads were placed in separate individually labelled plastic bags and transferred to the laboratory for measurement of fresh weight and diameter.

Field experiment 1 was a randomised complete block design consisting of four blocks (Figure 6). Each block included 10 randomised treatment plots. A plot comprised 36 broccoli plants spanning two beds (six rows with six plants in each row). Four plants per plot were randomly selected for cabbage root fly oviposition monitoring at the start of the trial. 10 plants were randomly selected for assessment. Oviposition monitoring plants were excluded from assessment. The outermost ‘margin’ plants separated plots from each other and were also excluded from assessment.

 

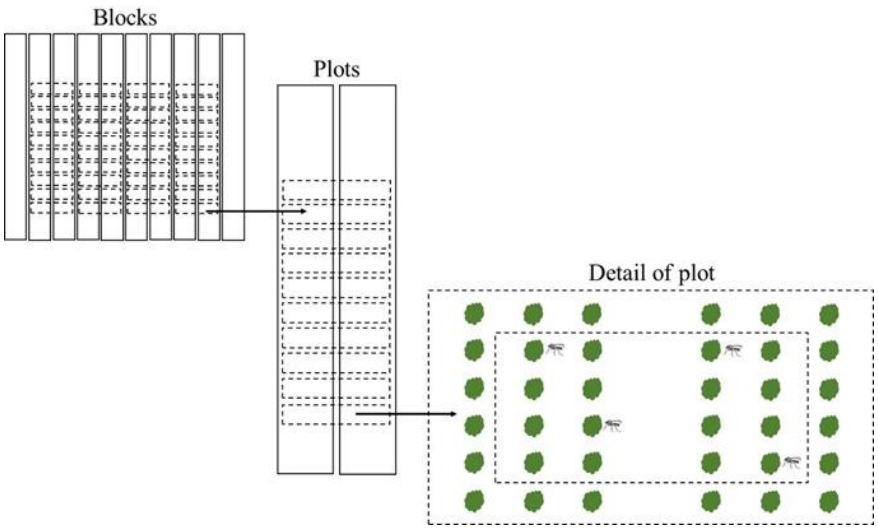


**Figure 6:** Schematic representation of the experimental design for field experiment 1 (2011). A treatment plot comprised 36 plants. Four plants were randomly chosen for cabbage root fly oviposition monitoring. 10 plants were selected for assessment. Oviposition monitoring and margin plants were excluded from assessment. Broccoli plant. Oviposition monitoring plant.

Statistical analysis was carried out using GenStat. When significant departure from homogeneity of variances or normality of residuals was found, data were square root transformed to approximate normality. A general parametric ANOVA was performed for the normally distributed variables; broccoli head fresh weight, head diameter, root fresh weight and cabbage root fly root damage. A GLM approach was used to calculate ANOVA for the Poisson distributed variables; number of cabbage root fly pupae and larvae, and number of eggs counted weekly. LSD at 5% were calculated for the determination of significant differences between treatments, including controls, when the *F*-ratio of the ANOVA was significant.

 

**2012 field trial (Experiment 2)**

The field trial site was located in Fife, Scotland (NO 40157 24978 UK Grid Reference) and was part of a commercial crop of broccoli (Figure 7a). Fertiliser was applied by the grower to the entire field, including the trial plots, immediately before transplanting with no further machine intervention to the plots beyond this. No insecticide or fungicide was used in the experimental plots. Beds containing the plots consisted of three rows of plants with a spacing distance of 33 cm between each plant.

**Figure 7:** Field trial 2012. **a,** Site Fife, Scotland. **b,** Felt trap cabbage root fly oviposition monitoring procedure.

Broccoli 'Parthenon' plants were used in the experiment. Both treated and untreated module grown transplants at the 3-4 true leaf stage were supplied by East of Scotland Growers Ltd. Table 6 represents a summary of the treatments used in field experiment 2. All experimental treatments, other than Chlorpyrifos (30 g in 5 L of water per 5, 000 plants) and Spinosad (Tracer®) (60 mL in 5 L of water per 5, 000 plants) applied by the plant producer, were made manually as root drenches using a 100 mL Plastipak syringe immediately post-transplanting. Garlic granules (ECOspray Ltd., UK) and Entonem [*Steinernema feltiae* Filipjev (Nematoda: Steinernematidae)] (Koppert, The Netherlands) were applied on a further two dates at 10 day intervals after the first treatment, while the remaining applications were only used once. For treatment preparation and application: MeJA 100 and 1000 ppm aqueous solutions with 0.01% Tween® 20 were made from 4.362 M concentration MeJA and applied at 50 mL per plant; 50 mL MeJA 100 ppm was combined with 70 mL of half the label recommended maximum rate for Chlorpyrifos (0.5 mL per L of distilled water) per plant; 50 mL MeJA 100 ppm was combined with 50 mL aqueous solution of 100 ppm D-Fructose and 0.01% Tween® 20; DMDS (Sigma-Aldrich®, UK) was diluted in ethanol to make a stock solution of 1.0 M (Huang *et al*., 2012) before subsequently diluting

 

the stock solution in distilled water to make a solution of 100 mM applied at 50 mL per plant; Garlic was applied at 0.5 g per plant; and Entonem consisting of 100 million nematodes (Schroder *et al*., 1996) per 10 L of distilled water was applied at 45 mL per plant (D. Foster, Koppert UK, personal communication, 2012).

**Table 6:** 2012 field trial (Experiment 2) treatments.

| Treatment | Application(s) |
|---|---|
| Untreated | |
| Chlorpyrifos (30 g in 5 L of water)/5, 000 plants | 1 root |
| 45 mL Entonem (100 million nematodes in 10 L of water)/plant | 3 root |
| Spinosad (60 mL in 5 L of water)/5, 000 plants | 1 root |
| 0.5 g Garlic/plant | 3 root |
| 50 mL 100 ppm MeJA/70 mL Chlorpyrifos (0.5 mL in 1 L of water)/plant | 1 root |
| 50 mL 100 ppm MeJA/50 mL 100 ppm D-Fructose/plant | 1 root |
| 50 mL 100 ppm MeJA/plant | 1 root |
| 50 mL 1000 ppm MeJA/plant | 1 root |
| 50 mL 100 mM DMDS/plant | 1 root |

Egg laying by cabbage root fly was monitored using felt traps (Ateliers Olbis, Switzerland) placed around the stem bases of the plants (Bligaard *et al*., 1999), using a protocol developed in the EU PURE project (Figure 7b). Four traps were used in each treatment plot and inspected weekly for the duration of the field study from 3 July to 17 September 2012. The same plants were used throughout. Hatched and unhatched eggs found inside were counted and removed before emptied traps were replaced on the plants. Traps in each plot were analysed for differences in the number of eggs between treatments.

The roots and surrounding soil of broccoli plants selected for assessment were hand-lifted from 19 to 20 September 2012 after heads were removed. Plant stems were first cut at soil level to collect heads for yield assessment, before the roots and adjoining soil (25 cm deep, 25 cm diameter) were removed using a spade and placed in separate individually labelled plastic bags for laboratory measurement of root fresh mass, visual scoring of cabbage root fly larval feeding damage and counting of pupae and larvae. To measure the fresh weight, roots (main and fine) were cleaned with water, dried with paper tissue and weighed. For external cabbage root fly damage assessment, washed roots were scored visually by placing into one of five categories outlined in Table 4. Pupae and larvae retrieved by flotation on water and sieving during the cleaning procedure were counted.

 

Experimental broccoli plant heads were manually harvested, in accordance with industry standards from 17 to 18 September 2012, corresponding with the commercial crop harvest. Five plants were randomly selected from the centre of each treatment plot for assessment. Harvested broccoli heads were placed in separate individually labelled plastic bags and removed to the laboratory for measurement of fresh weight and diameter.

Field experiment 2 was a randomised complete block design consisting of six blocks (Figure 8). Each block included 10 randomised treatment plots with treatments represented once per block. A plot comprised 36 broccoli plants spanning two beds (six rows of six plants each). Four plants per plot were selected randomly for oviposition monitoring. Five plants were randomly selected for assessment. Plants which had been used for cabbage root fly oviposition monitoring were excluded from assessment. Plants in the outermost ‘margin’ separating plots from each other were also excluded from assessment.

 

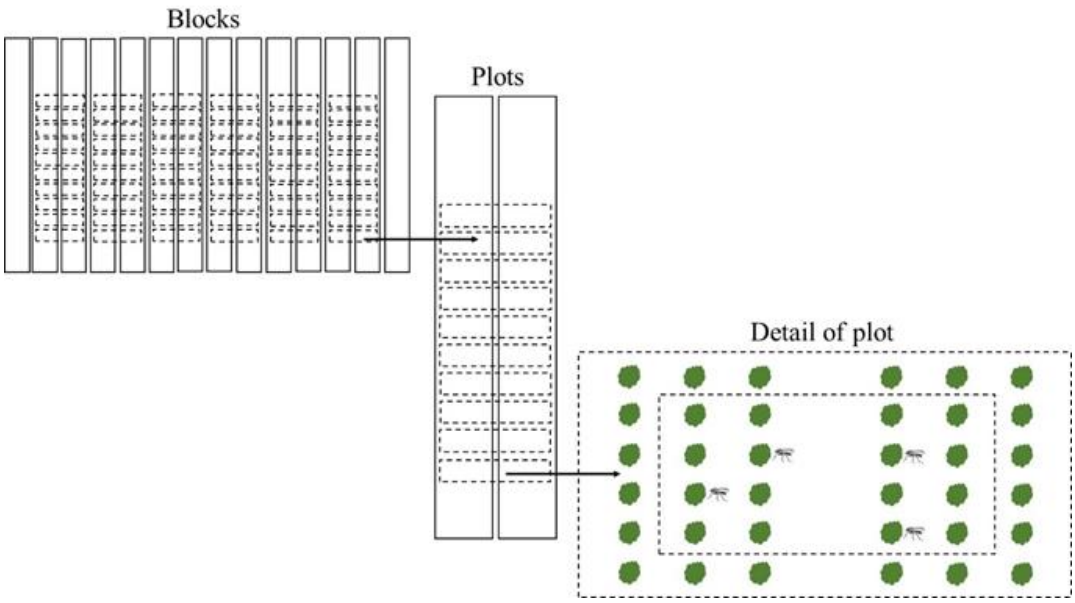


**Figure 8:** Schematic representation of the experimental design for field experiment 2 (2012). A treatment plot comprised 36 plants. Four plants were randomly chosen for cabbage root fly oviposition monitoring. Five plants were selected for assessment. Oviposition monitoring and margin plants were excluded from assessment. Broccoli plant. Oviposition monitoring plant.

Statistical analysis was carried out using GenStat. When significant departure from homogeneity of variances or normality of residuals was found, data were square root transformed to approximate normality. A general parametric ANOVA was performed for the normally distributed variables; broccoli head fresh weight, head diameter, root fresh weight, and cabbage root fly root damage. A GLM approach was used to calculate ANOVA for the Poisson distributed variables; number of cabbage root fly pupae and larvae, and number of eggs counted weekly. LSD at 5% were calculated for the determination of significant differences between treatments, including controls, when the *F*-ratio of the ANOVA was significant.

 

# Results

## Larval behavioural bioassays

**Table 7:** Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to ally isothiocyanate serially diluted. The control was diethyl ether. ($P \leq 0.05$).

| Serial dilution (%) | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|
| Neat | 30 | 24 | 0.318 |
| 10 | | | 0.954 |
| 1 | | | 0.995 |
| 0.1 | | | 0.438 |
| 0.01 | | | 0.147 |
| Control | | | 0.877 |
| Neat | 15 | 10 | 0.966 |
| 10 | | | 0.259 |
| 1 | | | **0.031** |
| 0.1 | | | 0.199 |
| 0.01 | | | 0.158 |
| Control | | | 0.059 |
| Neat | 5 | 10 | 0.223 |
| 10 | | | **0.001** |
| 1 | | | 0.229 |
| 0.1 | | | **0.001** |
| 0.01 | | | 0.089 |
| Control | | | 0.429 |

**Table 8:** Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to 2-Chlorophenyl isothiocyanate serially diluted. The control was diethyl ether. ($P \leq 0.05$).

| Serial dilution (%) | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|
| Neat | 30 | 24 | 0.980 |
| 10 | | | 0.880 |
| 1 | | | 0.999 |
| 0.1 | | | 0.981 |
| 0.01 | | | 0.152 |
| Control | | | 1.000 |

 

**Table 9:** Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to Dazitol™ serially diluted. The control was sterile distilled water. ($P \leq 0.05$).

| Serial dilution (%) | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|
| Neat | 30 | 24 | 0.943 |
| 10 | | | 0.645 |
| 1 | | | 0.674 |
| 0.1 | | | 0.231 |
| Control | | | 0.765 |

**Table 10:** Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to host and non-host plant root exudates. Exudates were collected in sterile distilled water from plants grown on Murashige and Skoog nutrient solution and 3% sucrose. The control was sterile distilled water. ($P \leq 0.05$).

| | Collection | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|---|
| Control | 1a | 40 | 24 | 0.976 |
| Broccoli | | | | 0.251 |
| Chinese cabbage | | | | 0.803 |
| Tomato | | | | 0.262 |
| Control | 1b | 40 | 24 | 0.912 |
| Broccoli | | | | 0.191 |
| Chinese cabbage | | | | 0.921 |
| Tomato | | | | 0.750 |
| Control | 2a | 40 | 16 | 0.885 |
| Broccoli | | | | 0.127 |
| Chinese cabbage | | | | 0.282 |
| Tomato | | | | 0.516 |
| Control | 2b | 40 | 16 | 0.929 |
| Broccoli | | | | **0.003** |
| Chinese cabbage | | | | 0.634 |
| Tomato | | | | 0.759 |

 

**Table 11**: Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to host plant root exudates. Exudates were collected in sterile distilled water from plants grown without sucrose on Ruakura nutrient solution. The control was sterile distilled water. ($P \leq 0.05$). damgd. denotes damaged.

| | Collection | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|---|
| Control | 1 | 40 | 30 | 0.650 |
| Broccoli | | | | **0.019** |
| Chinese cabbage | | | | 0.304 |
| Control | 2 | 40 | 30 | 0.562 |
| Broccoli | | | | 0.671 |
| Chinese cabbage | | | | 0.536 |
| Control | 3 | 40 | 30 | 0.527 |
| Broccoli | | | | 0.893 |
| Chinese cabbage | | | | 0.950 |
| Control | 4 | 40 | 30 | 0.939 |
| Broccoli | | | | 0.902 |
| Chinese cabbage | | | | 0.675 |
| Control | 5 | 40 | 20 | 0.157 |
| Broccoli | | | | 0.754 |
| Chinese cabbage | | | | 0.978 |
| Broccoli damgd. | 6 | 40 | 20 | 0.725 |
| Chinese cabbage damgd. | | | | **0.004** |
| Control (myrosinase) | 7 | 40 | 20 | 0.887 |
| Broccoli damgd. (myrosinase) | | | | 0.760 |
| Chinese cabbage damgd. (myrosinase) | | | | 0.838 |

**Table 12:** Rayleigh test of uniformity for resting positions of *Delia radicum* larvae in response to volatiles from macerated host and non-host plant roots. The control was distilled water. ($P \leq 0.05$).

| | Bioassay volume (µL) | Bioassay *n* = | *P* |
|---|---|---|---|
| Control | 40 | 20 | 0.411 |
| Tomato | | | 0.391 |
| *Brassica nigra* | | | **0.032** |
| Chinese cabbage | | | **0.010** |
| Broccoli | | | **0.001** |
| Cauliflower | | | **0.001** |

 

**Table 13:** Rayleigh test of uniformity for resting position of *Delia radicum* larvae in response to a sulfur compound. The controls were volatiles from macerated swede roots and untreated assay discs. ($P \leq 0.05$).

| | Bioassay *n* = | *P* |
|---|---|---|
| 0.05 µL | | 0.528 |
| 0.1 µL | | 0.520 |
| 1 µL | | **0.001** |
| 10 µL | 14 | 0.109 |
| 50 µL | | 0.373 |
| Swede (positive control) | | **0.001** |
| Control | | 0.691 |

**a** **b**

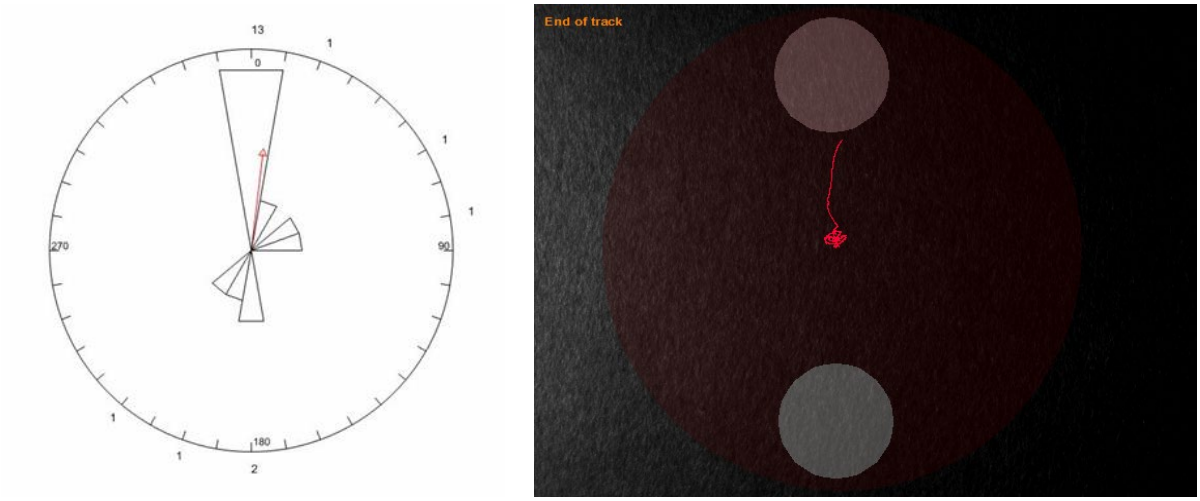


**Figure 9: a,** Directional responses of *Delia radicum* larvae to volatiles from macerated broccoli roots using the Rayleigh test of uniformity ($P = 0.001$; see Table 12). **b,** Newly hatched cabbage root fly larval tracks recorded using EthoVision® video-tracking software (Noldus; Tracksys Ltd., UK).

 

## Chemical analysis

Principal component analysis showed that i*n situ* SPME can detect broccoli root volatiles pre- and post-damage.

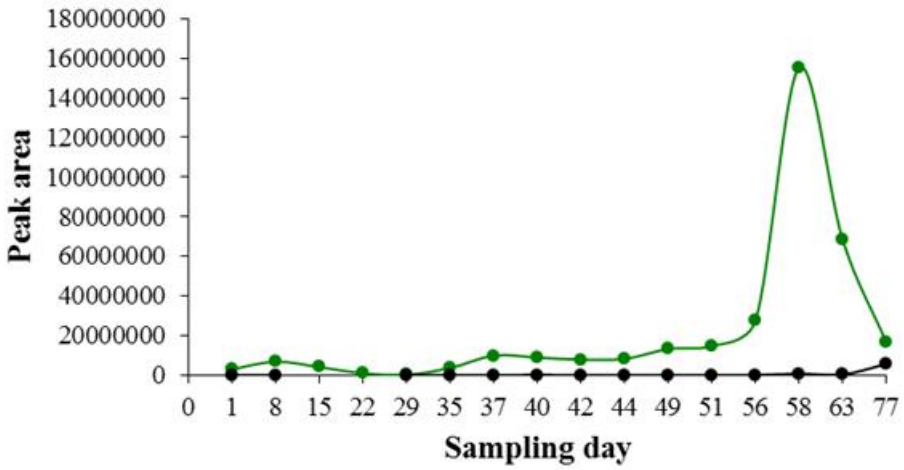


**Figure 10:** Time course of a selected sulfur compound identified from root volatiles samples collected from broccoli plants infested with cabbage root fly using *in situ* SPME. represent root volatiles samples, and represent soil control samples (*n* = 3).

## Glasshouse trials

### Glasshouse experiment 1

**Garlic**. Of the treatments tested in glasshouse experiment 1, Garlic proved superior at reducing *D. radicum* larval performance. Comparison with the untreated infested control plants showed that plants treated with Garlic had significantly less feeding damage (Figure 11) and significantly fewer larvae developing to pupal stage (Figure 12). Broccoli leaf fresh weight for infested Garlic treated plants was significantly higher than untreated infested controls (Figure 13), however there was no significant difference in root fresh weight between any of the treatments following ANOVA (Figure 14). Furthermore, the Garlic treatment did not adversely affect plant growth when compared with the untreated uninfested control plants (Figures 13 and 14).

**MeJA 1000 ppm**. MeJA induced plants reduced larval feeding damage and survival (Figures 11 and 12, respectively), but in contrast to plants treated with Garlic, leaf fresh weight did not differ significantly from untreated infested control plants (Figure 13). Similar to Garlic treated plants, leaf and root growth for the MeJA 1000 ppm treated uninfested

 

plants were not significantly different from untreated uninfested control plants (Figures 13 and 14, respectively).

**D-Fructose 10 ppm**. Plants treated with D-Fructose 10 ppm were not significantly different from untreated infested control plants in terms of damage and number of pupae (Figures 11 and 12, respectively). However, leaf fresh weight was significantly higher in D-Fructose 10 ppm treated infested plants compared with the untreated infested controls (Figure 13). Despite this, leaf and root fresh weight for uninfested D-Fructose 10 ppm treated plants were not significantly different from untreated uninfested controls (Figures 13 and 14, respectively).

**D-Fructose 100 ppm, DMDS 1 mM, DMDS 10 mM, and Ethanol**. Results for plants treated with these applications were not statistically different from D-Fructose 10 ppm treated plants.

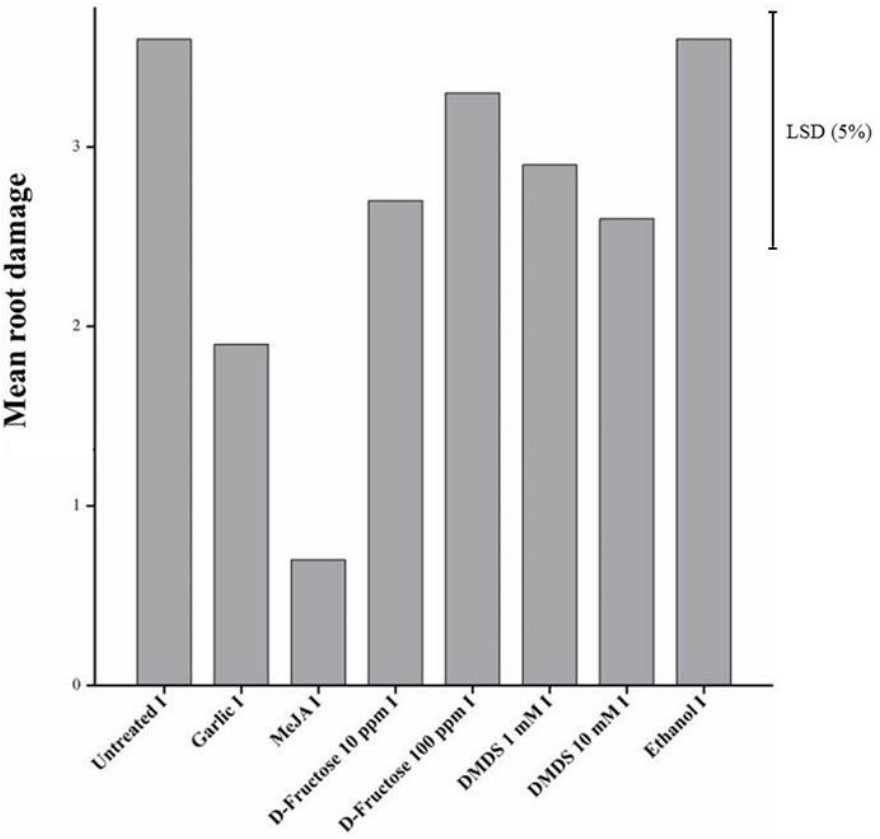


**Figure 11:** Glasshouse experiment 1 treatment means for *Delia radicum* root damage. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. (I) denotes infested plants. ($n$ = 10). Root damage was scored for each plant; 0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = >50%, 4 = >75%.

 

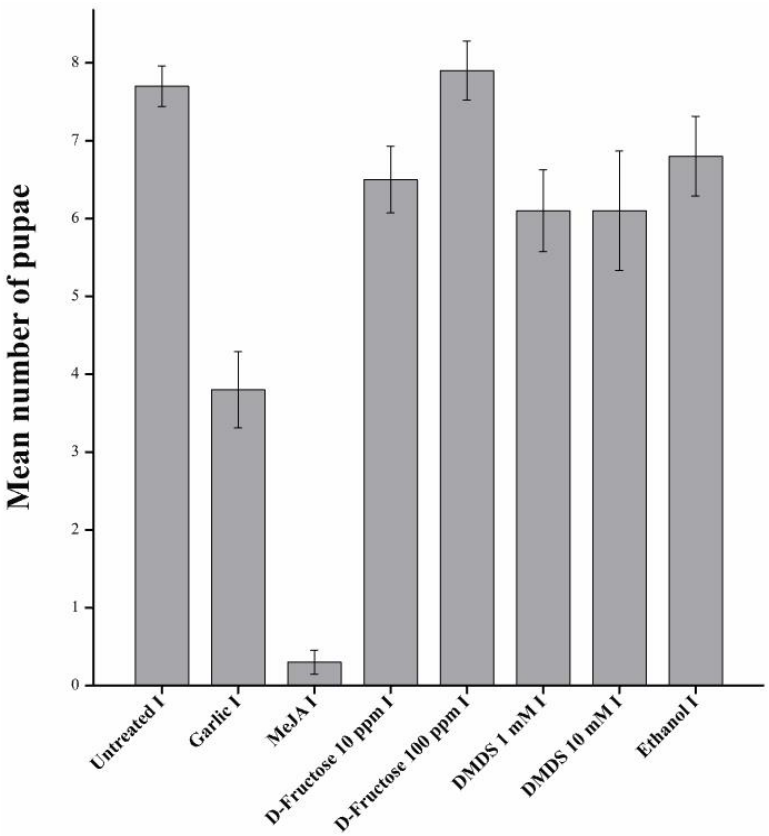


**Figure 12:** Glasshouse experiment 1 treatment means for number of *Delia radicum* pupae ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. (I) denotes infested plants. (*n* = 10).

 

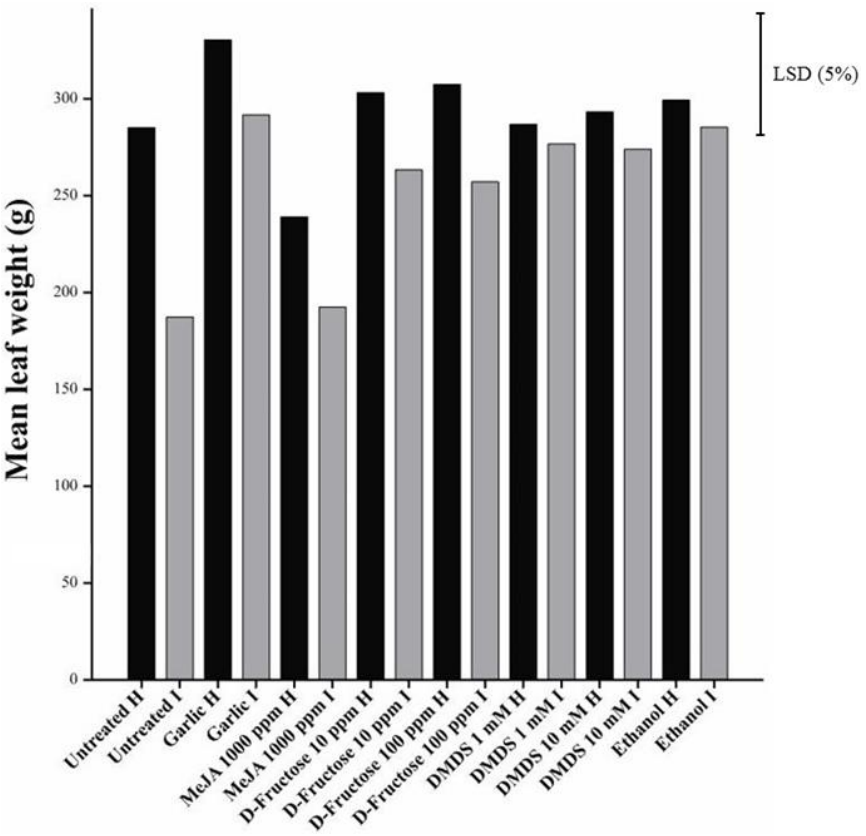


**Figure 13:** Glasshouse experiment 1 treatment means for broccoli leaf fresh weight (g). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. (H) and black bars denote healthy plants. (I) and grey bars denote infested plants. ($n$ = 10).

 

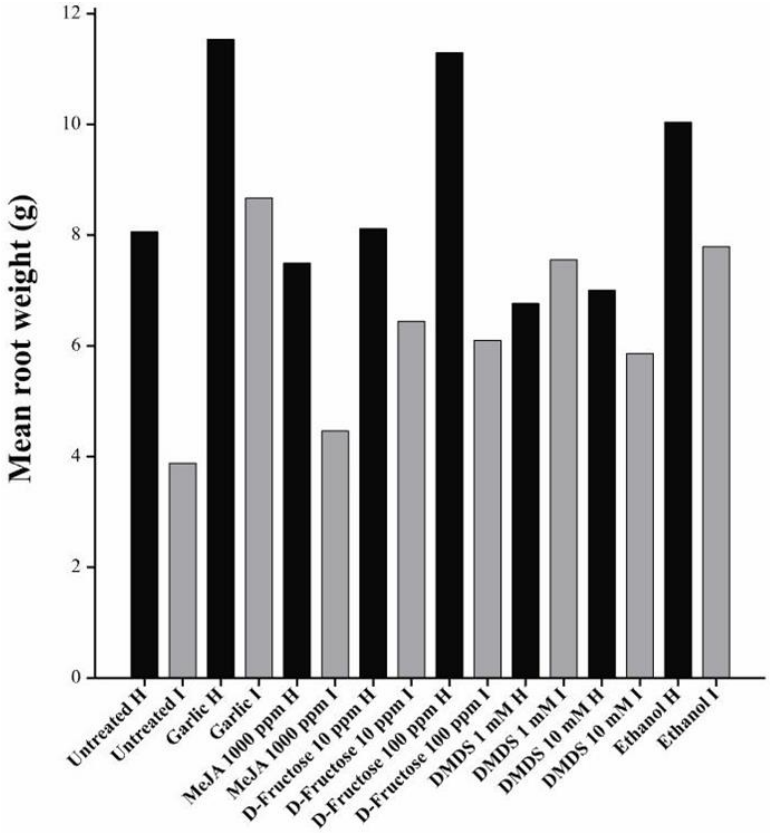


**Figure 14:** Glasshouse experiment 1 treatment means for broccoli root fresh weight (g). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. (H) and black bars denote healthy plants. (I) and grey bars denote infested plants. (*n* = 10).

**Glasshouse experiment 2**

**MeJA 100 ppm**. In glasshouse experiment 2, MeJA 100 ppm induced plants had significantly less *D. radicum* root damage (Figure 15) and fewer pupae (Figure 16) in comparison with the untreated infested control plants. In addition, broccoli leaf (Figure 17) and root (Figure 18) fresh weight were significantly higher than untreated infested controls. Leaf and root weight for treated uninfested plants were not significantly different from untreated uninfested plants (Figures 17 and 18, respectively) which indicated that plant growth was not affected at this dose and concentration.

**MeJA 1000 ppm**. While this higher concentration of MeJA did significantly reduce damage (Figure 15) and larval survival (Figure 16) compared with untreated infested plants, there was no significant difference in leaf and root fresh weight between the two (Figures 17 and 18, respectively). In this experiment, the treatment on its own without feeding larvae, significantly reduced leaf and root fresh weight compared to untreated uninfested control plants (Figures 17 and 18, respectively).

 

**MeJA 100 ppm/D-Fructose 100 ppm**. The combined treatment of MeJA 100 ppm and D-Fructose 100 ppm had similar damage levels to the untreated infested controls (Figure 15). However, significantly fewer larvae survived to pupal stage on the treated plants compared with the controls (Figure 16). Despite damage not being significantly different, leaf and root fresh weight were significantly higher than untreated infested controls (Figures 17 and 18, respectively). Comparison between leaf and root fresh weight for uninfested treated plants with untreated uninfested plants revealed that plant growth was not significantly different (Figures 17 and 18, respectively).

**D-Fructose 100 ppm**. D-Fructose 100 ppm treated plants did not differ significantly from the untreated infested control plants in terms of damage (Figure 15) and number of pupae (Figure 16). Furthermore, leaf and root fresh weight were not significantly different from untreated infested controls (Figures 17 and 18, respectively). There was no apparent application effect on leaf and root fresh weight when treated uninfested plants were compared with untreated uninfested controls (Figures 17 and 18, respectively).

**MeJA 100 ppm/DMDS 10 mM**. The MeJA 100 ppm and DMDS 10 mM combined treatment produced statistically similar results as D-Fructose 100 ppm.

**DMDS 100 mM**. The DMDS 100 mM treatment did not significantly reduce damage (Figure 15) nor the number of pupae (Figure 16) when compared with the untreated infested control plants. However, root fresh weight was significantly higher (Figure 18), despite leaf fresh weight not being significantly different from untreated infested controls (Figure 17). Treated uninfested plants and untreated uninfested plants did not differ significantly (Figures 17 and 18).

**DMDS 1 M and MeJA 100 ppm/DMDS 1 M**. DMDS 1 M, and MeJA 100 ppm and DMDS 1 M treatments were not analysed due to severe phytotoxicity.

 

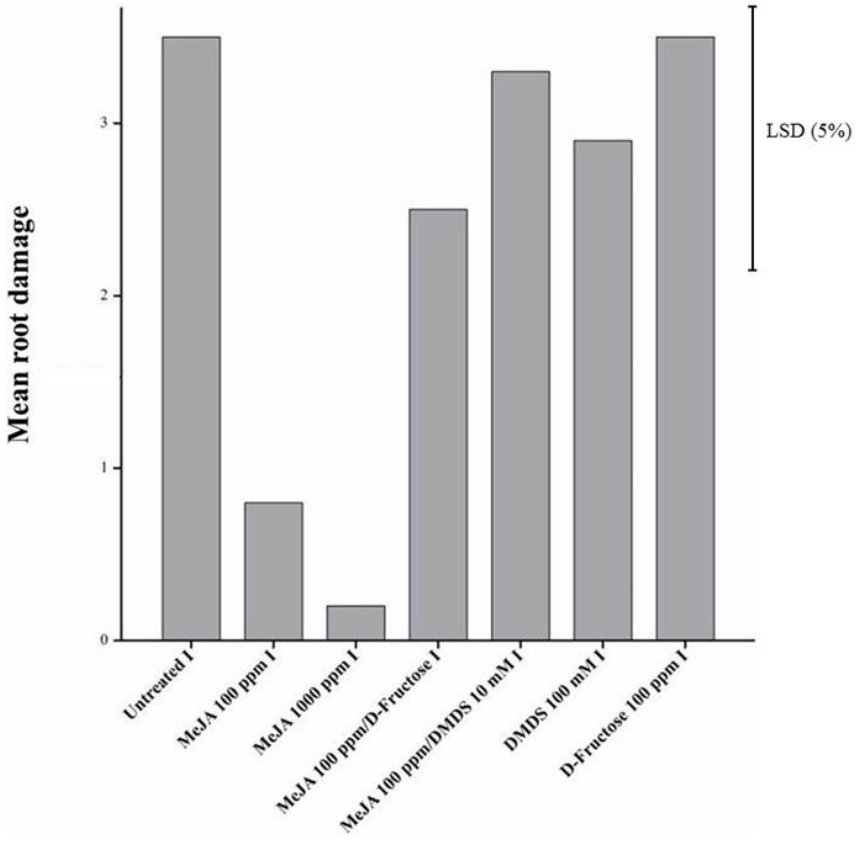


**Figure 15:** Glasshouse experiment 2 treatment means for *Delia radicum* root damage. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% (*P* = 0.05), shown as a single vertical bar, was calculated to compare means. (I) denotes infested plants. (*n* = 10). Root damage was scored for each plant; 0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = >50%, 4 = >75%.

 

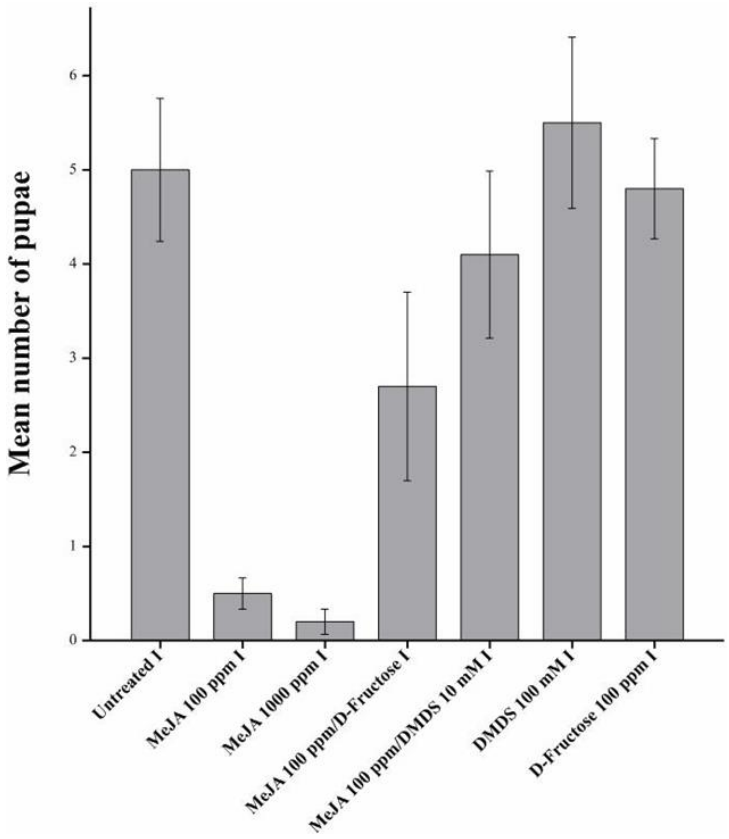


**Figure 16:** Glasshouse experiment 2 treatment means for number of *Delia radicum* pupae ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. (I) denotes infested plants. (*n* = 10).

 

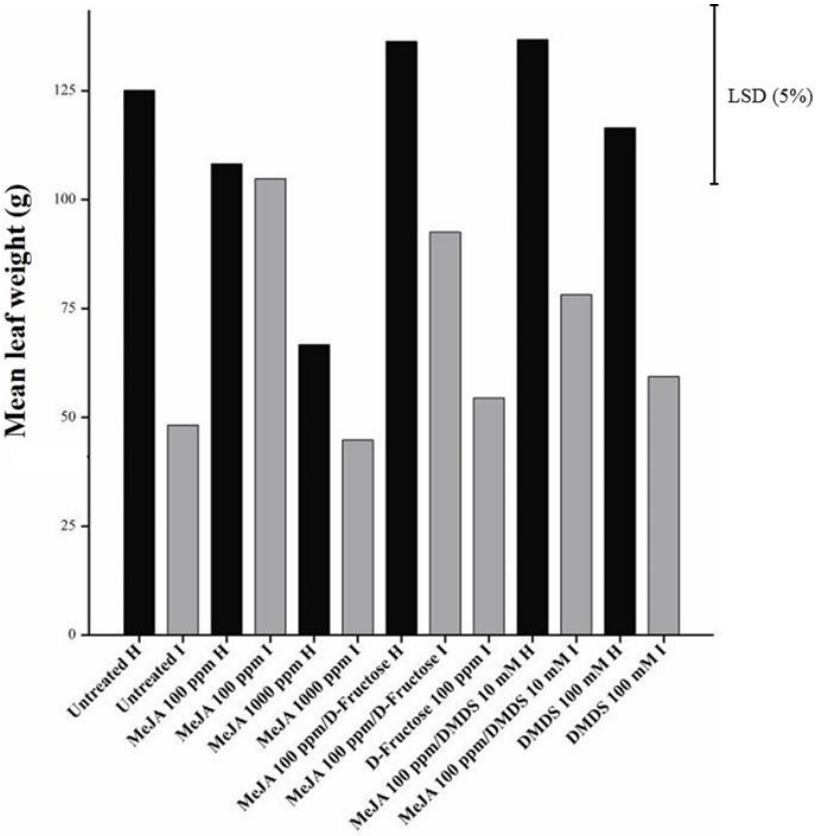


**Figure 17:** Glasshouse experiment 2 treatment means for broccoli leaf fresh weight (g). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. (H) and black bars denote healthy plants. (I) and grey bars denote infested plants. ($n$ = 10).

 

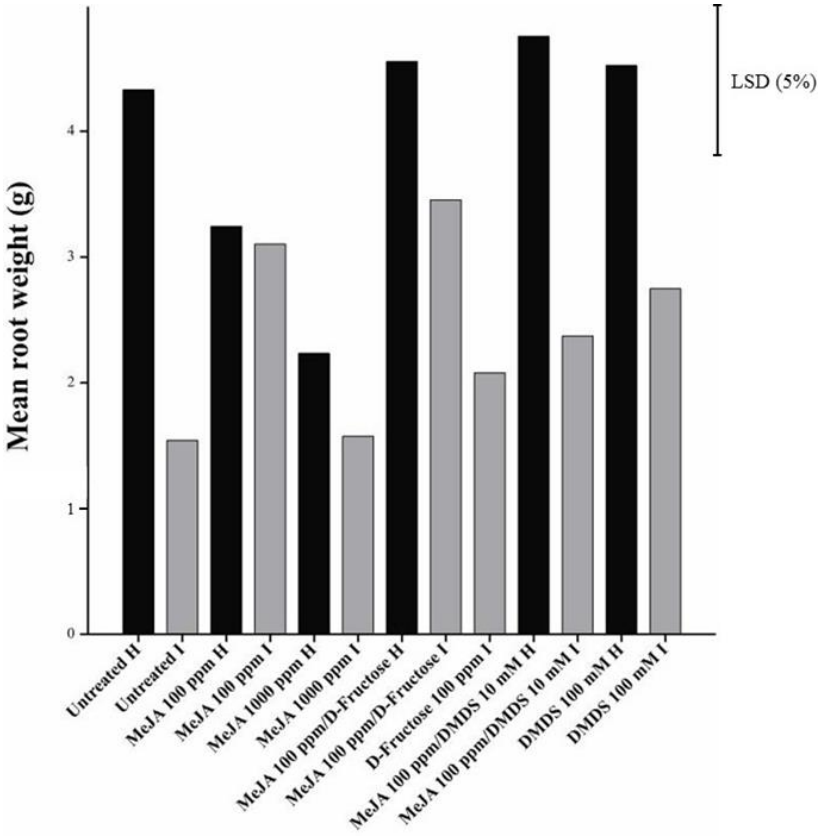


**Figure 18:** Glasshouse experiment 2 treatment means for broccoli root fresh weight (g). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. (H) and black bars denote healthy plants. (I) and grey bars denote infested plants. ($n$ = 10).

 

**Field trials**

Infestation by *D. radicum* was much greater in 2011 than in 2012 and the results should be considered in this context.

**Field experiment 1 (2011)**

Results from the 2011 field trial showed that all MeJA and D-Fructose leaf and root treated plants stimulated significantly higher *D. radicum* oviposition than both untreated control plants and Chlorpyrifos treated plants (Figure 19). Chlorpyrifos significantly reduced feeding damage (Figure 20) and the number of *D. radicum* pupae/larvae (Figure 21) compared with untreated controls. MeJA leaf and D-Fructose leaf treatments marginally, but not significantly, reduced larval damage compared with untreated plants. Only MeJA leaf treated plants significantly reduced the number of pupae/larvae when compared with untreated plants, but numbers were still significantly higher than plants treated with Chlorpyrifos. Yield (broccoli head fresh weight and diameter) was found to be statistically similar for both untreated and Chlorpyrifos treated plants, as well as D-Fructose leaf and root treated plants (Figures 22 and 23). MeJA leaf and root treatments, however, significantly reduced yield. Dazitol™ was severely phytotoxic which influenced results, particularly egg laying and yield.

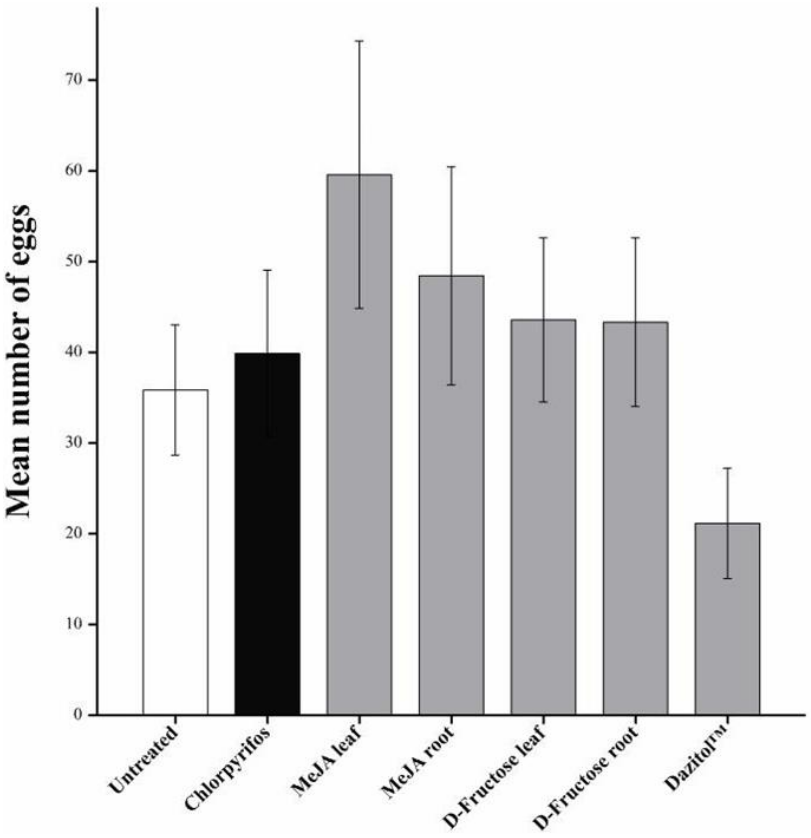


 

**Figure 19:** Field experiment 1 treatment means for number of *Delia radicum* eggs ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. (*n* = 16).

 

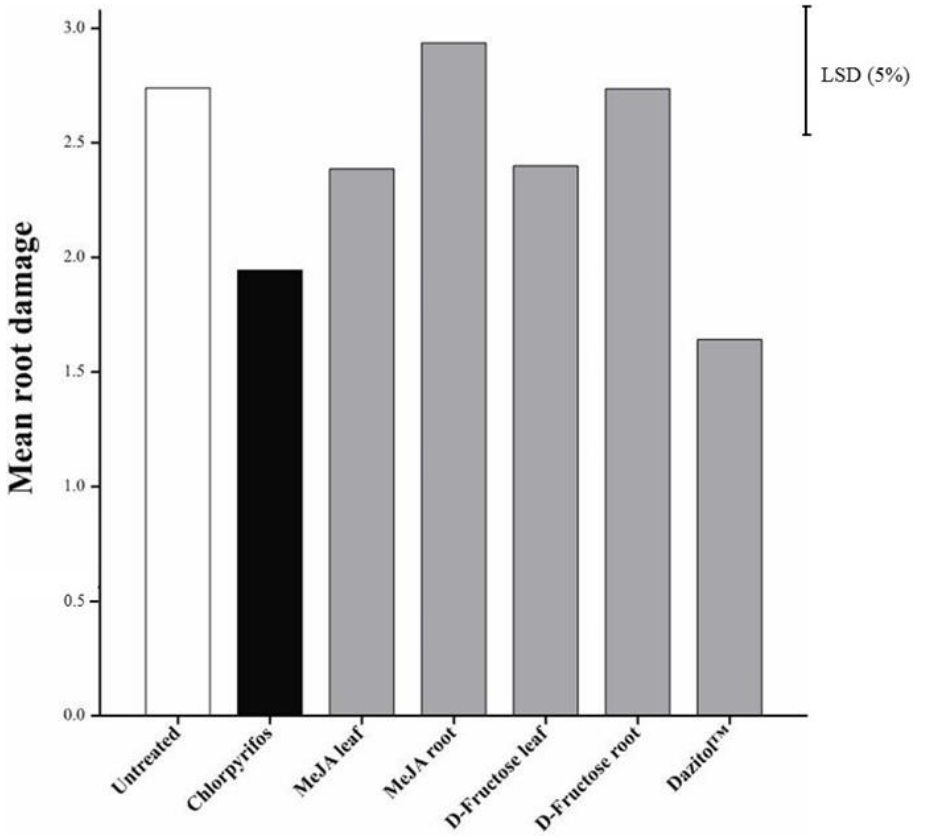


**Figure 20:** Field experiment 1 treatment means for *Delia radicum* root damage. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% (*P* = 0.05), shown as a single vertical bar, was calculated to compare means. (*n* = 40). Root damage was scored for each plant; 0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = >50%, 4 = >75%.

 

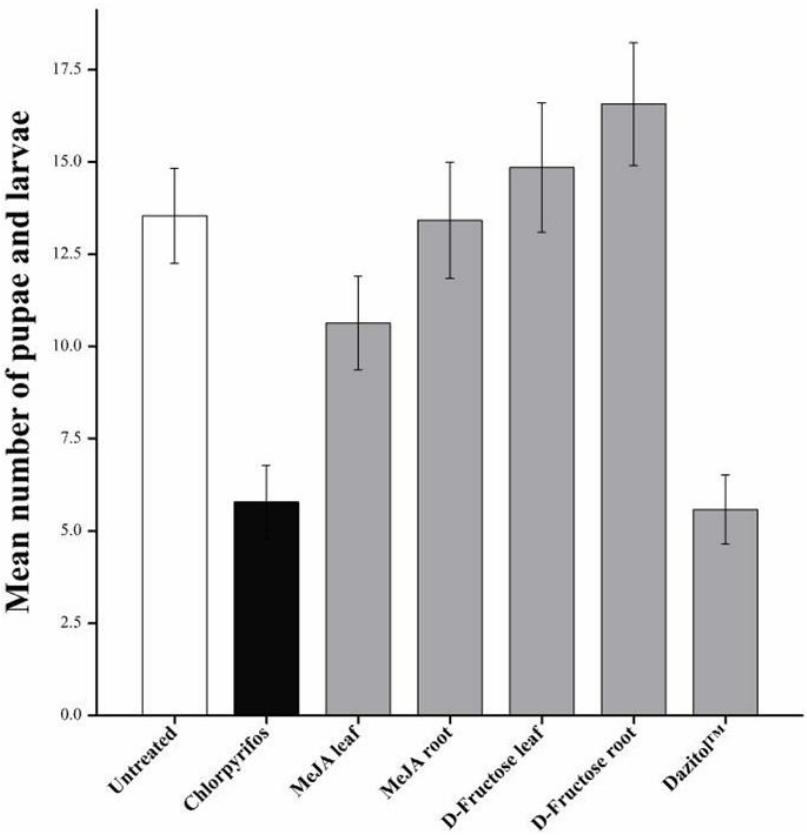


**Figure 21:** Field experiment 1 treatment means for number of *Delia radicum* pupae and larvae ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. ($n$ = 40).

 

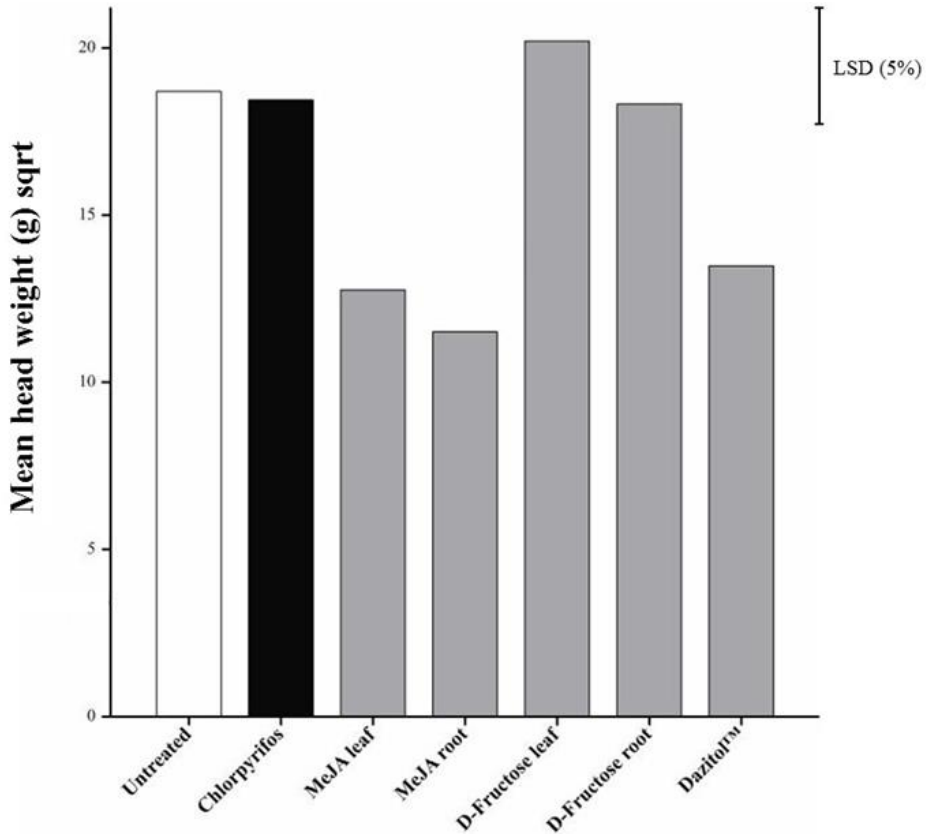


**Figure 22:** Field experiment 1 treatment means for broccoli head weight (g). Data were square root transformed (sqrt) to meet the assumptions of normal distribution and homogeneity of variance. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. ($n$ = 40).

 

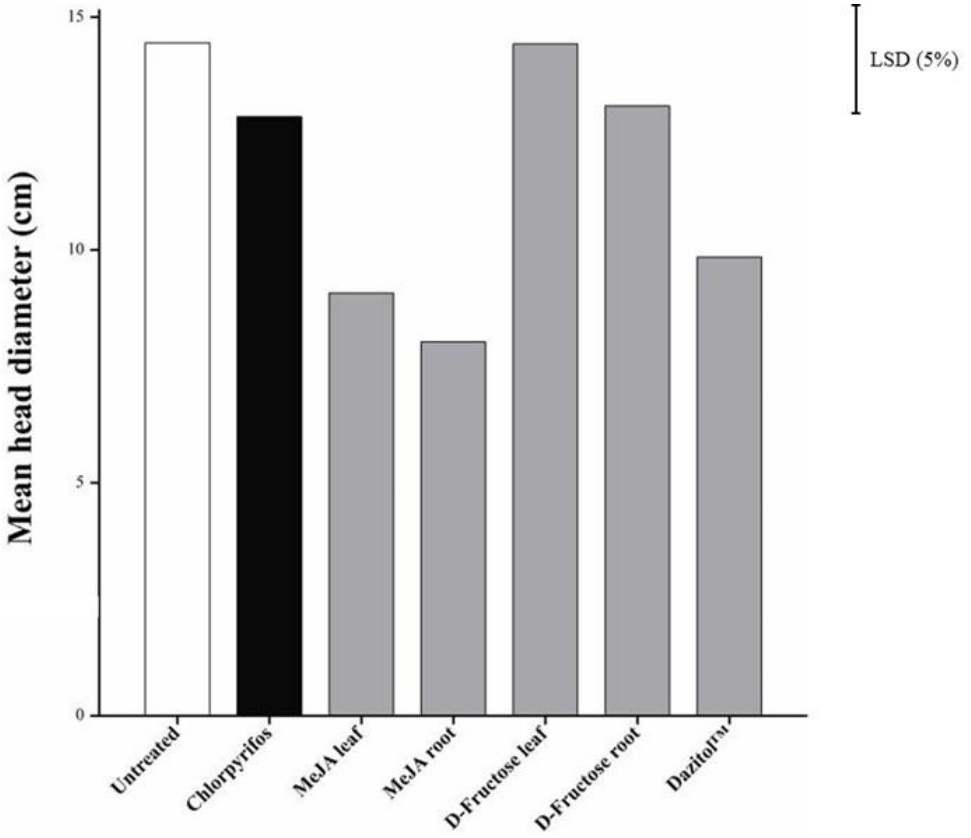


**Figure 23:** Field experiment 1 treatment means for broccoli head diameter (cm). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% (*P* = 0.05), shown as a single vertical bar, was calculated to compare means. (*n* = 40).

**Field experiment 2 (2012)**

Overall, oviposition monitoring results from the 2012 field trial (Figure 24) highlighted lower egg laying by *D. radicum* than in the 2011 field trial (Figure 19). The low egg numbers may be attributed to a combination of unseasonal weather compared with average weather data, influence of the experimental site, and efficacy of the felt traps employed for oviposition monitoring compared with soil sampling. Numbers of *D. radicum* pupae/larvae recovered at the end of the 2012 study (Figure 26) were lower than 2011 (Figure 21). Oviposition in 2012 for untreated plants, Chlorpyrifos, Entonem, Garlic, MeJA/Chlorpyrifos, and MeJA/D-Fructose treated plants did not differ significantly (Figure 24). Spinosad, MeJA 100 ppm, and MeJA 1000 ppm treated plants had significantly lower egg laying than control plants, whereas plants treated with DMDS had significantly more eggs laid than untreated and Chlorpyrifos treated plants. The lack of significant differences between all treated and control plants for *D. radicum* larval root damage potentially reflected the low number of eggs and consequently larvae present (Figure 25). Despite this, results indicated that Chlorpyrifos significantly reduced the number of pupae/larvae recovered compared with

 

untreated controls. In addition, the number of pupae/larvae for MeJA-/Chlorpyrifos, MeJA/D-Fructose, MeJA 100 ppm, and MeJA 1000 ppm treated plants were statistically similar to Chlorpyrifos treated plants, while plants treated with Entonem and Spinosad had significantly fewer pupae/larvae than Chlorpyrifos treated plants (Figure 26). Consistent with yield results for the 2011 field experiment, in 2012, yields for untreated and Chlorpyrifos treated plants did not differ significantly (Figures 27 and 28). Yield results for plants treated with Entonem, Spinosad, Garlic, and MeJA/Chlorpyrifos were statistically similar to both Chlorpyrifos and untreated plants. In contrast, MeJA/D-Fructose, MeJA 1000 ppm, and DMDS treated plants had significantly lower yields than untreated and Chlorpyrifos treated plants, while plants treated with MeJA 100 ppm had significantly smaller broccoli head diameter than Chlorpyrifos treated plants.

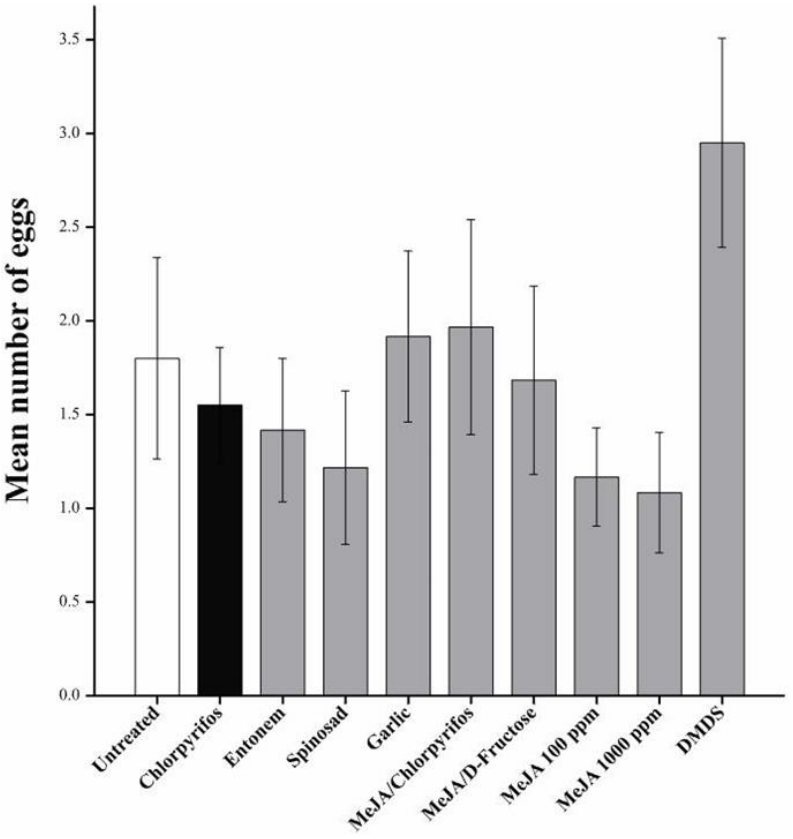


**Figure 24:** Field experiment 2 treatment means for number of *Delia radicum* eggs ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. (*n* = 16).

 

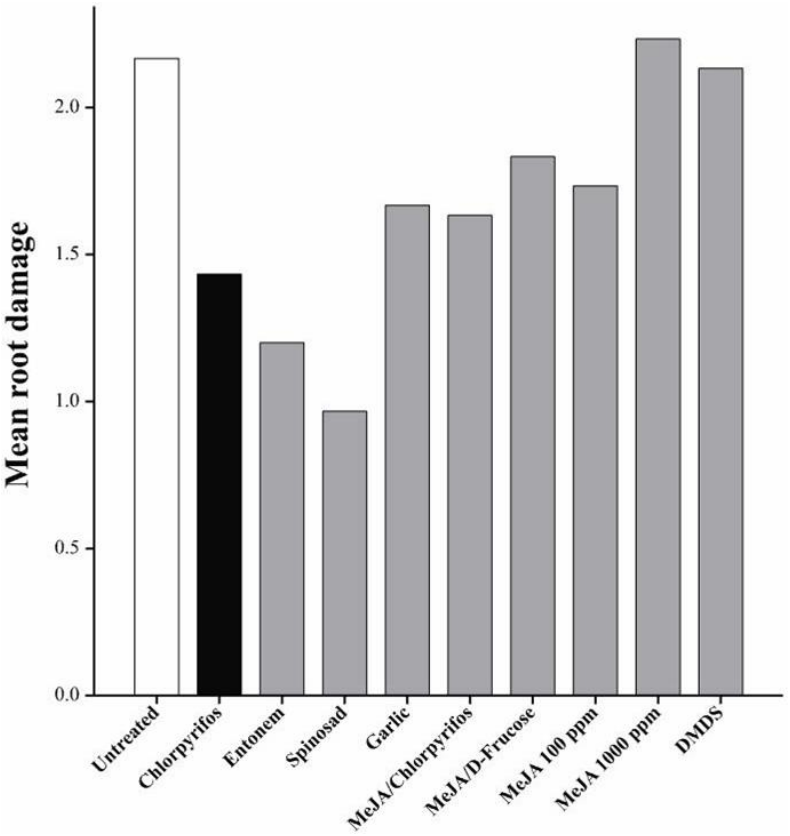


**Figure 25:** Field experiment 2 treatment means for *Delia radicum* root damage. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. (*n* = 30). Root damage was scored for each plant; 0 = undamaged, 1 = < 25%, 2 = 25-50%, 3 = >50%, 4 = >75%.

 

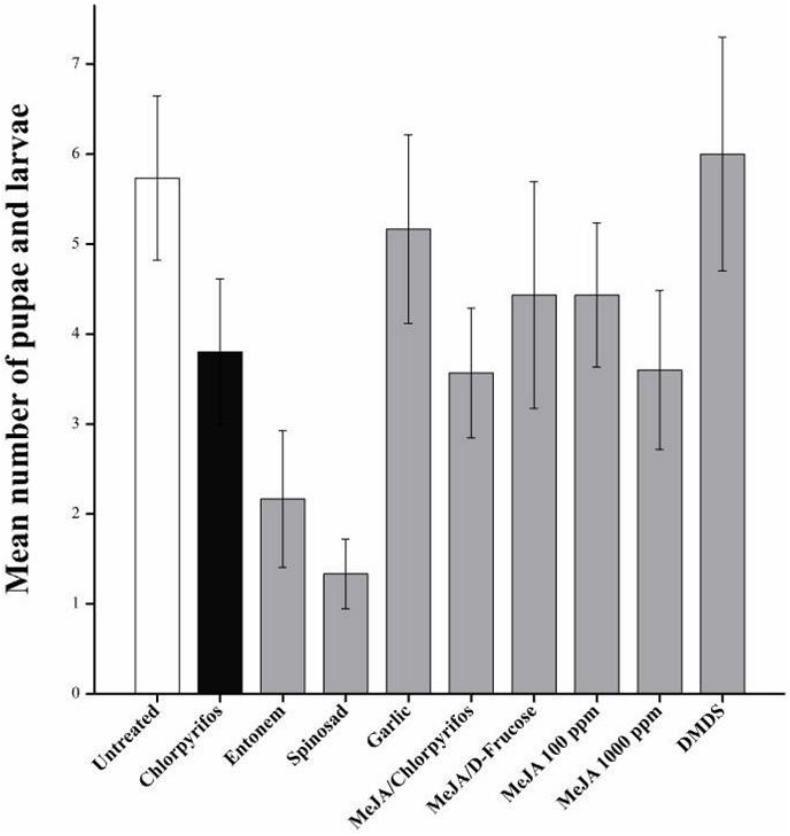


**Figure 26:** Field experiment 2 treatment means for number of *Delia radicum* pupae and larvae ± SE. $LSD_{(P = 0.05)}$ was calculated following a GLM calculation of ANOVA. Error bars represent SE around means. ($n$ = 30).

 

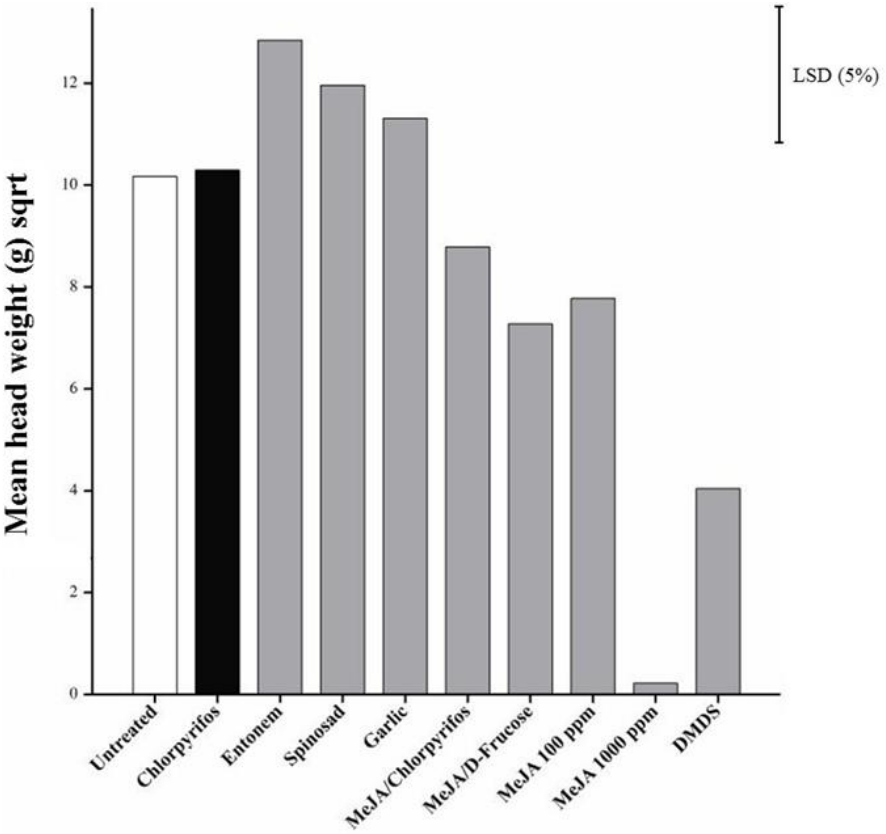


**Figure 27:** Field experiment 2 treatment means for broccoli head fresh weight (g). Data were square root transformed (sqrt) to meet the assumptions of normal distribution and homogeneity of variance. SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% ($P$ = 0.05), shown as a single vertical bar, was calculated to compare means. ($n$ = 30).

 

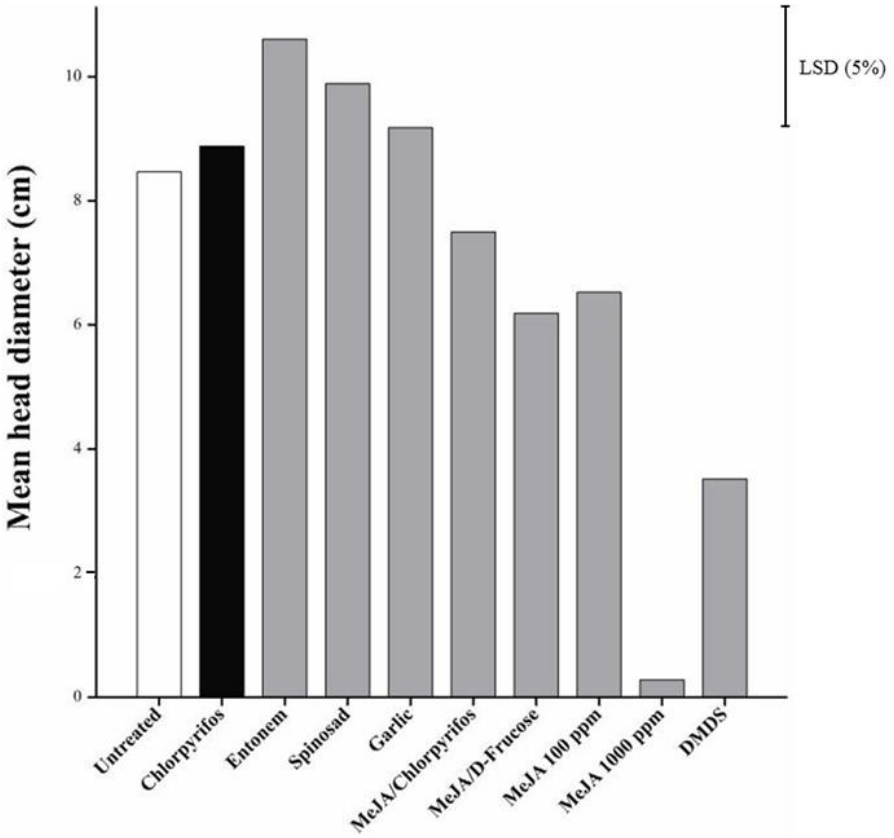


**Figure 28:** Field experiment 2 treatment means for broccoli head diameter (cm). SE for means are based on degrees of freedom for stratum at which treatments were compared using a general ANOVA. LSD at 5% (*P* = 0.05), shown as a single vertical bar, was calculated to compare means. (*n* = 30).

## Discussion

### Larval behavioural bioassays and chemical analysis

When presented with 30 µL volumes of both undiluted and diluted concentrations of allyl isothiocyanate, larvae did not display oriented movement. Reducing the dose to 15 µL resulted in positive taxis to the 1% concentration. Further reduction of the dose to 5 µL, overall, elicited the greatest level of attraction in larvae. Similar to allyl isothiocyanate 30 µL doses, no oriented responses were observed in the present study for 30 µL volumes of 2-Chlorophenyl isothiocyanate or Dazitol™ at any of the concentrations tested. These results indicate that nature-identical concentrations and volumes of compounds presented to larvae in bioassays are crucial. 30 µL doses therefore appeared too high for all compounds tested in these studies, even after serial dilutions. At 5 µL volumes, larvae displayed attracted movement to allyl isothiocyanate.

 

*D. radicum* neonate larvae were significantly attracted to host plant root exudates collected from 18 day old broccoli plants over a 72 hour exudation period, and exudates collected for 72 hours from 46 day old Chinese cabbage plants with mechanically damaged roots. Overall, however, wide variation in larval responses to host plant broccoli and Chinese cabbage root exudates existed. Root exudates were collected and tested in bioassays for 11, 18, 25, 32, 39 and 46 day old intact/undamaged broccoli and Chinese cabbage plants. (At 46 days, plants had 6-7 true leaves). Of these, only root exudates from 18 day old broccoli plants elicited a significant attracted response in larvae. When 46 day old broccoli and Chinese cabbage roots were damaged and exudates were collected over a further 72 hours, larvae showed a significant attracted response to Chinese cabbage root exudates but not to broccoli. The variation evident in larval responses to host plant root exudates might be attributed to (1) the lack of perception of any, or only low levels of host plant specific and general cues by larvae, particularly in exudates from intact/undamaged roots, (2) growing conditions and plant growth stage, (3) the diluted nature of the solution of root exudates presented to larvae, and (4) the distance between the introduced larva and the test stimulus.

GC-MS analysis of root exudates samples revealed quantitatively lower levels of exudation in intact/undamaged roots in comparison to the observed increase in compounds released following physical damage. GC-MS did not detect the presence of thiosulfinates, nor glucosinolates or their hydrolysis products in root exudates samples in this study. Larval attracted responses might be explained by their potential detection of gradients of other non-specific low-volatile or non-volatile components (e.g. fatty acids, sugars, amino acids) diffusing via air and the filter paper arena surface.

 

Beyond successfully developing and optimising the EthoVision® video-tracking method, this project has shown that larvae can orientate towards volatiles from *Brassica* host-plant roots. A dose range of an identified sulfur compound (SPME-GC-MS) was tested in bioassays to evaluate whether larvae show a dose dependent response. Larvae were found to be only significantly attracted to one dose, 1 µL. Similarly, larvae showed highly significant attracted responses to their host plant swede. There was no clear evidence of repelled response patterns in larval behaviour to any of the doses tested. Larvae instead were found to be randomly distributed in the arena similar to responses to the blank control. The maximum dose tested, 50 µL, was highly toxic to larvae, causing rapid immobilisation soon after introducing them into the arena and commencing the test.

**Glasshouse and field trials**

In both glasshouse and field experiments DMDS applied as a root drench did not affect larval host plant location nor reduce larval performance at the concentrations and volumes tested. Despite this, field results indicated that DMDS can stimulate *D. radicum* oviposition. Notwithstanding these results, the direct application of DMDS as a drench to broccoli roots can reduce growth and yield (at the concentrations and volumes tested). Considerable work, therefore, is still needed to identify biologically relevant or nature-identical concentrations and/or volumes of DMDS that influence larval and/or adult behaviour without impacting plant growth and/or yield.

Results showed that application of MeJA to broccoli plant roots pre-infestation with cabbage root fly can significantly reduce subsequent *D. radicum* larval performance and feeding damage under glasshouse conditions. Although the underlying induced resistance mechanisms remain to be elucidated, treatment of *Brassica* plants with jasmonates can reduce sugars and amino acids, and increase glucosinolates and proteinase inhibitors (Bodnaryk and Rymerson, 1994, Cipollini and Sipe, 2001, Liang *et al*., 2006, van Dam and Oomen, 2008, Pierre *et al.*, 2012, Tytgat *et al*., 2013). Field results indicated that MeJA applied to host plants had a less pronounced effect on *D. radicum* larval performance than in glasshouse studies, and can both positively and negatively influence adult oviposition preference. These effects on egg laying may reflect local and systemic induced changes in host plant or leaf surface chemistry and changes in emissions of volatiles (Loivamäki *et al*., 2004, Pierre *et al.*, 2012). Decreased oviposition may, on the other hand, indicate reduced preference for smaller plants due to treatment (concentration-dependent phytotoxic) effects on plant growth (Pierre *et al*., 2013). Of particular relevance when considering current crop protection policies and reducing reliance on pesticides (Hillocks, 2012), MeJA combined with half rate chlorpyrifos (industry standard pesticide) showed similar efficacy to full rate

 

chlorpyrifos for reducing numbers of *D. radicum* pupae/larvae without treatment elicited loss of yield. Overall, despite some promising results for MeJA induced resistance against *D. radicum*, recurring treatment related inhibitory (phytotoxic) effects on plant growth and yield in both glasshouse and field studies may limit the practical application of MeJA without further research on concentrations, formulation, timing and mode of application.

In contrast to MeJA, application of D-Fructose to broccoli plants did not result in any negative effects on larval performance in either glasshouse or field experiments here. Furthermore, there was no clear evidence of growth promotion nor induced tolerance. Notwithstanding this, results suggested that leaves treated with D-Fructose can stimulate cabbage root fly oviposition. While application of sugar may induce changes in plant chemistry that could influence oviposition acceptance (Gigolashvili *et al*., 2007), the stimulatory contribution of sugar on the leaf surface cannot be ruled out (Städler, 1978, Lombarkia and Derridj, 2002, Lombarkia and Derridj, 2008).

## Conclusions

Newly hatched *D. radicum* larvae can orientate using host plant root volatiles. Specially developed EthoVision® video-tracking bioassays showed that host and non-host plant root volatiles with sulfur containing functional groups play a key role in cabbage root fly larval orientation. Larval behaviour was characterised by more direct movement when strongly attracted. In the absence of a recognised host plant cue(s), larvae moved around randomly over greater distances.

*In situ* SPME collection of broccoli root volatiles revealed the identify of a number of candidate volatile host plant location cues that are released by actively growing and damaged roots. Studying the temporal changes in broccoli root volatiles emissions indicated that profiles of volatiles emitted are correlated with cabbage root fly larval feeding and developmental progress. Typically, broccoli roots emit glucosinolate and S-methyl-L-cysteine sulfoxide derived sulfur containing volatiles such as isothiocyanates and sulfides that show significant enhancement following mechanical or *D. radicum* larval feeding damage.

DMDS did not disrupt *D. radicum* larval host plant location nor negatively affect the feeding performance of larvae at the concentrations/volumes/modes of application tested. Field results suggested that DMDS at the concentration/volume tested can, however, stimulate oviposition by adult cabbage root fly females.

 

Glasshouse pot trials showed MeJA possesses good efficacy for reducing *D. radicum* larval performance and survival. In field studies, efficacy against larvae was less pronounced while adult oviposition was either positively or negatively influenced. For the most part, the concentrations and volumes tested here elicited inhibitory effects (phytotoxicity) on broccoli plant growth and yield. Broccoli plants treated with reduced rate MeJA and chlorpyrifos did, however, show similar levels of *D. radicum* control as full rate chlorpyrifos, without inhibition of yield.

D-Fructose treated broccoli plants, either in the glasshouse or field, showed no induced efficacy against *D. radicum* larvae. Results suggested adult ovipositon was stimulated.

Evaluation of a number of commercially available alternative cabbage root fly treatments with potential for use in future IPM systems showed that garlic granules possess good efficacy for controlling cabbage root fly larvae under glasshouse conditions. The same level of control was not observed in the field. Results indicated that spinosad (Tracer®) and entomopathogenic nematodes (Entonem) were at least as effective as chlorpyrifos (Dursban® WG) for reducing the number of *D. radicum* pupae retrieved under field conditions.

European Union policy is geared towards significant reductions in pesticide use and implementing IPM (Directive 91/414/EEC, Directive 2009/128/EC). The ongoing registration review of chlorpyrifos, the main pesticide used against cabbage root fly, has resulted in a need for IPM compatible alternative control strategies for *D. radicum*. This project has identified potential future tools for cabbage root fly IPM that require further development.

## Knowledge and Technology Transfer

- PPL meeting 2010 (oral presentation)
- HDC Technical Seminar 2011 (oral presentation)
- HDC Annual Studentship Conference 2011 (oral presentation) and 2012 (poster presentation)
- SRUC Research Postgraduate Conference 2011 (oral presentation) and 2012 (poster presentation)
- The James Hutton Institute Student Competition 2012 (oral presentation) and 2013 (oral presentation Sprent Prize winner)
- Inter-Institute PhD Student Competition 2013 (oral presentation)
- PURE IPM Congress 2013 (oral presentation)

 

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