Project title:	Baby-leaf Cruciferae and Watercress: Improved control of *Scaptomyza flava* (extension to FV 408)
Project number:	FV 408a
Project leader:	Gemma Hough, ADAS
Report:	Interim report, November 2014
Previous report:	N/A
Key staff:	Gemma Hough, Entomologist Jude Bennison Kerry Maulden, Rob Drummond (until leaving ADAS September 2014)
Location of project:	ADAS, Boxworth
Industry Representative:	Will Archer
Date project commenced:	February 2014
Date project completed (or expected completion date):	July 2015 (extended from 30 November 2014)

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[The results and conclusions in this report are based on an investigation conducted over a one-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.]

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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.

Gemma Hough

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GROWER SUMMARY

Headline

Potential products for the control of *Scaptomyza* species have been identified in laboratory based rocket leaf dip tests and in semi-field cage trials. Further work will determine the efficacy of insecticide spray applications in reducing pest damage in a commercial cruciferous baby-leaf crop.

Background

During summer 2009, leaf miners caused serious economic damage to watercress and Cruciferae grown as baby-leaf salads in central, eastern and southern England. In severe cases up to 40% of leaves have been damaged. As a result, growers incurred economic losses resulting from increased pesticide applications, crop rejection and additional packhouse labour inputs. In HDC project FV 376, *S. flava* was identified as the pest responsible for the damage.

In FV 376, crop covers were shown to be the only effective measure for the control of *S. flava* and, as in FV 376 the subsequent project, FV 408, did not identify any effective pesticides in a field trial. However, in HDC project FV 408, two experimental products and spinosad (Tracer) gave effective kill of adult *S. flava* in leaf dip laboratory tests. This project aims to build on previous work and identify insecticides which can provide reliable control of *S. flava* on baby-leaf Cruciferae. The project will also provide information on the behaviour of *S. flava.*

Summary

Culturing *Scaptomyza* sp.

A culture of *Scaptomyza* sp. is maintained at ADAS, Boxworth in insect-proof cages containing rocket. When there are few leaves left for new mines to develop, the adult flies are transferred to a new insect-proof cage containing pots of fresh rocket. The old cage of rocket is left to allow the larvae to mine the leaves and pupate. The new flies are then collected when they emerge and added to a new cage.

Due to difficulties in culturing *Scaptomyza* adults in high enough numbers for the experiments which require over 110-250 flies for good replication, visits to a

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commercial crop in East Anglia were made and *Scaptomyza* sp. were collected. *Scaptomyza* sp. were collected successfully by sweeping a net above the crop on a warm still day which resulted in catches of 140 *Scaptomyza* sp. over a two hour period. This method will continue to be used to provide flies for future work. As the adults were collected from the field it is impossible to confirm that each adult is *S. flava* and not another other closely related rocket leaf mining species such as *Scaptomyza pallida.* Therefore this project will now refer to *Scaptomyza* sp.

Objective 1: Determine the survival of *S. flava (*now *Scaptomyza* sp.) adults on pesticide-treated rocket leaves under controlled laboratory conditions.

The survival of *Scaptomyza sp.* adults on pesticide-treated rocket leaves under controlled laboratory conditions was determined. A fully expanded rocket leaf was dipped into the insecticide treatment to represent spraying to run-off. The leaf was allowed to dry on a mesh tray before being placed in a ventilated container with a piece of cotton wool soaked in 1:1 solution of honey and water as a food source for the adult flies. Two *Scaptomyza* sp. adult flies were added to each container.

The experiment consisted of 11 treatments (Table 1) with five replicate containers each (10 flies per treatment- 110 flies in total). After 24 and 48 hours the numbers of live and dead flies were counted.

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Table 1 Treatments used in Objective 1

Trt. Num	Treatment name/code	Active ingredient	Rate	Approval status on outdoor rocket
1	Untreated	Water	300 litres of water per hectare	-
2	HDCI 059	-	-	Not approved (approved on certain other crops in UK)
3	Hallmark	lambda-cyhalothrin	75 ml/ha in 300 litres of water per hectare	EAMU 0636/2006
4	Movento	spirotetramat	0.5 l/ha in 300 litres of water per hectare	EAMU 2410/2010
5	Tracer	Spinosad	200 ml/ha in 300 litres of water per hectare	EAMU 1290/2008
6	HDCI 045	-	-	Not approved in UK
7	HDCI 046	-	-	Not approved (approved on certain other crops in UK)
8	HDCI 047	-	-	Not approved in UK
9	HDCI 062	-	-	Not approved in UK
10	HDCI 060	-	-	Not approved in UK
11	HDCI 061	-	-	Not approved in UK

After 24 hours, HDCI 060 and Tracer were the best performing products reducing the mean number of live *Scaptomyza* flies to 0 and 0.2 respectively (Figure 1). After 48 hours, Tracer, HDCI 060, HDCI 045, HDCI 047, and HDCI 061 were the best performing products reducing the mean number of live *Scaptomyza* flies per pot to 0, 0, 0.2, 0.4, and 0.6 respectively (Figure 2).

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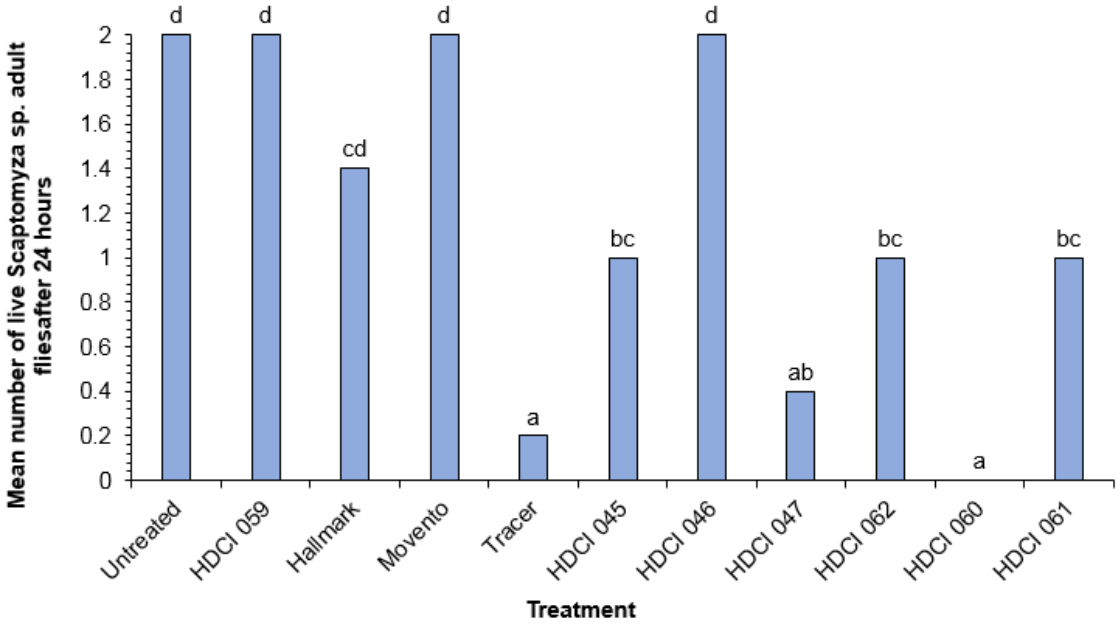

Figure 1 The mean number of live *Scaptomyza* sp. adult flies alive after 24 hours (treatments that share the same letter are not significantly different from each other).

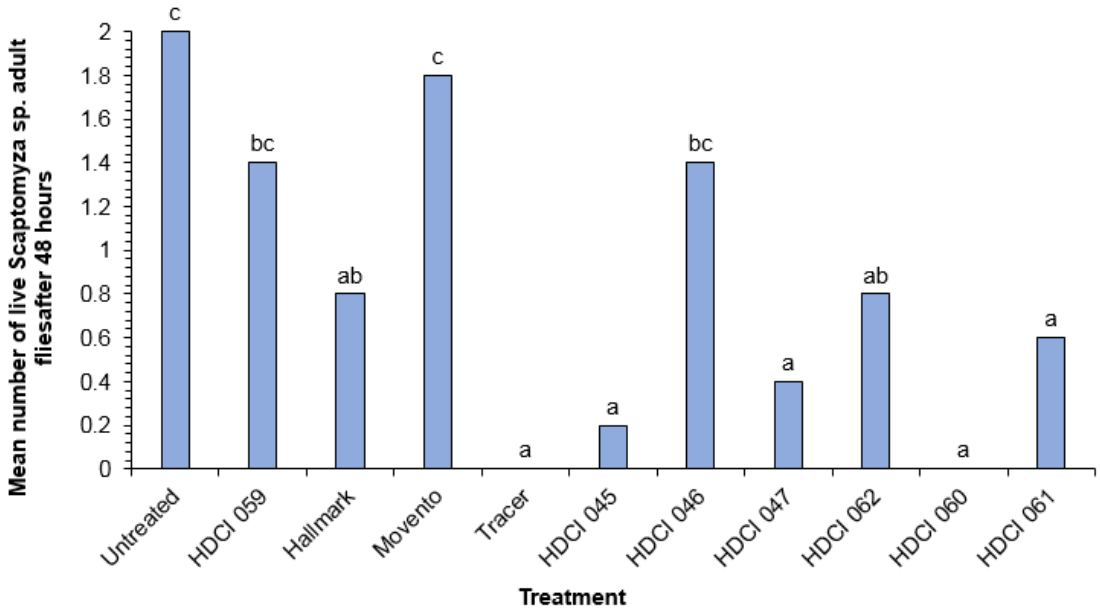

Figure 2 The mean number of live *Scaptomyza* sp. adult flies alive after 48 hours (treatments that share the same letter are not significantly different from each other).

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Objective 2: Record the survival and leaf puncturing damage of *S. flava (*now *Scaptomyza* sp.) adults on whole rocket plants following spray application under semi-field conditions.

The experiment consisted of five treatments including an untreated control. Four of the best performing products from Objective 1 were selected including Tracer, HDCI 045, HDCI 047 and HDCI 060. Each treatment had five replicate plots. Each plot consisted of an insect-proof cage containing four pots, each pot contained three rocket plants at the two true leaf stage (12 plants per cage). Plants were sprayed with a pot sprayer and arranged in the cages.

Ten flies were then added to each cage which was kept in a glasshouse at ADAS, Boxworth. Each cage had a dish containing cotton wool soaked in a 1:1 solution of honey and water as a food source for the adult flies. It was assumed that the flies added to the cage were an even mix of males and females due to the difficulties in identifying the sex of *Scaptomyza* without killing them or anaesthetizing them with carbon dioxide which can sometimes be fatal. As there were only 150 flies available for the experiment out of the 250 required, only three of the five replicates were completed.

After 72 hours the number of live *Scaptomyza* adult flies and the number of leaf punctures were counted on every plant in each cage.

The results from Objective 2 identified changes which are required to the experimental method to improve the robustness of the data. When looking at the mean number of leaf punctures per plant, the data (based on three of five replicates) showed that the mean number of punctures by HDCI060, Tracer and HDCI047 was reduced to 2, 4.5 and 6.3 punctures per plant compared with the untreated control which had 6.4 punctures per plant. HDCI 045 led to more punctures than the control with a mean of 10.3 punctures per plant. However, when looking at the raw data, in one of the four replicate untreated control cages (where we would expect the most damage), no leaf puncturing occurred. This suggests that all introduced flies may have been males that would not have made egg-laying punctures. Therefore, this highlights the need to confirm the sex of the flies being introduced otherwise some treatments may have more damage than others simply because they have more females. Therefore the experiment will be repeated using the same proportion of males and females in each cage. A method using carbon dioxide to anaesthetize

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the flies is being investigated.

Objective 3: Determine whether pesticide-treated plants are repellent to *S. flava (*now *Scaptomyza* sp.) adults under semi-field conditions*.*

This work is to be completed over the winter months if the culture provides enough flies (250 required). Alternatively it will be completed as soon as *Scaptomyza* sp. are active in commercial crops and can be collected.

Objective 4: Determine the efficacy of insecticide spray applications in reducing *S. flava (*now *Scaptomyza* sp.) puncturing damage on a commercial baby-leaf cruciferous crop.

This work is planned for completion in June using the most promising products.

Financial Benefits

If a crop was written off, the cost to buy replacement material would be approximately £2.40/kg (£2,400/tonne). Therefore, replacing a typical 20 tonne weekly programme during the summer would result in a grower cost of £48,000 per week. An effective additional method for reducing damage by *Scaptomyza* sp. in baby-leaf Cruciferous crops therefore has considerable financial benefits for growers at times of high pest pressure.

As a result of this project growers will benefit from improved knowledge on the effectiveness of selected pesticides in controlling this pest to avoid crop rejection.

Action Points

- Grower can use crop covers to protect plants from adult flies.

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SCIENCE SECTION

Introduction

During summer 2009, leaf miners caused serious economic damage on watercress and Cruciferae grown as baby-leaf salads in central, eastern and southern England. As a result, growers incurred economic losses resulting from increased use of pesticides, crop rejection and additional packhouse labour inputs. In severe cases up to 40% of leaves were damaged, causing crop rejection or extra packing costs. Prior to this little damage by leaf miners had been observed and they were not considered as a significant pest on these crops.

Research work completed in March 2011 (HDC project FV 376), confirmed that *Scaptomyza flava* was the leaf miner responsible for the damage. The large oval leaf punctures are made by adult females using their robust, saw-toothed ovipositors while the larvae hatch from the eggs and feed between the upper and lower leaf surfaces, causing broad 'blotch' mines.

Research using leaf dip and glass tube tests in this project indicated the development of resistance to pyrethroid pesticides (Hallmark, (lambda-cyhalothrin), which has an EAMU for use on outdoor rocket and baby-leaf brassicas). Pyrethroid pesticides are used on these crops for control of other pests and this likely resistance in *S. flava* may explain its recent increased status as a key pest.

Initial research in 2010, monitoring *S. flava* adults using white sticky traps showed that adults were active between April and September, with several (probably three or four) generations during the summer months (HDC project FV 376). Populations seemed to fluctuate between sites, but trapping data indicated that sudden, unpredictable and large increases can occur at different times on different sites. It is not known how these peaks in pest numbers relate to crop damage. Experiments in the subsequent HDC funded project FV 408 showed no clear relationship between numbers of *Scaptomyza* adults caught on traps and the amount of puncturing damage. It was concluded that more intensive monitoring using a greater number of traps over several seasons would be needed in order to develop the use of traps as a monitoring or prediction tool to aid *Scaptomyza* management strategies.

Currently, the most effective method of controlling *S. flava* damage is through the

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use of crop covers to prevent access by adult flies, however these are expensive and they can increase the incidence of diseases such as downy mildew (personal Communication, Will Archer). Field trials in HDC projects FV 376 and FV 408, testing the efficacy of a range of pesticides against *S. flava* on rocket, did not identify any insecticides with approval for use on baby-leaf Cruciferae that effectively reduced damage caused by the adult flies. Research in New Zealand suggests that the insecticide abamectin may be effective in reducing the number of leaf mines produced by this pest in baby-leaf Cruciferae (Martin *et al.* 2006). However, by the time leaf mines are visible in the crop, it is too late to prevent crop damage by pesticide application to kill larvae. In the UK, growers have recently reported some success using spinosad (Tracer) in spray programmes or in combination with crop covers. Results from the recent HDC-funded project FV 408 have shown that in laboratory tests on detached rocket leaves, HDCI 045, HDCI 047 and Tracer demonstrated a residual activity over a 48-hour period, killing 80-100% of adult *S. flava* within 48 hours. Further laboratory tests would be needed to confirm the maximum effective residual time after pesticide application for kill of *S. flava* adults and associated reduction in leaf puncturing.

Work in New Zealand has also shown that naturally-occurring parasitoids can be important in regulating *S. flava* populations. In the UK, parasitism of *S. flava* has also been recorded and in HDC funded project FV 408, naturally occurring parasitoids were collected and identified by the Natural History Museum as *Dacnusa temula* and *Chrysocharis pallipes.* Defra-funded project (PS2718) also noted the potential importance of hymenopteran parasitoids attacking *S. flava* within crops, particularly later in the season (Pope *et al* 2011). Further work on the potential of naturally-occurring parasitoids was suggested in the proposed project but was not supported by the SPGA.

Further work is justified on identifying a pesticide which might provide reliable control of *S. flava* on rocket. The objectives of this project are as follows:

(i) Project objective(s):

1: Determine the survival of *S. flava* adults on pesticide-treated rocket leaves

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under controlled laboratory conditions.

2: Record the survival and leaf puncturing damage of *S. flava* adults on whole rocket plants following spray application under semi-field conditions.

3: Determine whether pesticide-treated plants are repellent to *S. flava* adults under semi-field conditions.

4: Determine the efficacy of insecticide spray applications in reducing *S. flava* puncturing damage on a commercial baby-leaf cruciferous crop.

5: Communicate the results to the industry.

Materials and methods

Culturing *Scaptomyza* sp.

A culture of *Scaptomyza* sp. is maintained at ADAS, Boxworth in insect-proof cages containing rocket (Figure 1). When there are few leaves left for new mines to develop, the adult flies are transferred to a new insect-proof cage containing pots of fresh rocket. The old cage of rocket is left to allow the larvae to mine the leaves and pupate. The new flies are then collected when they emerge and added to a new cage.

Due to difficulties in culturing *Scaptomyza* adults in high enough numbers for the experiments which require over 110-250 flies for good replication, visits to a commercial crop in East Anglia were made and *Scaptomyza* sp. were collected. The most effective method involved sweeping a net above the crop on a warm still day which resulted in 140 *Scaptomyza* over a two hour period. *Scaptomyza* sp. adults were then collected from the net into tubes and brought back to ADAS Boxworth where *Scaptomyza* sp. were separated from other flies which had also been captured (Figure 2). As the adults were collected from the field it is impossible to confirm that each adult is *S. flava* and not another closely related rocket leaf mining species such as *Scaptomyza pallida.* Therefore this project will now refer to *Scaptomyza* sp.

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Figure 1 One of the Scaptomyza cultures maintained at ADAS Boxworth.

Figure 2 *Scaptomyza* sp. collected from a commercial crop by sweeping a net above the crop.

Objective 1: Determine the survival of *S. flava* adults on pesticide-treated rocket leaves under controlled laboratory conditions.

A fully expanded rocket leaf was dipped into an insecticide treatment to represent spraying to run-off. The leaf was allowed to dry on a mesh tray before being placed in a ventilated container with a piece of cotton wool soaked in 1:1 solution of honey

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and water as a food source for the adult flies. Two *Scaptomyza* sp. adult flies were added to each container.

The experiment consisted of 11 treatments (Table 1) with five replicate containers each (10 flies per treatment- 110 flies in total). The containers were kept in an incubator at 20°C 16hrs L:8hrs D. After 24 and 48 hours the numbers of live and dead flies were counted. Data were analysed using an analysis of variance (ANOVA) in GenStat (12th Edition).

Table 1 Treatments used in Objective 1

Trt. Num	Treatment name/code	Active ingredient	Rate	Approval status on outdoor rocket
1	Untreated	Water	300 litres of water per hectare	-
2	HDCI 059	-	-	Not approved (approved on certain other crops in UK)
3	Hallmark	lambda-cyhalothrin	75 ml/ha in 300 litres of water per hectare	EAMU 0636/2006
4	Movento	spirotetramat	0.5 l/ha in 300 litres of water per hectare	EAMU 2410/2010
5	Tracer	Spinosad	200 ml/ha in 300 litres of water per hectare	EAMU 1290/2008
6	HDCI 045	-	-	Not approved in UK
7	HDCI 046	-	-	Not approved (approved on certain other crops in UK)
8	HDCI 047	-	-	Not approved in UK
9	HDCI 062	-	-	Not approved in UK
10	HDCI 060	-	-	Not approved in UK
11	HDCI 061	-	-	Not approved in UK

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Objective 2: Record the survival and leaf puncturing damage of *S. flava* adults on whole rocket plants following spray application under semi-field conditions.

The experiment consisted of five treatments including an untreated control. Four of the best performing products from Objective 1 were selected including Tracer, HDCI 045, HDCI 047 and HDCI 060 (Table 2). Each treatment had five replicate plots. Each plot consisted of an insect-proof cage containing four pots; each pot contained three rocket plants at the two true leaf stage (12 plants per cage).

20 pots (each containing three plants) for each treatment were sprayed using a pot sprayer and the pots were then arranged in cages in a glasshouse at ADAS, Boxworth. Ten flies were then added to each cage. Each cage had a dish containing cotton wool soaked in a 1:1 solution of honey and water provided as a food source for the adult flies.

As there were only 150 flies available for the experiment out of the 250 required, only three of the five replicates were completed with a plan to complete the remaining replicates at a later date. It was assumed that the flies added to the cage were an even mix of males and females as identifying them is difficult without killing them or anaesthetizing them with carbon dioxide (which can be fatal).

Table 2 Treatments used in Objective 2

Trt. Num	Treatment name/code	Active ingredient	Rate	Approval status on outdoor rocket
1	Untreated	Water	300 litres of water per hectare	-
5	Tracer	Spinosad	200 ml/ha in 300 litres of water per hectare	EAMU 1290/2008
6	HDCI 045	-	-	Not approved in UK
8	HDCI 047	-	-	Not approved in UK
10	HDCI 060	-	-	Not approved in UK

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Once the flies had been added to each cage they were left for 72 hours and the number of live Scaptomyza adult flies and the number of leaf punctures were counted on every plant in each cage.

Objective 3: Determine whether pesticide-treated plants are repellent to *S. flava* adults under semi-field conditions.

This work is to be completed over the winter months if the culture provides enough flies (250 are required). Alternatively it will be completed as soon as *Scaptomyza* are active in commercial crops and can be collected.

Objective 4: Determine the efficacy of insecticide spray applications in reducing *S. flava* puncturing damage on a commercial baby-leaf cruciferous crop.

This work is planned for completion in June using the most promising products.

Results

Objective 1: Determine the survival of *S. flava* adults on pesticide-treated rocket leaves under controlled laboratory conditions.

After 24 hours, HDCI 060 and Tracer were the best performing products reducing the mean number of live *Scaptomyza* flies per pot to 0 and 0.2 respectively (Figure 3). After 48 hours, Tracer, HDCI 060, HDCI 045, HDCI 047, and HDCI 061 were the best performing products reducing the mean number of live *Scaptomyza* flies per pot to 0, 0, 0.2, 0.4, and 0.6 respectively (Figure 4).

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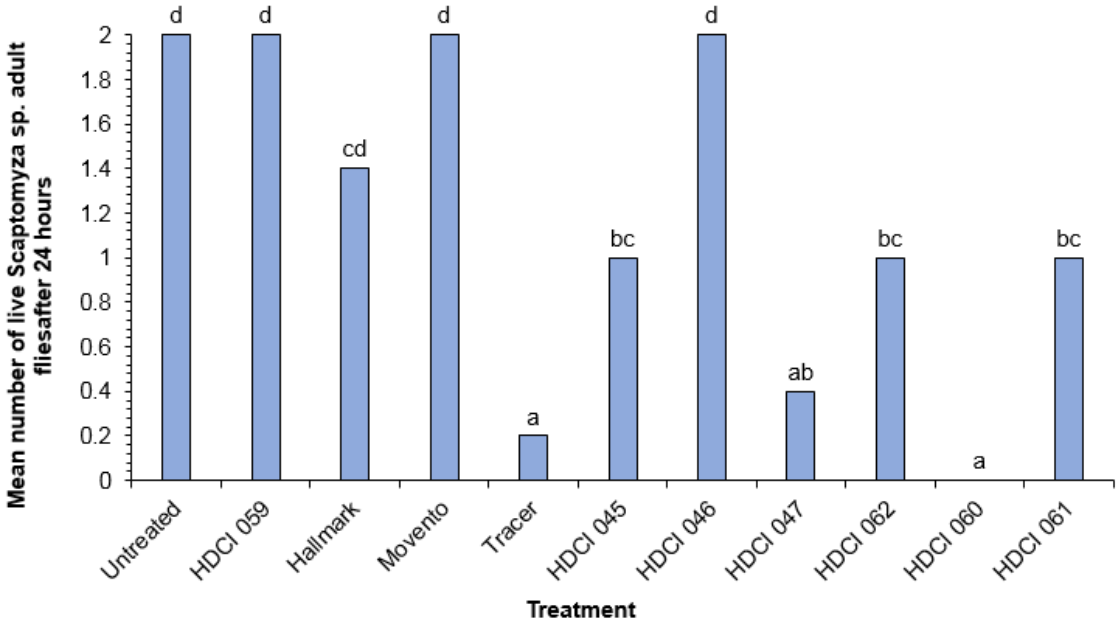

Figure 3 The mean number of live *Scaptomyza* sp. adult flies alive after 24 hours (LSD 5% = 0.648)

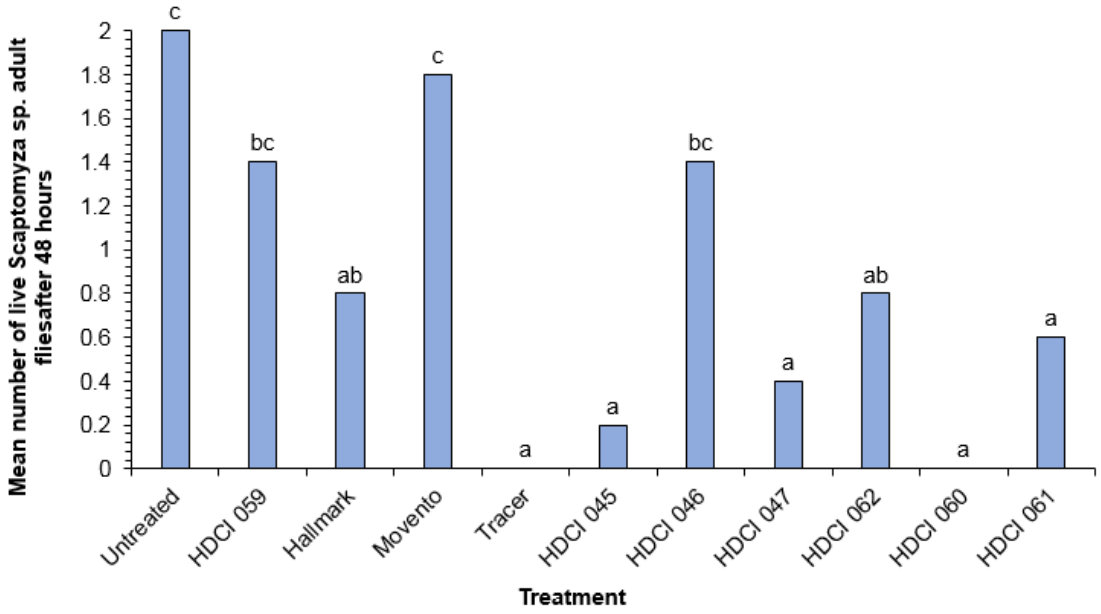

Figure 4 The mean number of live *Scaptomyza* sp. adult flies alive after 48 hours (LSD 5% = 0.782)

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Objective 2: Record the survival and leaf puncturing damage of *S. flava* adults on whole rocket plants following spray application under semi-field conditions

Seventy two hours after the flies were added to the cages containing the treated plants, the number of punctures per pot (three plants per pot) were counted and this number was divided by three to give an estimate of the average number of punctures per plant. Figure 5 shows the preliminary results of the trial based on three of the five replicates completed. Due to incomplete replication statistical analysis has not yet been carried out.

The untreated treatment had a mean number of 6.4 punctures per plant. The number of punctures on plants treated with HDCI060, Tracer and HDCI047 were reduced to 2, 4.5 and 6.3 punctures per plant. When looking at the raw data, in one of the three replicate untreated control cages (where we would have expected the most damage), no leaf puncturing occurred. This suggests that all introduced flies may have been males which would not have made egg laying punctures. Therefore, this highlights the need to confirm the sex of the flies being introduced otherwise some treatments may have more damage than others simply because they have more females. Therefore the experiment will be entirely repeated using the same proportion of males and females in each cage. A method using carbon dioxide to anaesthetise flies is being investigated.

The number of surviving adult *Scaptomyza* adults was also recorded but not all the dead flies could be found to confirm the count. Ten flies were added to each cage and the highest mean of live flies recorded was 7.3 per cage (Figure 6).

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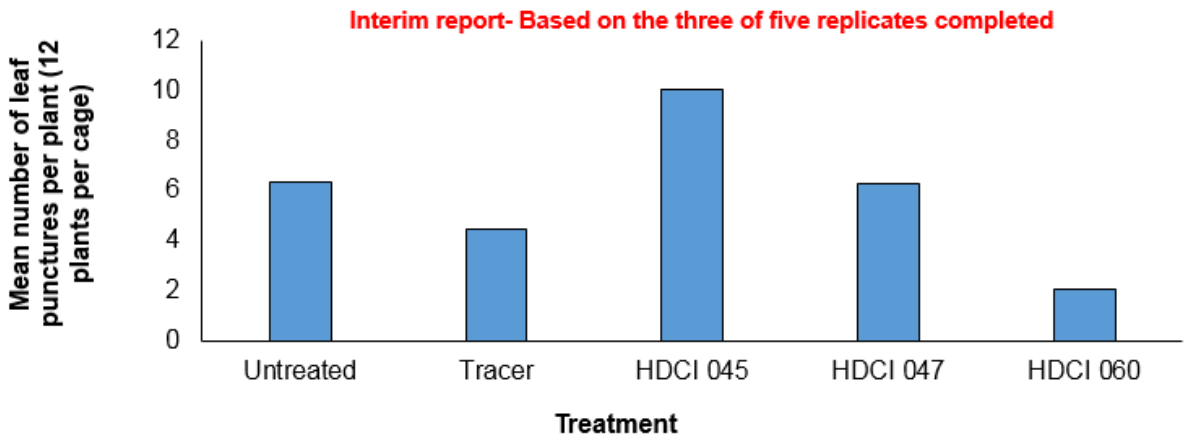

Figure 5 Mean number of leaf punctures per plant after 72 hours

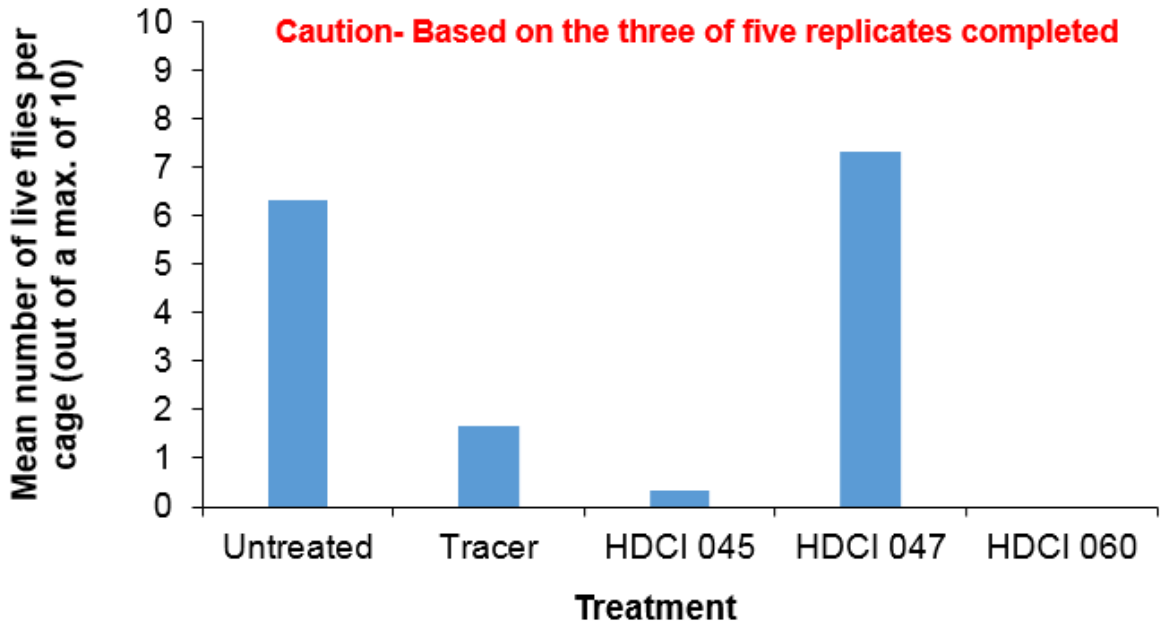

Figure 6 Mean number of live *Scaptomyza* adult flies found per cage after 72

Discussion

Work in Objective 1 has identified products which may have the potential to be used for the control of *Scaptomyza* species. It has confirmed that Tracer, which is currently available for use on rocket, is effective. It has also identified various other products which are not currently approved on rocket or approved in the UK which may also be useful for controlling *Scaptomyza* species if Extensions of Authorisations for Minor Uses (EAMU) can be sought.

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Scaptomyza sp. has been challenging to culture in high enough numbers to support the replication that the planned experiments require but visiting commercial grower sites and collecting them from rocket crops has been successful and will be continued as soon as they become active again. The work towards completing Objective 2 has identified changes that are required to the experimental methods so that the most robust data can be provided.

Conclusions

- After 24 hours, HDCI 060 and Tracer were the best performing products in reducing the mean number of live *Scaptomyza* flies.
- After 48 hours, Tracer, HDCI 045, HDCI 047, HDCI 060 and HDCI 061 were the best performing products.

Knowledge and Technology Transfer

- Project update written for the HDC Leafy Salads Roadshow 2014 booklet.

References

Martin, N. A., Workman, P. J. & Hedderley, D. (2006) Susceptibility of *Scaptomyza flava* (Diptera: Drosophilidae) to insecticide. *New Zealand Plant Protection*. **59**: 228-234.

Martin, N. & MacDonald, F. (2009) Evaluating the impact of insecticides on *Scaptomyza flava* and its parasitoid, *Asobara persimilis*. *New Zealand Journal of Crop and Horticultural Science*. **37**: 243-252.

Pope, T., Lole, M. & Bennison, J. (2011) Review of factors including pest biology and control strategies that may explain the increased importance of the leaf miner *Scaptomyza flava*. *Defra project PS2718*.

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