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DISCLAIMER:

AUTHENTICATION

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

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GROWER SUMMARY

Headline

- Of the chemical pesticides tested, only chlorpyrifos was effective in controlling blackberry leaf midge, but knowledge of the biology of the pest was improved.

Background and expected deliverables

To meet consumer demand for soft fruit over an extended season, there has been a substantial increase in UK production of protected blackberries and raspberries, mainly in 'Spanish' tunnels. This has led to increased problems with pests previously considered to be 'minor' pests of outdoor crops, including blackberry leaf midge, *Dasineura plicatrix*. This pest is now widespread and is often very damaging, particularly on blackberry. The larvae feed on the leaves of primocane tips, causing leaf twisting and distortion, cane stunting, cane branching and reduced yield in the following year's crop. Prior to this project, the life cycle of blackberry leaf midge on protected blackberry and raspberry was not fully understood.

The project aims and expected deliverables were to confirm the location and timing of key life-stage events of blackberry leaf midge on protected blackberry and raspberry crops and to test integrated control methods for use on protected blackberry, with a view to extrapolating the methods to protected raspberry.

Summary of the project and main conclusions

Objective 1: To confirm the location, timing and duration of key life-stage events of blackberry leaf midge on protected blackberry and raspberry

The biology of blackberry leaf midge was confirmed in two protected blackberry crops during 2009 and 2010, one grown in pots (Meadow field), the other grown in the soil (Chivers field).

In 2008/2009, the pest was shown to overwinter as cocoons in the soil. Most were found at a depth of 3 cm in the path in between the plant rows, at the edge of the ground-cover matting running under the crop canopy. They were also found in the soil in the planting hole, under the polythene covering the ground between plants and in plant debris on the polythene.

In 2009, the first male midge was found in water traps on 8 April in Meadow field and the first females on 15 April in both fields. In 2010, the first females were found on 12 and 19 May in

Meadow and Chivers fields respectively and the first males found on 19 May in both fields. The later midge emergence in 2010 is likely to have been due to the very severe winter of 2009/2010.

In 2009, the first midge eggs and larvae were found in leaf tips on 15 and 22 April in Meadow and Chivers fields respectively. In 2010, larvae were first found in Chivers field on 5 May (two weeks before adult midges were detected in this field). Thus the water traps did not detect the first adult midges emerging in 2010, probably due to midge numbers being much lower than in 2009.

Soil temperature data between March and June was modelled in a similar manner to the ADAS raspberry cane midge model to predict first generation blackberry leaf midge emergence and first egg-laying dates. In 2009, results were very promising as the predicted dates were only five days earlier than the actual dates when first eggs were found, i.e. 15 and 22 April in the two fields. This suggested that blackberry leaf midge emerges and lays eggs slightly earlier than raspberry cane midge, at a cumulative soil temperature of around 280°C days above a base temperature of 4°C.

In 2010, we found first eggs and larvae between 5 and 12 May, 11-18 days later than the predicted dates of 22 and 25 April in the two fields. However, the grower noticed the occasional infested leaf tip earlier than this. Midge numbers were much lower than in 2009, thus sampling random leaf tips must have missed the very first infested tips.

In 2009, numbers of midge adults remained low (below two per trap) until June and July. Mean numbers per trap peaked on 22 July in Meadow field (24 per trap) and on 8 July in Chivers field (132 per trap). In 2010, numbers of midge adults were much lower in both fields. Meadow field was only monitored until first adult emergence and on the final trapping date on 19 May there was a mean of only 0.2 per trap. In Chivers field, midge adults were monitored between March and September and mean numbers peaked on 15 July at 34 per trap. The lower numbers of midges occurring in 2010 could have been partly due to the severe winter of 2009/2010 killing a proportion of overwintered cocoons but also due to the plants in Chivers field suffering from *Verticillium* wilt so they produced fewer new leaf tips for egg-laying.

During peak adult activity in both years, numbers of males trapped were much higher than those of females. This could possibly be due to differences in the behaviour of males and females rather than an uneven sex ratio in the population.

In 2009, the percentage of leaf tips infested with eggs and larvae increased rapidly in both fields, from 30% and 5% in Meadow and Chivers fields respectively in mid-April, to 90% and

100% respectively in late June/early July. In 2010, the percentage of infested leaf tips in Chivers field increased from 5% in early May to 100% in late June. Larvae were found until late September in 2009 and until early September in 2010.

Midge larvae fed for approximately two weeks in the leaf tips, causing them to twist. Severely damaged leaves turned brown and withered.

When fully fed, the larvae dropped to the ground to pupate in cocoons.

Monitoring of three protected raspberry crops in June 2009 confirmed that the pest damaged raspberry but the percentage of infested leaf tips was much lower than on protected blackberry crops at this site. This may have been partly due to incidental midge control by chlorpyrifos used against other pests such as raspberry cane midge.

In 2009 there were four overlapping generations in protected blackberry between April and September, unlike on outdoor blackberry where only two generations are reported, in May/June and July/August. In 2010, first generation midges were not active until May/June and there were second and third generations in July/August and August/September. Thus on protected blackberry, depending on the season, first adult emergence can be earlier, there are more generations per season and the pest is active for a longer period than on outdoor blackberry.

Objective 2: To evaluate potential integrated control methods against blackberry leaf midge, for use in IPM programmes on protected blackberry

Biological control agents

In 2009, laboratory experiments indicated that both the predatory mites *Neoseiulus (Amblyseius) cucumeris* and *Amblyseius andersoni* predated midge eggs and young larvae.

In 2009, introductions of both *N. cucumeris* and *A. andersoni* to blackberry using release sachets in research tunnels led to some establishment of *N. cucumeris* in flowers but no apparent establishment of *A. andersoni*. Following grower releases of *A. andersoni* to protected blackberry, there was no apparent reduction in the pest when compared with untreated plants. In 2010, releases of the two species in sachets to blackberry in research tunnels indicated that *N. cucumeris* reduced the percentage of infested leaf tips whereas *A. andersoni* did not. Further work would be needed on a larger replicated scale to confirm the potential control given by each species.

In 2009, in laboratory pot experiments, neither the predatory mites *Macrocheles robustulus* nor the predatory beetles *Atheta coriaria* gave significant reductions in numbers of midge larvae successfully completing their development in compost and emerging as adults. In 2010, similar pot experiments compared the potential of *M. robustulus* (at higher release rates than in 2009) with that of the predatory mite *Hypoaspis aculeifer*. Adding each predator in equal numbers to those of leaf midge larvae did not significantly reduce numbers of adult midges emerging. However, in both years, the reduction given by *M. robustulus* was almost statistically significant, and further replication or adding higher numbers of predators might give a significant result. This species in now commercially available and justifies further investigation should any future funding be available.

In 2009, grower applications of *Beauveria bassiana* (Naturalis-L) to the commercial crop did not give reductions in numbers of midge larvae or infested leaf tips. *B. bassiana* is a contact-acting fungus and is unlikely to reach the target pest inside folded leaf tips. In a laboratory test, application of Naturalis-L to compost did not reduce numbers of blackberry leaf midge larvae successfully completing their development in the compost and emerging as adults.

In 2009, naturally-occurring anthocorid bugs (both *Anthocoris nemorum* and *Orius* sp. adults and nymphs) were observed feeding on blackberry leaf midge larvae in the commercial crop during July and August.

Orius laevigatus are commercially available and work on its potential against the pest was conducted in 2010. In laboratory studies, adult *Orius* established and produced nymphs in blackberry flowers where only blackberry pollen was available as food. In predation studies, adult *Orius* ate at least five midge larvae per day.

In 2010, releases of adult *O. laevigatus* were made to a commercial tunnel of blackberries in late March and early April. The crop was not yet in flower so flowering strawberry and pussy willow were tested as potential breeding ‘banker’ plants for *Orius*. A few *Orius* nymphs developed on the strawberry plants but establishment was poor and *Orius* were not detected on the blackberry plants when they began flowering. This was probably due to temperatures being too low for good predator reproduction. However, grower releases of *Orius* in late June led to good establishment on blackberry by early August, indicating that *Orius* could play a useful role in maintaining low midge numbers once temperatures are suitable. In Horticulture LINK project HL0117, ADAS research in 2010 demonstrated the potential of flowering alyssum as a ‘banker’ plant to aid establishment of *O. laevigatus* in everbearer strawberry, for improved biological control of western flower thrips (Cross *et al* 2011). Further research in this HortLINK project could also benefit midge control in protected blackberry.

Cultural control methods

In 2010, a pot experiment showed that using polythene or woven ground-cover matting over the substrate inhibited successful pupation of larvae dropping to the ground. Black polythene and Mypex ® reduced the numbers of midges emerging by 96% and 53% respectively compared with the compost control. It is possible that if the whole tunnel floor was covered in matting or polythene, and if this could be kept intact and free from plant debris, it might prevent adults successfully emerging from overwintered cocoons in the soil, or prevent first generation midge larvae successfully pupating and emerging as second generation adults. Further work would be needed to validate this potential cultural control method in a protected blackberry crop.

Pesticides

In 2009, grower applications of thiacloprid (Agrovista Reggae, SOLA 0467/2008) and abamectin (Dynamec, SOLA 2290/2007) did not reduce numbers of live midge larvae per leaf tip or percentage of infested leaf tips when compared with untreated plants.

In 2009, grower application of chlorpyrifos (Alpha chlorpyrifos, SOLA 1081/2009 for outdoor blackberry) to outdoor blackberry reduced numbers of live midge larvae per leaf tip by 87% and reduced the percentage of infested leaf tips by 92% when compared to untreated plants. By extrapolation, chlorpyrifos should give some control of the pest on raspberry. Various chlorpyrifos products are approved on both outdoor and protected raspberry. However, chlorpyrifos does not have a SOLA for use on protected blackberry and is not compatible with biological control agents used in IPM.

In 2010, application of a coded pesticide as a foliar spray to blackberries in research tunnels did not give reductions in numbers of midge larvae or infested leaf tips.

Financial benefits

The UK area of blackberries is estimated at 140 ha of which at least 70% is grown under protection, mostly under Spanish tunnels during the growing season. Average yields are estimated at 15 t/ha with a value of £4,800/t and a gross margin of about £16,000/ha. Blackberry leaf midge is present in approximately 70% of the blackberry area, with up to 100% leaf tips infested at peak activity. As there is currently no effective control measure for the pest on protected blackberry, it is estimated that in an average year, yields could be reduced by at least 30%. On infested farms, this would result in gross margin reductions of 60%, costing individual growers £9,600/ha and the industry £2.1m per annum. In the absence of an effective control measure for the pest, it is likely that the cumulative reduction in cane and plant growth on highly susceptible varieties such as Loch Ness, will force growers to grow other more profitable protected soft fruit crops.

This project demonstrated that blackberry leaf midge also attacked protected raspberry, but to a lesser extent than on blackberry. This was probably due to incidental midge control by chlorpyrifos used against other pests e.g. raspberry cane midge and raspberry beetle, and to the raspberry varieties monitored being less susceptible to the pest. The area of raspberries grown in the UK is 1,757 ha (Defra Basic Hort Stats 2010). Average yields are estimated at 14.2t/ha with a value of £6,000/t, giving a gross margin of about £34,500/ha. Blackberry leaf midge currently affects about 20% of the raspberry area, notably on farms where chlorpyrifos is no longer routinely used, or where particularly susceptible primocane varieties are grown and/or double cropped each year. In the absence of chlorpyrifos, e.g. where growers are using IPM, or should this pesticide be withdrawn, in an average year, on farms where the pest is present, raspberry yields are likely to be reduced by at least 15%. This would result in gross margin reductions of 15%, costing individual growers £4,500/ha and costing the industry over £22.5m per year.

This project has provided a significant amount of new information on the biology of blackberry leaf midge on protected blackberry. This knowledge will allow more effective planning and timing of currently available and future control measures should they be developed. Chlorpyrifos was shown to be the only currently effective pesticide and this will benefit control of the pest on outdoor blackberry and on both protected and outdoor raspberry, on which various chlorpyrifos products are approved. However, a SOLA for use on protected blackberry is unlikely to be supported. Discussions are currently being held with pesticide suppliers over possible testing of other potential active ingredients against the pest, which could not be included in this project due to limited resources.

For example, new knowledge on the location of overwintering cocoons in the soil offers the opportunity for targeting this life stage with a soil-applied pesticide, either at the end of the season or before first generation adults are likely to emerge. An effective soil drench could offer critical control of first generation midges and would be more compatible with biological control agents used on the plants in IPM programmes.

Knowledge on the location of overwintering cocoons also offers the opportunity for cultural control of the pest using ground-cover materials. Work to validate the potential control method demonstrated in this project is justified on a commercial crop scale.

Results in this project indicated that *Neoseiulus (Amblyseius) cucumeris*, *Orius laevigatus* and *Macrocheles robustulus* were the most promising biological control agents against the pest. Further research would be needed to develop an effective IPM strategy including prediction, monitoring, cultural, biological and compatible chemical control methods.

Action points for growers

- Monitor for first generation blackberry leaf midge from early April.

- If the pest occurs on outdoor blackberry or either outdoor or protected raspberry, consider a foliar application of chlorpyrifos which should give some control. However, this pesticide does not have a SOLA for use on protected blackberry and is not IPM- compatible.

SCIENCE SECTION

Introduction

In order to meet consumer demand for soft fruit over an extended season, there has been a substantial increase in UK production of blackberries and raspberries under protection, mainly using ‘Spanish’ tunnels. This has led to increased problems with what were considered previously in outdoor rubus crops as ‘minor’ localised pests, including blackberry leaf midge, *Dasineura plicatrix*. This pest has now become widespread and is often very damaging, particularly on blackberry crops. The pest damages crops in propagation in addition to fruiting crops. The larvae feed on the leaf tips, causing leaf twisting and distortion, cane stunting and branching and reduced yield in the following year’s crop. Prior to work in this project, the life cycle of blackberry leaf midge on protected *Rubus* crops was not fully understood. Results in year 1 demonstrated that on protected blackberry, the pest had four overlapping generations, rather than the two generations reported on outdoor crops. This led to high levels of midge infestation and extensive damage between April and September.

There is a shortage of approved, effective pesticides for use against the pest on protected *Rubus* crops, particularly on protected blackberry. In year 1, chlorpyrifos was shown to be the only effective pesticide of those tested and although this is approved on both outdoor and protected raspberry, it has a SOLA on outdoor blackberry only. Chlorpyrifos is not compatible with bees used for pollination, or biological control agents used against other pests in Integrated Pest Management (IPM) programmes. There is a need to develop integrated control methods for use within IPM.

In addition to the limited options for chemical control, growers often see damage by first generation midges too late to apply any pesticide effectively, as in year 1 the larvae were shown to feed in the twisted leaves for only two weeks before dropping to the ground to pupate. It would be useful for growers to be aware of the timing of first generation midge emergence. Modelling of soil temperature data to predict first generation emergence and egg laying gave promising results in year 1 and with further validation, might be used by growers in a similar way to the raspberry cane midge model to help time early season control measures.

In a current HDC-funded project, SF 117, EMR and NRI are doing research on the blackberry leaf midge pheromone, with a view to developing a trap to indicate first generation emergence. However, methods for the practical use of the pheromone have yet to be developed. Any prediction or detection method for first generation adults will only be of practical use if it can be used to trigger the application of an effective early season control measure and this has not yet been identified for protected blackberry.

An effective non-chemical strategy for control of the pest could include the establishment of one or more biological control agents in the crop, so that they are available to attack the pest at key times in the pest's life cycle. Potential biological control agents for use against blackberry leaf midge include those that target the leaf-dwelling stages (eggs and larvae on leaves) and those that target the ground-dwelling stages (larvae that drop to the ground to pupate inside cocoons). Recent Dutch laboratory research on biological control of the ground-dwelling stages of blackberry leaf midge showed that although three species of entomopathogenic nematodes were ineffective against the larvae in laboratory studies, the ground-dwelling predatory mite, *Hypoaspis aculeifer* and the predatory beetle, *Atheta coriaria* showed potential for control of the pest (Wenneker, 2008 and personal communication). These biological control agents are commercially available in the UK. The Dutch laboratory studies indicated that either higher numbers of *Hypoaspis* or *Atheta* than those tested would be needed for effective control, or a more aggressive predator would be needed, as the midge larvae are only vulnerable to predation before they spin a cocoon in the soil.

Additional Dutch research has demonstrated the potential of a large, aggressive soil-dwelling predatory mite species, *Macrocheles robustulus*, against the ground-based life stages of western flower thrips (WFT) (Messelink & van Holstein-Saj, 2008). The results showed that *M. robustulus* gave better control of WFT than *Hypoaspis* species. *M. robustulus* is known to feed on various soil-dwelling prey and could have potential against the larvae of blackberry leaf midge once they have dropped from the foliage to the ground. *M. robustulus* is native to the UK and has been found naturally occurring in large numbers in various commercial and research glasshouses (Bennison, unpublished). The predator became commercially available in the UK in 2010 and its potential against blackberry leaf midge larvae in the soil or substrate should be investigated.

In year 1 of the project, a range of biological control agents were tested against the pest. Preliminary laboratory experiments indicated that both the predatory mites *Neoseiulus (Amblyseius) cucumeris* and *Amblyseius andersoni* predated young blackberry leaf midge larvae. Releases of both species in controlled-release sachets to blackberry plants in research tunnels led to some establishment of *N. cucumeris* in the flowers but no apparent establishment of *A. andersoni*. Following grower releases of *A. andersoni* to commercial protected blackberry plants, there was no apparent reduction in the pest when compared with untreated plants. In laboratory pot experiments, neither the predatory mites *Macrocheles robustulus* nor the predatory beetles *Atheta coriaria* gave significant reductions in numbers of blackberry leaf midge larvae successfully completing their development in compost and emerging as adult midges. Results indicated that numbers of both predators may need to be equal to or higher than numbers of the target midge larvae in the soil to give significant control of the ground-dwelling stage of the pest.

An entomopathogenic fungus, *Beauveria bassiana* (Naturalis-L) was approved for use on all protected crops in the UK during 2009 and became available in spring 2010. The product can infect a wide range of invertebrate pests. The HDC soft fruit panel requested that this product should be tested for efficacy against the pest in this project. In year 1 of this project, grower applications of Naturalis-L to the commercial crop did not reduce the percentage of leaf tips infested or mean numbers of live larvae per leaf tip. *B. bassiana* is a contact-acting fungus and is unlikely to reach the target pest inside folded leaf tips. In a laboratory test, application of Naturalis-L to compost did not reduce numbers of blackberry leaf midge larvae successfully completing their development in the compost and emerging as adult midges.

Naturally-occurring anthocorid bugs (both *Anthocoris nemorum* and *Orius* sp.) were observed feeding on blackberry leaf midge larvae in the commercial crop during July and August. Both adults and nymphs were recorded, which showed that the predatory bugs were breeding on the blackberry crop. *Orius laevigatus* is commercially available and work on its potential against the pest is justified in year 2.

Overall aim of project

The aims of this project are to confirm the location and timing of key life-stage events of blackberry leaf midge on commercial protected blackberry and raspberry crops and to test integrated control methods for use on protected blackberry, with a view to extrapolating the methods to protected raspberry.

Objective 1: Confirm the location, timing and duration of key life-stage events

Materials and Methods

Experimental site: Sunclose Farm, Milton, Cambridge.

Experimental crops: Two blackberry crops grown in ‘Spanish’ tunnels: one crop grown in pots (Meadow Field) and one grown in the soil (Chivers Field).

Variety: Loch Ness in Meadow Field and both Loch Ness and Loch Tay in Chivers Field.

Crops in both fields were severely damaged by blackberry leaf midge in year 1 of the project (2009). The biology and life cycle of blackberry leaf midge was monitored in detail in Chivers

Field between 17 March and 22 September, and in Meadow Field from 17 March until first generation midge larvae were active.

Adult emergence and number and duration of generations

Water traps: Adult emergence was monitored with water traps in one tunnel in each of Meadow and Chivers Fields. Five water traps were set up on the floor beneath the crop canopy in each field on 17 March. The water traps were white, circular plastic dishes, 16.5 cm diameter and 6 cm deep. The traps were placed at equal distances along the length of the row of plants. The traps were filled to 1.5 cm below the top with water mixed with a drop of detergent ('Tween') to break the surface tension so that midges could not escape once trapped. One 'Campden' tablet was added to each trap to help preserve the fragile midges until collection. The trap contents were collected weekly from 17 March until 19 May in Meadow Field. In Chivers Field, the trap contents were collected weekly from 17 March until 16 June in a tunnel of cv. Loch Ness. On 16 June, five additional traps were set up in a tunnel of cv. Loch Tay in Chivers Field and the contents of these, in addition to those in cv. Loch Ness, were collected every fortnight until 22 September. The trap contents were collected by pouring and rinsing the contents of each trap through a 250 μm sieve, then washing the contents of the sieve with water into a labelled screw-top jar.

In the laboratory the contents of the jars were either examined on the day of collection or if necessary, refrigerated for a few days until they could be examined. The contents of each jar were poured through a 250 μm sieve. Any large insects and blackberry fruit retained on the sieve were washed on the sieve to remove any possible midges and were then discarded. The contents of the sieve were then washed with water into a Petri dish and examined under a binocular microscope. On some trapping dates there were large numbers of petals in the traps. These were examined in the Petri dish in alcohol before disposal, to ensure that no midges were attached to them. Numbers of male and female adult blackberry leaf midges were then recorded per trap.

Prediction of adult emergence from soil temperatures

Daily mean soil temperatures at two depths (3 and 10 cm) were recorded between 29 January and 7 June in the monitoring tunnels in both Meadow and Chivers Fields. As in year 1, this was done in order to determine whether first generation blackberry leaf midge emergence occurs when a particular cumulative temperature is reached, as with raspberry cane midge. Two replicate 'Tinytalk' dataloggers were buried at each depth in each tunnel. The data was used to model first generation blackberry leaf midge emergence and first egg-laying dates in a similar manner to the ADAS raspberry cane midge model.

Eggs and larvae

Monitoring of leaf tips for midge eggs and larvae was done on the same sampling dates as the water traps, from 28 April to 19 May in Meadow Field and from 28 April to 22 September in Chivers Field. Twenty random leaf tips were collected from each of the monitored tunnels in each field. The leaf tips comprised a group of unfolded leaves at the growing tip, and the number of individual leaves varied between two and eight. The leaf tips were placed into labelled plastic boxes which were placed in a cool box and returned to the laboratory. In the laboratory each individual folded leaf was examined under a binocular microscope and checked for midge eggs at the base of the hairs on the outside (under-surface) of the folded leaf. Then each folded leaf was gently unfolded using fine forceps and checked for midge larvae, which feed on the inside of the folded leaves.

Results and Discussion

Adult emergence and number of generations

The mean numbers of male and female blackberry leaf midges per water trap in Meadow and Chivers Fields are shown in Table 1 and Figures 1 and 2 respectively. The first female was caught in Meadow and Chivers Fields on 12 and 25 May respectively. The first male was caught on 19 and 25 May in Meadow and Chivers Fields respectively. Numbers of midge adults remained low in cv. Loch Ness (below one per trap) until 9 June. On this date, the water traps in Meadow Field were discontinued as it was only intended to monitor adult emergence in both fields until first adults and larvae were recorded, to obtain information on predicted and actual first generation emergence dates. On 9 June, the water traps in Meadow Field were moved to an additional blackberry variety, Loch Tay, in Chivers Field, as the grower had observed more midge-infested leaf tips in this variety than in the Loch Ness tunnel. This was thought to be due to the Loch Ness plants suffering from infection with *Verticillium* wilt, leading to very poor growth and very few new leaf tips being available for midge egg-laying. Numbers of midge adults in traps were higher in the Loch Tay tunnel than in the Loch Ness, although numbers were still much lower than in 2009. On 15 July, mean numbers of adults peaked at 34 per trap (Table 1, Figure 2), with a mean of 31 per trap being males. This was a similar result as in 2009, when numbers of adults peaked on 8 July in Chivers Field, although peak mean numbers per trap were higher (mean 132 per trap, 3 females and 129 males).

Table 1. Mean numbers of adult male and female blackberry leaf midges per water trap in cv. Loch Ness in Meadow Field and Chivers Field tunnels and in cv Loch Tay in a Chivers Field tunnel.

- indicates no trap collected.

Date	Meadow Field cv Loch Ness		Chivers Field cv Loch Ness		Chivers Field cv Loch Tay	
	Females	Males	Females	Males	Females	Males
17 March	0	0	0	0	-	-
24 March	0	0	0	0	-	-
31 March	0	0	0	0	-	-
14 April	0	0	0	0	-	-
21 April	0	0	0	0	-	-
28 April	0	0	0	0	-	-
5 May	0	0	0	0	-	-
12 May	0.2	0	0	0	-	-
19 May	0	0.2	0	0.2	-	-
25 May	-	-	0.1	0.1	-	-
2 June	-	-	0.5	0	-	-
9 June	-	-	0.2	0.4	-	-
16 June	-	-	0.2	0.2	2	0.8
30 June	-	-	0.4	0.2	1.2	0.6
15 July	-	-	1.4	1.2	2.8	30.8
29 July	-	-	2.4	0.8	3.4	5
11 Aug	-	-	1.2	0.8	1.8	4
25 Aug	-	-	1.2	0.6	0.8	1.4
8 Sep	-	-	-	-	0.6	1.8
22 Sep	-	-	-	-	0.2	0.2

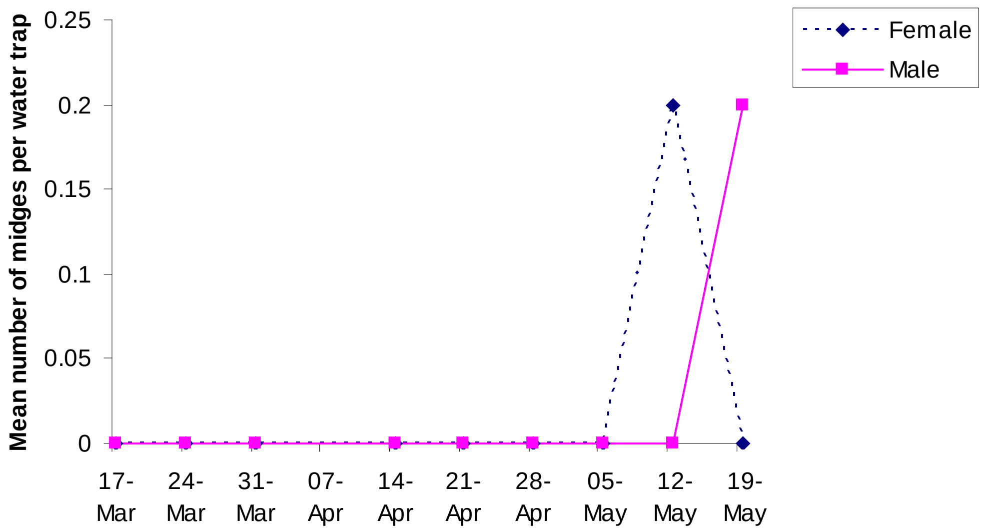

Figure 1. Meadow Field: Mean numbers of blackberry leaf midge females and males per water trap in 2010.

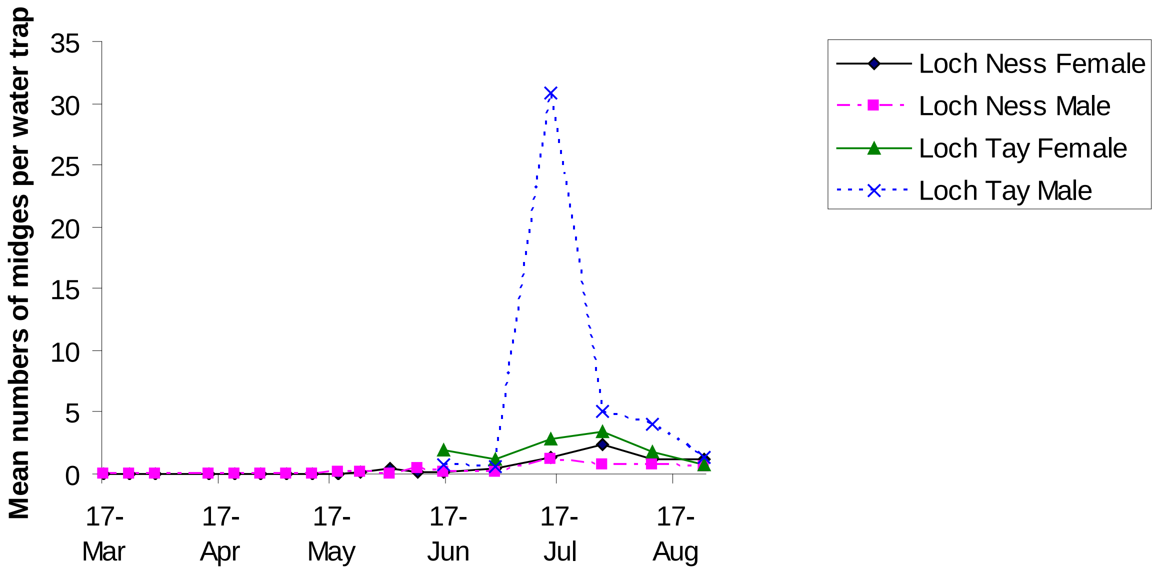

Figure 2. Chivers Field: Mean numbers of blackberry leaf midge females and males per water trap in 2010.

Eggs and larvae

No blackberry leaf midge eggs or larvae were found in leaf tips sampled from Meadow Field between 28 April and 19 May. Larvae were first found in Chivers Field on 11 May (one week before adult midges were detected in this field). However, the grower reported seeing occasional infested leaf tips earlier than this. Numbers of the pest were much lower in both fields than in 2009, thus the first adults in water traps must have been missed and sampling random leaf tips must have missed the very first infested tips. When larvae were first detected in the Loch Ness on 11 May, 15% of the leaf tips were infested with eggs and/or larvae. The percentage of infested leaf tips had increased to 50% by 16 June and 90% by 29 July (Figure 3). In the Loch Tay, 100% leaf tips were infested on 30 June. This pattern of increase in percentage infested leaf tips was similar to that in 2009 in the same field, when 100% were infested by 8 July.

The pattern of numbers of eggs and larvae in Chivers Field suggested that there were three generations in 2010; the first in May/June, in July/August and the third in August/September (Figures 3, 4). In 2009, there were four generations, the first in April/May. As in 2009, the generations were not discrete, but overlapped so that midge activity extended from May to September.

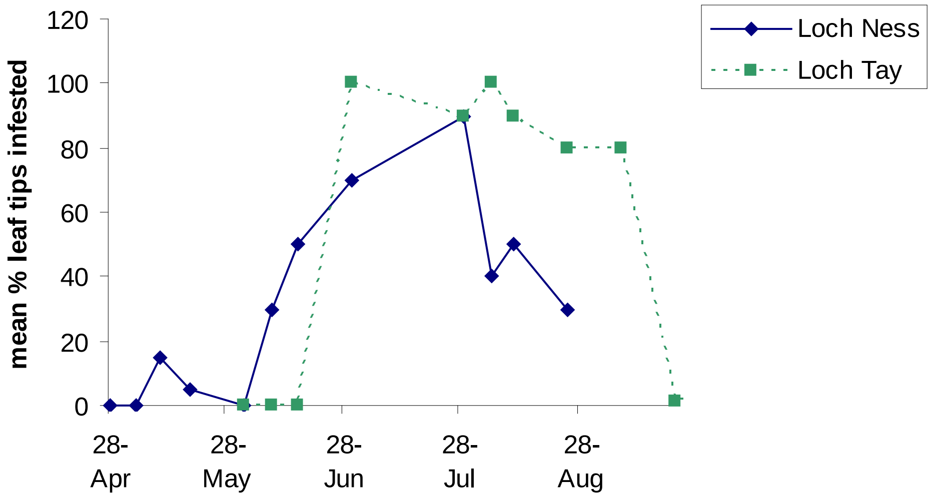

Figure 3. Percentage of leaf tips infested with eggs and/or larvae in cvs Loch Ness and Loch Tay in Chivers Field between 11 May and 22 September 2010.

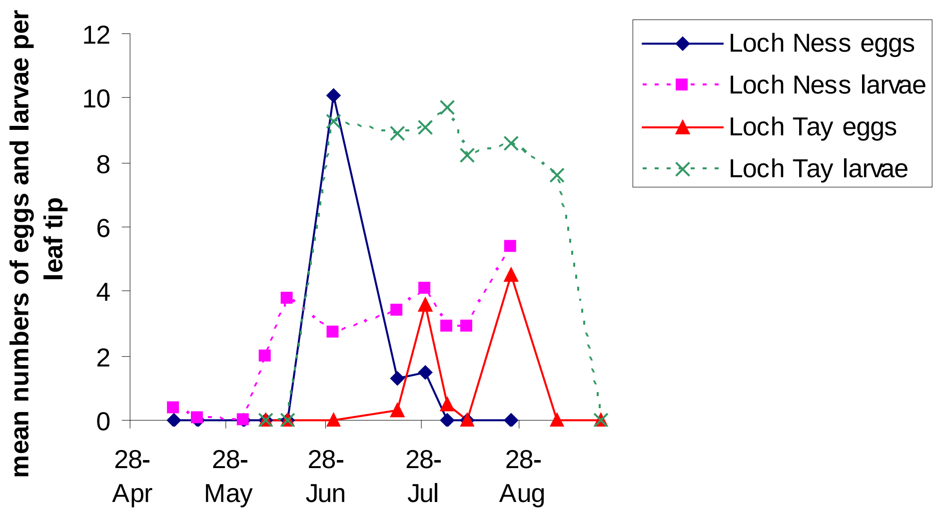

Figure 4. Mean numbers of eggs and larvae per leaf tip in Chivers Field between 11 May and 22 September 2010.

Prediction of first blackberry leaf midge eggs from soil temperatures

Modelling of the soil temperature data in tunnels in both Meadow and Chivers Fields in 2010 indicated that first generation blackberry leaf midge adults would emerge and eggs would be laid at a cumulative soil temperature of around 335°C days above a base temperature of 4°C. This was a similar prediction as for raspberry cane midge, which are predicted to lay first eggs when accumulated soil temperatures reach 339°C days above a base temperature of 4°C (Gordon *et al*, 1989). In 2010, the model predicted first eggs on 21 April in Meadow Field and on 5 May in Chivers Field. In Meadow Field first adults were caught in water traps on 12 May but we did not detect eggs or larvae in leaf samples during the monitoring period. However, the grower reported seeing low numbers of twisted leaf tips before we detected adults. In Chivers Field first adults were found on 19 May but we detected eggs and larvae on 11 May, only six days after the prediction date. Due to the very low numbers of midges at the start of their activity in 2010, sampling random leaf tips must have missed the very first infested tips.

In 2009, modelling of soil temperature data indicated that blackberry leaf midge adults emerge and lay eggs at a cumulative soil temperature of around 280°C days above a base temperature of 4°C, i.e. earlier than raspberry cane midge. In 2009, eggs were detected in Chivers Field five days after the predicted date, thus the model predicted first eggs within 5-6 days of finding first eggs in both years. As sampling was only done weekly, this result was very encouraging. More frequent sampling may have allowed detection of the first eggs closer to the predicted date.

Summary of findings from work in Objective 1

The findings from the results of work in both years of the project are given below:

- The biology and life stage events of blackberry leaf midge were confirmed in two protected blackberry crops during 2009 and 2010, one grown in pots (Meadow Field), the other grown in the soil (Chivers Field).
- In 2008/2009, the pest was shown to overwinter as cocoons in the soil. Most were found at a depth of 3 cm in the path in between the plant rows, at the edge of the ground-cover matting running under the crop canopy. They were also found in the soil in the planting hole, under the polythene covering the ground between plants and in plant debris on the polythene.
- In 2009, the first male midge was found in water traps on 8 April in Meadow Field and the first females on 15 April in both Meadow and Chivers Fields. In 2010, the first females were found on 12 and 19 May in Meadow and Chivers Fields respectively and

the first males found on 19 May in both fields. The later midge emergence in 2010 is likely to have been due to the very severe winter of 2009/2010.

- In 2009, the first midge eggs and larvae were found in leaf tips on 15 and 22 April in Meadow and Chivers Fields respectively. In 2010, larvae were first found in Chivers Field on 5 May (two weeks before adult midges were detected in this field). Thus the water traps did not detect the first adult midges emerging in 2010, probably due to midge numbers being much lower than in 2009.
- Soil temperature data between March and June was modelled in a similar manner to the ADAS raspberry cane midge model to predict first generation blackberry leaf midge emergence and first egg-laying dates. In 2009, results were very promising as the predicted dates were only five days earlier than the actual dates when first eggs were found, i.e. 15 and 22 April in the two respective fields. This suggested that blackberry leaf midge emerges and lays eggs slightly earlier than raspberry cane midge, at a cumulative soil temperature of around 280°C days above a base temperature of 4°C.
- In 2010, we found first eggs and larvae between 5 and 12 May, 11-18 days later than the predicted dates of 22 and 25 April in the two fields. However, the grower noticed the occasional infested leaf tip earlier than this. Midge numbers were much lower in both fields than in 2009, thus sampling random leaf tips must have missed the very first infested tips.
- In 2009, numbers of midge adults remained low (below two per trap) until June and July. Mean numbers per trap peaked on 22 July in Meadow Field (24 per trap) and on 8 July in Chivers Field (132 per trap). In 2010, numbers of midge adults were much lower in both fields. Meadow Field was only monitored until first adult emergence and on the final trapping date on 19 May there was a mean of only 0.2 per trap. In Chivers Field, midge adults were monitored between March and September and mean numbers peaked on 15 July at 34 per trap. The lower numbers of midges occurring in 2010 could have been partly due to the severe winter of 2009/2010 killing a proportion of overwintered cocoons but also due to the plants in Chivers Field suffering from *Verticillium* wilt and thus produced fewer new leaf tips for egg-laying.
- During peak adult activity in both years, numbers of males trapped were much higher than those of females. This could possibly be due to differences in the behaviour of males and females rather than an uneven sex ratio in the population.
- In 2009, the percentage of leaf tips infested with eggs and larvae increased rapidly in both fields, from 30% and 5% in Meadow and Chivers Fields respectively in mid-April, to 90% and 100% respectively in late June/early July. In 2010, the percentage of infested leaf tips in Chivers Field increased from 5% in early May to 100% in late June. Larvae were found until late September in 2009 and until early September in 2010.
- Midge larvae fed for approximately two weeks in the leaf tips, causing them to twist. Severely damaged leaves turned brown and withered.

- When fully fed, the larvae dropped to the ground to pupate in cocoons. No pupae were ever found in leaf tips, and dropped larvae were found in the water traps under the crop canopy that were use for monitoring adult activity.
- Monitoring of three protected raspberry crops in June 2009 confirmed that blackberry leaf midge larvae damaged raspberry leaves but the percentage of infested leaf tips was much lower than that in the protected blackberry crops at this site. This may have been due to the use of chlorpyrifos against other pests such as raspberry cane midge having given some control of blackberry leaf midge populations.
- In 2009 there were four overlapping generations in protected blackberry between April and September, unlike on outdoor blackberry when only two generations are reported, in May/June and July/August. In 2010, first generation midges were not active until May/June and the data suggested a second and third generation in July/August and August/September. Thus on protected blackberry, depending on the season, first adult emergence can be earlier, there are more generations per season and the pest is active for a longer period than on outdoor blackberry.

Conclusions from work in Objective 1

Conclusions from the results of work in both years of the project are given below:

- In the UK blackberry leaf midge overwinters as cocoons in the soil, under the polythene covering the ground between plants and in plant debris on the polythene.
- On protected blackberry, depending on the season, first adult emergence can be earlier than outdoors, there are more generations per season and the pest is active for a longer period than on outdoor blackberry.
- Water traps do not reliably detect the first emerging adult midges when midge numbers are low.
- The model using soil temperature data between March and June, in a similar manner to the ADAS raspberry cane midge model, to predict first generation blackberry leaf midge emergence and first egg-laying dates is promising. Results suggested that blackberry leaf midge emerges and lays eggs slightly earlier than raspberry cane midge, at a cumulative soil temperature of around 280°C days above a base temperature of 4°C.
- During peak adult activity numbers of males trapped were much higher than those of females, possibly be due to differences in the behaviour of males and females rather than an uneven sex ratio in the population.

Objective 2: Evaluate potential integrated control methods against blackberry leaf midge, for use in IPM programmes on protected blackberry

Small-scale experiments were done in the laboratory and in polythene tunnels at both ADAS Boxworth and the commercial farm to assess the potential of the following biological control agents, pesticides and a cultural control method for control of blackberry leaf midge:

- *Neoseiulus (Amblyseius) cucumeris* and *A. andersoni* against midge eggs and young larvae on foliage.
- *Macrocheles robustulus* and *Hypoaspis aculeifer* against midge larvae dropping to the ground.
- *Orius laevigatus* against midge eggs and young larvae on the foliage.
- Black polythene or woven ground-cover matting over the soil as a cultural control method to inhibit successful pupation of midge larvae dropping to the soil.
- A coded pesticide applied as a foliar spray against midges on foliage.

Amblyseius cucumeris* and *A. andersoni

Small-scale experiments were done in research polythene tunnels at ADAS Boxworth to assess the potential of the predatory mites *N. cucumeris* and *A. andersoni* against blackberry leaf midge eggs and young larvae.

Materials and Methods

Two identical research polythene tunnels at ADAS Boxworth were used for the experiment. In each tunnel there was a row of blackberry plants, cv. Loch Ness, kept from the previous year and infested with blackberry leaf midge. On 6 April, *N. cucumeris* were released to the plants in one tunnel and *A. andersoni* were released in the other tunnel. The mites were released at the rate of four release sachets per plant. In order to ensure that the pest was present in each tunnel, infested leaf tips were collected from the commercial farm hosting work in the project and equal numbers were placed onto the experimental plants on 17 May.

Assessment of predatory mites in sachets five weeks after release

On 11 May, the contents of three sachets from each tunnel were examined under a binocular microscope and numbers of predatory mites per sachet were recorded.

Assessment of leaf tip infestation with blackberry leaf midge

On 23 June, three leaf tips from each of six plants per tunnel were sampled and examined under a binocular microscope for blackberry leaf midge eggs and larvae. The percentage infested leaf tips were compared using regression analysis and the mean numbers of midge eggs and larvae per leaf tip were compared using analysis of variance.

Results and Discussion

Numbers of predatory mites in sachets five weeks after release

On 11 May, five weeks after release of both predator species, the mean numbers of *N. cucumeris* and *A. andersoni* remaining per sachet were 56 and 0.7 respectively. This indicated that most of the predatory mites had left the sachets and a further release should be made to ensure a continued release onto the plants. On 2 June, a second release of *N. cucumeris* was made to the first tunnel at four sachets per plant. A second release of *A. andersoni* was planned but not made as this species was not available from the supplier.

Leaf tip infestation with blackberry leaf midge

In the tunnel where *N. cucumeris* had been released, significantly less leaf tips (33%) were infested with blackberry leaf midge eggs and larvae then in the tunnel where *A. andersoni* had been released (89%), $P<0.05$ (Table 2). Mean numbers of midge eggs and larvae per leaf tip were significantly lower in the tunnel where *N. cucumeris* had been released (0.78) than in the tunnel where *A. andersoni* had been released (3.94), $P<0.05$. However, this result should be treated with caution, as a second release of *N. cucumeris* had been made on 2 June but *A. andersoni* were not available for a second release. In addition, the rows of blackberry plants and tunnels used for each treatment were not replicated. However, the results indicate that *N. cucumeris* might play a useful role in controlling the first generation of blackberry leaf midge eggs and young larvae. Further work would be needed to validate this on a larger, replicated scale.

Table 2. % leaf tips infested with leaf midge and mean numbers of midge eggs and larvae per leaf tip on 23 June, 11 weeks after first predator release

Treatment	% leaf tips infested with midge eggs & larvae	Mean no midge eggs & larvae per leaf tip
N. cucumeris	33 *	0.78 *
A. andersoni	89	3.94

Macrocheles robustulus **and** ***Hypoaspis aculeifer***

Materials and Methods

In year 1, a laboratory experiment showed that when two *M. robustulus* adults were added to replicate dishes, each with four midge larvae on compost, the predatory mites reduced numbers of adult midges emerging but the reduction was not quite significant at the 95% level ($P=0.053$). It was thought that if higher numbers of *M. robustulus* had been added, a significant result may have been given.

A laboratory experiment was set up on 11 August 2010 to assess the potential of the predatory mites *Macrocheles robustulus* added at a higher release rate and to compare it with *Hypoaspis aculeifer* against the midge soil-dwelling life stages, i.e. larvae that drop to the ground to spin their pupal cocoons. Large, fully grown midge larvae were removed from blackberry leaf tips sampled from the commercial crop used in Objective 1. Eight larvae per pot were added to 30 replicate 2cm deep Petri dishes with tight fitting lids, to which damp compost had been added to a depth of one cm. The base of each dish had a small hole drilled through it, plugged with cotton wool, to allow uptake of moisture by the compost but retaining the midges and predators within the dish. The lids of the dishes were fitted with ventilation holes screened with insect-proof mesh. Ten replicate dishes were used for each of three treatments:

1. Eight adult *M. robustulus* per dish
2. Eight adult *H. aculeifer* per dish
3. No predators (untreated control)

A small square portion of yellow sticky trap was placed inside the lid of each dish to catch emerging midge adults. The dishes were placed in a randomised block design on a large plastic tray lined with damp capillary matting. They were left in a controlled temperature room at 21°C and with a photoperiod of 16 hours light, eight hours dark for four weeks to allow the midge larvae to pupate and emerge as adults. The dishes were examined four weeks later on 7 September 2010 and numbers of midge adults per trap recorded. The data was statistically analysed using a t-test.

Results and Discussion

The mean numbers of adult midges emerging per dish over the 4-week period are shown in Table 3 and Figure 5. *M. robustulus* reduced the numbers of midge larvae surviving to the adult stage when compared with the untreated control, but as in year 1 this result was not quite statistically significant at the 95% level (P=0.098). Natural mortality of midges during the pupation stage was higher in this experiment (67%) than in year 1 (36%) and it is possible that with further replication or by adding even higher release rates of *M. robustulus*, a significant reduction may be given at the 95% level. No reduction in adult midge emergence was given by *H. aculeifer*. *M. robustulus* became commercially available during 2010 and is currently marketed for control of the ground-dwelling stages of sciarid flies, shore flies and thrips. *M. robustulus* has been reported as giving better control of western flower thrips ground-dwelling stages than *H. aculeifer* (Messelink & van Holstein-Saj, 2008).

Table 3. Mean numbers of adult blackberry leaf midges emerging per untreated dish and per dish treated with *Macrocheles robustulus* or *Hypoapsis aculeifer*

Treatment	Mean no. adult midges per dish	Mean % midge survival to adult stage
Untreated control	2.62	33%
Macrocheles robustulus	1.38	17%
Hypoaspis aculeifer	2.38	30%

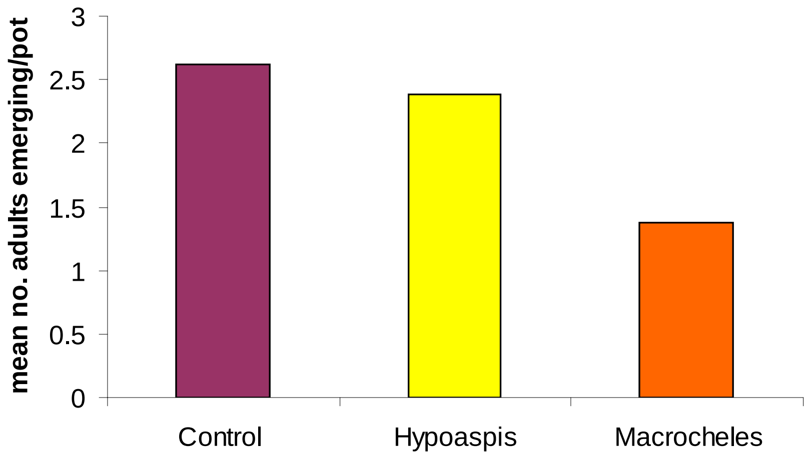

Figure 5. Mean numbers of adult blackberry leaf midges emerging per untreated dish and per dish treated with *Macrocheles robustulus* or *Hypoapsis aculeifer*

Orius laevigatus

In year 1 of the project, naturally-occurring anthocorid bugs (both *Orius* sp. and *Anthocoris nemorum*) were observed feeding on blackberry leaf midge larvae in the commercial crop during July and August. Both adults and nymphs were recorded, which showed that the predatory bugs were breeding on the blackberry crop. *O. laevigatus* is commercially available and work on its potential against the pest when released early in the season was included in year 2. Three experiments were done:

- Laboratory experiment on *O. laevigatus* establishment and breeding on blackberry flowers
- Laboratory experiment on *O. laevigatus* predation on blackberry leaf midge larvae
- Use of flowering 'banker plants' to aid early season establishment of *O. laevigatus* in a commercial blackberry crop

Laboratory experiment on O. laevigatus establishment and breeding on blackberry flowers

The aim of this experiment was to test whether *O. laevigatus* will breed on blackberry pollen in flowers, i.e. to test whether they could establish on flowering blackberry plants in the absence of leaf midge larvae or other invertebrate prey.

Materials and Methods

Untreated blackberry shoots with flowers and buds taken from plants in a research tunnel at ADAS Boxworth were used. The flowers were checked to ensure that no invertebrate prey were present. Water was added to four replicate specimen tubes and the tops of the tubes were sealed with Parafilm to prevent *Orius* from entering the tubes and drowning. Blackberry shoots with equal numbers of flowers and buds were pushed through the Parafilm covering each tube. The tubes were placed in four replicate plastic sandwich boxes with insect-screened ventilation holes in the lid. One male and one female *Orius* adult were added to each box using a ‘pooter’. The lid of each box was secured with sticky tape to ensure that the *Orius* could not escape. The boxes were placed in a controlled temperature laboratory at 25°C, with a photoperiod of 16:8 hrs light:dark and left for two weeks. Extra blackberry shoots were added to each tube as necessary during the experiment to ensure a continuous supply of pollen as food.

Assessment of Orius adults and nymphs

After two weeks, numbers of *Orius* adults and nymphs were recorded in each box.

Results and Discussion

Orius nymphs were found in three of the four tubes, in the fourth, only an adult was recorded. In the tubes with nymphs, there was a mean of 10.7 nymphs per box, ranging from first to fifth (final) instars. This result demonstrated that *O. laevigatus* can breed in blackberry flowers with only pollen available as food. Thus they should be able to establish in a flowering blackberry crop as soon as pollen is available and temperatures are suitable for reproduction.

Laboratory experiment on O. laevigatus predation on blackberry leaf midge larvae

Materials and Methods

Sixteen tight-fitting Petri dishes with insect-screened ventilation holes in the lids were used for the experiment, with eight replicate dishes for each of two treatments:

1. Untreated control
2. One *O. laevigatus* adult female per dish

A blackberry leaf tip, uninfested with midge larvae was placed in each replicate dish. The leaf petiole was wrapped in damp cotton wool covered and sealed with Parafilm to keep the leaf tip in good condition. Five medium sized blackberry leaf midge larvae, collected from the commercial farm were added to each leaf tip. One female *O. laevigatus* adult, taken from a fresh bottle received from the commercial supplier was added to eight of the dishes. The dishes were left in a randomised block design in a controlled temperature laboratory at 21°C and with a phototoperiod of 16:8 hrs light:dark, for 24 hours. Numbers of live *Orius* and both live and predated midge larvae were recorded after 24 hours.

Results and Discussion

All the blackberry leaf midge larvae survived in the untreated control dishes without *O. laevigatus.* In the dishes with *O. laevigatus*, the adult predator survived in all replicates and in five of the eight replicates, all five midge larvae had been predated. In the remaining three replicate dishes with *O. laevigatus*, four midge larvae had spun pupation cocoons and were thus protected from predation and the remaining larvae were missing, these might have been eaten but the shrivelled larval skins left behind after predation were not visible. The results demonstrated that each *O. laevigatus* can eat at least of five blackberry leaf midge per day.

Use of flowering 'banker plants' to aid early season establishment of O. laevigatus in a commercial blackberry crop

Materials and Methods

Blackberries grown in Spanish tunnels do not normally start flowering until May and blackberry leaf midge larvae can be active from April onwards. Thus the aim of this experiment was to test whether *O. laevigatus* could establish in the crop if flowering 'banker' plants were used to provide a pollen food source before blackberry flowers are available.

Experimental site

The experiment was set up on 31 March in a 'Spanish' tunnel of blackberries in Chivers field at Sunclose Farm, Milton, Cambridge. The experiment was set up as soon as crop canopy temperatures (monitored with dataloggers) had reached a minimum of 15°C for at least a few hours a day, as this is the minimum temperature for *O. laevigatus* egg-laying (Malais & Ravensberg 2003). The blackberry crop, cv. Loch Ness had been infested with blackberry leaf midge the previous year and had been used for monitoring the biology of the pest in year 1 of the project. The tunnel was approximately 90 metres long and 8.3 metres wide (747m^2) and contained three rows of blackberries planted in the soil.

Banker plants tested

The following potential 'banker plants' were used:

1. Pussy willow stems in flower
2. Flowering everbearer strawberry plants, cv. Eve's Delight

The pussy willow stems were collected from an ADAS research farm at ADAS Arthur Rickwood, Mepal, Cambridgeshire. The strawberry plants were provided by the host grower at Sunclose Farm.

Eight banker plant ‘stations’ were set up every 10 metres down the length of the tunnel, on the floor, under the crop canopy of the middle row of blackberry plants. Each banker plant station comprised one container filled with gravel and water, into which an equal number of pussy willow stems had been placed, and two grow-bags with strawberry plants, placed in plastic trays to which water was added as needed. One bag of strawberries was a second year crop and one was planted in March 2010. The different-aged strawberry plants were used to provide a continous source of flowers and pollen.

O. laevigatus release

O. laevigatus adults were released on 31 March to the pussy willow and on 9 April to the strawberry plants when flowers were available. The predators were released at 0.5 per m^2 (400 in the tunnel), with 25 *Orius* added to each of the eight pussy willow and strawberry ‘stations’. To facilitate equal release to each station, 25 *Orius* adults were added to sixteen specimen tubes using a ‘pooter’ in the laboratory before taking to the experimental site. One teaspoon-full of the buckwheat carrier for the predators was added to each tube. The tubes were sealed with the screw-top lids and taken to the experimental site in a cool box and the tubes were opened at each banker plant station. The tubes used at the pussy willow stations were placed next to the containers with the willow stems, thus allowing the *Orius* adults to fly out. The contents of the tubes used at the strawberry stations were emptied over the strawberry plants with open flowers.

A second release of *O. laevigatus* was made on 6 May, when 500 adults (0.7 per m^2) were released to both the banker plants and the blackberry plants, which had started to flower. A third release of *O. laevigatus* was made on 2 June, when 500 adults (0.7 per m^2) were released to both the strawberry banker plants and the blackberry plants. On this date, the *Orius* were released only to the newer strawberry banker plants, as the older strawberry plants and pussy willow stems had stopped flowering.

Assessments

The following assessments were made every week from 14 April then every fortnight from 19 May to 16 June:

1. Numbers of *Orius* adults and nymphs were recorded on one pussy willow stem per banker plant station, four fully open strawberry flowers per station and when the blackberry plants were in flower, on four blackberry flowers per station. To monitor for *Orius*, the pussy willow stem and strawberry/blackberry flowers were tapped over a

white plastic tray and any adults and nymphs recorded. Any *Orius* falling onto the tray were returned by tapping them back over the plants.

2. From 19 May when fruit were available on the strawberry banker plants, four white fruit per station were checked for *Orius* by examining the surface of the fruit and behind the calyx using a headband magnifier. Numbers of adults and nymphs per fruit were recorded.
3. Numbers of blackberry leaf midge eggs and larvae were assessed on 20 randomly sampled leaf tips from the tunnel where *Orius* were released and in an untreated tunnel of the same variety (Loch Ness) in Chivers field. Leaf tips were taken to the laboratory where they were examined under a binocular microscope. The leaf tips were checked for any *Orius* adults or larvae in addition to midge eggs and larvae.
4. Crop canopy air temperatures were recorded throughout the experimental period using two Tinytalk ® dataloggers hung from the wires so that the dataloggers were shaded by the blackberry foliage. A white paper cone was used as additional shade for each datalogger.

Results and Discussion

Orius establishment on banker plants and in blackberry flowers

Mean numbers of *Orius* adults and nymphs recorded on the banker plants on each assessment date are shown in Table 5. Although pussy willow is an early source of pollen, it was not considered to be a suitable banker plant for *O. laevigatus* as only one adult was recorded on the stems on one date, 28 April. The willow had stopped producing pollen by 19 May. *Orius* adults were found in the strawberry flowers from 21 April to 19 May, reaching a maximum mean of 0.3 adults per flower on 11 May (Table 5). *Orius* nymphs were found on strawberry fruits from 19 May when the first white fruit developed, reaching a maximum mean of 0.1 nymph per fruit on 2 June (Table 5). The presence of nymphs on the strawberry fruit demonstrated that the predators were breeding in the strawberries as only adult *Orius* had been released. Only one *Orius* adult (mean of 0.03 per assessed flower) was recorded in the blackberry flowers on 6 May, one week after the crop had started flowering. The experiment was finished on 16 June as it was concluded that although *Orius* was breeding to a limited extent on the strawberry banker plants, this had not helped them to establish on the blackberry crop. In Horticulture LINK project HL01107 (HDC project SF 120 - 'Biological, semiochemical and selective chemical management methods for insecticide resistant western flower thrips on protected strawberry'), ADAS research in 2010 demonstrated the potential of flowering alyssum as a suitable 'banker' plant to aid establishment of *O. laevigatus* in everbearer strawberry, for improved biological control of western flower thrips (Cross *et al* 2011). Further research in this HortLINK project could provide information to give an additional benefit to midge control in protected blackberry.

Table 5. Mean numbers of *O. laevigatus* adults and nymphs per willow stem, per strawberry flower and per white strawberry fruit between 14 April and 16 June (- not assessed due to lack of pussy willow pollen, blackberry flowers or white strawberry fruit).

Date	Mean number *Orius* per willow stem	Mean number *Orius* per strawberry flower	Mean number *Orius* per white strawberry fruit	Mean number *Orius* per blackberry flower
14 April	0	0	-	-
21 April	0	0.2 adults	-	-
28 April	1 adult	0.2 adults	-	0
6 May	0	0.1 adults	-	0.03 adults
11 May	0	0.3 adults	-	0
19 May	-	0.1 adults	0.03 nymphs	0
2 June	-	0	0.1 nymphs	0
16 June	-	0	0	0

Blackberry leaf midge infestation

Percentage leaf tips infested with blackberry leaf midge eggs and/or larvae and mean numbers of midge eggs and larvae per leaf tip are shown in Table 6. No *Orius* adults or nymphs were found in the leaf tips on any date. Mean numbers of leaf midge eggs and larvae per leaf tip and percentage of leaf tips infested were not lower in the *Orius* tunnel than in the untreated tunnel.

Table 6. Percentage leaf tips infested with blackberry leaf midge eggs and/or larvae and mean numbers of eggs and larvae per leaf tip in *Orius* tunnel and in untreated tunnel

Date	*Orius* tunnel		Untreated tunnel	
	% leaf tips infested	Mean no. eggs & larvae per tip	% leaf tips infested	Mean no. eggs & larvae per tip
28 April	0	0	0	0
5 May	5	0.4	0	0
12 May	15	0.8	15	0.4
19 May	0	1.5	5	0.1
2 June	25	0.3	0	0

Crop canopy temperatures

Mean, minimum and maximum crop canopy temperatures in the tunnel used for *Orius* release are shown in Figure 6. Although maximum day temperatures reached up to 34°C during April and May, minimum night temperatures were low (0.5 to 5°C) until the last week of April. Mean temperatures exceeded 15°C (the minimum temperature for *O. laevigatus* egg-laying) on only two dates (27 and 28 April) until 20 May. Mean temperatures did not reach 20-30°C (optimum temperatures for *O. laevigatus* egg-laying and population increase, Malais & Ravensberg 2003) until late June when the experiment was finished. Temperatures were thus too low for good *Orius* reproduction during April and May. Grower releases of *O. laevigatus* in late June led to good establishment on blackberry by early August (data not included in this report), indicating that the predator might play a useful role in maintaining low midge numbers once temperatures are suitable.

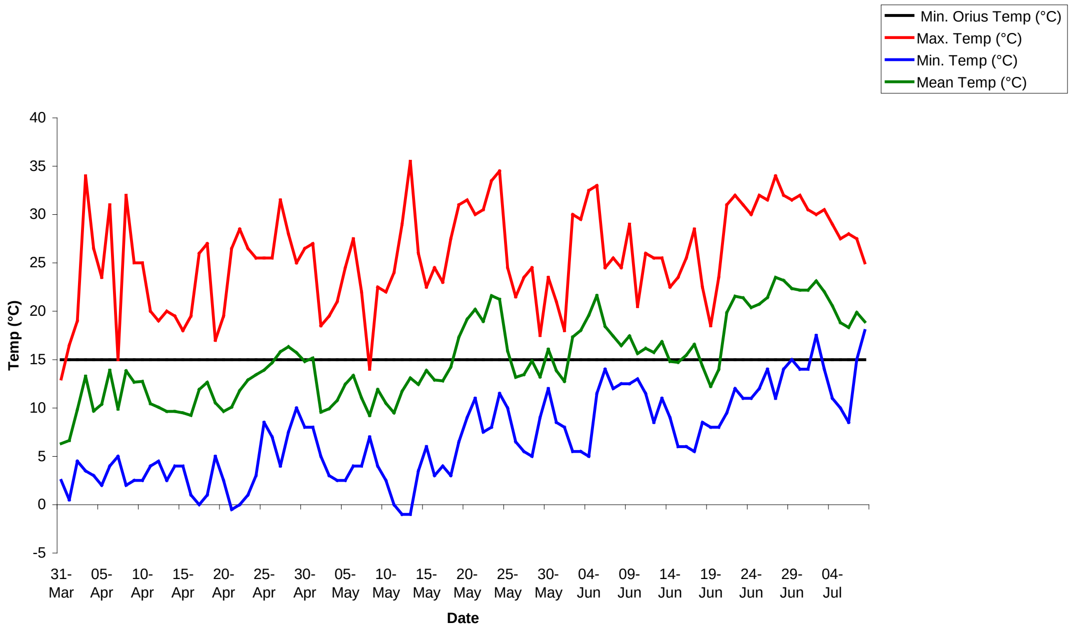

Figure 6. Mean, minimum and maximum crop canopy temperatures in the tunnel used for the experiment testing flowering banker plants for aiding early season establishment of *O. laevigatus*.

Cultural control with ground-cover materials

Materials and Methods

A similar laboratory experiment to that described earlier for ground-dwelling predatory mites was set up to test whether using ground-cover materials on the surface of the soil or compost could prevent successful pupation of blackberry leaf midge larvae dropping to the ground. Thirty replicate plastic plant pots were used for the experiment. These were half-filled with damp compost. Ten replicate pots were used for each of three treatments:

1. Untreated control (compost only)
2. Woven ground-cover matting (Mypex ®)
3. Black polythene

For treatments 2 and 3, the compost was covered with the material by using discs of polythene or Mypex slightly larger than the diameter of the pot and taping the material to the inner sides of the pots with duct tape, so that the material was flush with the surface of the compost. Eight large, fully grown midge larvae per pot were added to the surface of the compost or material. A strip of yellow sticky trap was secured with duct tape across the inside of each pot approximately one cm from the top and a square of insect-proof mesh was secured to the rim of each pot with a rubber band, to form a ventilated 'lid'. The pots were arranged in a randomised block design in large plastic trays lined with capillary matting. The pots were left in a controlled temperature room at 21°C and with a photoperiod of 16 hours light: eight hours dark for four weeks to allow the midge larvae to pupate and emerge as adults. The dishes were examined four weeks later on 7 September 2010 and numbers of midge adults per trap recorded. The data was subjected to analysis of variance.

Results and Discussion

The mean numbers of adult midges emerging per dish over the 4-week period are shown in Table 4 and Figure 6. Both Mypex ® and black polythene significantly reduced the numbers of midge larvae surviving to the adult stage when compared with the untreated compost (P<0.05). The reduction given by black polythene was not significantly better than that given by Mypex ®. Natural mortality of midges in the untreated compost during the pupation stage was similar in this experiment (74%) to that in the experiment described earlier with predatory mites.

It is possible that if the whole tunnel floor was covered in matting or polythene, and if this could be kept intact and free from plant debris, it might prevent adults successfully emerging from overwintered cocoons in the soil, or prevent first generation midge larvae successfully pupating and emerging as second generation adults. Further work would be needed to validate this potential cultural control method in a protected blackberry crop.

Table 4. Mean numbers of adult blackberry leaf midges emerging per pot of untreated compost and compost covered with Mypex ® or black polythene. * significantly fewer than in the control, P<0.05.

Treatment	Mean no. adult midges per dish	Mean % midge survival to adult stage
Untreated compost (control)	2.1	26%
Compost covered with Mypex ®	1.0 *	13%
Compost covered with black polythene	0.1 *	1%

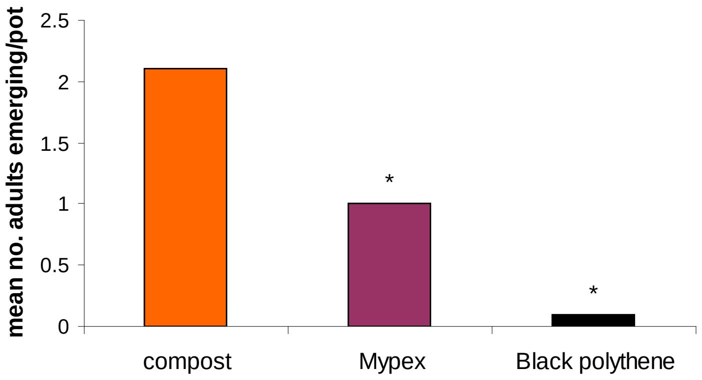

Figure 6. Mean numbers of adult blackberry leaf midges emerging per pot of untreated compost and compost covered with Mypex ® or black polythene. * significantly fewer than in the control, *P*<0.05.

Efficacy of coded pesticide

Materials and Methods

Three replicate rows of potted blackberry plants in a research poly tunnel at ADAS Boxworth were used for the experiment. The plants were infested with blackberry leaf midge.

Treatments

1. Untreated control
2. Coded pesticide with Codacide as adjuvant at 2.5 litres per ha.

Treatment 1 was applied on 23 July as a foliar spray to just before run-off and repeated after two weeks on 10 August.

Experimental design

Each of the three replicate rows was split into two plots, with the first plot being used for Treatment 1 and the second plot used for Treatment2.

Assessments

Seven days after each application, six replicate plants were assessed in each plot. Numbers of twisted leaf tips i.e. symptoms of leaf midge damage were recorded per plot. On each assessment date, up to 20 leaf tips (as available) were sampled and examined under a binocular microscope. Numbers of midge eggs and live and dead larvae were recorded.

Data analysis

The proportion of damaged leaf tips in the two treatments was compared using Regression Analysis and the mean numbers of midge eggs and larvae per leaf tip were compared using Analysis of Variance.

Results and Discussion

The coded pesticide did not reduce either the proportion of infested leaf tips or mean numbers of leaf midge eggs and larvae per leaf tip seven days after the first application (Table 5).

Table 5. Proportion of midge-damaged leaf tips and mean numbers of leaf midge eggs and larvae per leaf tip seven days after first application.

Treatment	Proportion of midge-damaged leaf tips	Mean numbers of midge eggs and larvae per leaf tip
Untreated	90%	8.9
Coded pesticide	81%	7.4

It was not possible to assess the effect of the second application as seven days after treatment there were insufficient leaf tips available on the plants to sample.

Summary of findings from work in Objective 2

The findings of work in both years of the project are summarised below:

Biological control agents

- In 2009, preliminary laboratory experiments indicated that both the predatory mites *Neoseiulus (Amblyseius cucumeris)* and *Amblyseius andersoni* predated young blackberry leaf midge larvae.
- In 2009, releases of both *N. cucumeris* and *A. andersoni* to blackberry using release sachets in research tunnels led to some establishment of *N. cucumeris* in flowers but no apparent establishment of *A. andersoni*. Following grower releases of *A. andersoni* to commercial protected blackberry, there was no apparent reduction in the pest when compared with untreated plants. In 2010, releases of the two species in sachets to blackberry in research tunnels indicated that *N. cucumeris* reduced the percentage of infested leaf tips whereas *A. andersoni* did not. Further work would be needed on a larger replicated scale to confirm the potential control given by each species.
- In 2009, in laboratory pot experiments, neither the predatory mites *Macrocheles robustulus* nor the predatory beetles *Atheta coriaria* gave significant reductions in numbers of midge larvae successfully completing their development in compost and emerging as adults. Results indicated that numbers of both predators may need to be equal to or higher than numbers of the target midge larvae in the soil to give significant control. In 2010, similar laboratory pot experiments compared the potential of *M. robustulus* with that of the predatory mite *Hypoaspis aculeifer*. Adding each predator in equal numbers to those of blackberry leaf midge larvae did not significantly reduce numbers of adult midges emerging. However, in both years, the reduction given by *M.*

robustulus was almost statistically significant, and further replication might give a significant result. This species is now commercially available and justifies further investigation should any future funding be available.

- In 2009, grower applications of *Beauveria bassiana* (Naturalis-L) to the commercial crop did not reduce the percentage of leaf tips infested or mean numbers of live larvae per leaf tip. *B. bassiana* is a contact-acting fungus and is unlikely to reach the target pest inside folded leaf tips. In a laboratory test, application of Naturalis-L to compost did not reduce numbers of blackberry leaf midge larvae successfully completing their development in the compost and emerging as adults.
- In 2009, naturally-occurring anthocorid bugs (both *Anthocoris nemorum* and *Orius* sp.) were observed feeding on blackberry leaf midge larvae in the commercial crop during July and August. Both adults and nymphs were recorded, which showed that the predatory bugs were breeding on the blackberry crop.
- *Orius laevigatus* are commercially available and work on its potential against the pest was done in 2010. In laboratory studies, adult *Orius* established and produced nymphs in blackberry flowers where only blackberry pollen was available as food. In predation studies, adult *Orius* ate at least five midge larvae per day.
- In 2010, early season releases of adult *O. laevigatus* were made to a commercial tunnel of protected blackberry in late March and early April. The crop was not yet in flower so flowering strawberry and pussy willow plants were tested as potential breeding 'banker' plants for *Orius*. A few *Orius* nymphs were produced on the strawberry banker plants but establishment was poor and *Orius* were not detected on the blackberry plants when they began flowering. This was probably due to temperatures being too low for good predator reproduction. However, grower releases of *Orius* in late June led to good establishment on blackberry by early August, indicating that *Orius* could play a useful role in maintaining low midge numbers once temperatures are suitable. In Horticulture LINK project HL01107, ADAS research in 2010 demonstrated the potential of flowering alyssum as a suitable 'banker' plant to aid establishment of *O. laevigatus* in everbearer strawberry, for improved biological control of western flower thrips. Further research in this HortLINK project could provide information to benefit midge control in protected blackberry.

Cultural control methods

- In 2010, a pot experiment testing potential cultural control of the pest using polythene or woven ground-cover matting over the soil or compost to inhibit successful pupation of larvae dropping to the ground showed a significant effect with both materials. Black polythene and Mypex ® reduced the numbers of midges emerging by 96% and 53% respectively compared with the compost control. It is possible that if the whole tunnel floor was covered in matting or polythene, and if this could be kept intact and free from plant debris, it might prevent adults successfully emerging from overwintered cocoons in the soil, or prevent first generation midge larvae successfully pupating and emerging as second generation adults. Further work would be needed to validate this potential cultural control method in a protected blackberry crop.

Pesticides

- In 2009, grower applications of thiacloprid (Agrovista Reggae, SOLA 0467/2008) and abamectin (Dynamec, SOLA 2290/2007) did not reduce numbers of live midge larvae per leaf tip or percentage of infested leaf tips when compared with untreated plants.
- In 2009, grower application of chlorpyrifos (Alpha chlorpyrifos, label recommendation for outdoor blackberry) to outdoor blackberry reduced numbers of live midge larvae per leaf tip by 87% and reduced the percentage of infested leaf tips by 92% when compared with those in untreated plants. By extrapolation, chlorpyrifos should give some control of the pest on raspberry and various chlorpyrifos products are approved for use on both outdoor and protected raspberry. However, chlorpyrifos is not approved for use on protected blackberry and is not compatible with biological control agents used in IPM.
- In 2010, application of a coded pesticide as a foliar spray to blackberries infested with leaf midge in research tunnels did not reduce the percentage damaged leaf tips or numbers of live midge eggs and larvae when compared with untreated control plants.

Conclusions from work in Objective 2

Conclusions from the results of work in both years of the project are given below:

Biological control agents

- Both of the predatory mites *Neoseiulus (Amblyseius cucumeris)* and *Amblyseius andersoni* predate young blackberry leaf midge larvae.
- *N. cucumeris* can reduce the percentage of infested leaf tips whereas *A. andersoni* appears less successful but further work would be needed on a larger replicated scale to confirm the potential control given by each species.
- Numbers of both the predatory mite *Macrocheles robustulus* and the predatory beetle *Atheta coriaria* may need to be equal to or higher than numbers of the target midge larvae in the soil to give significant control. *M. robustulus* is now commercially available and justifies further investigation.
- The predatory mite *Hypoaspis aculeifer* did not significantly reduce numbers of adult midges emerging.
- *Beauveria bassiana* (Naturalis-L) does not look promising as it did not reduce the percentage of leaf tips infested or mean numbers of live larvae per leaf tip nor reduce

numbers of blackberry leaf midge larvae successfully completing their development in the compost and emerging as adults.

- Naturally-occurring anthocorid bugs (both *Anthocoris nemorum* and *Orius* sp.) on the blackberry crop and feed on blackberry leaf midge larvae in the commercial crop during July and August.
- The commercially available predatory beetle *Orius laevigatus shows promise*. Adult *Orius* established and produced nymphs in blackberry flowers where only blackberry pollen was available as food. In predation studies, adult *Orius* ate at least five midge larvae per day.

Cultural control methods

- Black polythene or Mypex ® over the soil or compost show a significant effect of inhibiting successful pupation of larvae dropping to the ground. It is possible that if the whole tunnel floor was covered in matting or polythene, and if this could be kept intact and free from plant debris, it might prevent adults successfully emerging from overwintered cocoons in the soil, or prevent first generation midge larvae successfully pupating and emerging as second generation adults. Further work would be needed to validate this potential cultural control method in a protected blackberry crop.

Pesticides

- In 2009, grower applications of thiacloprid (Agrovista Reggae, SOLA 0467/2008) and abamectin (Dynamec, SOLA 2290/2007) did not reduce numbers of live midge larvae per leaf tip or percentage of infested leaf tips when compared with untreated plants.
- Chlorpyrifos (label recommendation for outdoor blackberry) appears to offer greater control than thiacloprid or abamectin. However, chlorpyrifos is not approved for use on protected blackberry and is not compatible with biological control agents used in IPM.

Technology Transfer

Outputs over the two years of the project:

- Project objectives presented to ADAS soft fruit growers' discussion group, Millets Farm Centre, Oxon, 23 March 2009
- Project objectives and images of pest provided to KG growers for their in-house training day, November 2009
- Update on project results to ADAS fruit consultants, EMR, 27 January 2010
- HDC News article March 2010
- Summary of project results presented at HDC/EMRA Soft fruit Day 24 Nov 2010
- Update on project results to ADAS fruit consultants, EMR, 26 January 2011

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- Belchim Crop Protection for the Naturalis-L
- Koppert UK for the *Macrocheles robustulus* and *Hypoaspis aculeifer*
- Syngenta Bioline for the *Neoseiulus (Amblyseius) cucumeris*, A*. andersoni* and *Orius laevigatus*.

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