Alternatives to Pesticides: Recent Advances in Fruit Crop Protection That Could Be Transferred to Ornamentals©

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INTRODUCTION

Research into alternatives to pesticides has been extensive over many decades, numerous and diverse alternative control methods have been developed and there is a vast literature. Biological control, including the use of introduced predators and parasites and the use of microbial biocontrol agents and nematodes, is perhaps the most widely exploited.

There are several well known and important established uses of introduced natural enemies to control pests of soft fruit crops in the U.K. — such as use of the predatory mite *Phytoseiulus persimilis* to control two spotted spider mite (*Tetranychus urticae*) and the predatory mite *Amblyseius cucumeris* to control strawberry mite (*Phytonemus pallidus* subsp. *fragariae*) and western flower thrips (*Frankliniella occidentalis*) — and the number and extent of use of these has increased in recent years in response to the increase in protected cropping, the development of pesticide resistance, loss of pesticides, and other factors.

There have been very few instances where natural enemies have been introduced to regulate an invasive pest in tree fruit crops. On apple, the best known is the introduction in the 1920s of the parasitoid *Aphelinus mali* to control woolly aphid, an invasive pest from America. *Aphelinus mali* is now an important natural enemy of woolly aphid present in most places where the pest occurs. It certainly greatly helps to regulate woolly aphid outbreaks, but is often not quite good enough on its own, requiring the assistance of earwigs. Inundative releases of arthropod predators or parasites as biocontrol agents to orchards are generally too costly and are often not successful because of climatic instability.

There are a small number of significant success stories of the use of microbial agents and nematodes for pest control in fruit growing. The widespread use of codling moth granulovirus is the most important. Formulations of the virus are approved in most European countries and are applied to the foliage as sprays. The virus is highly selective and virulent. In orchards, only codling moth can be infected. A single virus particle is sufficient to kill a first instar codling moth larva. The virus is safe to humans, plants, and the environment. It has to be ingested by the newly hatched larva when feeding on the skin of the apple before it penetrates the flesh.

Strains of the codling moth resistant to the virus have developed in some regions in continental Europe where the virus has been relied upon for control for many years. The problem has been overcome by using a different strain of the virus. However, this development highlights the need to use multiple suppressive control tactics to minimise the risk of resistance.

However, the subject is clearly too vast to cover in any depth here so for the purposes of this paper, I have concentrated on semiochemical-based control methods as these have been the subject of intensive ongoing collaborative research between East Malling Research (EMR) and the Chemical Ecology Group of the Natural Resources Institute (NRI) at the University of Greenwich. Semiochemicals are compounds such as pheromones which insects and other animals use to signal their presence to each other; and chemicals emitted by plants which influence the behaviour of specific insects and other animals.

SEMIOCHEMICAL-BASED CONTROL METHODS

Although numerous sex pheromones, aggregation pheromones and other semiochemicals of insect pests have been identified there have to date been relatively few commercially viable products and techniques to control only a limited number of pest species, mainly moths, though this number is now starting to increase.

There are three principle means of using sex pheromones, aggregation pheromones, and other semiochemicals for control of insect pests:

1) Mating disruption (MD)
2) Attract and kill (A&K)
3) Mass trapping (MT)

Semiochemical control methods usually have to be deployed on a large, preferably area-wide, scale and initial pest populations generally have to be low for control to be effective. Below are described two examples of ongoing work at EMR and NRI to exploit for control some of the non-lepidopteran semiochemicals we have discovered.

Mating Disruption and Attract and Kill. The use of sex pheromones for mating disruption or attract and kill are potentially very powerful tools in IPM. Most sex pheromones are produced by female insects and only attract males of the same species. The mating disruption (MD) technique is based on the premise that male insects are unable to locate females if the environment around the females is permeated with sex pheromone.

Three factors may act alone or in combination to produce mating disruption; sensory adaptation, habituation, and direct competition.

In theory, mating disruption may be accomplished in two principle ways: false trail following or confusion. False trail following results from placing many more point sources of pheromone per unit area than the anticipated numbers of females in the crop. The chances of males finding females at the end of the pheromone trail must therefore be greatly reduced. Males following these trails are thought to spend their mating energies in pursuit of artificial pheromone sources. In contrast, male confusion is thought to be the result of ambient pheromone concentrations sufficient to hide the trails of calling females (large doses from point or diffuse sources).

Attract and kill (A&K) is an extension of the false trail MD method, and is closely related to the mass trapping method described below. In false trail following, the males come in close vicinity of the artificial pheromone sources and may try to mate with them. In A&K formulations, the pheromone dispensers or the target devices on which they are held are coated with a contact insecticide, often formulated in a sticky carrier. The males become contaminated with insecticide and die. Females are thus less likely to encounter males by chance. However, in many cases the effectiveness and benefits of the added insecticide is largely unknown under field conditions.

A further combination of pheromones and insecticides is occasionally encountered. Dual applications of pheromone and insecticides (either separately or, in the case of sprayable pheromone formulations, in tank mixes) are applied, sometimes with the idea of increasing insect flight activity and thus increasing the chance of insecticide exposure.

The MD technique is widely used for control of a number of economically important lepidopteran pests including codling moth (*Cydia pomonella*), the oriental fruit moth (*C. molesta*) and the vine moth (*Eupoecilia ambiguella*). In comparison, there have been very few investigations of the use of sex pheromones of non-lepidopteran pests for MD or A&K. Our discovery of the sex pheromones of many of the midge pests of U.K. fruit crops has presented an important opportunity to attempt to exploit these for control. The midge pheromones were active at very low doses, several orders of magnitude lower than those of most Lepidoptera.

Experiments on Use of Pheromones in Orchards. We first attempted to exploit the female sex pheromone of apple leaf midge (*Dasineura mali*) for control in newly planted versus established apple orchards in 2004 and 2005 (Cross and Hall, 2007). This pheromone was present at less than 20 pg per female (Hall and Cross, 2006). The pheromone is extraordinarily active. Rubber septa lures containing 1 μg and emitting only a few pg of pheromone per hour are highly attractive. An experimental permit was obtained from the Pesticides Safety Directorate to conduct large-scale field trials of the pheromone without the need to destroy the crop from fruiting trees. By analogy with similar compounds, the sex pheromone was considered to be comparatively safe to humans and the environment but because no data was available, the experimental permit restricted the dose applied to 1 g per ha per season on the basis that the local concentration of the pheromone would not exceed the maximum concentration that occurs naturally.

In July 2004, a preliminary field experiment was done at EMR to investigate use of the apple leaf midge sex pheromone for control of apple leaf midge using an A&K strategy. The A&K devices were 20 cm × 20 cm squares of plastic laminated cardboard surface coated on both sides with a microencapsulated formulation of the insecticide lambda-cyhalothrin, developed for control of olive fly (AgriSense, BCS Ltd.). These were positioned 5 cm above ground level and baited with a rubber septum lure impregnated with 100 μg of apple leaf midge pheromone fixed centrally.

Four small heavily infested apple orchards at EMR were selected for the experiment. On 5 July, a single standard white delta trap baited with a standard 3-μg rubber-septum lure was deployed in the centre of each plot. Catches of males were counted on six occasions between 7–27 July 2004. On 7 July, 28 and 12 A&K devices respectively were deployed in lattices in two of the plots to give a density of approximately 100/ha.

On 27 July, 100-shoot terminals in the centre of each plot were examined for ovipositing females and presence of apple leaf midge galls.

The two orchards where the A&K treatment was deployed had considerably lower trap catches than the untreated plots, but the treatments failed to shut down catches completely. The shoots assessment on 27 July revealed that 100% of shoots were galled on three of the plots, with 84% galled on the other. Small numbers of ovipositing females were also recorded. The results showed the treatment was not able to adequately suppress mating and that a much higher dosage of pheromone would be required, probably with many more devices per ha.

Bioassays of the effect of contact of apple leaf midge adult males with the lambda-cyhalothrin target devices were conducted on 1 and 2 Sept. 2004. The A&K devices were observed in the field and at intervals. Ten attracted male midges were taken from the surface of the device or shortly after they had made contact, and held in tubes. Midges were taken in a similar way from the surface of a similar device not treated with lambda-cyhalothrin. After 1 h, all the midges that had been exposed to the lambda-cyhalothrin card, even for 5 min, were severely affected by the insecticide. After 3 h, all were dead. Similar results were obtained with cards from A&K devices that had previously been exposed for 2 months in the field.

A very large-scale field trial was carried out in commercial apple orchards in Kent during 2005 to evaluate the use of the apple leaf midge sex pheromone for control of apple leaf midge by MD or A&K approaches. The MD devices were polythene caps each initially loaded with 500 µg of the apple-leaf-midge sex pheromone. These caps each released the pheromone at approximately 10 ng/h at 27 °C. The A&K target devices were 10 cm × 6.7 cm oblongs of the microencapsulated lambda cyhalothrin surface treated cardboard with a polythene cap lure containing 100 µg of the apple leaf midge sex pheromone fixed to the centre with a drawing pin. These caps each released the pheromone at approximately 2 ng/h at 27 °C. Both MD and A&K devices were deployed at 500 devices/ha or 2000 devices/ha, fixed to tree stakes so that the lure was at a height of approximately 15 cm above the ground in a regularly spaced lattice.

Thus for the MD treatments, pheromone application rates were 0.25 $g \cdot ha^{-1}$ or 1 $g \cdot ha^{-1}$ respectively and release rates were approximately 5 $\mu g \cdot ha^{-1}$ per h and 20 $\mu g \cdot ha^{-1}$ per h. For the A&K treatments, pheromone application rates were 0.05 $g \cdot ha^{-1}$ or 0.2 $g \cdot ha^{-1}$ respectively and release rates were approximately 1 $\mu g \cdot ha^{-1}$ per hour and 4 $\mu g \cdot ha^{-1}$ per hour.

A fully randomised experimental design was used with six replicate 1-ha plots of each treatment, requiring 30 plots of 1 ha in 11 orchards on six different fruit farms in Kent. Three of the plots for each treatment were in newly planted orchards where leaf midge populations were low and three were in established orchards where leaf midge populations were high. Untreated plots were well separated from those which had MD or A&K treatments which themselves were adjacent. The effectiveness of the treatments was assessed by weekly monitoring of catches of adult male midges in a delta trap baited with a 3-µg rubber-septum lure in the centre of each plot and by counting the number of galls present in 200 shoots in the centre of each plot for each of the three main generations, at the peak of damage expression on 17–23 May, 20–25 June, 4–6 July, and 30–31 August 2005.

The only differences of significance were whether or not any "treatment" had been applied, with no differences between type and number of lures. All the MD and A&K treatments suppressed the catches of males in the traps in the centres of the plots compared to the untreated control. In the established orchards with higher populations, catches were decreased by >98% in April–May, by >99% in June–July, but by only >91% in August–September. In the newly planted orchards with very low populations of the midge, trap catches were zero in April–May in all plots, were very low but suppressed by 90% by the MD and A&K treatments in June–July, but rose somewhat in August September being suppressed by about 80% in the treated plots.

There was no evidence that either the MD or A&K treatments were suppressing numbers of galls in shoots in the established orchards. In the newly planted orchards, it appeared that the MD and A&K treatments were failing in July and certainly by August, no suppression of galling damage was evident.

Measurement of release of pheromone from the open polyethylene caps used in these trials for both MD and A&K treatments showed relatively uniform release for at least 270 days under laboratory conditions. However, lures recovered from the field at the end of the above experiments were found to contain no detectable pheromone.

This has subsequently been shown to be due to degradation of the pheromone, the lures being unprotected from direct sunlight in both MD and A&K treatments.

Why did these first attempts at exploiting the pheromone fail? In the Cecidomyiidae, unmated females are evidently not able to lay viable eggs (Gagne, 1984; 1989) though monogeny often occurs in some species (Murchie and Hume, 2003). The bioassays indicated the A&K devices did kill the males, albeit only after a few hours. Males are known to be able to mate several times, though females only need to be mated once to be fertilised. Clearly, sufficient mating was still occurring to provide enough fertilised females to oviposit in the shoots that were present.

There are several possible explanations for the failure:

- The dose (1 $g \cdot ha^{-1}$) was too small
- An insufficient number of dispensers/devices was deployed per ha
- There was rapid UV degradation of the pheromone
- The 1-ha scale of deployment of treatments was insufficient and there was ingress of mated females into the plots from the edges
- The initial populations of the midge were too high to prevent some mating from occurring

Experiments on Use of Pheromones in Raspberry Plantations. Unfortunately, our funding for working on exploiting the apple-leaf-midge sex pheromone was not continued to allow us to investigate further, but, as part of Horticulture LINK project HL0175, we did start work on exploiting for control the raspberry cane-midge (*Resseliella theobaldi*) sex pheromone, which we had already identified (Hall et al., 2009).

To date we have conducted 4 years of field trials testing a wide range of formulations of the raspberry cane-midge pheromone. At the outset, we determined the effect of the release rate of pheromone from rubber septum lures on attractiveness to male raspberry cane midge. The results were surprising.

Lures which released 600 pg of pheromone/h (in the NRI laboratory wind tunnel at 27 °C and 8 km/h wind speed) and initially loaded with 0.1 µg of the pheromone racemate were significantly attractive. Maximum attraction occurred at 600 ng/h though 60 ng/h (initial loading 10 µg) performed nearly as well. Attraction was significantly reduced at greater release rates. Two alternative control strategies were apparent:

1) MD or A&K using false trail following using large numbers of devices with release rates of ~60 ng/h.
2) Male confusion using high ambient pheromone concentrations sufficient to hide the trails of calling females using smaller numbers of devices with high release rates (>6 µg/h).

Each year for 4 years to date, large-scale (~1-ha plots) field experiments have been done in commercial raspberry plantations in southeast and east England to evaluate the efficacy of MD and A&K treatments in comparison with an untreated control for control of raspberry cane midge.

In 2006, we evaluated a MD treatment with 2,000 polythene cap dispensers per ha, each initially loaded with 1 mg of raspberry cane-midge sex pheromone racemate, deployed in a regular lattice through the crop at a height of 15 cm. The A&K treatment tested comprised 2,000 plastic laminated cards surface coated with a microencapsulated formulation of the SP insecticide lambda-cyhalothrin and which had a polythene cap dispenser (initially loaded with 100 µg of the pheromone) fixed to the centre per ha.

The MD and A&K treatments were effective outdoors where a high degree of trap suppression was achieved but were ineffective in the polytunnel crops where trap suppression was less effective. Possible explanations for this difference in efficacy are that pheromone release was too rapid from the dispensers when they were deployed in the polytunnels where temperatures were much higher than outdoors and/or that the pheromone did not disperse so effectively in the enclosed polytunnel environment.

In 2007, we tested 200 polythene sachet MD devices and 200 mass trapping (MT) devices per ha. The sachets were each initially loaded with 50 mg of the midge sex pheromone racemate released at rates of approximately 0.5 mg/d at 28 °C. The MT devices were Lynfield traps each baited with a rubber septa lure initially loaded with 200 µg of the pheromone racemate and released 60 ng pheromone/h at 28 °C.

The traps contained 50 ml of water + 50% glycol, and were suspended at a height of 15 cm from the ground. The MT rather than A&K devices were used because they gave records of whether or not attracted midges were killed (by drowning). Although the MD treatment gave fairly good suppression of trap catches the MT treatment was less effective in this respect and neither treatment prevented larval attack in artificial splits in the canes. It was concluded that 200 devices per ha was probably too small a number.

In 2008, a new MD treatment comprising 3 $kg \cdot ha^{-1}$ of 0.5% w/w pheromone racemate EVA granules (~150,000 granules/ha) broadcast by hand to the soil surface between the rows was tested in comparison an A&K treatment comprising 2,000 plastic laminated lambda cyhalothrin cards (similar to those tested in 2006) each with a rubber septum dispenser loaded with 200 µg of the pheromone racemate and an untreated control.

Although both the MD and A&K treatments failed at one site, very good control was achieved at another and intermediate results at a third. The reasons for the different results at the different sites are unclear. There was evidence that the MD treatment was losing its efficacy as the season progressed with better results for the first generation pests. Lab measurements of release rate indicated that the EVA granules used for the MD treatment released 60% of their pheromone in the first 31 days at 27 °C. One explanation of the decline in trap-catch reduction may be that the pheromone release rate from the EVA granules declined steeply through the season. Another possible explanation is that the granules progressively worked themselves into the soil surface, some being trampled by pickers as they walked through the tunnels. The trap-catch reductions achieved by the A&K treatments remained consistent through the season

The trial MD/A&K/MT formulations were either impractical for use by growers or were ineffective. In 2009, we developed and tested a new *R. theobaldi* sex pheromone MD/A&K method which we thought would be practical for use by growers, would not get lost in soil, and would have a large number of pheromone sources per ha so having a better chance of success. We also used the method in conjunction with a directed spray of a contact acting insecticide, deltamethrin. The aim was to use a competitive MD approach to attract the male midges with the sex pheromone to numerous artificial pheromone sources where they would then be killed by a surface deposit of insecticide, to be applied subsequently. The insecticide chosen was deltamethrin (Decis®), a product already approved for use in raspberry. It is a light stable synthetic pyrethroid with excellent knock down properties and good persistence. It was used as a separate spray to avoid registration difficulties.

A new "SPLAT" formulation of the cane midge pheromone was produced in the laboratory at NRI by mixing 4 g of the raspberry cane midge sex pheromone racemate per kg of SPLAT-base formulation supplied by ISCA technologies, California. The formulation was then transferred to caulking guns. The integrated pest and disease management (IPDM) plots were then treated with 2.5 kg of SPLAT containing 10 g of raspberry cane-midge pheromone racemate per ha. The SPLAT was applied in 5,000 0.5 g, 7-cm-long strings per ha to the polythene mulch or lay flat polythene irrigation pipe. Depending on the row spacing, approximately one SPLAT string was dispensed per metre of row. One to three days after SPLAT application, Decis was applied by the grower to the polythene at 600 ml of product in 200 L of water per ha to polythene mulch on which SPLAT has been applied.

Regrettably, the SPLAT plus deltamethrin treatment was unsuccessful in suppressing pheromone trap catches of males and at the one site where they were found, the treatment was ineffective in controlling larvae.

The weekly pheromone trap catches did show that the SPLAT formulation gave a very high degree of trap shut down for the first 2–3 weeks, but thereafter the suppression of catches declined sharply. Measurements of the amount of pheromone remaining in the formulation also showed a sharp decline in the first few weeks, which may be at least in part the explanation for the poor results.

So to date, despite much effort, we have not managed to produce a successful formulation for exploiting the *R. theobaldi* sex pheromone for control. The reasons for this are unclear but appear to be a combination of an inadequate sustained release rate and deployment where populations are too high initially. In 2010, we hope to test the SPLAT formulation again but at higher doses and possibly with repeated applications, and in fields with low to moderate *R. theobaldi* populations. We will devote more attention to behavioural observations of the effect of the formulation on the midges.

Mass Trapping. In MT, traps are deployed with the objective of destroying insects for population control. As stated above, most sex pheromones are female produced and attract males only. For mass trapping to be effective using female produced sex pheromones, enough traps have to be deployed to catch enough males to leave the females of the species without mates. But it is difficult to get good results because any untrapped males simply mate more frequently. Large numbers of traps ($>50 \cdot ha^{-1}$) are likely to be too costly.

However, aggregation pheromones often attract both sexes and are therefore better suited for MT applications. The strawberry blossom weevil (*Anthonomus rubi*) aggregation pheromone is male produced and attracts both males and females. We evaluated the aggregation pheromone in sticky stake traps for MT of *A. rubi* but achieved poor results (Cross et al., 2006b). Importantly, its attractiveness can be greatly enhanced by addition of host volatiles, especially the host volatile from wild strawberry flowers. Furthermore, bucket traps with white cross vanes are far superior to sticky stake traps used in our previous work and the combination of these factors has enabled us to develop a supertrap for this pest.

In 2010, we will start large-scale field trials to evaluate the use of this supertrap for control of *A. rubi* by MT.

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