Pheromones for Monitoring and Control of Pests of Woody Ornamentals©

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This paper presents and overview of recent collaborative work by East Malling Research and the Natural Resources Institute, U.K., to identify and exploit the sex pheromones and other semiochemicals ("signalling" substances) of important non-lepidopteran fruit pests. Though perhaps the majority of insect pests rely on sex pheromones and other semiochemicals to mediate reproduction, to date only the sex pheromones of lepidopteran (caterpillar) pests have been exploited to a significant extent in horticulture in Europe and even for these, exploitation is limited. The sex pheromones of many important non-lepidopteran pests were known to exist and pheromones of related species which were pests in other crops in other continents had already been identified. In some cases, it has been possible to use pheromones in integrated pest management by using them alongside volatile host plant compounds.

The first step in development of semiochemical monitoring systems for gall midge, capsid bug, and blossom weevil pests of fruit crops was to identify and synthesise the attractants and demonstrate their behavioural activity. Further work was required to develop practical lures and traps and to explore their use in pest management. Highly sensitive, pest-specific monitoring traps have been developed which are proving valuable for pest monitoring in fruit crops. For some pests, precision monitoring has allowed the local application of targeted sprays resulting in longer term reduction in pest populations. Pheromones of two species have been exploited for control through mating disruption, mass trapping or attract and kill. Opportunities for similar approaches in woody ornamentals are considered.

INTRODUCTION

Though many insect pests rely on sex pheromones and other semiochemicals ("signalling" chemicals) to mediate reproduction and aggregation, to date it is mainly the sex pheromones of lepidopteran pests that have been exploited to a significant extent in horticulture in Europe and, even for these, exploitation is limited. The two primary uses of insect pheromones are for detection and monitoring of populations and for control by mating disruption, attract and kill, or mass trapping.

Sex pheromone traps provide a quick, easy, pest-specific and sensitive way to monitor pest populations and are valuable tools for deciding if and when to treat – for example with pesticide sprays – for rational and effective pest management. They are preferable to population surveys by approaches such as beat sampling or visual inspection as these are time-consuming and therefore costly. However, most traps provide only relative population estimates and are prone to changes in environmental conditions, with the consequence that the data collected on different occasions may not be strictly comparable. This major drawback can be minimised by operating the traps over extended periods to provide total, integrated catches over extended periods of several days, a week, or longer periods.

Sex pheromone catches of males may not accurately reflect female or reproductive activity. The sex pheromones of practically every significant lepidopteran horticultural pest have already been identified and are commercially available. Sex pheromone traps have been widely used for monitoring moth pests in orchards, especially of codling moth (*Cydia pomonella*), fruit tree tortrix moth (*Archips podana*), and summer fruit tortrix moth (*Adoxophyes orana*) since the 1960s. Sex pheromone traps are also available for several important pests of woody ornamentals including carnation tortrix (*Cacoecimorpha pronubana*) and light brown apple moth (*Epiphyas postvittana*), a new invasive species now widespread on woody ornamentals in the U.K. and likely to spread throughout Europe. However, these traps do not appear to be widely used.

METHODS OF USING PHEROMONES AND OTHER SEMIOCHEMICALS IN INTEGRATED PEST MANAGEMENT

Although numerous sex pheromones and other semiochemicals of insect pests have been identified there have so far been relatively few commercially viable products and techniques to control only a limited number of pest species, mainly moths. This is in part due to problems with the technology, but also to the hurdles of product registration. However, these difficulties are now being overcome. The three principle means of using sex pheromones and other semiochemicals for control of insect pests are mating disruption (MD), attract & kill (A&K), and 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.

The use of sex pheromones for mating disruption or attract and kill are potentially very powerful tools in integrated pest management (IPM) (Witzgall et al., 2010). Most sex pheromones are produced by female insects and only attract males of the same species. The 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. In theory, MD 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. In contrast, male confusion is thought to be the result of ambient concentrations of the synthetic pheromone being sufficient to hide the trails of calling females and requires large doses from point or diffuse sources.

Attract & kill, and MT, are extensions of the false trail MD method (El-Sayed et al., 2006). The males come in close vicinity of the artificial pheromone sources and may try to mate with them. In MT they are trapped and in A&K the pheromone dispensers or the target devices on which they are held are coated with a contact insecticide so that the males become contaminated with insecticide and die. However, in many cases the effectiveness and benefits of the added insecticide are largely unknown under field conditions.

Volatile compounds produced by the host plant may be detected by female insects and used to find a host plant for egg laying after mating. In some cases it has proved possible to use host volatiles to attract females for monitoring purposes, though a major limitation is that the host volatile attractants have to compete with large amounts of the same substances produced by the host crop. However, in some insects the activity of pheromones is greatly enhanced by host volatiles.

A close collaboration between horticultural entomologists at East Malling Research and chemical ecologists at the Natural Resources Institute, University of Greenwich began in 1987 with the primary aim of identifying and exploiting the sex pheromones and other semiochemicals of U.K. non-lepidopteran fruit pests. The sex pheromones of many of these important pests were known to exist and pheromones of related species, which were pests in other crops in other continents, had already been identified. Finding ways of using the pheromones alongside host plant volatiles was an important quest. This collaboration led to the development of semiochemical monitoring systems for strawberry blossom weevil (*Anthonomus rubi*), gall midges, and capsid bugs.

In the next sections we briefly review our recent work and speculate how similar approaches might be helpful for developing new semiochemical-based management methods for pests of hardy ornamentals so that reliance on routine use of pesticides can be reduced. There is considerable scope. Common and damaging pest species with a wide host range are the obvious first targets for use of pheromones in management.

GALL MIDGES

Many horticultural crops, especially perennial fruit and ornamental crops, are subject to pest attack by various species of plant feeding gall midges (*Cecidomyiidae*). Many species attack and kill growing points, stunting growth and causing branch proliferation. Many have more than one generation per year. Plant-feeding gall midges are typically very short-lived as adults and highly specific for their host crop. They generally pupate in the soil under plants they infested previously. Many fruit pest species can be controlled with chlorpyrifos or synthetic pyrethroid insecticides but these do not work well unless they are timed to coincide with the start of attacks, before larvae and galls develop. For other species, such as the apple and pear leaf midges, there are currently no effective control methods and they cause extensive damage, especially in nurseries and young orchards. Recent work has shown that the novel insecticide spirotetramat can give effective control of midge larvae inside galls, though this insecticide is not yet available commercially for use in fruit crops. In any event, methods for deciding when and where to spray are needed.

The female sex pheromones of some 16 gall midge species have been identified worldwide to date (Hall et al., 2012). These include those of most of the major gall midge pests of fruit crops in the U.K. which have been identified by EMR and NRI over the last decade including apple leaf midge, pear midge, pear leaf midge, raspberry cane midge, blackcurrant leaf midge and blackberry leaf midge. We have also identified the pheromone of one species that is an important pest of an ornamental tree species, the honey locust gall midge (*Dasineura gleditchiae*) which causes disfiguring red galls on the foliage of the honey locust, *Gleditsia tricanthos*. We are currently in the process of identifying the blueberry gall midge sex pheromone.

The chemical structures of gall midge sex pheromones are related to each other. In several cases identification of the fruit gall midge pheromones was extremely challenging due to the very small amounts of chemical involved and the difficulties of carrying out laboratory bioassays with the small and delicate insects. The gall midge sex pheromones are highly attractive to males and are species-specific. Pheromone traps for many of these species are now commercially available and some are widely used in commercial fruit growing in the U.K.

Strong relationships between trap catch and subsequent numbers of galls and/or larvae have been demonstrated for both of the two fruit pest species where these relationships have been investigated, the apple leaf midge and the raspberry cane midge (Cross et al., 2008, 2009), demonstrating that for these species at least, pheromone trap catches can be used to estimate pest populations in a crop.

Our work on several fruit pests has demonstrated that sex pheromone traps are effective for monitoring the flight activity of successive generations of the target species. We have demonstrated that they are useful and effective for timing insecticide sprays. Control of early generations prevents or reduces damage later.

There have been a few attempts to exploit gall midge sex pheromones for control (Hall et al., 2012). Attempts to control apple leaf midge failed but trials with raspberry cane midge gave mixed results, some treatments working well in some trials but failing in others. Competitive attraction MD or A&K, using large numbers of sources with low release rates, appeared to be the most promising approach, but many of the formulations evaluated were impractical for use by growers. A commercially available polymer emulsion, known as “SPLAT”, applied at 5,000 drops per ha, each drop of 0.5 g, gave good results in crops in polythene tunnels in which the initial populations of midge were low. Adjustment of the formulation is needed to sustain an adequate release rate.

There are many species of gall midge that attack hardy ornamental plants. Many are only important on more mature plants after planting out, though several cause significant economic damage in the nursery production stage. Apple and pear leaf midges (*Dasineura mali* and *D. pyri*) are important pests of ornamental *Malus* and *Pyrus*, respectively, in nurseries. The existing sex pheromone traps for these pests are excellent for timing sprays. *Dasineura thomasiana* is a serious pest of young *Tilia* trees on nurseries (H. Helsen, pers. commun.). In past years, growers have been spraying many times per season with pyrethroids and neonicotinoids (up to eight applications per season in some cases), with limited results. Other common gall midge species which attack growing shoots include *Contarinia fagi*, *Arnoldiola quercus*, *Dasineura crataegi*, *Rhabdophaga terminalis*, and *Dasineura irregularis* (*D. acercrispans*) which attack the growing shoots of *Fagus*, *Quercus*, *Crataegus*, *Salix*, and *Acer*, respectively.

One of the most important gall midge pests of ornamentals is the hemerocallis gall midge (*Contarinia quinquenotata*). This affects the developing flower buds and stops them opening. It was new to Britain in the 1980s but is now widespread and a serious pest in gardens, ruining this commonly-grown, attractive garden plant. The honeylocust gall midge (*Dasineura gleditchiae*) which converts the leaflets into pod-like galls is also a disfiguring pest. The sex pheromone of this species has already been identified but is not yet commercially available. The violet leaf midge (*D. affinis*) causes abnormally swollen new leaves that fail to unfurl. Robinia gall midge (*Obolodiplosis robiniae*), a recent arrival in U.K., is spreading rapidly and causes a leaf fold gall on the leaf margins. Aquilegia gall midge (*Macrolabis aquilegiae*) larvae feed in the flower buds, causing affected blooms to be small and disfigured. Another midge that sometimes causes noticeable galling is the yew gall midge (*Taxomyia taxi*).

CAPSID BUGS

Capsid bugs (*Heteroptera*, *Miridae*, *Mirinae*) are common and important pests of many horticultural and some agricultural crops worldwide. Three of the most important species in Europe are the European tarnished plant bug (*Lygus rugulipennis*), the common green capsid (*Lygocoris pabulinus*), and the nettle capsid (*Liocoris tripustulatus*). Feeding by capsid bugs in growing points, flowers, or fruits of the wide range of crops they variously attack causes severe damage and crop loss. Capsid bugs can be readily controlled by sprays of broad-spectrum insecticides such as synthetic pyrethroids or chlorpyrifos, but these pesticides are incompatible with biocontrol and IPM and a reduction in their use is causing a resurgence of these pests. Sampling methods for them (sweep-net or beating-tray sampling) are time consuming and unsuitable for use by growers.

Several species of capsids have been shown to produce sex pheromones. During more than 30 years of work in the USA and Canada on the sex pheromones of *L. lineolaris* (Gueldner and Parrot, 1978; Aldrich, 1988; Wardle et al., 2003) and *L. hesperus* (Ho and Millar, 2002), several potential pheromone components were identified but attraction to a synthetic lure was never shown. Sex pheromone identification in capsid and related bugs has been hampered by the abundant defensive secretions, present in the metathoracic scent gland, that are released upon disturbance (Aldrich, 1988). Furthermore, in many species it is probable that certain compounds can function as components both of the pheromone and of defensive secretions, depending upon the blend and concentration (cf. Blum, 1981, 1996).

Work by EMR and NRI to identify and exploit the sex pheromones of capsid bugs started with *L. rugulipennis* in 1997 (Cross and Hall, 2003). Three female-specific pheromone components were identified and synthesised. However, in initial field trials, traps baited with blends of these chemicals dispensed from standard pheromone dispensers failed to catch significant numbers of males (Innocenzi et al., 2004). Intensive investigations in The Netherlands between 1996 and 2004, to identify the sex pheromone of *L. pabulinus* showed both female and male bugs produced the same compounds which caused responses in males but not females (Drijfhout et al., 2000, 2002; Drifout, 2001; Groot, 2000). We made an important breakthrough in subsequent field trials in which the chemicals were released from glass micro-capillary tubes, as reported by Kakizaki and Sugie (2001) for another insect pheromone. Different blends of the components were found to attract *L. rugulipennis* and its congener *L. pratensis*. This was the first time a *Lygus* bug pheromone had been identified and attraction in the field demonstrated

(Innocenzi et al., 2005). However, the reason why the pheromone blends are attractive when dispensed from glass micro-capillary was not known – for example it may have been that release rate and/or release from a small point source was critical.

Subsequently we made collections of volatile substances from females at different times of day and showed that single females of four different species (*L. rugulipennis*, *L. pratensis*, *L. pabulinus*, and *L. tripustulatus*) all produce different ratios of the same three compounds. The ratios were very different from those we obtained in our original work and which proved unattractive. It appears that at times of day when they are not "calling," and when females are in groups, they produce the same compounds for defensive purposes in different amounts and ratios from the ratios used for sexual attraction. It also appears that dead and dying insects produce large amounts of these compounds. These factors distorted the ratios in our collections from those that are used by the insects for sexual attraction and once we realised this it allowed us to overcome the problem and determine the correct ratios for sexual attraction. We have good evidence that species specificity of sex attraction for this group of insects is based on different ratios of these three compounds, plus other factors such as time of day of attraction and possibly host plant.

A further important advance has been the development of artificial point source lures which can release the required correct blends of the three components at a steady rate over long periods. Adjusting concentrations of the three components to get the desired release rates and ratios has been challenging, especially as one of them decomposes rapidly in daylight. The point source lures are each made from a polypropylene pipette tip containing a cigarette filter loaded with a blend of synthetic chemicals in sunflower oil and sealed at the wide end with a Teflon®-lined metal crimp seal. The pipette tip lures have proved much more attractive than the glass micro-capillary lures and are now commercially available for use by growers.

Another crucial advance has been the development of effective practical trap designs. We have shown that bucket traps with green cross vanes give vastly improved catches of *L. rugulipennis* compared to delta traps or other designs but they are totally ineffective for *L. pabulinus* for which sticky traps are most effective. Suitable traps designs are commercially available.

The common green capsid (*L. pabulinus*) is a common and often abundant pest of trees, shrubs, and herbaceous plants, attacking the foliage and tips of new shoots causing unsightly distortion and sometimes stopping growth. The European tarnished plant bug is a pest of many annual and herbaceous subjects and is especially troublesome under protection. The pheromone traps developed for these pests in fruit could be useful in ornamentals.

WEEVILS

The pheromones of many curculionid species had been identified, and several found to be useful in monitoring and control (Bartelt, 1999). We identified the components of the male-produced aggregation pheromone of the strawberry blossom weevil, *Anthonomus rubi*, as grandlure I, grandlure II, and lavandulol (Innocenzi et al., 2001). "Grandlures I-IV" are the four components of the aggregation pheromone lure of the closely-related cotton boll weevil, *Anthonomus grandis*. In *A. rubi*, the components occurred naturally in the ratio 1:4:1, respectively, and the blend of synthetic compounds attracted both male and female weevils to traps in the field. Germacrene-D, a known volatile from strawberry plants, was also collected in increased amounts in the presence of pheromone-producing weevils.

Innocenzi et al. (2001) used a simple prototype trap design for initial field evaluation and optimisation of the pheromone lure. This consisted of a horizontal white plastic board coated with adhesive on both sides and fixed to the top of a wooden stake. The lure was held on top in a small cage made from a hair curler. Although this first prototype proved satisfactory for the purposes of initial testing, no other work had been done to test alternative trap designs or improve on the prototype design. In subsequent collaborative research the first pheromone lure and trap were developed (Cross et al., 2006a,b). Low-cost, robust, and reliable polyethylene sachet dispensers containing 100 mg of a blend of grandlures I, II, and lavandulol in the naturally occurring 1:4:1 ratio were shown to have constant release rates and a life of more than 8 weeks in the field. Although germacrene-D slightly improved the effectiveness of the mixture when added to the pheromone components, inclusion in a commercial lure was uneconomic. A sticky stake trap design was used initially and the lure and trap was calibrated for pest monitoring. The lure and trap was commercialised in 2006.

A new collaboration with Atle Wibe, Bioforsk, Norway, led to two important new developments. Investigations by Anna-Karin Borg-Carlson, Group of Ecological Chemistry, Royal Institute of Technology KTH, Stockholm, had identified a number of host volatile substances produced by strawberry including one which was produced in large amounts by wild strawberry flowers. Field experiments in Norway in 2007 evaluated the use of this host volatile to improve effectiveness of traps using the *A rubi* pheromone. Fortuitously, white cross-vane green bucket traps, which were under development for monitoring raspberry beetle (*Byturus tormentosus*), by Agrisense BCS and the James Hutton Institute, were used. The combination of the *A. rubi* pheromone, the wild strawberry flower host volatile and the attractive trap design proved to be a winner, a kind of "supertrap." In one of the first field tests in Norway the new traps caught more than 200 weevils on average, far more than had ever been obtained with the pheromone sachet lures and sticky stake traps. The high attractiveness of the trap to both males and females has made it more likely that it can be exploited for control of the pest by MT and this is currently being investigated in a new EU-funded project "Softpest Multitrap".

Vine weevil (*Otiorhynchus sulcatus*) is by far the most important "non-crop-specific" pest of ornamentals. As this species consists only of reproducing females, no males, a sex pheromone is unlikely to exist. The weevil is known to aggregate, but it has not been demonstrated that this is pheromone-mediated. There have been a number of unfruitful attempts to identify aggregation pheromones of this species and new attempts are currently in progress. Recently, Van Tol et al. (2012) have demonstrated that volatile substances produced by host plants attract the weevils. These may be exploited for use for pest monitoring and/or control in future.

Several other *Otiorhynchus* species are serious pests of woody ornamental plants occasionally. Some reproduce sexually but many are parthogenetic, like vine weevils. Semiochemcial attractants for these have yet to be investigated. Leaf weevils (*Phyllobius* and *Polydrusus* spp.) are very common and moderately important pests of a wide range of woody ornamentals, adults causing marginal leaf notching on a wide range of subjects, especially trees. Semiochemical attractants for these have not been investigated though, interestingly, the adults of one species are reported to be caught incidentally in sex pheromone traps for another weevil pest. Clearly, there is considerable scope for developing and exploiting new attractants for pests of woody ornamental plants, a fertile field for future research.

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