PHEROMONES, SEX ATTRACTANTS AND KAIROMONES IN WEED AND INSECT BIOLOGICAL CONTROL: AN EMERGING FRONTIER OF TOOLS TO MANAGE RISK AND REWARD

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ABSTRACT.

Pheromone traps can help to determine the establishment and population size of new pest and beneficial organisms, as well their phenology, synchrony with their host, dispersal, and rate of spread. The application of lures for biocontrol agents introduced against either insects or weeds is emerging as an expanding use pattern for semiochemicals. The kairomonal trapping of biocontrol agents including parasitoids and predators of scale insects represent the earliest examples of this new application for studying population trends. The deliberate development of a sex attractant for gorse pod moth (*Cydia succedana*) was probably the first example of the use of attractants in weed biocontrol, although there have now been several others developed and used after release of new organisms. After the lure was identified in Hawaii, trapping showed that the gorse (*Ulex europaeus*) biocontrol agent *Agonopterix umbellana* was established in New Zealand, and enabled field experiments to determine the minimum number of moth pairs required for establishment. The recent discovery of a newly-introduced parasitoid with attraction to the obscure mealybug pheromone showed that both can be monitored in one trap. Bioprospecting for new biocontrol agents using kairomones such as mealybug pheromones in the centre of biodiversity could help to offer a new risk mitigation tool for classical biocontrol. These cases of pheromones for monitoring biocontrol agents suggest that the area warrants development, especially since it also informs us about pest incursion biology. *Catoblastis cactorum* in Mexico has proven that some insects can be both successful biocontrol agents and unwanted pest organisms.

INTRODUCTION.

Inadequate post-release monitoring of biocontrol agents has long been recognized as a critical failing of biocontrol programs. Challenges to the ecological safety of biocontrol (Simberloff & Stiling 1996) have highlighted the need to document impacts on target and non-target organisms (Delfosse 1999). Unfortunately, monitoring insects released for biocontrol of weeds usually relies on sampling immature stages from plant material (e.g. Nagata & Markin 1986). This method is labor-intensive and insufficiently sensitive when insect populations are at low density.

By contrast, a method widely used and cost-effective for pest management in horticulture, agriculture, and forestry involves sampling adult insects using sticky traps baited with attractants such as pheromones. Pheromone traps or the kairomone (species-species) equivalent have been long and widely used to report the establishment of many new pest organisms, with the largest application being gypsy moth (*Lymantria dispar*) (Linnaeus, 1759) in the USA, where hundreds of thousands of traps have been used over a long period (Sharov *et al.* 2002).

Attractants that work in the field, including moth sex attractants (which are essentially likely to be pheromones but where the female pheromone gland contents has not been confirmed) have been identified for a certain number of weed biocontrol agents or potential agents (e.g. Suckling *et al.* 1999). Furthermore, kairomones have long been known to be attractive for parasitoids (McLain *et al.* 1990; Morgan & Hale 1998) and predators of scale insects (e.g. Mendel *et al.* 1995; Dunkelbloom 1999), and there are at least 11 hymenopteran parasitoid pheromones known and many volatiles reported (El-Sayed 2008), representing potential for further examination. However, the exploitation of these findings through development into field programmes appears to be lacking momentum, with a few exceptions. Case studies will be used to illustrate a range of examples involving semiochemicals and biocontrol agents.

<u>BENEFITS TO IPM.</u>

There are various ways that attractants for biocontrol agents could be deployed within integrated pest management programs, but the additional complexity of the third trophic level makes the context very important to the outcome of pest suppression. It is possible to consider both monitoring tools and direct management of biocontrol agent populations, and there are important successes in both areas. As in the development and use of attractants for pests, usage in biocontrol may only result in increasing knowledge of ecosystems, rather than lead to direct uptake by growers and practitioners. Of course, there have been cases where odourant-based concepts have been tested without success at improving biocontrol. For example, attempts to attract a tachinid parasitoid into orchards with borneol did not result in reduced winter moth damage (Roland *et al.* 1995). Although there are so far relatively few examples of successful deployment of attractants in biocontrol, cases are emerging.

The identification of a kairomone inducing oviposition by the parasitoid *Aphytis melinus* DeBach (Hymenotpera: Aphelinidae)(Millar & Hare 1993) was later used to increase parasitisation of California red scale in the field (Hare *et al.* 1997). Hare *et al*. (1997) suggested that exposure of *A. melinus* reared in commercial insectaries to O-caffeoyltyrosine prior to release may be a means to improve the effectiveness of *A. melinus* used in augmentative release programs to control California red scale.

In another well-developed case, the synthetic pheromone formulated from chemicals found in the airborne secretion of *Podisus maculiventris* (Say)(Heteroptera: Pentatomidae), the spined soldier bug (Aldrich *et al.* 1984), was found to be highly effective in attracting nymphs and adults of this beneficial predatory insect to desired areas for (biocontrol of pests in the U.S.A. The dorsal abdominal glands are much smaller in adult females than males, and females produce a mixture of (*E*)-2-hexenal, (*E*)-2-octenal, (*E*)-2-hexenoic acid, benzaldehyde, and nonanal in these glands. The pheromone enables field collection for augmentative release (Thorpe and Aldrich 2005). There is similar work underway in this area in Japan (Mituzani 2005).

A relatively new area using the sampling efficiency of pheromone traps in IPM is their application in the area of biocontrol of horticultural pest insects. This was explored with the pheromone of a codling moth parasitoid *Ascogaster quadridentata* Wsml. (Delury *et al.* 1999), where phenology, synchrony, and presence of the pest and parasitoid were determined by pheromone trapping (Suckling *et al.* 2002). In this case, the nil tolerance of codling moth ultimately limited the scope of working with the parasitoid in New Zealand's export apple orchards. Parasitoid populations were not supported in these highly managed situations, where the host is kept rare. In practice, it would be necessary to offer growers specific benefits of monitoring their natural enemies before technology uptake could be expected. Instead, mating disruption of codling moth is increasingly being used to maintain levels of pest control. Hence the benefits of this approach may sometimes only be of new knowledge of phenology or regional geographic distribution.

Monitoring of a wider guild natural enemies has been examined using synthetic host induced plant volatiles, and ways of enhancing spring populations of natural enemies together with conservation biocontrol tactics have been proposed (James 2003). A wide range of predators and parasitoids (11 species) were attracted (James 2005).

Another example involving semiochemicals concerns self-introduced natural enemies gone wrong. The multi-colored Asian lady beetle, *Harmonia axyridis* (Pallas) became a considerable nuisance in the U.S.A. due to a habit of invading houses and other places in search of shelter to form overwintering aggregations. There is a search for repellents to prevent these unwanted invasions (Riddick *et al.* 2004). Similarly, the cactus moth *Catoblastis cactorum* (Berg), a biocontrol agent that provided spectacular control of *Opuntia* spp. cacti in Australia (Dodd 1940), has accidentally invaded the continental U.S.A., most likely from the Caribbean, and this moth now threatens indigenous cacti in the southern U.S.A. and Mexico (Hernandez *et al*. 2007). The pheromone has been identified as part of a management plan (Heath *et al.* 2006).

BENEFITS IN WEED BIOCONTROL.

There is obvious potential application for survey trapping in cases of weed biocontrol where Lepidoptera are involved. This order of insects is often used as weed biocontrol agents and there are many more attractants for Lepidoptera than other insects (El-Sayed 2008). The advantages of pheromone trapping in such biocontrol programs include low costs for materials and labor, ability to survey extensively in time and space, efficacy at low population density, high specificity for the target species, and high correlation with reproduction since adults are sampled (Suckling *et al.* 1999).

A low-cost method was tested to develop sex attractant traps for the gorse pod moth (*Cydia succedana*) and the gorse soft shoot moth (*Agonopterix umbellana*) (Suckling *et al.* 1999; 2000). This involved deductive screening of blends thought likely to be attractive, based on congeneric species (e.g. El-Sayed 2008), and tested as single components, binary, or ternary mixtures. In both cases, the method was successful. These new tools have been successfully used to support biocontrol of the gorse in New Zealand, representing the first time a sex attractant was used to document the successful establishment of a biocontrol agent within a country

(Suckling *et al.* 2000). This provides evidence that the biocontrol agent's life cycle was not optimally synchronized with the target weed with negative consequences for efficacy (Hill *et al.* 2000).

The identification of four sex attractants was attempted in Hawaii by deductive field screening of binary and ternary blends for potential use in the study of biocontrol agents against *Rubus* spp. and other weeds, but only two of the four experiments were successful. Attractants for *Croesia zimmermani* (Lepidotpera: Tortricidae) and *Schreckensteinia festaliella* (Lepidotpera: Heliodinidae) were thus further developed by deductive field screening of a variety of lures in sticky traps (Suckling *et al.* 2006). The subsequent use of the traps by Tracy Johnson (pers. comm.) has since included demonstration of the host range to include native Hawaiian *Rubus spp*. being attacked by the introduced agents at altitudes where only the native *Rubus argutus* is present (Gerrish *et al.* 1992), thus indicating range expansion geographically as well. The apparently beneficial risk-reward result of this low cost approach to providing a lure warrants further development for other Lepidoptera, but a study based on gland extracts is preferable. The approach has potential for other types of weed biocontrol agents (e.g. Cossé *et al.* 2006).

POST-RELEASE MONITORING.

Clear benefit can be identified from the development of monitoring systems based on sex attractants that can enable biocontrol programs to better evaluate the impacts of weed or insect control agents after release, which is widely requested by submitters to the Environmental Risk Management Authority of New Zealand (www.ermanz.govt.nz). The benefits were shown by researchers in a Landcare Research experiment where a mass release program investigated a range of *Cydia succedana* release densities (10 adults to 300 adults per site). Pheromone trapping established later that 5 pairs was sufficient, enabling many more sites to be targeted for release and establishment. An attractant also proved valuable for assessing the success of releases of *Agonopterix umbellana* around New Zealand, which depended on life stage (larvae or adults) released and size of release from 300-1000 adults and/or larvae (Gourlay pers. comm.). Recent examples of programs monitoring natural enemies with insect attractants are presented in Table 1.

Post-release monitoring of a new organism was serendipitously enabled when it was discovered that a mealybug parasitoid (*Pseudophycus maculipenis* Fachhandel), recently-released in New Zealand under the Hazardous Substances and New Organisms Act (Charles 2004) was attracted to obscure mealybug (*Pseudococcus viburni* Maskell) pheromone traps (Bell *et al.* 2008) that had been baited with synthetic pheromone (Millar and Midland 2007). In the North Island's Hawkes Bay, the phenology of the two species evident in the traps showed a mealybug generation without evident parasitoid catch in the middle of the winter. Traps recovered *P. maculipennis* from many sites in two regions where releases were been made, and its' rate of natural spread was determined from wider recoveries (Bell *et al.* in prep.).

Table 1. Benefits demonstrated of insect attractants in six case studies of biocontrol from New Zealand and Hawaii.

Case	Organism (family)	Benefits (lure type)	Reference
1	Gorse pod moth *Cydia succedana* (Tortricidae)	Presence/absence, synchrony with the host phenology (sex attractant), minimum release for establishment during national release programme (sex attractant)	Suckling *et al.* 1999 J. Memmott and H Gourlay pers. comm.
2	Gorse soft shoot moth *Agonoptrix ulicitella* (Oecophoridae)	Population presence in New Zealand for the first time, regional distribution, minimum release for establishment during national release programme (sex attractant)	Suckling *et al.* 2000, H. Gourlay pers. comm.
3	Codling moth egg parasitoid *Ascogaster quadridentata* (Braconidae)	Synchrony with the host phenology, national distribution, no IPM use (pheromone)	Suckling *et al.* 2002
4	Mealybug parasitoid *Pseudophycus maculipenis* (Encyrtidae*)*	Post-release survey distribution, spread (kairomone),	Bell *et al.* 2008
5	*Croesia zimmermani* (Tortricidae)	Distribution (sex attractant)	Suckling *et al.* 2006
6	*Schreckensteinia festaliella* (Heliodinidae)	Distribution (sex attractant)	Suckling *et al.* 2006

RISK REDUCTION IN CLASSICAL BIOCONTROL.

One under-exploited role for insect attractants in biocontrol that could be developed is based on the discovery of kairomonal attraction and the following hypothesis. If a cross species attraction has evolved between a natural enemy and its host/prey then the natural enemy may be more likely to be quite species-specific in host range. It seems unlikely that the trait of broadband reception of species-specific pheromone would occur at random, although it has to be noted that the phenomonen of attraction can occur in clusters within the same genera of predators (Mendel *et al.* 2004). On balance, the risk of a proposed new organism with the demonstrated evolutionary investment in detecting and responding to the pheromone of its exotic host species then tracking the habitat and/or the pheromone of an equivalent native species seems very low. Therefore the introduction of the new organism known to be tuned to the pheromone of its' intended target host should carry lower risk of non-target impacts. Species-specificity in kairomone communication is therefore likely to be a desirable trait for lowering risk in biocontrol.

This testable hypothesis leads to the idea of bioprospecting for new biocontrol agents using odourants, as a way of pre-screening candidates for biosafety.

The idea of bioprospecting for new biocontrol agents using kairomones is not new, since Mendel *et al.* (2004) suggested that it could lead to the discovery of new predators for pine blast scale which could be introduced into Israel. In their ecosystem, a whole guild of predators can be trapped. They found that two guilds of predators were attracted: flower bugs of the genus *Elatophilus* Reuter and brown lacewings of the genera *Hemerobius* and *Sympherobius*. Predators identified as attracted to the pheromone of the scale in Portugal were then evaluated to augment the natural enemy fauna of Israel.

We (DMS with S. Learmonth and J.G. Millar pers. comm.) have been exploring the potential of this concept in the centre of biodiversity of the long-tailed mealybug (*Pseudococcus longispinus*) in Western Australia. We have been using the newly-identified synthetic pheromone (J.G. Millar pers. comm.), although to date and possibly due to the long-term drought, no parasitoids have been trapped (Learmonth pers. comm.).

CONCLUSIONS.

The development and use of attractants for insect or weed biocontrol agents is emerging as an expanding use pattern for semiochemicals, and it is likely that new applications will be developed. Successful early cases of use of pheromones and other attractants for biocontrol agents suggest that the area warrants further consideration and development. Although weed biocontrol agents seldom have unpredicted non-target impacts, if they become unwanted organisms due to host range expansion for whatever reason, insect attractants can offer benefits in managing the reverse situation. *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) has emerged as a threat to native cactus biodiversity in the southern U.S.A. and Mexico, and illustrated that some insects can be both excellent biocontrol agents (Dodd 1940) and unwanted organisms. In this case, the pheromone was identified when it became an unwanted organism (Heath et al. 2006). In other cases with both pest and beneficial insects, pro-active identification of attractants could help to manage risks with new organisms that warrant this investment.

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