Autodissemination of Semiochemicals and Pesticides: a New Concept Compatible with the Sterile Insect Technique

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ABSTRACT ExoSex technology utilizes inert particles of materials that have the ability to adhere to the arthropod cuticle. The ExoSex Autoconfusion™ system has been developed as an insect control method that differs from all other mating disruption systems in contaminating the target pest with electrostatically chargeable powder formulated with pheromone. The technique has now been evaluated in the field against a range of lepidopteran species, including the codling moth *Cydia pomonella* (L.), the grape berry moth *Lobesia botrana* Denis and Schiffermueller, and the Asiatic rice borer *Chilo suppressalis* Walker. Research into the mechanisms of autoconfusion shows that habituation, inhibition of courtship and delay in mating are important mechanisms in confusion and population reduction. The ExoLure™ system utilizes adhesive particles as carriers for synthetic or biological pesticides and can be used as a lure-and-kill technique for insect pest control. Ways are suggested in which Exosect technology can be integrated with the sterile insect technique (SIT) to provide new powerful hybrid techniques for area-wide insect pest control.

KEY WORDS autodissemination, mating disruption, autoconfusion, electrostatic powder

1. Introduction

The use of charged particle technology (UK patent application No. EP0650322) was tested in preliminary trials against the codling moth *Cydia pomonella* (L.) and tephritid fruit flies using a pheromone lure to attract insects into dispensers and electrostatically charged powder formulated with slow-acting insecticide as a killing agent (Howse and Underwood 2000). The latter was spread by contact to non-contaminated insects. Having proven the potential of the technique (Chandler 2004a,b, Chandler and Howse 2005, Armsworth et al. 2006), regulatory approval has been granted for use against certain species in the USA and UK, and further applications are pending. This technology also suggests other applications, e.g. the dissemination of biopathogens from bait stations and, coupled with this, the use of sterile insects as carriers for semiochemicals or biopathogens.

2. Mechanism of Autoconfusion

In the proprietary powder formulation Entostat™ there are approximately 1.5 x 1010 particles per gram and the diameter particle size is in the range of 5-20 microns. Taking into account the known threshold response of *C. pomonella* to pheromone there is theoretically sufficient pheromone in one particle resting on the surface of the antenna to initiate habituation. An insect carrying approximately 1800 particles would then be liberating sufficient pheromone in the short term to constitute an attractive source for another male moth. Therefore one ExoSex

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dispenser (containing around three grams) is capable of contaminating about 21 million male moths with enough pheromone to make them attractive sources to other males. Autoconfusion is believed to be due to habituation of male responsiveness as a result of constant sensory stimulation, and males acting as false lures or mobile dispensers (Chandler 2004a). Possible components of autoconfusion in codling moth are listed below.

2.1. False Trail Following

This also occurs with conventional mating disruption, where discrete pheromone sources effectively act as female mimics thereby distracting males in their search for calling females. However, in the case of ExoSex Autoconfusion™, it is the contaminated males themselves that act as dispensers rather than discrete sustained release devices. The males are mobile, and so progressively increasing their density will greatly decrease the chances that newly arriving males will locate calling females.

2.2. Habituation

Again, this occurs in conventional mating disruption by raising the threshold for detection of aerial plumes of pheromone originating from calling females. Habituation may involve the sensory pathways or the central nervous system, or both. Thus the sensory receptors may "saturate", or the sustained input to the insect's brain may induce a long-lasting blockage of response. In ExoSex Autoconfusion™, the pheromone sources are in intimate contact with the sensory receptors on the antennae, which provide a sustained high level of sensory input. Reversal of the response (dishabituation) cannot therefore occur when the insects are in clean air outside the crop or in a windy situation.

2.3. Trail Masking

Together with habituation effects, the presence of a dense concentration of pheromone in the air will mask the individual pheromone trails from calling females. An analogy can be made with the so-called "cocktail party effect" in which a high level of background noise makes it impossible to pick out a voice a few feet away. This is, in other words, like habituation, a form of "chemical deafening". In ExoSex Autoconfusion™ this effect will be of minor significance compared with habituation, and there is therefore no requirement to release the extremely high quantities of pheromone into the environment to achieve trail masking.

2.4. Sensory Imbalance

With ExoSex Autoconfusion™, there may be a strong imbalance of sensory input due to unequal contamination of the two antennae, particularly where the amount of powder picked up is very small. This may interfere with the guidance mechanism of the male, causing it to deviate to the most heavily stimulated side.

2.5. Inhibition of Courtship

In the laboratory the percentage of females mating when confined in a small space with males is substantially reduced when the males are contaminated with Entostat™ powder (Howse and MacDonald 2005). Courtship in Lepidoptera normally involves an exchange of stimuli at close range, including release of male-produced pheromone (Howse et al 1998). This is less likely to occur. It has also been hypothesized that males releasing a high concentration of female pheromone are less sexually competent (Knight 2003).

2.6. The Role of Foliage

Suckling and Karg (2000) found that apple leaves could absorb and release sufficient pheromone from nearby dispensers to enhance mating disruption of the light brown apple moth *Epiphyas postvittana* Walker. This effect can also be seen after removal of dispensers. The same phenomenon is known to occur in the pea moth *Cydia nigricana* (F.), where the former sites of monitoring traps remain attractive for long periods (Wall et al. 1981).

2.7. Enhancement of Predation

Mating disruption when used alone does not interfere with the action of predators and parasites. Knight (1997) reported levels of egg predation around 20% higher in orchards treated for mating disruption of codling moth, compared with orchards under conventional insecticide treatment.

2.8. Delay in Mating

Recent evidence indicates that very low levels of pheromone in the environment, may delay mating. Brunner (2003) and Knight (1997) believe that this delay in mating results from interference in mate location.

3. Relevance to the Sterile Insect Technique (SIT)

In any mating disruption technique, the main constraints are the cost of the materials and the labour-intensive process of placing dispensers in the crop. In these respects, the Exosex system is considerably more cost-effective than other techniques. In the case of codling moth, for example, the total amount of pheromone dispensed per hectare is between 80 and 200 milligrams in 25 dispensers. By comparison, most conventional techniques dispense between 45 and 192 grams/hectare, i.e. around 1000 times as much, in 400-1000 dispensers (Chandler 2004a,b).

Area-wide integrated pest control programmes using mating disruption may be made more efficient by using sterile insects as mobile dispensers of adhesive particles, carrying behaviour-modifying chemicals. When they are coated with sex pheromone, they can achieve control by mating disruption, in which they act as female mimics, outcompeting the females (or males) in the natural population that are releasing their own sex pheromones. By using sterile insects as living dispensers of pheromone, the level of mating in the natural population is reduced, and when matings do occur they are likely to be between sterile males and females of the natural population.

Sterile insects can also be released at a density sufficient to raise the concentration of the sex pheromone throughout the crop to a level high enough to achieve mating disruption by trail masking. This would then compare with the spraying of pheromone in, for example, Hercon flakes or Consep Checkmate macrocapsules for pink bollworm *Pectinophora gossypiella* (Saunders), where saturation of the environment is achieved at between 12 000-50 000 sources per hectare (Hall and Marrs 1989).

Sterile insects of one species could be used to control populations of a different pest species. For example, in a zone where both Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and codling moth are established, sterile Mediterranean fruit flies can be released coated with particles containing the codling moth sex pheromone. The Mediterranean fruit fly does not pose a threat to the crop concerned but they act, as before, as mobile dispensers of codling moth pheromone, decoying males away from females. The main advantage here is that Mediterranean fruit flies are much cheaper to mass-rear than most Lepidoptera, including codling moth.

Sterile insects could be coated with powder containing a slow-acting chemical pesticide, or spores of a fungal entomopathogen. Males and females of the natural population attempting to mate with the released flies become contaminated with the pathogen, which they pass to partners in subsequent mating attempts.

Possible advantages of the above applications: (1) environmental contamination is reduced because the active ingredients are applied to insects released into the environment and are not sprayed onto the crop; (2) the amounts of active ingredients used are much

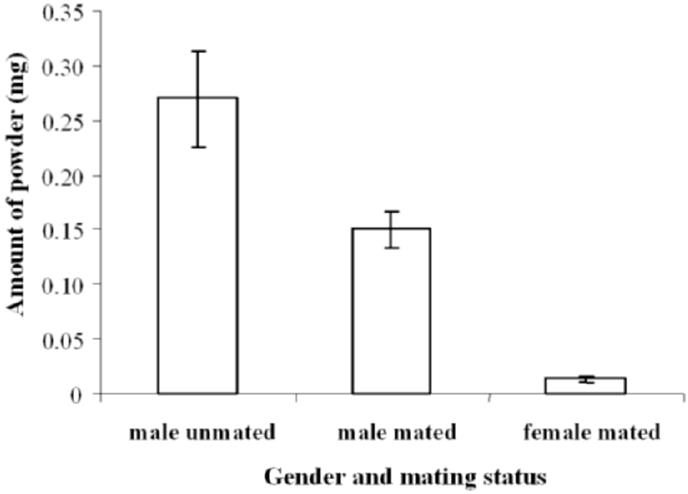

Figure 1. *Transfer of dyed adhesive particles to female Mediterranean fruit fly* Ceratitis capitata *during mating, measured using a fluorimetric assay. Ten virgin males artificially contaminated with adhesive particles were introduced to a fly cage with ten virgin females. Mating pairs were removed and the experiment terminated when five pairs had mated. Closed bars represent standard errors (n = 5).*

lower than in conventional mating disruption systems or lure-and-kill techniques; (3) as the method depends on the use of odours and visual stimuli to which the particular pest is strongly attracted, it is also highly selective, protecting, in particular, beneficial insects; (4) because sterile insects are used as carriers for insecticides or behaviour-modifying compounds they pose no risk to the build-up of pest populations: all mating result in sterile eggs; (5) the insects are biodegradable and do not need to be removed from the crop, unlike plastic traps and dispensers commonly used in insect control; (6) both sterile males and sterile females can be released (unless the females cause damage themselves); (7) quality control of sterile male competitiveness becomes less important if the main route of control is through transfer of materials between individuals. It should be borne in mind that dispersal and survival will strongly influence the efficacy of the technique; and (8) the numbers of sterile insects released per unit area can be substantially reduced while still achieving efficacy of control. This may give rise to substantial cost savings in production, although the more insects are produced in a rearing facility, the cheaper the insect becomes.

4. Lure-and-Kill Applications with Mediterranean Fruit Fly

The efficacy of a control method for the Mediterranean fruit fly (or any insect) depends on several factors: the number of particles picked up, the number transferred to another insect, the concentration of active ingredient in the particles, the rate of loss of particles from the cuticle of the secondarily contaminated insect, and (in a lure-and-kill system) the LT_{50} of the pesticide.

Entostat™, electrostatically chargeable powders are readily picked up by Mediterranean fruit flies. Entostat™ was dyed with a fluorescent dye and flies were artificially contaminated with the dyed powder in glass vials. The amount of dyed powder picked up by flies was determined by retriev-

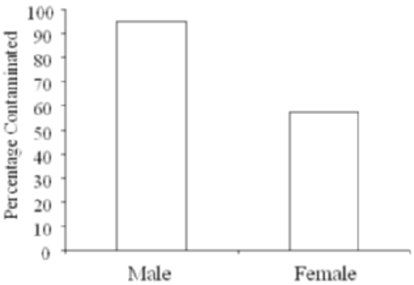

Figure 2. *Percentage of contaminated Mediterranean fruit fly* Ceratitis capitata *after five days exposure to an ExoLure™ dispenser in a field cage (Supplied courtesy of Karen Underwood).*

ing the powder in solvent and running samples in a fluorimeter and comparing the samples to a calibration curve. It was determined that Mediterranean fruit flies picked up a mean average of 130 micrograms of Entostat™ powder. This amounts to approximately 10 000 particles (P. Howse, unpublished). There is therefore little doubt that such insects can support quantities of attractants that are well above the threshold of attraction for others of the same species.

Transfer of electrostatically chargeable powders between individual Mediterranean fruit flies has been demonstrated both in the laboratory and in the field (P. Howse, K. Underwood, and C. Jackson, unpublished). In the laboratory, ten virgin males coated with dyed adhesive particles were introduced into a cage with ten virgin females. After 1 hour 40 minutes, half the flies had mated. It appears that males lost particles during mating and the mated females gained between 9.2 and 21.3 micrograms, i.e. around 10% of that retained by unmated males (Fig. 1). Similar results have been obtained with tsetse flies (*Glossina austeni* Newstead), (P. Howse and K. Underwood, unpublished). In field cage experiments carried out in Mallorca, male Mediterranean fruit flies made repeated visits to a bait station containing trimedlure and electrostatically chargeable powder, and the powder was also transferred to females (Fig. 2).

Fig. 3 shows the rate of loss of dyed adhesive particles from Mediterranean fruit flies in a controlled environment (laboratory conditions of 20-25°C and 60-80% relative humidity) assessed using a fluorimetric assay. This conforms to a pattern the authors have also observed in other Diptera of an initial loss of 50-60% of the material, followed by exponential loss over a period of from about one hour to at least one week. Most of the initial loss is due to larger particles being lost from the surface of cuticular hairs. These figures then suggest that approximately 40% of the initial amount transferred from one insect to another will remain on the secondarily contaminated insect for at least a week.

5. Conclusions

The Exosect technique can be used to coat different insect species with certain types of powder that readily adhere to the cuticle and studies are ongoing to assess the dynamics of powder pick-up and transfer between individuals. The final goal is to show that particles formulated with semiochemicals or insecticidal materials can be distributed throughout a pest population by the insects themselves. It is proposed that this technique can be extended to use sterile insects as mobile dispensers, or vectors, of semiochemicals or pesticides. Work has now begun to establish dosage and transfer rates so that the efficacy of combining Exosect technologies with the SIT can be investigated.

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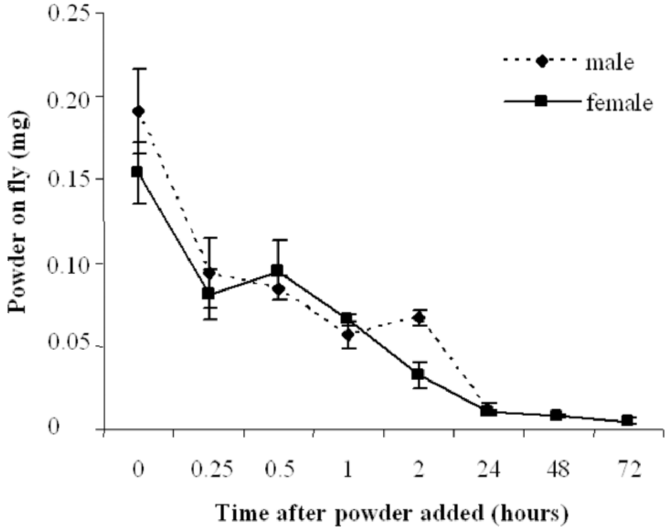

Figure 3. *Loss of dyed Entostat™ powder from artificially contaminated male and female Mediterranean fruit fly* Ceratitis capitata *over time in a laboratory fly cage, measured using a fluorimetric assay. Closed bars represent standard errors (n = 5).*

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