Canterbury Research and Theses Environment

Canterbury Christ Church University's repository of research outputs

http://create.canterbury.ac.uk

Please cite this publication as follows:

Link to official URL (if available):

Contact: create.library@canterbury.ac.uk

Canterbury Christ Church University

The Effect of Exposure to Synthetic Pheromone Lures on Male *Zygaena filipendulae* Mating Behaviour – Implications for Monitoring Species of Conservation Interest

Ashen Oleander[1], David Thackery[2] and Joseph Burman[3]

Canterbury Christ Church University, North Holmes Road, Canterbury, Kent, CT1 1QU

[1]a.oleander131@canterbury.ac.uk

[1]Tel 07794 897 117

[2]d.w.thackery9@canterbury.ac.uk

[3]joseph.burman@canterbury.ac.uk

The Effect of Exposure to Synthetic Pheromone Lures on Male Zygaena filipendulae Mating Behaviour – Implications for Monitoring Species of Conservation Interest

Abstract

Pheromone based monitoring of insects of conservation value has the potential to revolutionise the way in which surveys are carried out. However, due to their effective use in pest management, concerns have been raised about potential negative effects of pheromone exposure on populations of rare insects. The effect of exposure to synthetic pheromone lures on male mating behaviour was examined in laboratory and field conditions using the six spot burnet moth Zygaena filipendulae (Linnaeus, 1758). For the laboratory experiment larvae were collected and cultured separately under controlled conditions. Virgin males were exposed to a synthetic pheromone lure for 24 hours; then tested for responsiveness immediately after this exposure, 1hour and 24 hours later. Control males were tested three times: initially, 1 hour later and 24 hours later. The time taken for males to detect females, shown by exposure of their anal claspers, and the time taken for males to locate females were recorded. No significant difference was found between the time taken for control and exposed males to detect or locate females, and no significant difference between the proportions of males that successfully located females in exposed and control groups was found. In the field experiment the time males spent in the presence of contained females, both with and without a pheromone lure present, was recorded. Males spent more time in the presence of the females when the pheromone lure was present. Both experiments indicate male Z. filipendulae mating behaviour is not adversely affected by exposure to synthetic pheromone lures.

Keywords: Pheromone; Monitoring; Conservation; Mating Disruption; Zygaenidae.

1. Introduction

Insect sex pheromones are a well-established form of pest management and are used across the world (Witzgall et al. 2010; Miller et al. 2010; Onufrieva et al. 2010; Stelinski et al. 2013). The potential of using synthetic insect pheromones to monitor and control pest insects in an environmentally friendly way was proposed soon after the commercial silk moth, Bombyx mori (Linnaeus, 1758) pheromone was identified in 1959 (Hansson 1995; Bergmann et al. 2009; Witzgall et al. 2010). Pheromones have been used in pest management of insects for decades, the main applications being trapping insects to monitor their activity and mating disruption (El-Sayed and Suckling 2005; Larsson and Svensson 2009; Andersson 2012; Andersson et al. 2014). Historically, insect pheromone research has focussed on species considered to be commercially important; in particular agricultural, stored product and forestry pests (Campbell et al. 2002; Stelinski et al. 2005; Bergmann et al. 2009). However more recently the potential use of pheromones to monitor insects for conservation purposes is being explored with Osmoderma eremita (Scopoli,1763) becoming the first insect species whose sex pheromone was identified purely for conservation (Larsson and Svensson 2009; Svensson et al. 2009; Andersson2012; Andersson et al. 2014).

Just as in the case of monitoring pest species, the incredible attractiveness and species-specificity of pheromones makes them potentially useful tools in monitoring insects for conservation purposes (Musa et al. 2013). Once the specific pheromone blend has been identified, pheromone based trapping can be used to monitor rare insects of conservation concern and those that have been identified as indicator species (Andersson 2012; Andersson et al. 2014). Currently sampling methods such as pitfall traps and sweep netting are used to survey areas of conservation interest; however these are time consuming and dependent on the presence of taxonomic experts to identify the species found (Svensson et al. 2009; Andersson et al. 2014). The species-specific nature of pheromone blends overcomes the need for experts to be present and the attractiveness of pheromones yields a higher degree of accuracy in the data obtained: an empty pheromone trap suggests the absence of a species much more strongly than an empty pit fall trap, for example (Musa et al. 2013). Pheromone-based monitoring can result in more effective surveys, even with bold day flying moths such as Zygaena spp., which are attracted

to lures in far greater numbers, establishing occupancy in places where standard searching cannot (unpublished data). Consequently pheromone based monitoring of insects has the potential to revolutionise the way in which conservation surveys are carried out and greatly improve the accuracy of data obtained.

However, the behaviour modifying properties of pheromones which have been utilised so effectively in insect pest control by mating disruption cannot be ignored when considering using pheromones to monitor insects for conservation purposes. Mating disruption strategies are most effective at low population densities (Sharov et al. 2002). Consequently exposure to synthetic pheromones could prove detrimental to populations of rare insects occurring at low population densities and jeopardise their conservation (Sharov et al. 2002; Witzgall et al. 2008). The synthetic pheromone has to be present in sufficient quantities to successfully disrupt mating; for example a density of 27,300 wax droplet pheromone dispensers per ha is needed to effectively disrupt mating in Grapholita molesta (Busck, 1916) (Stelinski et al. 2005; Tcheslavskaia et al. 2005; Witzgall et al. 2008). While the density of pheromone lures used in monitoring for conservation purposes is considerably lower, this is not to say that the populations will not be affected. Pheromones are used to disrupt mating in a number of ways including false plume following (Stelinski et al. 2004; Welter et al. 2005; El-Sayed and Suckling 2005; Stelinski 2007; Huang et al. 2010), competitive attraction (Welter et al. 2005; Stelinski 2007), camouflage of plumes produced by females (Stelinski et al. 2004; El-Sayed and Suckling 2005) and impaired or eliminated normal responses to the pheromone (Stelinski et al. 2004; Welter et al. 2005; El-Sayed and Suckling 2005; Stelinski 2007). The change in response is caused by sensory overload and results in adaptation of the peripheral receptors or habituation of the central nervous system (Stelinski et al. 2004; El-Sayed and Suckling 2005; Evenden et al. 2005; Stelinski 2007; Witzgall et al. 2008).

This study examines whether male Zygaena filipendulae (Linnaeus, 1758) become desensitised to the pheromone produced by the female after exposure to synthetic pheromone lures and whether the presence of synthetic pheromones in the field affects males' normal responses to the female. Desensitisation was investigated by testing whether mating behaviour has been affected as a consequence of exposure. The effects of adaptation of the peripheral receptors are usually reversible and short lived (Stelinski et al. 2004) so males in this study were tested on three occasions to determine how long-lived desensitisation was and if it was reversible. To establish whether exposure to synthetic pheromone lures affects mating behaviour in male Z. filipendulae, mating behaviour was considered from three perspectives: whether exposure interferes with a male's ability to detect the female; whether exposure impedes a male's ability to locate the female; and whether a higher proportion of males were unable to detect and locate females after exposure. A field experiment examined whether the synthetic pheromone lure competed with males' attraction to female produced pheromone plumes. The amount of time a male spent in the presence of a contained female was recorded both with and without a synthetic pheromone present.

2. Method

Study Species

Burnet moths (Zygaenidae) have declined throughout Western Europe and have been considered as indicator species for semi-natural grassland habitats (Franzén 2002; Franzen and Ranius 2004; Franzen and Nilsson 2012; Hein et al. 2007). Z. filipendulae is the most common species found in Great Britain and was chosen for this study due to its status and accessibility.

Pheromone Lures

The pheromone compounds for Z. filipendulae are Z7-12:Acetate, Z9-14:Acetate and Z5-12:Acetate in a ratio of 100:10:3 (Priesner et al. 1984). The pheromone compounds were obtained from Pherobank and hexane was used as a solvent to make up the blend to the correct concentration. Supelco butyl rubber septa with a 13mm diameter sourced from Sigma-Aldrich were used as lures and 200µl of the blend was added to each. Each 200µl hexane,

and therefore each lure, contained 0.1mg of Z7-12Ac, 0.01mg of Z9-14Ac and 0.003mg of Z5-12Ac. Once the hexane had evaporated lures were wrapped in tin foil and placed in the freezer until required.

2.1 Desensitisation

2.1.1 Larvae Culturing

104 3rd, 4th and 5th instar Z. filipendulae larvae were collected from a small wild flower garden at Canterbury Christ Church University, Kent. The larvae were separated into individual petri dishes so they would pupate and emerge separately, thus ensuring that all moths tested would be virgins. The larvae were fed their food plant Lotus corniculatus (Linneaus, 1758) three times daily and excrement was removed from the petri dishes once a day. Once the larvae pupated the lid was removed from the petri dish and the petri dish placed in a clear plastic container measuring 17.8×11.5×4.4 cm. Holes 1.5 cm apart were made in the lid of the container using a pin. Removing the lid of the petri dish and placing it in a larger container ensured that the adult moths would be able to emerge properly (as the petri dish itself was too small to allow the moths to fan their wings out on emergence). Moths emerged between 30/06/2013 and 19/07/2013. Once emerged the moths remained in the plastic containers and were fed on a very dilute sugar solution of approximately 1g/l administered on cotton wool pieces. After emerging, the moths were sexed by sight: females have a shorter and rounder abdomen than males.

2.1.2 Exposure to Pheromone Blend

The males were divided into two groups: one that would be exposed to the pheromone blend and a control. In field studies males would be likely to be exposed to a pheromone lure in a trap for a maximum of 24 hours, consequently in this experiment, males were exposed to the pheromone lure for 24 hours in order to reflect a realistic scenario. In pheromone traps commonly used to trap moths in the field the insect is not in direct contact with the lure. However in this study to test the effect of maximum exposure, the lure was placed in the plastic container with each individual, enabling the moth to make direct contact with it, thus receiving maximum exposure. Males were exposed to the pheromone lure within 72 hours of emergence. An empty rubber septum was also placed in the plastic container with the control males for 24 hours within 72 hours of emergence. Exposed males were kept in a separate area to the control group in order to ensure that the controls did not receive any exposure to the pheromone lure.

2.1.3 Mating Tests

To test the effect of exposure to pheromone lures on male mating behaviour the following set up was used. A male was placed in the front left side of aBugDorm-44545F insect cage measuring 47.5 × 47.0 × 47.5 cm and the female was in a plastic container measuring 17.8 × 11.5 × 4.4 cm at the rear right corner of the tent. The lid was removed from the female's container and replaced with a plastic mesh cover. Keeping the female in the container ensured she was unable to move towards the male. This set up can be seen in figure 1.
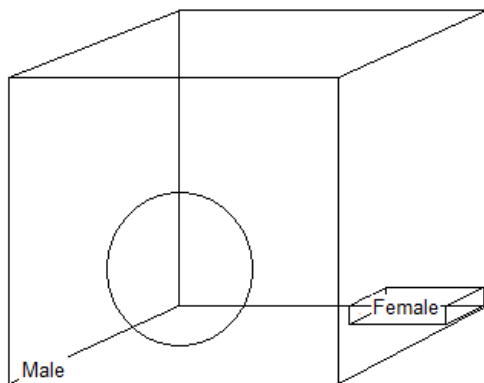
Male and female moths were paired for the experiment so each male was tested with the same female on each occasion. The contained female was placed in the cage before the male was released. The time taken for a male to reach the female was recorded to determine the males' ability to locate the females. The time taken for a male to detect the female (shown by the exposure of the anal claspers) was also noted, as were other calling behaviours such as wing fanning. The test was stopped if the male did not expose his claspers or display calling behaviours within 30 minutes. Tests were carried out on exposed males 24 hours after their initial exposure to the pheromone lure, then again 1 hour and 24 hours later to determine whether the possible desensitising effect of exposure would decrease over time. Control males were also tested three times: an initial test, 1 hour later and 24 hours later. Exposed and control males were tested in separate areas using separate cages. Between each test the cage was thoroughly cleaned using industrial methylated spirit. Tests were conducted between 12.00 and 17.00 as the moths are most active during this period of time. A total of 14 exposed males and 14 control males were tested.

2.2 Competitive Attraction in the Field

The experiment was carried out at Reculver Country Park, Herne Bay, Kent. 7 Z. filipendulae females were caught on site and placed in individual plastic containers measuring $17.8 \times 11.5 \times 4.4$ cm. The lid of each container was removed and replaced with plastic mesh to allow aeration. A small amount of damp foliage was added to each container to provide moisture and shelter. These were referred to as mating stations. Each mating station was attached to a bamboo cane elevating it to a height of 1 metre above the ground, as can be seen in figure 2. The mating stations were spaced out at 2 metre intervals in a semi-circle around a central observation point. The mating stations were allocated a number (1-7) to aid in recordings. This set up is illustrated in figure 2.
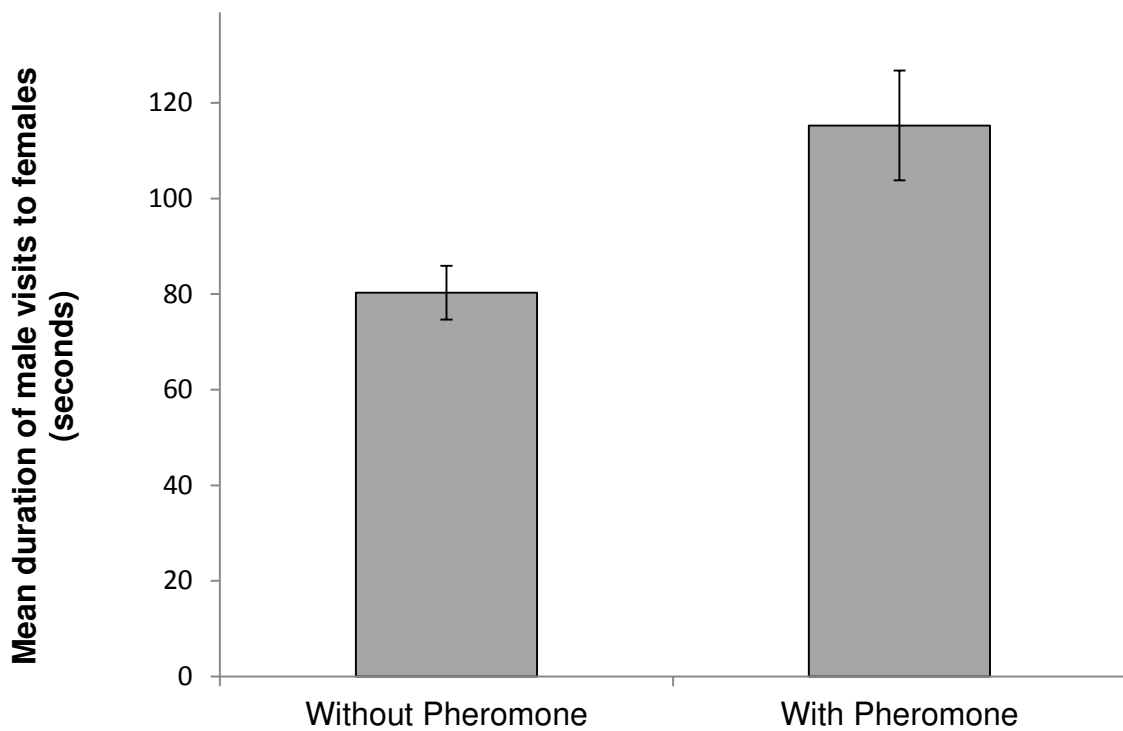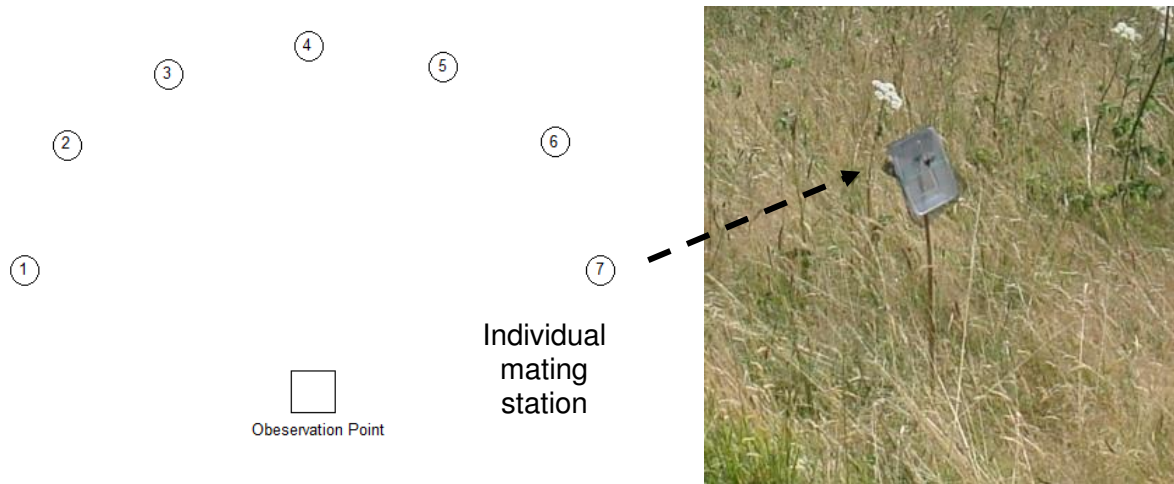
Figure 2

The time at which males entered into an approximately 20cm radius of a mating station and the time of leaving this area were recorded so the amount of time males spent at the mating station could be calculated. Where multiple males were present at the same mating location the first entry for arrival was assumed as the first to leave. Though this may have not always been the case it was the standard used throughout. 2 days of recording on 16/07/2014 and 17/07/2014 between 13.30 and 17.30 were carried out: the first day without a synthetic pheromone lure present, the second in the presence of a synthetic pheromone lure.

When recording in the presence of the pheromone lure the same mating stations were placed in the same locations. Females were assessed for health and subsequently used again. The pheromone lure was attached to a bamboo cane to raise it 1 metre from the ground and placed 2 metres behind the observation point. The same

criterion for recording was used at the mating stations. A recording of the number of individuals at the pheromone lure was taken at 15 minute intervals throughout the observation period.

2.3 Statistical Analysis

Anderson-Darling normality tests were carried out on the data. General Linear Model analyses were performed on the time taken for males to expose their anal claspers and the time taken for males to reach the female in order to determine whether the males' ability to detect and locate females was affected by exposure to pheromones. A chi squared test was used to compare the proportions of males successfully locating the female in exposed and control groups. A two sample T-test was carried out on the amount of time males spent at mating stations with and without the pheromone present.

3. Results

3.1 Desensitisation

3.1.1 Males' Detection of Females

The mean times taken for exposed and control virgin males to detect virgin females in each test period are shown in figure 3a.

No significant difference ($F_1=0.93$, p = 0.339) was found between the time taken for exposed males and control males to detect the female and expose their anal claspers. No significant difference ($F_2=0.69$, p = 0.508) was found between the time males spent detecting the female when comparing immediate, 1 hour and 24 hour time points.

3.1.2 Males' Location of Females

The mean times taken for exposed and control virgin males to locate virgin females in each test period are shown in figure 3b. No significant difference ($F_1=0.05$, p = 0.819) was found between the time taken for exposed males and control males to locate the female. No significant difference ($F_2=0.67$, p = 0.513) was found between the time males spent locating the female when comparing immediate, 1 hour and 24 hour time points.

3.1.3 Proportion of Successful Locations of Female

The proportion of exposed and control virgin males that successfully located virgin females in each test period is shown in figure 3c. No significant difference (X^2 (1, N=28) = 0.159, p = 0.690) was found between the proportions of males which successfully located the females in exposed and control groups.

3.2 Competitive Attraction in the field

The duration of males' visits to females with and without a synthetic pheromone lure present is shown in figure 2. A significant difference (t(268) = -2.98, p = 0.003) was found between the duration of the males' visits to females when the synthetic pheromone lure was present and when it was absent. Males' visits to females had the longest duration when the synthetic pheromone lure was present.
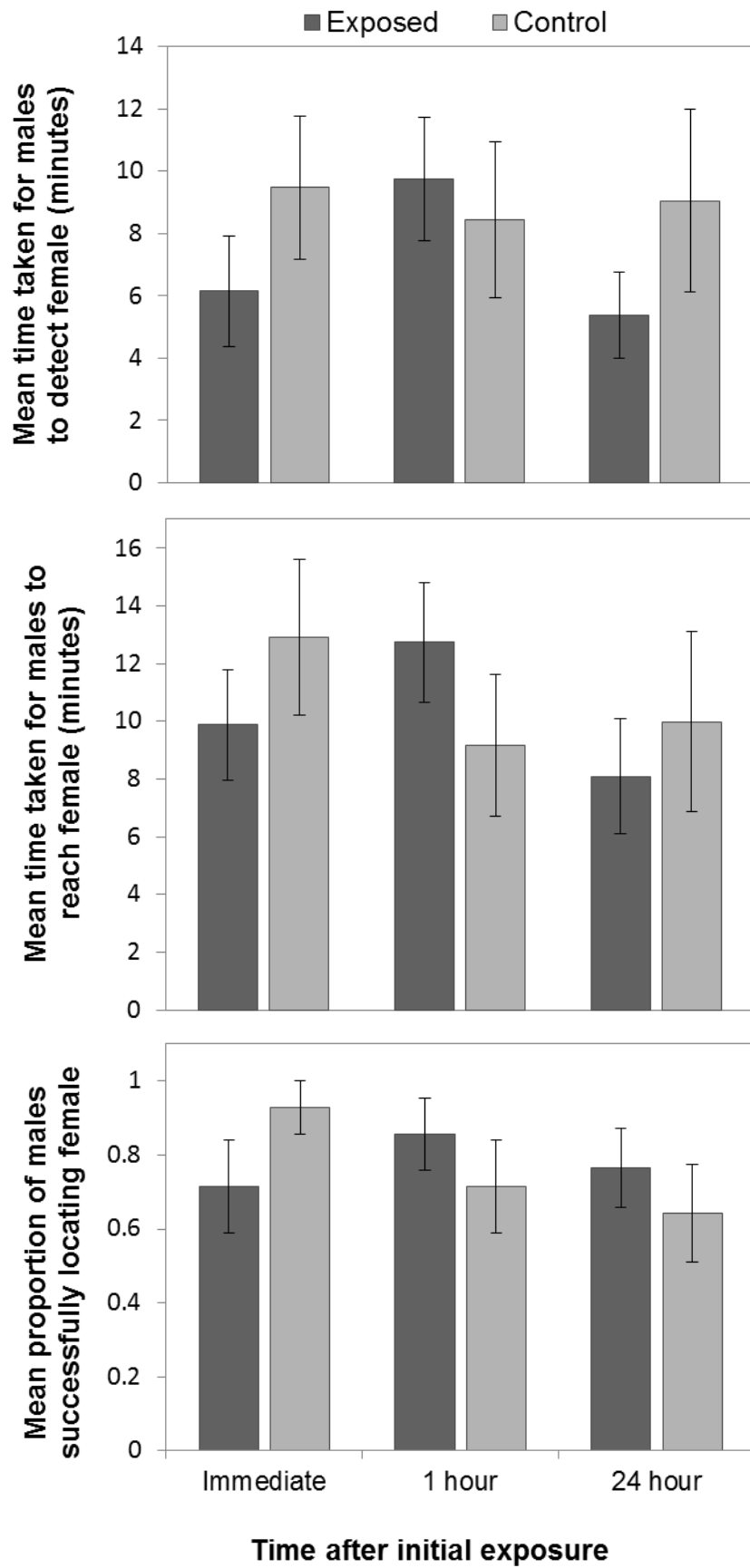
Figure 3

4. Discussion

A general linear model showed no significant difference between the time taken for males to detect and locate females in exposed and control groups. This shows ability to detect and locate females is not impaired by exposure to synthetic pheromones. A chi squared test found no significant difference between the proportions of males in exposed and control groups successfully locating females. Thus control males were no more likely to locate the female than exposed males. This demonstrates that not only can a male detect and locate a female in the same amount of time whether exposed or not but a male is equally likely to locate the female whether exposed or not. Thus no desensitising effect resulted from exposure to synthetic pheromones in this experiment and the male was not desensitised to the presence of the female. This study therefore found no direct evidence that synthetic pheromone exposure desensitises males to female-produced pheromones.

A two-sample T-test found a significant difference between the time males spent in the presence of females when a synthetic pheromone lure was present and when it was absent. Males spent longer periods of time in the presence of females when the pheromone lure was present. This could indicate that males were attracted to the area by the synthetic pheromone lure and then became aroused by the presence of the strong synthetic blend. Nevertheless it is clear that they are capable of locating a female. Therefore no evidence was found of competitive attraction and the presence of a synthetic pheromone lure did not adversely affect male mating behaviour.

Lepidoptera are sensitive indicators of habitat quality and environmental change and burnet moths in particular are considered to be important umbrella species for semi-natural grassland habitats (Schmitt 2003; Spitzer et al. 2009; Rakosy and Schmitt 2011). Strong correlations have been found between burnet moths and species richness of butterflies and they have been suggested as indicator species for semi-natural grasslands (Franzén 2002; Franzen and Ranius 2004; Hein et al. 2007). Monitoring their populations accurately using pheromones without adverse effects would provide information regarding the moths' distribution and population dynamics. Additionally, insights into the presence and absence of other species of the same habitats and their population dynamics and distribution could be gained based on the presence or absence of burnet moths. Semi-natural grassland habitats have substantially decreased across Europe over the last 100 years and burnet moths have been in decline throughout Western Europe for the past 50 years (Franzen and Ranius 2004; Franzen and Nilsson 2012). Given the decline in burnet numbers and the fact that many species inhabiting semi-natural grassland are now red listed, it is essential to monitor these species accurately to provide the most effective conservation strategies (Franzen and Ranius 2004; Franzen and Nilsson 2012). This is particularly important as the rate of habitat change and loss of semi-natural grasslands in Europe is placing a great selection pressure on the species occupying these areas and it will be vital to understand the effects of these changes in order to promote survival of the species affected (Franzén 2002; Franzen and Nilsson 2012).

Currently knowledge of the ecology of burnet moths is patchy, creating a problem with using them as biodiversity indicators (Franzen and Ranius 2004). Knowledge of their distribution across Europe is incomplete and the temporal and spatial responses of burnets to environmental changes are unknown (Franzen and Ranius 2004). Pheromone based monitoring without impacting populations has the possibility of rectifying this as many sites can be surveyed with much less effort and expense than traditional sampling methods. Once understanding of the burnet moths' ecology has increased, their potential as biodiversity indicators for semi-natural grasslands can be realised.

Pest management use of pheromones exploits other aspects of mating disruption that are also of interest. Males that have landed directly on the pheromone lure can become contaminated with the pheromone and consequently act as false females attracting males (Nansen et al. 2007). This is utilised to disrupt mating in Lobesia botrana (Denis and Schiffermüller, 1775) (Nansen et al. 2007). Females are also affected by exposure to synthetic pheromone lures (Stelinski et al. 2004). Currently little is known about the effects of exposure but female calling behaviour has been found to be inhibited or disrupted (Stelinski et al. 2004). Calling behaviour in female Spodoptera littoralis (Boisduval, 1833) is significantly disrupted by exposure to synthetic pheromones and in Adoxophyes sp. and Homona magnanima (Diakonoff, 1948) exposure leads to a delay in calling

behaviour and consequently a delay in mating (Stelinski et al. 2004). It would be beneficial for future studies to investigate these effects.

Other potential impacts of pheromone exposure besides those associated with mating disruption may be worthwhile to consider. The presence of female sex pheromones has been found to interfere with male moths' normal responses to predation risk (Skals et al. 2005). A study carried out by Skals et al. (2005) concurrently exposed male S. littoralis moths to female sex pheromones and bat sounds, replicating a predation risk. It was found that males became less responsive to the predation risk as the amount of pheromone increased (Skals et al. 2005). This shows that if a male has detected con-specific female sex pheromone he will not attempt to avoid an approaching bat (Skals et al. 2005). This increased vulnerability to predation could prove damaging to populations being monitored using pheromones. Recently it has been found that exposure to pheromones can adversely affect lifespan (Gendron et al. 2014). Decreased fat stores, reduced resistance to starvation and a reduced lifespan were observed in male Drosophila melanogaster (Meigen, 1830) exposed to female sex pheromones (Gendron et al. 2014). Although these effects were reversed by mating (Gendron et al. 2014); the possible impacts on lifespan should be considered when proposing to expose insects of conservation value to pheromones.

Conclusions

While this study found Z. filipendulae males were not desensitised by exposure to synthetic pheromone lures nor did the presence of a lure in the field adversely affect their ability to locate a female, other mechanisms of mating disruption may impact populations being monitored by pheromones for conservation purposes. These are worthy of investigation before declaring pheromone monitoring of rare insects to be completely safe and to have no impact on the population. Nevertheless a lack of desensitisation and competitive attraction affecting mate location in burnet moths has been demonstrated. This is encouraging given the potential value of pheromones to conservation.

Acknowledgements

References

Andersson K (2012) Pheromone-based monitoring of Elater ferrugineus as an indicator for species-rich hollow oak stands. Masters Thesis, SLU, Sweden.

Andersson K, Bergman KO, Andersson F, Hedenstrom E, Jansson N, Burman J, Winde I, Larsson MC and Milberg P (2014) High-accuracy sampling of saproxylic diversity indicators at regional scales with pheromones: the case of Elater ferrugineus (Coleoptera, Elateridae). Biol. Conserv. 171:156-166. doi: 10.1016/j.biocon.2014.01.007

Bergmann J, Gonzalez A and Zarbin PHG (2009) Insect pheromone research in South America. J. Braz. Chem. Soc. 20:1206-1219. doi:10.1590/S0103-50532009000700003

Campbell JF, Mullen MA and Dowdy AK (2002) Monitoring stored-product pests in food processing plants with pheromone trapping, contour mapping, and mark-recapture. J. Econ. Entomol. 95:1089-1101. doi: 10.1603/0022-0493-95.5.1089

El-Sayed AM and Suckling DM (2005) Behavioural observations of mating disruption in three lepidopteran pests. Behaviour, 142:717-729. doi: 10.1163/1568539054729114

Evenden ML, Mclaughlin JR and Czokajlo D (2005) Effects of exposure to pheromone and insecticide constituents of an attracticide formulation on reproductive behaviour of oriental fruit moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 98:334-341. doi: 10.1603/0022-0493-98.2.334

Franzén M (2002) Occurrence patterns of butterflies (Rhopalocera) and burnet moths (Zygaenidae) in seminatural pastures in southeastern Sweden and their potential and their potential as indicators of species richness. Masters Thesis, SLU, Sweden.

Franzen M and Nilsson SG (2012) Climate dependent dispersal rates in metapopulations of burnet moths. J. Insect Conserv. 16:941-947. doi: 10.1007/s10841-012-9481-4

Franzen M and Ranius T (2004) Habitat associations and occupancy patterns of burnet moths (Zygaenidae) in semi-natural pastures in Sweden. Entomol. Fenn. 15:91-101

Gendron CM, Kuo TH, Harvanek ZM, Chung BY, Yew JY, Dierick HA and Pletcher SD (2014) Drosophila life span and physiology are modulated by sexual perception and reward. Science 343:544-548. doi: 10.1126/science.1243339

Hansson BS (1995) Olfaction in lepidoptera. Experientia 51:1003-1027.doi: 10.1007/BF01946910

Hein S, Binzenhöfer B, Poethke HJ, Biedermann R, Settele J and Schröder B (2007) The generality of habitat suitability models: a practical test with two insect groups. Basic Appl. Ecol. 8:310-320. doi: 10.1016/j.baae.2006.09.012

Huang J, Stelinski LL and Gut LJ (2010) Mating behaviours of Cydia pomonella (Lepidoptera: Tortricidae) as influenced by sex pheromone in electrostatic powder. J. Econ. Entomol. 103:2100-2106. doi: 10.1603/EC10063

Larsson MC and Svensson GP (2009) Pheromone monitoring of rare and threatened insects: exploiting a pheromone–kairomone system to estimate prey and predator abundance. Conserv. Biol. 23:1516-1525. doi: 10.1111/j.1523-1739.2009.01263.x

Miller JR, McGhee PS, Siegert PY, Adams CG, Huang J, Grieshop MJ, Gut LJ and Hildebrand JG (2010) General principles of attraction and competitive attraction as revealed by large-cage studies of moths responding to sex pheromone. P. Natl. Acad. Sci. USA 107:22-27. doi: 10.1073/pnas.0908453107

Musa N, Andersson K, Burman J, Andersson F, Hedenström E, Jansson N, Paltto H, Westerberg L, Winde I, Larsson MC, Bergman KO and Milberg P (2013) Using sex pheromone and a multi-scale approach to predict the distribution of a rare saproxylic beetle. PloS one 8:1-10. doi: 10.1371/journal.pone.0066149

Nansen C, MacDonald KM, Rogers CD, Thomas M, Poppy GM and Baxter IH (2007) Effects of sex pheromone in electrostatic powder on mating behaviour by Lobesia botrana males. J. Appl. Entomol. 131:303-310. doi: 10.1111/j.1439-0418.2007.01167.x

Onufrieva KS, Thorpe KW, Hickman AD, Tobin PC, Leonard DS and Roberts EA (2010) Effects of SPLAT[®] GM sprayable pheromone formulation on gypsy moth mating success. Entomol. Exp. Appl. 136:109-115. doi: 10.1111/j.1570-7458.2010.01009.x

Priesner E, Naumann CM, and Stertenbrink J. (1984). Specificity of synthetic sex-attractants in Zygaena moths. Z. Naturforsch. C. 39:841-844.

Rakosy L and Schmitt T (2011) Are butterflies and moths suitable ecological indicator systems for restoration measures of semi-natural calcareous grassland habitats? Ecol. Indic. 11:1040-1055. doi: 10.1016/j.ecolind.2010.10.010

Schmitt T (2003) Influence of forest and grassland management on the diversity and conservation of butterflies and burnet moths (Lepidoptera, Papilionoidea, Hesperiidae, Zygaenidae). Anim. Biodivers. Conserv. 26:51-67

Sharov AA, Thorpe KW and Tcheslavskaia K (2002) Effect of synthetic pheromone on gypsy moth (Lepidoptera: Lymantriidae) trap catch and mating success beyond treated areas. Environ. Entomol. 31:1119-1127. doi: 10.1603/0046-225X-31.6.1119

Skals N, Anderson P, Kanneworff M, Löfstedt C and Surlykke A (2005) Her odours make him deaf: crossmodal modulation of olfaction and hearing in a male moth. J. Exp. Biol. 208:595-601. doi: 10.1242/jeb.01400

Spitzer L, Benes J, Dandova J, Jaskova V and Konvicka M (2009) The large blue butterfly Phengaris [Maculinea] arion as a conservation umbrella on a landscape scale: the case of the Czech Carpathians. Ecol. Indic. 9:1056-1063. doi: 10.1016/j.ecolind.2008.12.006

Stelinski LL (2007) On the physiological and behavioural mechanisms of pheromone based mating disruption. Pestycydy 3:27-32

Stelinski LL, Gut LJ, Ketner KC and Miller JR (2005) Orientational disruption of codling moth, Cydia pomonella (L.) (Lep.,Tortricidae), by concentrated formulations of microencapsulated pheromone in flight tunnel assays. J. Appl. Entomol. 129:481-488. doi: 10.1111/j.1439-0418.2005.01005.x

Stelinski LL, Gut LJ, Mallinger RE, Epstein D, Reed TP and Miller JR (2005) Small plot trials documenting effective mating disruption of oriental fruit moth by using high densities of wax-drop pheromone dispensers J. Econ. Entomol. 98:1267-1274. doi: 10.1603/0022-0493-98.4.1267

Stelinski LS, Gut LJ and Miller JR (2013) An attempt to increase efficacy of moth mating disruption by co-releasing pheromones with kairomones and to understand possible underlying mechanisms of this technique. Environ. Entomol. 42:158-166. doi: 10.1603/EN12257

Stelinski LS, Gut LJ, Vogel KJ and Miller JR (2004) Behaviours of naïve vs. pheromone-exposed leafroller moths in plumes from high-dosage pheromone dispensers in a sustained-flight wind tunnel: implications for mating disruption of these species. J. Insect Behav. 17:533-554. doi: 10.1023/B:JOIR.0000042540.09188.eb

Svensson GP, Oleksa A, Gawronski R Lassance JM and Larsson MC (2009) Enantiomeric conservation of the male-produced sex pheromone facilitates monitoring of threatened European hermit beetles (Osmoderma spp.). Entomol. Exp. Appl. 133:276-282. doi: 10.1111/j.1570-7458.2009.00923.x

Tcheslavskaia KS, Thorpe KW, Brewster CC, Sharov AA, Leonard DS, Reardon RC, Mastro VC, Sellers P and Roberts EA (2005) Optimization of pheromone dosage for gypsy moth mating disruption. Entomol. Exp. Appl. 115:355-361. doi: 10.1111/j.1570-7458.2005.00266.x

Welter SC, Pickel C, Millar JG, Cave F, Van Steenwyk RA and Dunley J (2005) Pheromone mating disruption offers selective management options for key pests. Calif. Agric. 59:16-22. doi: 10.3733/ca.v059n01p16

Witzgall P, Kirsch P and Cork A (2010) Sex pheromones and their impact on pest management. J. Chem. Ecol. 36:80-100. doi: 10.1007/s10886-009-9737-y

Witzgall P, Stelinski L, Gut L and Thomson D (2008) Codling moth management and chemical ecology. Annu. Rev. Entomol. 53:503-522. doi: 10.1146/annurev.ento.53.103106.093323

FIGURE TITLES

Fig. 1 Diagram illustrating the set up used for the mating tests.

Fig. 2 Top Set up of mating station in field competitive attraction experiment **Bottom** Mean duration, in seconds, of males' visits to females with and without a pheromone lure present with standard error bars

Fig. 3a Mean time taken for virgin males to detect virgin females (shown by exposure of their anal claspers) in immediate, 1 hour and 24 hour test periods in exposed and control groups with standard error bars **b** Mean time taken for virgin males to locate virgin females in immediate, 1 hour and 24 hour test periods in both exposed and control groups with standard error bars **c** Proportion of virgin males that successfully located virgin females in both exposed and control groups in initial, 1 hour and 24 hour test periods with standard error bars