PRELIMINARY EVIDENCE FOR A FEMALE SEX PHEROMONE IN PORINA (*WISEANA COPULARIS*)

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ABSTRACT

Porina larvae (*Wiseana* spp.) are a pest of pasture in New Zealand. Preliminary observations of adult *W. copularis* in a laboratory wind tunnel revealed that female moths fan their wings during dusk just prior to copulation. Females stopped wing-fanning upon arrival of a male moth. Mating lasted two to three minutes and no multiple mating was observed. Gas chromatography analysis of the air surrounding wing-fanning females showed the presence of four volatile compounds. Gas chromatography/electro-antennogram (GC-EAD) studies indicated that male antennae were strongly responsive to one of these compounds. These findings suggest that female moths release a sex pheromone to attract male moths prior to copulation.

Keywords: Hepialidae, sex pheromones, mating behaviour, porina, *Wiseana copularis*.

INTRODUCTION

Porina (*Wiseana* spp.) are a pest of pasture in New Zealand. Early instar larvae are surface-dwelling while later instar larvae dig more or less vertical burrows to the soil surface. The caterpillar emerges at night, cuts pasture (typically grasses and clovers) at its base and draws it back into its silk-lined burrow to consume. Economic injury level is estimated at 20 to 30 larvae per m² but infestations can reach as many as 200 larvae per m² (Chapman 1984). Damage appears from February through to August. Traditionally, control methods have focussed on treatment of larvae with insecticides (Barratt *et al.* 1990).

No studies have examined porina mating behaviour, with information restricted to some anecdotal observations (e.g. French 1973). Adults fly between September and April depending on the species and locality, with a single generation occurring each year (Dugdale 1994). The porina moth lives for a matter of days and has no functional mouthparts. Its primary role is copulation and oviposition. Each female moth is capable of laying approximately 2000 eggs (C.M. Ferguson pers. comm.).

The use of sex pheromones to monitor and control insect pests is becoming increasingly common in insect pest management programmes. Advantages of such a method include target specificity, decrease in pesticide use, ease of application and reduced expenditure (Howse *et al.* 1998). Lepidopteran pests are often targeted with this technology, although there are no reports of research on *Wiseana* spp. Anecdotal observations however, do suggest that female porina moths emit pheromones that are attractive to males (Dugdale 1994; French 1973). Here, we investigate whether female *W. copularis* emit sex pheromones prior to copulating with males.

METHODS

Insect collection and maintenance

Porina pupae and larvae were collected by digging in pasture at the Ross na Clonagh farm between Pahiatua and Ballance, 50km east of Palmerston North in late January 1999. Approximately 250 pupae and 50 larvae were collected from a 4 m² area. Larvae and pupae were brought into the laboratory at Massey University where they were placed vertically in plastic vials (10 cm x 4 cm) that were half filled with moistened

vermiculite and fitted with a wire mesh lid. The larvae were watered every two days and fed with an artificial diet based on Dodgshun (1970) and modified by A. Popay (pers. comm.). The pupae were sprayed with water every two days. Half the pupae and all the larvae were kept at 5°C for one month, after which the temperature was increased to 15°C. The remaining pupae were kept at 15°C. The pupae were checked daily for emerging adults. All moths reared from the pupae and larvae were identified as *W. copularis*. Emerging adults were removed from the temperature control rooms and placed in a laboratory wind-tunnel (2.5 m x 1 m x 1 m). The floor of the wind tunnel was made uneven by placing two loosely stretched pieces of 1 m² cotton mesh inside it. Adult porina were observed in the laboratory wind-tunnel at a wind speed of 0.2 m/sec.

Behavioural observations

Male and female moths became active during sunset for approximately 90 min. As adults only live for approximately three days, males and females were observed for two nights after emergence. This resulted in 27 observation nights. Moths of both sexes were present during 17 observation nights. In total, 45 males and 43 females were observed during sun-set between the hours of 6 pm and 11 pm under a red florescent 36 watt light stationed above the wind tunnel. Moth behaviour, including flying time, duration of wing-fanning and mating were recorded. In addition, humidity, air pressure, air flow, time of day, temperature, female orientation and position, and male presence or absence were recorded.

Pheromone collection and gas chromatography-electroantennogram detection (GC-EAD)

Immediately after a female started wing-fanning in the wind-tunnel and males responded by flying towards the female, the male and female moths were removed from the wind-tunnel. Female moths were transferred to a glass aeration chamber (18 cm x 10 cm) containing three aluminium mesh stands. Up to five female moths were placed inside the aeration chamber at any one time, where they climbed the mesh stands and began wing-fanning. Air was passed through two charcoal filters to remove possible contaminants prior to entering the aeration chamber at a constant flow rate of 300 ml/min. Any volatile compounds emitted from the moths whilst wing-fanning passed out of the chamber and through a 30 cm long glass U-tube immersed in liquid nitrogen. The cold temperature caused the volatile compounds to condense out of the air current onto glass wool packed inside the U-tube. The glass wool and U-tube was then washed with 1 ml pentane and this solution was stored in a 2 ml brown glass vial at 4°C until analysis, when it was evaporated to 10 µl under a stream of nitrogen. The above process was repeated each night that both female and male moths were present in the wind-tunnel resulting in nine samples for gas chromatographic analysis.

Gas chromatographic analyses were carried out on each sample using an HP6890 gas chromatograph (GC) equipped with a flame ionization detector (FID) and fitted with a 5% phenyl and 95% methyl siloxane stationary phase column (HP5, 30 m x 0.32 mm i.d., phase thickness 0.25 mm). The injection mode was splitless for 60 seconds. The temperature program increased from 50°C to 200°C at 10°C/min where it was held for five minutes, resulting in a total retention time per sample of 20 minutes. The injector temperature was set at 150°C and the detector temperature at 275°C. The carrier gas was hydrogen.

The responses of eleven antennae from eleven different male moths to the compounds produced by the females were investigated using a coupled GC-EAD system, in which the effluent from the GC capillary column was delivered simultaneously to the antennal preparation and the GC detector (Wadhams 1990). The response signals from both the FID and the EAD were recorded simultaneously by a PC computer. The one or two day old unmated males that responded to a wing-fanning female in the wind tunnel were removed and anesthetized using carbon dioxide. One of their antennae was excised and suspended between two glass electrodes filled with a 10% KCl solution. Signals from the antenna were passed through an amplifier and data storage and processing were carried out with a PC-based interface and software package (Syntech, Hilversum, The Netherlands). The effluent from the gas chromatograph was delivered into a constant humidified air stream that passed over the antennal preparation. As a 15 cm x 1 cm piece

of glass tubing was inserted between the GC and the antenna, there was a short delay between the FID response and the corresponding antennal response.

RESULTS

Behavioural observations of adult moths

Prior to wing-fanning, a female moth would begin to walk on the floor of the wind tunnel and climb in search of an elevation point. Once settled, females would vibrate their wings before fanning them. The hind wings remained stationary lying along the abdomen. Occasionally, the abdomen was also observed to rotate in circles brushing up against the hind wings. The mean length of time before a male responded by flying upwind towards a wing-fanning female was 3.38 ± 0.6 (se) minutes ($n = 15$). Wing fanning ceased upon arrival of a male moth. On the three occasions when the sexes were not separated for pheromone collection, copulation was observed. The male sat beside the female and curled his abdomen until their genitalia made contact. On all three occasions, copulation lasted between two and three minutes. Males then flew approximately 45 cm above ground level for up to 5 min before settling, while females remained stationary and began to oviposit almost immediately. When laying eggs, the females spun in small circles (5 cm diameter) occasionally fanning their wings. Approximately 1500 eggs were released per moth over a 12 hour period. The mated moths were not observed to mate a second time either with each other, or with another moth.

Females would only wing-fan if the temperature was less than 20°C and during the hours between 6pm and 11pm. If the pressure in the aeration chamber was increased above atmospheric pressure, wing-fanning ceased. Wing-fanning was observed only when the air flow ≤ 300 ml/min. The presence of a male moth was not required for the female to fan her wings. Wing-fanning continued for 21.08 ± 6.02 (se) minutes ($n = 11$) in the wind-tunnel when male moths were not present.

Pheromone collection and gas chromatography-electroantennogram detection (GC-EAD)

A representative gas chromatogram of the air surrounding wing-fanning female moths is presented in Fig. 1. A representative GC-EAD trace of the response of a male antenna to the four volatile compounds is presented in Fig. 2. In both figures, the four volatile compounds are labelled 1, 2, 3 and 4. The male antenna only responded by eliciting a response (labelled with an asterix) to the first of the four female volatile compounds emitted whilst wing-fanning.

FIGURE 1: A gas chromatogram of the four volatile compounds emitted from female *W. copularis* whilst wing-fanning.

FIGURE 2: The GC-EAD response (*) of a male antenna from *W. copularis* to the four volatile compounds emitted from female moths whilst wing-fanning prior to copulation.

DISCUSSION

The preliminary observations and GC-EAD results suggested that the first of the four volatile compounds released by female *W. copularis* when wing-fanning, may be a sex pheromone used to attract males prior to copulation. Confirmation of whether the compound is used as a sex pheromone will be obtained only after further analysis using gas chromatography-mass spectral analysis (GCMS), synthesis of the compound and behavioural experimentation in both the field and laboratory.

Observations made by French (1973), Dugdale (1994) and Q. Wang (pers. observ.) support this study, in that they described a female-released volatile compound(s) that attracted males. In contrast, in the only study that has identified pheromones in hepialids, the male swift moth (*Hepialus hecta*) swarms to attract females. Three male pheromones have been identified from conspicuous tibial scent organs on the front legs of *H. hecta* (Francke *et al.* 1985; Schulz *et al.* 1990; Sinnwell *et al.* 1985). This study did not examine whether males released sex pheromones, as females appeared to attract males when engaged in wing-fanning and lacked tibial scent organs. Of interest however, is that the primary male sex pheromone in *H. hecta* is an unstable compound ((*R*)-6-Ethyl-2-methyl-2,3-dihydro-4H-pyran-4-one). In this study, the suspected female sex pheromone compound could not be traced by GC one month after collection, also suggesting that it is unstable. In support of our observations, Kuenen *et al.* (1994) found that female *Korscheltellus gracilis* (Hepialidae) also initiated wing-fanning as the light intensity reduces, and males downwind of wing-fanning females responded by flying upwind. Kuenen *et al.* (1994) also provided behavioural bioassay evidence to show that females release a sex pheromone from their hind wings.

Female hepialids appear to mate with the first male to arrive (Wagner and Rosovsky 1991). If multiple mating does not occur in porina moths, then a sex pheromone-based method of control could be very useful. In terms of monitoring porina, a pheromonal trap may also have the advantage of targeting individual species. Current methods rely on light traps, which are not specific in the species they attract. Design of a pheromonal trapping system generally depends on the structure, volatility and stability of the pheromone itself (Howse *et al.* 1998). To establish whether porina can be effectively controlled using pheromone traps, the identity of the pheromone, and the mating system and dispersal patterns of porina need to be examined thoroughly.

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