

SEX PHEROMONE COMMUNICATION IN THE APPLE LEAFCURLING MIDGE (*DASINEURA MALI*)

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ABSTRACT

The use of a pheromone for sexual communication by the apple leafcurling midge (ALCM) was demonstrated in field and laboratory tests. In field tests, traps baited with virgin ALCM females caught large numbers of ALCM males. Empty traps and traps baited with ALCM males caught no males. When flight responses of male ALCM to various odour sources were tested in a wind tunnel, males flew upwind and landed on cages that contained virgin ALCM females and on filter papers treated with a pentane extract of virgin ALCM females. Males did not fly upwind to mated ALCM females, odours released by apple foliage, filter papers treated with pentane, or pear leafcurling virgin females. Male ALCM that emerged between the hours of 7:00 and 9:00 a.m. responded to pentane extracts of virgin ALCM throughout the day of their emergence.

Keywords: Cecidomyiidae, mating, monitoring, leaf curling midge

INTRODUCTION

The apple leafcurling midge, *Dasineura mali* Kieffer (Diptera: Cecidomyiidae), is a specialist herbivore which attacks apple trees. It is found in Europe, Great Britain, North America and New Zealand. While formerly of little concern to orchardists in New Zealand, the apple leafcurling midge (hereafter referred to as ALCM) has recently become a serious pest because of large increases in populations (Wilton 1994; Smith and Chapman 1995). Although pesticide resistance is suspected, the cause of these population increases is not known. Adult female ALCM lay their eggs on young apple foliage. These eggs give rise to larvae whose feeding causes distortions in leaf growth (curled leaves) and early senescence of leaves. In addition to a reduction in the productivity of apple trees (Allison *et al.* 1995), ALCM larvae and pupae found on harvested fruit can lead to the rejection of fruit for export to countries without ALCM, such as Japan (Lowe 1994).

A convenient method for monitoring ALCM phenology and population size would contribute to the development of an integrated management programme for this pest. Information about peak emergence of ALCM adults would be particularly useful. For example, adults, and the eggs that adult females produce, may be more susceptible to pesticides than ALCM larvae feeding in leaf shelters or pupae buried in leaf litter and soil beneath apple trees. At present, monitoring of these life stages of ALCM is achieved by counting eggs, a time-consuming method which requires expertise and magnification. In other pest species, particularly Lepidoptera, traps baited with sex pheromone have provided a convenient method for monitoring pests (Wall 1990). Sex pheromone communication has been demonstrated behaviourally in a small number of cecidomyiids (McKay and Hatchett 1984; Williams and Martin 1986).

The present study was initiated to establish if ALCM uses species-specific sex pheromone communication, to determine if apple odours play a role in sexual communication, and to investigate methods that might be used to isolate and identify the sex pheromone of this pest species.

MATERIALS AND METHODS

Insects

Curled leaves of apple (cultivar "Red Delicious") and pear (cultivar unknown) infested with late instar midge larvae were collected from orchards in Palmerston North and Auckland in January-April 1995 and 1996. In the laboratory, the curled part of leaves (pear or apple) was broken open and placed on the surface of petri dishes (10 cm diam) or plastic boxes (15 x 10 x 5 cm) filled with a moist peat-soil mixture. This encouraged mature larvae to exit leaves and to burrow into soil. Containers were covered, placed in a controlled environment chamber (20 ± 1 °C and 60-80% r.h.), and watered when the soil surface dried out. Twelve to 30 days later, adults emerged (between the hours of 6:00 and 10:00 a.m.) and were immediately captured and placed singly in glass vials (2 cm diam x 9.5 cm high). The virgin status of females was confirmed by observing their calling behaviour. As in other cecidomyiids (Bergh *et al.* 1992), the ovipositor of ALCM is elongate-protrusible and is extruded to its full length during calling. Males and females were held in separate rooms (22 ± 2 °C, 50-70% r.h.).

Orchard tests

Two tests were run in a block of "Red Delicious" apples at Huarau Orchards, Palmerston North. In the first test, "Delta" traps (HortResearch, Auckland) were loaded with cylindrical plastic cages containing: (1) 10 ALCM males, (2) 10 virgin ALCM females, (3) 10 ALCM females and 10 ALCM males, or (4) no ALCM. For all treatments, insects were placed in cages (4 cm diam x 7 cm long with cotton screening across both ends) at 10:30 am. At 12:05 pm, three blocks of these treatments were positioned within the orchard using a randomised complete block design, with each block arranged within a single row of trees and separated from other blocks by at least three rows of trees. Traps were hung from the lowest branches of young trees, ca. 20 cm above the ground and 20-25 meters away from traps within the same block. Twenty minutes later, traps were removed from the orchard and taken to the laboratory to count the numbers of trapped flies. In a second test, the same procedures were used to test two treatments: (1) cages with 7 virgin ALCM females and (2) empty cages. Four blocks of this test were placed in the orchard at 11:45 am and removed at 12:15 pm.

Wind tunnel bioassays

Behavioural responses of male ALCM to various odours were tested in a wind tunnel based on the design of Miller and Roelofs (1978). The wind tunnel (Plexiglas walls and aluminium frame) measured 0.95 m high x 0.95 m wide x 2 m long, had a screen at the upwind end, and contained a 10 cm deep layer of moist sand. The 0.7 m diam. fan (Woods Air Movement, G.E.C., Wellington) was connected to a variable motor speed controller. Wind speed in the tunnel was set at 0.3 m/sec. Fourteen full-spectrum composition fluorescent tubes (Biolux, Hamburg) with high-frequency control circuits (Quicktronic Deluxe, Biolux, Hamburg) provided light within the tunnel (310 microwatts/cm²). Room temperature was held at 23 ± 1 °C.

Soon after eclosion, male ALCM were either placed (1) individually or (2) in groups of 10 in cylindrical plastic release cages (4 cm diam x 6 cm long) which had cotton mesh across one end and a screw cap at the other end. During tests, empty cages or cages containing females (treatment cages) were placed 30 cm downwind of the upwind end of the wind tunnel. One to two minutes later, the release cage containing the male or males was positioned in the wind tunnel so that the screw cap side of the release cage was 50 cm downwind of the treatment cage. The screw cap of the release cage was removed and the male(s) within the cage was scored for the following behaviours: flight out of the cage within 2 minutes, upwind flight over a distance of at least 20 cm, and contact with the treatment (the cage or the filter paper). Observations were terminated 5 minutes after the (first) male left the cage or before 5 minutes if the male (1) remained in the release cage for more than 2 mins, (2) contacted the walls of the tunnel, or (3) flew out of the back of the tunnel. Each male was tested only once. Except where noted, all males were tested between the hours of 10:00 a.m. and noon.

In the first test of individual male responses ($n = 20$ individual males/treatment), the following treatments were presented: (1) an empty plastic cage placed downwind

of the screen and 6 branches of artificial foliage placed 10 cm upwind of the screen, (2) a plastic cage with 10 virgin ALCM females placed downwind of the screen and 6 branches of artificial foliage placed upwind of the screen, (3) an empty plastic cage placed downwind of the screen and 6 branches of apple foliage (cultivar "Gala") placed upwind of the screen, and (4) a plastic cage with 10 virgin ALCM females placed downwind of the screen and 6 branches of apple foliage placed upwind of the screen. In a second test, male ALCM ($n=5$ individual males/treatment) responses were tested using the following treatments: (1) an empty plastic cage placed downwind of the screen, (2) a plastic cage containing 5 virgin ALCM females, or (3) a plastic cage containing 5 virgin pear leafcurling midge females. In a third test, male ALCM ($n=20$ individual males/ treatment) were presented with the following treatments: (1) an empty plastic cage placed downwind of the screen, (2) a plastic cage with 10 virgin ALCM females placed downwind of the screen, and (3) a plastic cage with 10 mated ALCM females placed downwind of the screen. In this test, mated females were obtained by placing 10 virgin ALCM females in a cage with 10 male ALCM between the hours of 9:00 and 10:00. None of the females which were left with males prior to testing were observed calling during the wind tunnel test. Females that had not been exposed to males were observed calling throughout the wind tunnel test. In a fourth test, male ALCM ($n=21$ individual males/treatment) responses to an extract of virgin ALCM females were quantified. Pheromone extract was prepared by collecting virgin females between the hours of 6:00 and 9:00 am and then placing females in pentane (Foster *et al.* 1991b) at 9:00 am. One hour later, 5 female equivalents (FE) of this extract or an equivalent volume of solvent was applied to the downwind edge of a rectangular strip of filter paper (*ca.* 0.5 x 2 cm) mounted on a partially straightened paper clip, 3 cm above the soil surface. In a fifth test, male ALCM were placed in cages in groups of 10 ($n=6$ groups of males/treatment) soon after eclosion (8:30 to 9:30 a.m.) and were exposed to 5 FE of virgin ALCM extract at 10:00 a.m., 1:00 p.m., or 5:00 p.m. on that same day or at 10:00 a.m. on the following day.

Statistical Analysis

A randomised complete block design was used for all tests. In orchard tests, the spatial order of treatments was randomised within each block. In wind tunnel tests, the order in which treatments were presented was randomised within each block. Before being subjected to two way analysis of variance, data were tested for homogeneity of variances (Levene's test, JMP, SAS Institute, 1989) and, if heterogeneous, transformed (square root + 0.5). If ANOVAs revealed significant differences among treatments, means were separated by the LSD test.

RESULTS

Orchard tests

When traps were baited with empty cages or cages with 10 males, 10 males and 10 virgin females, or 10 virgin females (Fig. 1), there were significant differences in numbers of ALCM males caught in traps (treatment $F=10.46$, $df=3$, $P<0.009$; block $F=1.09$, $df=2$, $P<0.40$). While traps with virgin females caught a mean of 886 males ($SE=199.09$) over the 20 minute test, traps baited with empty cages, males, or males and females caught 0, 0, and 3.3 males, respectively. In a second orchard test (Fig. 1), traps baited with 7 virgin female ALCM caught a mean of 274 males ($SE=73.6$) over the 30 minute test while traps with empty cages caught no males (treatment $F=20.70$, $df=1$, $P<0.02$; block $F=1.0$, $df=3$, $P<0.50$).

Wind tunnel tests

When male ALCM were exposed to odours from virgin female ALCM and/or apple odours in the wind tunnel (Fig. 2), odours from females but not apple odours influenced numbers of males flying out of cages (virgins $F=192.2$, $df=1$, $P<0.00001$; apple $F=0.18$, $df=1$, $P<0.68$; virgins x apple $F=0.18$, $df=1$, $P<0.68$), flying upwind (virgins $F=380.3$, $df=1$, $P<0.00001$; apple $F=0.25$, $df=1$, $P<0.34$; virgins x apple $F=0.25$, $df=1$, $P<0.34$), and contacting the cage (virgins $F=1083$, $df=1$, $P<0.0001$; apple $F=3.0$, $df=1$, $P<0.11$; virgins x apple $F=3.0$, $df=1$, $P<0.00001$). While almost all of the males exposed to virgin female or virgin females and apple odour flew upwind

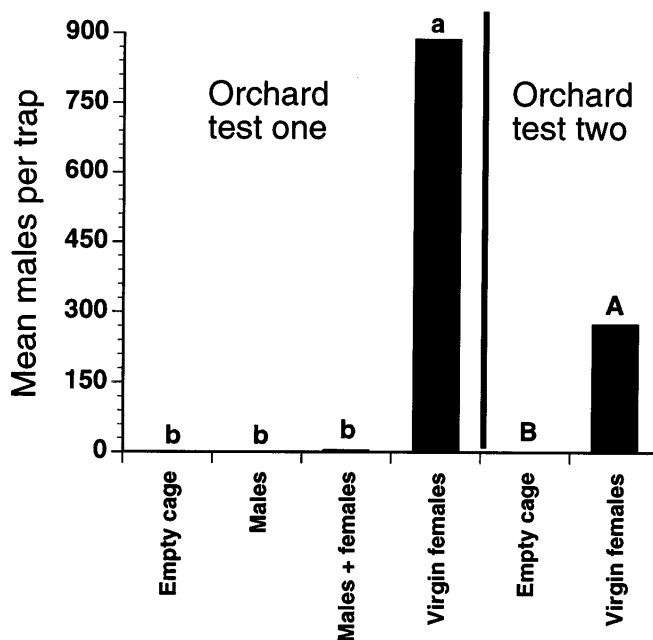


FIGURE 1: Orchard trap catches of male apple leafcurling midges (ALCM) in traps baited with an empty cage, caged ALCM males, ALCM males and females caged together, or caged ALCM virgin females (Orchard test one) and in traps baited with an empty cage or caged ALCM virgin females (Orchard test two). Within each test, means accompanied by different letters are significantly different at $P < 0.05$ (ANOVA and LSD tests).

and contacted the cage that held the females (Fig. 2), none of the males exposed to empty cages or apple odour alone flew upwind or contacted the cage. Pheromone-mediated flight of male ALCM occurred 5-10 cm above the soil surface and varied, sometimes consisting of narrow zig-zags and at other times appearing to be straight flight upwind (track angles near 0°). Upwind flight was sometimes interrupted by casting, which in ALCM consisted of wide sweeps in the horizontal plane. Males were also observed hovering in the windtunnel, without significant progress upwind, for periods of up to three minutes.

In the second wind tunnel test, all of the male ALCM exposed to virgin ALCM females (5/5) flew upwind and contacted the cage containing virgin female ALCM. While some of the ALCM males exposed to empty cages and cages containing virgin pear leafcurling midge females flew out of the release cage (3/5 and 2/5, respectively), none of these males flew upwind or contacted the cage.

In the third test male ALCM were exposed to virgin ALCM females, mated ALCM females, or empty cages. There were significant differences in numbers of males flying out of cages (treatment $F = 57.00$, $df = 2$, $P < 0.0001$; block $F = 2.25$, $df = 3$, $P < 0.18$), numbers of males flying upwind (treatment $F = 361.00$, $df = 2$, $P < 0.00001$; block $F = 1.0$, $df = 3$, $P < 0.45$), and numbers of males contacting the cage (treatment $F = 243.00$, $df = 2$, $P < 0.0001$; block $F = 1.0$, $df = 3$, $P < 0.45$). While an average of 15 and 25% of males exposed to empty cages or mated females flew out of the cage, respectively,

none of these males exhibited upwind flight. Ninety-five percent of the males exposed to virgin females flew out of the cage and initiated upwind flight; 90% contacted the cage.

In a fourth test in which males were exposed to filter papers treated with pentane or a pentane extract of virgin females (5 FE), there was no significant difference in numbers of males flying out of cages (treatment $F=2.49$, $df=1$, $P<0.21$; block $F=1.0$, $df=3$, $P<0.50$); however, there were significant differences in numbers of males flying upwind (treatment $F=48.0$, $df=1$, $P<0.006$; block $F=1.0$, $df=3$, $P<0.50$) and numbers of males contacting the cage (treatment $F=48.0$, $df=1$, $P<0.006$; block $F=1.0$, $df=3$, $P<0.50$). Eighty percent of all males exposed to the extract of virgin females flew upwind and contacted the filter paper while none of the males exposed to solvent flew upwind.

In the fifth and final test, male responses to virgin ALCM extract were tested in the wind tunnel at various times (10:00 a.m., 1:00 or 5:00 p.m.) during the day males eclosed (males eclosed between the hours of 8:00 to 9:30 a.m.) or at 10:00 a.m. on the day following eclosion. Time of testing did not have a significant effect on numbers of males flying out of the cage (treatment $F=1.79$, $df=3$, $P<0.19$; block $F=2.35$, $df=5$, $P<0.09$), numbers of males flying upwind (treatment $F=0.45$, $df=3$, $P<0.72$; block $F=1.22$, $df=5$, $P<0.34$) or numbers of males contacting the cage (treatment $F=0.81$, $df=3$, $P<0.51$; block $F=0.19$, $df=5$, $P<0.96$). Mortality of males during the ten hours that followed eclosion was insignificant (<1%); however, by 10:00 a.m. the following day, 42% of males were dead.

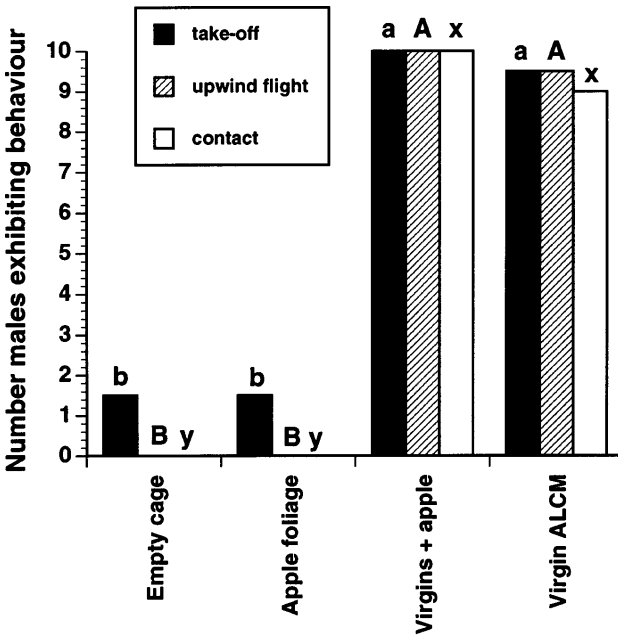


FIGURE 2: Males exhibiting various behaviours when presented with an empty cage, an empty cage and apple odours, caged ALCM virgin females and apple odours, and caged ALCM virgin females. Within means for a single behavioural response, means accompanied by different letters are significantly different at $P<0.05$ (LSD test).

DISCUSSION

Both field and laboratory tests confirmed that female ALCM use a sex pheromone to elicit sexual responses by conspecific males. Communication by sex pheromones may be common in the family Cecidomyiidae but it has only been demonstrated behaviourally in a small number of species, one of which, the brassica pod midge, *Dasineura brassicae* (Williams and Martin 1986), is in the same genus as ALCM. Only one cecidomyiid sex pheromone component has been identified, that from the Hessian fly, *Mayetiola destructor* (Foster *et al.* 1991b). In the Hessian fly, pheromone production is permanently shut down by mating and pheromone titre is significantly reduced one hour after mating (Foster *et al.* 1991a). The latter also appears to be the case in ALCM: in both the orchard and wind tunnel, male ALCM did not fly to females mated one to two hours previously.

Behavioural responses of male ALCM to the female-produced pheromone were similar to those documented for many moth species (Kennedy 1986) as well as the Hessian fly (Harris and Foster 1991). Males were more likely to take flight in the presence of sex pheromone and flew to pheromone sources either by straight flights upwind or by narrow zigzagging flights. Upwind flight was often punctuated by wide casting flights; such flights are also exhibited by moths when the odour plume is lost (Kennedy 1986). Apple odours appeared to have no effect on male ALCM when presented alone or in conjunction with virgin females. This contrasts with the upwind flight responses of mated female ALCM, which are triggered by odours from young apple foliage (Galanihe and Harris unpublished results).

Although we were able to test the responses of only a small number of ALCM males to virgin female pear leaf curling midges, it appears that these species use different sex pheromones. While male ALCM exhibited consistent responses to virgin female midges reared from apple, they did not respond to virgin female midges reared from pear trees that grew in the same orchard. Although apple and pear leafcurling midges have been designated as species on the basis of their different host plants, the two midges do not show obvious differences in traits commonly used to distinguish cecidomyiid species (i.e., genitalia) and are not regarded as well-defined species (R.J. Gagne, Systematics Entomology Laboratory, U.S.D.A., pers. comm.). Identification of sex pheromones of these two leafcurling species, as well as other *Dasineura* species, may contribute to a further understanding of the systematics of this poorly understood, but important, cecidomyiid genus (R.J. Gagne, pers. comm.).

Research is currently underway to identify the sex pheromone of ALCM. We have shown that the first step of this identification, making a behaviourally active extract of the pheromone, can be achieved by placing virgin ALCM females in pentane for periods of 1-2 hours. We have also shown that a wind tunnel can be used to assay the behavioural responses of male ALCM during pheromone isolation and identification. While assays using y-tube olfactometers are generally quicker to run (as in the Hessian fly, Foster *et al.* 1991b), wind tunnel assays require fewer males, an important consideration given the labour-intensive nature of rearing adult ALCM. For bioassays, ALCM males have two advantages over Hessian fly males: they live longer and exhibit responses to sex pheromone throughout the day rather than only during a period of 2-3 hours.

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