Kinky sex and suicidal mating behaviour in *Aphidoletes aphidimyza*

Mass production of *Aphidoletes aphidimyza* is often difficult because usually a large proportion of the females are infertile or lay very few eggs. By accident we found that 'dirty' rearing cages containing spider webs resulted in a much higher reproduction. When we analysed this problem, it became clear that *A. aphidimyza* use spider webs for mating. After a female has landed in a spider web she starts to emit a pheromone. Males react to this pheromone, land in the spider web, move to the female and mate. Both males and females will leave the spider web after mating. We discuss why *A. aphidimyza* uses spider webs for mating even though it runs the risk of being discovered and devoured by the spider*.

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Introduction

The family gall midges, Cecidomyiidae (Diptera), contains both very serious herbivorous pest species as well as important zoophagous predators for biological control of pests (Harris & Foster 1999). There are over 5000 described species of Cecidomyiidae but, likely, many more species await discovery. Gall midges show a wide variety in feeding habits, including mycetophagy, zoophagy and phytophagy (Barnes 1956). Most phytophagous and zoophagous species are oligophagous or monophagous (Gagné 1989).

The species *Aphidoletes aphidimyza* (Rondani) is entomophagous and is used in commercial inundative or seasonal inoculative biological programmes for control of several aphid species in greenhouse crops, such as sweet pepper and tomato, since the 1970's (Rabasse & van Steenis 1999, Van Lenteren 2000, Van Schelt & Mulder 2000). Larvae of this gall midge prey on almost all true aphids, so the species can be considered as polyphagous in its feeding habits.

Earlier, rearing methods have been studied by Havelka & Zemek (1999) and Kulp *et al*. (1989). Still, mass rearing is cumbersome, and *A. aphidimyza* is therefore expensive in comparison with other natural enemies (Gilkeson 1987, Van Lenteren *et al*. 1997). One of the problems is the mating behaviour, as unmated females do not oviposit. During mass rearing the gall midges are often kept in small containers, in which reproduction is poor. Increasing the size of the rearing containers resulted in somewhat better reproduction, but presence of suitable mating sites seems more important than container size. Van Schelt & Mulder (2000) found under laboratory conditions a strong correlation between availability of spider webs and mating frequency. However, little is known about mating behaviour of *A. aphidimyza* and even less about the role of sex pheromones in Cecidomyiidae.

In this paper we summarize the rather spectacular and risky mating behaviour of *A. aphidimyza*. Information about anatomy of the pheromone glands and experiments on attractiveness of the pheromone can be found in Van Lenteren *et al*. (2002).

Materials and methods

Aphidoletes aphidimyza was reared by a commercial supplier (Koppert Biological Systems, Berkel en Rodenrijs) until the pupal stage and then shipped in plastic bottles filled with vermiculite to the Laboratory of Entomology, Wageningen University. Shipment from the supplier to the laboratory took c 24 hours. Cages (46 x 37 x 32 cm) with three metallic grid walls and a plexiglas front wall and plastic cylinders (15 x 8 cm) were used to observe adult emergence and mating behaviour. Spiders (*Pholcus phalangioides* (Fuesslin) (Pholcidae)) were released in the cages or cylinders for a few hours until they had made several webs. The cages and cylinders were kept in a climate room at a temperature of 23 ± 2 °C, 65-75% relative humidity, and with a reversed photoperiod of L16-D8 (dark: 09.00 - 17.00 hrs). To create a sunset and sunrise effect, lights started to decrease in intensity at 08.30 hrs and were switched off completely at 09.00 hrs, at 16.30

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hrs lights were switched on and reached full intensity at 17.00 hrs.

To determine the period of emergence of adult gall midges, pupae were put into a cage in a room with 20 ± 3 °C, 60 ± 10% relative humidity and a natural photoperiod. The cage was checked once every 30 minutes from sunset until the gall midges had emerged. To study courtship and mating behaviour *A. aphidimyza* pupae were put in cages after the spiders had been removed. Upon emergence, courtship and mating behaviour were observed by video recording. Mating behaviour was studied during the dark period; a 20-Watt spotlight was used for illumination to check whether mating occurred. After the experiments without spiders, observations of matings were made while spiders were present. In addition to the spider webs, various other materials (twigs, cotton wool, electricity wires, synthetic silk, and cotton strings) were offered to the gall midges as mating sites.

Results

Most adults emerged between midnight and 02.00 hrs. After emergence, the gall midges stood still during *c* 30 minutes, presumably while the exoskeleton was hardening. They discharged liquid meconium which dried in a few minutes. Then they took flight and landed after a few seconds in the spider web. Both males and females first touched the web with the tarsi of the front legs. Sometimes they held the web only with the front legs (figure 1); in other cases they also used one or two legs of the second pair. Males were flying more actively then females. Flights lasted usually a few minutes and the insects hung in the spider web between flights. The diel flight period of *A. aphidimyza* started after sunset and ended approximately two hours before sunrise

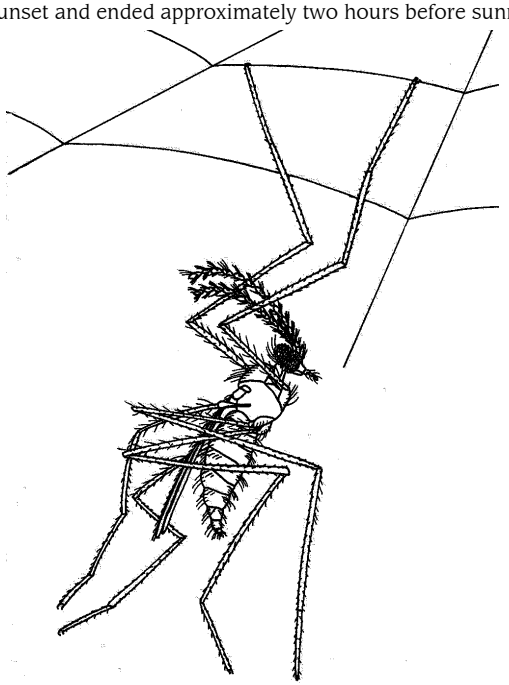

Figure 1. Schematic drawing of *Aphidoletes aphidimyza* female hanging in spider web. Drawing: R. Romani (after G. Grandi)
Schematische afbeelding van een vrouwtje Aphidoletes aphidimyza *in een spinnenweb.*

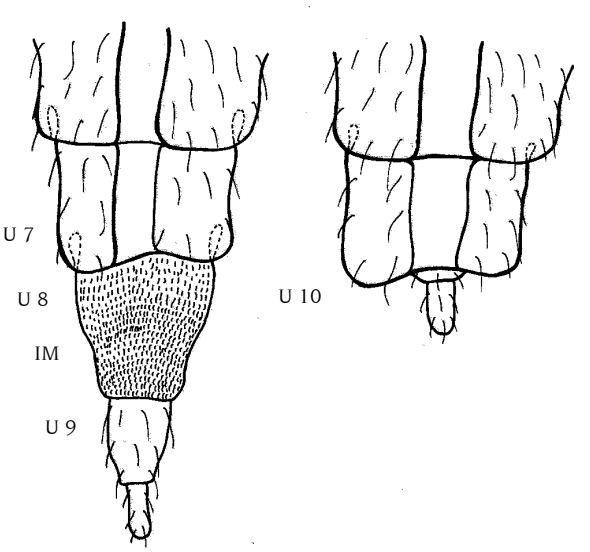

Figure 2. Schematic drawings of terminal abdominal segments with exposed pheromone glands of 'calling' female (left) and idem in non-'calling' female (right). U7- U10 = 7th-10th uromeres; IM = 8th-9th intersegmental membrane. Drawing: R. Romani (after G. Grandi)
Schematische afbeelding van laatste achterlijfsegmenten van een 'roepend' vrouwtje met uitgestulpte feromoonklieren (links) en idem voor een niet-'roepend' vrouwtje (rechts).

under a natural photoperiod in the laboratory. A similar activity period was observed under an artificial photoperiod. During the light period, the males and females were generally inactive and hung in the spider web.

Shortly after having landed in the spider web the females started 'calling' (*ie* emitting a pheromone): they extruded the terminal part of the abdomen and moved it slowly (figure 2 left). Mating behaviour was recorded in the cages during several consecutive nights. Males which had landed in the web as described above approached females frontally while wing-fanning and by moving the front legs along the spider web. In front of females males bended the abdomen towards them and then clasped the female's genitalia with the *forceps copulatrix* and mated in a face-to-face position (figure 3). While mating male wing fanning was interrupted, with wings initially extended and then folded against the body, as in the female. Mating lasted two to three minutes. When there were many males in the cage, mating was often interrupted prematurely. After mating males flew away while females stayed in the web for several hours. Mated females were not observed to extrude the ovipositor or to call. Shortly after mating, females were still approached by males, but no matings occurred because the females flew away. After *c* one hour the females were no longer approached by males.

More than 100 matings were observed in spider webs. Matings in the presence of spiders follow the same pattern, although premating and mating behaviour is interupted as soon as the gall midges percieve any movement. In addition, matings were observed five times in twigs to which the spider webs were attached, or on the metal grid of the cages. Females were observed to 'call' while clinging to the other offered materials, but here no matings were observed, even though these tests lasted seven days during which the fema-

les were observed once an hour during scotophase.

A peculiar behaviour was regularly observed: males were observed to be attracted to and tried to copulate with other males that had just mated. We suppose that these latter males are contaminated with the sex pheromone of the female.

Discussion

Mating behaviour in Cecidomyiidae

Peak emergence of adult gall midges is generally either at dawn or at dusk (Gagné 1989). In *A. aphidimyza* it occurs at dusk. The adult life span of most gall midges, including that of *A. aphidimyza*, is short. Without food and water *A. aphidimyza* males and females live up to four days.

The literature on 'calling' and other mating behaviour and on reproduction in gall midges was recently reviewed by Harris & Foster (1999). In *A. aphidimyza* extension of the ovipostitor is short compared to other cecidomyiid species. Also, abdominal movement in *A. aphidimyza* is limited compared to other species (Isidoro *et al*. 1992, Isidoro & Colazza 1994; Isidoro unpubl.). Very little information is available on 'calling' periods and duration of 'calling' bouts in gall midges. 'Calling' may occur during day or night. Calling bouts can last from less than one minute in the Douglas fir cone midge *Contarinia oregonensis* Foote to up to 20 hours in the Hessian fly *Mayetiola destructor* (Say) (Harris & Foster 1999). In *A. aphidimyza* females were observed to 'call' continuously up to eight hours during several nights.

Harris & Foster (1999) state that courtship behaviour in gall midges appears to be remarkably uncomplicated: the male attempts copulation, and generally succeeds, immediately after finding a virgin female, without any apparent movement by the female. Male and female mate either in parallel position or the male climbs on her back. Mating behaviour of *A. aphidimyza* is, however, rather complex. Females and males first have to land safely in the spider web, then the male approaches and mates in a face-to-face position. Copulation lasts 10-20 seconds for several gall midge species, but lasts up to 170 seconds for the orange wheat blossom midge *Sitodiplosis mosellana* (Gehin) (Harris & Foster 1999), which is similar to what we found for *A. aphidimyza*.

Male cecidomyiids are able to mate many times, but females appear to be monogamous (Harris & Foster 1999). This monogamy is the result of the termination of calling behaviour and production of sex pheromone (Foster *et al.* 1991). After this, females are no longer attractive to males. The changes in behaviour of *A. aphidimyza* females after mating fit into this pattern: withdrawal of the abdominal part and the pheromone gland, termination of 'calling' movements, refusal to mate, and flying away from the spider web when males keep approaching. Females no longer attract males one hour after mating. These observations strongly suggest that females mate just once. This might partly explain the low reproduction of *A. aphidimyza* in mass production situations where good mating sites are absent or where a high proportion of males is present. At such conditions mating is often interrupted and females are not fertilized, thus preventing reproduction.

Why do Aphidoletes aphidimyza *mate in spider webs?*

Gall midges are often found as prey in spider nests (Isidoro, pers. comm.) and spider webs. *Aphidoletes aphidimyza* seems to have developed a range of adaptations in order to use spider webs as a site for 'calling' and mating. We observed that the gall midges fly with their front legs protruded; when they touch a thread of the spider web the spider perceives the landing and moves towards the gall midge. Because of the very delicate motions of the gall midges in the web, the spider quickly loses interest as the web is hardly moving. Also during 'calling' barely any movement occurs. Other gall midge species may show extensive abdominal movement to aid pheromone dispersal (Isidoro unpubl.). We speculate that *A. aphidimyza* females make use of the position of spider webs - which are usually at sites exposed to air currents - to be able to release pheromones without having to make these abdominal movements.

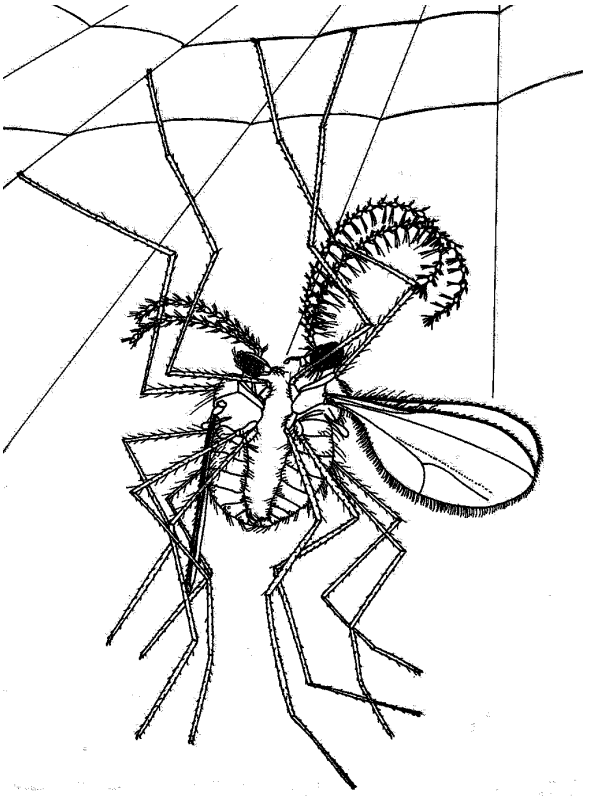

Figure 3. Schematic drawing of face-to-face mating behaviour of male and female *Aphidoletes aphidimyza*. Drawing: R. Romani (based on drawings by G. Grandi)
Schematische afbeelding van tegenover elkaar hangende parende Aphidoletes aphidimyza *in een spinnenweb.*

The face-to-face mating behaviour could be interpreted as another adaptation, which makes it possible to mate in a web without extensive movements. Other species of gall midges are showing more action during mating (Isidoro, unpubl.). Directly after mating the gall midges show no movement. During the presence of gall midges in the web, spiders are seen walking about the web without discovering the gall midges. However, when gall midges perceive movement of the spider, they immediately become quiet.

Yet another adaptation of these gall midges is that they are capable of landing in and leaving from a spider web without getting caught. It is unclear how *A. aphidimyza* females find the spider webs; they may use visual cues, which is un-

likely because they are active at night, or chemical information, which seems more plausible. Schulz & Toft (1993) found that females of several species of Linyphiidae (the second-largest spider family) produce sex pheromones which are emitted via the spider web. In several species of linyphids, pheromones are composed of 'volatile acids that may form a less specific long-range pheromone, that is embedded in a species-specific matrix that accounts for close-range recognition' (Schulz & Toft 1993). Particularly the less specific long-range components of these pheromones may play a role in the web-location behaviour of female gall midges, and this will be a topic of further research.

Finally, the risk for *A. aphidimyza* of being eaten by the spider may be compensated by the benefit of being 'protected' by the spider from other predators.

Mating sites and mass production of Aphidoletes aphidimyza

Mass rearing of *A. aphidimyza* is difficult and makes these predators very expensive biological control agents. Our knowledge of adult emergence, mating period and mating behaviour - making use of spider webs - leads to several suggestions for improvement of current mass production methods. The short life-span, and the fact that most of the males emerge before the females, make a proper timing for provisioning spider webs necessary. It is suggested to introduce pupae ready to emerge in large cages (50 x 50 x 50 cm or larger) containing spider webs. The sex ratio of the emerging adults should not be biased towards males, as excessive numbers of males lead to interrupted matings and to females unable to reproduce. An alternative for spider webs which would be easier to produce and manipulate has not yet been found.

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Samenvatting

'Kinky seks' en suïcidaal paargedrag van de galmug *Aphidoletes aphidimyza*

De galmug *Aphidoletes aphidimyza* is een heel goede biologische bestrijder van bladluizen. Het kweken van grote aantallen galmuggen is echter niet eenvoudig, aangezien veel vrouwtjes onvruchtbaar zijn of weinig eieren leggen. Bij toeval werden in 'vieze' kweekkooien met spinnenwebben veel meer galmugna-komelingen gevonden. Bij nader onderzoek bleek dat de galmuggen spinnenwebben gebruiken om in te paren. Nadat een galmugvrouwtje in een spinnenweb is geland begint ze een feromoon te verspreiden. Mannetjes komen op het feromoon af, landen in het spinnenweb, bewegen al hangend in het web naar het vrouwtje en paren. Zowel mannetjes als vrouwtjes verlaten het spinnenweb na paring. Aanpassingen van galmuggen om in spinnenwebben te kunen paren worden besproken en getracht wordt de vraag te beantwoorden waarom galmuggen in spinnenwebben paren, gezien het risico om door de spin ondekt en opgegeten te worden.