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Host-finding Behaviour of Female Apple Leaf Curling Midge, *Dasineura mali* Kieffer (Diptera: Cecidomyiidae)

A thesis presented in partial fulfilment of the requirements

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Dedicated to my loving parents

who showed me the way to succeed in my life

Abstract

In the past few years New Zealand populations of apple leaf curling midge, *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) (hereafter referred to as ALCM) have exploded, making control in commercial apple orchards more difficult. The present studies were initiated to generate information about the basic biology and behaviour of adult female ALCM. Experiments were conducted in the laboratory during two consecutive summer seasons in 1994/95 and 1995/96 at Massey University, Palmerston North and at HortResearch, Mt. Albert Research Center, Auckland, respectively.

The diel emergence patterns of adult ALCM males and females were synchronized. Adults of both sexes started emerging at 05.00 h, with approximately 95% of adults emerging before noon. Females exhibited calling behaviour (a posture associated with the release of sex pheromone) within minutes after emergence. After mating females ceased calling. When tested in a wind tunnel containing apple foliage, mated females were rarely active before 10.00 h. After this, greater numbers of females flew upwind and landed on apple foliage, with peak responses to apple foliage occurring after 14.00 h.

When given a choice between apple and pear foliage, female ALCM oviposited four times more eggs on apple than on pear. However, when given no choice between plant species females oviposited similar numbers of eggs on the two plant species. Female ALCM laid more eggs on immature apple leaves and buds than on mature apple leaves.

Chemical cues from apple foliage were found to be of major importance in the host-finding behaviour of ALCM females. Volatile

chemicals from apple foliage triggered upwind flight, approach and landing. Volatile chemicals from a non-host plant, pear, stimulated only half as many females to fly upwind and rarely stimulated approach or landing. Females were more responsive to chemical stimuli from immature foliage of apple than to stimuli from mature foliage.

A dichloromethane extract of apple leaves increased the percentages of females flying upwind and approaching extract treated filter papers six and thirty times, respectively, over filter papers treated with dichloromethane. Out of the females that flew upwind to apple foliar extracts, 48.7% landed and 23% exhibited post-landing plant-examination behaviours. No females landed on filter papers treated with dichloromethane.

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Chapter I: Introduction

The apple leaf curling midge, *Dasineura mali* Kieffer (hereafter refered to as ALCM), is a dipteran pest of apples. By feeding on apple foliage, ALCM larvae cause developing leaves to curl rather than unfold. Curled leaves eventually turn brown and drop prematurely. Larval feeding thus stunts shoot growth, especially on young trees. A second problem occurs with ALCM infestations when mature larvae exiting leaf curls enter the stalk or calyx end of mature fruit and pupate there instead of in the soil (Tomkins et al., 1994). Such infested fruit cannot be exported and can jeopardise the export of fruit grown in the same orchard block. Specific tolerances to the presence of live ALCM larvae or pupal cocoons on fruits apply for some markets. Japan, for example, which imported 400 tonnes of New Zealand apples in 1994 (Anon., 1995a), has a zero tolerance for ALCM (Anon., 1994). For other export markets, a 20 percent tolerance is allowed (Anon., 1994), e.g., the USA and Europe. However, even when ALCM populations are not high enough to exclude fruit from export to these markets, economic returns to the grower may be reduced because of costs associated with careful examination of fruit during quality control inspections and with labelling, documenting and segregating affected lines of fruit (Anon., 1994). As apples are one of New Zealand's major export crops, rejection of apples from the export market due to contamination of ALCM can have serious effects on the economy of the country.

ALCM was introduced to New Zealand from the Netherlands in the 1950s. During the decades that followed its introduction, it was only a minor pest in New Zealand. However, in recent years ALCM populations have increased greatly, making it one of the most important pests of apples in New Zealand (Anon., 1993). Pesticide resistance was suspected to be the

cause of the population outbreak of ALCM, but a recent study found that resistance to azinphos-methyl was not quantifiable (Chapman and Evans, 1995). Theoretically, pesticide resistance may be promoted in ALCM because of the protected nature of the larval stage within the leaf curls. This may result in sub-lethal exposure to insecticides, with susceptible individuals killed but individuals with heterozygous or homozygous genes for resistance surviving and contributing to the development of insecticide resistance (Dent, 1991; Anon., 1993).

Little is known about factors regulating populations of ALCM. It has been documented that ALCM is attacked by three species of natural enemies, two predators, *Sejanus albosignatus* (Anon., 1994) and an anthocorid bug, probably *Orius (Triphleps) insidiosa* Say (Whitcomb, 1934), and a hymenopterous egg parasite, *Platygaster demades* Walker (syn. *Prosactogaster demades*), which was introduced to New Zealand in 1925 to control the pear leaf midge, *Dasineura pyri* Bouche (Todd, 1956). Although the parasite *Platygaster demades* is well-established throughout New Zealand, its life-cycle is not well-synchronised with that of ALCM. The parasite generally attains high levels of parasitism (44-95%) at the beginning and during the later part of the season, but is present only at low levels (0.7-2.8%) when the second generation of the midge occurs (Todd, 1959). This lack of a generation in the middle of the summer may restrict the effectiveness of *P. demades* as a control agent. No apple varieties are resistant to the pest (Todd, 1959); however, the degree of susceptibility varies among apple varieties. Infestation is in part determined by the quantity of terminal growth present.

In the recent past, ALCM management had been incidental to standard New Zealand orchard pest control practices. These practices are based on

insecticidal control of key apple pests: leafrollers and codling moth. However, full season programmes of insecticide applications are no longer able to effectively control ALCM (Tomkins, 1995). In 1993/94, a survey of pip fruit growers in the Waikato and Bay of Plenty revealed that increased use of diazinon and dimethoate sprays by growers for the control of ALCM had not resulted in effective control of the pest (Tomkins et al., 1994). A similar situation also was revealed in a survey conducted in 1994/95 in the Nelson district (Smith and Chapman, 1995). The increased use of insecticides in apple orchards for control of ALCM has many obvious disadvantages: higher residue levels at harvest, increased costs of production, and increased risk of the development of insecticide resistance in ALCM and other apple pests.

The research conducted on ALCM in New Zealand has dealt mainly with the pest status of ALCM, its seasonal history, varietal susceptibility, parasitism, and chemical control (Todd, 1956 & 1959; Woon and Haydon, 1973; Anon., 1993 & 1994; Tomkins et al., 1994). Several research projects are currently underway at Lincoln University and HortResearch to study its phenology and life cycle, factors affecting the triggering of emergence of the adult flies from the soil, resistance and the efficacy of insecticides, and the identification of sex pheromones (Anon., 1994).

One aspect of ALCM biology that has not been studied is the host-finding behaviour of the female. Like many other *Dasineura* species, ALCM is believed to be monophagous, having only one host, apple (Whitcomb, 1934). This specialisation on apple may be due to monophagy of ovipositing females or monophagy of larvae or monophagy of both adult females and larvae. However, as there is no evidence in the literature for the movement of ALCM larvae from their original feeding sites to other feeding

sites, monophagy of the adult female for apples will give larvae no choice but to develop on apple. Host-plant location and accurate egg placement by adult females are therefore critical aspects of the survival of the species. Furthermore, damage to the apple plant caused by the larval stage of the insect occurs only after the adult female has found, identified and accepted the plant as a host.

Understanding key factors involved in the insect-plant relationship between ALCM females and the apple plant is important for the development of alternative control techniques. As regards behaviour, the responses of a pest to its host-plant are fundamental to understanding the relationship between the pest and a crop. For example, information on the stimuli that are used by adult ALCM females to locate their host-plant may be useful in developing novel methods of controlling ALCM, such as varieties resistant to the pest via antixenosis. A second application would be the use of kairomones for monitoring populations of adult ALCM females. Finally kairomones used by female ALCM might also be used by the parasitoid *P. demades* to find ALCM eggs, and therefore might be useful in improving the efficacy of parasitoids (Lewis and Martin, 1990).

The present study was undertaken to document sensory inputs mediating host-finding behaviour of ALCM. More specifically, this research programme addressed the question: what plant stimuli influence host-finding by ALCM females? To answer this question, it was necessary to develop rearing and handling methods for adult ALCM and also to study reproductive behaviour in general, as well as the life history of adult midges. The latter studies are also discussed herein.

Chapter II: Literature Review

Apples are one of New Zealand's principal horticultural crops. The area planted in apples in New Zealand was estimated in 1994 to be 22,437 hectares (Anon., 1995b). Apples are a major export crop of New Zealand: 209,765 tonnes of fresh fruit valued at 326.7 million NZ dollars were exported in 1994 (Anon., 1995c). Hence, apple is an important crop for the export-oriented economy of New Zealand.

In New Zealand, apple crops are damaged by about 12 different insect pest species (Penman, 1978). The key pests are codling moth and the leafrollers: *Epiphyas postvittana* (Walker), *Ctenopseustis obliquana* (Walker), *Ctenopseustis herana* (Felder & Rogenhofer), *Planotortrix octo* (Dugdale), and *Planotortrix excessana* (Walker). Wooly apple aphid, mealy bugs, apple leaf hoppers, bronze beetle, European red mite, scales and apple leaf curling midge (ALCM) are secondary pest species. In the past, the key pests of apples, as well as the secondary pests, were successfully kept under control by regular applications of chloropyriphos and azinphos-methyl (Anon., 1994).

The incidence of the ALCM has been increasing over the last few seasons. In several districts it has become a very difficult pest to control. The reasons for this are not clear. Hence, it is necessary to develop alternative methods for controlling this pest.

The aim of this research project was to document basic information on the sensory cues mediating host-finding behaviour of apple leaf curling midge. This chapter presents relevant information on all aspects of apple leaf curling midge biology, with an emphasis on sensory cues involved in

host-finding and acceptance by phytophagous insects, sensory mechanisms and sense organs of insects.

II.1 General characteristics of cecidomyiid flies

Cecidomyiidae is a very large family in the order Diptera and contains a large number of minute, fragile flies. These flies are characterized by having reduced wing venation and long moniliform antennae with whorls of bristles (Hill, 1987). Cecidomyiid flies are short-lived as adults, with most species probably living less than a day or two (Gagné, 1989). The majority of species belonging to the family Cecidomyiidae are phytophagous. Among these phytophagous species, most are monophagous, restricted to one host plant species, or oligophagous, restricted to few host species. Few species are polyphagous, living on hosts that are not closely related (Gagné, 1989). Most midge species cannot be easily identified, as many adult and larval forms of different midge species show similar morphological characteristics. Typically, midge species are identified by the distinctive damage they produce in a specific host-plant species.

Different species of the family Cecidomyiidae vary to a large extent, both anatomically and biologically (Gagné, 1989). However, some generalities can be made. For example, cecidomyiid larvae generally are spindleform and legless. All or most feeding is done by the larval stage. Larvae feed by sucking plant juices, which are broken down by salivary secretions and then ingested (Gagné, 1989). The larval stage may last from less than two weeks to more than two years. Species with long-lived larval forms spend most of their larval stage in diapause. Some cecidomyiid larvae leave the host when full-grown; others pupate in the gall and leave only as adults. Cecidomyiid larvae that leave the gall to pupate always have a

spatula, an elongate, sclerotized epidermal structure on the venter of the prothorax. Such larvae are usually found in simple leaf folds or live freely in plant parts. When fully grown they simply crawl out and drop to the soil. Once on the ground, larvae move to a suitable site and then dig into the soil with the spatula (Gagné, 1989). Larvae of cecidomyiids survive unsuitable dry or cold periods by diapause. In the temperate zone, diapause most commonly occurs in overwintering, full-grown larvae (Gagné, 1989).

II.2 Apple leaf curling midge

II.2. 1 Taxonomic relationships of apple leaf curling midge, *Dasineura mali*

Out of the large number of phytophagous species in the family Cecidomyiidae, *Dasineura* species are classified under cecidogeneous species which are true gall formers, and are further categorized as species that form leaf and leaflet semi-galls (Hill, 1987). The genus *Dasineura*, which is the largest genus within the family Cecidomyiidae, has 136 species recorded in the UK (Penman, 1978) and 101 species recorded in North America (Gagné, 1989). Most *Dasineura* species are host specific and produce distinctive larval damage (Penman, 1978). Buds, leaves, petioles, terminal shoots, flowers, fruits and grafted buds of many fruit crops, viz. apple, apricot, plum, pear, olive, pitanga, mango, myrobalan, black currant, blackberry, raspberry, dewberry, blueberry, cranberry, bilberry, gooseberry, barberry, are attacked by species of gall midges in the genus *Dasineura* (Barnes, 1948).

There are four species of gall midges which attack apples grown in various parts of the world: namely, *Dasineura mali* Kieffer, associated with leaves; *Contarinia mali* Barnes, with flowers; *Thomasiniana oculiperda*

(Rubsaamen) with grafted buds and an unidentified gall midge species with stems. Besides these gall midge species, larvae of a cecidomyiid species are sometimes found on the surface of mite-infested leaves of apple. These species are free-living and predatory on mites (Barnes, 1948). *Dasineura mali,* the apple leaf curling midge (ALCM), is the only midge species damaging apples in New Zealand.

II.2.2 Description of apple leaf curling midge

Adult ALCM are minute, delicate, two-winged flies with long legs and iridescent wings. The adult female midge is slightly larger than the male and has body and wing lengths of 1.5 to 2.5 mm and 1.5 to 2.0 mm, respectively. The abdomen of adult ALCM midges is covered with black scales dorsally, while the legs and thorax are brownish. The female midge has a reddish colour on her abdomen (Barnes, 1948) and a long, telescoped, and tapering soft ovipositor with fused cerci. The later is characteristic to the genus *Dasineura* and is suitable for placing eggs in crevices between buds or unfolded leaves. Eggs are elliptical in shape, being about five times as long as wide, and are orange in colour. Following hatching, the larvae are white to creamy white. This colour deepens to orange when larvae mature. Mature larvae are 2 to 4 mm long. The pupa, which is also orange in colour, is enclosed in a tough white, silken cocoon (Barnes, 1948).

II.2.3 Life history

Adult ALCM emerge from the soil and swarm approximately 2 ft. above the ground immediately below the tree canopy (Todd, 1956). This swarming of adults may be related to the production of a sex pheromone by virgin females and male flight responses to this sex pheromone (Harris et al.,

1996). Swarms eventually disperse and female ALCM commence oviposition (Todd, 1956). Females lay their eggs in groups on the margins or upper surfaces of uncurled or partly uncurled leaves (Barnes, 1948). The numbers of eggs laid in each egg batch has not been documented. A large number of eggs (as many as 50-70) has been observed in a single group of eggs (personal observation). The number of eggs per attacked shoot varied from 198 to 404 in the first generation of ALCM in America (Barnes, 1948 and references therein). The incubation period for eggs ranges from 3 to 5 days (Barnes, 1948).

Neonate ALCM larvae commence feeding on the unopened leaf, damaging the growing tissue of the leaf, and as a result prevent it from uncurling (Whitcomb, 1934). Infested leaf curls become tighter as the growth of the leaf proceeds. As larvae continue to feed, the leaf becomes hard, brittle and brown in colour and drops prematurely. There is little direct evidence of feeding on the leaf other than the presence of small red blisters on the exterior of the curled leaf (Whitcomb, 1934; Todd, 1956). The number of larvae found in a single leaf varies considerably. In New Zealand, Todd (1956) recorded 147 - 491 larvae per leaf (mean of 265.5) during February and 27-209 (mean of 92.8) larvae in April. Larval development takes about three weeks (Todd, 1956). However, spells of dry weather can keep larvae imprisoned in leaves for up to 10 days longer than normal and thereby delay pupation (Barnes, 1948). Softening of tightly rolled leaves caused by rainfall appears to trigger movement of mature larvae out of leaf curls. Studies conducted by Whitcomb from 1936 to 1942 (Barnes, 1948) suggested that the time of descent of the larva, and therefore time of pupation, is probably influenced by rainfall as well as the length of time required for larval feeding and development.

Whether triggered by rain or by maturation, the majority of larvae leave foliage and fall to the ground to pupate either among fallen leaves or just below the surface of the soil (Barnes, 1948). A smaller number of larvae (1) crawl down the trunks and, under these circumstances, may pupate under the rough bark or in any other shelter on the tree (Todd, 1956), (2) fall onto fruit and pupate in the calyx or stalk cavities (Anon., 1993), or (3) pupate within rolled leaves on the tree (Barnes, 1948). Mature larvae collected in the field and held in the laboratory, required a minimum of 14 days for adult emergence (Todd, 1956). Little is known about whether midges enter diapause in the winter or which life stage (larval or pupal) enters diapause.

II.2.4 Phenology

In New Zealand, there are normally five generations of the midge between early October and the late April (Todd, 1959). First generation adults emerge from the soil near the time of flowering of apple trees in mid October. The second generation emerges at the end of November and the third generation from early to mid January. The fourth and fifth generations overlap and emerge during February, March and early April (Anon., 1994). The first generation of ALCM is generally small and causes only slight damage, while the second generation causes considerable damage. From the third generation onwards all stages of the midge are present in the field at all times and cause severe defoliation (Todd, 1959).

II.2.5 Damage

ALCM can cause considerable damage to the terminal shoots of apple trees which can lead to stunted growth of the tree. This is particularly important on young trees and scions. It is believed that damage mainly results from

the curling of the leaves. Curled leaves then cease to function normally and may drop prematurely. In 1994, a survey of 30 Waikato and one Bay of Plenty apple (cv. Braeburn) blocks, Tomkins et al. (1994) found that 27% of survey trees had 100% shoot damage and up to 41% of leaves on individual trees were infested. In 1994, a similar severity of ALCM damage to leaves was found in a survey of 30 apple orchards in Nelson (Smith and Chapman, 1995).

The effect of ALCM on the profit realised by New Zealand orchardists has yet to be measured. Although severe stunting of apple stocks occurred in NZ nurseries, no noticeable reduction in the abundance of fruit was observed on infested mature trees (Todd, 1956). Developing fruitlets can be damaged by the ALCM larvae when population pressures are high. Incidences of fruit damage tend to be sporadic, although up to 30% damage has been seen. In a study conducted in Havelock North, New Zealand, photosynthetic rate was not altered when less than 60% of the leaf area in the apple cv. 'Braeburn" was damaged (Allison et al., 1995). However, due to the amount of leaf area that was lost, the amount of carbon accumulation by the tree was reduced (Allison et al., 1995). The effect of leaf damage by ALCM on the fruit yield of apple is unknown. Trials in Germany suggest that leaf damage caused by a related pest, the pear leaf curling midge, reduces fruit yield by an average of 10% (Anon., 1994).

Several of New Zealand's export markets have specific tolerances for the presence of ALCM pupae or larvae on apple fruits. Therefore, ALCM also poses a threat to orchard profits by being a quarantine pest (Anon., 1994). Extra costs to the individual grower and the pipfruit industry are incurred during careful examination of fruit during quality control inspections and for labelling, documenting, and segregating affected lines of

fruit (Anon., 1993). In the Waikato up to 10% of apples were contaminated with ALCM pupae or larvae at harvest during 1993/94 season, while up to 1% were contaminated in Hawkes Bay (Anon., 1994).

II.2.6 Control of apple leaf curling midge

In the past ALCM was regarded as a secondary pest in New Zealand, that is, it was mainly controlled by insecticides applied against key pests such as codling moth and leafrollers. However, in recent years orchardists have reported increasing difficulties in trying to control this pest and have added insecticide sprays specifically targeted at ALCM (Anon., 1993).

In New Zealand, there are 3 insecticides with label registration for the control of ALCM: Diazinon®, Carbaryl®, and Supracide® (Anon., 1993). Diazinon is the most effective chemical among these (Anon., 1994) but must be added to the regular spray schedule because it is less effective against the major pests of New Zealand apples. At the same time, diazinon has to be used with care after flowering as there is potential for russet development under slow drying conditions (Anon., 1993). If Carbaryl® is applied during November it will thin apples while Supracide® is lethal to predatory mites and therefore, cannot be used in combination with integrated mite control programmes (Anon., 1993). In addition to these registered chemicals, chloropyriphos, azinphos methyl and dimethoate have been used against ALCM. Dimethoate has little effect on ALCM and is lethal to predatory mites (Anon., 1994).

In a survey conducted in 30 apple blocks in the Waikato and Bay of Plenty, 74% had been treated with one or more applications of diazinon and/or dimethoate (Tomkins et al., 1994). Blocks treated with soil or foliar applications of diazinon had less leaf damage than blocks which had not

been treated with diazinon; however, differences were not significant. Leaf damage from ALCM was higher in blocks treated with dimethoate than in blocks treated with diazinon or in untreated blocks (Tomkins et al., 1994). In a study examining the efficacy of organic pest control agents against ALCM in organic orchards, treatment with *Bacillus thuringiensis*, plant oils, Naturoil® and Defender® or rotenone did not control ALCM (Epenhuijsen et al., 1990).

Few studies have been done to identify natural enemies attacking ALCM in New Zealand. A hymenopteran egg parasitoid, *Platygaster demades* Walker, was introduced to New Zealand to control pear leaf curling midge, but also causes a generally high level of parasitism of ALCM (Todd, 1956). During the 1955-1958 seasons, the level of parasitism ranged from 0.7-95.6% (Todd, 1959). However, the failure of *P. demades* to check the second generation of ALCM restricts its effectiveness as a control agent (Todd, 1959). Whitcomb (1934) reported that an anthocorid bug, *Orius (Triphleps) insidiosa* Say, attacks ALCM larvae in curled leaves. However, the identification was unsure. The predator, *Sejanus albosignatus* found in organic orchards has been observed preying on ALCM larvae (Anon., 1994).

Host plant resistance to ALCM has not been studied in detail. A study in 1956/57 in New Zealand investigated the varietal susceptibility of the most commonly grown apple varieties (Todd, 1959). On the basis of mean larval population per infested leaf and the percentage of infested shoot tips, out of 12 varieties tested (Gravenstein, Cox's Orange, Kidd's Orange, Jonathan, Red Delicious, Golden Delicious, Sturmer, Granny Smith, Rome Beauty, Statesman, Dougherty, and Ballarat) none appeared to be resistant to ALCM. However, there was variation among varieties in the percentage

of leaves infested. This variation appeared to be related to the quantity of terminal growth present. In the UK it has been reported that all varieties can be attacked though they differ in susceptibility (Anon., 1983).

II.3 Ecology and behaviour of cecidomyiids

No studies have been done on the behaviour of adult ALCM. However, the behaviour of a related species, the Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is well documented (see references below). In the Hessian fly, the entire adult life of the male is devoted to finding mates. The life of the adult female can be partitioned into three major phases: the attraction of a mate by the release of a sex pheromone, a post-mating pre-ovipositional transition phase, and oviposition.

II.3.1 Patterning of emergence and onset of activities

In most cecidomyiid species, adults emerge at dawn or dusk; however, in some species adults emerge at midday or at night (Gagné, 1989). Emergence of a population can last a week, but emergence usually peaks on a single day. During each day of emergence, cecidomyiids usually emerge over a period of hours, with two separate peaks, one at dawn, and one at dusk, with peak male eclosion preceding that of females by one to several hours (Gagné, 1989). This phenomenon is evidenced by the studies done on Hessian flies and a number of other cecidomyiid flies (Barnes, 1930 cited in Bergh et al., 1990). Emergence of adult female Hessian flies was unimodal, 99.5% emerging from 01.00 to 09.00 hours. The majority of male Hessian flies (93.7%) emerged 12 h before the single daily peak of female emergence. A second smaller peak (6.9%) of male emergence coincided with the period of peak female emergence (Bergh et al., 1990). Females

exhibited calling behaviour minutes after emerging and continued to call until 10.00-11.00 h. Males remained inactive for more than 10 hours after emergence and only became active 1-3 hours before females emerged and began calling. Thus, the onset of male flight activity appeared to be synchronised with peak emergence of females (Bergh et al., 1990).

II.3.2 Mating behaviour

Hessian fly is one of the cecidomyiids for which mating behaviour has been studied in detail. The mating behaviour of Hessian flies is mediated by a sex pheromone. Within minutes after emergence, the adult female extends her ovipositor and emits a sex pheromone (Mckay and Hatchett, 1984). This behaviour is referred to as 'calling'. After mating, females cease emitting sex pheromone and continue to sit inactively with the ovipositor retracted for 20 min to 5 h before making the transition to a more active phase of flying and ovipositing (Harris and Rose, 1991). The length of this pre-ovipositional transition period is influenced by temperature and the age of the female (Harris and Rose, 1991). If mating does not occur on the first day after eclosion, females stop calling at about 11.00 hours. Calling behaviour is resumed the next morning and continues for two more days until the female dies (Bergh et al., 1990; Harris and Rose, 1991).

II.3.3 Oviposition behaviour

Mated female Hessian flies exhibited the following behaviours: flying, landing on leaves, arching of the abdomen to bring it in contact with the leaf surface, antennation, movements of the tip of the abdomen across the leaf at right angles to leaf veins, oviposition, sitting with the ovipositor straight but still extended, and sitting with the ovipositor telescoped into the body

(Harris and Rose, 1989). In the presence of a suitable substrate (a wheat leaf), females continued to fly and oviposit until death occurred several hours later (Harris and Rose, 1991).

Investigations into the effects of plant characters on the host finding and acceptance behaviour of mated females showed that Hessian flies use their chemical, visual and tactile senses to locate and identify host plants (Harris and Rose, 1990). Changes in information received by any of these three sensory modalities resulted in major reductions in the number of eggs laid on a treated substrate. Significant interactions among stimuli were reported when chemical, colour and tactile cues were combined. Visual cues from plants consisted of both spectral (colour) and spatial information (Harris et al., 1993). For example, targets with vertical rather than horizontal contour lengths and a higher density of vertical contour lengths were approached and landed on more frequently. At least two different chemicals in the foliar waxes of wheat and related grasses (rye, barley and oats) influenced the oviposition behaviour of female Hessian fly (Foster and Harris, 1992).

One other cecidomyiid that has been studied in detail is the brassica pod midge, *Dasineura brassicae* Winn.. Experiments done under field conditions with wild populations in Sweden (Ahman, 1985) showed that flying females respond to plant olfactory and/or visual stimuli from the preferred host (summer oilseed rape). A non-preferred host (brown mustard) was not landed on as frequently and thus presumably lacked these olfactory and/or visual stimuli. Female *D. brassicae* stayed longer and laid more egg batches on rape than on mustard plants. This suggests that females detected further differences between plant species after landing. These studies on Hessian fly and brassica pod midge indicate that interactions among

chemical, visual and tactile stimuli are critical to the host recognition process of these cecidomyiids.

II.4. Host-selection by herbivorous insects

II.4.1. Sensory cues in host-selection

The behaviour of insects is mediated by a large number of internal and external stimuli (Kennedy, 1978). Thus all sensory organs of the insect, i.e., those that detect their external environment (compound eyes, ocelli, different mechanoreceptive sensilla) as well as those that detect their internal environment (sensors detecting physiological states, mating states, egg maturity etc.) are involved in host-selection behaviour (Städler, 1977). Less is known about influences of internal stimuli as these stimuli are more difficult to manipulate experimentally (Harris and Foster, 1995).

An insect searching for a host-plant goes through a series of behavioural steps (e.g., long-range searching, short-range orientation, landing, host-recognition and acceptance) before, during and after landing on the host-plant (Kogan, 1977; Feeny et al., 1983; Renwick and Radke, 1988; Prokopy and Owens, 1983; Harris et al., 1993). Plant stimuli influence all of these steps. Past studies on host-selection in a range of insect species revealed that an insect typically uses an array of senses (Courtney and Kibota, 1990; Dethier, 1982; Städler, 1977). These senses can be either visual, olfactory, contact chemosensory, or tactile or a combination of any or all of these cues (Städler, 1977; Dethier, 1982; Prokopy and Owens, 1983; Harris and Foster, 1995). Sensory cues interact during host-selection behaviours, either by acting simultaneously to trigger a single behaviour or sequentially to trigger a series of behaviours (Courtney and Kibota, 1990). All behavioural processes involved in host-

finding and acceptance by a herbivore represent outputs of the central nervous system, which processes the integrated inputs of one or more of the above sensory stimuli (Dethier, 1982; Harris and Foster, 1995). This integration of inputs theoretically can occur in three forms: the simultaneous integration of the inputs; the successive integration of inputs; or a combination of these two (Harris and Foster, 1995).

II.4.1.1 Vision

Vision plays a major role in the orientation of flying insects to the host-plant. Typically, insects are able to perceive via visual receptors the part of the electromagnetic energy spectrum that ranges from 300-650 nm (Prokopy and Owens, 1983). However, certain coleopterans (e.g., *Melanophila acuminata* DeGeer) are also capable of perceiving and orienting to infrared energy of wavelengths greater than 750 nm by receptors located on the thorax (Evans and Kuster, 1980) or on the vertex of the head (Meyer, 1977). The physical stimulus of light provides colour cues in the form of brightness (intensity of perceived reflected light), hue (dominant wavelength of reflected light), and saturation (spectral purity of reflected light) (Prokopy and Owens, 1983). The quality and quantity of natural radiation received by an insect may be extremely variable depending on environmental factors (e.g., time of the year and day, latitude and longitude, altitude, atmospheric conditions) and the insect's position within a habitat (Prokopy and Owens, 1983).

Three principal properties of individual plants or their component parts serve as visual cues to foraging insects: spectral quality, dimensions and pattern (Prokopy and Owens, 1983). The dominant reflectance-transmittance hue of foliage ranges from 500-580 nm and does not vary

much from plant species to species. However, degree of saturation does vary substantially among plants and depends on surface glaucousness, pubescence, specular reflectance (glare), high cellular water content, and chlorophyll content (Prokopy and Owens, 1983). Depending upon the plant species and the growth stage of the plant, spectral reflectance properties of plant buds, bracts, blossoms, seeds, fruits, stems or twigs and thorns may be similar to or very different from that of foliage (Gates, 1980 cited in Prokopy and Owens, 1983).

During host-finding, insects may perceive visual cues either from a long distance (several meters or more), a short range (within a few meters or less) or within the plant canopy. When finding a host-plant over longer distances, flight orientation and stabilisation are strongly influenced by characteristics of the horizon (Wehner, 1981 cited in Prokopy and Owens, 1983). Movement of ground patterns across the visual field of flying insects provides visual cues for the optomotor reactions which help to maintain flight stability and velocity (Kennedy et al., 1961; Wehner, 1981 cited in Prokopy and Owens). Colvin et al. (1989) revealed that females of tsetse fly, *Glossina morsitans morsitans* Westwood fly upwind to host odour plumes by responding to the visual flow fields which arise from their movement over the ground (optomotor anemotaxis). Visual input from the apparent movement of the ground is also used to assess wind direction during upwind flight in an odour plume (Colvin et al., 1989).

According to Prokopy and Owens (1983), as an insect approaches a plant (within a few meters or less) the spectral reflectance characteristics of foliage may facilitate discrimination of the plant from other objects. Plant spectral quality (e.g., hue and intensity) appears to be the principal stimulus eliciting landing on plants by many herbivorous insects

(Kennedy et al., 1961; Prokopy and Owens, 1983). Many herbivores show attraction towards yellowish hues (which range from 520-600 nm) (Harris and Miller, 1983; Moericke et al., 1975; Harris et al., 1993) and this may enable them to discriminate foliage-like hues (which peak at 500-580 nm) from non-foliage like hues (peaking at less than 500 nm or at greater than 580 nm) (Prokopy et al., 1975; Kennedy et al., 1961; Owens, 1982 cited in Prokopy and Owens, 1983; Städler, 1977). In some aphid species (e.g., *Hyalopterous pruni* (Geoffroy)) discrimination between host and non-host plants, or between plant parts may be at least partly accomplished on the basis of differences in saturation or intensity of reflected light (Kennedy et al., 1961; Prokopy and Owens, 1983). Some aphids are more attracted to higher reflectance from newly developing leaves than to the lower reflectance of mature leaves (Moericke, 1955 cited in Prokopy and Owens, 1983). Certain tephritid flies are attracted most strongly to green and yellow pigments that most closely resemble plant foliar hues (Boller and Prokopy, 1971).

There are probably only a few herbivore species that identify their host plants on the basis of only plant dimensions or growth pattern characteristics. The insect eye perceives the angular size of an object as the object enters the visual field, but not the absolute size of the object (Saxena and Khattar, 1977). Landing of onion flies, *Delia antiqua* (Meigen) was enhanced by narrow, vertical cylinders (Harris and Miller, 1984). Lepidopterous larvae, orthopterans, tephritid flies and scolytid beetles respond to the tall, narrow dimensions of vertically growing stems, and butterfly adults are attracted toward plants bearing leaves of a characteristic shape (Prokopy and Owens, 1983; Rausher, 1978).

The ability of an insect to detect an individual tree or plant at close range is probably strongly influenced by background composition (Prokopy and Owens, 1983). Background can affect the visual appearance of a particular stimulus by changing colour contrast or overall illumination, or by providing or removing contrasting optical patterns (Hailman, 1977 cited in Prokopy and Owens, 1983). For example, ovipositing females of the butterfly, *Battus philenor* (Linnaeus) detected host plants more readily early in the season when host plants were surrounded by little or no vegetation, rather than later in the season when plants were intermixed with other vegetation (Rausher, 1981). Similarly, *Pieris* butterflies, aphids and whiteflies landed on plants surrounded by bare soil more often than on plants surrounded by weeds (Smith, 1976). Visual discrimination of plant structures, such as fruits growing within the plant canopy, appears to rely on shape, intensity of reflectance, and size (Owens, 1982 cited in Prokopy and Owens, 1983; Boller and Prokopy, 1976).

II.4.1.2 Chemoreception

When locating resources herbivorous insects use their senses of smell (olfaction) and taste (contact chemoreception). Upon perceiving chemical stimuli, the insect performs a series of responses based on a combination of internal and external factors (Hansell, 1985). A herbivore searching for feeding or oviposition sites perceives plant chemicals by the chemo-sensory organs that evoke positive or negative sensory signals. The balance of this sensory information, along with information from other sensory systems (e.g., vision, mechanoreception) determines whether a

plant is accepted or rejected by the insect (Huang and Renwick, 1993). Perception and recognition thus involve assessment of a complex multiple sensory input (Dethier, 1982).

Compounds that arise through secondary plant metabolism have been exploited by insects and provide an adaptive advantage to herbivorous insects. Host-finding for feeding and oviposition by the adult female as well as host-finding by immature stages for feeding, growth and development are basic behavioural and physiological processes mediated by these compounds (Kogan, 1977). Secondary plant metabolites, e.g., n-aliphatic alcohols and aldehydes, phenyl propanes, isoprenoids, certain cyanogenetic and other glycosides, play a role as attractants and arrestants for insect herbivores (Kogan, 1977). Primary plant compounds (e.g., sugars and salts) can act as stimulants or deterrents for insect feeding (Hanson, 1983). Many authors have given experimental evidence for butterflies having tarsal contact receptors sensitive to sugars and also to salt solutions (Feeny et al., 1983). The primary function of these contact receptors is related to feeding.

II.4.1.2.1 Contact chemoreception

Contact chemoreceptive sensilla have been identified on the major parts of the insect body such as wings, antennae, ovipositor, tarsi, palpi, proboscis and food channel (Städler, 1984). Females of a cecidomyiid, the Hessian fly, repeatedly antennate foliar surfaces before ovipositing (Harris and Rose, 1990) suggesting that chemical cues perceived by contact chemoreceptors on the antennae might be important in host evaluation for oviposition. In addition to antennation, Hessian fly females also extend the ovipositor, arch the abdomen, and move the abdomen from side to side

feeling the surface of the plant while walking (Harris and Rose, 1989). Harris and Rose (1990) suggested that this may bring chemoreceptors on the ovipositor into contact with foliar chemicals and furthermore, may stimulate mechanoreceptors on the ovipositor.

II.4.1.2.2 Olfaction

Volatile plant chemicals that are released into the air can affect the behaviour of herbivorous insects before and after landing on a plant. Many insects respond to volatile chemicals by orienting to the source of chemical. Odour-conditioned anemotaxis is important for flight orientation to odour sources at a distance in the field. Volatile plant chemicals are detected by olfactory organs present on the surface of the antennae or other parts of the insect body.

Olfaction in insects is best studied in lepidopterans. Past studies on olfaction in Lepidoptera have revealed that many morphological, physiological, biochemical and molecular characteristics of both peripheral and central nervous neurons are involved in the olfactory sense (Hansson, 1995). As is common to many insects, the main olfactory organ of both adult and larval Lepidoptera is the antenna.

Different morphological types of sensilla present on the antennae of butterflies and moths are sensitive to odours in the environment (Hansson, 1995). Behan and Schoonhoven (1978) identified four types of sensilla on the antennae of male and female *Pieris brassicae* (L.) butterflies. Two of these were considered to have an olfactory function. These antennal receptors can distinguish a wide range of volatile plant compounds and can also detect a host-marking pheromone associated with conspecific eggs (Rothschild and Schoonhoven, 1977). It has been shown that female

Spodoptera littoralis (Boisd.) have highly sensitive olfactory detectors tuned to odours of oxidised plant compounds produced in the larval frass, compounds that deter oviposition (Anderson et al., 1993).

The ability to discriminate conspecific females from females belonging to other species by sex pheromones is another important feature of the olfactory system of an adult male moth (Hansson, 1995). Sex pheromone molecules are perceived by receptor sites on antennal receptor neurons. These receptors are extremely sensitive to the chemicals involved in sexual communication. Receptor neurons with different specificities are present on the antenna of the insect (Hansson, 1995). Both moth and butterfly females also possess receptor neurons tuned to the male-produced close-range pheromones (Grant, 1971).

Cecidomyiid flies have sensorial appendages on their antennae having an olfactory function (Mamaev, 1975). Antennae of flies belonging to some genera of family Cecidomyiidae (e.g., sorghum midge, *Contarinia sorghicola* (Coquillet)) are covered with looped hair-like sensilla called circumfila which are considered to be olfactory in function (Slifer and Sekhon, 1971 cited in Harris and Rose, 1990). In addition to these sensoria, olfactory organs are also situated on the radial veins of the wings and at the base of the palps (Mamaev, 1975).

II.4.1.3 Mechanoreception

Mechanoreception plays an important role in host-plant recognition (Städler, 1977). Mechanoreceptors of plant feeding insects are probably involved in the monitoring of leaf geometry, the consistency of the leaf or portions thereof (Heinrich, 1971). This may also provide information on the distance and contact between the leaf and the chemoreceptive sensilla

(Städler and Hanson, 1975 cited in Städler, 1977). There are numerous bristly hairs acting as tactile organs on the antennae of cecidomyiid flies (Mamaev, 1975). The mechanoreceptive sensilla on the ovipositor are necessary for the regular positioning of eggs in *Bombyx mori* L. (Yamaoka et al., 1971). In other insects these may also be involved in the perception of surface shape and texture as well as the internal consistency of the oviposition substrate (Boller and Prokopy, 1976; Harris and Rose, 1990).

II.4.1.4 Temperature and humidity receptors

Because insects are small cold-blooded animals, their behaviour is strongly influenced by temperature and humidity. There is some evidence that insects have sensory receptors sensitive to temperature and humidity (Städler, 1977 and references therein). Lepidoptera larvae have temperature and humidity receptors located in the antennae and maxillary palpi which are sensitive to changes in temperature and relative humidity. These receptors may provide information to the central nervous system about the turgidity of leaves (Schoonhoven, 1967; Dethier and Schoonhoven, 1968).

II.4.2 Generalist vs specialist insects

Herbivorous insects are often classified as generalists or specialists, depending on the range of plant species that the insect can feed on. Insects that consume plants of several different families are considered generalists, whereas those that consume plants within a single family are considered specialists. A parallel scheme for classifying the feeding habits

of insects divides insects into polyphagous (many hosts), oligophagous (several hosts) and monophagous (a single host species) species.

It has been suggested that the host ranges of insect herbivores are related to the predictability and availability of plant species or tissues. Insect species whose resources are relatively unpredictable are more likely to be generalists, whereas insect species whose resources are relatively abundant in time and space are more likely to be specialists (Prokopy and Owens, 1983). In contrast to this, Lance (1983) proposed that specialist herbivores are adapted to utilize host plants that are relatively unpredictable in time and space, and because of this often require specialized host-location mechanisms that operate over relatively long distances (Lance, 1983; May and Ahmad, 1983). Furthermore, according to Lance (1983), food resources of generalist herbivores tend to be more predictable. Their host-location mechanisms would expected to be less specific, bringing the insect into contact with a wide variety of potential host-plant species. Based on this contact, the generalist herbivore should then be capable of selecting superior hosts (Lance, 1983).

Kogan (1977) described six distinct models of host-selection strategies ranging from highly generalized to highly specialized host-plant interactions. Insects that were categorized as model I species were highly polyphagous insects, such as species of Acrididae. In these insects, orientation to the host plant is triggered by generalized mechanisms. Plant recognition usually involves contact chemoreception. Host-acceptance results from the presence of feeding stimulants of common occurrence (e.g., sucrose, lipids, vitamins) and the absence of active deterrents.

Most of the generalist insects that have been studied in detail (e.g., aphids, whiteflies and thrips) orient to and land on plants at random, and

during this process respond to generalised visual cues such as yellow-green light. Kogan (1977) categorized these insects as model II insects. For example, gypsy moth larvae disperse randomly (passive dispersion by wind) and subsequently orient to vertical objects (Lance, 1983). The desert locust orients to vertical patterns and is stimulated by walk upwind by plant odours (Wallace, 1958). Japanese beetles respond to a wide range of plant volatiles (Lance, 1983). After landing, such generalists examine sites and during this examination, are exposed to various chemical and mechanical stimuli.

Model III of Kogan (1977) consists of the insects for which adult food-finding probably depends on generalized cues. Their food sources produce strong, specific arrestants and feeding excitants but weak attractants. After the first colonisers feed on acceptable host-plants, they produce aggregation pheromones which induce large numbers of conspecific insects to orient to the food source (e.g., several chrysomelid species; *Diabrotica* spp., and *Acalymma* spp.).

In certain specialist insect species that Kogan (1977) categorized as model IV insects (e.g., *Lema trilineata daturaphila* Kogan and Goeden, *Leptinotarsa decemlineata* Say and *Manduca sexta* (L.)) host-finding for oviposition occurs as a result of responses to specific stimuli perceived directly from the host-plant species. For example, adult females display positive anemotaxis if exposed to a wind stream carrying host plant odours. Contact chemoreceptors permit a high degree of discrimination after contact with the plant has been made and oviposition usually occurs on suitable host plants. Larvae of these specialist species tend to be less discriminating.

According to Kogan (1977) in some other specialist insects, host-finding for oviposition and feeding are highly selective for both adults and larvae (model V insects). The biochemical basis of host-plant selection sometimes being the same for adults and larvae. Specific attractants and feeding and oviposition stimulants are characteristic of plants within their host range (e.g., *Plutella maculipennis* (Curt.), *Brevicoryne brassicae* (L.), and *Erioachia brassicae* (Bouché)).

The insects that were categorized into model VI insects are highly specialised herbivores, such as *Heliconius* species. Adult host finding for feeding and oviposition sites is highly selective (Kogan, 1977). The host-plant ranges of adults and larvae of insects of this type are very different. Consequently selection mechanisms used by adults and larvae are also very different. Complex mechanisms of sensory organs permit adult females to locate larval host-plants for oviposition and their own hosts for feeding (Kogan, 1977).

Host specificity in phytophagous insect species may depend on the length of the discrimination phase of an insect, i.e., the period when one plant species is preferred over others (Papaj and Rausher, 1983). Courtney and Kibota (1990) argue that sensory inputs from host-plants determine their acceptability to insect herbivores. The balance of sensory inputs from attractants and deterrents can yield a net positive or negative stimulation, and therefore result in acceptance or rejection of the host, respectively. Rank order of hosts is determined solely by their stimulatory effects relative to each other. However, specificity of an insect to a particular host-plant depends on internal factors (e.g, physiological condition, age, nutritional states, travel time since last encountered host, etc.) which set a motivational threshold for acceptance. As internal factors change, this

critical threshold will also change. Thus, the specificity of an insect herbivore is a function of the interaction of motivational state of an insect with acceptabilities of various host-plants (Courtney and Kibota, 1990). Furthermore, there is evidence that olfactory stimuli from non-host plants can confuse or repel specialist insects (Tahvanainen and Root, 1972). The mechanisms underlying this confusion may be visual as well as tactile and chemotactic. Learned associative behaviour may also be involved (Kogan, 1977).

II.4.3 Host-selection mechanisms

II.4.3.1 Habitat and plant searching

In many insect species visual orientation is important in long-range host-location. For example, in insects such as aphids, whiteflies, thrips, and butterflies, vision appears to play a dominant role in searching (Kogan, 1977; Feeny et al., 1983; Renwick and Radke, 1988). In these species, landing is elicited by precise spectral characteristics. Colour seems to be the most important stimulus eliciting landing (e.g., *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli (Kennedy et al., 1961), cabbage butterfly, *Pieris brassicae* (Renwick and Radke, 1988)). In the case of the Hessian fly, visual cues have a greater effect on orientation during flight than plant chemicals (Harris et al., 1993). Shape and size of the foliage are also important stimuli for plant search by certain insects (e.g., pipevine swallowtail, *Battus philenor* (L.), Rausher, 1978).

Host-location by many herbivorous insects is also mediated by olfaction. The generalist herbivores (e.g., grasshoppers, aphids, and beetles), the insects that lay their eggs on leaves (e.g., lepidopterans), and flies whose larvae feed on the roots or shoots all respond by orientation to

commonly occurring green leaf volatiles (Bernays and Chapman, 1994). Similarly, insects that feed in the wood or bark of pine trees respond to a range of chemicals produced by these trees in their resins. In contrast, the specialist herbivores (e.g., the onion fly, *Delia antiqua* and carrot fly, *Psila rosae* F.), respond to the odours specific to their host-plants. Host-location by adult females of several oligophagous insects is mediated mainly by olfaction (Kogan, 1977; May and Ahmad, 1983): *Lema trilineata daturaphila* Kogan and Goeden; *Leptinotarsa decemlineata* (Say) and *Manduca sexta* (L.) associated with solanaceous hosts; *Erioachia brassicae* (Bouché), *Brevicoryne brassicae* (L.) and *Pieris brassicae* associated with cruciferous hosts (Städler, 1977). Frequently plant odours are only detectable over short distances (Städler, 1977).

May and Ahmad (1983) reviewed the literature and concluded that olfaction is very important for many polyphagous and oligophagous insect species, e.g., vegetable weevil, *Listroderes obliquus* is attracted to mustard oils; cotton boll weevil, *Anthonomus grandis* (Boh.) is attracted to several of the headspace volatiles over cotton plants; cabbage rootflies, *Delia brassicae* (Bouché) attracted to volatiles of crucifers; Colorado potato beetle is attracted to a group of closely related volatiles called 'green-leaf volatiles' (May and Ahmad, 1983). Several monophagous insects that use olfactory cues during host-plant location (e.g., bark beetles) (Byers, 1995) also use olfaction in many of their other behavioural processes (e.g., mating).

Long-range host-location must be mediated at least in part by visual and/or olfactory cues from plants (May and Ahmad, 1983). Many of the species known to use long-range cues for host-selection are oligophagous. An apparently monophagous olive fruit fly, *Dacus oleae* (Gmel.), does not

rely on olfaction to locate host-plant, but responds to the distinctive colour of the olive foliage and the colour and shape of fruits at later stages (Prokopy and Haniotakis, 1975; 1976). In contrast, certain highly polyphagous species (e.g., *Schistocerca* and *Listroderes)* appear to use olfactory stimuli in long-range host location. A highly polyphagous insect, the Japanese beetle, is attracted to a range of host-plants by a variety of unrelated plant compounds (Lance, 1983 and references therein).

Usually, generalist insects respond to generalized sensory cues such as odours common to a variety of different plant species (green leaf volatiles), and yellow-green light. Specialist herbivores respond to host-specific odours or visual cues distinctive to the host.

II.4.3.1.1 Orientation during flight

During host-finding, herbivorous insects may orient in response to visual and/or olfactory stimuli. Host-finding by odour occurs in two stages: arousal and orientation (Bernays and Chapman, 1994). Insects aroused by odour-carrying air are then in a state to respond to further stimuli. Wingless insects that respond to odour have been observed walking upwind in a wind tunnel. In some winged insects arousal by odours leads to take-off and upwind orientation. After take-off, many insects maintain their flight speed by maintaining a certain rate of image movement across the eye (optomotor reaction). This enables the insect to maintain an orientation at any angle to the wind, including up- or down-wind. Image movement that arises from the pattern of objects on the ground is important in orientation during flight (Bernays and Chapman, 1994).

Due to air turbulence, plant odours or sex pheromones do not reach the insect as a continuous flow from the source to the insect. The air

carrying an odour from the source is carried downwind as pockets in a mass of nonodourous air (Bernays and Chapman, 1994). Hence, an insect at a distance from the odour source perceives a series of bursts of odour, separated by periods without odour. The concentration of odour within a burst is very variable. Thus there is no odour gradient leading to the odour source that the insect can follow (Bernays and Chapman, 1994). The mechanism of insect orientation to an odour source is direct upwind flight when in contact with a packet of odour-laden air of the plume (optomotor anemotaxis) (David et al., 1982). When contact with the odour plume is lost, the insect casts (flies across the wind from side to side) until it again contacts the odour. The angle of the odour plume becomes narrower close to the odour source. Hence, as long as the insect continues to be stimulated, it progresses through the wind towards the odour source.

II.4.3.2 Landing

In many herbivorous insects, plant spectral quality (particularly hue and intensity of colour) appears to be the principal stimulus for landing on host-plants (Prokopy and Owens, 1983). There is evidence from numerous insect species that colour influences landing by herbivorous insects (eg., cherry fruit flies (Boller and Prokopy, 1971); whitefly, *Trialeurodes vaporariorum* (Coombe, 1982); onion fly, *Delia antiqua* (Harris and Miller, 1983); Hessian fly, *Mayetiola destructor* (Harris et al., 1993)).

As mentioned before, there is a small number of herbivore species that identify their host plants at a close range at least partly by plant dimensions or growth pattern characteristics (Prokopy and Owens, 1983). Stanton (1979) suggested that the butterfly, *Colias meadii* Edwards, may visually discriminate between *Trifolium* spp.. Wyatt et al. (1993) have

shown in a predator, *Rhizophagous grandis* Gyllenhall, that the physical effects caused by structures (e.g., tree trunk) lying in an air stream (e.g., turbulence, changes in wind velocity, and eddies) can be more important than the visual stimuli presented by the structure.

Although chemical stimuli are used by many insect species in both long and short range orientation to the host-plant, there is little evidence for their direct involvement in eliciting landing. Renwick and Radke (1988) suggested that the involvement of olfaction in the landing of butterflies appears to be restricted to an avoidance response to non-hosts and the absence of negative signals from potentially acceptable plants. According to Byers (1995) at least some species of bark beetle avoid non-host trees due to specific odour. However, Bursell (1990) reported that landing of male savanna tsetse flies appeared to be triggered when steep odour gradients were encountered as flies flew upwind and downwind across the edge of an odour plume. This occurred both when visual targets were present and when they were absent.

II.4.3.3 Host-plant evaluation

Final acceptance of a host-plant by a herbivore for feeding or oviposition depends on the stimuli perceived during post-landing host-plant examination (Mitchell et al., 1991). Many studies have provided evidence that chemical stimuli play a key role in host-plant recognition and acceptance after the feeding or ovipositing insect has made contact with the plant (Renwick and Radke, 1988; Feeny et al., 1983; Foster and Harris, 1992). Leaf-surface chemistry may contribute very significantly to host-plant selection. In many species of butterflies, stimuli perceived by tarsal chemoreceptors during drumming behaviour are important for the

acceptance of a particular host either for feeding or for oviposition (Feeny et al., 1983 and references therein).

According to Thorsteinson (1960) olfactory and gustatory stimuli, sensed after landing, are usually considered the primary mediators of host-plant acceptance. However, Harris and Miller (1983) reported that the colour yellow positively influenced post-landing behaviours of the onion fly, *Delia antiqua* in the laboratory. Studies on European corn borer, *Ostrinia nubilalis* (Hubner) (Udayagiri and Mason, 1995), indicated that plant phenology affects its chemically-mediated oviposition response.

The physical properties of the plant (e.g., size, shape, and surface texture) appear to be important factors in host-plant evaluation by insects, especially in oviposition behaviour. In oviposition behaviour of the onion fly, size, shape, and orientation of the surrogate leaves strongly influenced the acceptance for oviposition (Harris and Miller, 1988). In cabbage root fly, *Delia radicum* (L.), in addition to leaf surface texture, size, and colour, flies also evaluated veins or irregularities in the leaf or the folds on an artificial leaf (Roessingh and Städler, 1990). In the absence of the latter stimulus, the transition from leaf evaluation to stem runs and oviposition did not occur. Hessian fly females often move the tip of the abdomen across the leaf surface at right angles to leaf veins (Harris and Rose, 1990). The tactile stimuli that are perceived during this behaviour appeared to influence egglaying responses. The presence or absence, as well as orientation, of leaf venation are important in the oviposition of Hessian flies (Harris and Rose, 1990).

II.5 Non-plant factors influencing the behaviour of herbivorous insects

In addition to stimuli originating from host plants, the behaviour of herbivorous insects is modified by a large number of internal and external environmental factors. Among internal factors influencing the behaviour of insects are host-plant deprivation, previous experience, age, mating status, and physiological state. External factors such as temperature, photoperiod and other climatic factors, and conspecific insect density also affect the behaviour of herbivorous insects (Papaj and Rausher, 1983).

II.5.1 Internal factors

II.5.1.1 Host-plant deprivation period

Host-plant deprivation, nutrient imbalance and hydration are physiological states of insects that alter host-plant selection for food and feeding-behaviour of insects (Bernays and Chapman, 1994). For example, after increasing periods of food deprivation, Australian plague locust, *Chortoicetes terminifera* (Wlk.) was found to show increasing acceptability of plants that are normally rejected (Bernays and Chapman, 1973). Females of other insect species show declining specificity (strength of preference) for oviposition sites if they are prevented from oviposition, e.g., sphingid moths, *Macroglossum stellatarum* (Knoll, 1922 cited in Hinton, 1981). If deprived of egglaying sites, female sphingid moths that usually only approached and landed on objects that exuded host-plant odour eventually deposited eggs on anything with the appropriate colour. Female Hessian flies deprived of host-plants and later released into wheat or oats showed greater frequencies of oviposition behaviours and deposited more eggs (Harris and Rose, 1989). The effect of hydration on

food selection had been demonstrated in nymphs of the locust, *Schistocerca gregaria* Forskål (Roessingh, et al., 1985).

II.5.1.2 Experience

Previous experience of a phytophagous insect can influence its response to a plant in many ways (Bernays and Chapman, 1994). Changes in host-selection as a result of experience usually occur within the normal host range of the insect. These behavioural changes occur by several different mechanisms: (1) habituation or decline of a response to a stimulus with repeated exposure, (2) sensitization or increase of a response to a stimulus on repeated exposure without any learned association, (3) aversion learning or learning of an insect to develop a negative association with a stimulus, and (4) induction of preference or learning of an insect to develop a positive association with a stimulus (Bernays and Chapman, 1994). Searching behaviour of many insect species is modified by experience (e.g., parasitoids, *M. croceipes* (Cresson) (Wäckers and Lewis, 1993); *L. heterotoma* (Thomson) (Papaj and Vet, 1990)) or by learning (e.g., *Brachymeria intermedia* (Nees) (Drost and Cardé, 1993)).

II.5.1.3 Age

Host-finding behaviour of herbivorous insects also can be influenced by the age of the individual. Older individuals show lower oviposition rates (Papaj and Rausher, 1983). This may be due to reduced responsiveness to chemotactile stimuli from host-plant or variability in flight ability. Conversely in *Eucarcelia rutilla* Vill, a tachinid parasite of the pine looper moth that orients to olfactory cues of the looper's host-plant, the female

becomes increasingly responsive to pine odour as the pre-oviposition period progresses, (Herrebout and van der Veer, 1969).

II.5.1.4 Physiological states of the female

The physiological state of an insect also influences its behaviour. These physiological states include sexual maturity of females and egg load (Bernays and Chapman, 1994). For example in the grasshopper, *Oedaleus senegalensis* Krauss, reproductive females require more nutrients than males or non-reproductive females and choose the protein-rich seed of millet while males eat more leaves (Boys, 1978). The activity level of gravid females of cabbage rootfly, *Delia brassicae,* increases as the length of time that female has been gravid (with fully developed eggs) increases (Traynier, 1967). Ovipositing cabbage butterflies with a large daily complement of eggs showed a higher frequency of visiting to the same host, higher responsiveness to host-plants and shorter average flight length than females with only a few eggs left to lay (Jones, 1977).

In ovipositing females of many insect species, host-plant selection is influenced by egg load. When the egg load is large, females of many phytophagous insects become much less selective about their host plants (e.g., *Battus philenor, Euphydryas editha, Dacus* Spp., and *Rhagoletis* spp.) (Bernays and Chapman, 1994). Studies with apple maggot flies showed that when the egg load becomes very high, females accepted fruits that were previously rejected, such as fruits which had been marked with pheromones by previous insects (Mulkenberg et al., 1992).

II.5.2 External factors

II.5.2.1 Effect of climatic factors

External climatic factors, especially temperature and photoperiod, also influence host-location behaviour of insects (Papaj and Rausher, 1983). Insect behaviour is to a large extent limited by temperature. Many insect species exhibit behaviours which tend to optimize body temperature, e.g., when air temperature is low, insects expose themselves to maximum radiation. When temperatures are high, insects tend to move to the shade or to a greater height above ground (Bernays and Chapman, 1994). Many insect species (e.g., butterflies, beetles and cicadas) regulate their body temperature by basking (Matthews and Matthews, 1978). Such insects require a high thoracic temperature to start flight. Butterflies cannot fly until their bodies are above certain range of temperature. Hence, female butterflies cannot search for host-plants for oviposition below that particular range of temperature (Rawlins, 1980). Unlike relatively large insects such as butterflies, small flies such as midges and fruit flies have rapid heat loss and little build up of body heat during flight. Therefore, the wing beat frequency and flight speed of these small flies varies with varying ambient temperature conditions (Matthews and Matthews, 1978).

II.5.2.2 Conspecific insect density

In many herbivorous insects, host-finding behaviour is influenced by the density of conspecifics present in the host-plant. For example, certain butterflies avoid laying eggs on plants that are already infested with conspecific eggs (Rothschild and Schoonhoven, 1977; Rausher, 1979). The marking pheromones of two butterfly species, *P. brassicae* and *P. rapae,* inhibit oviposition by either species on leaves with previously-laid

eggs (Schoonhoven, 1990). Oviposition by apple maggot fly, *Rhagoletis pomonella* (Walsh) is deterred by the presence of a marking pheromone (Prokopy, 1972). A tephritid fly, *Rhagoletis cerasi* L., marks cherry fruits after oviposition with a marking pheromone that prevents oviposition by the same individual or by conspecific females (Städler et al., 1994).

II.6 Concluding remarks

The variety of behaviours involved in insect life, the mechanisms underlying these behaviours, the sensory cues mediating these behaviours, and the sensory receptors perceiving these sensory cues have been reviewed in this chapter. The external and internal factors that influence these behaviours were also discussed. While little of this information was generated by research on ALCM or insects closely related to ALCM, information on other herbivorous insects presented in this chapter provides background for understanding the behaviour exhibited by female ALCM in the experiments discussed in the following chapters.

Chapter III

Studies on rearing techniques for apple leaf curling midge larvae and biology of adult midges

III.1 Introduction

Although the primary aim of my research was to study the host-finding behaviour of mated apple leaf curling midge females, several preliminary studies on ALCM had to be conducted first. First, because I did not have access to a laboratory colony of ALCM and yet needed large numbers of adult midges for experiments, a study was undertaken to determine an efficient method for rearing adults from mature larvae collected from orchards. Second, because I needed to develop methodologies to study the host-finding behaviour of mated females, studies were carried out on diel emergence patterns of adult males and females, pre-ovipositional behavioural sequences of virgin and mated females, specificity of ovipositing females for the host-plant, and choice of oviposition sites by females. Several of the experiments presented in this chapter were done as observational studies and conducted with few replications. These experiments provide only preliminary results but are included here because, while inconclusive, they provide a modicum of information on a poorly understood insect pest species.

III.2 Methods common to experiments

The studies presented in this chapter were conducted during two consecutive summer seasons, 1994/1995 and 1995/1996. Experiments done in 1994/95 were conducted at the Plant Growth Unit, Massey

University, Palmerston North, using foliage of the apple cultivar 'Fuji' and the pear cultivar 'Pakchams Triumph' from the Massey University Fruit Crops Unit. Experiments done in 1995/96 were conducted at the Insect Science Laboratory of HortResearch, Mt. Albert Research Center, Auckland, using the apple cultivars "Gravenstein" and "Dunn's Favourite", and the pear cultivar "Beurre Bosc" and a seedling of European pear, *Pyrus communis*.

The mature larvae (identified as mature by their size 2-4 mm, and deep orange colour) used for rearing adult midges were collected from apple orchards in Palmerston North in 1994/1995 and in Kumeu and Henderson in 1995/1996. Mated females used in experiments were obtained by the following method. Adult females (30-50) were collected from laboratory cultures by aspirator in the morning (between 07.00 to 08.00 h) and introduced into a plastic container (13 cm height, 13 cm diameter) which contained a similar number of newly eclosed males. Females were continuously observed for one hour during mating. The females that were mated and of a similar body size were removed from the container immediately after mating and individually placed in glass vials (10 cm height, 2.5 cm diameter with a fitted plastic lid having a 1.5 cm diameter mesh window).

Except for observational studies, all experiments presented in this chapter were conducted using either a completely randomized design (CRD) or a randomized complete block design (RCBD) with three to ten replicates. Data from these studies were subjected to Bartlett's test for homogeneity of variance (Sokal & Rohlf, 1981; Steel & Torrie, 1980). When variances were shown by this test to be heterogeneous, data were transformed to achieve homogeneity of variances. Transformed data were

then analyzed by analysis of variance (ANOVA) using SAS Release 6.10, SAS system for Windows (SAS Institute Inc., Cary, NC, USA) and were tested for significance of means using the least significant difference (LSD) test.

III.3 Experiments on rearing technique

III.3.1 Materials and Methods

III.3.1.1 Rearing Method

In the field, mature ALCM larvae normally leave their leaf curls and fall to soil to pupate. The aim of this study was to determine: (1) the best way to introduce larvae to rearing media for pupation and (2) the best medium for pupation of mature larvae for large scale rearing. To study this, three methods of introducing larvae to the rearing media and three types of media were tested in a preliminary study in November, 1994. For the first method of introducing larvae, infested leaves were collected from the field and taken to the laboratory where their stalks were wrapped individually in strips of tissue paper and held in small stoppered vials (5 cm height, 2 cm diameter) which contained water. The vials were then positioned horizontally in a test tube rack which itself lay on a layer of bark medium (2 cm deep) in a plastic container (30 x 25 cm). In this setup, mature larvae exiting leaves were expected to drop naturally to the bark medium. A second group of infested leaves was also placed in vials (containing a nutrient solution) in the same setup as above. Vials were then positioned at a 45° angle relative to a 2 cm deep layer of moist culture medium held in a plastic container. Here the container was covered with a plastic lid to keep leaves moist. A third group of infested leaves was broken into pieces and

placed on a flat surface. Larvae exiting from these leaves were transferred, using a wet camel's hair brush, into vials (7.5 cm long, 2.5 cm diameter) containing a 2 cm deep layer of a culture medium and fitted with plastic lids with a mesh window. In all containers, the culture medium was kept moist by spraying water either every three days or at more frequent intervals if the surface of the media appeared dry. Three culture media (bark material, soil and sawdust) were tested using each of the three methods for setting up infested leaves (nine treatments in total). Each treatment was replicated three times, with one block of each set of treatments set up each day over a three day period (RCBD).

In the first two methods for setting up infested leaves (where leaves were set in vials), leaves were checked 10 days later to see whether the larvae were still present. The numbers of adults that emerged from each replicate of each treatment were counted daily throughout the period of adult emergence.

III.3.1.2 Identification of a suitable culture medium

Three materials (bark, soil and sawdust) were tested for their suitability as culture media for the rearing of mature larvae and pupae. ALCM infested leaves were collected from apple orchards in Palmerston North in January, 1995. In the laboratory leaves were broken open so that larvae exited leaf curls. Twelve mature larvae were then transferred, using a wet camel's hair brush, to the surface of a 1.5 cm deep layer of culture medium, held in petri-dishes (8.5 cm diameter x 2 cm depth). After larvae had disappeared into the medium, the medium was lightly sprayed with water. Five dishes (= five replicates, CRD) of each rearing medium were prepared in this manner (n = 60 larvae/ treatment) and the dishes were held in an incubator

(21 ± 2 °C and 14:10 L:D). When adult emergence started ten days later, the total number of adult flies emerging from each petri-dish was recorded daily.

A third experiment was conducted in March, 1995 to compare bark as a culture medium for ALCM against two other materials, pumice and horticultural river sand. For each culture medium, ten small pots (7.5 cm diameter, 6 cm height) were filled with a similar volume (approx. 250 cu.cm) and weighed (weight of medium: bark, 50 g; pumice, 80 g; and sand, 160 g). Samples of each medium were tested for moisture content at the beginning of the experiment and for field capacity using the Pressure plate technique at 0.1 bar pressure (Department of Soil Science Laboratory, Massey University, Palmerston North).

In each pot, fifty mature larvae were placed on the surface of the medium. After larvae had disappeared below the surface of the medium, pots were sprayed with water until the moisture content was equal to 60 percent of the field capacity. The weight of each pot was recorded. Pots were covered with mesh tops and then randomly arranged (CRD design) on a large aluminium tray and held in controlled temperature of 22 ± 1 °C and 75% ± 5% RH. Moisture contents of the different media were adjusted by spraying water once every three days until each pot reached its initial weight. Starting ten days after introducing larvae, the following were recorded:

1. date of the commencement of adult emergence,
2. numbers of adults that emerged daily, and
3. date of the termination of adult emergence.

III.3.2 Results

III.3.2.1 Rearing method

Transferring larvae from the leaves into the culture medium using a wet brush was the most efficient method for rearing ALCM. Even though transferring larvae was time consuming, more adults were produced by this method. In terms of the three culture media tested in the above experiment with larvae physically transferred to media by use of a brush, an average of 48.72% of the larvae placed on bark emerged as adults as opposed to 46.15% from soil and 58.97% from saw dust. Setting the leaves in vials in the first two methods tested was more time consuming and therefore was not an appropriate method for rearing large numbers of larvae. Furthermore, leaves held in vials with only water dried out over a ten day period. Larvae were trapped inside leaves; thus, no adults emerged. When leaves were held in vials with a nutrient solution, leaves were kept fresh for a longer period. Larvae were able to exit the leaves but crawled down the leaf stalk instead of dropping onto the culture media and pupated among the wet tissue paper that surrounded the leaf petiole and plugged the hole in the lid of the vial. The total numbers of adults that emerged from all three replicates in these two methods (where leaves were held in vials) were 0 and 12. Initial numbers of larvae in the leaves were not possible to estimate. Hence, data were not statistically analysed.

Lower success with rearing larvae by setting up infested leaves may be due to (1) the absence of any stimulating effect for the larvae to exit from their leaf curls or (2) the drying of the leaf causing the larvae to be trapped in curled leaves. Under the field conditions, rainfall influences the exact time of larval movement from the leaf curls (Barnes, 1948) and lack of rains delays larval descent.

These results indicated that mechanically removing mature larvae from leaves and transferring them to a suitable culture medium was the most efficient technique for rearing ALCM through to the adult stage in the laboratory.

III.3.2.2 Identification of a suitable culture medium

When mature ALCM larvae (n = 12) were introduced onto three different media (bark, soil, and sawdust), adult emergence from bark commenced 15 days after placement of larvae on the medium and continued for five consecutive days. Adults commenced emergence from both soil and sawdust after a range of 15 - 19 days; however, patterns of emergence were irregular and continued for a period of 10 -14 days in soil and 14 days in saw dust. Greater numbers of adults eclosed from soil (X ± S. E, 68.33% ± 6.30) and from bark (63.33% ± 8.97). The lowest number of adults eclosed from sawdust (48.33% ± 10.99); however, emergence from sawdust was extremely variable, ranging from 33.33% to 91.66% among different replicates. Among media, mean percentages of eclosed adults were not significantly different (two-way ANOVA, $F = 1.54$, $df = 2$, $P < 0.27$). During this study, room temperature ranged from 19.2 ^{0}C - 25.7 ^{0}C during daytime.

In a third experiment comparing bark to two other media, pumice and sand (Table 1), more adults emerged from sand than from bark and pumice (two-way ANOVA, $F = 18.11$, $d.f = 2$, $P < 0.00001$). The percentage of adult emergence from bark was significantly lower than that from sand but significantly greater than that from pumice (Table 1).

Bark and pumice took shorter periods of time for the commencement of adult emergence while sand took a slightly longer time (two-way

ANOVA, F = 7.69, df = 2, P < 0.0038). The period that adult flies emerged over was shorter for ALCM reared in bark than in pumice or in sand (two-way ANOVA, log transformed data, F = 19.42, df = 2, P < 0.00001). A problem with sand was moisture retention: sand had to be sprayed once every 3 days whereas bark and pumice only needed to be sprayed once a week.

Table 1. Rate of adult emergence from different culture medium in laboratory

Culture medium	Mean percent adults eclosed ± Standard error	Mean pupation Period (Days)	Mean duration of adult emergence
Experiment 3 (March 1995)			
Sand	56.0 ± 5.87 a	14.1 ± 0.36 a	12.6 ± 1.60 a
Bark	45.6 ± 2.71 b	12.6 ± 0.15 b	7.2 ± 0.93 b
Pumice	28.0 ± 5.27 c	12.9 ± 0.26 b	4.1 ± 0.50 c

Within a coloumn, the treatments with the same letter are not significantly different.

These results indicated that mature larvae of ALCM can be reared through to the adult stage in the laboratory by breaking infested leaves into pieces, thereby stimulating the larvae to crawl out of leaves, and by transferring these larvae to a suitable culture medium. Moisture levels of medium also appeared to be important. When culture media were too moist, emerging

adults were trapped because their wings stuck to moist media. When media were too dry, larvae and pupae dessicated and died (personal observation).

Sand, bark and soil appear to be better culture media than sawdust and pumice because more larvae and pupae survived to the adult stage. These experiments indicated that, in terms of the adult numbers, sand is the best medium for rearing larvae and pupae in the laboratory. However, sand had the very important drawback of moisture retention, and also was more difficult to handle because of its weight. Although production of adults in bark was significantly lower than in sand, it was more convenient as a culture medium because of its lighter weight and moisture retention ability. Finally, the shorter periods of time that adults emerged over were more convenient for rearing.

III.4 Diel emergence pattern of adult apple leaf curling midges

III.4.1 Materials and Methods

Information on the diel emergence pattern of adult male and female midges and on time of mating was necessary to determine the best time to run experiments on the host-finding behaviour of mated female midges. Twenty mature larvae were placed on bark material (1.5 cm depth) held in a petri-dish (12.5 cm diameter, 2 cm height), with ten replicates set up on the same day (n = 200 larvae). These petri-dishes were held in an incubator (20 °C and 14:10 L:D) until adults started emerging. Thereafter, on the days of peak adult emergence (16 - 17 days after placement of larvae on the medium), the numbers of males and females that emerged at room

temperature were recorded at 30 min intervals, starting at 04.00 - 04.30 h and ending at 21.30 - 22.00 h. The number of insects that emerged during each 30 min interval was plotted against each time interval with the time given as 4.30-5.00, 5.00-5.30 etc.

III.4.2 Results

Figure 1C shows the daily emergence pattern of male and female ALCM reared in the laboratory. Both male and female emergence were synchronous and unimodel; 95.6% of the total females (Fig. 1A, n = 91) and 97.4% of the total males (Fig. 1B, n = 77) emerged before noon. Seventy-nine percent of the total females emerged between 06:00 h and 08:30 h, with peak emergence during this time occurring between 06:30 h and 07:30 h (45.05% of the total) (Fig. 1A). For males, 88.31% emerged between 05:30 h and 08:30 h, with peak emergence between 06:00 h and 07:00 h (50.65% of the total) (Fig. 1B). A small number of females and males emerged in the afternoon (12:00 h until 19:00 h). During the afternoon there was no obvious peak of emergence.

III.5 Pre-ovipositional behaviour of females

III.5.1 Materials and Methods

III.5.1.1 Pre-ovipositional behaviour of females in wind tunnel

To identify behavioural units exhibited by females from the time of their emergence, through mating and up to their first oviposition, an observational study was conducted in a large wind tunnel in November, 1994. The wind tunnel (plexiglass walls and aluminium frame), which measured 2 m (length) x 1 m (width) x 1 m (height) with a layer of moist

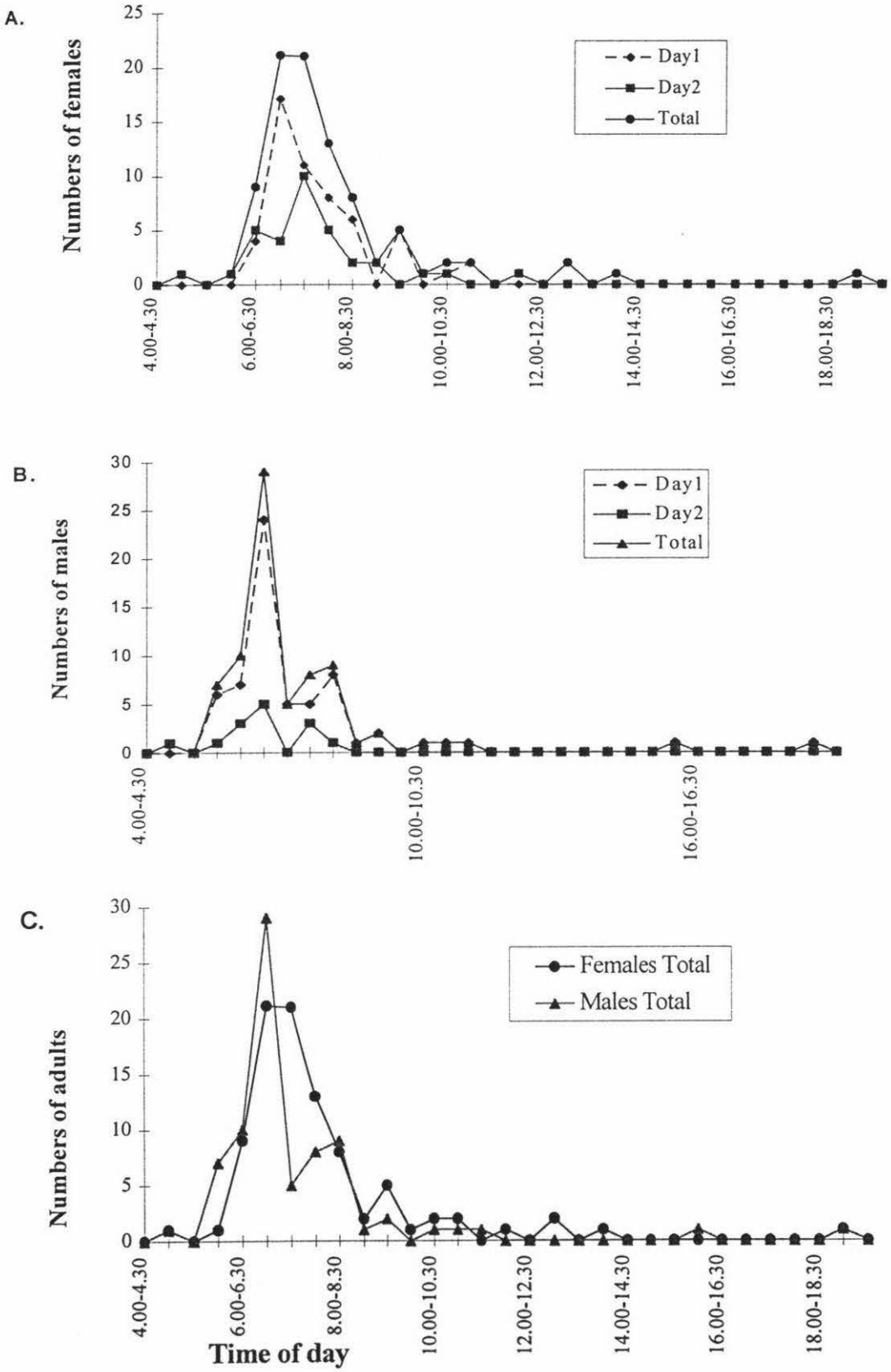

Fig. 1. Temporal patterns of adult apple leaf curling midge emergence in the laboratory, January 1995.

soil (5 cm depth) on the floor, was illuminated from above by a light bank of fourteen full-spectrum composition fluorescent tubes (36 W, 230 LUX, 1.2 m long, BIOLUX, Hamburg, Germany) with high-frequency control circuits (Quicktronic Deluxe, BIOLUX, Hamburg).

Three apple branches (50 cm height) were set 50 cm apart from each other in a triangular formation in the center of the wind tunnel. As the fan in the wind tunnel was not turned on, there was minimal air movement in the tunnel. The soil layer covering the floor of the wind tunnel was sprayed with water. An open vial containing a virgin female was placed on the soil in the center of the wind tunnel and in the middle of the three apple branches. Approximately ten minutes later, when the female walked or flew out of the vial, landed and started calling, a single newly eclosed male was released into the arena. Thereafter, all the movements of the female were recorded over a three hour observation period. Three females were observed, one female per day over three consecutive days. Temperature and relative humidity in the wind tunnel were recorded hourly.

III.5.1.2 Pre-ovipositional behaviour of virgin females in vials

Information about the peak period of virgin ALCM female sexual receptivity was necessary to obtain high numbers of mated females for experiments. It was also necessary to identify specific behaviours of unmated females. These would be helpful to separate unmated females from mated females when ALCM were allowed to mate in a group. Furthermore, I wanted to test whether virgin females of ALCM lay eggs before death. Therefore, with these aims in mind, a study was conducted using females held in vials with an apple leaf. The advantage of such an arena over the wind tunnel was that the behavioural postures of females

could be observed easily and timed accordingly. Furthermore, a greater number of females could be studied simultaneously. This experiment was conducted in January 1995 in Palmerston North.

At 08.30 h. ten virgin females of a similar body size, which had eclosed between 07.30 and 08.30 h, were collected and placed individually in glass vials (7.5 cm height, 2.5 cm diameter) with fitted plastic lids with a mesh window. Each vial contained an immature apple leaf. Every 15 minutes, starting at 08.45 h and ending at 20.00 h, the behavioural activities identified in the previous experiment (sitting, walking, flying, antennation, probing, and oviposition) (see III.5.1.1) were recorded for each female. Observations continued the next day, starting at 07.00 h and ceasing at 18.00 h. After the death of the fly, numbers of eggs deposited on the leaf and on the lid and walls of the vials were counted. Dead flies were dissected under 10 x 20 magnification so that the number of eggs remaining in the ovaries could be recorded. Room temperature and relative humidity were recorded every hour.

III.5.1.3 Pre-ovipositional behaviour of mated females in vials

Similar to the above study on virgin females was a study conducted on mated females (January 1995, Palmerston North). A group of females that eclosed between 08.00 and 09.00 h were placed in a plastic container (13 cm height x 13 cm diameter) along with newly eclosed males (approx. 1:1 ratio of females to males). Thirty minutes later, at 09.30 h, ten females that had ceased calling were removed from the container, individually placed in glass vials and observed in the same manner used for virgin females. This test was repeated under controlled environmental conditions over three consecutive days in November 1995 using 15 individual females per day

and again in January 1996, using 15 females. The females used in the later two tests eclosed between 06.30 and 07.30 h and mated between 07.30 and 08.30 h.

III.5.2 Results

III.5.2.1 Pre-ovipositional behaviour of females in wind tunnel

Immediately after eclosion, females were released onto the soil substrate of the wind tunnel. Two of the three females walked on the soil until they encountered a vertical object (apple branch or wall of the wind tunnel) and then climbed the object to a height of approximately 30 cm. The third female flew and landed on the underside of an apple leaf. A few minutes later, females extruded the ovipositor to its full length, parallel to the surface on which the female sat, and started waving the terminal segments of the ovipositor. This type of behaviour, which is commonly referred to as calling behaviour, is typical of insect species that produce long-range sex pheromones. Several members of the family Cecidomyiidae have been observed exhibiting such calling behaviour, including a *Dasineura* spp. (Isidoro et al., 1992). The females that first climbed up the apple branch or the wind tunnel wall, sat and called for a period that varied between the two females (20 min and 75 min). After this, they flew onto an apple branch, settled on the underside of an apple leaf and again exhibited calling.

Immediately upon their release into the wind tunnel males flew to and landed on the wall of the wind tunnel. They continued doing this for 30 min to 1 hour and eventually landed on an apple leaf in the same shoot where female was sitting. Thereafter they hovered around the shoot until they located the females and mated. With the commencement of mating

females retracted their ovipositors and after mating sat motionless. Twenty to ninety minutes later females became active again and started flying around the wind tunnel. The three females I observed were mated at two different times of the day (Table 2) and showed variable times to flight and landing on apple foliage.

Table 2. Activities of the mated females in relation to the time of the day

Activity	Time of the day		
	Female #1 Hr:Min	Female #2 Hr:Min	Female #3 Hr:Min
Time of mating	09:34	09:32	13:56
Time to first flight	1:21	1:30	0:20
Time to first landing on foliage	3:30	1:31	1:11
Time to first probing	4:36	1:31	1:11

When a mated female approached a shoot containing both young and old leaves, she flew around it for several seconds, hovering within 5 cm of the leaves. She then either landed or flew again and subsequently hovered near another shoot. After landing on the shoot she either (1) sat for a few minutes, took a short flight, and then landed elsewhere on the same branch or (2) proceeded to examine the surface of the foliage. Examining behaviours were similar to those of a related species, the Hessian fly (Harris and Rose, 1989). The female bent her head towards the leaf surface and walked forward while her antennae touched or moved closer to the leaf surface (antennation). Then she extended her ovipositor and bent the last few abdominal segments, so that the tip of the ovipositor contacted the leaf

surface. In this posture (while walking forward), the female moved her ovipositor from side to side over the surface of the leaf (probing). During oviposition, the female stopped walking, lifted her head and antennae off the leaf surface and extended her ovipositor to its full length. While holding the ovipositor at a right angle to the leaf surface the female inserted her ovipositor through unopened buds or leaf axils or leaf hairs and deposited eggs. After ovipositing in one place, she retracted her ovipositor and continued antennation, probing and oviposition on the same foliage or flew away and continued to search for new oviposition sites. The duration of flights between two oviposition sites on the same branch ranged from 15 to 228 sec. Up to 15 min were spent during flights between two different apple branches. A description of the pre-ovipositional behaviours and associated body postures of the female midge is given in Table 3.

III.5.2.2 Pre-ovipositional behaviour of virgin females (in vials)

After introduction into the vial at 8.30 h, all virgin females (n = 10) sat at the top of the vial until 09.00 h. At 09.00 h, 30% of the females were observed calling. The percentage of calling females gradually increased to 100% by 10.00 h. During this period of peak calling, females periodically retracted the ovipositor and sat without calling for a few minutes or occasionally walked for short periods. From 14.30 h onwards, calling was observed less frequently. By 16.00 h 100% females had ceased calling and proceeded to sit for the rest of the day.

When observations recommenced at 07.00 h the next morning, females were observed sitting without calling. Soon after females exhibited brief periods of walking or flying. At 08.30 h calling behaviour

Table 3. Pre-ovipositional behaviours exhibited by ALCM females and associated body postures

Behavioural category	Description of the body posture
Sitting	Female sits with her body axis parallel to the surface she is sitting on with ovipositor retracted into the abdomen.
Calling	Female sits with her body parallel to the surface with the terminal segments of the ovipositor extended and waving perpendicular to the surface.
Walking	Female moves forward while ovipositor is fully or halfway retracted into the abdomen. Head, antennae and abdomen are held parallel to the surface she is walking on.
Antennation	Female walks forward all the while moving the head and antennae down towards the leaf surface so that they either approach or touch the leaf surface.
Probing	Abdomen is arched so that ovipositor is positioned perpendicular to and touching the surface, with the head and antennae also touching or close to the leaf surface. Female walks while in this posture, all the while moving the ovipositor from side to side.
Oviposition	Female stops walking, head and antennae are lifted off and held parallel to the leaf surface. Abdomen arched so that the distal segments of the ovipositor are pressed to the surface. Eggs are laid.

was again observed and became more frequent until 09.40 h when 100% of the females were observed calling. Unlike the first day post-eclosion, females on the second day post-eclosion frequently stopped calling and either sat, walked or flew for brief periods during the calling period. From 11.00 h onwards calling was observed less frequently and by 14.00 h all females had ceased calling. After this most of the females entered into a more active phase of behaviour and walked, flew, antennated and probed the leaf surface more frequently. Oviposition was never observed. A small number of females remained inactive and some females appeared to be close to death. Seventy-five percent of the females were dead by 16.15 h. The rest were still alive at 21.30 h. These behaviours are summarised in the ethogram shown in Figure 2.

When vials and apple leaves were examined for eggs, only two females were found to have laid a small number of eggs (2 and 5 eggs). When the ovaries of dead females were checked for eggs, a mean of 178.5 eggs (range 145-223) were found. Day-time temperatures during the period of study ranged from 20-29 ^{0}C, with relative humidities of 40-45%.

III.5.2.3 Pre-ovipositional behaviour of mated females in vials

A group of females (n = 10) was observed in January 1995 in Palmerston North. These females eclosed between 08.00-09.00 h, mated between 09.00-09.30 h, became active starting from 11.00 h, and by 12.30 h, 100% of the females were active. Walking and flying were the first activities observed. Antennation, probing and oviposition were not observed until 12.00 h. The percentage of females that engaged in oviposition gradually increased up to 50% by 13.30 h. By 17.00 h 100% of the females were

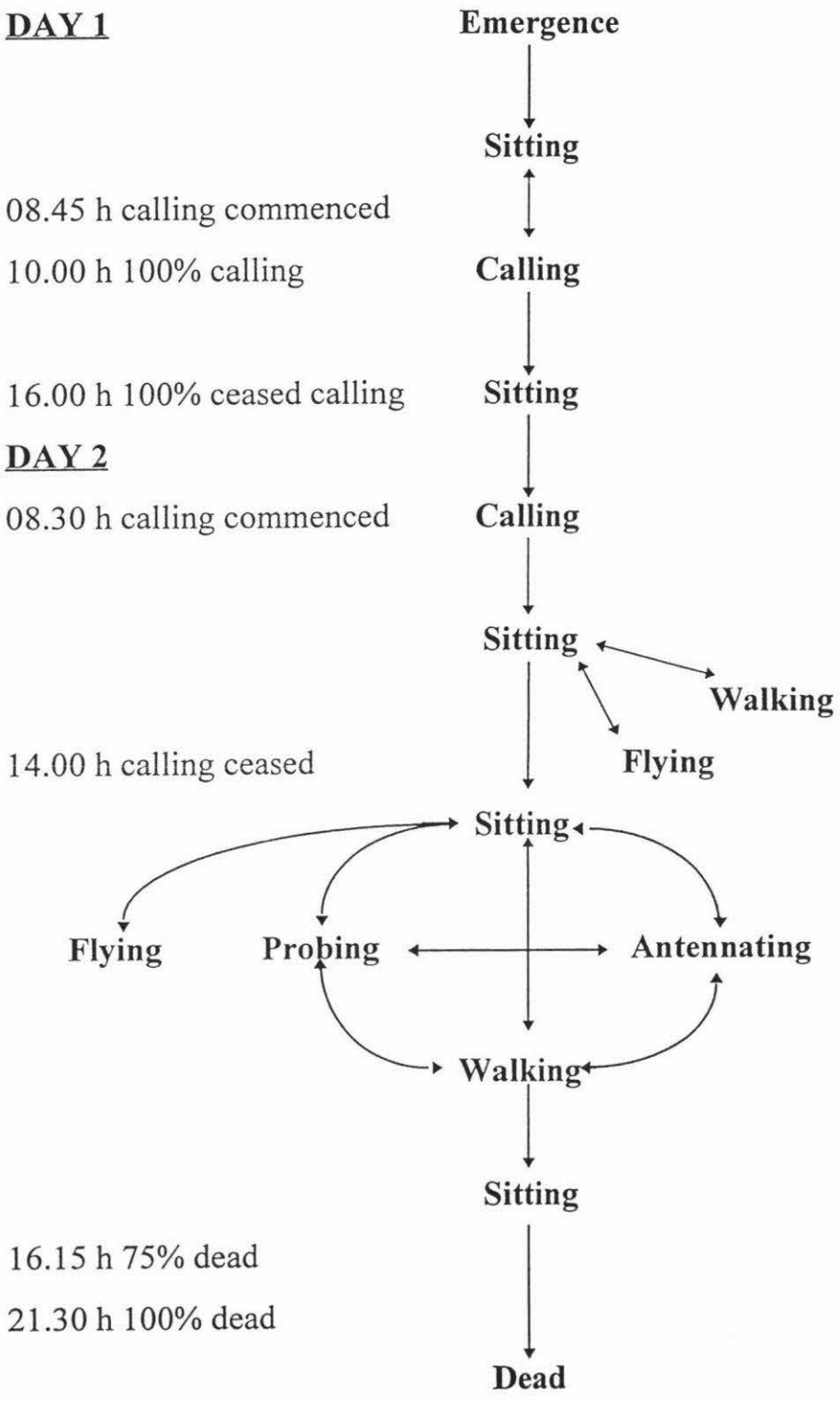

Fig. 2. Behaviour of virgin ALCM females in vials with an apple leaf observed under laboratory conditions.

observed ovipositing. From 17.30 h onwards a smaller percentage of females were observed ovipositing. By 21.00 h, 30% females were dead and all others sat without moving. All females were dead by 06.00 h the next morning. Numbers of eggs deposited by females were recorded only for a single group of females (n = 10) in January 1995. The results were variable among individual females, ranging from 11 to 142 eggs laid on apple. A range of 40 - 150 eggs were found remaining in the ovaries of individual females after death. Thus, the eggs laid on apple represented 6.8% to 77.1% of the potential fecundity of individual females. Females (n = 33) that had eclosed between 06.30 to 07.30 h and mated between 07.30 to 08.30 h were observed during three consecutive days in November 1995. Results were pooled over the three days. Mated females became active approximately 90 min. after mating. By 12.00 h approximately 90% of the females were actively flying and walking. Antennation and probing were not observed until 12.00 h and 14.30 h, respectively, approximately two hours later than the groups of females observed in January 1995. This difference may have arisen from colder temperatures during November (18-26 ^{0}C) than during January (20-29 ^{0}C). An increase in ambient temperature decreased the post mating pre-ovipositional transition period of the Hessian fly (Harris and Rose, 1991). In the present study, oviposition was first observed at 15.30 h and continued until the final observation at 16.30 h, when 40% of the females were observed to be either ovipositing or probing. These behaviours of mated females are summarised in Figure 3.

Results of observations on the group of females (n = 15) that eclosed between 06.30 and 07.30 h and mated between 07.30 and 08.30 h (January 1996, Auckland) were similar to those in 1995. However, females became

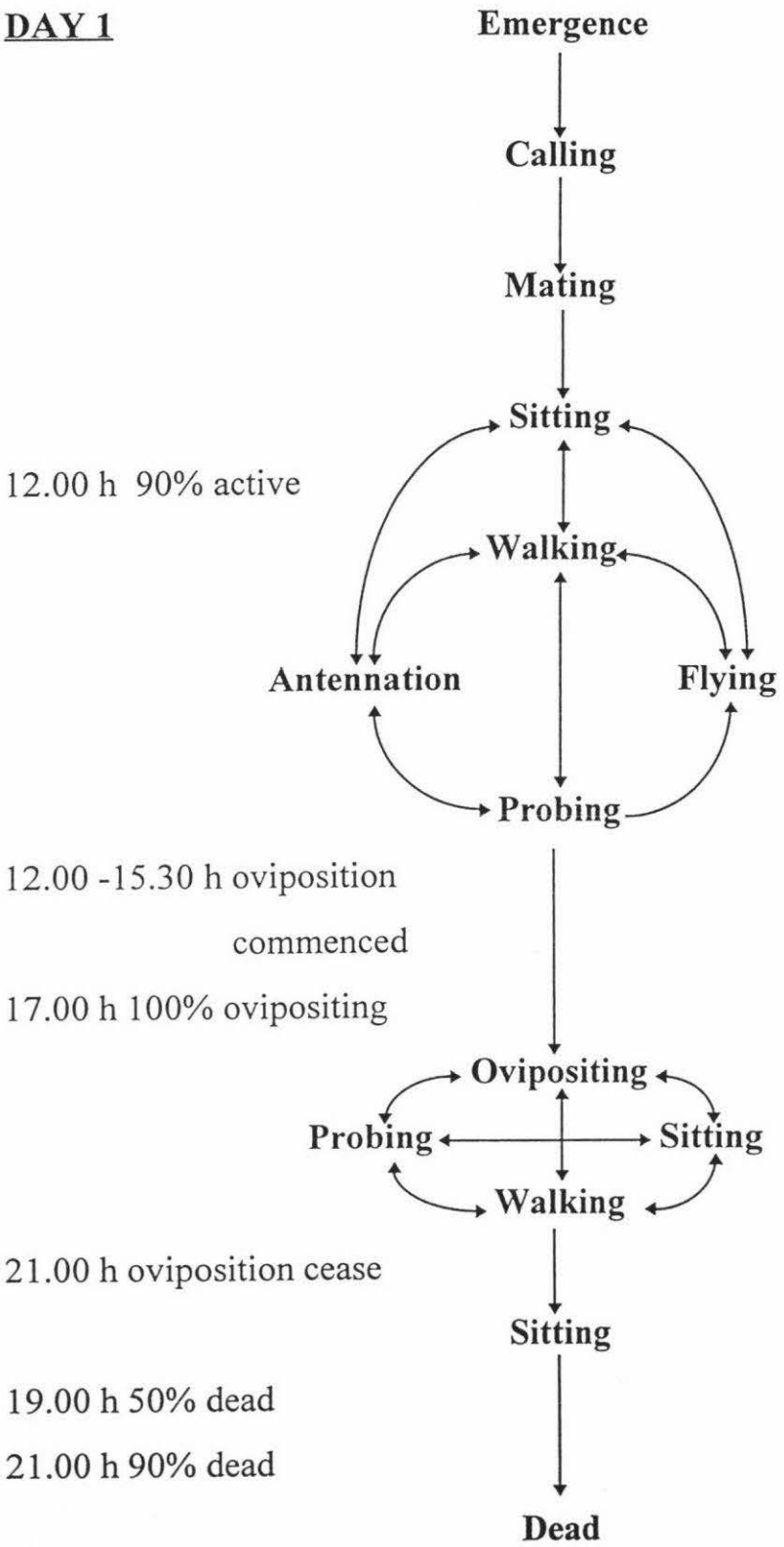

Fig. 3. Behaviour of mated ALCM females in vials with an apple leaf observed under laboratory conditions, November 1995.

active earlier than the previous group: 50% rather than the 0% of the females were walking by 10.00 h. In summary, virgin ALCM females either do not lay eggs before death or lay only a small number of eggs. Virgin females appear to live longer than mated females, two days versus one day, respectively. After passing through a post-mating inactive period which lasts approximately 90 min, mated females spend the first day of their adult life in flight and oviposition.

III.6 Host-plant specificity of ovipositing females

III.6.1 Materials and Methods

III.6.1.1 Host-plant specificity of ovipositing females

Choice and no-choice bioassays were conducted in February 1995 (Palmerston North) using host (apple, cv. Fuji) and non-host (pear, cv. Pakchams Triumph) plant species to determine how specialized ALCM females are in their oviposition behaviour. For the no-choice test, a single shoot (four leaves and the bud) of apple or pear, free of previously laid ALCM eggs (checked under microscope) was positioned vertically in a vial (7.5 cm height, 2.5 cm diameter) with water and supported by a rubber stopper. Five shoots of each plant species were prepared and each shoot was randomly placed on moist soil in two rows 15 cm apart between and within rows (five shoots per row) in a CRD design, in the wind tunnel described in section III.5.1.1. Each shoot was covered with a mesh cage (30 cm height, 13 cm diameter) and illuminated by full-spectrum fluorescent tubes from above (see III.5.1.1). Light conditions were 14:10 L:D. A single mated female was introduced into each cage. Forty-eight

hours later, when all females were dead, numbers of eggs laid on individual shoots were recorded.

A choice test using shoots of the same apple and pear cultivars was conducted using a group of mated females (n = 18) under the same conditions as in no-choice test. As before, single shoots of apple and pear were set in vials, and three vials of each treatment were arranged randomly in a circular formation in a large mesh cage (45 cm x 45 cm x 45 cm). Females were released into the center of the cage. Twenty-four hours later, numbers of eggs deposited on each shoot were recorded. This test was not replicated, therefore the results were averaged over the three samples.

III.6.1.2 Temporal patterns of oviposition on host and non-host plant species

This study was done to determine the temporal distribution of egglaying on host (apple) and non-host (pear) foliage, when ALCM females were given a choice between the foliage of two plant species. This study was conducted using the same apple and pear cultivars used in the previous experiments in February 1995 (Palmerston North). Apple and pear shoots (30 cm long) were collected from the field and were thoroughly checked for ALCM eggs under the microscope. Shoots free of eggs were selected and trimmed so that only 2 immature leaves and the unopened bud remained. Each shoot was supported in a vial of water by a rubber stopper.

One shoot of apple and one of pear were placed 4 cm apart in a plastic pot (12.5 cm diam, 12 cm height) containing moist sand and covered with a mesh cage (13 cm diameter, 30 cm height). Twelve such pots (twelve replicates) were randomly placed on a moist sand layer in RCB design. A single female, mated between 08.00 and 09.00 h, was

introduced into each cage at 10.00 h. In each cage, apple and pear shoots were changed at 14.00 h and 20.00 h on this first day. On the second day of the test, shoots were changed at 08.00 h, 14.00 h, and 20.00 h, and on the third day, at 08.00h or until the female died. Shoots were not changed after 20.00 h during the scotophase. Numbers of eggs laid on apple and pear shoots were recorded for each time interval for each cage. The twelve replicates were held in a controlled environment room (22 ± 1° C, 75 ± 5% RH, 14:10 L:D).

To more precisely define the patterning of oviposition on host and non-host foliage over time a second experiment was conducted with numbers of eggs on shoots recorded at four hour intervals. Here, apple and pear shoots were changed at 14.00 h, 18.00 h, and 22.00 h on the first day. On the second day, shoots were changed at 06.00 h, 10.00 h, 14.00 h, 18.00 h and 22.00 h and on the third day at 06.00 h or until the female died. Here also shoots were not changed after 22.00 h in the scotophase. Numbers of eggs laid on the apple and pear shoots were recorded for each cage for each time interval. Eighteen replicates were run. Percentages of eggs laid on apple shoot and on pear shoot by each female were calculated based on the total number of eggs laid by each female on both apple and pear shoots.

III.6.2 Results

III.6.2.1 Host-plant specificity for oviposition

When females were given only apple or pear shoots for oviposition (no-choice), similar numbers of eggs were laid on each ($F= 7.89$, $df = 1$, $P < 0.1068$, Table 4). When a single group of females was placed in an

arena with both apple and pear shoots (choice), more eggs were deposited on apple shoots than on pear shoots (Table 4).

Table 4. Mean numbers of eggs deposited on host and non-host plant foliage under choice and no-choice conditions

Treatment	Mean Number of eggs per shoot ± S.E.	
	No choice	Given choice *
Apple	75 ± 42	172 ± 49
Pear	78 ± 76	23 ± 26
* For choice test all shoots were placed in a single cage with a single group of females. Thus, this test was not replicated with different populations of ALCM		

These results suggest that, although ALCM is considered as a specialist herbivore on apple some ALCM females may lay a number of their eggs on pear plants, especially when deprived of their host-plant species: in the no-choice test females laid a range of 0 to 166 eggs on pear. Although attempts to breed the closely related species *D. pyri* on apple were unsuccessful (Barnes, 1948), the survival of *D. mali* on pear has not been tested.

III.6.2.2 Temporal patterns of oviposition on host and non-host plants (choice)

In the first experiment, the majority of females (n=7) laid from 68 to 197 eggs while a smaller number of females laid either no eggs (n=2) or less

than 5 eggs (n=2). Hence, to reduce variation, data for females laying less than five eggs were discarded.

Females started ovipositing between 10.00 and 14.00 h on the day of mating, but at this time laid only a small number of eggs on both apple and pear (Fig. 4A). Between 14.00 and 20.00 h, numbers of eggs laid on apple and pear increased, reaching a peak on both apple and pear shoots (Fig 4A). However, during this time ten times more eggs were laid on apple than on pear. After this no more eggs were laid on pear. A small number of eggs were laid on apple by 08.00 h the next morning. Overall a significantly greater numbers of eggs ($X \pm S.E.$, 109.75 ± 21.83) (two-way ANOVA, log transformed data, $F = 5.05$, $df = 1$, $P < 0.0595$) were deposited on apple shoots than on pear shoots (19.50 ± 8.21).

In the second experiment six females laid either zero ($n = 4$) or less than three eggs ($n = 2$). Hence, as in the previous experiment data from these females were not included in analyses. Females given both an apple and a pear shoot, again started oviposition within four hours after mating (between 10.00 and 14.00 h) (Fig. 4B). During this period more eggs were deposited on apple than on pear shoots. Egg deposition on apple peaked during the next time interval (14.00 to 18.00 h) (Fig. 4B). Numbers of eggs laid on apple decreased between 18.00 and 22.00 hrs. Numbers of eggs laid on pear decreased after 14.00 h. Thirty three percent of the females ($n = 4$) were dead by 06.00 h the next morning. The remaining females ($n = 8$) laid a small number of eggs on apple but did not lay again after 06.00 h on the second day. The females that had not oviposited on the first day started ovipositing 24 to 32 hours after mating, and laid less than a total of 50 eggs. Three females, that delayed oviposition, deposited a range of 22-79 eggs on apple and 0 - 56 eggs on pear after 10.00 h on the second

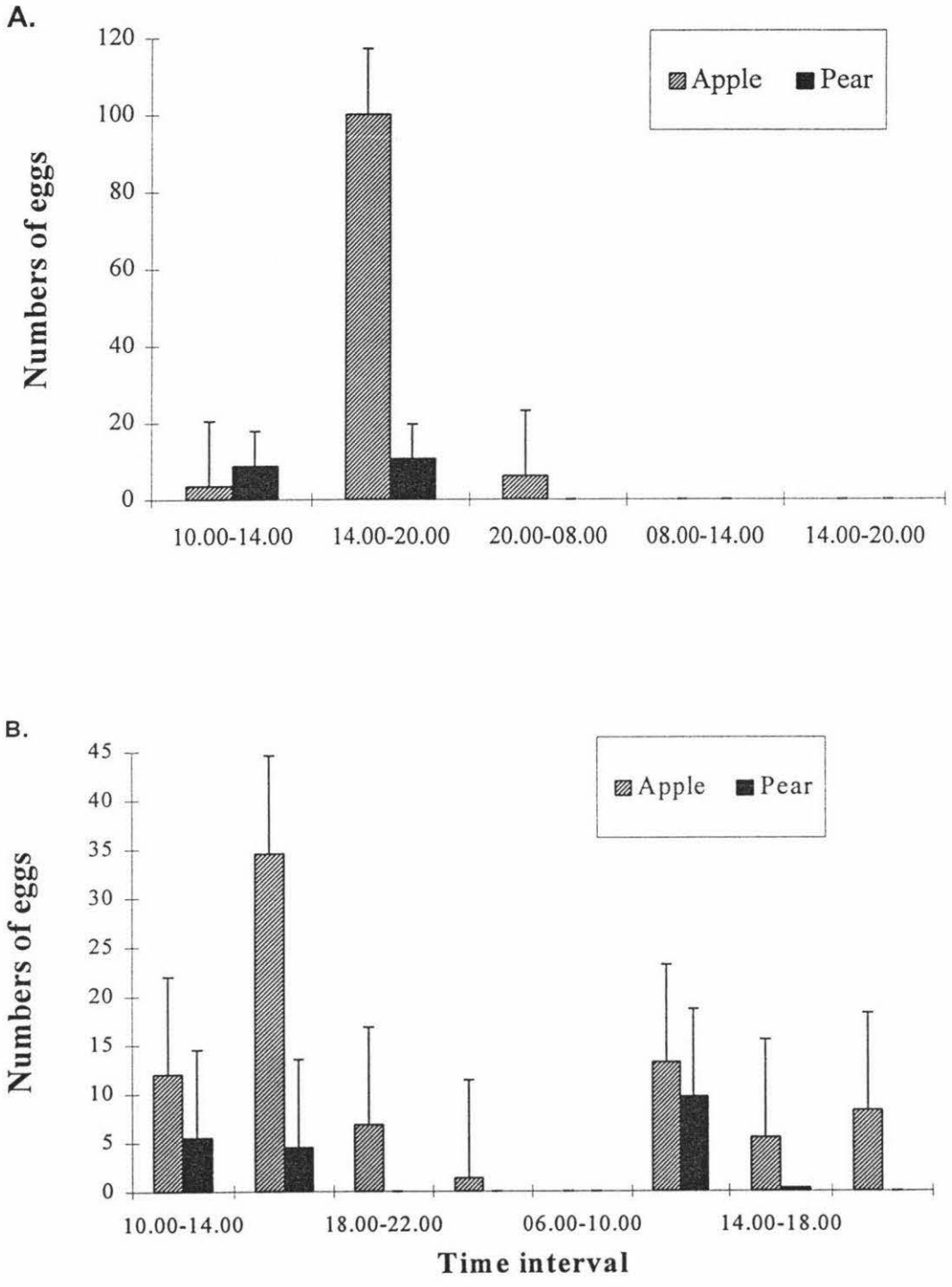

Fig. 4. **Temporal patterning of egglaying of apple leaf curling midge on host- and non-host plant foliage in two separate assays (A) and (B).**

day. In total, significantly more eggs were deposited on apple shoots (X ± S.E., 66.42 ± 14.80) than on pear shoots (15.00 ± 6.97) (two-way ANOVA, log transformed data, $F = 8.72$; $df = 1$; $P < 0.0131$). In these two tests (choice), a large number of females (n = 11) laid 100% of their eggs on apple while a smaller number of females (n = 7) laid the majority of their eggs on apple (ranging from 52.21% to 86.95%) with the rest of their eggs being laid on pear. Two-females laid 100% of their eggs on pear.

III.7 Choice of oviposition sites by mated females

With the aim of determining the choice of females for oviposition sites in cages in the laboratory, several preliminary tests were conducted. Specifically, preliminary tests helped to identify the best way to present treatments in experiments on factors influencing host-finding and oviposition behaviour of ALCM.

III.7.1 Materials and Methods

III.7.1.1 Height of oviposition sites

A choice test was conducted in the laboratory to test whether ALCM females choose oviposition sites at a particular height within a cage. Apple (cv. Fuji) shoots of similar growth stage were cut from the orchard and brought to the laboratory where all were checked for previously laid eggs. Of these, 20 shoots that were free of eggs were cut to five different lengths (four shoots each at: 10, 20, 30, 40, and 50 cm). The mature leaves of these 20 shoots were trimmed, leaving only the bud and three adjacent leaves. Each trimmed shoot was set in a plastic vial as in the previous experiments. The 20 vials containing shoots of each of the five lengths (choice test) were then embedded in the moist soil layer lining the bottom

of a mesh cage (45 cm x 45 cm x 60 cm), 4 cm apart from each other in a random arrangement inside the wind tunnel. The cage was illuminated by the light bank (see III.5.1.1) described previously on a 14:10 D:L cycle. A group of ten mated females were released into the cage at 12.00 h. Fourty-eight hours later, numbers of eggs on individual shoots were counted. This test was not replicated with other groups of ALCM females. Data were analysed by simple linear regression analysis.

III.7.1.2 Developmental stage of foliage

A choice test was conducted to test whether females prefer to oviposit on apple foliage of a particular developmental stage. To prepare these treatments 30 cm long apple shoots (cv.Fuji) of a similar growth stage were taken to the laboratory where they were thoroughly checked for previously laid eggs. Each shoot was trimmed leaving:

(1) only the unopened bud and four leaves below the bud at the end of a 30 cm shoot (positive control),

(2) only one mature leaf at the end of a 4 cm length of shoot,

(3) only one immature leaf at the end of a 4 cm length of shoot, and

(4) only the unopened bud and one immature leaf at the end of 4 cm length of stem.

Each shoot was placed in a plastic vial with water (vials 5 cm length x 1.5 cm diameter or 10 cm length x 2.5 cm diameter for 4 cm or 30 cm long shoot, respectively). For treatments 2, 3 and 4, plastic vials carrying foliage were individually fixed on top of a 30 cm length of metal rod by adhesive tape so all treatments were held at the same height (30 cm) above the floor of the cage.

Two shoots of each developmental stage were arranged randomly in a circular formulation 10 cm apart from each other on a layer of moist sand which covered the bottom of a mesh cage (45 cm x 45 cm x 45 cm). A group of ten mated females were released into the center of the cage. Numbers of eggs deposited on each treatment were recorded 48 hours later. This experiment was repeated on four separate occasions with four different groups of shoots and ALCM females.

III.7.2 Results

III.7.2.1 Height of oviposition sites

When female ALCM were given apple shoots at different heights above the cage floor, mean numbers of eggs laid increased with the height of the shoot. Regression analysis indicated that height of shoots had a significant effect on the numbers of eggs laid on them ($r^2 = 0.5087$, $F = 13.46$, $df = 1$, $P < 0.0028$). Females most preferred oviposition sites at the 50 cm height (Fig. 5). These results may have been obtained because ALCM females tend to fly upwards and sit on the mesh top of the cage. Whether such a bias for higher shoots occurs under field conditions is not known; however, these results suggest that in the laboratory keeping targets at a greater height within the cage (greater than 30 cm) may give better results.

III.7.2.2 Developmental stage of foliage

When females were given apple foliage at different developmental stages, the highest mean number of eggs (Fig. 6) were deposited on whole shoots (positive control). Immature leaves and unopened buds received fewer eggs but more eggs than mature leaves. The lowest numbers of eggs were

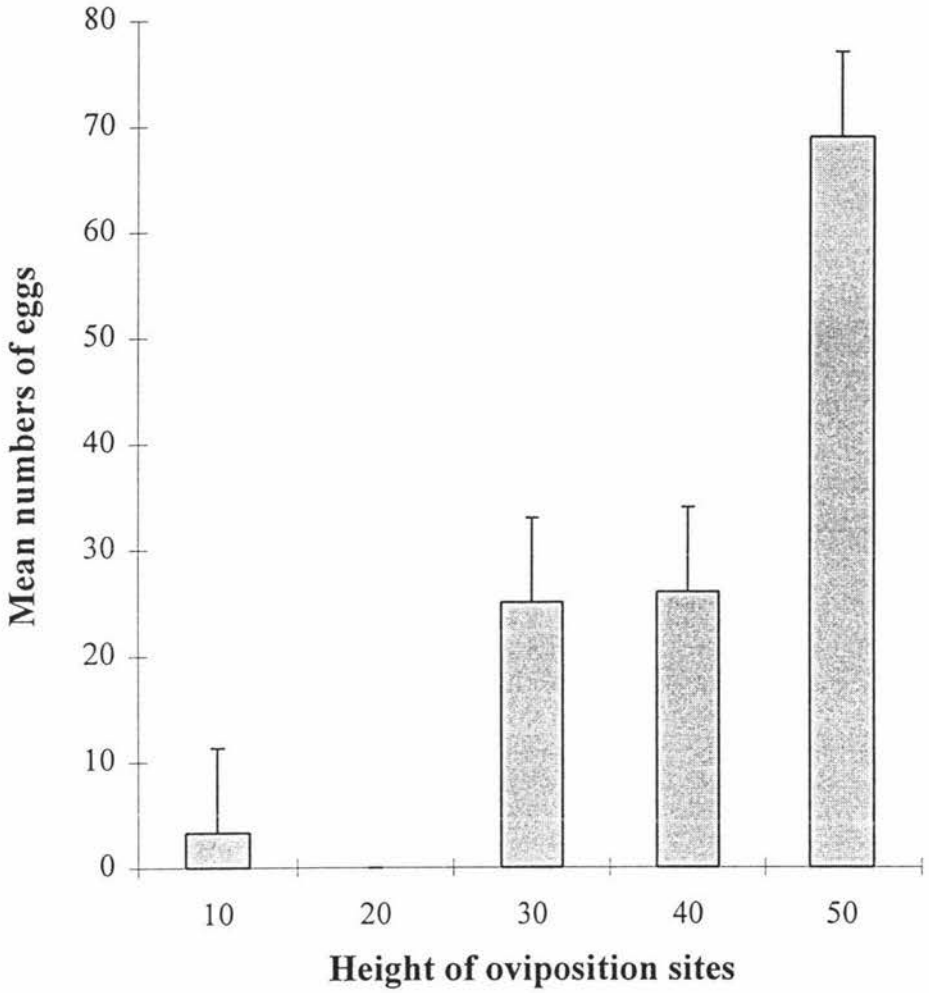

Fig. 5. Numbers of eggs (Mean ± S.E.) laid by ALCM females on oviposition sites at different heights.

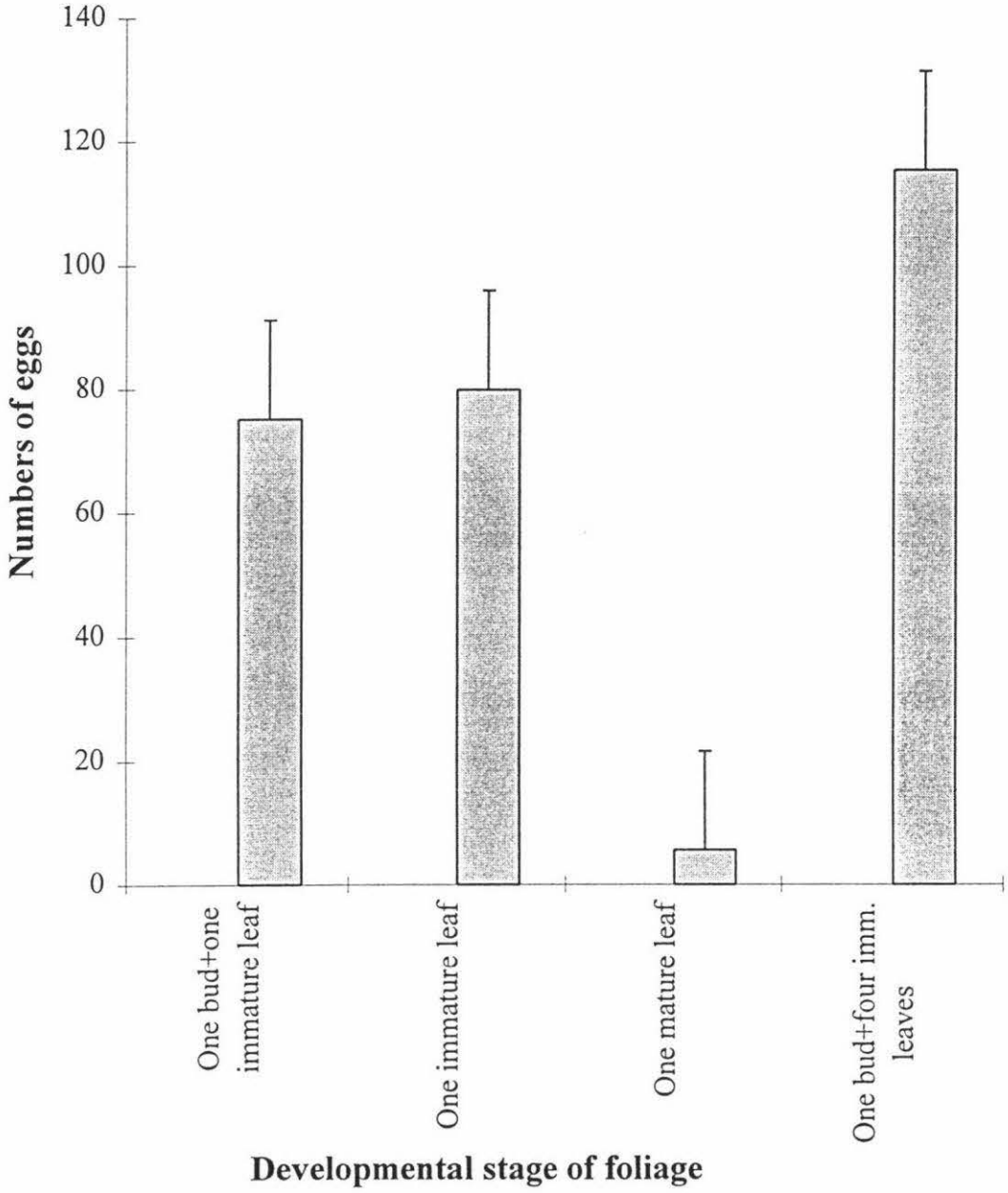

Fig. 6. **Numbers of eggs (Mean ± S.E.) laid by ALCM females on foliage of different developmental stages.**

found on mature leaves (log transformed data, $F = 5.84$; $df = 3$; $P < 0.0170$).

These results show that ALCM females prefer buds or immature leaves over mature leaves. In field studies it has been shown that the degree of ALCM infestation depends on the presence of fresh shoot growth of the plant (Todd, 1959). Results of the present study suggest that the preference of adult females for unopened buds and immature leaves may contribute to this pattern of infestation.

Chapter IV

Host-finding behaviour of apple leaf curling midge females

IV.1 Introduction

In the field, ALCM adults have been observed emerging from the soil, and then swarming below the canopy of apple trees (Todd, 1959). Soon after, presumably after mating occurred, females were observed laying eggs on buds and uncurled leaves of apple. How female ALCM found these apple buds and young leaves was not known.

In my preliminary studies on the behaviour of mated ALCM females (Chapter III) I observed that, once mated females became active after a post-mating transition period, they often flew around the wind tunnel for a long period of time before approaching apple foliage. Once females approached apple foliage, they hovered within a 5 cm radial zone of the foliage, and then either landed or flew onto another shoot. If the female landed and oviposited, she remained on a single shoot for approximately 20-30 mins and continued walking, probing and ovipositing, taking short flights between two oviposition sites on the same branch. Between oviposition bouts on two different apple branches, females often flew for long periods.

The studies reported in this chapter were conducted with the aim of identifying the plant stimuli that influence host-finding behaviours of mated ALCM females. To do this, I needed an arena in which plant stimuli could be presented in a more controlled, but also a more natural manner. In previous experiments (Chapter III), small vials were used to study female midges. The advantage of this small arena was that body postures related to different behavioural activities could be easily observed. The

disadvantage of a small arena was that it was not possible to observe flight responses to host-plant stimuli because of restricted space and still air inside the vial. Therefore, a wind tunnel, which provided a large space and controlled air movement, was used to conduct the studies on host-finding behaviour of ALCM that will be presented here in Chapter IV. Apple was used in these wind tunnel experiments as it is the only known host-plant species of ALCM. Pear was selected as a representative non-host plant species because pears are damaged by a closely related midge species (pear leaf curling midge, *Dasineura pyri)* and are grown in close proximity to apples in many New Zealand fruit orchards, but are not known to be damaged by ALCM. These studies were conducted during the 1995/1996 summer season at the Insect Science Laboratory at HortResearch, Mt. Albert Research Center, Auckland.

IV.2 Materials and Methods

IV.2.1 Insect rearing

Apple leaves infested with ALCM larvae were collected from a HortResearch experimental orchard in Kumeu and from a commercial apple orchard in Henderson, Auckland in 1995/1996. In the 1995/1996 season, the rearing method described in chapter III was modified slightly to make it less time consuming. Instead of collecting mature larvae and placing them on medium, infested leaves were broken into pieces and placed on top of a 3 cm deep layer of rearing medium held in plastic boxes (22 cm x 13 cm x 8 cm). Infested leaves were then sprayed with water and left until adults emerged. In this method, some of the larvae exited the broken leaves and pupated in the medium while others remained in curled

leaves and pupated there. Boxes were held in a controlled environment room (21 °C ± 2 °C, 14:10 L:D, and 70-80% RH).

IV.2.2 Experimental arena - the wind tunnel

A wind tunnel (plexiglass walls and aluminium frame), which measured 2 m (length) x 1 m (width) x 1 m (height), with a layer of moist soil (5 cm depth) on the floor, was used in the experiments. A fan (0.7 m diameter, Woods Air Movement, G.E.C., Wellington) connected to a variable motor-speed controller generated air movement through the wind tunnel. Fourteen full-spectrum composition fluorescent tubes (36W, 230LUX, 1.2 m long, BIOLUX, Hamburg, Germany) with high-frequency control circuits (Quicktronic Deluxe, BIOLUX, Hamburg) illuminated the wind tunnel from above. The plastic side walls of the wind tunnel were covered on the outside by a light blue cloth to make visual stimuli viewed from inside the tunnel more homogeneous. The upwind end of the wind tunnel was separated from the chamber holding the fan by a white mesh screen.

IV.2.3 General procedures

Unless otherwise specified, midges used in experiments were of similar body size and eclosed during the hour of peak emergence (06.30 to 07.30 h) (see section III.4.2). Mated females were obtained by the following method. At 06.30 h culture boxes were cleared of all midges (these midges were discarded). One hour later adult midges were collected by aspirator and introduced into a plastic container (13 cm height, 13 cm diameter) with males and females at approximately a 1:1 ratio. At 08:30 h, females were placed individually in a glass vial (10 cm height, 2.5 cm diameter), closed with a fitted plastic lid having a 1.5 cm diameter mesh window.

When all females had been placed in vials, females were scanned at 10 minute intervals for the next 30 minutes. Females that resumed calling during this time were assumed to be virgins (Bergh et al., 1992) and were discarded. Until the experiment commenced, mated females were held either at 24 ± 3 °C and 62-72% RH (wind tunnel experiments) or at 21 ± 2 °C and 80% RH (all other experiments).

All experiments in the wind tunnel (Fig.7) were carried out as no-choice bio-assays. Except for experiments examining responses of females to colour cues and to chemical foliar extracts, where groups of females were used, mated females were tested individually. All tests discussed in this chapter were carried out between 13.00 and 18.00 h, a time interval in which females are in the active phase after mating (see III.5.2.3). A wooden pole (30 cm height) with Blu tack® on the top end was positioned vertically in the middle of the wind tunnel, halfway between the two longitudinal sides and 50 cm downwind of the screened upwind end of the wind tunnel. Test insects were released from platforms placed on top of this pole. When foliage was used as a treatment, it was placed midway across the upwind end of the tunnel, 10-20 cm downwind of the mesh screen, so that the distance between the wooden pole and the edge of the foliage was 30 cm (Fig. 7).

A single mated female was released (Fig. 8) from a vial into the wind tunnel by maintaining the vial in an upright position (lid side up) so that the female sat on the lid, removing the lid of the vial, and moving it to the rod, where the lid was gently turned over (so that the top of the lid was bottommost) and attached to the rod by the Blu tack®. Females that did not fly away from the vial cap within the ten minute observation period were recorded as non- responders. Females that flew away from the vial before

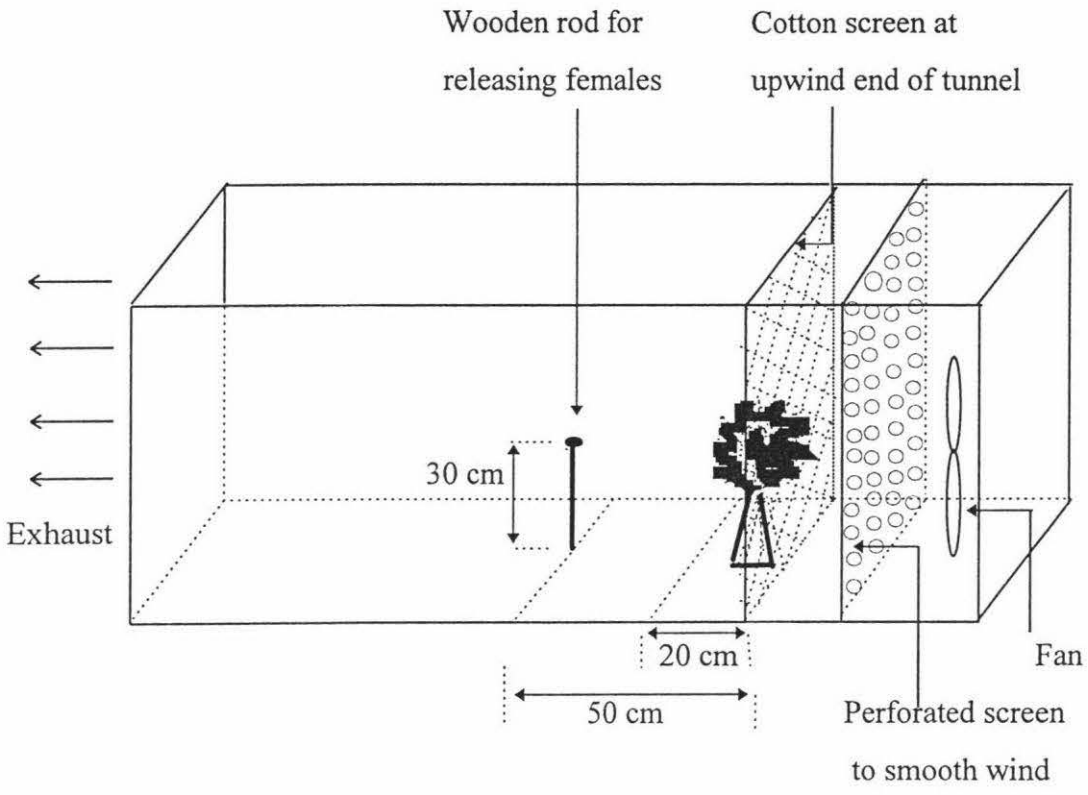

Fig. 7. Diagram of the wind tunnel and arrangement of the treatment and wooden pole inside the tunnel.

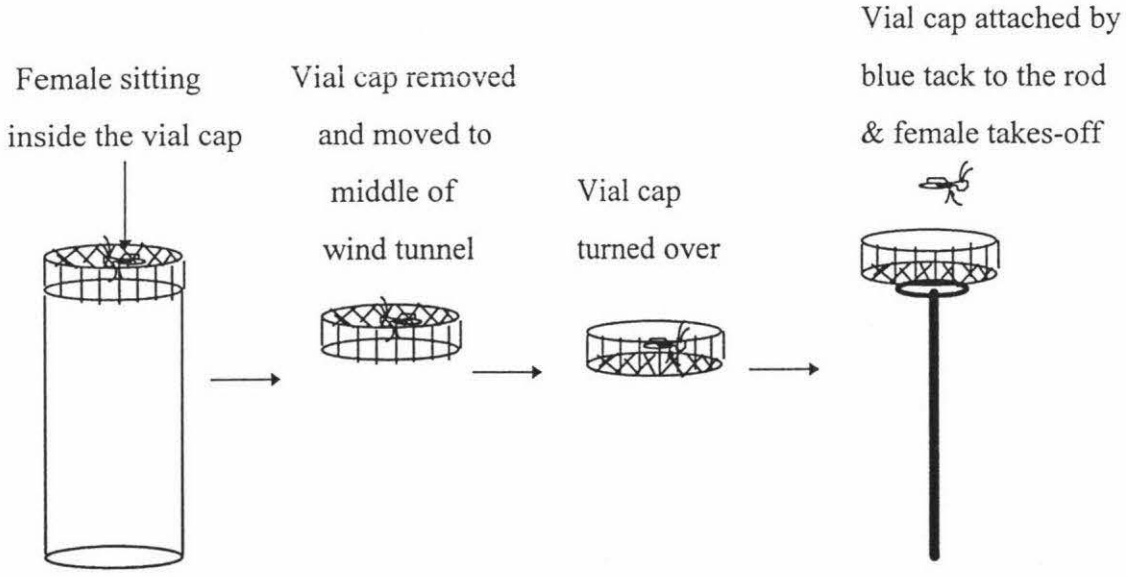

Fig. 8. Procedure followed in releasing test females into the wind tunnel.

it was placed on the rod or that were lost from view immediately after take-off from the vial cap were not included in analysis.

Wind speed at the point where females were introduced into the tunnel was checked using a Turbometer (Davis Instruments, Hayward, California, USA) and adjusted to 0.2-0.3 m/sec prior to introducing flies into the wind tunnel. Unless otherwise specified, each female was scored for the following behaviours:

1. time that the female was placed on top of the rod,
2. time that flight was initiated from the vial cap (take-off),
3. direction of movement after take-off: upwind, downwind, or any other direction,
4. directional changes during flight: downwind to upwind, other direction, etc.,
5. time of commencing upwind flight,
6. whether the female entered a 5 cm radial zone surrounding the target,
7. time of entering the 5 cm zone,
8. whether the female landed on the target,
9. time of landing,
10. place of landing, and
11. approximate distance of upwind flight from the starting point downwind to ending point upwind.

Three different stop-watches were used to record the timing of behaviours for an individual female. Data were recorded manually onto a data sheet.

IV.2.4 Experimental designs and data analysis

All tests (factorial and non factorial) conducted in the wind tunnel were done using a randomized complete block design (RCBD). In these experiments, a single block represented the group of individuals (always 5 individual females tested per treatment) that was tested at a similar time on a single day. The order of presentation of treatments within each block was randomized. In data anlysis, the percentage of females that responded to each treatment was calculated as the number of females that responded out of the five females tested for each treatment in each block. These percentage data were used in the final data analysis. Data on the timing of behaviours by individual females were analysed without being averaged within a block. All data were subjected to Bartlett's test for homogeneity of variance by JMP® (Version 2, SAS Institute Inc., USA). When variances were shown by this test to be heterogeneous, data were transformed to achieve homogeneity of variances. Transformed data were reanalysed for homogeneity of variance and if homogeneous, analysed by two-way ANOVA for RCBD or for factorial experiments by ANOVA for factorial design. Data that did not achieve homogeneity by data transformation were subjected to a Welch-ANOVA (JMP® User's Guide), a test that is valid when group sample variances are heterogeneous. When an ANOVA indicated significant treatment differences, treatment means were separated by the least significant difference (LSD) test using JMP® (Version 2, SAS Institute Inc., USA). When sample sizes were small because only small numbers of females were observed engaging in a behaviour, data were pooled over all blocks and subjected to a Chi-square test.

IV.3 Time of peak activity of mated females

IV.3.1 Materials and methods

The objective of this study was to identify the time of day that females become active after mating and exhibit the behavioural sequence leading to oviposition. It was conducted in the wind tunnel with a low windspeed (0.2 - 0.3 m/sec) and fresh apple shoots (40 cm height, ca.100 leaves), placed upright in a container with water, as a source of plant stimuli. The container (with shoots) was placed downwind of the screen at the upwind end of the tunnel as explained earlier (see Fig. 7, section IV.2.3).

Both before and during testing, females were treated as explained previously (see section IV.2.3) and were released individually from the vial top at the top of the wooden rod, 50 and 30 cm downwind from the upwind screen and shoots, respectively. The behavioural responses of each individual female to apple foliage were recorded, beginning from the time that the female was released into the wind tunnel and ending either 15 minutes later, or if the female landed on the apple foliage within these 15 minutes, five minutes after this landing occurred. In addition to the observations listed in section IV.2.4, behavioural activities of the female after landing on foliage and the timing of each post-landing behavioural activity were also recorded. Five females were observed during each hour, starting at 09.00 h and ending at 18.00 h daily. The 45 females observed over these hours on a single day constituted a block. Four blocks were run over four consecutive days in January 1996.

IV.3.2 Results

After being released into the windtunnel, females exhibited shorter times to take-off as the day progressed (Fig. 9) (two-way ANOVA, treatment

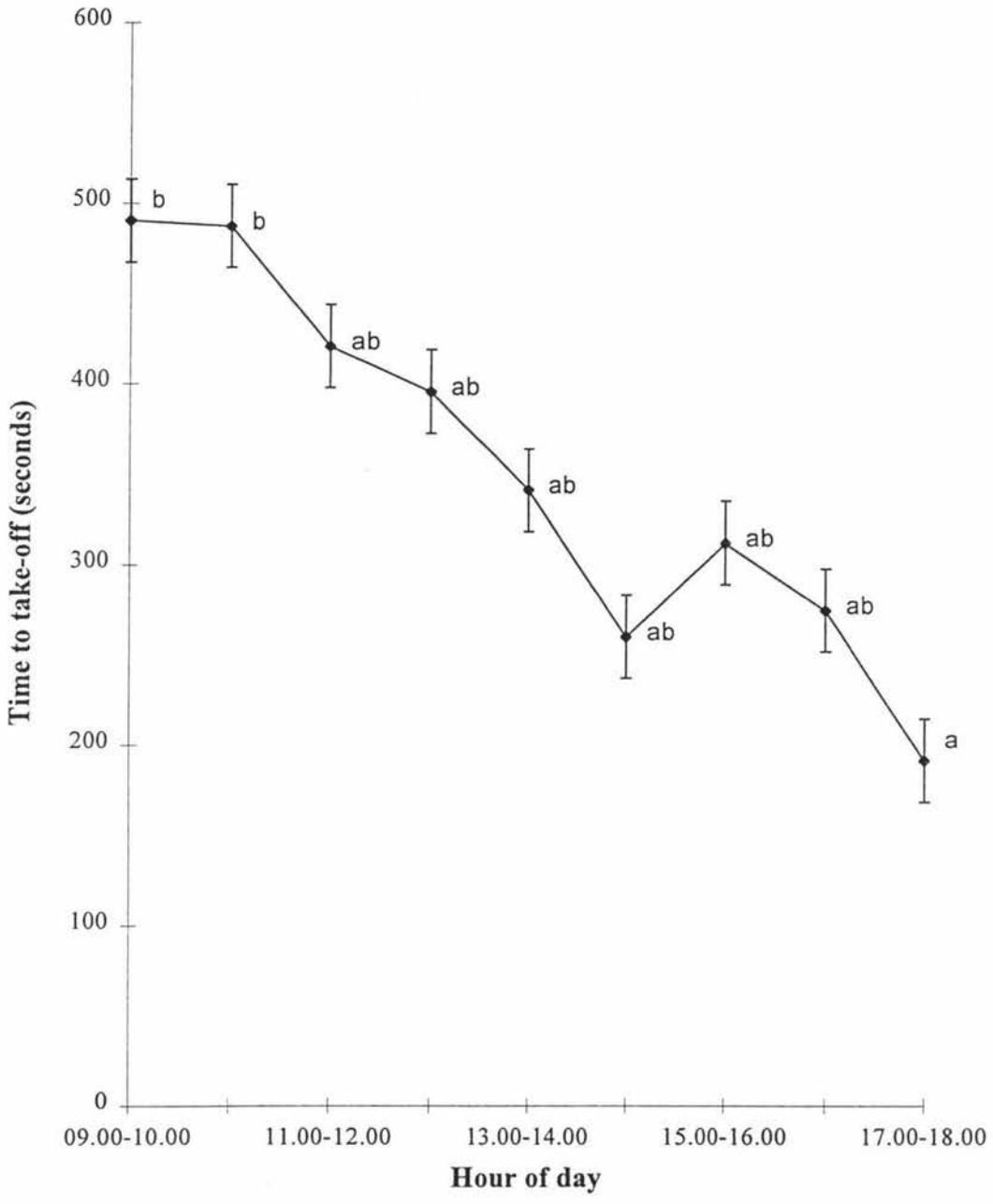

Fig. 9. **Take-off times from the release platform exhibited by females released into the windtunnel at different times of day. Means accompanied by different letters are significantly different at $P < 0.05$.**

F =3.60, df = 8, P < 0.0007). The block effect was significant (F = 3.15, P < 0.0267). Percentages of females that exhibited behaviours at various times during the day were analysed in two different ways. First, percentages of females that responded were calculated as numbers of females responding/females tested per block (Fig. 10). The percentage of females that took-off within 15 minutes of their release into the windtunnel showed a steady increase over the day (Fig. 10) (two-way ANOVA, F = 4.05, df = 8, P < 0.0048; block effect not significant F= 2.19, P < 0.12). While means were not significantly different over the day, the percentage of females that flew upwind (F = 1.36, P < 0.2702) and that entered a 5 cm radial zone around foliage and landed (F = 1.68, P < 0.1630) tended to increase over the day (Fig. 10).

Data were also analysed with percentages of females responding calculated as numbers of females exhibiting the behaviour out of the number of females that took-off in each block (Fig. 11). Percentages of females that entered a 5 cm radial zone surrounding the foliage and then landed on the foliage were significantly different over the day (two-way ANOVA, F = 2.85, df = 8, P < 0.0291). Percentages of females that entered and landed were lower during the 09.00-10.00 h time interval than at times after 10.00 h (Fig. 11).

Percentages of females that exhibited post-landing behaviours (antennation, probing and oviposition) were heterogeneous. Welch ANOVA showed the mean differences were not significant. However, these percentages were generally lower at times before 14.00 h (0 to 8%) than at times after that (16 to 26%)

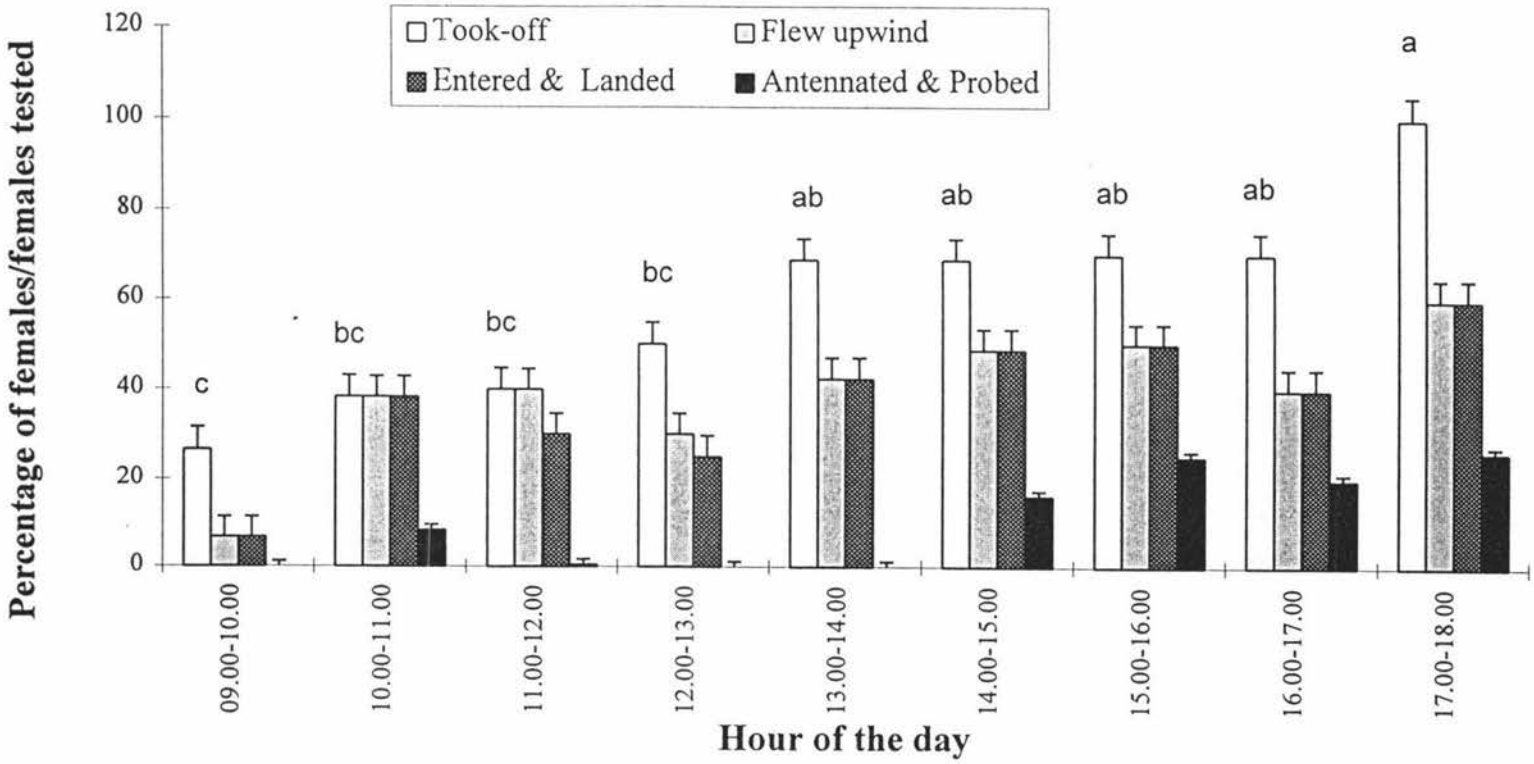

Fig. 10. **Percentages of females (X ± S.E.) that exhibited behaviours in a wind tunnel at various times of the day. For percentages of females that took-off, means accompanied by different letteres are significantly different at $P < 0.05$.**

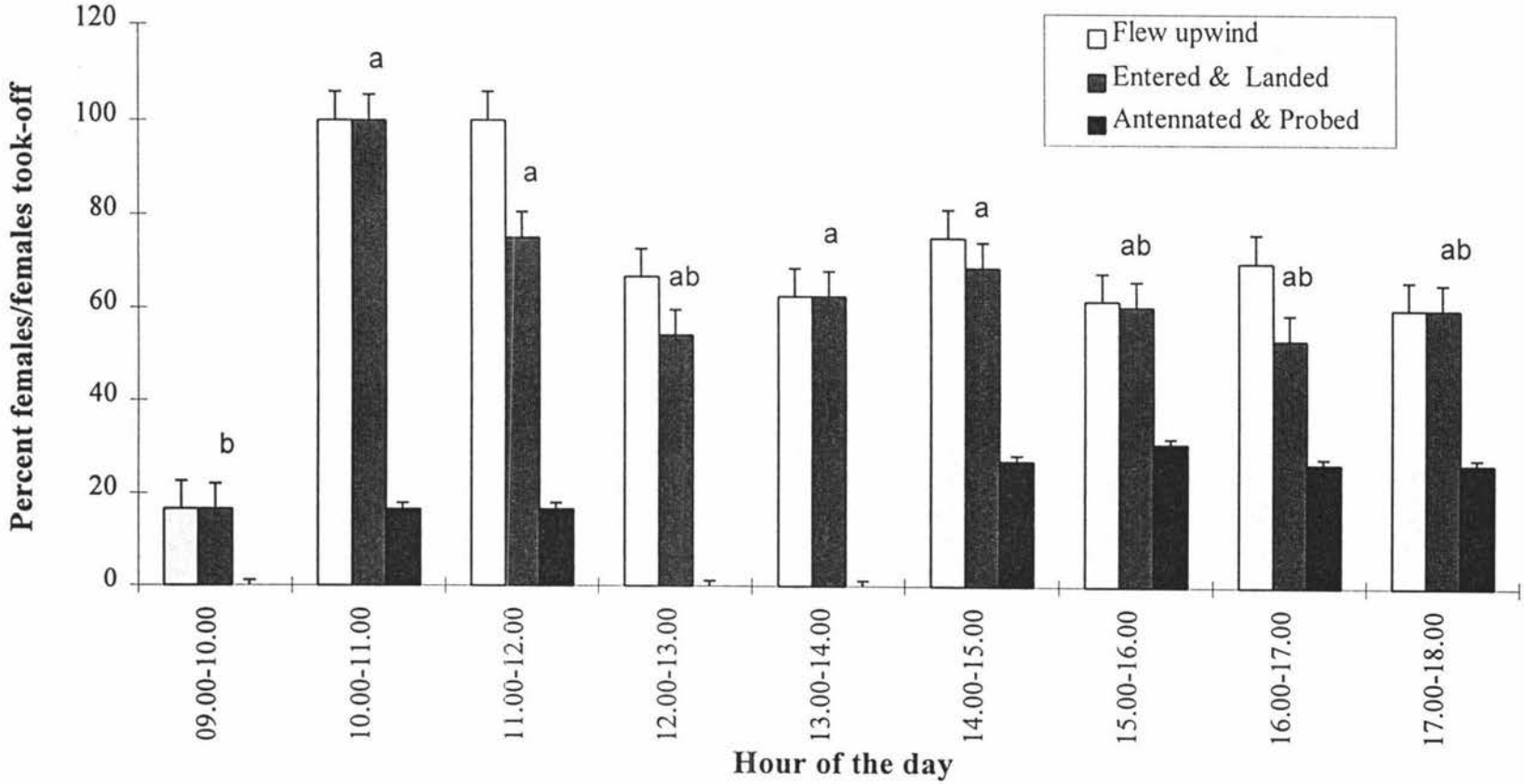

Fig. 11. **Percentages of females out of the females that took-off (X ± S.E.)that exhibited behaviours in a wind tunnel at various times of the day. For percentages of females that entered and landed, means accompanied by different letters are significantly different at $P < 0.05$.**

IV.4 Flight orientation of mated females exposed to host and non-host plant foliage

IV.4.1 Materials and Methods

This test was done to determine whether ALCM females orient to apple foliage (host-plant) but not to pear foliage (non-host plant) in a wind tunnel. The test was carried out as a no-choice bioassay in December 1995. Three fresh shoots (30 cm in height, ca. 30 leaves) of a single plant species (apple or pear) held in a flask containing water were placed 20 cm downwind of the upwind screened end of the wind tunnel (see Fig. 7). A single mated female was placed at the point of release, 30 cm downwind of foliage.

Observations (see IV.2.3) were made from the time that the female took-off from the release point until she landed. After five females were tested with the foliage of a single plant species, the foliage was replaced with foliage of the other plant species and five more females were tested. These ten females constituted a block. Nine blocks were run, with a total of 45 females per plant species tested.

IV.4.2 Results

Similar percentages out of the total number of females tested on apple (n=59) and pear (n=55) initiated flight within 5 min. after release into the tunnel (76.2 and 81.8, respectively). Latency to take-off after release showed no statistical significance either in the presence of apple or pear foliage (56 sec. and 34 sec., respectively) (two-way ANOVA, $F = 1.45$, $df = 1$, $P < 0.2330$; block effect also not significant, $F = 0.54$, $P < 0.8199$).

After take-off from the release point, the females performed three patterns of flight:

1. upwind flight directly towards the foliage,
2. flight in a direction other than upwind, or
3. an initial random flight followed by upwind flight towards the foliage.

Females exposed to apple foliage were more likely to exhibit upwind flight responses than females exposed to pear foliage. Out of the females that took-off (Fig. 12), more females flew upwind towards the apple foliage than flew upwind towards pear foliage (two-way ANOVA, $F = 5.97$, $df = 1$, $P < 0.0404$; block effect was significant, $F = 3.95$, $P < 0.0344$).

Nine times more females entered a 1 cm radial zone (Fig. 12) surrounding the apple foliage, than entered the same zone around pear foliage (two-way ANOVA on square root transformed data, $F = 69.65$, $df = 1$, $P < 0.00001$; block effect not significant, $F = 2.71$). Similarly, a greater percentage of the females given apple (Fig. 12) landed on the foliage than females given pear ($F = 120.72$, $df = 1$, $P < 0.00001$; block effect not significant, $F = 2.45$). These results indicated that during flight, mated ALCM females are more responsive to sensory stimuli specific to their host-plant species, apple, than to stimuli from a non-host plant species, pear.

While the site of landing on foliage was not under investigation in this experiment, females showed strong preferences for landing sites. Out of the total number of females that landed on apple foliage, 74 percent landed on buds or immature leaves, nine percent landed on the stalk, and 17 percent landed on mature leaves ($Chi = 26.59$, $n = 23$, $P < 0.00001$).

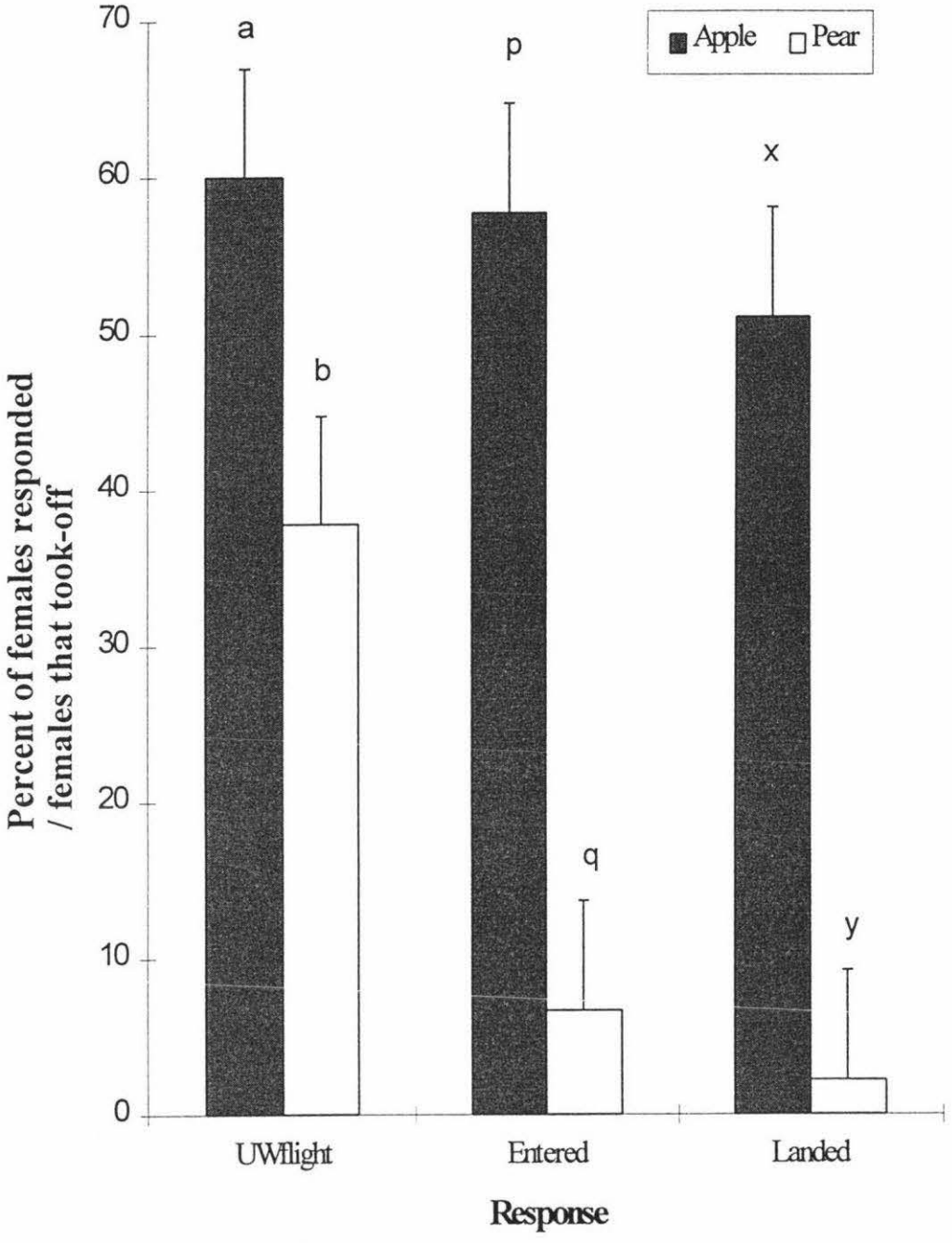

Fig. 12. **Responses of females to host and non-host plant foliage in the wind tunnel. Within a behaviour (upwind flight, entered or landed), means that are accompanied by different letters are significantly different at $P < 0.05$.**

IV.5 Responses of mated females to chemical cues

IV.5.1 Responses to volatile foliar chemicals from host and non-host plant species

IV.5.1.1 Materials and Methods

In the experiment discussed above, female ALCM were more responsive to apple foliage than to pear foliage. A question that arose from these results was: which plant stimuli caused more females to fly to apple than to pear? To begin to answer this question, varied odour sources were presented to female ALCM in the presence of constant visual stimuli. Odours came from apple or pear foliage that was placed in a chamber at the upwind end of the wind tunnel, a chamber that was separated from the main body of the wind tunnel by two white mesh screens (Fig. 13). A model of foliage placed in this same upwind chamber served as a control treatment. The screens prevented females in the wind tunnel from seeing apple, pear and model foliage placed upwind of the screens. Within the main body of the wind tunnel at the upwind end, three identical models of an apple shoot (ca. 10 leaves) were used to provide constant visual cues (Fig. 13). Three treatments were tested.

1. Odour from apple foliage was provided by apple shoots (ca. 50 leaves) held in a flask of water. A model (ca. 10 leaves) was placed 20 cm downwind from the upwind screen of the wind tunnel.
2. Odour from pear foliage was provided by pear shoots (ca. 50 leaves) held in a flask of water. A model was placed 20 cm downwind from the upwind screen.
3. A model of apple foliage (ca. 50 leaves) was held in the upwind chamber of the wind tunnel. A model was placed 20 cm downwind

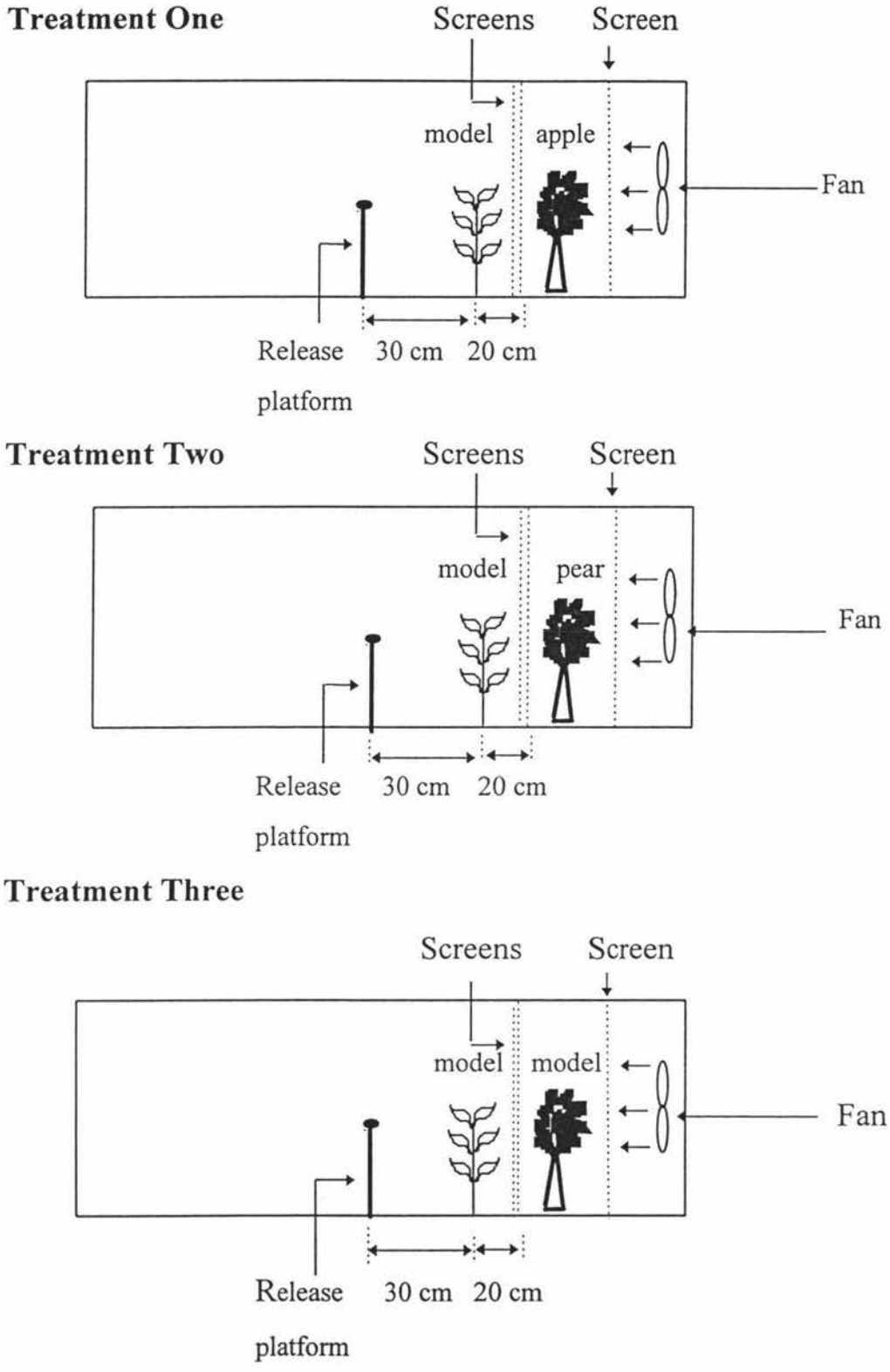

Fig. 13. Experimental setup used for testing responses of female ALCM to apple and pear odour (upwind and downwind models = 50 and 10 leaves, respectively).

from the upwind screen (control).

The foliar model (ca. 50 leaves) used in the control treatment placed in chamber upwind of the screen provided no odour but created turbulence similar to that created by the foliage in the pear and apple treatments. Models were made by fixing green ready-made cloth leaves (similar to apple leaves) on plastic rods.

In a block, five females were tested individually for each treatment (fifteen females/block in total for the three treatments). Fifteen blocks were run, using a total of 75 females per treatment. This experiment was conducted during 15 consecutive days in January 1995, during the time of the day that females forage most actively (between 13.00 and 18.00 h).

IV.5.1.2 Results

Latencies to take-off were not significantly different at $P < 0.05$ when females were given odour from apple or pear foliage (means = 127, 80, and 126 sec. for apple, pear, and the control, respectively; $F = 2.85$, $df = 2$, $P < 0.0603$). Variances of percentages of females that flew upwind (out of numbers that took-off), entered a 5 cm zone downwind of the screen and landed were heterogeneous and could not be made homogeneous by data transformation. However, analysis by a Welch ANOVA test, which is valid when variances are heterogeneous (JMP®, SAS Institute) showed significant treatment effects for percentages of females that flew upwind ($F = 26.20$, $df = 2$, $P < 0.00001$) with greater numbers of females flying upwind to sources of apple odour than to pear odour. The lowest percentage flew upwind in the control treatment (Fig. 14).

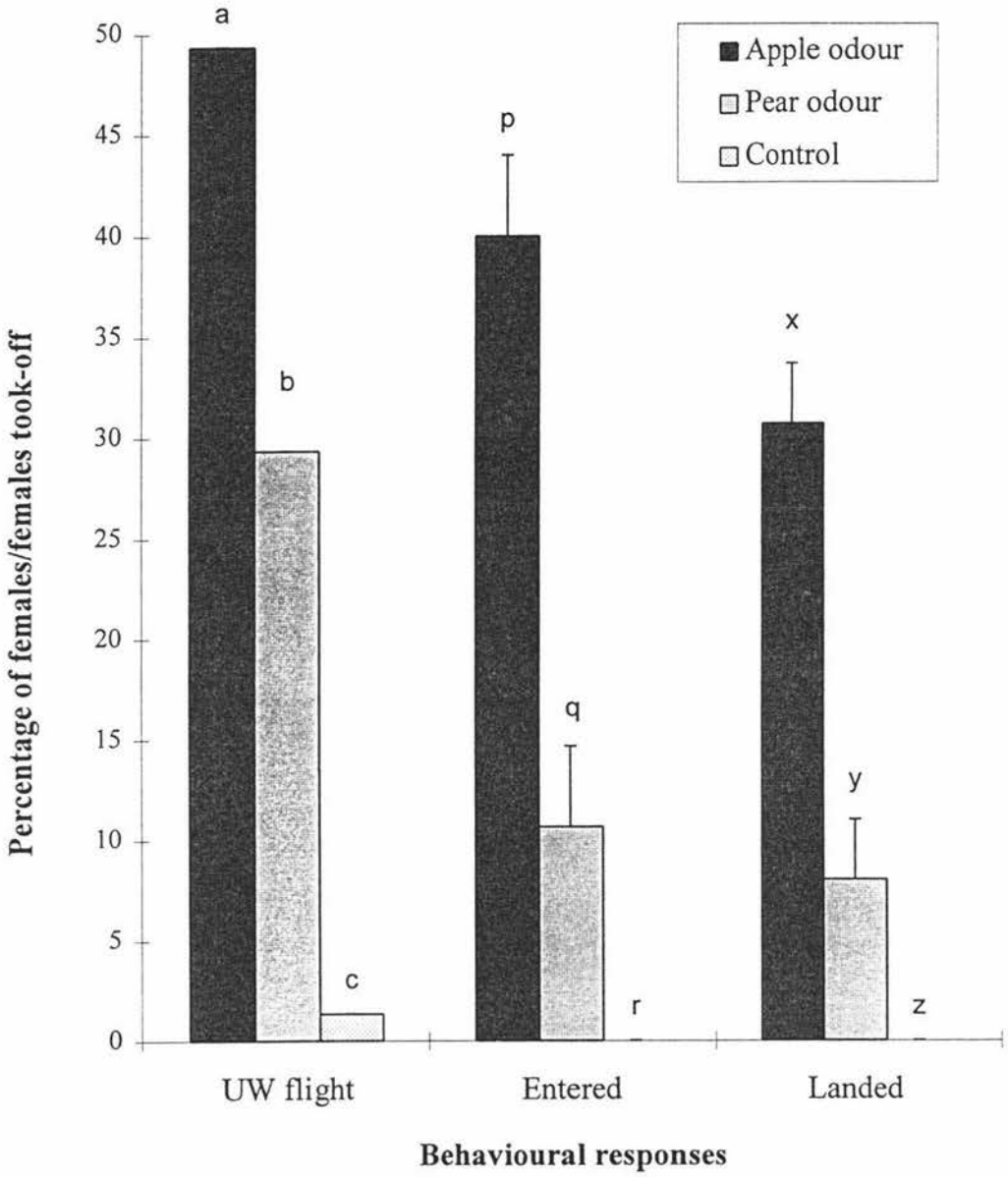

Fig. 14. **Responses of mated ALCM females to host and non-host plant odours (means ± S.E.). Within a single behavioural response, means accompanied by different letters are significantly different at $P < 0.05$.**

Of the females that flew upwind, only those flying to apple or pear odour approached and landed on the screen at the upwind end of the wind tunnel. In these treatments, females rarely landed on plant models placed downwind of odour sources, in the main body of the wind tunnel. When there was no odour (control treatment) females that flew upwind did not approach or land on the model or the screen. The latter data on the numbers of females approaching and landing in the control treatment (all zero values) therefore were excluded from further data analysis. After data for this control treatment were excluded, variances of data for apple and pear on the percentages of females that approached and landed reached homogeneity after square root transformation. Percentages of females that approached and then landed on the upwind screen (Fig. 14) when odour was from apple foliage were significantly greater than percentages that approached and landed when odour was from pear foliage (two-way ANOVA on square root transformed data, $F = 7.79$, $df = 1$, $P < 0.0144$, and $F = 4.69$, $df = 1$, $P < 0.0481$ respectively, for percentages of females that approached or that landed). When apple or pear odour was given with a model in the main body of wind tunnel, 85.71% of females flew upwind passing the model and landed on the upwind screen while only 14.29% landed on the model.

A question that arose in this analysis was whether there were any significant differences between the effect of pear odour and the control on the numbers of females that entered or that entered and landed. A Chi-square test (Likelihood ratio test) was done to compare the effect of these two treatments on the numbers that entered and then landed when given pear odour or given no odour (control). Greater numbers of females approached and landed on the screen when pear odour was given ($n = 8$

and 6 respectively) than when there was no odour (n = 0) (Chi = 11.54, $P < 0.0007$, n = 75, for numbers that entered, and Chi = 8.57; $P < 0.0034$, n = 75, for numbers that landed).

These results indicated that females fly upwind to volatile chemical stimuli from both apple and pear foliage but show greater responses to chemical stimuli from apple foliage than from pear foliage. In the absence of chemical stimuli (control), females rarely flew upwind indicating that chemical stimuli are necessary for host-finding behaviour of ALCM females. Relative to no odour source, pear odour appeared to have a slight stimulatory effect on females approaching and landing on odour sources. Because females that flew upwind to odour sources rarely landed on the model foliage but, instead flew beyond the model and landed on the screen itself, it appeared that ALCM females are more responsive to chemical stimuli than to visual stimuli from foliage.

IV.5.2 Responses to chemical stimuli from young and mature apple foliage

IV.5.2.1 Materials and Methods

In the experiments reported in sections III.7.1.2 and IV.4.2, females were more likely to land on immature leaves and buds of apple than on mature leaves of apple. A test was carried out in January 1996 to determine whether volatile chemical stimuli from young foliage mediated this response. Apple shoots (40 cm height), with a single bud and three newly opened leaves on each (ca. 50 leaves), held in a container of water were used to provide volatile chemical cues from young foliage. Similarly, chemical cues from mature foliage were provided by shoots (40 cm height) with only mature leaves (ca. 50 leaves).

Foliage was placed in a chamber upwind of the main body of the wind tunnel, behind a double screen. Because there was no plant or plant model within the main body of the wind tunnel, the behaviour of female midges was scored relative to the screen at the upwind end of the tunnel (i.e., approach the screen, landing on the screen, and times of approach and landing on the screen). Females were tested individually. After five females were tested for young foliage, the treatment was changed to mature foliage and five more females were tested. These ten females constituted a block. The order of testing treatments was alternated for each block. Fifteen blocks were run, testing a total of 75 females per treatment.

IV.5.2.2 Results

Females given odour of immature or mature apple foliage showed no significant difference either in latencies to take-off (110 and 104 sec., respectively) ($F = 0.08$, $P < 0.7741$), percentages that took-off (89% and 91%, respectively) ($F = 0.10$, $P < 0.7541$) or in the percentage of females that flew upwind (44% and 35% to the odours from immature and mature foliage respectively) ($F = 2.91$, $P < 0.11$; block effect was significant, $F = 4.50$, $P < 0.0040$). However, after flying upwind, a significantly higher percentage of females entered the 5 cm zone downwind of the screen (Fig. 15) when chemical stimuli were from young rather than mature foliage (two-way ANOVA, $F = 6.14$, $df = 1$, $P < 0.0266$; block effect significant, $F = 9.14$, $P < 0.0001$). A significantly greater percentage of females landed on the screen (Fig. 15) in response to chemical stimuli from immature foliage than from mature foliage (two- way ANOVA, $F = 5.09$, $df = 1$, $P < 0.0406$; block effect also significant, $F = 6.25$, $P < 0.0008$).

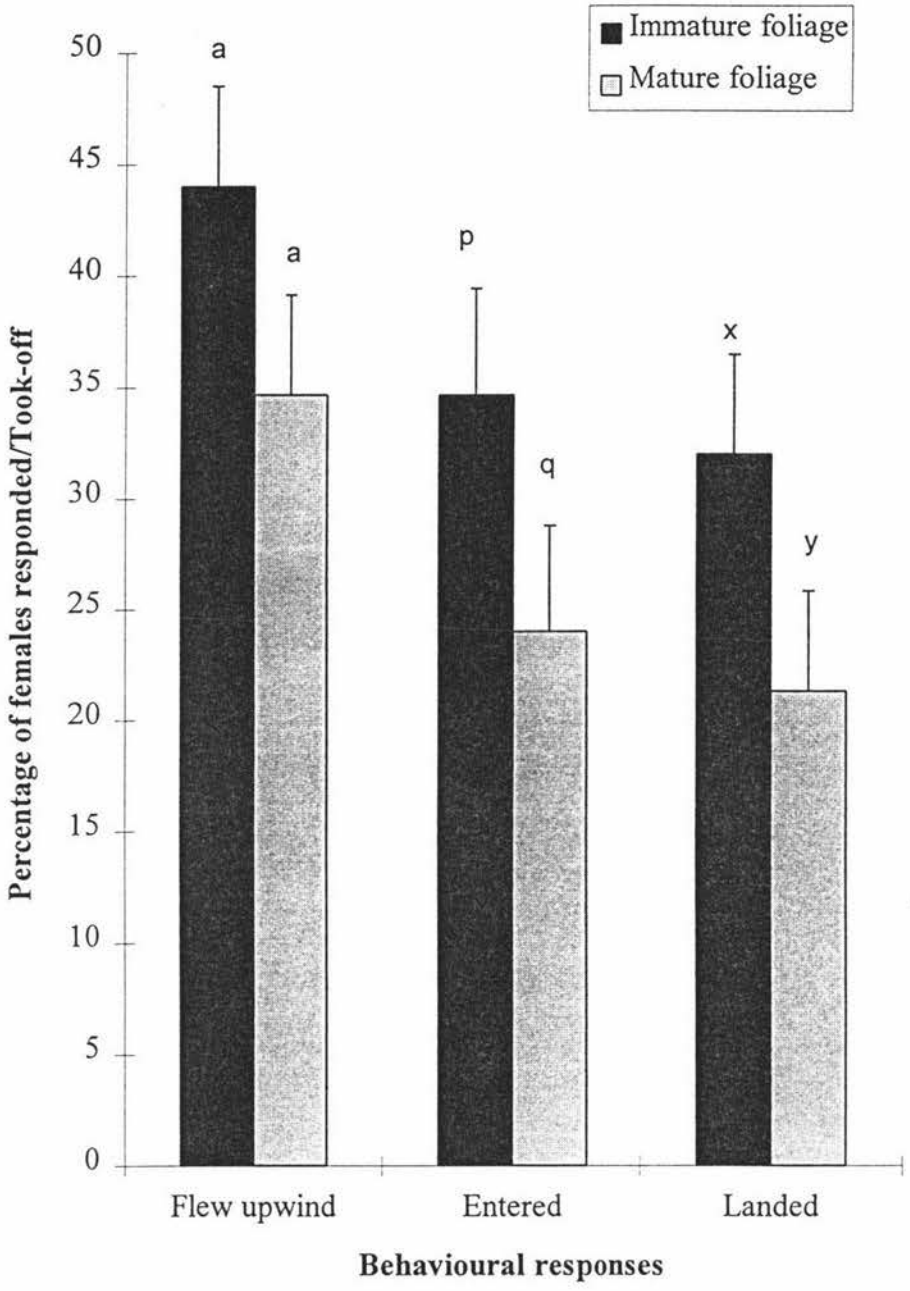

Fig. 15. **Responses of females to odours from mature and immature apple foliage (X ± S.E.). Means accompanied by different letters were significantly different at $P < 0.05$.**

Furthermore, the flight speed of females tended to be faster in the presence of odour from immature foliage (2.45 cm/sec) than in the presence of odour from mature foliage (1.82 cm/sec); however, these means were not significantly different. These results suggest that mated females may respond similarly to immature and mature foliage in taking-off and flying upwind to the odour source but respond differently at a closer range (< 5 cm), being more stimulated to approach and land by immature foliage than by mature foliage.

IV.6 Responses of mated females to a combination of visual and chemical cues

IV.6.1 Materials and Methods

The objective of this test was to determine whether females would orient to visual stimuli from a model representing apple foliage and if so, whether this response would be enhanced by chemical stimuli from apple odour. This test was conducted in February 1996. The layout of treatments in the wind tunnel was similar to that described in the section IV.5.1.1 (Fig. 13). Five treatments tested for the flight response of mated females in this experiment are shown in Table 5.

Model leaves with a shape and area similar to apple leaves (surface area 49 cm^2 per leaf) were made out of green construction paper. Models of apple shoots were made by fixing, at a 45^0 angle, five model leaves to a single wooden rod (6 mm diameter x 30 cm length). Two containers filled with water were prepared, one with 10 real apple shoots and the other with 10 model apple shoots (ca. 50 leaves in each) to keep downwind of the screen to provide test females with visual cues. Similarly a second set of

containers were prepared, one container with real apple shoots (ca. 100 leaves) to keep upwind of the screen to provide the test insect with volatile chemical cues, and the other container with model apple shoots to provide turbulence similar to that created by the real shoots placed upwind of the screen.

Table 5. Design of the treatments used to test the effect of chemical and visual stimuli on flight responses of ALCM females

Treatment	Upwind of screen		Downwind of screen	
	Chemical stimuli	Source of odour	Visual stimuli	Source of stimuli
A+M	Present	Apple	Present	Model
M+M	Absent	Model	Present	Model
A+N	Present	Apple	Absent	Nothing
M+N	Absent	Nothing	Absent	Nothing
M+A	Present	Model	Present	Apple

This experiment was conducted as a no-choice bioassay in a wind tunnel using individual females. A single mated female held in a vial was released onto the rod. Observations on the behaviour of the female (see III.6.5.1) were recorded from take-off to landing. Five females were tested individually for a single treatment, with the five treatments changed one after another (in a random order). The 25 females that were tested for each of the five treatments constituted a block. Ten blocks were run using a total of 50 females per treatment.

The results were analysed by ANOVA for two-factor factorial design using SAS Release 6.10, SAS System for Windows (SAS Institute Inc.,

Cary, NC, USA). Means were tested for differences using the least significant difference (LSD) test.

IV.6.2 Results

The visual model placed downwind of the screen, in the main body of the wind tunnel, reduced the percentages of females that took-off ($F = 12.33$, $df = 4$, $P < 0.0001$) (Fig. 16A, Table 6). After taking-off from the release point, the effects of visual stimuli ($F = 20.41$, $P < 0.0001$), chemical stimuli ($F = 23.16$, $P < 0.0001$), and the interaction of visual and chemical stimuli ($F = 43.93$, $P < 0.0001$) were highly significant for the upwind flight of females (Fig. 16B). When chemical stimuli were absent, only 2% of females flew upwind regardless of whether the visual model of foliage was present (M+M) or absent (M+N, negative control). This result indicated that females did not respond to visual stimuli from the model of apple foliage. The response of the females by upwind flight was much higher (40 to 66%) when chemical stimuli from apple foliage were present (Treatments A+N and A+M). This clearly indicated that during upwind flight the females were responding to chemical stimuli rather than to visual stimuli from models. This result agrees with the results reported in section IV.5.1.2. Furthermore, a greater percentage of females responded by upwind flight to apple odour without visual stimuli from a model (A+N) (58%) than to apple odour in the presence of a visual model (A+M) (40%). These responses were further enhanced (70%) when real apple shoots were presented in the main body of the wind tunnel (M+A, positive control). The latter treatment may have been more attractive because the odour source was closer to the females or because chemical stimuli and visual

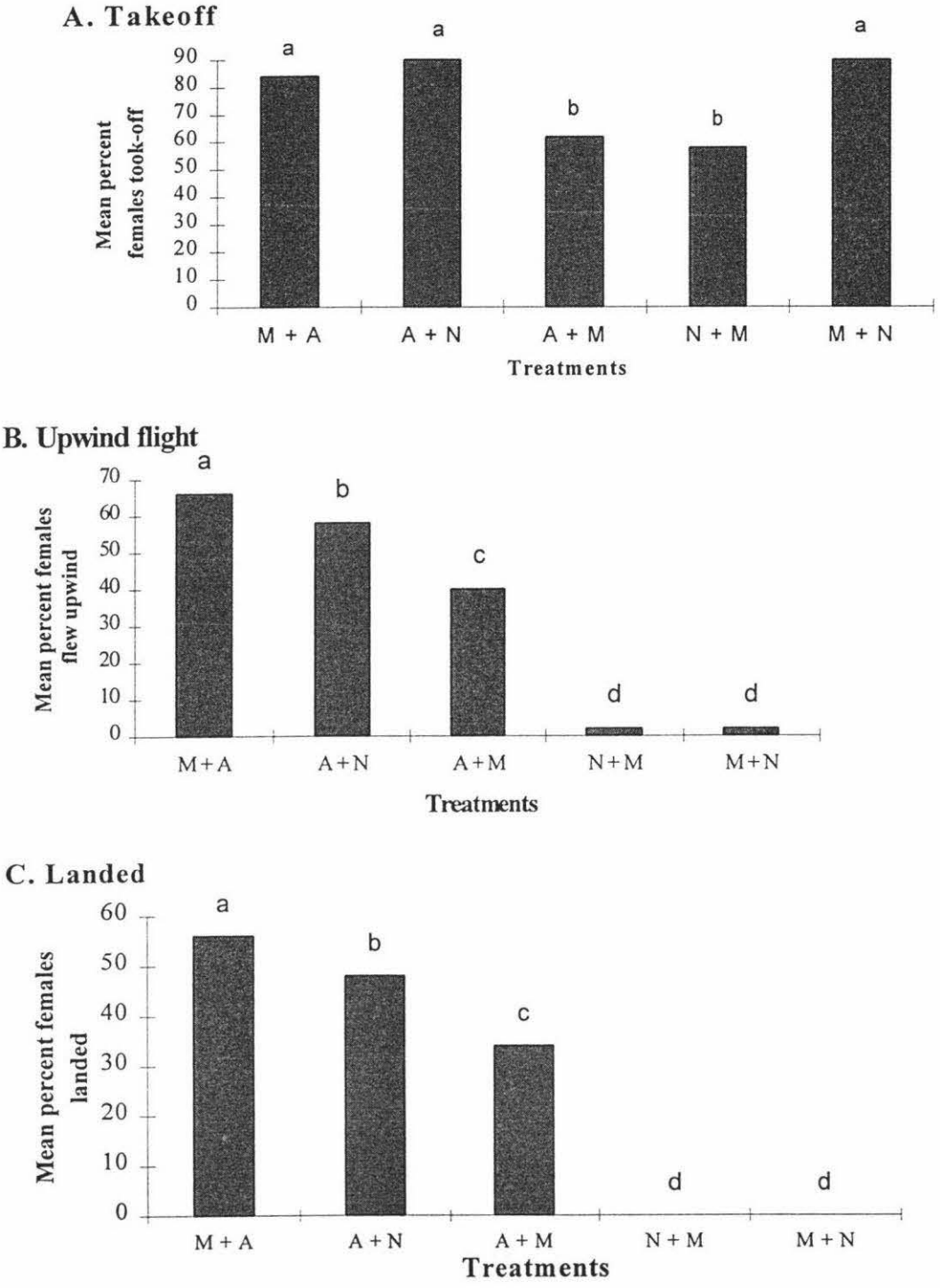

Fig.16. Flight responses of ALCM females to visual and/or chemical stimuli. Treatments are shown with item in chamber upwind of wind tunnel first and item at upwind end of the main body of the wind tunnel second (M = model, A = apple foliage, N = nothing). Within each graphs, means accompanied by same letter were not significantly different at $P < 0.05$.

Table 6. Responses of the mated ALCM females to combinations of visual and chemical stimuli tested in a wind tunnel.

Treatments		Mean percentage of total number tested		
upwind	downwind	Took-off [1]	Flew upwind [1]	Entered [1] & landed
Model	Apple	84.00 a	66.00 a	56.00 a
Apple	Nothing	90.00 a	58.00 b	48.00 b
Apple	Model	62.00 b	40.00 c	34.00 c
Model	Model	58.00 b	2.00 d	0.00 d
Model	Nothing	90.00 a	2.00 d	0.00 d
P <		0.0053	0.0001	0.0001
F =		2.94	9.21	8.86

[1] Figures accompanied by the same letter are not significantly different

stimuli from real apple shoots were both stimulatory. There was no significant difference in the flight speeds of females flying towards the five treatments.

Entering the 5 cm zone of either the model or the upwind screen and landing on any of these two targets (Fig. 16C) showed a pattern similar to upwind flight responses. In the case of landing, significant effects of chemical stimuli from the foliage held in the chamber upwind of the main body of the wind tunnel ($F = 21.79$, $P < 0.0001$) and of visual stimuli from the upwind end of the main body of the wind tunnel ($F = 38.95$, $P > 0.0001$) were found. However, there was no significant interaction effect between chemical and visual stimuli for landing. The highest percentage of females landed (56%) when real apple foliage was present in the main body of the wind tunnel (M+A, positive control). None of the females entered or landed when there was no apple odour present, either when the model was present (M+M) or absent (M+N, negative control) in the main body of the wind tunnel. In addition, significantly higher numbers of females (48%) responded by landing on the screen at the upwind end of the wind tunnel when apple odour was presented alone (A+N) than when apple odour was presented with a model in the main body of the wind tunnel (A+M) (34%).

From this experiment it appears that mated females of apple leaf curling midge responded to chemical stimuli from apple foliage with increased orientation and landing in the presence of visual stimuli from real apple shoots. However, they did not respond to visual stimuli from a model of apple foliage. This indicated that chemical cues are more important than visual cues in orientation of ALCM females during flight and landing.

Apple odour from the upwind of the screen or the model in the upwind end of the main body of wind tunnel or the interaction of these visual and chemical stimuli had no stimulatory effect on the take-off of the females. It can be suggested that the reduction in the percentage of females that took-off when a model was placed in the main body of the wind tunnel downwind of the screen may have been due to turbulence caused by models.

IV.7 Responses of mated females to colour stimuli

IV.7.1 Response of mated females to colour stimuli (cage study)

IV.7.1.1 Materials and Methods

In this study I tested whether mated females would respond to particular reflected wavelengths during behaviours leading to host-finding and oviposition. Five colours reflecting light in the range of wavelengths that are visible to the insect eye (350-650 nm) were tested in a choice bioassay in a cage.

Discs (3 cm diameter) were cut out of coloured construction paper (blue, green, yellow, red, or orange). Targets were made by gluing these discs onto toothpicks (15 cm long) with the flat side of the disc positioned perpendicular to the floor of the arena. A layer of apple leaves (ca. 30) was placed on top of the sand that lined the floor of a cylindrical arena (50 cm diameter and 35 cm height, clear plastic walls and a mesh top) and covered with a mesh screen. This layer of apple leaves provided volatile chemical stimuli for the test females. Coloured targets stood 15 cm above the floor of the arena in a circular formation, equidistant from each other and 5 cm away from the arena wall.

Twenty actively-flying females were released into the center of the arena at 13.00 h. Behaviour of the females in relation to the coloured targets (entering to 1 cm zone, landing, probing, and oviposition) was recorded for 15 minutes.

IV.7.1.2 Results

Upon their release into the cage, females flew upwards and sat on the walls or on the mesh top of the cage. They continued walking and flying near the mesh top throughout the observation period and did not fly towards or land on any of the coloured paper discs. Coloured discs were checked for eggs 24 hours later. No eggs were found on the discs; however, eight eggs were found on one of the toothpicks which was attached to a yellow disc, close to the patch of glue used to fix the disc to the toothpick. These results suggested that in ALCM females, behaviours leading to oviposition require other stimuli or stimuli additional to those presented here.

IV.7.2 Responses of mated females to colour stimuli (wind tunnel)

IV.7.2.1 Materials and Methods

This study was done to further test whether female ALCM respond to colour stimuli during flight. Two different colours (blue and yellow), were tested in the presence and absence of chemical stimuli (factorial design) (Table 7). Nylon mesh screening material was dyed using Blue (No.07, Turquoise) or yellow (No.08, Yellow) Dylon machine liquid dye (Dylon International Ltd., London). The coloured screen was placed at the upwind end of the wind tunnel either with chemical stimuli provided from fresh apple shoots (ca. 50 leaves) placed in a chamber upwind of the screen, or with a model of apple shoots (ca. 50 leaves) placed in the upwind chamber. The coloured screens obscured visual stimuli from the foliage placed in the

chamber upwind of the screen but allowed foliar volatile chemicals to pass through.

Table 7. Description of treatment layout for testing the effect of colour stimuli on the response of ALCM females

Treatment	Upwind of the screen	Screen colour
A+B	Apple (ca. 50 leaves)	Blue
A+Y	Apple (ca. 50 leaves)	Yellow
M+B	Model (50 leaves)	Blue
M+Y	Model (50 leaves)	Yellow

Five females were tested individually on each of the four treatments, with these 20 females constituting a block. Ten blocks were run using 50 individual females per treatment. This test was conducted in February 1996.

IV.7.2.2 Results

In all four treatments (Table 7), females spent similar times on the release platform before taking-off, indicating that the colour of the upwind screen and/or chemical stimuli had no effect on take-off time ($F = 1.25$, $df = 3$, $P < 0.2936$). Differences among mean percentages of females that took-off also were not significant ($F = 1.41$, $P < 0.2624$). Block effects for take-off percentage were significant ($F = 3.08$, $P < 0.0112$).

None of the females flew upwind, entered or landed in the two treatments in which chemical stimuli were absent (M+B and M+Y) (Fig. 17), indicating that these two treatments had no effects on behavioural responses of females. As data for these two treatments were all zero

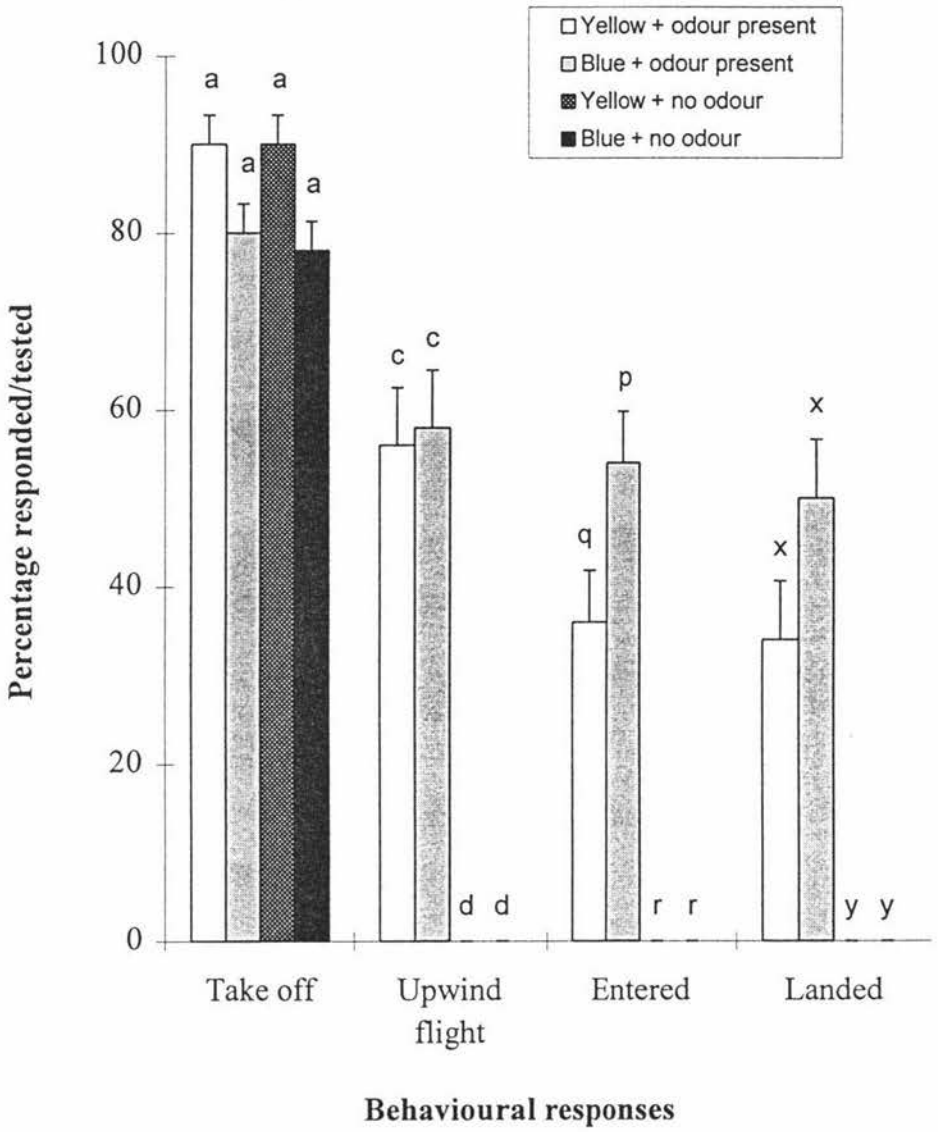

Fig. 17. Mean percentages of females that responded (X ± S.E) to visual stimuli from blue and yellow coloured screens, in the presence and absence of odours from apple foliage. Within a behavioural response, means accompanied by different letters were significantly different at $P < 0.05$.

values, these treatments were excluded from further analyses of upwind flight, entering a 5 cm zone downwind of the screen, and landing. The two treatments remaining (A+B and A+Y) varied only in the colour of the screen at the upwind end of the tunnel. Here the analysis was a two-way ANOVA for RCB design, instead of an ANOVA for factorial design.

More than 55% of the females that were tested flew upwind when chemical stimuli were present. There was no significant difference in upwind flight to chemical stimuli presented with blue or yellow screens ($F = 0.04$, $P < 0.8321$; block effects also not significant, $F = 2.01$, $P < 0.1555$). Relative to the yellow screen, significantly greater numbers of females entered the zone extending 5 cm downwind of the blue screen ($F = 4.89$, $P < 0.0543$; block effects not significant, $F = 1.91$, $P < 0.1740$). Similar percentages of females landed on the blue screen or the yellow screen when chemical stimuli were present ($F = 2.94$, $P < 0.1206$; block effects not significant $F = 1.51$, $P < 1.5102$).

This test again confirmed that chemical stimuli are essential for the triggering of flight behaviours of mated ALCM females, starting with upwind flight and ending with landing. Females did not discriminate between the two colour spectrums, blue and yellow during landing. However, females flying towards blue screens were more likely to progress further up the wind tunnel (to the 5 cm zone). This experiment was repeated (see next section) with slight modifications in the presentation of colour stimuli to clarify these results.

IV.7.3 Effect of background colour stimuli

IV.7.3.1 Materials and Methods

In this study, conducted in February 1996, there were two treatments, which differed only in the colour of the screens used. In the first treatment, an apple shoot (ca.10 leaves) was placed 20 cm downwind of the blue screen while additional apple shoots (ca. 50 leaves) were placed in a chamber upwind of the blue screen. The second treatment was identical except that a yellow screen rather than a blue screen was used.

Test females were individually released into the wind tunnel and observations were recorded as described earlier (see III.6.5.1). In addition, the following observations were recorded:

1. time when the female reached 50 cm mark downwind of the screen,
2. time when the female terminated its flight upwind.

IV.7.3.2 Results

Equal percentages of females took off (Fig. 18) (two-way ANOVA, $F = 0.00$, $df = 1$, $P < 1.0000$) after spending similar times on the release platform ($F = 0.11$, $P < 0.7442$). Initial flight speeds to a point 50 cm downwind of the screen, (i.e., 30 cm downwind of the apple shoot) or flight speed from the 50 cm mark, to the shoot, or the screen were not statistically significant when blue and yellow screens were used. This indicated that the background colour of foliage has no effect on the initiation of take-off or on the speed of upwind flight.

Furthermore, out of the females that took-off, the percentages of females that flew upwind, entered a 5 cm zone, or that landed on the apple shoot or on the screen showed no significant differences when different-coloured backgrounds were used ($F = 1.21$, 0.10 and 0.45, respectively).

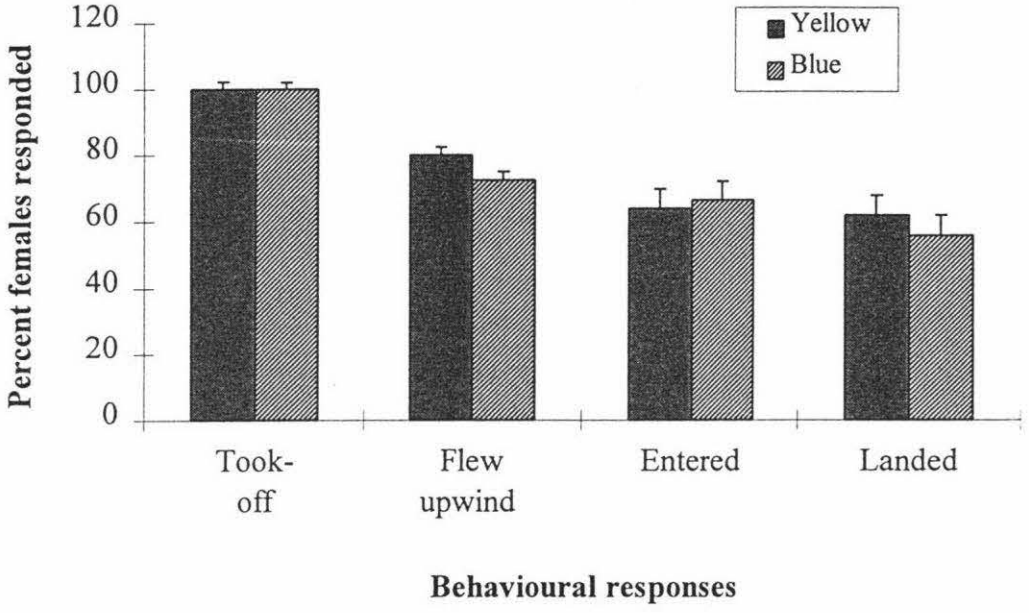

Fig. 18. Responses of females to an apple shoot with yellow or blue background colours.

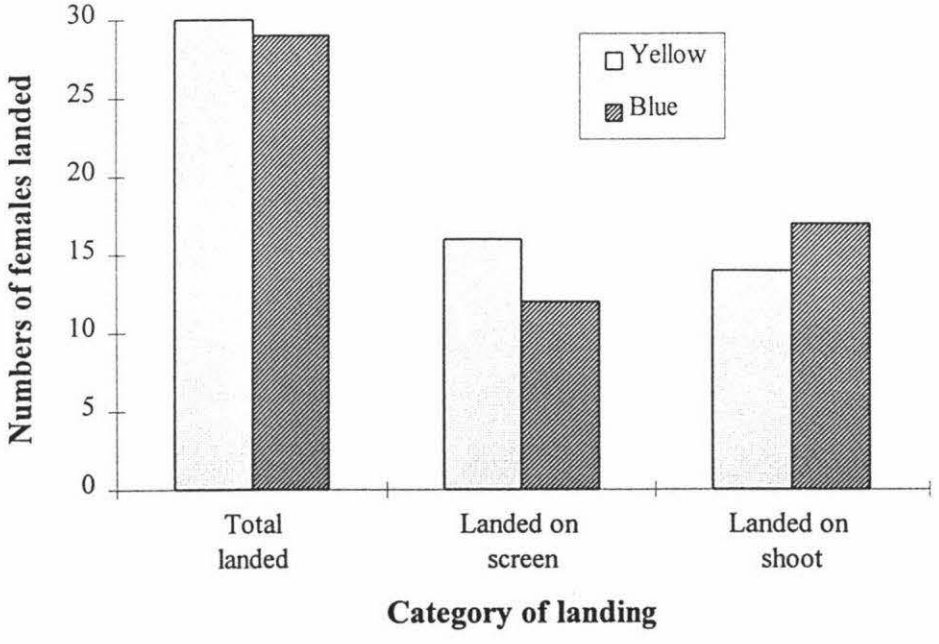

Fig. 19. Total numbers of females landed on the shoot or on the screen when the background colour was either blue or yellow.

These results showed that the females do not respond differently to host odours when apple foliage is presented in front of a contrasting (blue) or less contrasting (yellow) colour.

Total numbers of females that landed on the screen or on the shoot were analysed without considering the background colours, using a Chi-square test because the overall sample size was small (n = 59). Similar numbers landed on the screen (47.46%) or on the shoot (52.54%) (Chi = 0.85). Total numbers that landed on the shoot when the background colour was either blue or yellow, and similarly, total numbers that landed on the screen with the two background colours were further analysed separately using chi-square test to see whether the colour has any effect on the place of landing. Differences were not significant (Fig. 19) (Log likelihood ratio test, Chi = 2.77).

IV.8 Responses of mated females to foliar extracts

IV.8.1 Responses to foliar extract in host-finding

IV.8.1.1 Materials and Methods

The objective of this study was to determine the effects of apple foliar extract on the host-finding behaviours of mated ALCM females. Two treatments were presented to females in a wind tunnel:

1. four filter paper discs (No.1, 7.0 cm diameter) each treated with 12.5 leaf equivalents of a dichloromethane extract of apple leaves, and
2. four filter paper discs treated with an equivalent volume of dichloromethane.

The extract of foliage was made according to the method described by Harris and Rose (1990). Fifty immature apple leaves (cv. Gravenstein)

collected from the field were extracted in dichloromethane for 50 sec, in groups of 4-5 leaves. The cut end of the leaf petiole did not contact the dichloromethane; thus, only intact leaf tissue was extracted. This crude extract was concentrated down to 2.5 leaf-equivalents/ml by passive evaporation in a fume cupboard and stored at - 15 ^{0}C. Extracts were applied at a concentration of 12.5 LE per disc to each of four filter paper discs (No.1, 7.0 cm diameter). Dichloromethane controls were prepared with a similar volume of solvent (5 ml/disc x 4 discs). Both extract and solvent-treated filter papers were kept in the fume cupboard for 5 min before use in experiments, so that the solvent could evaporate.

The four filter paper discs of one treatment type (extract or solvent) were held in metal clips and hung side by side on two parallel strings (5 cm apart) tied across the wind tunnel at a height of 40 cm above the wind tunnel floor, at the upwind end of the wind tunnel. Groups of mated females (n = 10) were held in cylindrical plastic containers (6 cm height, 4 cm diameter) with a mesh covering on one end and a plastic lid at the other end. This container was attached by Blu tack® to the top of the rod (30 cm height, 30 cm downwind), with the plastic lid oriented upwind. The lid was removed and the following observations were recorded for a period of 15 minutes:

1. numbers of females flying out of the container,
2. numbers of females that flew upwind,
3. numbers of females that entered a 5 cm radial zone around the filter paper discs,
4. numbers of times that each female entered this zone,
5. numbers of females that landed on the filter paper discs, and

6. numbers of females that exhibited post-landing examining behaviours (antennation, probing & oviposition).

The ten females in each group tested for each of the two treatments constituted a block. Ten blocks were run, testing a total of 100 females per treatment during five consecutive days in March 1996.

IV.8.1.2 Results

Similar percentages of females flew out of the container when exposed to foliar extract or the solvent, indicating that chemicals in the extract had no effect on the initiation of flight (Fig. 20) (two-way ANOVA, $F = 0.44$, $df = 1$, $P < 0.5217$). The mean percentage of females (out of total tested) that flew upwind in response to foliar extract was significantly higher than for the solvent (square root transformed data; $F = 13.26$, $df = 1$, $P < 0.0054$). Similarly, significantly higher percentages of females entered (log transformed, $F = 95.82$, $df = 1$, $P < 0.00001$) a 5 cm radial zone downwind of extract-treated filter paper discs. Greater numbers of females (33%) landed on the extract treated filter paper discs. No females landed on the filter paper discs treated with the solvent.

Out of the total females that landed on the discs treated with foliar extracts, 47% exhibited post-landing behaviours. No eggs were found on these discs. These results showed that dichloromethane extracts of apple foliage contain chemicals that stimulate the behavioural sequence leading to host acceptance.

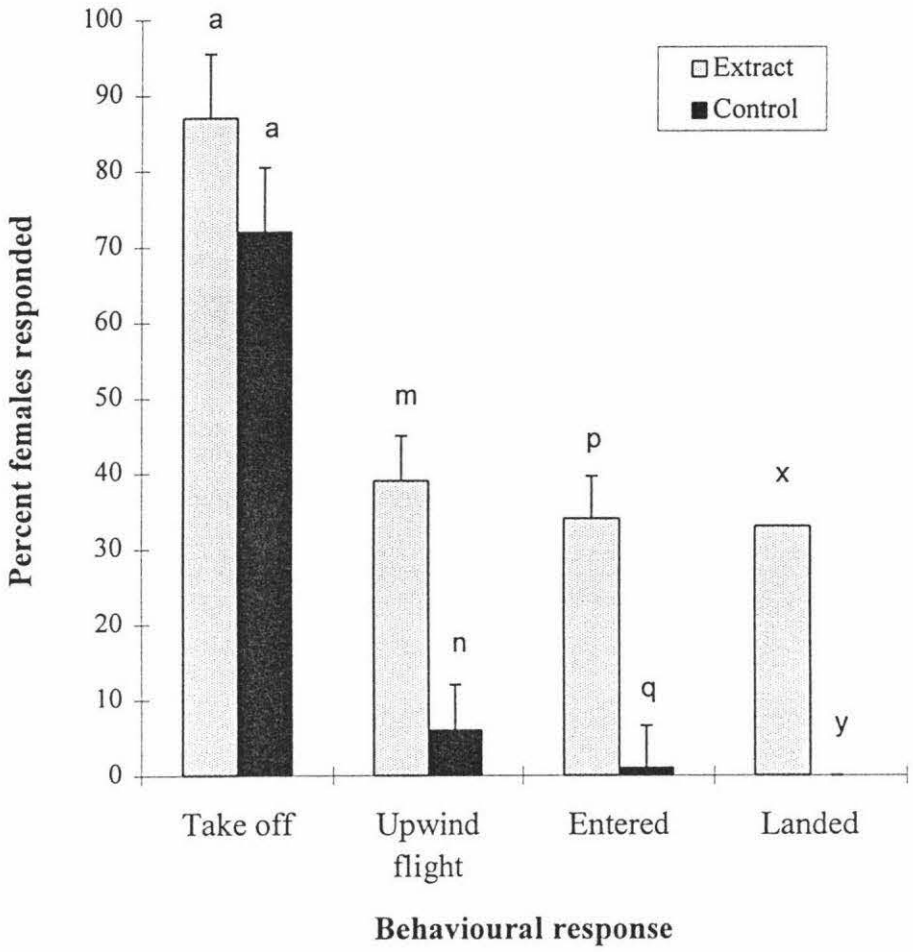

Fig. 20. **Mean percentages of females that exhibited behavioural responses to foliar chemical extracts of apple (X ± S.E.). Within a behavioural response, means accompanied by different letters were significantly different at $P < 0.05$.**

IV.8.2 Effects of chemical extracts on post-landing behaviour

IV.8.2.1 Materials and Methods

A final test was conducted to quantify the effects of chemical extracts of apple foliage on post-landing behaviours of ALCM females. There were two treatments:

1. a filter paper strip (6 cm x 2 cm) treated with a dichloromethane extract (10 LE) of apple foliage, and
2. a filter paper strip treated with an equivalent volume of dichloromethane.

The test was run in vials containing treated filter paper strips, so that the behaviours of more than one female could be recorded simultaneously over a longer observation time.

The foliar chemical extract was made as described previously. Filter paper strips (6 cm x 2 cm) were prepared with a 2 cm longitudinal cut lengthwise in one end. Five filter paper strips were prepared with 10 LE of extract per strip or a similar volume (2.5 ml) of dichloromethane (controls). Before being introduced into the vial, all treated papers were allowed to evaporate for 5 min in the fume cupboard. A single treated filter paper was placed in a glass vial (10 cm height, 2.5 cm diameter) and hung vertically by bending the two cut strips over the side of the vial (Fig.21).

A single mated female was introduced into each glass vial at 15.30 h. Commencing 15 min after placement of the filter paper strips, the behaviour of individual females was recorded every 5 min for two hours. After observations ended, females were held in vials for 22 hours. After this the filter paper strip, the lid of the vial, and the walls of the glass vial were examined for eggs. The test was conducted using a RCB design replicated over time. The females tested on each treatment (n = 5) tested on

a single day (n = 10) constituted a block. Nine blocks were run over nine consecutive days during April 1996.

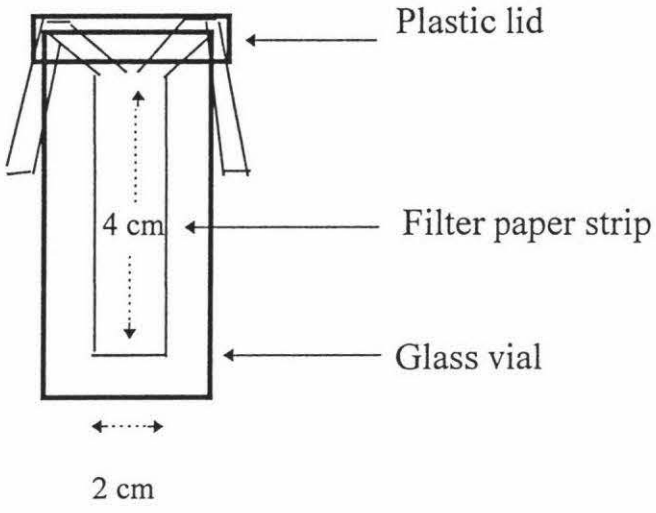

Fig. 21. The experimental set up of extract and solvent treated filter papers in vials.

The numbers of females that walked, antennated, probed, and oviposited were summed over the five individuals tested for each treatment in each block. These total numbers (n = 5) were analysed using two-way ANOVA for RCBD. Numbers of eggs laid by each individual were also analysed by two-way ANOVA.

IV.8.2.2 Results

Numbers of females that exhibited each behaviour (walked, antennated, probed, and oviposited) on filter paper strips treated with foliar extract or the solvent control were not statistically significant (Fig. 22) (F = 1.00, 1.70, 0.40, and 1.00, respectively). However, percentages of females (percentages calculated based on the total numbers, n = 45) that exhibited antennation, probing and oviposition in the vials containing paper strips

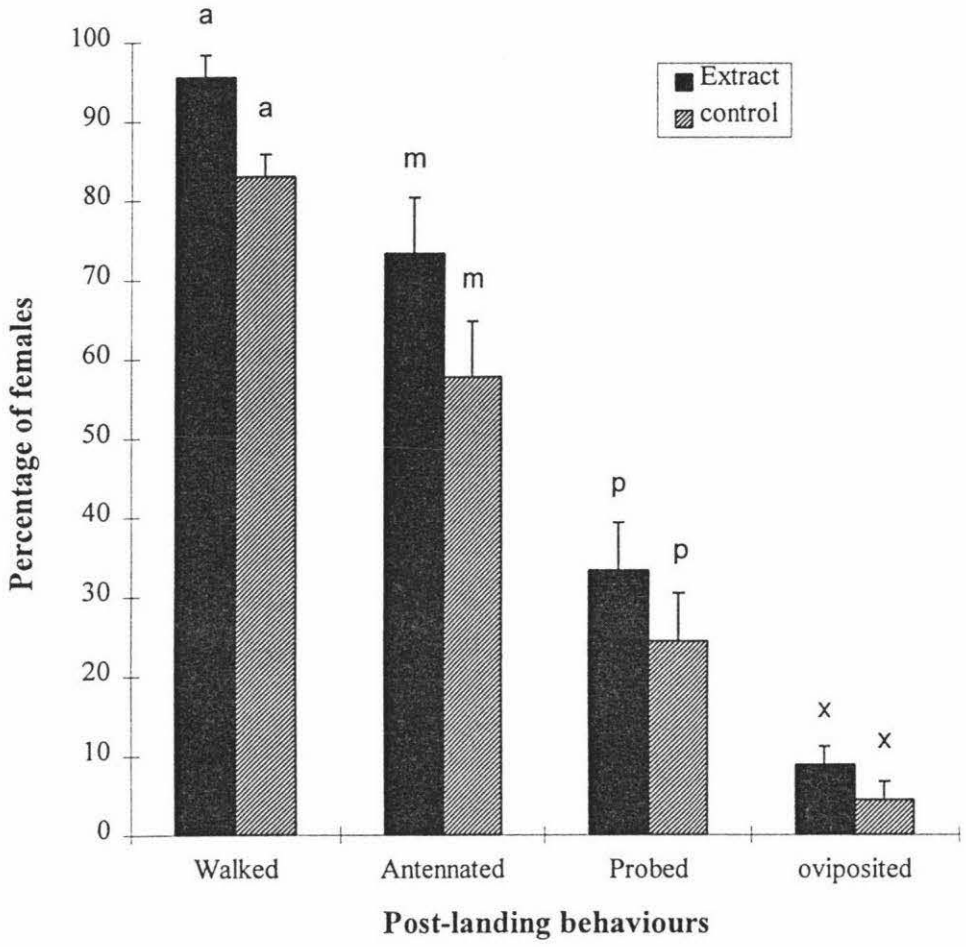

Fig. 22. **Mean percentages of females (X ± S.E.) exhibited post-landing behaviours on filter paper strips treated with a dichloromethane extract of apple foliage or with dichloromethane.**

treated with foliar extract were comparatively higher than those exhibited by the females in the vials containing control treatment.

Numbers of eggs laid by females on vials containing extract-treated filter paper strips (5 eggs/female) or solvent-treated filter paper strips (4 eggs/female) were not significantly different (two-way ANOVA, $F = 0.08$, $df = 1$, $P < 0.7773$).

These results indicated that 10 leaf equivalents of apple foliar extract did not enhance plant examination behaviours or oviposition of ALCM females. It can be suggested that sensory stimuli (perhaps a tactile stimulus) which were missing in this experiment are necessary in combination with chemical stimuli to trigger ALCM females to exhibit plant examination and oviposition behaviours.

Chapter V: Discussion

V.1 Rearing

Studies on rearing adults from field-collected ALCM larvae indicated that three materials, bark, soil and sand, could be used as rearing media. Bark appeared to be the most convenient culture medium. The advantages of using bark were (1) a shorter period of time from pupation to adult emergence, (2) occurrence of adult emergence for a period of medium duration, and (3) ease of use because of its lighter weight and better moisture retention.

For the mass rearing of ALCM larvae up to the adult stage, spreading larvae-infested leaves on a layer of bark medium appeared to be a more convenient method of rearing than breaking leaves and manually transferring larvae exiting from the broken pieces onto bark medium. However, while the first method was more convenient, the period over which adults emerged was longer. This prolonged emergence was less convenient because rearing boxes had to be checked over long periods of time. It may be possible to manipulate adult emergence in a timely manner by selectively introducing leaves containing mature or immature larval stages into separate culture boxes. Moisture levels of the medium also appeared to be important.

V.2 Diel emergence pattern of adult ALCM

The availability of a rearing method for ALCM adults allowed me to study several important aspects of adult life. For example, diel emergence patterns of adult males and females could be studied and were found to be synchronized. Adult midges of both sexes started emerging at 05.00 h and

continued emerging for several hours, with approximately 95 percent of adults emerging before noon. Smaller numbers (< 5%) of adults of both sexes eclosed from 12.00 to 14.00 h. A very small number (< 3%) of adults emerged between 14.00 and 19.00 h. Emergence of males reached a peak between 06.30 and 07.00 h with females peaking between 06.30 and 07.30 h.

This emergence pattern contrasts with the diel emergence pattern of another cecidomyiid species, the Hessian fly, *Mayetiola destructor.* In this species peak male and female emergence time does not coincide (Bergh et al., 1990). However, the emergence pattern of ALCM is typical of gall midges in that ALCM adults emerge over a period of hours, with small numbers initially emerging and peak numbers emerging a short time later. In different gall midge species peaks of emergence occur at different times of the day (Gagné, 1989).

V.3 Behaviour of virgin and mated ALCM females

Soon after eclosion, virgin ALCM females exhibited calling behaviour, and extruded the ovipositor to its full length so that the pheromone gland was exposed. This behaviour is similar to that of *Mayetiola destructor* (Bergh et al., 1990). Sex pheromone communication has been documented for a small number of cecidomyiids, including *Mayetiola destructor* (Mckay and Hatchett, 1984) *Dasineura brassicae* (Williams and Martin, 1986), and recently for ALCM females (Harris et al., 1996). By 10.00 h, almost all virgin females exhibited the calling posture. If females did not mate during this first day, they ceased calling at approximately 16.00 h and sat without calling for the remainder of the day. At approximately 09.00 h the next day, virgin females resumed calling and also exhibited

more active behaviours that were rarely exhibited the day before (walking and flying). Unless they mated on this second day, females ceased calling at approximately 14.00 h and thereafter were more likely to exhibit walking and flying. A similar calling pattern has been reported for virgin Hessian fly females (Bergh et al., 1990). Subsequently virgin ALCM females exhibited behaviours associated with examining the plant surface prior to oviposition (e.g., antennation and probing). These behaviours were similar to those exhibited by three-day old virgin Hessian fly females (Harris and Rose, 1989).

However, in spite of exhibiting examining behaviours, virgin ALCM females laid only a small number of eggs (< 5% of potential fecundity) before dying at the end of the second day of adult life. Similarly, gravid virgin females of the BWAMBA strain of mosquito, *Aedes aegypti,* rarely lay any eggs until they are mated (Fuchs and Kang, 1978). There is evidence for a naturally occuring, genetically controlled, endogenous inhibitor for oviposition in virgin mosquitoes, *Aedes aegypti* (Fuchs and Kang, 1978). Such an inhibitory factor might also occur in virgin ALCM and Hessian fly females. Virgin Hessian fly females initiated egglaying approx. 48 h later than mated females and laid 3-4 times fewer eggs before death (Harris and Rose, 1991). A male reproductive tract secretion is involved in triggering flight and oviposition behaviour of Hessian fly females (Bergh et al., 1992). Some internal factor related with mating also may regulate oviposition by virgin ALCM females. Further studies are required to resolve how exogeneous and endogeneous factors influence oviposition behaviour of ALCM females.

The behaviour of mated ALCM females was observed using two different types of arenas: small glass vials and a large wind tunnel. In

vials, females ceased calling immediately after mating and sat without moving for a period that ranged from 20 - 90 minutes. This represents a transition period after mating and before oviposition. Similar post-mating inactive periods of 2-3 hrs are evident in the two cecidomyiids, the Hessian fly, *Mayetiola destructor* (Harris and Rose, 1991) and the swede midge, *Contarinia nasturtii* (Readshaw, 1966). By 10.00 h some mated ALCM females had initiated active foraging behaviours and by 12.00 h, 100% of females were active. The first activity observed was walking, which was followed by flying, antennating, probing, and ovipositing. Once these activities began, they continued until the female died between the hours of 18.00 and 21.00 h.

Unlike virgins, mated females lived only for one day in a glass vial, almost always laid eggs before dying (90% of the females), and laid 6.79 to 67.54% of the eggs in their ovaries. Why some females laid such small numbers of eggs before dying, in spite of having access to apple foliage, is not known. After landing, ALCM females exhibited probing during which the surface texture of apple leaves and other surfaces was sensed by moving the ovipositor across the surface while walking forward. Hence it seemed that ALCM females might be searching for particular tactile stimuli which were important to the acceptance of the substrate for oviposition. Similar effects of tactile stimuli on the oviposition behaviour of the Hessian fly have been documented (Harris and Rose, 1990). In tests conducted without chemical stimuli from wheat extract, female Hessian flies laid 28 times more eggs on a veined surface than on a smooth surface. The vials used in the present experiment had a smooth inner surface which might have not provided acceptable tactile stimuli to trigger oviposition of ALCM females. Furthermore, the single leaves used in this

experiment had been removed from the tree and may not have emitted the same volatile chemicals that are emitted by leaves still attached to the tree. Thus ALCM females may have laid smaller numbers of eggs due to the artificial nature of the test arena or problems with the quality or quantity of plant material given as oviposition sites. Unsuccessful mating could be another cause of low egg numbers as has been reported for Hessian fly females (Bergh et al., 1992). Hessian fly females mated by multiply-mated (depleted) males did not deposit eggs on the day of mating and deposited fewer eggs before death than did females mated by undepleted males.

Mated ALCM females were presented with apple foliage in a wind tunnel at various times on the day of eclosion to determine the best time to run behavioural assays. Females were inactive between 09.00 and 10.00 h. This inactive period coincides with the post-mating pre-ovipositional transition period, as reported in the previous experiment. Relative to the period before 10.00 h, six times more females flew upwind to host plant odour and landed on the foliage after 10.00 h. Numbers of females that flew upwind and landed peaked between 17.00 and 18.00 h and were ten times greater than numbers of females exhibiting these behaviours between 09.00 and 10.00 h. This increase in behavioural responsiveness may result from physiological changes that occur in mated females, with sensitivity to apple odour increasing with time after mating. Increasing responsiveness to host or host associated odours has been documented in females of the tachinid fly, *Eucarcelia rutilla,* as the pre-ovipositional period progresses (Herrebout and van der Veer, 1969), and in females of *Delia brassicae* as the length of time the female has been gravid increases (Traynier, 1967).

After landing on foliage, female ALCM either sat for long periods of time (i.e., longer than the 15 minute observation period) or proceeded to examine plant surfaces by antennation and probing with the ovipositor. Before 14.00 h, females always exhibited the first of these behaviours (i.e., sit on foliage) but after 14.00 h showed an increased probability (> 16%) to proceed to examining behaviours. This might be due to some endogeneous factor that prevented ALCM females from oviposition until a certain period of time after mating. Physiological factors underlying ALCM behavioural transitions and information about egg maturation have not been studied. It can be suggested that progress through the different stages of post-mating behaviours of ALCM females might be controlled by one or more endogeneous factors that change over time. These factors might also be related to the development of physiological states of mated females leading to oviposition. If there is an inhibitor present in virgin females that prevents them from ovipositing, mating might cause inactivation of such an inhibitor and the sequential triggering of plant examining and oviposition behaviours. Fuchs and Kang (1978) suggested a similar scenario for the oviposition behaviour of *Aedes aegypti*. In Hessian fly females, the mechanical stimulus associated with the act of mating and the chemical stimulus passed to the female during copulation appeared to act sequentially, leading the female to respond by ceasing to call, rejecting mating attempts by males, and sequentially exhibiting antennation, probing and eventually oviposition (Bergh et al., 1992).

V.4 Host-plant specificity of ALCM females for oviposition

Experiments exploring the host-plant specificity of ALCM females indicated that, given a choice between apple and pear foliage, females laid

four times more eggs on apple than on pear. However, when given no choice between the two plant species, similar numbers of eggs were deposited on both apple and pear foliage. Expansion of specificity for host-plants due to deprivation of specific host-plant species has been demonstrated in a sphingid moth (Knoll, 1922 cited in Hinton, 1981) and in the Hessian fly (Harris and Rose, 1989). Other specialised species of gall midges will sometimes lay eggs on plants related to their hosts, plants on which the larvae do not develop (Gagné, 1989 and references therein).

The pattern of oviposition on apple and pear foliage was studied in a choice bioassay. After being introduced to cages holding foliage at 10.00 h, females commenced oviposition within the first four hour period. Peak numbers of eggs were deposited on apple foliage between 14.00 and 18.00 h, while eggs on pear foliage reached a peak earlier between 10.00 and 14.00 h. After that egg deposition decreased steadily. During scotophase no eggs were laid. A small second group of eggs were laid on the second day between 10.00 and 14.00 h by females that had not oviposited on the previous day. The cause of this delay in egglaying is not known.

Further studies on choice of oviposition sites revealed that females preferred immature foliage and buds over mature foliage and sites at a greater height within the cage. The former result is not surprising given that younger apple leaves are always more heavily infested than mature leaves by ALCM larvae in the field (Whitcomb, 1934; Todd, 1956). The later result is less straight-forward but may be related to the behaviour of ALCM females in cages. In cages, females are typically found walking or flying at the top of the cage and are rarely observed flying in the centre of the cage. In general, this indicates that ALCM females do not behave naturally in small enclosed spaces. This behaviour contrasts with the

behaviour of Hessian fly females tested in similar cages (Harris et al., 1993; Harris and Rose, 1990). This observation and other similar observations prompted me to conduct the rest of my experiments in a more natural setting, a large wind tunnel, where females could fly freely.

V.5 Effect of plant stimuli on host-finding behaviour of ALCM females

Studies in the wind tunnel on the flight responses of mated females to host (apple) and non-host (pear) plant foliage indicated that females respond to plant stimuli over distances of at least 30 cm. Numbers of females that flew upwind to apple foliage were approximately double the number that flew upwind to pear foliage. Females also appeared to distinguish host and non-host plant foliage at a closer distance (5 cm) as fewer females approached and landed on pear foliage. Nine times more females approached and more than twenty times more females landed on apple foliage than on pear foliage. Thus, discrimination between apple and pear foliage improved as females approached the foliage. The mechanism underlying improved discrimination is not clear. It may be that females respond to a sensory cue which is common to both apple and pear foliage at greater distances (> 30 cm), but at closer distances distinguish apple from pear by sensory stimuli active over shorter distances.

Further studies in the wind tunnel indicated that these stimuli are volatile chemicals. Females discriminated between odours from pear foliage and odours from apple foliage even in absence of plant visual stimuli. These chemicals may occur in higher concentrations close to the source and may therefore be discriminated better by females at this distance. Previous reports investigating volatile foliar chemicals of apple

(cv. Delicious) and pear (cvs. Bartlett and Bradford) revealed that certain compounds are common to both plant species. (Z)-3-hexenyl acetate and α-farnesene were present in moderate levels in the volatile chemicals of both plant species (Ebel et al., 1995; Miller et al., 1989). According to Metcalf (1987 cited in Miller et al., 1989), terpene and sesquiterpene hydrocarbon type compounds may be important semiochemicals influencing insect behaviour. A sesquiterpene, α-farnesene, and a series of headspace odours of apple fruits including (E)-2-hexen-1-yl acetate attract two specialised pest species of apple fruit, larval and adult codling moth, *Laspeyresia pomonella* (Sutherland et al., 1974) and the apple fruit fly, *Rhagoletis pomonella* (Fein et al., 1982). Phloridzin is a chief phenolic compound in surface wax of apple leaves (Richmond and Martin, 1959). Two aphid species, *Aphis pomi* and *Rhopalosiphum insertum* react to silica gel treated with phloridzin by walking and probing (Klingauf, 1971 cited in Städler, 1986). These three chemicals might be components of a blend of chemicals that attracts ALCM females, with qualitative or quantitative differences in volatile blends given off by apple and pear foliage.

Responses of females to chemical stimuli from young apple foliage were also tested relative to mature apple foliage. Females responded to stimuli from both immature and mature foliage by orientation and landing on the foliage. However, more females responded to immature than mature foliage. Richmond and Martin (1959) revealed that higher deposits of surface wax and phenolic materials are associated with young (second to fifth leaves from the tip) apple leaves (cvs. Merton Worcester and Cox's Orange Pippin) than with older leaves (sixth and tenth leaves). The greater responses of ALCM females to immature apple foliage than to

mature foliage observed in the present study may have occurred because volatile chemicals that attract ALCM are emitted in higher concentrations by immature than by mature foliage.

In experiments on ALCM responses to volatile chemicals from host and non-host plant species it was observed that females flew past a visual model of apple foliage when volatile chemical stimuli originated from a source hidden behind a screen, upwind of the model. Thus, females flying upwind in an odour plume of apple volatiles may not simply fly upwind until a visual target enters their visual field but continue flying to the source of the odour. This was further tested in a factorial experiment in which a visual model of apple foliage was placed in an odour plume of apple volatiles. Visual stimuli from the model used in these studies had no effect on its own and did not enhance the behavioural effects of volatile chemical stimuli.

Furthermore, in another experiment females did not respond to yellow or blue colour fields in the absence of chemical stimuli. When chemical stimuli were present upwind of these blue and yellow screens, similar numbers of females took off from the release platform, flew upwind, and approached the blue and yellow screens. In a second experiment with the blue and yellow screens, females given an apple shoot downwind of a blue or yellow screen did not exhibit different behaviours. These studies revealed that chemical stimuli play a more important role than visual stimuli in the host-finding behaviour of mated ALCM females.

These results contrast with results on stimuli effecting the host-finding behaviour of the cecidomyiid, the Hessian fly, and many other insect herbivores. However, similar results were reported by Fein et al. (1982) for the apple maggot fly, *Rhagoletis pomonella.* The response of

apple maggot flies to the synthetic blend of the natural components in apple volatiles (cvs. Red Delicious and Red Astrachan) was not enhanced by testing the blend with artificial apples, indicating that visual stimuli were not necessary for the flies to be attracted to volatiles. Olfactory responses to rape plants have been documented for a species closely related to ALCM, *Dasineura brassicae* (Pettersson, 1976). Sinigrin, a volatile component of rape plants has a considerable arrestant effect on *Dasineura brassicae* females, and also is part of long distance stimuli facilitating the finding of suitable host-plants.

As a first step towards isolating and identifying behaviourally-active volatile chemicals from apple foliage, a dichloromethane extract of apple foliage was tested. Extracts of surface waxes of apple foliage were made by dipping immature leaves in dichloromethane for 50 seconds. Extracts were bioassayed in the wind tunnel and in glass vials. In the wind tunnel, six times more females flew upwind to apple extracts applied at a concentration of 50 leaf equivalents (LE) than to the solvent control. Relative to the solvent control, thirty times more females approached and landed on filter papers treated with the foliar extract. In a bioassay run in glass vials, foliar extract at a concentration of 10 LE did not stimulate post-landing examining behaviours or oviposition any more than the solvent control. This may have occurred because females were confined to a small space which did not allow them to exhibit normal host location and post-alightment behaviours or because oviposition responses to chemical stimuli only occur in the presence of other stimuli, perhaps tactile stimuli (Harris and Foster, 1995).

In conclusion, the host-finding behaviour of female ALCM is strongly influenced by volatile chemicals from apple foliage. Either qualitative or quantitative differences in these volatiles cause females to respond to immature foliage more than to mature foliage. Visual stimuli from plant models appeared to have no effect on the host-finding behaviour of females either during long range orientation or during landing. Behaviourally-active apple foliar chemicals were extracted from apple foliage by dipping leaves in dichloromethane for a short period of time. At a concentration of 50 leaf equivalents this extract triggered behavioural sequences leading to host-finding and landing.

V.6 Future prospects for behavioural research on the host-plant relationships of ALCM

In choice experiments on host-plant specificity, I hypothesized that ALCM females would discriminate between apple and pear foliage and would not oviposit on pear. However, ALCM females did lay a small number of eggs on pear. Whether this happens in orchards is not known. It would be interesting to study the survival of ALCM larvae hatching out from eggs laid on pear foliage and to determine whether female adults or larvae are more specific in their host range.

In future experiments, apple foliar extracts could be used to isolate volatile foliar chemicals that trigger host-finding behaviour of ALCM. Upon identification and synthesis of such chemicals, it might be possible to use these chemicals for the purpose of monitoring ALCM populations in the field. These chemicals could also be used directly as an attracticide for the control of adult females (Phelan and Baker, 1987). Furthermore, it could be a useful semio-chemical to enhance the effectiveness of

parasitoids in controlling ALCM in the field. Such semiochemicals have been used to stimulate increased rates of parasitization (e.g., *H. zea* by *Trichogramma* spp. in soybean, cowpea, tomato, and cotton, Lewis and Martin, 1990 and references therein). Furthermore, it might be possible to breed apple varieties emitting volatiles that are less attractive to female ALCM.

Many questions remain about the host-finding behaviour of ALCM females. For example, in the present study, the time of the day that mated females actively forage and proceed to oviposition was determined in a wind tunnel under laboratory conditions. More females took-off after 14.00 h than did before 14.00 h. Furthermore, out of the females that took-off, greater percentages approached and landed on apple foliage after 10.00 h than before 10.00 h. Although the females flew, landed and settled on foliage, only a few females went on to exhibit post-landing behaviours before 14.00 h. After 14.00 h, the latter behaviours were observed more frequently. From these data, it can be suggested that mated females are responsive to host-plant odour and capable of orienting to, approaching and landing on plants even before they are physiologically ready to lay eggs. Thus, until a particular physiological state is attained, females may not exhibit post-landing plant examining behaviours and may not proceed onto oviposition. Further studies on these aspects will have to be conducted under field conditions. Control programmes then might be timed to coincide with the peak time that females fly before proceeding onto oviposition (during the peak time that females settle on foliage).

The reason that some females did not lay eggs in the presence of apple foliage (e.g., small cages tests) was not clear. One possible reason for this is that females may need stimuli other than or in addition to

chemical stimuli, perhaps tactile stimuli, for oviposition to be triggered. Studies to explore this will provide useful information that might be used in developing resistant varieties with less attractive leaf surface structures. Second, there might be some relationship between the mating status of females and the numbers of eggs that they oviposit. This is a phenomenon that has to be explored to better plan future experiments on oviposition. Third, reduced oviposition may have occurred because leaves given in small cages were removed from the trees. Volatile chemicals emitted by leaves may change when leaves are damaged and be less attractive or act as inhibitors to females for oviposition.

Although the findings of this research programme may not provide direct applications for the purpose of ALCM control in the field, they provide basic information on the biology and behaviour of ALCM that will be useful in future experiments on this poorly understood pest species.

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