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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
for the degree
of Masters of Applied Science in Plant Health at
Massey University, Palmerston North,
New Zealand**

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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 referred 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.