FINE STRUCTURE OF THE OVIPOSITOR AND STUDIES OF FEEDING AND OVIPOSITION SITE SELECTION BY THE SERPENTINE LEAFMINER, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

by

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(ABSTRACT)

Ovipositors of female leafminers were examined microscopically to locate and describe types of sensory receptors present. Two basic types were found: trichoid sensilla and basiconic sensilla. These receptors are believed to function as mechano- and chemoreceptors to evaluate the suitability of a host plant for feeding, oviposition, and subsequent larval development.

Two greenhouse experiments were conducted to evaluate the effects of photoperiod and application of plant growth regulators on two factors: 1) feeding and oviposition site selection and 2) spatial distribution of feeding and oviposition sites on individual chrysanthemum plants. In the photoperiod experiment, leafminers fed and oviposited more intensely on chrysanthemums grown under short days (SD) than on plants grown under long days (LD). Densities of feeding punctures and larval mines on chrysanthemums grown

under SD was positively related to leaf height on the plant, and negatively related to leaf trichome density. Spatial distribution of feeding and oviposition sites within plants under LD was variable, and no specific pattern of preference was discerned. An experiment to determine the effect of plant growth regulators on site selection by female leafminers revealed no significant differences ($P > 0.05$) in feeding and oviposition densities on chrysanthemums treated with plant growth regulators napthaleneacetic acid, gibberellic acid, and daminozide.

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INTRODUCTION

Chrysanthemum, *Chrysanthemum morifolium* Ramat is among the most widely produced commercial flowering crops in the United States (Price 1981). Commercial shipments of chrysanthemums to other regions of the world has led to the intercontinental dispersion of agromyzid leafminers. In the United States, the major leafminer pest on chrysanthemums is *Liriomyza trifolii* (Burgess), a serpentine leafminer. The adult female punctures a host leaf tissue with its sclerotized ovipositor for feeding and egg laying, resulting in a stippling effect on leaf surfaces. Leafminer larvae which hatch from eggs deposited in the leaf, produce a serpentine shaped mine by feeding on the chlorophyll bearing leaf tissues. Larval mines and stipples reduce the photosynthetic ability of the plant, the aesthetic quality of the foliage, and the ornamental value of the crop. Therefore, low leafminer populations are critical for quality flower production.

Efforts to control the leafminer have been hampered by the insect's ability to rapidly develop resistance to insecticides and by the lack of a wide range of control methods. Much of the unpredictability of present alternative control strategies arises from the lack of behavioral and physiological information about leafminers.

Female leafminers may make "exploratory probes" with the ovipositor by touching but not penetrating the host leaf surface prior to feeding or egg laying. The initial probing and stippling activities by the female associated with feeding may also be a means of gathering sensory information for evaluating the suitability of a potential host for oviposition and larval development. Thus, the objectives of this project were: 1) to examine the types of sensory receptors on the ovipositor and 2) to measure the preference for and spatial distribution of oviposition and feeding sites within individual chrysanthemum plants as influenced by photoperiod and application of plant growth regulators. An understanding of how leafminers evaluate the potential of host plants for oviposition could improve crop protection strategies and decrease losses.

LITERATURE REVIEW

Taxonomy

Liriomyza trifolii (Burgess) was first described in the United States by Burgess in 1880 and was discovered mining *Trifolium repens* L. (Burgess 1880). The genus *Liriomyza* is homogenous; adult morphology and coloration is similar for all species in the genus. Unfortunately, this has frequently caused misidentifications in significant papers dealing with the genus (Spencer 1973). Smith et al. (1962) and Webb and Smith (1969, 1970) studied the biology and damage to greenhouse chrysanthemums caused by a leafminer species originally identified by Frick (Spencer 1973) as a new *Liriomyza* species. Later, Stegmaier and Steyskal (Spencer 1973) identified the species as *L. munda* Frick. Further examination of specimens by Spencer revealed that this species was *L. trifolii.* Numerous examples of taxonomic ambiguity such as the one above appear in the literature dealing with *Liriomyza.* Spencer (1961) stated that the morphology of the male genetalia is the only character which can by used to conclusively identify similar *Liriomyza* species. However, Knodel-Montz and Poe (1982) found that external morphology of the female ovipositor can be used to decisively distinguish between three economically important

Liriomyza species. Sasakawa (1958, 1961) also found external morphology important in his study of female terminalia of Japanese Agromyzidae.

Physical description

The adult *L. trifolii* is smaller than a fruit fly and approximately 2.5 mm long (Fig. 1). Spencer (1973) described the holotypic *L. trifolii.* The head has yellow orbits, both inner and outer vertical bristles on yellow background, and yellow antennal segments. The thorax has a matte gray mesonotum, acrostichals in 3 or 4 rows in front and reduced to two rows behind, mesopleura with a small gray patch on the lower margin, and the stenopleura is black with upper margins yellow. The abdomen lacks distinguishing characters. The legs have yellow coxae, yellow femora with brown striations, and dark brown tibiae and tarsi. The male genetalia is described by distiphallus constricted apically, epiphallus narrowed distally, and surstyli with a single distal spine (Spencer 1973).

Biology

McClanahan (1980) and Parrella et al. (1981) found a positive linear relationship between temperature and *Liriomyza* development in greenhouses. Similar developmental rate-temperature relationships have been recorded for other genera of leafmining agromyzids (Oatman and Michelbacher 1958, Tauber and Tauber 1968, Mellors and Helgeson 1978).

Figure 1. Lateral view of adult female *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) with everted ovipositor, scale = 1 mm.

Webb and Smith (1969) found a negative correlation between length of larval development and larval mortalities with temperature. Musgrave et al. (1978) formulated a model to predict the number of degree days necessary to complete a generation at 20 C.

Parrella et al. (1983) found that the development rate for each stage of *L. trifolii* was dependent on species of host plant. They concluded that the biology of *L. trifolii* must be determined for each specific host. Therefore, the remainder of this review will specifically examine papers dealing with aspects of the life cycle of leafminers on chrysanthemums.

The adult female fly lacerates leaf tissues by plunging a sharp, sclerotized ovipositor into the leaf for the purpose of feeding or oviposition. Repeated puncturing forms numerous stipples from which plant sap exudes, as well as providing many ports for entry of plant viral pathogens (Costa et al. 1958). Male flies lack puncturing apparatus and feed on sap from wounds made by females and on natural plant exudations (Oatman and Michelbacher 1958). Despite the high frequency of puncturing, Wolfenbarger (1947) reported that only 1% of all stipples contain viable eggs. Parrella et al. (1981) observed that feeding and oviposition puncture densities were lowest at temperature extremes (15.6 and 37.8 C) and highest at 26.7 C. Leafminer eggs are

ovate, cream colored, and of microscopic size (0.25 mm in length, 0.10 mm in width) (Dimetry 1971). Eggs hatch in 2-3 days into a legless, headless cyclorrhapus larvae at moderate temperatures (Price 1981).

Larvae feed by extruding a pair of sclerotized mandibles and rasping leaf mesophyll in a vertical plane (Zitter and Tsai 1980). As the larvae pass through three larval instars, mines caused by feeding increase in size. Mines become very conspicuous and reduce the decorative value and light interception ability of leaves. Larvae are fully grown (2 mm in length) in 7-10 days depending on temperature (van de Vrie and Dirkse 1981), and exit the mines by cutting a cresent shaped hole in the leaf's lower epidermis falling to the soil. Price (1981) observed larvae crawling several centimeters on the surface of the soil before pupating. The pupae are coarctate type (pupal stage is passed inside a puparium). The yellow puparium is approximately 1.5 mm long (Price 1981). In greenhouses, the pupal stage lasts 10-12 days in summer, and 15-20 days in winter (van de Vrie and Dirkse 1981). Van de Vrie and Dirkse (1981) found that one generation of *L. trifolii* on chrysanthemums under greenhouses could by completed in 3 weeks during the summer and 6-7 weeks during the winter.

Charlton and Allen (1981) described a positive linear relationship between relative humidity and the percentage of puparia surviving to adulthood. Adult flies mate soon after eclosion, and females usually oviposit within 2 days of mating (Price 1981). Parrella et al. (1981) found that fertility decreased at temperature extremes (15.6 and 37.8 C) and peaked at 26.7 C with an average of 279 eggs per female. Vercambre (1980) showed that fecundity was higher when female flies were provided honey; enabling females to lay from 134 to 256 more eggs than those feeding on plants alone. Parrella et al. (1981) stated that the fecundity of *L. trifolii* was generally higher than that reported for other *Liriomyza* species (Dimetry 1971, Hendriske et al. 1980, McClanahan 1980, Speyer and Parr 1949). Other *Liriomyza* species also exhibit increased fecundity when provided supplemental carbohydrates (Oatman and Michelbacher 1959).

The longevity of *L. trifolii* increases when flies are provided a supplemental carbohydrate food source. Female flies lived an average of 23 days when provided honey and 17 days without honey (Vercambre 1980). Males live only 10 days when supplied honey, and 4 days without honey (Vercambre 1980). Parrella et al. (1981) found that longevity of *L. trifolii* decreased with increasing temperature.

History of the Leafminer Problem

Liriomyza trifolii is a polyphagous leafminer (Spencer 1973). Although it has been recorded attacking over 47 genera of plants in 10 families (Stegmaier 1966), it is chiefly known as a pest on chrysanthemums. *Liriomyza trifolii* is probably native to eastern North America but now occurs on the west coast of the United States (Parrella et al. 1981, Trumble 1981). The original distribution of *L. trifolii* in the United States is unclear because the fly usually becomes prevalent only where chrysanthemums are grown (Price 1981). Invasion of *L. trifolii* into countries outside the United States was caused by importation of chrysanthemum cuttings or cut flowers from infested areas (Price 1981). For example, outbreaks of *L. trifolii* occurred in Kenya in 1977 following importation of chrysanthemum cuttings from Florida (de Lima 1979). Recent infestations of *L. trifolii* have been recorded in the United Kingdom (Anon. 1977), Italy (Arzone 1979), France (d'Aguilar and Martinez 1979), Reunion (Vercambre 1980), the Netherlands (van de Vrie and Dirkse 1981), and Columbia (Price 1981). Thus, commercial shipment of chrysanthemums has led to intercontinental dispersion of *L. trifolii*. Once introduced, *L. trifolii* has become a serious pest on chrysanthemums threatening an important ornamental industry.

Leafminers have most likely achieved primary pest status due to growers' unfamiliarity with their damage and rapid proliferation. When *L. trifolii* was accidentally introduced into greenhouses, growers had no natural enemies to control leafminers and were forced to apply insecticides, thus upsetting a balance already established between natural enemies and other chrysanthemum pests (i.e., aphids, thrips, spider mites). Unfortunately, chemical control is seldom effective because leafminers are often resistant to insecticides. In light of these characteristics, most countries receiving chyrsanthemums now require a zero tolerance level of leafminer damage. Such intolerance has intensified leafminer control efforts to prevent signs of stippling and mining. Most greenhouse operators in Europe are concentrating their efforts on integrated or biological control for chrysanthemum pests (Bennett and Greathead 1981). The intercontinental dispersion of *L. trifolii* has subsequently elevated this leafminer to the current status of major pest on chrysanthemums (Price 1981).

Chrysanthemum Propagation Techniques

Chrysanthemums are grown in either ground beds or raised benches in greenhouses. A definite sequence of practices must be used to ensure profitable production of chrysanthemums. First, soil conditions must be determined

and necessary adjustments made to ensure that the soil is optimum for chrysanthemums. The ideal soil for growing chrysanthemums is sandy loam with a neutral or slightly acid pH (6.5 to 7.0) and high organic content (Machin and Scopes 1978). The soil is then sterilized with methyl bromide (Maw and Kempton 1973) or other chemical means, or pasteurized by steaming. The plants are placed in beds, and fertilized and watered according to precise schedules. As plants mature, buds are removed (disbudding) and plant growth regulators applied to control growth. Disbudding, application of plant growth regulators, and artificial short day treatments cause chrysanthemums to develop at similar rates, and ultimately cause formation of flower buds of similar size and quality. Chrysanthemums are then harvested and the area sanitized to prevent spread of any plant diseases to future crops.

Plant Growth Regulators

Plant growth regulators are defined as endogenous or synthetic compounds which promote, inhibit, or modify plant physiological processes. These compounds ultimately control growth and development (i.e., auxins). Plant hormones (phytohormones) are naturally produced organic compounds of low molecular weight which, in low concentrations, regulate physiological processes. Hormones and plant growth regulators influence the growth of chrysanthemums from rooting to senescence (Machin and Scopes 1978).

There are five basic types of natural plant hormones (Leopold and Kriedemann 1964). These are auxins, gibberellins, cytokinins, abscisic acid and ethylene. Auxins are substances which generally resemble indoleacetic acid and induce cell elongation of coleoptiles. Gibberellins are compounds that have a gibbane ring carbon skeleton and stimulate cell division, cell elongation or both (Paleg 1965). Gibberellins can cause striking elongation in stems of certain dwarf mutants and rosette type plants. Cytokinins are usually substituted adenines which resemble zeatin and stimulate cell division in plants. Abscisic acids participate in a diverse group of processes ranging from senescence, stomatal closure and cold acclimation. Ethylene is a gaseous regulator which stimulates isodiametric growth of stems and roots. Each of the five types of regulators have overlapping functions in plant growth, but each is distinctive both chemically and in stimulating characteristic growth responses (Leopold and Kriedemann 1964).

Growth inhibitors, synthetic growth retardants in particular are used regularly by chrysanthemum growers to control plant growth. Phosfon (chlorphonium chloride) and B9 (N-dimethylaminosuccinamic acid) are two growth retardants used during chrysanthemum production (Machin and

Scopes 1978). Chemical retardants suppress plant height via internode length inhibition without affecting foliage density. Plants treated with retardants are compact and more aesthetically pleasing; and often appear to have darker green foliage than untreated plants. This may be caused by the development of thicker leaves, or from higher density of chloroplast containing cells in the leaves (Machin and Scopes 1978).

As a result of the control of plant height and shape afforded by growth retardants, they have become quite useful in chrysanthemum production. Growth retardants, such as Phosfon or B9, may also inhibit gibberellin synthesis in plants (Ryugo and Sachs 1969, Sachs and Kofranek 1963, Wylie et al. 1970). Interference with gibberellin synthesis allows growers to produce uniform crops of flowering chrysanthemums with improved timing. If gibberellin synthesis is not altered, normal shoot elongation results in undesirable growth and delayed flower bud development (Weaver 1972).

Chapter I

SENSORY RECEPTORS ON THE OVIPOSITOR OF *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

INTRODUCTION

Female agromyzid leafminers damage host plants by puncturing the leaf tissue with their sclerotized ovipositor for feeding and egg laying. These punctures produce unsightly stipples and provide a port for plant pathogens (virus, bacteria, fungi). Larvae hatch from eggs in the punctures, and mine the leaf mesophyll which causes plant desiccation and defoliation, especially at high densities.

Feeding behavior of female leafminers has been described in detail (Ahmad and Gupta 1941, Bollow 1955, Dureseau and Jeandel 1977, Oatman and Michelbacher 1958, Smulyan 1914, Speyer and Parr 1949). First, the leafminer selects a suitable feeding site. She then raises the abdomen and protrudes the sclerotized ovipositor to pierce the leaf surface vertically. The tip of the ovipositor is forced under the epidermis horizontally, then alternately withdrawn and reinserted. The piston-like thrusting of the ovipositor macerates the parenchyma cells forming a tiny circular "blotch". Following withdrawal of the ovipositor, the circular cavity becomes filled with oozed leaf sap. Males (incapable of making feeding holes) as well as females feed upon exuded juices.

Behavior during oviposition is similar to that of feeding described above. It differs from feeding behavior by the frequency of the thrusting motion and the deposition of a single egg (Ahmad and Gupta 1941, Cory 1916, Smulyan 1914, Speyer and Parr 1949, Tilden 1950). The ovipositor is again forced vertically through the leaf surface, and pushed horizontally with one or two piston-like thrusts through the parenchyma. It is partially retracted for a few seconds, then protruded for a final time, and slowly withdrawn leaving an egg at the distal end of the tunnel. The female withdraws the ovipositor without pressing the leaf cells upon the egg. She then imbibes the small amount of sap which is collected in the tunnel between the puncture and egg.

The behavior of leafminers in selecting food sites, oviposition sites and use of the ovipositor as a means of acquiring food implies a sensory role for the ovipositor. Consequently experiments were designed to locate and describe the sensory receptors on the ovipositor of *Liriomyza trifolii*.

MATERIALS AND METHODS

Liriomyza trifolii pupae obtained from a leafminer colony were reared at 26 C in plastic rearing dishes (50 mm dia. x 36 mm depth) filled to a depth 1 cm with sterile dampened sand. Dishes were covered with Saran filter cloth (42 x 42 mesh). Drops of water were added as needed to provide a humid environment. Rearing containers were checked daily for emerged adults. Emerged adults were kept alive for 24 hours to attain mature form and coloration.

Flies were anesthetized with Fly-nap (Carolina Biological Supply Co., Burlington, North Carolina) and removed from the rearing vials for microscopic examination at 40X. The ovipositor on each fly was everted by lightly compressing the tip of the abdomen with a forceps. This compression was repeated until the ovipositor remained fully extended.

Ovipositors were excised from the fly body and prepared for scanning electron microscope (SEM) examination in the following manner. Each ovipositor was fixed in 3% glutaraldehyde in 0.1 M Na cacodylate buffer. Following overnight fixing, the ovipositor was placed in 1% OsO_4 in 0.1 M cacodylate fixative for 15 minutes, and then dehydrated 15 minutes in each of six concentrations of ethanol (15, 30, 50, 70, 95, and 100%). After dehydration,

specimens were dried in a Ladd critical point dryer (Ladd Research Ind., Burlington, Vermont). Ovipositors were mounted on silver studs with copper taping or Avery Self-adhesive Paper Tacks (Avery Label System, Division of Avery Products Corp., Elmhurst, Illinois), coated with a 200 Å thick layer of gold in an SPI sputter coater (SPI Supplies Division of Structure Probe, West Chester, Pennsylvania). Ovipositors were examined with an Advance Metal Research-900 SEM (Amray Inc., Bedford, Massachusetts) or JEOL JSM-35C SEM (JEOL Inc., Peabody, Massachusetts). Morphological features of the ovipositors were photographed with a Polaroid camera (Polaroid Corp., Cambridge, Massachusetts), and later sensory structures were described.

RESULTS

The ovipositor of *L. trifolii* consists of abdominal segments VII-IX and is approximately 417 um long (Fig. 1). Segments X and XI are reduced in the higher Diptera. Normally these segments are telescoped within the anterior part of the abdomen, and are extended by haemolymph pressure for puncturing the leaf tissue.

The anterior part of segment VII is modified into an ovipositor sheath. A spiracle and numerous minute trichoid

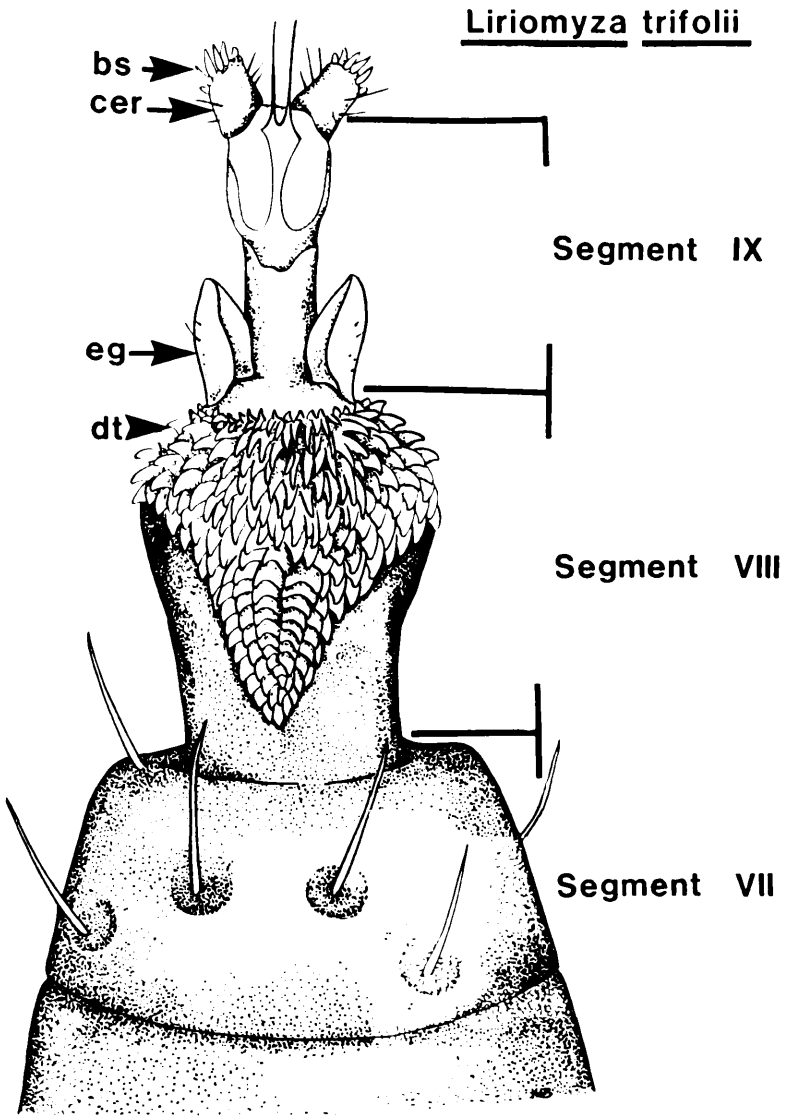

Figure 1. Diagram of dorsal view of ovipositor of *Liriomyza trifolii*. Segment VII to IX illustrated (bs- basiconic sensilla, cer - cercus, dt - denticle, and eg - egg guide)

sensillae (9 um) are located posterior to the ovipositor sheath (Fig. 2). Segment VII is approximately 235 um long. Tactile sensillae are randomly scattered distally on this segment. The length of the sensilla averaged 50 um.

Segment VIII (112 um) is formed by a sclerotized tergite and sternite with anteriorly directed denticles (Fig. 3). The tergite and sternite are V-shaped, 85 um long, and separated by a pilous region. Numerous trichoid sensillae averaging 18 um are found in this region. Two suboval egg guides extend distally from the eighth segment.

The main sense organs on the ovipositor are located at the tip of the paired cerci on the terminal abdominal segment IX. The accumulation of sense organs on the terminal segment of the ovipositor is believed to serve as sensory probe in determining the suitability of oviposition sites. Each cercus (35 um) has six cone shaped basiconic sensillae (1b-6b) located distally. (Fig. 4). Sensillae 1b and 6b are the smallest cones averaging 3 um and sensillae 2b-5b are larger, averaging 5 um (Fig. 5). Five trichoid sensillae are located laterally on the medial side of each cercus. Two trichoid sensillae are found on the opposite side of each cercus. Eggs that are being deposited pass between the cerci and progress can be monitored by laterally located sensillae.

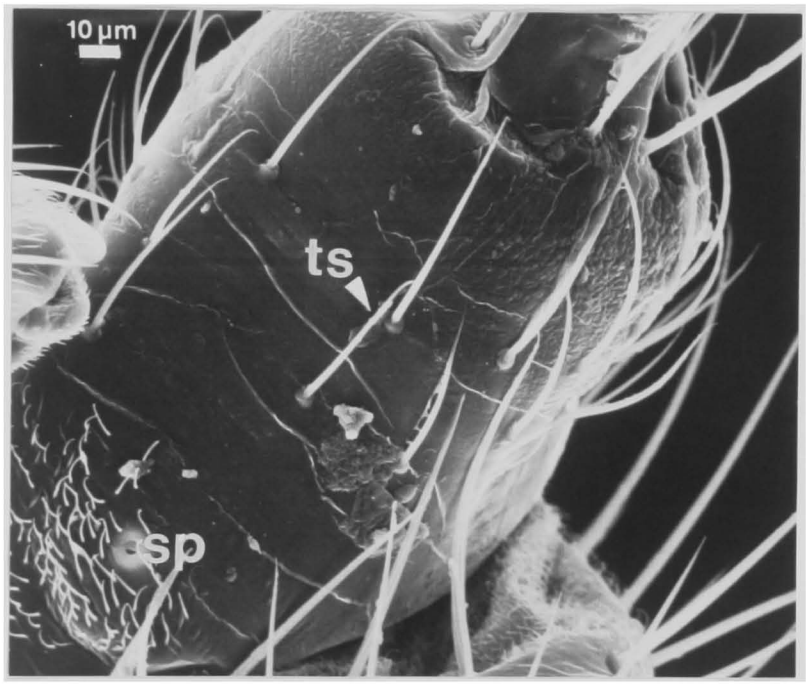

Figure 2. Scanning electron micrograph (SEM) of lateral view of Segment VII of ovipositor. Spiracle (sp) and trichoid sensilla (ts) present. (x500)

Figure 3. SEM of dorsal view of sclerotized tergite of Segment VIII on ovipositor. Anteriorly directed denticles (dt) present. (x2,000)

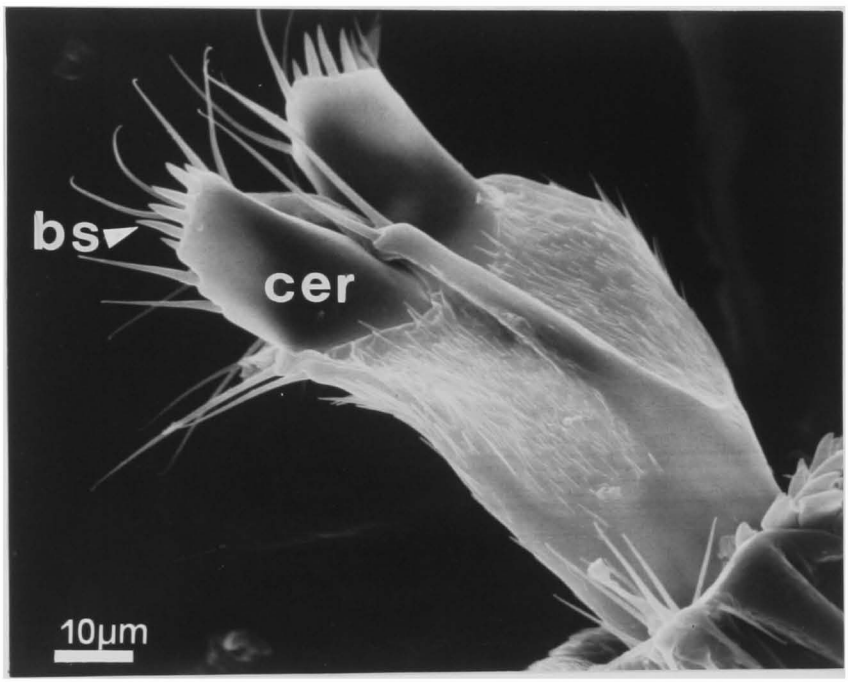

Figure 4. SEM of lateral view of cercus (cer) and basiconic sensillae (bs) on Segment IX of ovipositor. (x1,000)

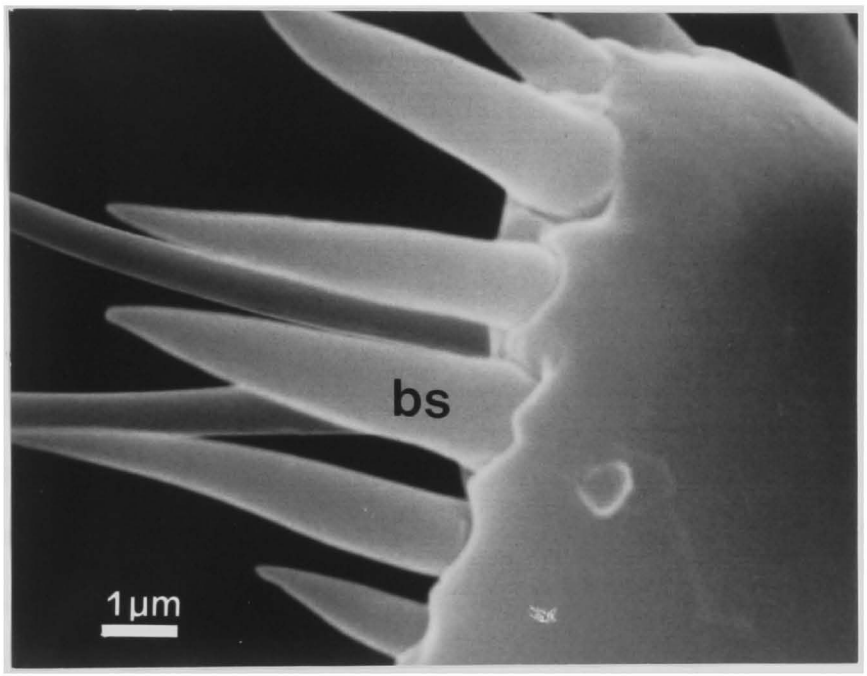

Figure 5. SEM of lateral view of basiconic sensillae (bs) of Segment IX on ovipositor. (x10,000)

DISCUSSION

Two types of sensillae have been described on the abdominal segments of the ovipositor of *L. trifolii.* They are trichoid sensillae and basiconic sensillae (Chapman 1971).

The upper epidermal layer of leaf tissue is believed to be initially pierced by egg guides extending from segment VIII. Then, the rearward-directed denticles on segment VIII may cut and rasp the leaf tissue to form a puncture. Female leafminers have been observed macerating the leaf tissue by making several rotatory and pumping motions with the ovipositor (Dimetry 1971, Oatman and Michelbacher 1958, Smulyan 1914, Speyer and Parr 1949, Tilden 1950). After oviposition, the egg guides probably play a role in separating the leaf tissues to prevent crushing the egg during ovipositor withdrawl.

The terminal position of the basiconic sensillae on the cerci suggests that they might provide the initial chemosensory information in host plant selection. Other insect behavioral investigations have strongly implicated contact chemoreception in the selection of oviposition sites (Hendry et al. 1976, Schoonhoven 1968, Stadler 1978). In some cases, oviposition is guided by chemoreceptors located on the ovipositor. Locusts perceive salt content and soil moisture with these organs (Norris 1968), and the

entomophagous wasp *Venturia* is able to discriminate between parasitized and nonparasitized hosts with its ovipositor (Ganesalingam 1974). Rice (1976) found contact chemoreceptors on the ovipositor may play a role in regulating egg-laying behavior of the sheep blowfly. The numerous sensillae on segment IX are likely to be implicated in monitoring the physical conditions at the site of piercing, and leaflet contact when ovipositing.

The factors governing oviposition and feeding site preference in agromyzid leafminers are unknown (Ibrahim and Madge 1977, Sasakawa et al. 1970, Yoshida and Sasakawa 1975). Tryon (1979) observed the female leafminer making "observatory probes" with its ovipositor by touching but not penetrating the host leaf surface prior to feeding or oviposition. Coaker (1973) observed the female *Phytomyza rufines* "testing" leaf surfaces by making punctures with the ovipositor during egg laying. Observations of oviposition behavior as indicated by discrimination in egg laying imply that females can determine the "suitability" of a host plant for oviposition and larval development (Oetting 1982, Schuster and Harbaugh 1979a,b, Schuster et al. 1981). The ratio of feeding to oviposition punctures varies for different varieties of host plants. For example, some are heavily mined but have few stipples, while others are

heavily stippled with few mines. Musgrave et al. (1975) found oviposition increased during vegetative growth but declined during senescence. Poe et al. (1977), Poe and Green (1974), Woltz and Kelsheimer (1958), Schuster and Harbaugh (1979b), Tryon (1979) and Speyer and Parr (1948) reported that leafminer activities were influenced by the condition and age of host plants.

The ovipositor may serve as a potential sensory probe in host plant selection. Initial probing and stippling activities by female leafminers might not only be a method of obtaining food and gathering more sensory information, but also a means of evaluating the suitability of a potential host for subsequent larval growth.

Chapter II

EFFECTS OF PHOTOPERIOD ON FEEDING AND OVIPOSITION SITE SELECTION BY *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

INTRODUCTION

Cultural factors known to influence leafminer populations in a production area are host plant susceptibility, plant nutrition, pinching (removal of apical buds of young plants), and harvest sanitation (Price and Harbaugh 1981). One particular host, *Chrysanthemum morifolium* Ramat., exhibits various degrees of susceptibility. This photoperiodic short day (SD) plant forms flowers when given 12 hours or less of light per day and remains vegetative during long days (LD) (Machin and Scopes 1978). The ease of photoperiod manipulation within greenhouses, coupled with the decorative aspects of the flower, has made the chrysanthemum one of the most valuable of all floricultural crops. Although Schuster and Harbaugh (1979b) found that chrysanthemums were less susceptible to leafminer attack when completely vegetative (LD), the use of cultural manipulations for leafminer control requires more research (Price and Harbaugh 1981).

Investigations on the distribution of leafminer larvae within a host plant suggest foliage age is an important factor (Musgrave et al. 1976, Hoskinson 1979, Beck et al. 1981). Beck et al. (1981) stated that the spatial distribution of leafminer larvae and adults should be considered in pest management programs so that optimum sampling sites for monitoring can be established. Thus, an experiment was designed (1) to measure the influence of photoperiod on leafminer feeding and oviposition preferences and (2) to determine the spatial distribution of leafminer punctures and mines within individual chrysanthemum plants.

MATERIALS AND METHODS

The chrysanthemum cultivar white Iceberg was chosen for the experiment since it is commonly grown and also highly susceptible to leafminer damage (Schuster and Harbaugh 1979a). Twelve cuttings were potted in 10-cm square plastic pots using Promix (Wetsel Seed Co., Harrisonburg, Virginia), a commercial potting medium. Each pot contained one cutting. Chrysanthemums were grown in a glass reinforced plastic greenhouse (18.3 x 8.2 m) on raised wire benches (3.7 x 1.2 m). Plants were fertilized weekly with 15-10-30 (15N:4.4P:24.9K) fertilizer, and watered daily. Temperature and relative humidity were recorded with a hygrothermograph

(Cole Parmer Instrument Co., Chicago, Illinois). This greenhouse experiment was conducted from February to April to avoid the confounding effects of high temperature on photoperiodism.

Plants were acclimated for two weeks under a LD photoperiod provided by night interruption (2200-0200 hours) with incandescent bulbs (60 watts) spaced 1.2 m apart and 1.5 m above the greenhouse benches. All plants were pinched at two weeks to promote the development of lateral branches. Plants were kept vegetative for two additional weeks under the LD cycle and then separated into different photoperiod groups. Six pots of chrysanthemums remained under the LD exposure and six were placed under a SD photoperiod. Cages (1 m^3) covered with black plastic were used to simulate the SD cycle of 8 light hours. The cages were placed over the plants each day at 1700 hours and removed at 0800 hours the following day. A black, 100% cotton cloth partition was also drawn each day from 1700-0600 between LD and SD areas as a further precaution against any light "contamination" during the night interruption period. Chrysanthemums were exposed for 4 weeks under their respective photoperiod regimes. At 8 weeks of age and just prior to insect introduction, 3 of the 6 plants from each photoperiod were randomly selected and placed together in a cage. This

procedure was replicated twice. Each cage was 1 m^3 and covered with Saran filter cloth (42 x 42 mesh). Plants were then exposed to natural daylengths (approximately 8 light hours) for the remainder of the experiment.

Liriomyza trifolii pupae obtained from a laboratory leafminer colony were reared at 26 C in a plastic rearing dishes (50 mm dia. x 36 mm in depth). Each dish was filled with sterile dampened sand to a depth of 1 cm, and was covered with Saran filter cloth (42 x 42 mesh). Drops of water were added as needed to provide a humid environment for emergence. Rearing containers were checked daily for emerged adults. One day old adults were refrigerated for 5 to 10 minutes for purposes of counting and selection of flies for experimental use. Seventy-two flies (36 males and 36 females) were randomly selected and released into the center of each cage (experiment day 0).

Water mats on the bottom of each cage and on top of the greenhouse bench prevented flies from escaping while providing plant irrigation. Honey was provided as a carbohydrate source for the flies. Flies remained in the cage for 6 days and were then removed with a vacuum. Data collected on experiment day 6 included: 1) visual counts of the number of punctures per leaf (both feeding and egg punctures) and 2) leaf length. Measurements were taken on

every fourth leaf starting at the plant base and continuing up the plant to the top. Six leaf positions were recorded. Each leaf was marked with a white string to identify it for future reference.

Larvae within punctures were allowed to develop mines for 4 days (experiment day 10). Marked leaves were then removed, and the following data were collected from each: 1) visual counts of the number of mines per leaf, 2) measurement of the leaf area, and 3) leaf length. Leaf area was measured by two methods: 1) an automatic leaf area meter (Type AAM-5 Tokyo Hayashi Denko, Inc., Tokyo, Japan), and 2) by multiplying the leaf length squared by a "leaf area constant" (Lyon 1948). The actual leaf area measurements from the area meter were used to calculate the leaf area constant, which, by definition, was the actual leaf area divided by the square of the leaf length. This leaf area constant was also used to calculate leaf area on experiment day 6. After data collection, the sampled leaves were frozen until scanning electron microscopy (SEM) examination to determine numbers of leaf trichomes.

Chrysanthemum leaves were prepared for SEM examination by the following steps. Leaf sections between the edge and midrib in the center of the leaf were excised for tissue preparation. Leaf sections (5 mm^2) were placed in 3%

glutaraldehyde in 0.1 M Na cacodylate buffer overnight. The next day, each section was fixed in 1% OsO_4 in 0.1 M buffer for 15 minutes, and then dehydrated in ethanol (15, 30, 50, 70, 95, 100%) for 15 minutes each. After dehydration, leaf sections were dried in a Ladd critical point dryer (Ladd Research Ind., Inc., Burlington, Vermont). Leaf sections were mounted on silver studs using Avery Self-adhesive Paper Tacks. The upper epidermis layer of leaf tissue was coated with a 200 Å thick layer of gold in a SPI sputter coater (SPI Supplies Division of Structure Probe, West Chester, Pennsylvania). Leaf tissues were examined with a JEOL JSM-35C SEM. Leaf trichomes were photographed with a Polaroid camera during SEM examination, and later trichome densities were calculated.

A microscopic inspection was made on experiment day 12 to locate any eggs which were not visible to the eye during larval mine examination on day 10. The number of punctures, and mines plus eggs were counted with the assistance of a staining technique specifically developed for egg counts of *L. trifolii* on chrysanthemums (Parrella and Robb 1982). Five leaves were randomly selected from the top, middle (leaf position 8) and bottom of the SD and LD exposed chrysanthemums, taken to the laboratory and refrigerated. The staining solution consisted of one part each of

distilled water, phenol, lactic acid, and two parts glycerine. Acid fuchsin (0.25 g) was added to 250 ml of the above solution. Leaves were boiled in the lactophenol-acid fuchsin stain for 3 to 5 minutes and then transferred to petri dishes (90 mm in diameter) to cool for 3 to 5 hours. Excess stain was removed with a warm water rinse and leaves were examined for egg punctures, mines, and feeding punctures under a dissecting microscope at 20X. Eggs in punctures appeared darker than feeding punctures and the surrounding leaf tissue (Parrella and Robb 1982). Average leaf area measurements from the area meter on day 10 were used to calculate puncture and mine plus egg densities for each of the leaf positions (top, middle, bottom).

Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test procedures available in the Statistical Analysis System (SAS) computer package (Helwig and Council 1979). Leaf areas measured using the leaf area meter were used in the calculation of all densities described herein. However, both methods of leaf area determination were compared statistically using a paired t-test. Significance levels were $P < 0.05$ unless stated otherwise.

RESULTS

Environmental conditions in the greenhouse averaged 23 C and 54% relative humidity during the day, and 21 C and 68% relative humidity during the night.

Chrysanthemums grown under SD conditions were selected more often by leafminers for puncturing and egg laying than chrysanthemums grown under LD. SD grown chrysanthemums had significantly higher puncture, mine, and mine plus egg densities than LD grown chrysanthemums (Table 1). Densities measured on day 12 were higher than densities on days 6 and 10 because of improved visibility of punctures and mines using leaf staining techniques and 20X magnification. Results of ANOVA for puncture densities on day 6, mine densities on day 10, puncture densities on day 12, and mine plus egg densities on day 12, are given in Appendix I Tables 1 through 4. These tables also confirm that oviposition and feeding depend not only on photoperiod but also on the position of a leaf on the plant.

The distribution of puncture densities of plants grown under SD and LD photoperiods is summarized in Tables 2 and 3. Leaf position 20 (top) of SD grown chrysanthemums had significantly more punctures than did other leaf positions within SD grown chrysanthemums. By contrast, no such relationship was found on LD grown chrysanthemums (Tables 2 and 3).

Table 1. Mean *Liriomyza trifolii* puncture, mine, and mine plus egg densities on chrysanthemums grown under short and long day photoperiods.

Photoperiod	Mean density			
	Punctures/ cm^2 (day 6)	Mines/ cm^2 (day 10)	Punctures/ cm^2 (day 12)	Mines + eggs/cm^2 (day 12)
Short day	1.95a[1]	0.33a	5.28a	0.64a
Long day	0.68b	0.11b	0.61b	0.16b

[1]Means within a column followed by the same letter are not significantly different at the $P < 0.05$ level, Duncan's Multiple Range Test.

Table 2. Within plant distribution of mean *Liriomyza trifolii* puncture densities on chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 6.

Leaf positions	Punctures/cm^2	
	Short day	Long day
20 (Top)	5.88a[1]	0.69a
16	1.57bc	1.08a
12	2.57b	0.67a
8	1.02bc	0.94a
4	0.46bc	0.28a
1 (Bottom)	0.18c	0.43a

[1]Means within a column followed by the same letter are not significantly different at the $P < 0.05$ level, Duncan's multiple range test.

Table 3. Within plant distribution of mean *Liriomyza trifolii* puncture densities on stained chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 12.

Leaf positions	Punctures/cm^2	
	Short day	Long day
20 (Top)	12.85a[1]	0.53a
8 (Middle)	2.74b	1.04a
1 (Bottom)	0.26b	0.25a

[1]Means within a column followed by the same letter are not significantly different at the $P < 0.05$ level, Duncan's multiple range test.

Distribution of mine and mine plus egg densities for each leaf position of short and long day photoperiods is given in Tables 4 and 5. The upper leaf positions (12 to top) of SD grown chrysanthemums had higher mine and mine plus egg densities than lower leaf positions (8 to bottom) (Table 5). LD grown chrysanthemums showed no significant differences in the distributions of mine and mine plus egg densities per leaf.

A significant interaction between leaf positions and photoperiods for both puncture and mine densities (Appendix I Tables 1 through 4) occurred because both puncture and mine densities increased consistently from bottom to top leaf positions within SD grown plants. However, these densities in LD grown plants remained relatively constant, regardless of leaf position.

Estimated trichome densities for leaf position 1 (bottom), 8 (middle), and 20 (top) of SD and LD grown chrysanthemums are shown in Table 6. Under LD, chrysanthemums had higher mean trichome densities than SD grown chrysanthemums. The top leaf position of LD grown chrysanthemums had a trichome density (38 trichomes/mm^2) approximately four times higher than the trichome density of SD grown plants (10 trichomes/mm^2) (Fig. 1). Trichome densities of bottom and middle positions differed less between photoperiods (Table 6).

Table 4. Within plant distribution of mean *Liriomyza trifolii* mine densities on chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 10.

Leaf positions	Mines/cm^2	
	Short day	Long day
20 (Top)	0.46a[1]	0.01a
16	0.39ab	0.09a
12	0.49a	0.05a
8	0.24ab	0.07a
4	0.22ab	0.25a
1 (Bottom)	0.18b	0.18a

[1]Means within a column followed by the same letter are not significantly different at the $P < 0.05$ level, Duncan's multiple range test.

Table 5. Within plant distribution of mean *Liriomyza trifolii* mine plus egg densities on stained chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 12.

Leaf positions	Mines plus eggs/cm^2	
	Short day	Long day
20 (Top)	1.15a[1]	0.01a
8 (Middle)	0.44b	0.20a
1 (Bottom)	0.35b	0.27a

[1]Means within a column followed by the same letter are not significantly different at the $P < 0.05$ level, Duncan's multiple range test.

Table 6. Density of trichomes observed on upper leaf epidermis of chrysanthemum foliage using scanning electron microscope examinations of bottom, middle, and top leaf positions.

Photoperiod	Trichomes/mm^2			
	Bottom Leaves	Middle Leaves	Top Leaves	Mean
Short day	7	13	10	10
Long day	11	7	38	19

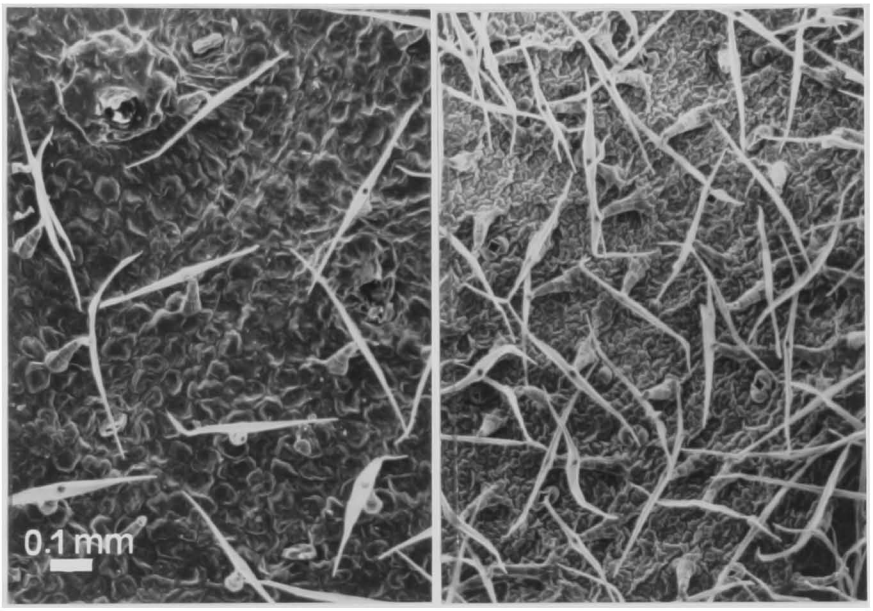

Figure 1. Scanning electron micrograph of t-shaped trichomes on the top leaves of short (left) and long day grown chrysanthemums (right). A leafminer puncture is visible in upper left corner and left center of micrograph. (x54)

Comparisons of estimated leaf area to actual leaf area measurements showed that 52% of the estimated measurements significantly underestimated the actual leaf area. The standard deviations of leaf area measurements using the leaf area meter were 0.008% of the mean. Lyon (1948) found the standard deviation of the mathematical formula for estimating tomato leaf areas was 3.5% of the mean. However, he reported that the form of tomato leaves remains essentially constant regardless of growth. By contrast, the standard deviations of SD and LD exposed chrysanthemum leaves using the formula were 23% and 11% of the mean, respectively. This indicates a large variability in the area of chrysanthemum leaves.

DISCUSSION

Leafminers are more attracted to chrysanthemums grown under SD than LD for feeding and oviposition. These results clarify earlier observations made by Schuster and Harbaugh (1979b) who found that different chrysanthemum cultivars had lower levels of leafminer damage at vegetative rather than flowering growth stages. Chrysanthemum leaves which developed under SD were less pubescent (lower trichome density) than those under LD. This difference in surface texture is at least one factor accounting for the preference

of flies for SD grown chrysanthemums. Mechanical barriers have figured in preference choices by other leafminer species. MacLean and Byers (1983) found a negative correlation between trichome densities and the presence of alfalfa blotch leafminer eggs. Rogers (1979) discovered high leaf trichome density, thin stems, and long internodes were associated with low viable egg counts of the bean leafminer, *Ophiomyia phaseoli*. Valencia and Campos (1979) observed that the trichome density of different potato cultivars appeared to be part of the mechanism of cultivar resistance to oviposition by *L. huidobrensis*. Nowakowski (1962) theorized that host plant selection by mining flies is influenced by phytochemical affinity of plants, the evolutionary origin of plants and flies, and morphological and anatomical features of the plant cells.

The distribution of feeding punctures and mines indicates that the upper half of chrysanthemums were selected for feeding and egg-laying in SD grown chrysanthemums. One theory which may explain differences in puncture densities within the plant is that younger tissue (upper half of the plant) may be physically easier to penetrate with the ovipositor. Or, more eggs are laid because nutritional quality for larval development is better. Hoskinson (1979) postulated that the new apical

plant growth provided fresh oviposition sites, which were more desirable than sites on lower foliage. Other research on oviposition and feeding site selection by agromyzid leafminers support these findings. *Agromyza frontella* preferred the upper leaflets of alfalfa for egg laying and pinholing (Andalora 1981, MacLean and Byers 1983), *Liriomyza solani* deposited the greatest number of eggs in the upper leaves of tomatoes (Speyer and Parr 1951), and *Phytomyza ilicicola* preferred newly expanding holly leaves for puncturing (Hartzell 1943).

No significant differences in puncture and mine distribution were detected in LD grown plants. Wyatt (1970) reported that the presence of deterrent compounds in chrysanthemum leaves determined whether they were suitable for oviposition by aphids. Thus variations in phytochemicals and plant structures caused by photoperiods may account for the variability in interplant site selection by female leafminers on SD and LD grown plants.

The mathematical formula for estimating leaf area resulted in substantial underestimations of leaf area. Hence, this limits the use of such mathematical relationships in the calculation of certain plant leaf areas. Because of the large variability in area estimates of chrysanthemum leaves, use of mathematical relationships in calculation of leaf areas should be viewed with caution.

Chapter III

EFFECTS OF PLANT GROWTH REGULATORS AND PHOTOPERIOD ON FEEDING AND OVIPOSITION SITE SELECTION BY *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

INTRODUCTION

Intensively grown greenhouse ornamentals, such as chrysanthemums, are high production cost crops. Precise environmental control is required to produce high quality plants, on schedule, throughout the entire year. Photoperiod, temperature, fertilization, pinching (removal of terminal bud), and application of plant growth regulating (PGR) chemicals have all been utilized to optimize growth and development of chrysanthemums. However, leafminer populations within a production area are also affected by many of these common cultural practices. In chrysanthemums, pinching resulted in increased leafmining, (Poe et al. 1976, 1977, Shuster and Harbaugh 1979b); higher fertility levels favored leafminer larval development (Hussey and Gurney 1962, Poe et al. 1976, Woltz and Kelsheimer 1958); and the number of mines increased rapidly during vegetative growth but declined sharply during senescence (Musgrave et al. 1975). Chrysanthemum cultivars have been bred to produce either many small flowers (sprays) or fewer large flowers (standards). Oetting (1982) observed that standards have a higher larval damage index than sprays.

Traditional cultural practices, such as pinching and the application of growth retarding chemicals, indirectly affect the level of endogenous plant hormones (Weaver 1972). It has been suggested that insects can utilize the disruptive effects caused by application of PGRs to enhance the chances of egg and larval survival (Webb 1972). Thus, the goal of this study was to investigate the role which endogenously applied plant growth regulators play in feeding and oviposition site selection by leafminers.

MATERIALS AND METHODS

The chrysanthemum cultivar white Iceberg was chosen for the experiment since it is commonly grown and also highly susceptible to leafminer damage (Schuster and Harbaugh 1979a). Forty cuttings were potted in 10-cm square plastic pots using Promix (Wetsel Seed Co., Harrisonburg, Virginia), a commercial potting medium. Each pot contained one cutting. Chrysanthemums were grown in a glass reinforced plastic greenhouse (18.3 x 8.2 m) on raised wire benches (3.7 x 1.2 m). Plants were fertilized weekly with 15-10-30 (15N:4.4P:24.9K) fertilizer, and watered daily. Temperature and relative humidity were recorded with a hygrothermograph (Cole Parmer Instrument Co., Chicago, Illinois). This greenhouse experiment was conducted from February to April

to avoid the confounding effects of high temperature on photoperiodism.

Plants were acclimated for two weeks under a long day (LD) photoperiod provided by night interruption (2200-0200 hours) with incandescent bulbs (60 watts) spaced 1.2 m apart and 1.5 m above the greenhouse benches. All plants were pinched at two weeks to promote the development of lateral branches. Plants were kept vegetative for two additional weeks under the LD cycle and then separated into different photoperiod groups. Twenty pots of chrysanthemums remained under the LD exposure and twenty were placed under a short day (SD) photoperiod. Cages (1 m^3) covered with black plastic were used to simulate the SD cycle of 8 light hours. The cages were placed over the plants each day at 1700 hours and removed at 0800 hours the following day. A black, 100% cotton cloth partition was also drawn each day from 1700-0600 between LD and SD areas as a further precaution against any light "contamination" during the night interruption period. Chrysanthemums were exposed for 4 weeks under their respective photoperiod regimes. At seven weeks, PGRs were sprayed to the point of "run-off" using a 0.5 liter plant sprayer (Dexol Ind., Torrance, California). The PGRs used were NAA (naphthaleneacetic acid) applied as a 10 parts per million (ppm) foliar spray, $GA_{4,7}$ (mixture of

two gibberellins) applied as a 25 ppm foliar spray, and B9 (daminozide) applied as a 2,500 ppm foliar spray. Water was sprayed as a check. Prior to fly introduction, plants were grown one additional week after spray application to allow time for PGR absorption and mobilization. A total of 10 cages (5 under short days, 5 under long days) were established such that one randomly selected plant from each treatment (NAA, $GA_{4,7}$, B9, water) was placed in each cage. Each cage was 1 m^3 and covered with Saran filter cloth (42 x 42 mesh). Plants were then exposed to natural daylengths (approximately 8 light hours) for the remainder of the experiment.

Liriomyza trifolii pupae obtained from a laboratory leafminer colony were reared at 26 C in a plastic rearing dishes (50 mm dia. x 36 mm in depth). Each dish was filled with sterile dampened sand to a depth of 1 cm, and was covered with Saran filter cloth (42 x 42 mesh). Drops of water were added as needed to provide a humid environment for emergence. Rearing containers were checked daily for emerged adults. One day old adults were refrigerated for 5 to 10 minutes for purposes of counting and selection of flies for experimental use. Forty-eight flies (24 females and 24 males) were randomly selected and released into the center of each cage (experiment day 0).

Water mats on the bottom of eacn cage and on top of the greenhouse bench prevented flies from escaping while providing plant irrigation. Honey was provided as a carbohydrate source for the flies. Flies remained in the cage for 6 days and were then removed with a vacuum. Data collected on experiment day 6 included: 1) visual counts of the number of punctures per leaf (both feeding and egg punctures) and 2) leaf length. Measurements were taken on every fourth leaf starting at the plant base and continuing up the plant to the top. Six leaf positions were recorded. Each leaf was marked with a white string to identify it for future reference.

Larvae within punctures were allowed to develop mines for 4 days (experiment day 10). Marked leaves were then removed, and the following data were collected from each: 1) visual counts of the number of mines per leaf, 2) measurement of the leaf area, and 3) leaf length. Leaf area was measured by two methods: 1) an automatic leaf area meter (Type AAM-5 Tokyo Hayashi Denko, Inc., Tokyo, Japan), and 2) by multiplying the leaf length squared by a "leaf area constant" (Lyon 1948). The actual leaf area measurements from the area meter were used to calculate the leaf area constant, which, by definition, was the actual leaf area divided by the square of the leaf length. This leaf area

constant was also used to calculate leaf area on experiment day 6. After data collection, the sampled leaves were frozen until scanning electron microscopy (SEM) examination to determine numbers of leaf trichomes.

Chrysanthemum leaves were prepared for SEM examination by the following steps. Leaf sections between the edge and midrib in the center of the leaf were excised for tissue preparation. Leaf sections (5 mm^2) were placed in 3% glutaraldehyde in 0.1 M Na cacodylate buffer overnight. The next day, each section was fixed in 1% OsO_4 in 0.1 M buffer for 15 minutes, and then dehydrated in ethanol (15, 30, 50, 70, 95, 100%) for 15 minutes each. After dehydration, leaf sections were dried in a Ladd critical point dryer (Ladd Research Ind., Inc., Burlington, Vermont). Leaf sections were mounted on silver studs using Avery Self-adhesive Paper Tacks. The upper epidermis layer of leaf tissue was coated with a 200 Å thick layer of gold in a SPI sputter coater (SPI Supplies Division of Structure Probe, West Chester, Pennsylvania). Leaf tissues were examined with a JEOL JSM-35C SEM. Leaf trichomes were photographed with a Polaroid camera during SEM examination, and later trichome densities were calculated.

A microscopic inspection was made on experiment day 12 to locate any eggs which were not visible to the eye during

larval mine examination on day 10. The number of punctures, and mines plus eggs were counted with the assistance of a staining technique specifically developed for egg counts of *L. trifolii* on chrysanthemums (Parrella and Robb 1982). Five leaves were randomly selected from the top, middle (leaf position 8) and bottom of each PGR treated, SD and LD exposed chrysanthemums, taken to the laboratory and refrigerated. The staining solution consisted of one part each of distilled water, phenol, lactic acid, and two parts glycerine. Acid fuchsin (0.25 g) was added to 250 ml of the above solution. Leaves were boiled in the lactophenol-acid fuchsin stain for 3 to 5 minutes and then transferred to petri dishes (90 mm in diameter) to cool for 3 to 5 hours. Excess stain was removed with a warm water rinse and leaves were examined for egg punctures, mines, and feeding punctures under a dissecting microscope at 20X. Eggs in punctures appeared darker than feeding punctures and the surrounding leaf tissue (Parrella and Robb 1982). Average leaf area measurements from the area meter on day 10 were used to calculate puncture and mine plus egg densities for each of the leaf positions (top, middle, bottom).

Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test procedures available in the Statistical Analysis System (SAS) computer package (Helwig

and Council 1979). Leaf areas measured using the leaf area meter were used in the calculation of all densities described herein. However, both methods of leaf area determination were compared statistically using a paired t-test. Duncan's multiple range test was employed to distinguish differences between means of plant growth regulator treatments within photoperiods. Differences between photoperiods were measured using t-tests. Leaf positions of treatments within SD or LD photoperiods were analyzed using Duncan's multiple range test. Significance levels were $P < 0.05$ unless stated otherwise.

RESULTS

Environmental conditions in the greenhouse

Environmental conditions averaged 23 C and 54% relative humidity during the day, and 21 C and 68% relative humidity during the night.

Plant growth regulator effects

Mean *Liriomyza trifolii* puncture, mine and mine plus egg densities on chrysanthemums treated with PGRs were not significantly different from densities of control chrysanthemums in the majority (75%) of the analyses (Table 1). When differences in density between treatments and controls were observed, plants treated with plant regulators

Table 1. Mean Liriomyza trifolii puncture, mine, and mine plus egg densities on chrysanthemums treated with plant growth regulators and grown under short and long day photoperiods.

Photoperiod	Treatment	Mean Density			
		Punctures/ cm^2 (day 6)	Mines/ cm^2 (day 10)	Punctures/ cm^2 (day 12)	Mines + Eggs/ cm^2 (day 12)
Short day	Water	2.01a[1]	0.29a	8.04a	0.56a
Short day	NAA	2.87a	0.23a	5.44a	0.23b
Short day	$GA_{4,7}$	1.80a	0.29a	7.81a	0.43ab
Short day	B9	1.69a	0.23a	4.92a	0.25b
Long day	Water	1.02a	0.16a	2.32a	0.15a
Long day	NAA	0.61a	0.20a	0.84b	0.26a
Long day	$GA_{4,7}$	0.67a	0.19a	0.80b	0.19a
Long day	B9	0.42a	0.27a	1.92a	0.17a

[1]Means within a column and photoperiod group followed by the same letter are not significantly different ($P < 0.05$), Duncan's multiple range test.

had lower puncture and mine plus egg densities than untreated plants (Table 1). However, these differences are probably due to chance, because no consistent differences in the densities of PGRs were observed. Results of the ANOVAs are given in Appendix II Tables 1 through 8.

Photoperiod effects

Chrysanthemums exposed to SD photoperiod generally had higher puncture, mine, and mine plus egg densities than LD grown chysanthemums (Table 2). Although this difference was not significant in all cases, damage was generally more intensive on SD grown chrysanthemums than LD grown chrysanthemums. The inability to detect differences among all means may have been due to the relatively large variability in densities, and small sample sizes.

Spatial distribution of puncture densities within individual chrysanthemums

A summary of the distribution of mean puncture densities within individual plants measured on day 6 (visual count) and day 12 (leaf staining count) are given in Tables 3 and 4, respectively. Under SD growing conditions, puncture densities measured on day 6 were significantly greater at the top leaf position than lower leaf positions on plants treated with water, NAA, and $GA_{4,7}$ (Table 3). On day 12, the top leaf postions of SD grown plants also had

Table 2. Photoperiod effects on mean Liriomyza trifolii puncture, mine, and mine plus egg densities on chrysanthemums treated with plant growth regulators.

Photoperiod	Treatment	Mean Density			
		Punctures/ cm^2 (day 6)	Mines/ cm^2 (day 10)	Punctures/ cm^2 (day 12)	Mines + Eggs/ cm^2 (day 12)
Short day	Water	2.01a[1]	0.29a	8.04a	0.56a
Long day	Water	1.02b	0.16a	2.32b	0.15b
Short day	NAA	2.87a	0.23a	5.44a	0.23a
Long day	NAA	0.61b	0.20a	0.84b	0.26a
Short day	$GA_{4,7}$	1.80a	0.29a	7.81a	0.43a
Long day	$GA_{4,7}$	0.67b	0.19a	0.80b	0.19b
Short day	B9	1.69a	0.23a	4.92a	0.25a
Long day	B9	0.42b	0.27a	1.92a	0.17a

[1]Means within a column and treatment group followed by the same letter are not significantly different ($P < 0.05$), t-test.

Table 3. Within plant distribution of mean Liriomyza trifolii puncture density on chrysanthemums grown under short and long day photoperiods and measured on experiment day 6.

Photoperiod	Leaf Position	Punctures/cm^2 Water	NAA	$GA_{4,7}$	B9
Short day	20 (Top)	6.33a[1]	5.68a	5.59a	3.16a
Short day	16	2.31b	4.07a	1.18b	3.45a
Short day	12	1.53b	3.49ab	2.07b	1.81a
Short day	8	1.03b	1.91b	0.94b	1.13a
Short day	4	0.28b	1.80b	0.42b	0.27a
Short day	1 (Bottom)	0.59b	0.28b	0.62b	0.34a
Long day	20 (Top)	0.01a	0.00a	0.00a	0.16a
Long day	16	1.35bcd	1.43b	1.01ab	0.29a
Long day	12	1.50cd	0.41a	1.07b	0.44a
Long day	8	0.87bc	0.40a	0.34ab	0.47a
Long day	4	1.86d	0.80ab	1.02b	0.43a
Long day	1 (Bottom)	0.54ab	0.64ab	0.59ab	0.74a

[1]Means within a column and photoperiod group followed by the same letter are not significantly different ($P < 0.05$), Duncan's multiple range test.

Table 4. Within plant distribution of mean Liriomyza trifolii puncture densities on stained chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 12.

Photoperiod	Leaf Position	Punctures/cm^2 Water	NAA	$GA_{4,7}$	B9
Short day	20 (Top)	20.35a[1]	15.93a	21.14a	12.61a
Short day	8 (Middle)	1.88b	2.40b	2.23b	1.58b
Short day	1 (Bottom)	0.35b	0.08b	0.07b	0.56b
Long day	20 (Top)	4.86a	0.00a	0.00a	3.38a
Long day	8 (Middle)	2.02b	1.76a	2.06b	1.47b
Long day	1 (Bottom)	0.59b	0.78a	0.34a	0.91b

[1]Means within a column and photoperiod group followed by the same letter are not significantly different ($P < 0.05$), Duncan's multiple range test.

higher puncture densities than lower leaves, regardless of treatment (Table 4). By contrast, the distribution of puncture densities within LD grown plants was highly variable, and no leaf positions had consistently higher densities than others. These differences in puncture densities were observed on both days 6 and 12 (Tables 3 and 4).

Spatial distribution of mine densities within individual chrysanthemums

The distribution of mines and mines plus egg densities within plants measured on day 10 (visual count) and day 12 (stained leaf count) are given in Tables 5 and 6, respectively. On SD grown chrysanthemums, the upper leaves (12, 16 and top) generally had higher mine densities compared to lower leaves (bottom, 4 and 8) regardless of treatment (Table 5). Similar distributions of mine plus egg densities on SD grown chrysanthemums were measured on day 12 using leaf staining; mine plus egg densities were generally greater in top leaves than in middle and lower leaves (Table 6). By contrast, LD grown chrysanthemums had highest mine densities in lower foliage but differences in mine densities were significant only in LD grown chrysanthemums treated with B9 (Table 5) and NAA (Table 6).

Table 5. Within plant distribution of mean Liriomyza trifolii mine densities on chrysanthemums grown under short and long day photoperiods and measured on experiment day 10.

Photoperiod	Leaf Position	Mines/cm^2 Water	NAA	$GA_{4,7}$	B9
Short day	20 (Top)	0.63a[1]	0.53a	0.54a	0.13b
Short day	16	0.38b	0.35b	0.32b	0.46a
Short day	12	0.28bc	0.16cd	0.34b	0.35a
Short day	8	0.17c	0.04c	0.20bc	0.12b
Short day	4	0.17c	0.23bd	0.09c	0.22b
Short day	1 (Bottom)	0.12c	0.05cd	0.26bc	0.11b
Long day	20 (Top)	0.00a	0.00a	0.00a	0.00a
Long day	16	0.06a	0.04a	0.04a	0.01a
Long day	12	0.12a	0.04a	0.18a	0.07a
Long day	8	0.15a	0.08a	0.05a	0.12a
Long day	4	0.49a	0.54a	0.49a	0.25a
Long day	1 (Bottom)	0.16a	0.52a	0.36a	1.19b

[1]Means within a column and photoperiod group followed by the same letter are not significantly different ($P < 0.05$), Duncan's multiple range test.

Table 6. Within plant distribution of mean Liriomyza trifolii mine plus egg densities on stained chrysanthemum leaves grown under short and long day photoperiods and measured on experiment day 12.

Photoperiod	Leaf Position	Mines plus eggs/cm^2 Water	NAA	$GA_{4,7}$	B9
Short day	20 (Top)	1.14a[1]	0.24a	0.84a	0.32a
Short day	8 (Middle)	0.16b	0.22a	0.28b	0.23a
Short day	1 (Bottom)	0.35b	0.24a	0.19b	0.19a
Long day	20 (Top)	0.07a	0.00a	0.00a	0.09b
Long day	8 (Middle)	0.21a	0.13a	0.35b	0.11a
Long day	1 (Bottom)	0.15a	0.66b	0.22a	0.30a

[1]Means within a column and photoperiod group followed by the same letter are not significantly different ($P > 0.05$), Duncan's multiple range test.

SEM examination of leaf trichome densities

Overall, LD grown chrysanthemums averaged higher trichome densities than SD grown chrysanthemums regardless of treatment (Table 7). The top leaves of LD grown chrysanthemums had at least twice as many trichomes per mm^2 as the top leaf position of SD grown chrysanthemums regardless of treatment (Fig. 1). The bottom and middle leaf position of SD and LD grown plants exhibited less differences in trichome densities with no trends apparent between treatments. No measurable differences in trichome densities were detected among treatments within photoperiods (Table 7).

Comparison of leaf estimates using the mathematical formula with leaf area meter measurements

The mathematical formula for estimating leaf area underestimated 55% of leaf areas compared to actual leaf areas measured using the leaf area meter. The standard deviation of leaf area measurements using the leaf area meter was 0.0008% of the mean. By contrast, the standard deviation of the leaf area of SD grown chrysanthemums treated with water, NAA, $GA_{4,7}$, and B9 was 17, 22, 25 and 16%, respectively. LD grown chrysanthemum leaves treated with water, NAA, $GA_{4,7}$, and B9 displayed the following standard deviations of mean leaf area: 14, 14, 22, and 25%,

Table 7. Density of trichomes observed on upper leaf epidermis of chrysanthemum foliage using scanning electron microscope examinations of bottom, middle, and top leaf positions.

Photoperiod	Treatment	Trichomes/mm^2			
		Bottom Leaves	Middle Leaves	Top Leaves	Mean
Short day	Water	9	7	20	12
Long day	Water	14	11	40	22
Short day	NAA	9	9	9	9
Long day	NAA	8	10	28	15
Short day	$GA_{4,7}$	6	6	5	6
Long day	$GA_{4,7}$	7	15	39	20
Short day	B9	4	11	9	8
Long day	B9	8	10	44	21

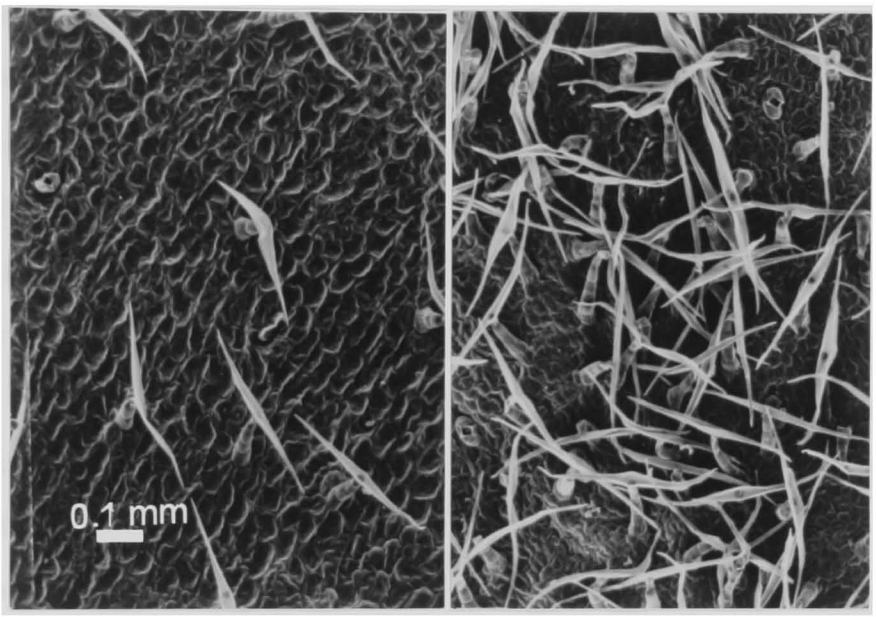

Figure 1. Scanning electron micrograph of t-shaped trichomes on the top leaves of short (left) and long day grown chrysanthemums (right) treated with B9. (x54)

respectively. This indicates a large variation in the area of chrysanthemum leaves using the formula.

DISCUSSION

Plant growth regulators are widely used on chrysanthemums for height control and for other purposes (Machin and Scopes 1978). Webb (1972) indicated that application of PGRs may affect the physiological balance between crop plants and their pests. In this study, application of the plant growth regulators NAA, $GA_{4,7}$ and B9 on chrysanthemums grown within a particular photoperiod regime had little measurable effect on densities of punctures, mines, and mines plus eggs created by leafminers. Webb (1977) studied the effects of PGRs on alteration of resistance levels of chrysanthemum cultivars to aphid attack. He found that maleic hydrazide (growth inhibitor) and ethylene altered the resistance of chrysanthemum cultivars to the advantage of aphids. He also observed that GA and SADH (B9) had no apparent effect on aphid-chrysanthemum interactions. The present study found little effect of $GA_{4,7}$ and B9 on desirability of chrysanthemums for leafminers.

Leafminers fed and oviposited more heavily on SD grown chrysanthemums than on LD grown chrysanthemums, regardless

of treatment. This observation is consistent with feeding and oviposition preferences observed in Chapter II. Schuster and Harbaugh (1979b) observed that certain cultivars of chrysanthemums had higher numbers of mines at harvest than before harvest which indicates that flowering chrysanthemums (SD) are preferred for puncturing by leafminers. One factor affecting the attack rate of leafminers on chrysanthemums may be the density of leaf trichomes. LD grown chrysanthemums had higher mean densities of leaf trichomes than SD grown chrysanthemums and also had lower feeding and oviposition puncture densities. This suggests that high densities of trichomes might form a physical barrier preventing puncturing by female leafminers. More research on the effects of cultural manipulations on plant metabolism are needed before the effects of such manipulations on insect-host plant interactions can be fully understood.

The density of feeding punctures and mines within SD grown plants was positively related to leaf height. Hoskinson (1979) and Speyer and Parr (1948) report that the texture of foliage may play a mechanical role in host plant site selection by leafminers. New apical plant growth may provide more palatable puncturing sites than on lower foliage. *Agromyza frontella* laid significantly more eggs on

upper two-thirds of alfalfa leaflets than on the lower third (Andalora 1981, MacLean and Byers 1983). *Phytomyza ilicicola* and *Liriomyza solani* also selected younger leaves over older leaves for puncturing and oviposition (Hartzell 1943, Speyer and Parr 1951).

The distribution of feeding puncture densities within LD grown chrysanthemums was quite variable for all treatments, and no consistent trends in density were observed. However, mine densities within LD grown chrysanthemums were not as variable. Densities were usually highest in lower leaves, regardless of treatment. Trichome density on the leaves of LD grown plants might have influenced the location of punctures and mines within these plants. Top leaves of LD grown chrysanthemums (low mine densities) had the highest trichome density. This suggests that the abundance of trichomes in the top leaves may have inhibited leafminers from ovipositing. By contrast, the top leaves of SD grown plants had low trichome densities, and were also the leaves most heavily punctured for feeding and oviposition. Thus, high trichome densities or pubescent leaves seem to adversely influence leafminer puncturing activities on chrysanthemums. Mechanical barriers have influenced preferences choices by other leafminer species. Perez-Nieto (1979) postulated that the high trichome density

on tomato leaves inhibited the feeding and oviposition behavior of *Liriomyza sativae* by acting as a mechanical barrier. MacLean and Byers (1983) found a negative correlation between trichome density and alfalfa blotch leafminer (*Agromyza frontella*) eggs. Rogers (1979) observed that *Ophiomyia phaseoli*, the bean leafminer, had low viable egg counts on leaves with high trichome density, thin stems, and long internodes. Valencia and Campos (1979) found the number of trichomes of different potato cultivars accounted for resistance of some cultivars to oviposition by *L. huidobrensis*.

Leaf areas calculated by the mathematical formula resulted in substantial underestimations of leaf areas compared to areas measured using a leaf area meter. Because of the relatively large variation in the areas of chrysanthemum leaves using the formula, the potential for estimating leaf areas from such formulas is limited for chrysanthemums.

Chapter IV

CONCLUSIONS

The results of the experiments described previously can be interpreted in a context relevent to flower growers concerned with minimizing the damage to chrysanthemums by leafminers. The following is a summary of specific conclusions which emerged from this study.

1. Ovipositors of female leafminers were endowed with numerous mechano- and chemoreceptors which might be used to discriminate host plant suitability for feeding, oviposition, and larval development. This basic sensory information is important in understanding insect responses to host plants. Such information can be used, for example, in trials in which chemicals derived from chrysanthemums are applied directly to the ovipositor to assess the behavioral response of insects to a variety of plant chemicals. Secondary plant chemicals known to adversely affect leafminers can be used in developing plants resistant to leafminers.
2. Short day grown chysanthemums were more attractive for feeding and oviposition than long day grown chrysanthemums. A physical property of chrysanthemum leaves influencing the preference for short day grown plants was identified as leaf pubescence. Short day

grown chrysanthemums were less pubescent (lower trichome density) than long day grown chyrsanthemums. Trichome density is part of the host resistance mechanism against other species of agromyzid leafminers in certain cultivars of host plants. Such mechanical resistance features can also be useful in breeding programs to improve resistance of commercial cultivars of chrysanthemums by developing insect-resistant types. Other factors, such as the presence of deterrent chemicals in chrysanthemum leaves or leaf cuticle thickness and toughness, are likely to play a role in host plant resistance and need to be investigated.

3. Plant growth regulators are known to alter the physiological balance and interaction between host plants and pests, often affecting their susceptibility. However, no effects could be demonstrated on the desirability of chrysanthemums for feeding and oviposition by leafminers after treatment with plant growth regulators. The fact that B9 will not alter leafminer damage is important to chrysanthemum growers because B9 is routinely used as a growth retardant on chrysanthemums.
4. The upper foliage of short day grown chrysanthemums had higher densities of feeding and oviposition punctures

compared to lower foliage. Such a trait would have an adaptive survival value since it would ensure optimal nutrition and adequate development time for the larvae, because older leaves at the bottom of the plant senesce and abscise before leafminer larvae mature. In addition, if the nutrient levels in older leaves are suboptimal, it may increase the time required to reach larval maturity. A second theory which might help explain the variations in feeding and oviposition is that the younger, softer tissue at the plant apex is easier for the female leafminer to pierce with the ovipositor than the more mature basal tissue.

5. The spatial distribution of both feeding and oviposition punctures within long day grown chrysanthemums was inconsistent, and no specific pattern of density in particular plants was evident. The spatial distribution of patterns of leafminer punctures and larvae within plants is important in sampling to monitor the pest in fields. Scouting programs use a minimum of labor to obtain relative density estimates for making control decisions. A non-uniform distribution of punctures such as that observed in short day grown chrysanthemums might bias estimates of larval densities if the scout did not scrupulously sample foliage in the same region of the plant (preferrably the top leaves).

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APPENDIX I

ANOVA TABLES SUMMARIZING DATA PRESENTED IN CHAPTER II

ANOVA Tables Summarizing Data Presented in Chapter II

The following ANOVA tables summarize statistical analyses performed on the data in Chapter II.

Appendix Table 1. ANOVA table for chyrsanthemum leaf puncture density created by *Liriomyza trifolii* and measured on experiment day 6.

Variable	df	Mean square	F-value	Pr>F
Photoperiod	1	28.72	5.72	0.04
Cage	1	1.34	0.27	0.62
Photoperiod*cage	1	1.17	0.23	0.64
Plant(photoperiod*cage)[1]	8	5.03		
Leaf position	5	14.37	5.00	0.001
Photoperiod*leaf position	5	12.79	4.45	0.003
Leaf position*cage	5	10.50	3.65	0.02
Photoperiod*leaf position*cage	5	4.50	1.57	0.20
Error[2]	40	4.15		

[1]Error term used to test whole plot main effects and interaction.
[2]Error term used to test subplot main effect and interactions.

Appendix Table 2. ANOVA table for chrysanthemum leaf puncture density created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
Photoperiod	1	163.83	15.11	0.0007
Leaf position	2	111.81	10.31	0.0006
Photoperiod*leaf position	2	111.24	10.26	0.0006
Error	24	10.84		

Appendix Table 3. ANOVA table for chrysanthemum leaf mine density created by *Liriomyza trifolii* and measured on experiment day 10.

Variable	df	Mean square	F-value	Pr>F
Photoperiod	1	0.89	22.15	0.002
Cage	1	0.08	2.03	0.19
Photoperiod*cage	1	0.002	0.06	0.82
Plant(photoperiod*cage)[1]	8	0.04		
Leaf position	5	0.03	0.58	0.71
Photoperiod*leaf position	5	0.14	3.19	0.02
Leaf position*cage	5	0.06	1.32	0.28
Photoperiod*leaf position*cage	5	0.05	1.14	0.36
Error[2]	40	0.43		

[1]Error term used to test whole plot main effects and interaction.
[2]Error term used to test subplot main effect and interactions.

Appendix Table 4. ANOVA table for chrysanthemum leaf mine plus egg densities created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
Photoperiod	1	1.76	24.55	0.0001
Leaf position	2	0.23	3.24	0.05
Photoperiod*leaf position	2	0.82	1.49	0.0003
Error	24	0.07		

APPENDIX II

ANOVA TABLES SUMMARIZING DATA PRESENTED IN CHAPTER III

ANOVA Tables Summarizing Data Presented in Chapter III

The following ANOVA tables summarize the statistical analyses performed on the data in Chapter III.

Appendix Table 1. ANOVA table for short day grown chrysanthemum leaf puncture density created by *Liriomyza trifolii* and measured on experiment day 6.

Variable	df	Mean square	F-value	Pr>F
PGR	3	8.57	1.07	0.39
Cage(PGR)[1]	16	8.02		
Leaf position	5	61.54	8.74	0.0001
PGR*leaf position	15	3.40	0.48	0.94
Leaf position* cage(PGR)[2]	80	7.04		

[1]Error term used to test whole plot main effect.
[2]Error term used to test subplot main effect and interaction.

Appendix Table 2. ANOVA table for long day grown chrysanthemum leaf puncture density created by *Liriomyza trifolii* and measured on experiment day 6.

Variable	df	Mean square	F-value	Pr>F
PGR	3	1.88	1.53	0.25
Cage(PGR)[1]	16	1.23		
Leaf position	5	2.81	6.91	0.0001
PGR*leaf position	15	0.61	1.50	0.12
Leaf position* cage(PGR)[2]	80	0.41		

[1]Error term used to test whole plot main effect.
[2]Error term used to test subplot main effect and interaction.

Appendix Table 3. ANOVA table for short day grown chrysanthemum leaf puncture density created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
PGR	3	26.45	0.43	0.73
Leaf position	2	1706.71	27.98	0.0001
PGR*leaf position	6	26.46	0.43	0.85
Error	48	61.00		

Appendix Table 4. ANOVA table for long day grown chrysanthemum leaf puncture density created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
PGR	3	9.99	5.03	0.004
Leaf position	2	11.14	5.60	0.007
PGR*leaf position	6	9.62	4.84	0.001
Error	47	1.99		

Appendix Table 5. ANOVA table for short day grown chrysantheumum leaf mine density created by *Liriomyza trifolii* and measured on experiment day 10.

Variable	df	Mean square	F-value	Pr>F
PGR	3	0.04	0.20	0.90
Cage(PGR)[1]	16	0.20		
Leaf position	5	0.37	6.17	0.0001
PGR*leaf position	15	0.07	1.15	0.33
Leaf position* cage(PGR)[2]	80	0.06		

[1]Error term used to test whole plot main effect.
[2]Error term used to test subplot main effect and interaction.

Appendix Table 6. ANOVA table for long day grown chrysanthemum leaf mine density created by *Liriomyza trifolii* and measured on experiment day 10.

Variable	df	Mean square	F-value	Pr>F
PGR	3	0.07	0.24	0.86
Cage(PGR)[1]	16	0.28		
Leaf position	5	1.10	5.15	0.0004
PGR*leaf position	15	0.21	0.98	0.48
Leaf position* cage(PGR)[2]	80	0.21		

[1]Error term used to test whole plot main effect.
[2]Error term used to test subplot main effect and interaction.

Appendix Table 7. ANOVA table for short day grown chrysanthemum leaf mine plus egg density created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
PGR	3	0.33	2.86	0.05
Leaf position	2	1.03	9.00	0.0005
PGR*leaf position	6	0.29	2.57	0.03
Error	46	0.11		

Appendix Table 8. ANOVA table for long day grown chrysanthemum leaf mine plus egg density created by *Liriomyza trifolii* and measured using leaf staining techniques on experiment day 12.

Variable	df	Mean square	F-value	Pr>F
PGR	3	0.04	1.06	0.37
Leaf position	2	0.42	11.32	0.0001
PGR*leaf position	6	0.14	3.86	0.003
Error	46	0.04		

VITA

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