Differential Probing Response of Serpentine Leafminer, *Liriomyza trifolii* (Burgess), on Cos Lettuce[1]

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ABSTRACT Leaf mining damage by serpentine leafminers, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), is a major problem of many leafy vegetables especially lettuce. A hierarchy of leaf probing preference by *L. trifolii* on romaine lettuce (*Lactuca sativa* L.) cultivars 'Floricos 83' (FC), 'Parris Island Cos' (PI), 'Tall Guzmaine' (TG), and 'Valmaine' (VL) was determined. Based on stipple counts (puncture wounds in the leaf surface), *L. trifolii* preferred TG by an experiment-wide average of 3:1 over the other cultivars. In choice tests where *L. trifolii* were able to select their preferred cultivar, TG was preferred 2.2:1 to 5.5:1 over the over varieties. On 12-leaf stage TG plants, eight female flies produced means ± SEM of 664.2 ± 165.8, 1,581.8 ± 333.8, and 2,084.5 ± 242.6 stipples per plant after 24, 48, and 72 h exposures, respectively. Preference for TG was maintained in no-choice tests where TG was preferred 1.8:1 to 2.6:1 over the other cultivars. Stipple counts on FC, PI, and VL did not vary significantly between choice and no-choice tests, but nearly twice as many stipples per plant were found on TG in choice than in no-choice tests. More probing occurred on all cultivars on the youngest fully expanded leaves in the middle of the plants than on leaves toward the bottom or top of the plants. Preference for these middle leaves was more pronounced on TG than on the other cultivars. The differences in stipple rates followed the pedigrees of the cultivars tested. The character(s) preferred by *L. trifolii* were apparently introduced into the lineage with a cross to 'Paris White.'

KEY WORDS Vegetable breeding, host plant resistance, leafminers.

Serpentine leafminer, *Liriomyza trifolii* (Burgess), is a major pest of lettuce. Damage to lettuce occurs in two ways. First, the female probes the leaf surface with her ovipositor to deposit eggs and to feed on the resulting exudates from the leaf tissue (Bethke and Parrella 1985). Males feed from puncture wounds created by the females. Wounding results in scars called stipples. The stipples are usually a sunken depression, but may develop into raised scars. The second way damage occurs is by the larvae tunneling through the leaf mesophyll producing obvious leaf mines and subsequent leaf necrosis. Both types of leaf damage reduce plant vigor by affecting photosynthetic activity (Trumble et al. 1985) and reduce the quality of the harvested product. Product grade may be reduced if damage is evident (Anon. 1961).

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Major outbreaks of *L. trifolii* in vegetables have occurred in Arizona and California since the late 1980's (Pryor 1990) and in Florida since the late 1940's (Genung and Janes 1975). Control of this insect pest has historically been achieved through the application of chemical insecticides (Parrella and Keil 1984). Evidence of resistance in *Liriomyza* spp. in Florida to insecticides was first recognized during the early 1950's (Wolfenbarger 1958). Despite development and release of new chemical compounds to control leafminers, control in Florida vegetables has been transitory, because resistance to new materials has developed within several years of registration (Genung and Harris 1961, Genung and Janes 1975, Leibee 1981, Parrella and Keil 1984, Foster 1986). A celery integrated pest management program based on sampling, judicious use of pesticides, and natural control of *L. trifolii* was developed in the late 1970's in response to a lack of effective materials and to toxic residue concerns (Genung et al. 1978, Guzman et al. 1979, 1980). The program was not well adopted probably because overall yields and quality of stalks produced still did not meet grower and consumer expectations. New insecticides registered in the 1980's again allowed the growers to produce high quality celery. However, the future of the lettuce and celery industries was jeopardized again in late 1989 when local *L. trifolii* populations in the Everglades Agricultural Area of southern Florida became resistant to the most effective registered material, cyromazine (a substituted melamine, Trigard®, Ciba-Ceigy, Greensboro, NC) (Nuessly, unpublished data). Although a successful statewide program is now in place to manage this resistance and prolong the effectiveness of cyromazine, the program is dependent on an emergency exemption for use of abamectin (an avermectin, Agri-mek®, MSDAGVET, Rahway, NJ). This material provides excellent control at very low rates (Leibee 1988). Emergency registrations of cyromazine and abamectin in Arizona, and abamectin in California and Florida, have averted more serious losses to lettuce by this pest (Natwick 1990).

There is a need to develop effective nonchemical alternatives to leafminer control which will help to preserve current pesticide effectiveness and reduce pressure for insecticide resistance development, while meeting grower and consumer expectations for lettuce quality. Host plant resistance to leafminers would fit well into this scenario (Anon. 1992). Preliminary field trials with romaine (i.e., cos) lettuce indicated a wide range of damage levels resulting from differential varietal preferences in leaf probing (Nuessly and Nagata 1993). The purpose of this research was to quantify the probing response of *L. trifolii* on four cos lettuce cultivars.

Materials and Methods

Testing for differential probing responses by *L. trifolii* to lettuce cultivars was initiated after receiving reports of extensive stipple damage on 'Tall Guzmaine' cos lettuce grown in a field trial in California (Dean Gregg, pers. commun. Royal Sluis Inc., San Juan Bautistia, CA). 'Parris Island Cos' planted in adjacent plots had noticeably less stipples. 'Tall Guzmaine' is seldom grown in California, but it is widely grown in Florida. When similar results were observed with these and other cultivars in a broad screen field trial at Belle

Glade, FL (Nuessly and Nagata 1993), the current laboratory study was begun. The cultivars 'Floricos 83' (FC), 'Parris Island Cos' (PI), 'Tall Guzmaine' (TG), and 'Valmaine' (VL) were selected based on differences in *L. trifolii* probing responses observed in a field trial and on their pedigrees (Fig. 1). All have similar color, shape, and leaf size as young (i.e., < 15 leaves) plants. Seeds of each cultivar were sown in a transplant tray and grown for 3 wk. Seedlings were transplanted to a 12.7-cm diam plastic azalea pot containing 700 cm^3 of soilless planting media and then grown for an additional 3 wk. Plants were grown in an insect-free greenhouse under ambient light maintained between 18.3 and 27.8°C. At the end of this period, all cultivars had 10 to 12 leaves. Individual test plants were selected for size uniformity and transferred into an insectory room maintained at 28 ± 1°C and a photoperiod of 14:10 (L:D) h. Experimental units were screened cages 35 × 30 × 50 cm (W × D × H) that contained four individually potted plants. Lighting was supplied by four 40-watt cool white flourescent bulbs suspended 2 cm above each bank of cages.

Leafminers used in all experiments were from a laboratory colony maintained on southern pea seedlings, *Vigna ungiculata* L. ('California #5', Upjohn & Co., Kalamazoo, MI). Adults used to start the colony were collected from a lettuce field at Belle Glade, FL, 2 mo before the experiments were begun. It was difficult to quickly and accurately determine the sex of *L. trifolii,* so we selected mating pairs to insure the desired number of female flies in each cage. Flies less than 24 h old were released into a screened cage and allowed to mate. Mating pairs (i.e., in copulo) were aspirated from the cage into a glass tube fitted with a cotton plug. Pairs were then transferred to 50 cc holding vials and provided with a honey water solution (25%) for 20 to 24 h before release into screened cages containing the test plants.

Feeding choice and no-choice, as well as feeding duration experiments were conducted. A randomized complete block design was used with cultivars and feeding duration as treatments. In the no-choice tests, one cage was used for each of the four cultivars with four plants of a single cultivar placed into individual cages. No-choice tests were repeated six times. The choice tests had one plant of each cultivar placed within each of four cages. Each of the four possible plant arrangements within a cage were represented. A group of four cages was used for each choice test, and each test was repeated six times. Eight mated pairs of *L. trifolii* were released into each cage and held for 24, 48, or 72 h. At the end of the exposure period the plants were removed from the cages, flies were removed from the plants, and probe events were counted within 48 h using a stereo-microscope. The mean ± SEM number of stipples was calculated by plant and by leaf number (i.e., position). Leaves were numbered from 1 to 12 starting with the first true leaf at the bottom of the plants. Statistical analysis was conducted using analysis of variance and chi-square analysis (PROC GLM and PROC FREQ, SAS Institute 1985). Fisher's unprotected least significant differences (LSD) was used for post-hoc means separation tests.

Results and Discussion

L. trifolii preferred 'Tall Guzmaine' over the other cultivars in the choice tests by a 2.2:1 to 5.5:1 ratio (Table 1). Significantly more stipples were found

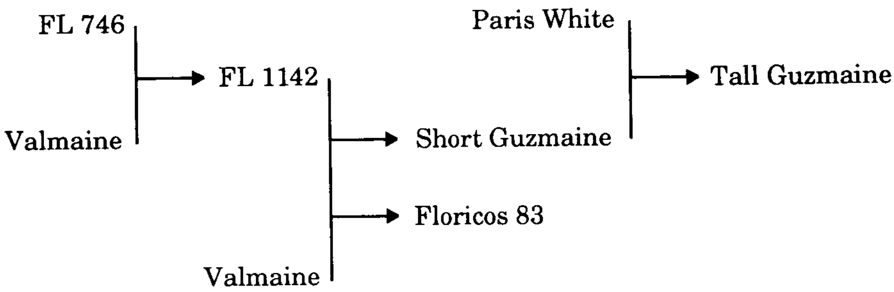

Fig. 1. Pedigree relationship of 'Floricos 83', 'Tall Guzmaine', and 'Valmaine'.

on TG than on the other cultivars at all time exposures. Feeding preference was in the order of TG > PI ≥ VL ≥ FC, but stipple counts did not vary significantly among FC, PI, or VL at any time exposure.

The same pattern of feeding preference was evident in the no-choice tests as in the choice tests, but stippling rates were lower than the level in the choice tests. Significantly more stipples per plant were found on TG than on the other cultivars in the no-choice tests. There were no significant differences in stipple counts among the other cultivars. The number of stipples on TG was significantly higher in the choice than in the no-choice tests after the 48 h (F = 8.249, $P < 0.01$) and 72 h (F = 15.236, $P < 0.001$) exposures (Table 1). Stipple counts on FC, PI, and VL were not significantly different between choice and no-choice tests at any time exposure. Because the plants were exposed to the same number of flies in both choice and no-choice tests, the results suggest that something beyond simple preference occurred in the choice cages.

A chi-square test was used to evaluate whether the plants in the choice tests elicited more probing than would be expected based on the results of the no-choice tests. The mean number of stipples per plant from each of the four cultivars in the no-choice tests were summed to produce a value for the total number of stipples that would be expected from exposing one plant of each cultivar in a single cage for each of the six replicates. These were compared with the total number of stipples per cage in the choice tests from the corresponding six replicates. Significantly more stipples per cage were found on plants in the choice tests than would be expected based on the expected values from the no-choice tests at all three time exposures (Table 2). Since the rate was greater for the TG plants in the choice tests than in the no-choice tests, the results of the chi-square analysis suggest that stippling in the choice test was stimulated by the presence of the TG plants.

Table 1. Comparisons of mean ± SEM *L. trifolii* stipples per cos lettuce plant between cultivars and time exposures.

Time exposure	Cultivars*				F†
	FC	PI	TG	VL	
Choice tests					
24 h	145.3 ± 26.4 aA	259.3 ± 65.5 aA	664.2 ± 165.8 bA	305.8 ± 101.2 aA	4.70 a
48 h	394.2 ± 117.4 aAB	588.8 ± 153.2 aAB	1,581.8 ± 333.8 bB	323.7 ± 81.2 aA	8.78 b
72 h	377.5 ± 68.4 aB	754.3 ± 209.5 aB	2,084.5 ± 242.6 bB	448.2 ± 111.9 aA	21.13 c
F**	3.79 a	4.07 a	7.87 a	1.27 n.s.	
No-choice tests					
24 h	290.5 ± 31.3 aA	257.1 ± 33.1 aA	560.5 ± 54.7 bA	236.2 ± 24.2 aA	16.21 c
48 h	469.8 ± 44.3 aB	404.1 ± 68.8 aAB	869.0 ± 94.6 bB	351.8 ± 34.8 aB	13.14 c
72 h	517.1 ± 75.5 aB	465.9 ± 55.9 aB	1,150.8 ± 103.6 bC	434.8 ± 42.2 aB	21.76 c
F**	4.96 b	3.85 a	11.54 c	8.33 c	

Four plants and eight *L. trifolii* pairs per cage. Means followed by different letters are significantly different, LSD, $P < 0.05$. Small letters used for horizontal comparisons between cultivars; capital letters used for vertical comparisons between exposure times within test type.

* FC, 'Floricos 83"; PI, 'Parris Island Cos'; TG, 'Tall Guzmaine'; VL, 'Valmaine'.

** ANOVA, choice tests df = 17, no-choice tests df = 71; not significant, n.s.; $P < 0.05$, a; $P < 0.01$, b, $P < 0.001$, c.

† ANOVA, choice tests df = 23, no-choice tests df = 95; $P < 0.05$, a; $P < 0.01$, b; $P < 0.001$, c.

Stipple counts increased significantly with increased time exposure on FC, PI, and TG for both choice and no-choice tests (Table 1). In the choice tests, the increase in stipple counts from the 24 h to the 72 h exposure was more than twice as much on PI than on FC or VL, with little or no increase in stipples with time on VL (Table 1 and Fig. 2). There was a large increase in stipple counts with increased time exposure on TG. Stipple counts on TG increased more than twice as much in the choice than in the no-choice tests between the 24 h and the 72 h exposures. This further indicates feeding preference by *L. trifolii* for TG. As time progressed in the choice tests the females apparently spent more time probing TG plants and less time on the other cultivars.

Table 2. Comparisons of mean ± SEM *L. trifolii* stipples per cage of cos lettuce plants between choice tests (i.e., observed) and expected values from no-choice tests.

	Time (h)		
	24	48	72
Observed (Choice tests)*	1,374.7 ± 226.6	2,888.5 ± 600.9	3,664.5 ± 417.1
Expected (No-choice tests)**	1,344.3 ± 95.5	2,094.6 ± 269.8	2,556.0 ± 227.5
X^2	271.01	12.81	59.03
P	<0.001	<0.001	<0.001

* Mean no. stipples per cage containing one plant of each of four cultivars.
** Mean no. stipples per plant for each cultivar in no-choice tests summed to estimate no. stipples expected per cage in a choice tests for each of six replicates.

Female probing was greatest on the fully expanded leaves in the upper middle portion of the plants (i.e., leaf positions 5 to 9) on all four cultivars (Fig. 2). This was particularly true for the TG plants. Stipple counts on leaves 1 to 4 and 10 to 12 were not significantly different among cultivars. However, stipple counts on leaves 5 to 9 were much greater on TG than on the other cultivars $F = 10.46$, df = 3; $P < 0.0001$). Although these middle to upper leaves were the largest on the plants, leaf surface area did not completely explain the observed differences. Leaves in positions 5 to 9 had more stipples per cm^2 leaf surface than the other leaves, and a polynomial equation, $y = -0.1460 + 0.2176x - 0.0179x^2$ (y = stipples per cm^2 leaf surface; x = leaf position), fit the data best ($r^2 = 0.7469$). If leaf surface area was the only factor that influenced stipple density, then the relationship would have been described by a linear equation with a slope close to zero. Therefore, within plant feeding and oviposition site preference by female *L. trifolii* is probably dependent on other factors (e.g., nutrient availability, leaf thickness, and cuticle composition or microstructure) that may vary as much between leaves as between cultivars. The nitrogen content of tomato leaves was shown to effect *L. trifolii* probing with a preference in choice tests for leaves and plants with higher total nitrogen concentration (Minkenburg and Ottenheim 1990).

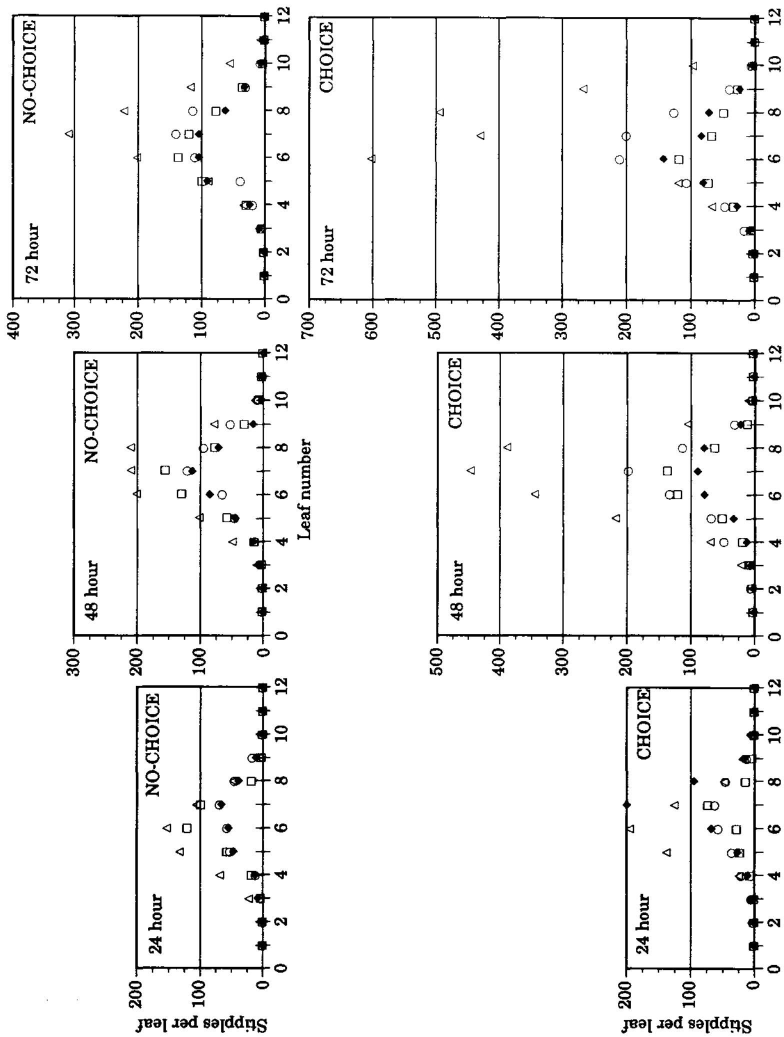

Fig. 2. Mean stipples per leaf over time produced by *L. trifolii* on cos lettuce plants in choice or no-choice tests. Cultivars: 'Floricos 83' (□), 'Parris Island Cos' (o), 'Tall Guzmaine' (Δ), 'Valmaine' (◆).

Differences in probing responses of *L. trifolii* appears to closely follow the pedigrees of the tested cultivars (Fig. 1). 'Valmaine' and 'Floricos 83' had similar stipple rates that were both low compared to rates on 'Tall Guzmaine'. Both 'Floricos 83' and 'Tall Guzmaine' have 'Valmaine' as a relative in their pedigrees. 'Floricos 83' is the result of a cross between 'Valmaine' and FL 1142 (Guzman and Zitter 1983) and is 3/4 'Valmaine'. 'Tall Guzmaine' was developed from a cross between 'Short Guzmaine' and 'Paris White' (Guzman 1986) and is 3/8 'Valmaine'. 'Short Guzmaine' is a full sib with 'Floricos 83'. Therefore, the differences observed in stipple counts between 'Tall Guzmaine' and 'Floricos 83' were probably introduced with the cross to 'Paris White'.

The results of this trial underscore the importance of insect resistance criteria in the breeding and selection process. 'Valmaine' (Leeper et al. 1963) was the leading cultivar of romaine lettuce grown in Florida organic soils before 'Tall Guzmaine' was released. 'Tall Guzmaine' was selected for characters that were needed in the warm, humid climate of southern Florida, but insect resistance was not included in the selection criteria. As a result 'Tall Guzmaine' is less susceptible to thermodormancy and premature bolting, is more resistant to lettuce mosaic virus, and is more tolerant to corky root rot than 'Valmaine' (Guzman 1986). However, 'Tall Guzmaine' is preferred by serpentine leafminer and appears to stimulate elevated levels of stippling over the other available commercial cultivars.

This trial demonstrated the potential of *L. trifolii* for producing stipples that reduce crop quality, and the dramatic difference that can be made by simply changing the cultivar. *L. trifolii* probed TG leaves an average of over 80 times per day in choice tests and 70 times per day in no-choice tests. Many fewer stipples per day were produced on the other cultivars. Methods used in this test could be adopted for large scale screening of promising selections in the field under natural conditions, or in the greenhouse using young plants in screened enclosures. Information is needed on whether differences in probing rates translate into predictable differences in oviposition and larval development, and on the source and regulation of any nonpreference or antibiotic mechanisms in order to engineer specific crosses that would result in reduced leaf miner damage in horticulturally acceptable commercial cultivars.

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