



Interspecific and intraspecific differences in two *Liriomyza* leafminer species in California

Stuart R. Reitz^{1,2} & John T. Trumble¹

Department of Entomology, University of California, Riverside, CA 92521, USA; ²Present address: USDA-ARS, Center for Biological Control, 310 Perry-Paige Bldg. Florida A&M University, Tallahassee, FL 32307-4100, USA (Phone: 850-412-7062; Fax: 850-412-7263; E-mail: sreitz@nettally.com)

Accepted: December 6, 2001

Key words: host plant performance, reproductive success, Agromyzidae, biotypes, assortative mating, cryptic species

Abstract

In recent years, the pest status of *Liriomyza trifolii* (Burgess) and *L. huidobrensis* (Blanchard) (Diptera: Agromyzidae) has changed in California, as well as other areas of the world. In California, *L. huidobrensis* has become the predominant *Liriomyza* species in valleys along the central coast, while *L. trifolii* remains the predominant species in southern California. To investigate possible reasons for this change in status, differences in host plant use and reproductive success of intraspecific populations were examined for *Liriomyza trifolii* and *L. huidobrensis* from both central and southern California. The southern *L. trifolii* fed, oviposited and reproduced successfully on all five hosts tested, but the central population fed significantly less on all hosts and was restricted to reproducing on pepper only. With the exception of pepper, southern *L. trifolii* had significantly greater larval survival on all hosts than central *L. trifolii*. In contrast, the central *L. huidobrensis* population had greater reproductive success than the southern population of that species on all hosts plants tested. However, pepper was not a suitable host for either *L. huidobrensis* population. Both species showed positive assortative mating, with homotypic mating occurring more frequently than heterotypic mating; however, the difference between *L. trifolii* populations was much more pronounced than between *L. huidobrensis* populations. These data indicate that central and southern California populations of each species are distinct biotypes. Furthermore when coupled with previous genetic data, our results suggest the possible existence of cryptic species within *L. trifolii*.

Introduction

Leafmining flies of the genus *Liriomyza* (Diptera: Agromyzidae) are among the most important insect pests of vegetable crops and ornamental flowers grown in California (Trumble, 1990) and elsewhere in the world (Spencer, 1973). The three species of *Liriomyza* that have been major economic pests in California are *L. huidobrensis* (Blanchard), *L. sativae* Blanchard, and *L. trifolii* (Burgess). The pest status of these species results, in part, from their multivoltine nature, polyphagous feeding habits, and ability to develop insecticide resistance rapidly. They have been considered classic secondary outbreak pests in various agroecosystems (Oatman & Kennedy, 1976; Johnson

et al., 1980). At optimum temperatures, a generation can be completed within 21 days (see Parrella, 1987), making it possible for several generations to be completed within one cropping season. In addition, their broad host ranges allow for migration among different crops and wild hosts. The host ranges of *L. huidobrensis* and *L. trifolii* each encompass over 400 species of plants in some 12 families, and there is considerable overlap in the ranges of the two species (Spencer, 1973; Morgan et al., 2000). This extreme degree of polyphagy is unusual among species of *Liriomyza*; only five of the more than 300 species of the genus are considered to be truly polyphagous (Spencer, 1973).

The history of these species as crop pests is complex. This complexity has resulted from the wide-

spread and overlapping distributions of species and from taxonomic confusion (Spencer, 1973). In addition, there have been at least two dramatic shifts in the *Liriomyza* species complex in the last 30 years in California resulting from the displacement of one species by another. Until the mid 1970s, *L. sativae*, a species native to the southern USA and South America, was considered the most significant *Liriomyza* pest in California (Spencer 1973, 1981). Then in the mid 1970s, *L. trifolii*, a native of the southeastern USA, was introduced, most likely on infested plant material from Florida (Spencer, 1981; Zehnder et al., 1983). Following its introduction, *L. trifolii* displaced *L. sativae* to become the predominant *Liriomyza* species in California (Trumble & Nakakihara, 1983; Zehnder & Trumble, 1984). Although never critically examined, this displacement has been attributed to differential insecticide resistance (Palumbo et al., 1994) but competition, differential host plant use, and differential effects of natural enemies may have been contributing factors.

In 1992, a second significant shift in the *Liriomyza* leafminer complex began in the coastal valleys of central California where *L. huidobrensis* has displaced *L. trifolii* to become the predominant leafminer species (Dlott & Chaney, 1995; Heinz & Chaney, 1995). This displacement is unusual in that *L. huidobrensis* is native to California and South America (Spencer, 1973), and hence it is not an invasive species. In addition it had been only a minor, sporadic pest in California prior to 1992 (Lange et al., 1957). *Liriomyza huidobrensis* also appears to be extending its predominance southward in California (Reitz et al., 1999). At the same time the distribution of *L. trifolii* has become limited in the central coast region. From 1996 through 1998, we found *L. trifolii* infesting no other field crops than peppers (*Capsicum annuum* L.) in the Salinas valley region of the central coast, whereas we found *L. huidobrensis* infesting numerous cultivated hosts (S.R. Reitz & J.T. Trumble, unpubl). During that period in extreme southern California (Orange, Riverside, San Diego counties), we found *L. trifolii* infesting numerous hosts and *L. huidobrensis* infesting some, but not all, of the same hosts as in central California. Overall population levels of *L. huidobrensis* during this time were much lower in southern than in central California.

This outbreak of *L. huidobrensis* in California coincides with outbreaks in numerous regions of the world, including Europe (Hume et al., 1990; De Goffau, 1991; Cheek et al., 1993), the Middle East

(Weintraub & Horowitz, 1995; Abou-Fakhr Hammad & Nemer, 2000), southern and eastern Asia (Shepherd et al., 1998; Zhiying et al., 1999), and several Pacific islands (Johnson, 1993). The broad scale and rapidity of these outbreaks suggest the possible widespread introduction of a common, highly pestiferous strain of *L. huidobrensis*. If so, such a strain may have originated in South America, where populations of *L. huidobrensis* have developed significant levels of pesticide resistance (see Chavez & Raman, 1987). High levels of insecticide resistance also have been reported in European and Middle Eastern populations of *L. huidobrensis* (MacDonald, 1991; Weintraub & Horowitz, 1995). Based on variation in sequence data from mitochondrial cytochrome oxidase genes, Scheffer (2000) has determined that flies from many of these outbreak areas are derived from a South American strain of *L. huidobrensis*. That study also showed that populations of *L. huidobrensis* from central and southern California are genetically distinct from those of South American origin and form a relatively homogeneous clade, which maybe a distinct but cryptic species, *L. langei* Frick (Scheffer & Lewis 2001).

Although California populations of *L. huidobrensis* form a separate, relatively homogeneous clade compared with the South American strain, Morgan et al. (2000), using random amplified polymorphic DNA polymerase chain reactions, showed that central and southern California populations of *L. huidobrensis* are genetically distinct from one another. In addition, corresponding populations of *L. trifolii* show even greater genetic variation. This genetic difference and the recent host range limits of the central *L. trifolii* indicate the existence of distinct populations or biotypes of this species.

The existence of different populations or biotypes in geographically widespread populations is relatively common (Diehl & Bush, 1984; Gilbert, 1990). Although populations can be differentiated genetically, they may or may not show appreciable phenotypic differences. Therefore we were interested in assessing how divergent central and southern California populations of *L. huidobrensis* and *L. trifolii* may be in regard to certain biological traits. One objective of our study was to determine if central and southern California populations of these species show differences in host plant performance. Greater reproductive success across multiple hosts could contribute to the enhanced pest status of central *L. huidobrensis* populations. Alternatively, the central population of *L. trifolii* may not have as wide a host range as the

southern population. A second objective was to determine if central and southern California populations of these species, which are genetically distinct, are reproductively isolated and therefore constitute distinct biotypes, or even incipient species (Diehl & Bush, 1984).

Materials and methods

Colony establishment

Colonies of *L. huidobrensis* and *L. trifolii* from southern and central California were established from field-collected larvae and adults. The central population of *L. huidobrensis* was collected from lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), broccoli (*Brassica oleracea* L. var. botrytis), spinach (*Spinacia oleracea* L.), and onion (*Allium cepa* L.) in Monterey and San Benito counties. The southern *L. huidobrensis* population was derived from material collected from gypsophila (*Gypsophila paniculata* L.) and cucurbits (*Cucurbita* spp.) in Riverside and San Diego counties. The central population of *L. trifolii* was derived from material collected from pepper (*Capsicum annuum* L.) in Monterey and San Benito counties. Pepper was the only open field host on which we found *L. trifolii* in that region. The southern *L. trifolii* population was derived from material collected from tomato (*Lycopersicon esculentum* Mill.) and celery in Orange County. Colonies were maintained in sleeve cages located in separate rooms at the Department of Entomology, UC Riverside. Rearing procedures followed those described by Parrella & Trumble (1989). The central and southern *L. huidobrensis* populations were reared primarily on peas (*Pisum sativum* L.) and celery. Eggplant (*Solanum melongena* L.), fava bean (*Vicia faba* L.), and spinach were used as hosts occasionally. The central *L. trifolii* population could be reared on pepper only. The southern *L. trifolii* population was reared on beans (*Phaseolus lunatus* L.), celery, and pepper. Except for the central *L. trifolii* population, different types of hosts were used for rearing over the entire duration of the study. Experiments were conducted from 1996 to 1998.

Identification of adults was confirmed by E. Fisher (California Department of Food and Agriculture). Voucher specimens are deposited in the Department of Entomology, University of California, Riverside.

Host plant performance

We evaluated inter- and intraspecific variation in host plant performance on celery, peas, peppers, spinach, and tomatoes. These host plants were selected because they represent a cross section of hosts on which these species are recorded as developing (Spencer, 1973), and they are hosts common to the areas from which all of the original source populations were derived. To evaluate the performance of each population, we analyzed the number of feeding and oviposition punctures, and the number of larvae, pupae, and adults produced. The number of feeding punctures reflects the suitability of plants for adult foraging. In addition, females usually feed before ovipositing (Reitz, pers. obs.). Although there is not a strict numerical correlation between these behaviors (Bethke & Parrella, 1985; Smith & Hardman, 1986), plants on which females do not feed are unlikely to be used for oviposition.

Plants used in these experiments were grown under similar conditions in the greenhouse. Plants received similar applications of fertilizer (Scott's Miracle-Gro, Maryville, Ohio). All plants, except for peas, were grown individually in 10 cm wide pots. Peas were grown in groups of five plants per pot.

Plants were exposed to flies by placing the plants individually in 11.4-l cylindrical containers. Plants of each species were standardized by age and leaf area to the extent possible. A group of five female and five male flies was introduced into each container. Because of their 1–2 day preoviposition period, flies were 3–5 days old when used in these tests. Plants were exposed to flies for 4 h. At the end of the exposure period, flies were removed from the containers. Each host plant and population combination was replicated 20–30 times. Two days after exposure, the numbers of feeding and oviposition punctures on each plant were counted by examining plants under stereo microscopes. Seven days after exposure, the numbers of larval mines were counted. Following that, plants were placed on Styrofoam trays to collect larvae as they emerged from their mines to pupate. Puparia from each plant were collected daily, and the numbers recorded. Puparia were placed in 30-ml plastic cups and held until adult eclosion. The number and sex of adults emerging each day were recorded. To determine if host plants affected size of adult flies, metathoracic tibial length was measured to the nearest 0.01 mm for a subset of females of each population and host combination.

The numbers of feeding and oviposition punctures, larvae, puparia, and adults, and mean tibial length, were compared among populations for each host plant. We used analysis of variance (ANOVA) to test for population by host interaction and overall population effects for each dependent variable. Where ANOVA showed a significant difference, we compared differences between populations on each host plant through least squares means comparisons (SAS, 1989b). Because of interspecific plant variation, direct comparisons were not made among host plants. To satisfy the assumption of homogeneity of variances, leaf puncture data were transformed to $\log_{10}(y + 1)$ prior to analysis, and data on the number of larvae, pupae, and adults were transformed to the square root of $(y + 0.375)$ prior to analysis (Sokal & Rohlf, 1995). Untransformed means are presented with their standard errors.

Reproductive success of intraspecific crosses

We examined reproductive differences between central and southern populations of each species and potential reproductive isolation by comparing the frequency of matings within and between populations on two different host plants, and the number of offspring produced from successful matings.

Puparia from each colony were individually isolated in microcentrifuge tubes. A small drop of honey was placed in the cap of the tube as a carbohydrate source to sustain emerging adults. Following emergence, two males from the same population were introduced into an arena. Five minutes later an individual female was introduced into the arena. Flies remained caged together for four days. The status of the flies was inspected daily. Replicates in which the female and at least one male did not survive the four days were not included in analyses. All possible crosses between populations of each species were attempted, using celery and pepper as host plants. Each type of cross was replicated 24–36 times.

Arenas for these trials consisted of a 40-dram clear plastic vial, which enclosed host plant foliage. A slit was cut in the cap of the vial to accommodate the plant petiole. This slit was lined with foam weatherstripping to prevent flies from escaping. Two 2.5-cm diameter screen-lined holes were made in opposite sides of the vial to allow for ventilation.

After four days, adults were removed from the arenas. Plants were examined for the presence of feeding punctures and larval mines. Plants without mines were

also examined for the presence of eggs. We examined the spermathecae of females that did not produce larvae for the presence of sperm to determine their mating status. All puparia and adults emerging from each cross were collected, as described in the host plant tests. Data on the frequency of mating were analyzed via categorical analysis of variance (SAS, 1989a; Sokal & Rohlf, 1995; Agresti, 1996). Data on the numbers of progeny from successful matings were subjected to ANOVA following square root transformation. Untransformed means are presented with their standard errors.

Results

L. trifolii host plant performance. Females of both populations of *L. trifolii* fed on all host plants to some extent, although there was substantial variation in the numbers of feeding and oviposition punctures made on the various types of plants. In all cases, the southern females made significantly greater numbers of leaf punctures than did females of the central population (test for population difference: $F_{1,225} = 139.4$, $P < 0.0001$). This result suggests that females of the southern population more readily accepted the different hosts for feeding, and possibly oviposition, than do females of the central population. The only host on which these two populations made remotely comparable numbers of punctures was pepper. Even in that case, the southern population made on average over two times as many punctures as the central population.

Females of the southern *L. trifolii* population did oviposit on all hosts offered. There were significantly more southern *L. trifolii* larvae than central *L. trifolii* larvae produced on all hosts (test for population difference: $F_{1,225} = 17.3$, $P < 0.0001$, Figure 1a). The only host on which the central population consistently produced larvae was pepper. Although they produced a mean of 6.7 ± 1.70 larvae, that is less than 21% of the mean number produced by southern *L. trifolii* on pepper. For the other hosts tested, no larvae were recorded on peas, and less than 15% of the replicates for the other three hosts produced any larvae. A closer examination of plants not producing larvae did not reveal the presence of unhatched eggs. Therefore, the low level of larvae present for this population results from females not ovipositing rather than a plant-induced inhibition of egg hatch.

Survivorship of larvae to the pupal stage ranged from 74% to 95% for the southern *L. trifolii* popula-

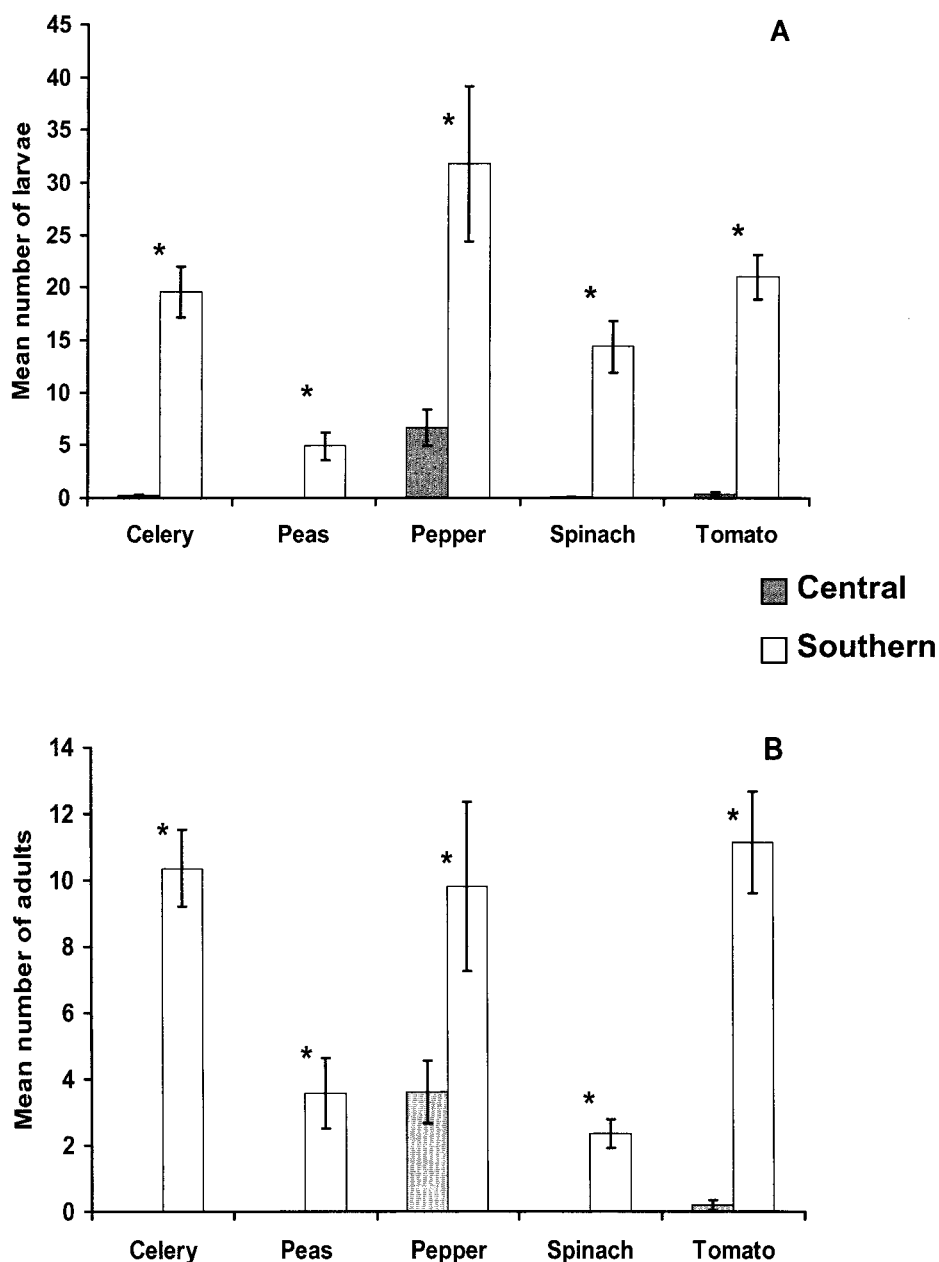


Figure 1. Performance of central and southern California *L. trifolii* on five different host plants in no choice tests. Significant differences ($P < 0.05$) between the populations in numbers of larvae (a), and number of adults (b) for each host plant are indicated by asterisks (*). Bars indicate standard error of the means.

tion on the various hosts; however, the survivorship of central population larvae reached a comparable level only on pepper (87%). Mortality increased for both populations during the pupal stage. Hence the overall survivorship to adult for southern *L. trifolii* ranged from 22% on spinach to 70% on peas. On peppers the central population had higher survivorship (57%) than

the southern population (35%). Even with the variation in survivorship, the southern *L. trifolii* produced significantly more adults on all hosts than the central population (test for population difference: $F_{1,225} = 14.9$, $P < 0.0001$, Figure 1b).

Despite the differences in numbers of progeny produced by the *L. trifolii* populations on the various

hosts, we found no significant differences in progeny size or development between the populations on pepper ($P > 0.05$). These comparisons were limited to pepper because it was the one host that produced significant numbers of central *L. trifolii*.

L. huidobrensis host plant performance

As with *L. trifolii*, females of both populations of *L. huidobrensis* fed on all host plants, although there was substantial variation in the numbers of feeding and oviposition punctures made on the different host plants. Overall the central *L. huidobrensis* population made significantly more leaf punctures than the southern population (test for population difference: $F_{1,230} = 4.53$, $P = 0.034$), but this result was primarily driven by the significant difference in punctures made on peas. Otherwise, these populations found the remaining hosts equally suitable in terms of feeding activity.

The central population of *L. huidobrensis* always produced significantly more larvae on the various hosts than did the southern *L. huidobrensis* (test for population difference: $F_{1,230} = 10.4$, $P < 0.0001$, Figure 2a). There was no significant population by host plant interaction ($F_{4,230} = 1.08$, $P = 0.37$). The greatest disparity in numbers of larvae occurred on pepper, followed by tomato and spinach, but even on celery and peas the central population produced over twice as many larvae as did the southern population. As with *L. trifolii*, differences in numbers of larvae reflect differences in oviposition. Closer examination of plants showed that virtually all eggs that were oviposited did hatch.

The survivorship of these larvae to the pupal stage was relatively constant, except in the case of pepper. An average of 70% of the central *L. huidobrensis* larvae from the four hosts other than pepper pupated. The corresponding average for southern *L. huidobrensis* was 72%. Pepper was not as suitable a host for either population. Only 39% of the central larvae pupated successfully, and none of the southern larvae survived to pupation. Survivorship of central *L. huidobrensis* pupae was lower (46%), and more variable across hosts, than for the southern *L. huidobrensis* (58%). The numbers of adults produced by the two populations also differed ($F_{1,230} = 8.46$, $P < 0.004$, Figure 2b). Again the primary difference was on peas, where the central population produced over two times as many adults as did the southern population.

Although female progeny size was variable across host plants for *L. huidobrensis*, there was no significant difference between the central and southern populations in size or development time ($P > 0.05$). The smallest adults were from pepper and tomato. The low survivorship and small size of *L. huidobrensis* from pepper indicate again that it is not a suitable host for *L. huidobrensis*.

Mating behavior within and between populations of *L. trifolii*

The courtship and mating behavior is relatively stereotypical for both *L. trifolii* and *L. huidobrensis*. Mating takes place on the host plant. Males have a stridulatory apparatus on their abdomen and metathoracic femur (Von Tschirnhaus, 1971). In a typical courtship, the males approach the female, after the female has made a feeding puncture and is in the process of feeding. If the female is receptive, she makes a bobbing motion with her body, which is followed by the male stridulating. These stridulations are made only in proximity to females, and may be used to convey substrate-borne vibratory signals to the female. Following the stridulating, males will attempt to mount and copulate with females. Females that are still receptive will allow males to mate. Unreceptive females will aggressively kick at males to prevent them from copulating. Males of both *L. trifolii* and *L. huidobrensis* will court conspecific as well as heterospecific females. Males will also attempt to mount females, but females will reject some of these attempted copulations. Based on these observations, females of *Liriomyza* appear to be the sex that chooses mates.

L. trifolii reproduction

For *L. trifolii*, more successful matings occurred between members of the same population (homotypic matings) than between members of the different populations (heterotypic matings) (male \times female interaction $X^2 = 56.8$, 1df, $P < 0.0001$, Figure 3a). Central females mated over five times more often with central males than with southern males (Figure 3a). In addition, the host plant appears to play a role in mating for females of the central population. Significantly more females of the central population mated on pepper than on celery ($X^2 = 20.9$, 1df, $P < 0.0001$, Figure 3a).

Southern *L. trifolii* females also mated significantly more often with homotypic males than with heterotypic males (Figure 3a). Southern females mated over twice as often with southern males as central

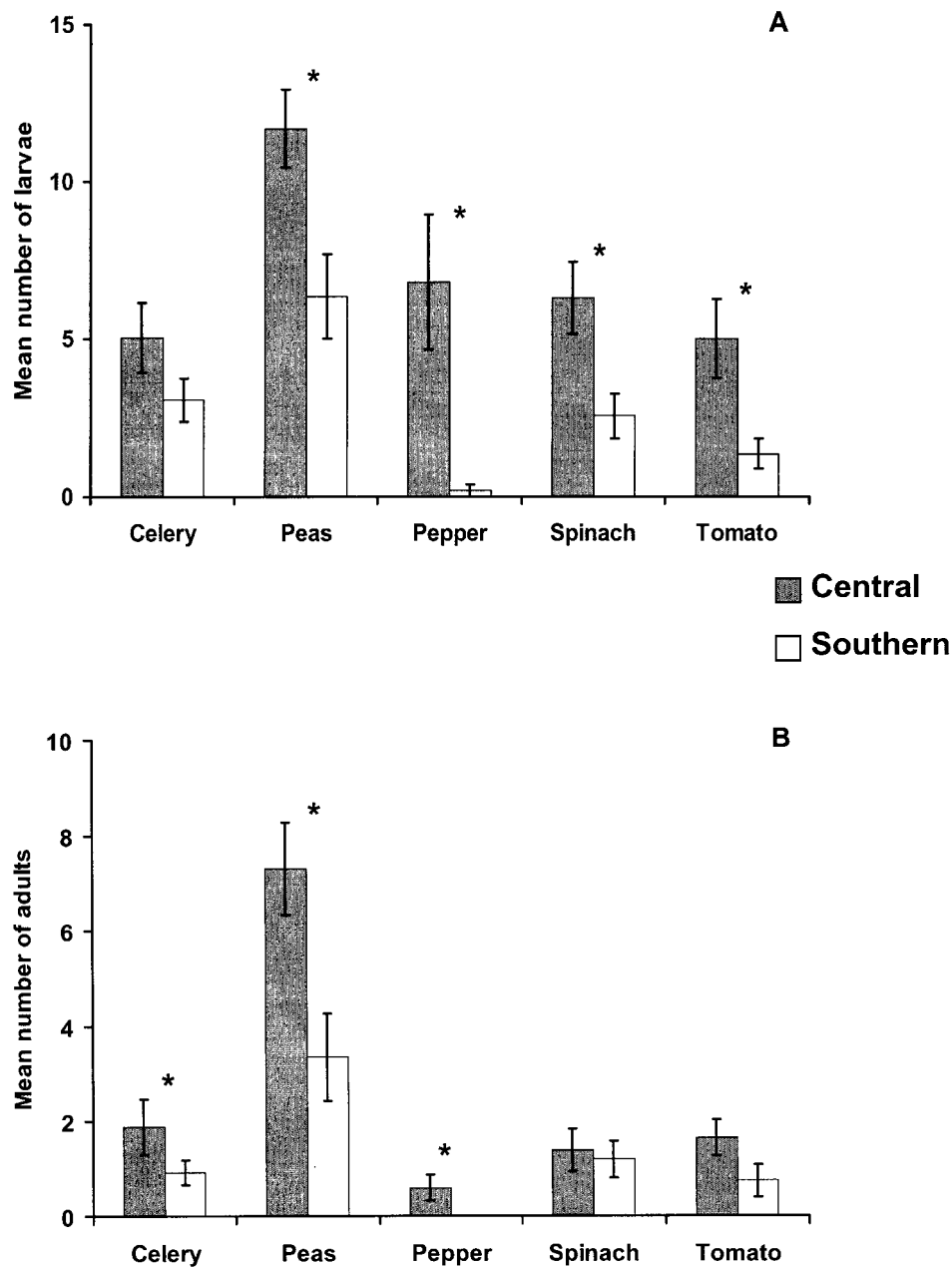


Figure 2. Performance of central and southern California *L. huidobrensis* on five different host plants in no choice tests. Significant differences ($P < 0.05$) between the populations in numbers of larvae (a), and number of adults (b) for each host plant are indicated by asterisks (*). Bars indicate standard error of the means.

males. In contrast to the central females, there was no significant effect of host plant on the frequency of mating for southern *L. trifolii* females. Similar numbers of southern females mated on pepper and celery.

Our analysis did not reveal significant postmating factors in terms of number of offspring produced or their viability. Although there were differ-

ences between host plants, the pattern of survival for progeny from homotypic matings was comparable with progeny survival from the host plant performance trials (Figure 3b). Even though homotypic matings were more frequent than heterotypic matings, numbers of adults produced by homotypic matings were not greater than the number of adults produced by

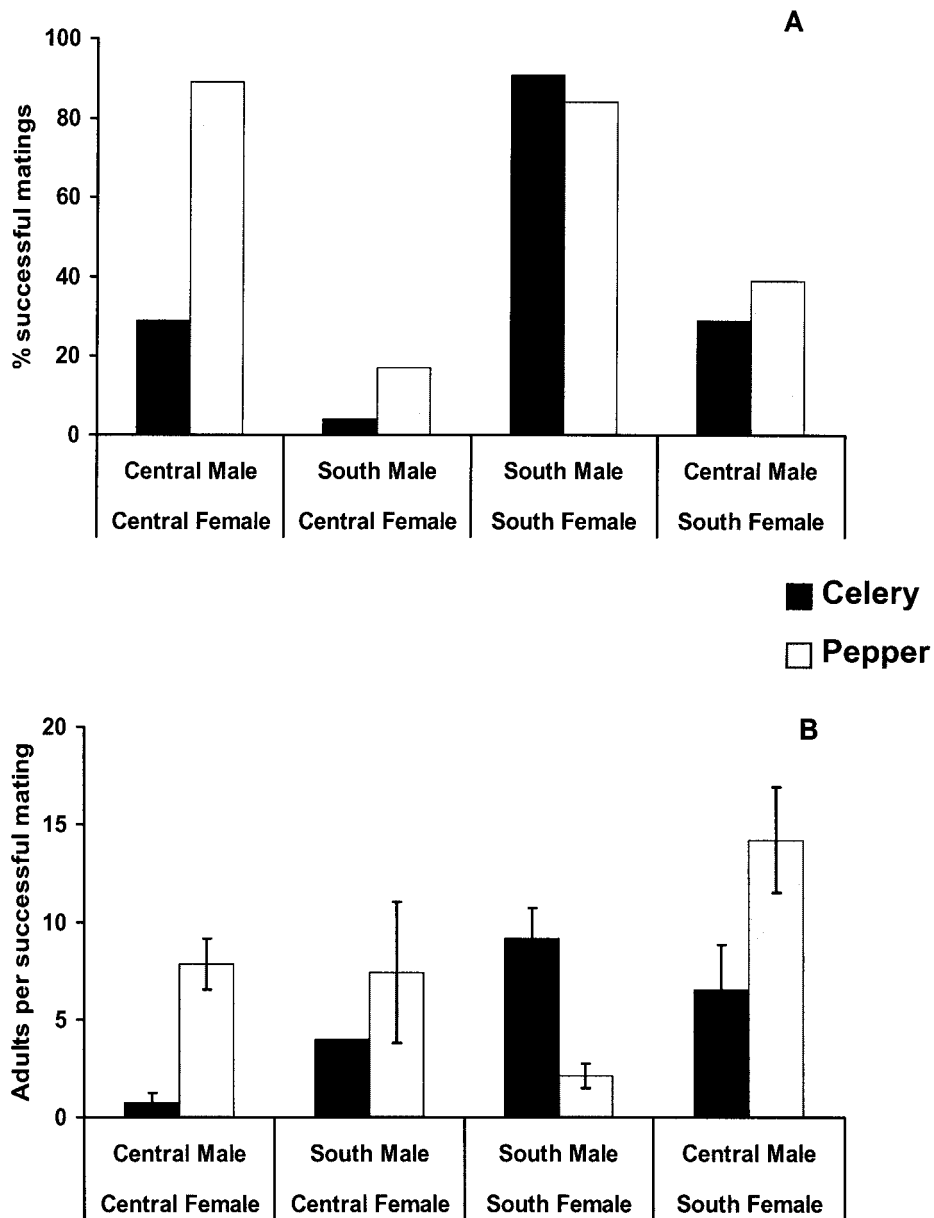


Figure 3. Results of mating trials for *L. trifolii*. (a) Proportion of successful matings between males and females from central and southern populations of *L. trifolii* when placed on celery or pepper. (b) Mean number of adults from successful matings of *L. trifolii* on celery and pepper. Bars indicate standard error of the means.

heterotypic matings. There was a significant difference in progeny numbers among the different crosses ($F_{3,120} = 2.69, P = 0.05$). This result was because females mated with central males on pepper actually produced significantly more adults than southern females mated with southern males (Figure 3b).

Mating behavior within and between populations of L. huidobrensis

The frequency of successful matings for *L. huidobrensis* varied consistently with the host plant. There were significantly more successful matings on celery than on pepper (overall plant effect: $X^2 = 8.55, 1 \text{ df}, P = 0.0034$, Figure 4a). However the disparity was not as great as that seen for *L. trifolii*. *Liriomyza*

huidobrensis also had more successful matings in homotypic pairings than in heterotypic pairings (male x female interaction: $X^2 = 5.78$, 1df, $P = 0.017$). Still, the differences in frequencies of heterotypic and homotypic matings for *L. huidobrensis* were not as extensive as for *L. trifolii*, with approximately 65% of heterotypic pairings resulting in successful matings (Figure 4a).

L. huidobrensis reproduction

Consistent with the host plant performance trials for *L. huidobrensis*, more pupae and adults were produced on celery than pepper (host plant effect on adult progeny: $F_{1,153} = 36.8$, $P < 0.0001$, Figure 4b). There was a significant male by female interaction in terms of progeny production ($F_{3,153} = 3.25$, $P < 0.024$). However, there was no consistent relationship between the type of mating (homotypic or heterotypic) and the number of progeny produced. For example, an unexpectedly high number of adults was produced from central male and southern female crosses on celery compared with southern male and southern female crosses on celery. This difference seems to result from differences in the number of ovipositions on the two types of host plants. There was no significant difference in the emergence rate between host plants ($F_{1,107} = 0.55$, $P = 0.46$) or mating type ($F_{1,107} = 1.03$, $P = 0.38$). Therefore, reproductive isolation between these populations of *L. huidobrensis* is more likely the result of premating rather than postmating mechanisms, as is the case for *L. trifolii*.

Discussion

We have addressed our overall objective by showing that the central and southern California populations of both *L. huidobrensis* and *L. trifolii* have distinct intraspecific biological differences. These biological differences corroborate analyses of intraspecific genetic differences among these populations of each species (Morgan et al., 2000). Specifically for *L. huidobrensis*, we found (1) that for the host plants tested, the central population has significantly greater reproductive success than southern population, and (2) that these populations show positive assortative mating, with homotypic matings occurring more frequently than heterotypic matings. For *L. trifolii* we found (1) that the central population reproduces on pepper to the near exclusion of other host plants, whereas the southern

population accepts and reproduces on all hosts offered, (2) that this limited host range of the central population is driven largely by females not accepting alternative hosts for oviposition, and (3) that these populations of *L. trifolii* show an even greater degree of positive assortative mating than those of *L. huidobrensis*. In addition to females mating more often with homotypic males than heterotypic males, we found that the host plant plays a significant role in mating success, especially for the central *L. trifolii* population.

Although *L. huidobrensis* is native to California, the recent elevation in its pest status in central California led us to predict that there would be significant biological differences between central and southern populations of it. The recent, ongoing outbreak of *L. huidobrensis* in central California differs from previous ones in California, which were sporadic, localized, and short-lived (typically one growing season) (Lange et al., 1957). The current outbreak is widespread, severe, and appears to be expanding southward (Dlott & Chaney, 1995; Reitz et al., 1999). The differences in host plant performance of the California populations of *L. huidobrensis* could contribute to the differences in their pest status. Improved host plant performance and greater reproduction across a range of hosts would result in larger populations over time in the valleys of the central coast where many vegetable crops are grown throughout the year. Variations in host plant use by populations of phytophagous insects over large geographic areas is expected to occur as adaptations to local conditions (Gilbert, 1990). Such local adaptations coupled with other factors such as increased pesticide resistance, could account for the recent surge in *L. huidobrensis* populations.

Still we are uncertain why these population differences have developed so suddenly and led to increasing pest problems with *L. huidobrensis* in central California. Although this recent outbreak coincides with other outbreaks of *L. huidobrensis* worldwide, flies from central and southern California are genetically distinct from other outbreak populations, which are derived from South American populations (Scheffer, 2000; Scheffer & Lewis, 2001). Therefore the introduction of an exotic strain of *L. huidobrensis* would not account for the situation in California.

The differences in *L. trifolii* populations are more extensive and more challenging to explain. Because *L. trifolii* was introduced recently to California (approximately 25 years ago), and historic California populations were genetically similar, based on allozyme patterns (Zehnder et al., 1983), we expected

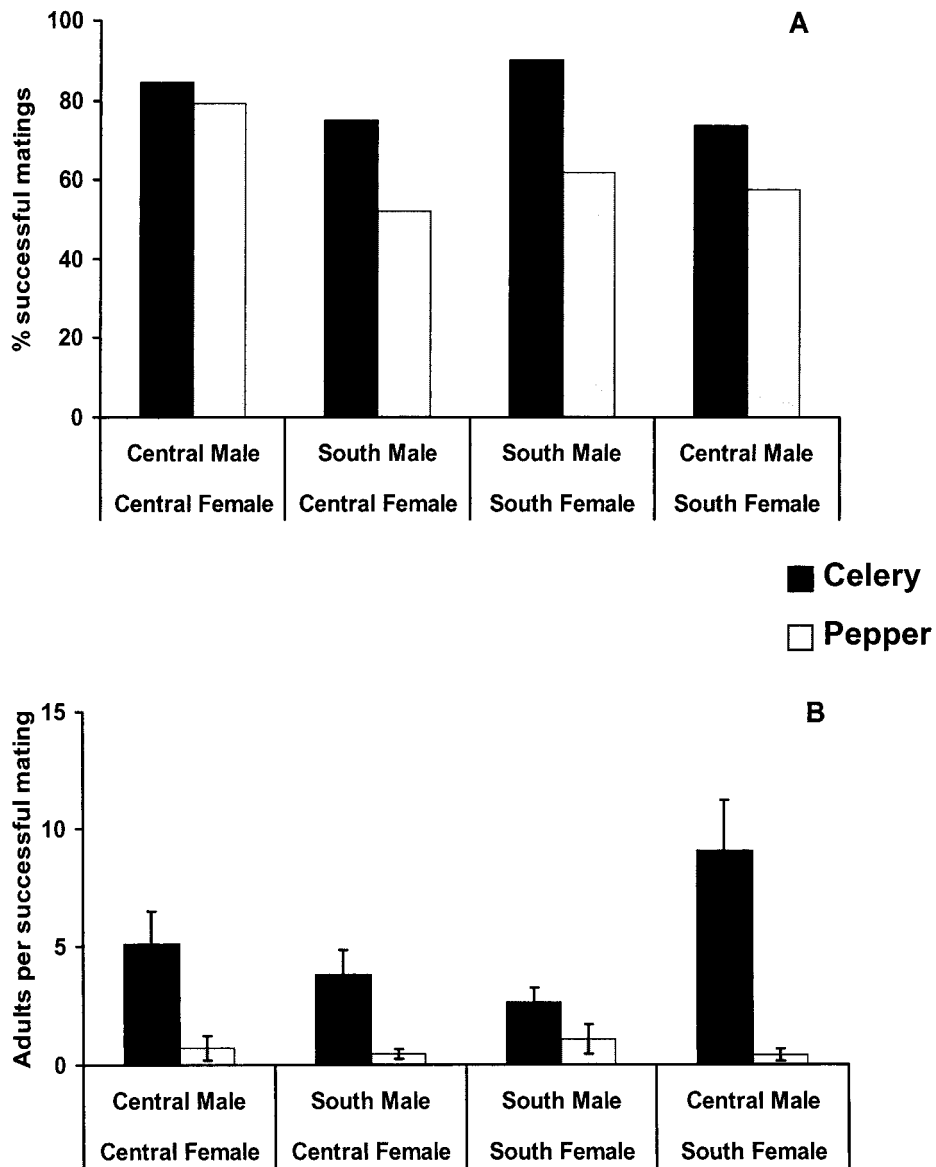


Figure 4. Results of mating trials for *L. huidobrensis*. (a) Proportion of successful matings between males and females from central and southern populations of *L. huidobrensis* when placed on celery or pepper. (b) Mean number of adults from successful matings of *L. trifolii* on celery and pepper. Bars indicate standard error of the means.

there to be less difference expressed between California populations of *L. trifolii* than between corresponding *L. huidobrensis* populations. However, the recently observed host range contraction in central California and the substantial reproductive isolation between the central and southern populations indicate considerable intraspecific variation is present currently within *L. trifolii*. Crop pests can undergo rapid evolution by adapting to widely planted hosts and may specialize on a particular host to the exclusion of other former

hosts (Futuyma, 1983). The host fidelity in mating and oviposition present in the central *L. trifolii* population indicates that this population is a separate biotype from the polyphagous southern *L. trifolii* (Diehl & Bush, 1984).

Female oviposition behavior appears to be the determinant of host range in *Liriomyza* rather than the nutritional or allelochemical suitability of the host plant for larval development. The central *L. trifolii* did survive on hosts other than pepper in an extremely

few select cases. These cases indicate that this population of *L. trifolii* still retains an ability to develop on alternative hosts. Likewise, larvae of the specialist *Liriomyza helianthi* Spencer can survive when transferred to novel hosts not oviposited in by females (Gratton & Welter, 1998). While our results are similar to those for *L. helianthi*, they differ from previous research with polyphagous *Liriomyza*. Smith & Hardman (1986) showed that females of another population of *L. trifolii* readily accept hosts other than the one on which they were reared, and that their larvae would survive on these alternate hosts. Tavormina (1982) found that while *Liriomyza brassicae* (Riley) reproduced better on the host species from which it was reared, females still retained the ability to oviposit on alternative hosts, and their larvae could survive on those hosts.

In the case of the central *L. trifolii*, its host range has contracted, even in the presence of apparently suitable alternative hosts. Refusal of hosts that are suitable to the southern population by central population females indicates that there is some advantage in terms of progeny survival or development (Lawton & McNeil, 1979; Jeffries & Lawton, 1984; Berdegue et al., 1996). The question of what triggered such a host range contraction remains. A tentative explanation for this situation could be that separate types of *L. trifolii* had been present in central California, a specialized pepper form and a more generalized polyphagous type, or types. Spencer (1973) has suggested that polyphagy arose twice in the genus *Liriomyza*, once among the *huidobrensis* species group and once among the *trifolii* species group, and that further speciation should be expected with new species developing the more typical monophagous or oligophagous feeding habit. Therefore, the existence of different forms within *L. trifolii*, as we have shown, or even cryptic species, should be expected. A limitation to the maintenance of sympatric host races should be gene flow and migration of individuals among hosts (Via, 1984), but if different forms of *L. trifolii* were present, the polyphagous *L. trifolii* form could have been displaced by *L. huidobrensis*, leaving a type that specializes on hosts that are poor for *L. huidobrensis*.

In the case of the allopatric populations of *L. trifolii* that we studied, a lack of gene flow could reinforce selection for differences between populations. Furthermore, subtle changes in mating behavior could reinforce reproductive isolation among allopatric populations of widespread herbivorous species. Therefore, an intriguing alternative hypothesis is that because of

interspecific competition leading to displacement on other hosts, the central *L. trifolii* have restricted their host range to hosts on which *L. huidobrensis* performs poorly. Via (1984) has shown that individual variation in host plant use exists in a population of *L. sativae* and has suggested that in a relatively homogeneous host plant environment, a greater degree of host plant specialization could occur. Such an environment could exist if competition from *L. huidobrensis* displaces *L. trifolii* from other hosts. We do not have data on competitive superiority of *L. huidobrensis* or if in fact there are differences in competitive abilities of *L. huidobrensis* populations; however, the earlier replacement of *L. sativae* by *L. trifolii* suggests that competitive displacement among leafminers is possible. Although many questions remain, recognition of intraspecific differences in *L. huidobrensis* and *L. trifolii* is important. The limited host range of the central *L. trifolii* population, host plant based mating, and premating reproductive isolation with another population indicate that *L. trifolii*, as a species, is more complex than previously thought. Of special interest is how these intraspecific differences are affected by rapid evolutionary shifts in a potential competitor, *L. huidobrensis*.

Acknowledgements

This research was supported in part by grants from the California Department of Natural and Agricultural Resources, the California Celery Research Advisory Board, and the California Tomato Commission. We are grateful to William Carson, Gregory Kund, Kristina White, and Jessica Young, University of California, Riverside, for their assistance in conducting these studies. We thank William Chaney, Franklin Dlott, and Karen Robb, University of California Cooperative Extension, for their assistance with collecting populations. Bradley Mullens and Joseph Morse, University of California, Riverside, and two anonymous reviewers provided valuable comments on an earlier draft of this manuscript.

References

- Abou-Fakhr Hammad, E. M. & N. M. Nemer, 2000. Population densities, spatial pattern and development of the pea leafminer (Diptera: Agromyzidae) on cucumber, swisschard and bean. *Journal of Agricultural Science* 134: 61–68.
- Agresti, A., 1996. *Introduction to Categorical Data Analysis*. John Wiley and Sons, New York.

- Berdegue, M., J. T. Trumble, J. D. Hare & R. A. Redak, 1996. Is it enemy-free space? The evidence for terrestrial insects and freshwater arthropods. *Ecological Entomology* 21: 203–217.
- Bethke, J. A. & M. P. Parrella, 1985. Leaf puncturing, feeding and oviposition behavior of *Liriomyza trifolii*. *Entomologia Experimentalis et Applicata* 39: 149–154.
- Chavez, G. L. & K. V. Raman, 1987. Evaluation of trapping and trap types to reduce damage to potatoes by the leafminer, *Liriomyza huidobrensis* (Diptera, Agromyzidae). *Insect Science and its Application* 8: 369–372.
- Cheek, S., O. C. MacDonald & P. W. Bartlett, 1993. Statutory action against *Liriomyza huidobrensis* (Blanchard) in the United Kingdom. *Liriomyza: Conference on leafmining flies in cultivated plants*, Montpellier, France, CIRAD.
- De Goffau, L. J. W., 1991. *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) a new economically important leaf miner in the Netherlands. *Nederlandse Entomologische Vereniging (N.E.V.)*, Amsterdam, the Netherlands.
- Diehl, S. R. & G. L. Bush, 1984. An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology* 29: 471–504.
- Dlott, J. W. & W. E. Chaney, 1995. Identifying management techniques and research needs for pea leafminer cultural, chemical, and biological control in celery. *Dinuba, California, California Celery Research Advisory Board*, pp. 79–91.
- Futuyma, D. J., 1983. Selective factors in the evolution of host choice by phytophagous insects. In: S. Ahmad (ed.), *Herbivorous Insects: Host-Seeking Behavior and Mechanisms*. Academic Press, New York, pp. 227–244.
- Gilbert, F. S., 1990. *Insect Life Cycles: Genetics, Evolution, and Co-Ordination*. Springer-Verlag, New York.
- Gratton, C. & S. C. Welter, 1998. Oviposition preference of larval performance and *Liriomyza helianthi* (Diptera: Agromyzidae) on normal and novel host plants. *Environmental Entomology* 27: 926–935.
- Heinz, K. M. & W. E. Chaney, 1995. Sampling for *Liriomyza huidobrensis* (Diptera: Agromyzidae) larvae and damage in celery. *Environmental Entomology* 24: 204–211.
- Hume, H., R. Dunne & J. P. O'Connor, 1990. *Liriomyza huidobrensis* new record (Blanchard) Diptera Agromyzidae an imported pest new to Ireland. *Irish Naturalists' Journal* 23: 325–326.
- Jeffries, M. J. & J. H. Lawton, 1984. Enemy-free space and the structure of biological communities. *Biological Journal of the Linnean Society* 23: 269–286.
- Johnson, M. W. 1993. Biological control of *Liriomyza* leafminers in the Pacific Basin. *Micronesica: Supplement* 4: 81–92.
- Johnson, M. W., E. R. Oatman & J. A. Wyman, 1980. Effects of insecticides on populations of the vegetable leafminer [*Liriomyza sativae*] and associated parasites on summer pole tomatoes. *Journal of Economic Entomology* 73: 61–66.
- Lange, W. H., A. A. Grigarick & E. C. Carlson, 1957. Serpentine leaf miner damage. *California Agriculture* 12: 3–5.
- Lawton, J. H. & S. McNeil, 1979. Between the devil and the deep blue sea: on the problem of being a herbivore. *Symposium of the British Ecological Society* 20: 223–244.
- MacDonald, O. C., 1991. Responses of the alien leaf miners *Liriomyza trifolii* and *Liriomyza huidobrensis* (Diptera: Agromyzidae) to some pesticides scheduled for their control in the UK. *Crop Protection* 10: 509–513.
- Morgan, D. J. W., S. R. Reitz, P. W. Atkinson & J. T. Trumble, 2000. The resolution of Californian populations of *Liriomyza huidobrensis* and *Liriomyza trifolii* (Diptera: Agromyzidae) using PCR. *Heredity* 85: 53–61.
- Oatman, E. R. & G. G. Kennedy, 1976. Methomyl induced outbreak of *Liriomyza sativae* on tomato. *Journal of Economic Entomology* 69: 667–668.
- Palumbo, J. C., C. H. Mullis, Jr. & F. J. Reyes, 1994. Composition, seasonal abundance, and parasitism of *Liriomyza* (Diptera: Agromyzidae) species on lettuce in Arizona. *Journal of Economic Entomology* 87: 1070–1077.
- Parrella, M. P., 1987. Biology of *Liriomyza*. *Annual Review of Entomology* 32: 201–224.
- Parrella, M. P. & J. T. Trumble, 1989. Decline of resistance in *Liriomyza trifolii* (Diptera: Agromyzidae) in the absence of insecticide selection pressure. *Journal of Economic Entomology* 82: 365–368.
- Reitz, S. R., G. S. Kund, W. G. Carson, P. A. Phillips & J. T. Trumble, 1999. Economics of reducing insecticide use on celery through low-input pest management strategies. *Agriculture Ecosystems & Environment* 73: 185–197.
- SAS., 1989a. SAS/STAT User's Guide, vol. 1. SAS Institute, Cary, NC.
- SAS., 1989b. SAS/STAT User's Guide, vol. 2. SAS Institute, Cary, NC.
- Scheffer, S. J., 2000. Molecular evidence of cryptic species within the *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* 93: 1146–1151.
- Scheffer, S. J. & M. L. Lewis, 2001. Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America* 94: 648–653.
- Shepard, B. M., Samsudin & A. R. Braun, 1998. Seasonal incidence of *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoids on vegetables in Indonesia. *International Journal of Pest Management* 44: 43–47.
- Smith, R. F. & J. M. Hardman, 1986. Rates of feeding, oviposition, development, and survival of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) on several weeds. *Canadian Entomologist* 118: 753–759.
- Sokal, R. R. & F. J. Rohlf, 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*. Freeman, New York.
- Spencer, K. A., 1973. Agromyzidae (Diptera) of Economic Importance. Dr W. Junk, The Hague.
- Spencer, K. A., 1981. A Revisionary Study of the Leaf-Mining Flies (Agromyzidae) of California. Division of Agricultural Sciences, University of California, Berkeley, CA.
- Tavormina, S. J., 1982. Sympatric genetic divergence in the leaf-mining insect *Liriomyza brassicae* (Diptera: Agromyzidae). *Evolution* 36: 523–534.
- Trumble, J. T., 1990. Vegetable insect control with minimal use of insecticides. *Hortscience* 25: 159–164.
- Trumble, J. T. & H. Nakakihara, 1983. Occurrence, parasitization, and sampling of *Liriomyza* species (Diptera: Agromyzidae) infesting celery in California. *Environmental Entomology* 12: 810–814.
- Via, S., 1984. The quantitative genetics of polyphagy in an insect herbivore. I. Genotype-environment interaction in larval performance on different host plant species. *Evolution* 38: 881–895.
- Von Tschirnhaus, M., 1971. Unbekannte Stridulationsorgane bei Dipteran und ihre Bedeutung für Taxonomie und Phylogenetik der Agromyziden. *Beiträge zur Entomologie* 21: 551–579.
- Weintraub, P. G. & A. R. Horowitz, 1995. The newest leafminer pest in Israel, *Liriomyza huidobrensis*. *Phytoparasitica* 23: 177–184.
- Zehnder, G. W. & J. T. Trumble, 1984. Host selection of *Liriomyza* species (Diptera: Agromyzidae) and associated parasites

- in adjacent plantings of tomato and celery (*Liriomyza sativae*, *Liriomyza trifolii*). Environmental Entomology 13: 492–496.
- Zehnder, G. W., J. T. Trumble & W. R. White, 1983. Discrimination of *Liriomyza* species (Diptera: Agromyzidae) using electrophoresis and scanning electron microscopy. Proceedings of The Entomological Society of Washington 85: 564–574.
- Zhiying, Z., W. Jianwen & S. Yuping, 1999. Population occurrence of *Liriomyza huidobrensis* in fields and its damage to vegetable crops in Kunming area [China]. Journal of Southwest Agricultural University (China) [Xinan Nongye Daxue Xuebao (China)] 21: 158–161.