

FOR INFORMATION AND
ACTION

DA-2008-76

December 19, 2008

SUBJECT: Request for Comments on Regulatory Options for Pea Leaf Miner

TO: STATE AND TERRITORY AGRICULTURAL REGULATORY OFFICIALS

The Animal and Plant Health Inspection Service (APHIS) is soliciting your comments to help us determine options for the continued regulation of *Liriomyza huidobrensis*, commonly known as pea leaf miner.

Pea leaf miner is an exotic, highly polyphagous leaf miner capable of inflicting severe damage to crops such as field- and glasshouse-grown vegetables and flowers. Pea leaf miner is a mining fly of the insect family *Agromyzidae*. Pea leaf miner can be found in Asia, Africa, Central and South America, Europe, the Middle East, and Oceania. This fly was also considered to be present in the States of California, Hawaii, Oregon, and Washington. However, there is now evidence that the fly in the United States previously believed to be pea leaf miner is not *Liriomyza huidobrensis*. Research has identified the fly present in the United States as *Liriomyza langei*. The fly was differentiated by molecular diagnosis from pea leaf miner but is morphologically almost identical. This research is summarized in the attached Center for Plant Health Science and Technology (CPHST) report entitled, "Is *Liriomyza langei* a real species or a biotype of *L. huidobrensis*?"

For regulatory purposes, APHIS believes that pea leaf miner and *L. langei* can be considered different species and distinct taxa. However, we recognize that we should not regulate one fly and not the other without scientific evidence indicating biological differences affecting pest character between species so taxonomically similar. In the attachment entitled, "Regulatory Options for Pea Leaf Miner and *Liriomyza langei*", we have presented three regulatory options that we are considering to address pea leaf miner and *L. langei*. The options are: (1) Regulate imported commodities for pea leaf miner into all 50 States; (2) Regulate imported commodities for pea leaf miner and interstate movement of commodities for *L. langei* into the State of Florida (current regulatory process); or (3) Provide no regulation for pea leaf miner or *L. langei*.

Since this is the first case for APHIS where a population of an organism with U.S. distribution was believed to be the same as an exotic pest but was subsequently differentiated from an exotic pest as a distinct species by molecular diagnosis without support of morphological characters, we wish to obtain comments from State Departments of Agriculture as to how APHIS should regulate for pea leaf miner. We also welcome the submission of any published scientific article(s) for our review and consideration regarding the relationship of pea leaf miner and *L. langei*. You may submit your comments and any supporting information by e-mail to

SPRO.PLM.Comments@aphis.usda.gov or by fax to (301) 734-3396. We ask that you provide your comments by January 16, 2009.

If you have any questions, please contact Janel Barsi, Special Assistant, Office of the Executive Director, Plant Protection and Quarantine, at (301)-734-0947.

/s/ Michael A. Lidsky for

Rebecca A. Bech
Deputy Administrator
Plant Protection and Quarantine

Attachments:

Regulatory options for pea leaf miner and *Liriomyza langei*

Is *Liriomyza langei* a real species or a biotype of *L. huidobrensis*?, August 2008

Regulatory Options Under Consideration for Pea Leaf Miner (*Liriomyza huidobrensis*) and *Liriomyza langei*

Pea leaf miner is an exotic, highly polyphagous leaf miner capable of inflicting severe damage to crops such as field- and glasshouse-grown vegetables and flowers. Pea leaf miner is a mining fly of the insect family Agromyzidae. Pea leaf miner can be found in Africa, Asia, Central and South America, Europe, the Middle East, and Oceania. This fly was also considered to be present in the States of California, Hawaii, Oregon, and Washington. As pea leaf miner was previously thought to be present in several States, pursuant to our plant pest authorities under the Plant Protection Act and obligations under the provisions of the International Plant Protection Convention, APHIS determined that it was best to regulate pea leaf miner under an official control program in a manner consistent with the pest controls necessary for the State or region of the port of entry. That is, APHIS supports actions taken by States to exclude pea leaf miner on shipments moving interstate into noninfested States. For instance, APHIS currently takes action on shipments destined for Florida because that State employs an effective exclusion program to prevent introduction of the pest.

Leaf mining flies of the insect family Agromyzidae, which includes pea leaf miner, arrive in commerce almost exclusively as immature larvae or pupae. Because these immature insects cannot be identified using morphological characters alone, APHIS usually takes action only when *Agromyzidae* are found on commodities in order to exclude exotic pests that the unidentifiable immature flies may represent. Only in the few cases where surveys on specific crops in specific countries determined that pea leaf miner was virtually the only species of *Agromyzidae* on those crops (i.e., snow peas and onions from Guatemala and chrysanthemum from the Dominican Republic, Costa Rica and Colombia) can we appropriately assume that the intercepted, immature flies are pea leaf miner. Accordingly, APHIS takes action for movement into Florida on any intercepted pea leaf miner identified to *Agromyzidae* on the numerous imported vegetable and flower hosts from countries throughout pea leaf miner's distribution in Africa, Asia, Central and South America, Europe, the Middle East, and Oceania. However, when immature leaf miners that may or may not be pea leaf miner are intercepted on commodities from other countries (or other commodities from Guatemala, the Dominican Republic, Costa Rica or Colombia), we take action for all States since, as previously stated, in the absence of surveys, we do not know which *Agromyzidae* the unidentifiable immature flies represent.

Below, we have presented three regulatory options that APHIS is considering for regulating pea leaf miner. With each option, we have provided the results of the option if selected. When evaluating these options, please note that APHIS usually takes action to prevent the introduction of intercepted pests identified only to the family level, or Agromyzidae in this case. Options 1-3 pertain to cases where an intercepted pest can be identified to either *L. huidobrensis* or *L. langei*.

1. Regulate imported commodities for pea leaf miner into all US States.

This option presumes the flies are different species and distinct entities for regulatory purposes. As such, pea leaf miner is not known to occur in the United States and is considered an exotic, quarantine significant pest.

If pea leaf miner is detected on any imported commodity from any country that has pea leaf miner, APHIS would take action on the commodity by requiring treatment for pea leaf miner, regardless of the destination of the commodity or port of importation into the United States.

(At this time, *L. langei* is known only to occur in the United States. If, in the future, *L. langei* is shown to be present in foreign regions, APHIS would only take action on *L. langei* on imports destined to States that have effective exclusion or control programs for *L. langei*.)

2. Regulate imported commodities for pea leaf miner into Florida only.

This option presumes the flies are not distinct entities for regulatory purposes.

APHIS would continue to regulate the importation of commodities that pose a risk of introduction of pea leaf miner into Florida. APHIS would also support actions by the State of Florida to exclude *L. langei*. APHIS would take action on *L. langei* on imports destined to Florida or any other State with effective exclusion or control programs for *L. langei*. However, at this time, *L. langei* is known to only occur in the United States.

3. Provide no regulation of imports for pea leaf miner or *L. langei*

APHIS would not take action on imports for pea leaf miner and/or *L. langei*.

This option, as with the preceding option, is based on a determination that pea leaf miner and *L. langei* are not distinct entities for regulatory purposes. However, under this option, APHIS would no longer consider pea leaf miner to be a quarantine pest because of its determined close association with *L. langei*, a presumably endemic species. Accordingly, States would be free to regulate commodities moving interstate for the presence of *L. langei* or pea leaf miner, if found to be present in the United States, but not imports.



United States Department
of Agriculture

Animal and Plant Health
Inspection Service

August 2008

Is *Liriomyza langei* a real species or a biotype of *L. huidobrensis*?



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Is *Liriomyza langei* a real species or a biotype¹ of *L. huidobrensis*?

Liriomyza huidobrensis is a leafminer that originated in Latin America and has since established populations in temperate and subtropical regions worldwide. There is evidence that a population found in California is not merely a biotype of *L. huidobrensis* but a distinct species called *L. langei*. While the Latin American and Californian populations cannot be differentiated based on morphology, differences in their invasive histories, host preferences, and feeding and oviposition behavior suggest they may have evolved into different species. Furthermore, molecular evidence and a possible reproductive isolating mechanism indicate that the two populations are individual species. This document discusses evidence that the Californian population may be distinct species rather than a biotype of *L. huidobrensis*.

What is a species?

From the time of Plato and Aristotle until Linnaeus, the word “species” simply meant a class of a class of objects that shared certain defining properties (Mayr, 1996). Philosophers applied this definition, which is often referred to as the morphological or typological species concept, to a wide range of animate and inanimate objects (Mayr, 1996). Naturalists, during this period, recognized biological species by morphological differences (Mayr, 1996).

Some early prophetic naturalists, like Buffon and Darwin, hinted at the biological species concept, but the concept was not clearly articulated until the late 19th and early 20th century, when studies revealed that similar looking “species” could behave very differently (Mayr, 1996). Widespread recognition of the many cryptic species that occur in nature, and recognition that many morphotypes can occur in a single species because individuals differ in sex, age, or genetics, eventually led to the almost complete replacement of the typological species concept by the biological species concept (BSC) (Mayr, 1996).

According to Ernst Mayr (1996), a staunch supporter of the BSC and critic of those who try to redefine it, “species are groups of interbreeding natural populations that are reproductively isolated from other such groups.” The isolating mechanism is a property of individuals, so geographic isolation, by itself, does not qualify populations as distinct species. Biologists must ask themselves whether or not their study population would interbreed with geographically isolated populations should the populations meet in nature. The biologist must then use morphology, geography, ecology, behavior, and molecular information, along with inference because “during a period of geographic isolation the presence of species specific isolating mechanisms can only be inferred,” to answer the question and demarcate species.

Do we have enough information to demarcate populations of *Liriomyza huidobrensis* that originate in either California or Latin America as separate species?

Unequal rates of evolution and a lack of information on the mating potential of isolated populations have always been problems for the BSC and species demarcation (Mayr, 1996). Biologists should expect to find populations in nature that are on their way to becoming new species because evolution is a gradual process (Mayr, 1996). Spencer predicted speciation of polyphagous agromyzid populations in 1973. He speculated that polyphagy arose independently only twice in the genus *Liriomyza*, and that the five known polyphagous species would, with some degree of isolation and host plant restriction, diverge into new “more typical monophagous or oligophagous” species; the vast majority of agromyzids are host specialists with less than one percent regularly feeding on more than two plants in different taxonomic orders or subclasses (Spencer, 1990). Speciation may actually be occurring in Californian populations of *L. huidobrensis*. Morgan *et al.* (2000) did not find any evidence of genetic mixing between central and southern Californian populations, and Reitz and Trumble (2002) concluded that the populations were distinct biotypes based on differential reproductive success rates on a variety of hosts and a higher frequency of homotypic than heterotypic mating in laboratory studies. The following sections of this paper discuss what science knows about Californian and Latin American *L. huidobrensis* populations in terms of what a biologist can use to demarcate species.

Morphology

Adult members of the genus *Liriomyza* all look very similar; they are small black flies that often have bright yellow patterns on their scutellum (EPPO, 2005). Species identification is difficult and often relies on a close examination

¹ Biologists can not designate either the Californian or Latin American population as an incipient species or a subspecies of *L. huidobrensis* because there are no known morphological differences in the two populations.

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of small external differences, such as the relative lengths of wing veins, the presence and position of a particular seta, or the color of the cuticle from which a seta arises (EPPO, 2005; Masetti *et al.*, 2006; Weintraub and Horowitz, 1995). Identification of closely related *Liriomyza* species is only possible by examining male genitalia (EPPO, 2005; Masetti *et al.*, 2006; Shiao, 2004). Even then, it is difficult to distinguish species; the genitalia must be properly prepared and viewed from the correct angle by an experienced identifier (Shiao, 2004; Shiao and Wu, 2000).

The immature stages of agromyzid flies are even more difficult to identify than the adults. The eggs can not even be identified to genus (EPPO, 2005). The larvae and pupae can be separated into species groups by morphological characteristics, but species determination requires electrophoretic or molecular analysis (EPPO, 2005).

There are no known morphological differences between members of the Californian and Latin American *L. huidobrensis* populations (Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001). Shiao (2004) recently evaluated the usefulness of some morphological characters to separate *Liriomyza* species of quarantine importance to Taiwan. He included specimens from California in his study of abdominal color patterns but found no differences between the Californian and Taiwanese *L. huidobrensis* populations. He did not include the Californian specimens in his study of thoracic microsetae, and it is not clear if he included them in the wing morphometrics or genitalia studies.

Geography

Molecular evidence suggests that the Californian and Latin American populations diverged about two million years ago (Scheffer, 2000). Since then, the Latin American population has spread to temperate and subtropical regions throughout the world (Chen and Kang, 2004; He *et al.*, 2002; Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001). In sharp contrast, the Californian population, which is frequently intercepted in Florida on vegetables from California (Halbert, 2006), has not spread since damage was reported by Lange *et al.* in 1957 (Borchert, 2006). The fly was introduced into Hawaii from California and established before 1952 (Hardy and Delfinado, 1980; Scheffer, 2000; Spencer, 1973). It was first detected in Washington state in 1918, where it is still occasionally classified as a serious pest (Gary *et al.*, 1986); the last reported outbreak occurred in 2003 (Pelter, 2003). Specimens in the Oregon State Arthropod Collection suggest that this fly was introduced into Oregon before 1938 (Marshall, 2007). Stegmaier (1968) listed the U.S. distribution as California, Oregon, and Washington. Gary *et al.* (1986) listed the U.S. distribution as California, Florida, Utah, and Virginia. Poe and Montz (1981) recorded the fly's presence in California, Virginia, and Florida. Steck and Dixon (2006) explained that the fly never established a permanent population in Florida, and CABI (2006) states that it is now absent from Florida, Utah, and Virginia.

Differences in the invasive histories of the two populations could be explained a number of ways but almost certainly involve differences in their abilities to survive diverse climatic conditions; introduction into areas with unfavorable climates is one of the leading reasons that introduced organisms fail to establish (Lodge, 1993), and scientists believe that thermal adaptation is a heritable characteristic (Chen and Kang, 2004).

Chen and Kang (2004) proved that Chinese populations, which originated from Latin American stock (He *et al.*, 2002), could survive cold temperatures and extend their range through supercooling. The absolute minimum temperatures that tested pupae survived increased with latitude from $\approx -18^{\circ}\text{C}$ at 25° latitude to $\approx -21^{\circ}\text{C}$ at 31 to 34° latitude (Chen and Kang, 2004). Because an insect's supercooling point is not a reliable indicator of cold hardiness (Martin *et al.*, 2005a), Chen and Kang (2004) estimated that members of the Chinese populations could not over-winter in areas where the mean monthly temperature was near -5°C , based on lethal time and temperature studies. Martin *et al.* (2005a) supported Chen and Kang's (2004) estimate by demonstrating that members of a Canadian population, which also originated from Latin American stock (Scheffer *et al.*, 2001), could not survive 16 consecutive days at -5°C . Some authors, who studied *L. huidobrensis* cold hardiness, recognized color variation in the puparia and suggested that dark colored puparia may over-winter in colder areas than light colored puparia (Weintraub and Horowitz, 1995). Other authors, who studied descendants of Latin American populations, suggest that all life stages are fairly cold tolerant (Lanzoni *et al.*, 2002), but Lange *et al.* (1957) indicate that cold winters tend to decrease fly populations in the Salinas Valley of California, where the mean low temperature during the coldest winter month is about 2°C (NOAA, 2007).

Although researchers report low *L. huidobrensis* populations, less female activity, and low fecundity at temperatures above 30°C (Weintraub, 2001), members of the Latin American population may adapt to warm climates. Lanzoni *et al.* (2002) showed that an Italian population, which originated from Latin American stock, could not complete development at a constant temperature of 30°C , but the authors recognized that other populations, such as those in

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Reunion, which probably also originated from Latin American stock, may complete development at this temperature. The diverse climatic situations that Latin American populations experienced as they migrated south along the Andes to Argentina (Spencer, 1973) may have equipped individuals with thermal adaptations that are not found in members of the Californian population.

Ecology

Ecology is a broad field that studies the interactions between and among organisms and their environment. As such, many ecological interactions are discussed throughout this paper. One ecological topic of paramount importance that has not yet been discussed is plant-herbivore interactions. For this reason, the following section concentrates on known differences in the host ranges of Californian and Latin American *L. huidobrensis* populations.

Interactions between *Liriomyza* species and their hosts are complex (Wei *et al.*, 2000). Their host ranges appear to be determined by female feeding and oviposition preferences rather than the host's suitability for larval survival and development (Reitz and Trumble, 2002). For example, Martin *et al.* (2005b) suggested that members of a Canadian *L. huidobrensis* population, which originated from Latin American stock (Scheffer *et al.*, 2001), prefer cucumber (*Cucumis sativus* cv. Calypso) to lettuce (*Lactuca sativa* cv. Ithaca) based on the proportion of eggs in feeding and oviposition punctures from three subpopulations, but the authors also demonstrated that larvae develop significantly faster and grow into larger pupae on lettuce than on cucumber. Wei *et al.* (2000) explained *L. huidobrensis* host selection in terms of leaf morphology; they suggested that females prefer to feed from and oviposit in leaves that have a thin epidermis and a low density mesophyll layer. These conditions make it easy for the female to puncture a leaf with her ovipositor (Wei *et al.*, 2000).

The primary literature contains numerous records of members or recent descendants of the Latin American population attacking crops, ornamentals, and weeds under natural conditions that are not known hosts of the Californian population. Some of these plants, such as Indian aster (*Kalimeris indica*), sponge gourd (*Luffa aegyptiaca*) (Shiao and Wu, 2000), and Ceylon spinach (*Basella albe*) (Rauf *et al.*, 2000) do not occur in areas of California where *L. huidobrensis* is present (Borchert, 2006; CDFA, 2006; NASS, 2007; USDA-NRCS, 2007). Other recorded natural hosts of the Latin American population are present in California and apparently unattractive to the Californian population; research and extension personnel in California did not indicate that any of the plants listed in Table 1 are attacked by *L. huidobrensis* (Colpetzer, 2007).

Table 1: Plants attacked under natural conditions by members or descendants of the Latin American population that are not known hosts of the Californian population

Plant	Country	Reference
<u>Asteraceae</u>		
<i>Ageratum conyzoides</i> (tropical whiteweed)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Ageratum</i> sp. (whiteweed)	Indonesia	Shepard <i>et al.</i> , 1998
<i>Arctium lappa</i> (greater burdock)	Philippines	Scheffer <i>et al.</i> , 2006
<i>Calendula</i> sp. (marigold)	Chile	Spencer, 1990
<i>Conyza canadensis</i> (Canadian horsetail)	China	Wei <i>et al.</i> , 2000
<i>Dahlia</i> sp. (dahlia)	Indonesia Venezuela	Rauf <i>et al.</i> , 2000 Spencer, 1973
<i>Emilia sonchifolia</i> (lilac tasselflower)	Indonesia Philippines Sri Lanka	Rauf <i>et al.</i> , 2000 Scheffer <i>et al.</i> , 2006 Scheffer <i>et al.</i> , 2001
<i>Erechtites hieraciifolia</i> (American burnweed)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Galinsonga parviflora</i> (gallant soldier)	China	Wei <i>et al.</i> , 2000
<i>Galinsonga quadriradiata</i> (shaggy soldier)	Venezuela	Spencer, 1973
<i>Ganzania</i> sp.(ganzania)	Colombia Europe South America	Spencer, 1990 Spencer, 1990 Spencer, 1990
<i>Gerbera jamesonii</i> (Barberton daisy)	Italy	Lanzoni <i>et al.</i> , 2002

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Plant	Country	Reference
	Indonesia	Rauf <i>et al.</i> , 2000
	China	Wei <i>et al.</i> , 2000
	Lebanon	Hammad <i>et al.</i> , 2000
	Poland	Górski, 2005
<i>Luecanthemum</i> sp. (daisy)	Netherlands	Kox <i>et al.</i> , 2005
<i>Tagetes erecta</i> (Aztec marigold)	China	Wei <i>et al.</i> , 2000
<i>Tagetes patula</i> (French marigold)	China	Wei <i>et al.</i> , 2000
<i>Tagetes</i> sp. (marigold)	Argentina	Spencer, 1990
<u>Brassicaceae</u>		
<i>Barbarea</i> sp. (yellowrocket)	Taiwan	Shiao and Wu, 2000
<i>Nasturtium officinale</i> (watercress)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Caryophyllaceae</u>		
<i>Stellaria media</i> (common chickweed)	China	Wei <i>et al.</i> , 2000
<u>Convolvulaceae</u>		
<i>Calystegia sepium</i> (hedge false bindweed)	China	Wei <i>et al.</i> , 2000
<i>Ipomoea batatas</i> (sweetpotato)	Indonesia Indonesia	Shepard <i>et al.</i> , 1998 Rauf <i>et al.</i> , 2000
<u>Cucurbitaceae</u>		
<i>Sechium edule</i> (chayote)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Fabaceae</u>		
<i>Lathyrus odoratus</i> (sweetpea)	Argentina	Spencer, 1973
<i>Vigna unguiculata</i> (blackeyed pea)	China Indonesia Indonesia	Wei <i>et al.</i> , 2000 Shepard <i>et al.</i> , 1998 Rauf <i>et al.</i> , 2000
<u>Gentianaceae</u>		
<i>Eustoma exaltatum</i> ssp. <i>russellianum</i> (showy prairie gentian)	China	Wei <i>et al.</i> , 2000
<u>Iridaceae</u>		
<i>Gladiolus hybridus</i> (gladiolus)	China	Wei <i>et al.</i> , 2000
<u>Lamiaceae</u>		
<i>Ocimum basilicum</i> (sweet basil)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Malvaceae</u>		
<i>Malva verticillata</i> (cluster mallow)	China	Wei <i>et al.</i> , 2000
<i>Sida</i> sp. (fanpetals)	Philippines	Scheffer <i>et al.</i> , 2006
<u>Oxalidaceae</u>		
<i>Oxalis</i> sp. (woodsorrel)	Argentina	Spencer, 1990
<u>Poaceae</u>		
<i>Setaria viridis</i> (green bristlegrass)	China	Wei <i>et al.</i> , 2000
<u>Polemoniaceae</u>		
<i>Phlox</i> sp. (phlox)	Argentina	Spencer, 1973
<u>Ranunculaceae</u>		
<i>Ranunculus sceleratus</i> (cursed buttercup)	China	Wei <i>et al.</i> , 2000

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Plant	Country	Reference
<u>Scrophulariaceae</u>		
<i>Antirrhinum majus</i> (garden snapdragon)	Canada	Martin <i>et al.</i> , 2005b
<u>Solanaceae</u>		
<i>Datura</i> sp. (jimsonweed)	South America	Spencer, 1990
<i>Physalis angulata</i> (cutleaf groundcherry)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Solanum americanum</i> (American black nightshade)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Solanum tuberosum</i> (Irish potato)	Canada	Martin <i>et al.</i> , 2005b
	Indonesia	Shepard <i>et al.</i> , 1998
<i>Solanum tuberosum</i> (Irish potato) continued	Indonesia	Rauf <i>et al.</i> , 2000
	Indonesia	Hidayani <i>et al.</i> , 2005
	Israel	Weintraub and Horowitz, 1996
	Israel	Weintraub, 2001
	South Africa	Scheffer <i>et al.</i> , 2001
	Venezuela	Spencer, 1973
<u>Tropaeolaceae</u>		
<i>Tropaeolum</i> sp. (nausturtium)	Argentina	Spencer, 1973
<u>Violaceae</u>		
<i>Viola</i> sp. (violet)	Argentina	Spencer, 1973

Some researchers recognized that the Californian and Latin American populations seemed to differ in host plant preference and insecticide resistance (Lanzoni *et al.*, 2002; Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001); the Californian population is “relatively easily controlled,” while pesticide resistant strains occur in other areas of the world (Lanzoni *et al.*, 2002). The over use of pesticides in South America during the 1970s is supposedly responsible for the resistant strains (Lanzoni *et al.*, 2002), but it may also partially explain why the Latin American population is recorded on a wider range of hosts than the Californian population. The Latin American population may have evolved either more efficient or different detoxification mechanisms than the Californian population due to extreme evolutionary pressures. These new or improved detoxification mechanisms could allow individuals to exploit a wide range of hosts with very diverse phytochemicals.

Behavior

All *Liriomyza* adults exhibit similar feeding behavior (Ameixa *et al.*, 2007; Parrella, 1987), but their larvae make characteristic leaf mines, and individual species differ in how they exploit the mesophyll layer (Parrella *et al.*, 1985). The mining behavior of an individual species may vary on different hosts, but numerous observations of *L. huidobrensis* in California suggest that the Californian population prefers to mine the spongy mesophyll layer of chrysanthemum leaves (Parrella and Bethke, 1984; Parrella *et al.*, 1985). In stark contrast to the Californian population, Spencer (1973) observed that *L. huidobrensis* mines occur with “almost equal frequency” in the upper (*i.e.*, palisade mesophyll) and lower (*i.e.*, spongy mesophyll) layers of chrysanthemum leaves in Timotes, Venezuela. Numerous conditions, including high population pressures, could have influenced the mining behavior that Spencer observed in Venezuela, or the Latin American population may actually have a slightly different mining behavior than the Californian population. EPPO (2005) supports Spencer’s observation by stating that *L. huidobrensis* mines undulate between upper and lower leaf surfaces. Additional scientific literature from around the world does not help answer whether different populations exhibit different mining behaviors because most publications either directly (*e.g.*, Weintraub and Horowitz (1996)) or indirectly (*e.g.*, Civelek *et al.* (2004)) regurgitate the findings of Parrella *et al.* (1985).

Female oviposition behavior is another potential difference between the Californian and Latin American populations. Blanchard (1926) wrote in the original description of *L. huidobrensis* from *Cineraria* in Argentina that, “females deposit small white oval eggs in the tissues of the leaf from the underside.” Weintraub and Horowitz (1995) also state that female *L. huidobrensis* deposit eggs into the lower surface of leaves, but it is not clear whether they are citing another researcher’s work, or stating what they observed in an Israeli population that originated from Latin American stock (Scheffer, 2000; Scheffer and Lewis, 2001). In sharp contrast, Parrella and Bethke (1984)

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showed that female *L. huidobrensis* from California make more feeding and oviposition punctures on the upper than lower surfaces of aster, chrysanthemum, and pea leaves. This fact is reflected in their observation that a larval mine “usually begins on the upper surface and moves to the lower surface after a few millimeters” (Parrella and Bethke, 1984). This behavioral difference may be real, or it may be an artifact of observations on different host plants.

Molecular Information

In 2000, Scheffer published a paper that analyzed a 941 base pair segment of the mitochondrial cytochrome oxidase I and II genes from various *L. huidobrensis* populations. Scheffer included specimens from various host plants and various locations (*i.e.*, California, Ecuador, Guatemala, Hawaii, Indonesia, Israel, and Sri Lanka) in this study, and from the results, she suggested that *L. huidobrensis*, as currently defined, contains, at least, two cryptic species. She suggested this because maximum parsimony analysis sorted the DNA sequence data into two well-defined monophyletic clades that differed in sequence divergence by a magnitude found in other agromyzid species; one clade contained sequence data from Californian and Hawaiian specimens, while the other contained sequence data from Latin American and non-American specimens. As mentioned above, Scheffer used a generalized insect mitochondrial DNA molecular clock estimate of 2.3% sequence divergence per million years to estimate that the Californian and Latin American populations diverged about two million years ago.

In 2001, Scheffer and Lewis published a paper that analyzed segments of two nuclear genes (*i.e.*, a 171 base pair segment of β -tubulin and a 921 base pair segment of elongation factor-1 α) from various *L. huidobrensis* populations. They also included specimens from various host plants and various locations (*i.e.*, Argentina, California, Colombia, Ecuador, Guatemala, Hawaii, Indonesia, Israel, Peru, and Sri Lanka) in their study. Again, maximum parsimony analysis sorted the DNA sequence data into two well-defined monophyletic clades (*i.e.*, one clade contained sequence data from Californian and Hawaiian specimens, while the other contained sequence data from Latin American and non-American specimens). Scheffer and Lewis (2001) used these results to formally resurrect the name *Liriomyza langei* for flies belonging to the Californian/Hawaiian clade and restricted the name *L. huidobrensis* to flies belonging to the Latin American clade. They did this because analyses of the sequence data from three independent gene regions (*i.e.*, a 941 base pair segment of the mitochondrial cytochrome oxidase I and II genes, a 171 base pair segment of β -tubulin, and a 921 base pair segment of elongation factor-1 α) all showed deep between clade divergence and low within clade variation, which suggests that the clades represent distinct species.

Isolating Mechanism

In laboratory studies, Reitz and Trumble (2002) observed a higher frequency of homotypic than heterotypic mating in two Californian *L. huidobrensis* populations that do not interbreed in nature (Morgan *et al.*, 2000). Reitz and Trumble (2002) did not find any relationship between the type of mating (*i.e.*, homotypic or heterotypic) and the number of offspring produced, so they concluded that the natural reproductive isolating mechanism must occur prior to mating. Observations of mating behavior in their laboratory colonies indicate that females choose a mate and “aggressively kick” at undesirable males (Reitz and Trumble, 2002). Their data also indicate that females are more likely to mate on certain hosts; homotypic and heterotypic pairs successfully mated more frequently on celery than on pepper (Reitz and Trumble, 2002). Given differences in the known host ranges of Californian and Latin American *L. huidobrensis* populations (see Ecology above) and the many reasons that a female may choose one potential mate over others (see Alcock, 2001), it seems likely that the Californian and Latin American populations may not interbreed if they meet in nature.

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