PLANT–INSECT INTERACTIONS

Preference and Performance of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on Three Citrus Hosts: Laboratory and Field Assessment

LUCÍA GOANE,[1,2] GRACIELA VALLADARES,[3] AND EDUARDO WILLINK[1]

ABSTRACT The relationship between preference and performance is crucial to the ecology and evolution of plant–insect interactions. Oviposition preference and offspring performance were evaluated for a citrus pest, the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), on three of its host plants: lemon (*Citrus limon* L. Burm.), orange (*Citrus sinensis* L. Osbeck), and grapefruit (*Citrus paradisi* Macfadyen) in Tucumán province (northwest Argentina). Choice and no-choice tests were performed in open and enclosed environments, and performance parameters (development time, survival, pupal size, and sex ratio) were estimated from laboratory rearing and 3-yr field sampling data. Parasitism rates were studied in laboratory choice test and field assessments. Preference trends were inconsistent, with lemon receiving more eggs in some tests, whereas no preference was observed in others. Patterns of host use in the field did not show significant differences among species. Leafminer performance, including parasitism and predation rates, was generally homogeneous among host plants. From these results, lemon, orange, and grapefruit seem to represent intrinsically similar resources for *P. citrella* populations in northwest Argentina, a trend that was accompanied by a lack of consistent oviposition preferences in foraging females. Ecological conditions might be more important than physiological adaptation in shaping a probably labile host ranking in this pest species.

RESUMEN La relación entre preferencia y rendimiento es crucial para la ecología y evolución de las interacciones insecto-planta. Se evaluó la preferencia de puesta y el rendimiento de la descendencia para una plaga de cítricos, el minador de la hoja *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), sobre tres de sus plantas hospederas: limonero (*Citrus limon* [Linn.] Burm.), naranjo (*Citrus sinensis* [Linn.] Osbeck) y pomelo (*Citrus paradisi* Macfadyen) en la provincia de Tucumán (Noroeste de Argentina). Se realizaron pruebas con y sin elección en ambientes abiertos y cerrados, y se estimaron parámetros de rendimiento (tiempo de desarrollo, supervivencia, tamaño pupal y proporción de sexos) a partir de individuos criados en laboratorio y datos de tres años de muestreos de campo. Las tasas de parasitismo se estudiaron en pruebas de laboratorio con elección y evaluaciones en campo. Las tendencias de preferencia fueron inconsistentes, con limonero recibiendo más huevos en algunas pruebas, mientras que no se observó preferencia en otras. Los patrones de uso de hospedero en campo no mostraron diferencias significativas entre especies. El rendimiento del minador, incluyendo las tasas de parasitismo y depredación, fue generalmente homogéneo entre plantas hospederas. Con estos resultados, las plantas de limonero, naranjo y pomelo representarían intrínsecamente recursos similares para las poblaciones del minador de los cítricos en el NO de Argentina, tendencia que fue acompañada por la ausencia de preferencias consistentes en la puesta de huevos por parte de las hembras. Las condiciones ecológicas podrían ser más importantes que la adaptación fisiológica al moldear un rango de hospederos probablemente voluble en esta especie plaga.

KEY WORDS citrus leafminer, host selection, tritrophic interactions, *Ageniaspis citricola*, *Cirrospilus neotropicus*

Plants as resources can have an overriding influence on geographic distribution, abundance, and diversity of herbivorous insects (Craig et al. 1986, Preszler and Price 1988, Lewinsohn et al. 2005). Key determinants of resource use in herbivore insects involve behavioral traits that shape host preferences of ovipositing females, as well as physiological traits that determine offspring growth and survival (Courtney and Kibota 1990). Female host choice can carry significant ecological and evolutionary implications for the progeny,

[1] Estación Experimental Agroindustrial Obispo Colombres, Sección Zoología Agrícola, CC 9, Las Talitas (T4101XAC), Tucumán, Argentina.
[2] Corresponding author, e-mail: lgoane@eeaoc.org.ar.
[3] Centro de Investigaciones Entomológicas–IMBIV, Edificio de Investigaciones Biológicas, FCEFYN, Universidad Nacional de Córdoba, Córdoba, Argentina.

and larval performance should have strong feedback on female choice, as stated by the optimal oviposition hypothesis (Thompson 1988). Therefore, insects feeding on a range of plant species tend to show preference hierarchies, preferring to lay eggs on particular species where offspring fitness will be higher (West and Cunningham 2002). Selective pressure on ovipositing females to place eggs on high-quality hosts should be particularly strong for insects like leafminers, which are constrained to feed at the oviposition site (Price 1997).

However, good or poor performance on a plant species need not result from direct interactions between plant and herbivore. Interactions with competitors or natural enemies could also play an important role in herbivore performance on particular plant species, because plants differ not only in nutritional quality but also in their provision of enemy-free space (Stamp 2001, Fritz et al. 2003, Videla et al. 2006). Thus, an herbivore may select plants on which parasitism rates are predictably low even if they are not the most nutritive hosts (Dicke 2000). Selection of oviposition site should therefore reflect the interactions and trade-offs among many ecological factors. Consequently, analyses of performance in the field with the presence of potential competitors, predators, and parasitoids are necessary to fully understand preference–performance relationships (Thompson 1988, Kursar et al. 2006).

The citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was first described in 1856 from India and has since expanded to nearly all citrus-growing regions in the world (Hoy and Nguyen 1997). Females deposit eggs singly on the adaxial and abaxial sides of young leaves. Eggs, measuring only 0.3 by 0.2 mm and looking like tiny droplets of water, hatch in 2–10 d depending on temperature conditions (Knapp et al. 1995). On hatching, four consecutive larval instars feed in the leaf parenchyma, finally forming a pupal chamber from which the adult leafminer emerges. It is a multivoltine species, with total generation time fluctuating between 13 and 52 d depending on temperature (Knapp et al. 1995). Larvae of *P. citrella* feed in the mesophyll beneath the leaf epidermis on a variety of citrus cultivars and many related species in the Rutaceae family, including some ornamentals (Knapp et al. 1995, Jacas et al. 1997). As a result of direct damage caused by the leafminer feeding activity, growth can be slowed on young trees, and the yield can be reduced in mature trees (Peña et al. 2000). Citrus leafminer damage has also been related to an increase in citrus canker (Sohi and Sandhu 1968).

Argentina is the most important lemon (*Citrus limon* L.) producer in the world (Federcitrus 2007). A substantial part of its lemon production (~83%) originates in Tucumán province placed in the northwestern region of Argentina (ATC 2006). Orange (*Citrus sinensis* L.) and grapefruit (*Citrus paradisi* Macfadyen) are also produced in Tucumán, although in smaller proportion (Palacios 2005). Flushing patterns are different for these three citrus species. In north-

west Argentina, the main flush is observed in early spring in all cases, whereas orange and grapefruit show three to four flushes between spring and autumn, lemon flushes are continually present throughout the year. *P. citrella* invaded Argentina in 1995, spreading rapidly throughout commercial citrus growing areas (Willink et al. 1996, Cáceres 2000). Severe damage by this pest has been since reported on lemon, orange, and grapefruit cultivars (Cáceres 2000, Diez et al. 2006). However, published accounts point at grapefruit and orange as the most preferred cultivars for the leafminer in Florida, Australia, and Ecuador (Wilson 1991, Heppner 1993, Bermudez et al. 2004), with severe damages being also reported on lime and grapefruit in Florida (Knapp et al. 1995). These statements, resulting only from field observations, raise the question of whether actual preferences for particular host cultivars exist. Thus far, we know that abundance of *P. citrella* differs between cultivars, but variations in host use as indicated by infestation levels may result from differences in either herbivore preference or performance (Martin et al. 2005, Videla et al. 2006).

In northwest Argentina, parasitoids and predators are an important source of mortality on *P. citrella*, with >20% of immature leafminers being lost to these factors (L.G., unpublished data). Natural enemies could therefore represent another factor affecting infestation levels on different plant hosts. The introduced species *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) and the native *Cirrospilus neotropicus* Diez and Fidalgo (Hymenoptera: Eulophidae) have been reported as the main parasitoids acting on *P. citrella* populations in the region (Fernandez et al. 1999).

The aim of this study was to evaluate preference and performance of *P. citrella* on three citrus hosts in laboratory and field conditions. In addition, parasitism and predation rates were evaluated from field samples and a laboratory choice test to detect possible tritrophic relationships that could influence the interactions between the leafminer and its host plants.

Materials and Methods

Biological Material. All *P. citrella* and parasitoid specimens used in this study were reared at "Estación Experimental Agroindustrial Obispo Colombres" (EEAOC), in Tucumán city, northwest of Argentina. The *P. citrella* colony was initiated from pupae collected on lemon plants in Tucumán province and the *A. citricola* colony from pupae specimens imported from Perú (Willink et al. 1998). Citrus leafminer and *A. citricola* colonies have been maintained since 1997 and 1998, respectively, in a 28-m² greenhouse at 25 ± 5°C, 75 ± 15% RH, and L 16:D 8 photoperiod, on 1- and 2-yr-old citrus plants with 30–50 cm height (Zaia et al. 2004). The rootstocks used for *P. citrella* and *A. citricola* rearing included Volkameriano lemon (*Citrus volkameriana* Pasquale), Rangpur lime (*Citrus limonia* Osbeck), and, less frequently, Cleopatra mandarin (*Citrus reshi* Hort. ex Tanaka) and Swingle citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* L. Raf.).

Thirty seedlings bearing young, 0.5- to 2-cm-long leaves were placed into each of 14 PVC pipe-framed cages (0.80 m wide by 1 m long by 1 m high), and 600 *P. citrella* unsexed moths were added for oviposition. A mixture of honey and water (1:16) was directly sprayed onto the plants as feeding supplement for the moths. Leaves with pupal chambers were collected 15 d later, placed in plastic bags with tissue paper to avoid humidity condensation, and maintained in an indoor rearing chamber at 22 ± 4°C and permanent ligh to accelerate emergence. Adults were collected, using a vacuum-aspirator, into vials with internal walls finely streaked with honey. For *A. citricola* rearing, 600 parasitoid adults (unknown sex ratio) were introduced in each cage when *P. citrella* eggs or first-instar larvae were detected (3–4 d after plants were exposed to leafminers). All leaves were collected 12 d after parasitoid release, and *A. citricola* adults were obtained as described for *P. citrella*. For more specific rearing details, see Zaia et al. (2004).

For the experiments described below, 2-yr-old lemon (*C. limon* cultivar Eureka), sweet orange (*C. sinensis* cultivar Valencia), and white grapefruit (*C. paradisi* cultivar Duncan) potted plants obtained from a commercial nursery were pruned, fertilized with urea N 46% (5 g/liter), and maintained outdoors within a mesh-covered structure to avoid *P. citrella* natural infestations.

Citrus Leafminer Preference: Enclosed Outdoor Experiments. Five PVC pipe-framed cages (0.60 m wide by 0.80 m long by 1.50 m high) with fine polyester mesh covers were placed at the EEAOC in an outdoor shaded area. In the choice test, three potted plants, one of each citrus species, with approximately the same amount of young expanding leaves (flushes), were placed inside each cage, and 150 unsexed *P. citrella* adults (≈2 d old) were released. After 48 h, all leaves of each plant were collected and observed under a stereoscopic microscope to record the number of eggs per leaf. Length and width of all leaves were measured, and the number of eggs per square centimeter was calculated. Number of infested leaves was also considered as an indicator of egg distribution among available resources.

In the no-choice test, one potted seedling per citrus species was placed individually in each cage with 50 *P. citrella* adults, and the procedure above described was followed. For each host plant, three replicates were performed.

Citrus Leafminer Preference: Open Field Experiments. Two potted plants (2 yr old) of each of *C. limon* (cultivar Eureka lemon), *C. sinensis* (cultivar Valencia orange), and *C. paradisi* (cultivar Duncan grapefruit) were simultaneously pruned and planted in lemon, orange, and grapefruit orchards. Experimental plants, thus showing similar flushing states, were planted in spring of 2002, and 200 g/plant of urea (N 46%) was applied after planting. Plants were irrigated when required, and a glyphosate application was made to control weeds. The experiment was conducted during December 2002 in two commercial plantations: Las Salinas and El Ojo (15 km from each other, in Bur-

ruyacú Department, Tucumán Province, Argentina). In Las Salinas, there were ≈2,000 lemon plants (7 yr old), 800 orange plants (9 yr old), and 500 grapefruit plants (6 yr old). There were 2,000 lemon and orange plants and 1,200 grapefruit plants (6, 10, and 8 yr old, respectively) in El Ojo. Plants placed at each orchard were supervised until they started to flush. Fourteen days after new flushes appeared, all young leaves on each plant were pruned and transported to the laboratory, where 25 leaves were randomly chosen for each plant species and orchard, and the number of *P. citrella* eggs was recorded. Because mines are considered the result of viable eggs (Parrella et al. 1983), *P. citrella* mines were included in egg assessments. This procedure was repeated 2 wk later using the regrowth on the same plants, and data from the two dates were combined.

In addition, new flushes of the established trees from the orchards where the trials were carried out were also sampled at the time of the experiment. One flush with five leaves was randomly sampled from each of 10 trees in each orchard to assess the amount of *P. citrella* eggs deposited. Number of mined leaves and number of eggs per leaf were assessed as above.

Citrus Leafminer Preference: Field Sampling. At the EEAOC research farm, an experimental plantation consisting of 28 10-yr-old orange trees, 60 10-yr-old grapefruit plants, and 90 8-yr-old lemon plants was sampled every 2 wk throughout September 2002 to September 2005. One randomly selected flush (9.8 ± 0.3 leaves per flush) from the middle region of 10 trees was collected on each occasion. Samples were placed in plastic bags and transported to the laboratory to observe them under a stereoscopic microscope. All *P. citrella* stages were registered for each leaf. Average number of eggs per leaf and proportion of mined leaves were calculated for each sample season. Proportion of infested leaves at each sampling date was used to compare *P. citrella* population fluctuations on each host throughout the sampling period.

Citrus Leafminer Performance: Greenhouse Experiments. A potted plant was placed in a cage (as described for enclosed outdoor experiments) within a greenhouse (25 ± 5°C, 75 ± 15% RH, 16:8-h light:dark) and exposed to 150 *P. citrella* adults. Four replications were made for each citrus species. Two days after adults were released, 30 eggs per plant were identified, and the remaining eggs were removed from the leaves using a pin to prevent larval competition, leaving up to two eggs per leaf (on opposite leaf sides). Larval development was daily assessed with the aid of a 10× lens. Larval stage duration, overall development time from egg to adult, and percentage survival were recorded. A separate set of leaves containing pupal chambers was examined to evaluate sex proportion following the methodology proposed by Jacas and Garrido (1996), and pupal size was measured under a stereoscopic microscope for female pupae (*n* = 315).

Citrus Leafminer Performance: Field Sampling. Leafminer performance on lemon, orange, and grapefruit was evaluated from the leaves sampled at EEAOC research for preference studies. Number of live and

dead *P. citrella* larvae (four instars) and pupae per leaf were recorded. Only the 16 sampling dates in each season (2002/03, 2003/04, and 2004/05), when all citrus species were simultaneously infested by *P.* citrella, were considered for comparisons. For each sampling season, the number of dead specimens in each life cycle stage was divided by the total number of *P. citrella* individuals to estimate stage-specific mortality rates. Moreover, 50 pupae per citrus species were collected in the EEAOC citrus farm in December 2006, and female pupae were measured in the laboratory under a stereoscopic microscope.

Parasitism and Predation: Parasitoid Choice Test. Five lemon, orange, or grapefruit plants together with 600 *P. citrella* adults were placed in a rearing cage inside a greenhouse (25–35°C, 70–80% RH, 16:8-h light: dark). Four replications were made for each citrus species. After 48 h, *P. citrella* eggs were counted, and three plants (one per citrus species) containing approximately the same number of eggs were transferred to new cages (four in total) into which approximately two unsexed *A. citricola* adults for every *P. citrella* egg were released (between 200 and 400 per cage). After 15 d, leaves containing pupal chambers were collected, and *P. citrella* and parasitoid pupae were counted to estimate parasitism rates by *A. citricola.*

Parasitism and Predation: Field Sampling. Predation and parasitism rates were also compared among *P. citrella* host plants using data from three seasons of fortnightly samples taken at the EEAOC research farm, as described above for preference and performance studies. Numbers of parasitized and predated *P. citrella* were recorded. A leafminer was considered parasitized when endo-parasitoid prepupae or pupae were present within *P. citrella* exoskeleton or inside the leafminer pupal chamber or when eggs, larvae, or pupae of ecto-parasitoids were observed inside the mine or pupal chamber. A leafminer was considered predated when the mine or pupal chamber was empty and showed a small irregular hole or when the mine contained part or the entire leafminer cadaver and showed a large ragged-edge opening. Deaths by host-feeding were not identified and might have been included as "miscellaneous deaths." Parasitized immature of *P. citrella* were isolated in hermetic plastic bags (Ziploc Thai Griptech, Bangkok, Thailand) and placed in a controlled temperature chamber at 22 ± 4°C to obtain parasitoid adults for taxonomic identification. Parasitism rates were calculated from second-instar larvae onward, because parasitized first-instar larvae were too difficult to recognize. Total number of parasitized or predated *P. citrella* divided by total *P. citrella* numbers in each of 16 sampling dates per sampling season (2002/03, 2003/04, and 2004/05) were used to estimate yearly average parasitism and predation rates on lemon, orange, and grapefruit. Parasitism rates by the introduced species *A. citricola* and the native *C. neotropicus* were also separately analyzed.

Data Analysis. In field preference experiments, the χ^2 test was performed on the pooled samples of 50 leaves per citrus species at each orchard and locality to assess whether total frequency of eggs or mined leaves was independent of plant species. Analysis of variance (ANOVA) was used to compare oviposition trends (as egg density per leaf and per area unit, and as number of mined leaves) among species, for the established plants of the orchards where field experiments had been carried out, for results of choice and no choice enclosed tests, and for data from field sampling (yearly average number of eggs per leaf and % mined leaves). ANOVA was also used to compare the various performance estimations of *P. citrella* growing on lemon, orange, and grapefruit as well as parasitism and predation rates. When significant effects were found, differences were identified by Tukey honestly significant difference (HSD) test a posteriori. All percentage data were arcsine square-root transformed before statistical analysis.

Results

Citrus Leafminer Preference. Leaf length by width measurements provided a good and easily obtained indicator of actual leaf area, as shown by regressions against leaf area measurements from digital images (lemon: $r^2 = 0.97$; orange: $r^2 = 0.99$; grapefruit: $r^2 = 0.99$; $P < 0.0001$, $n = 60$ for each citrus host). Consequently, leaf length by width values were used to estimate egg density for preference comparisons. *P. citrella* did not show any clear preference for a particular host plant species in either choice or no choice tests in enclosed experiments (Fig. 1). Lemon, orange, and grapefruit plants showed similar values for number of *P. citrella* eggs per leaf (ANOVA, choice test: $F_{2,12} = 2.14$, $P = 0.16$; no-choice test: $F_{2,6} = 4.08$, $P = 0.07$), eggs per square centimeter (ANOVA, choice test: $F_{2,12} = 0.15$, $P = 0.86$; no-choice test: $F_{2,6} = 0.66$, $P = 0.55$), and percentage infested leaves (ANOVA, choice test: $F_{2,12} = 0.42$, $P = 0.67$; no-choice test: $F_{2,6} = 2.57$, $P = 0.15$).

In the open field experiments, potted lemon plants were preferred over those of orange or grapefruit, independently of the citrus species surrounding the experimental plants (Fig. 2). There were more *P. citrella* eggs on lemon than on orange or grapefruit plants than would be expected from a random distribution in Las Salinas ($\chi^2 = 55.1$, 159.1, and 23.9 in lemon, orange, and grapefruit orchards, respectively; $P < 0.001$ in all cases) and El Ojo ($\chi^2 = 58.2$, 69.0, and 27.5, respectively; $P < 0.001$ in all cases). The frequency of infested leaves was also generally higher for lemon potted plants, but statistically significant differences were only found within lemon ($\chi^2 = 28.6$; $P \leq 0.001$) and orange ($\chi^2 = 28.1$; $P \leq 0.001$) orchards at El Ojo and within the orange orchard at Las Salinas ($\chi^2 = 16.5$; $P \leq 0.001$; Fig. 2). The higher *P. citrella* values observed in potted plants placed at Las Salinas compared with those at El Ojo reflected the pattern observed in the established plants from the orchards in these locations, as can be seen in Fig. 3.

In the established plants from the different orchards where the experiment was run, the apparent prefer-
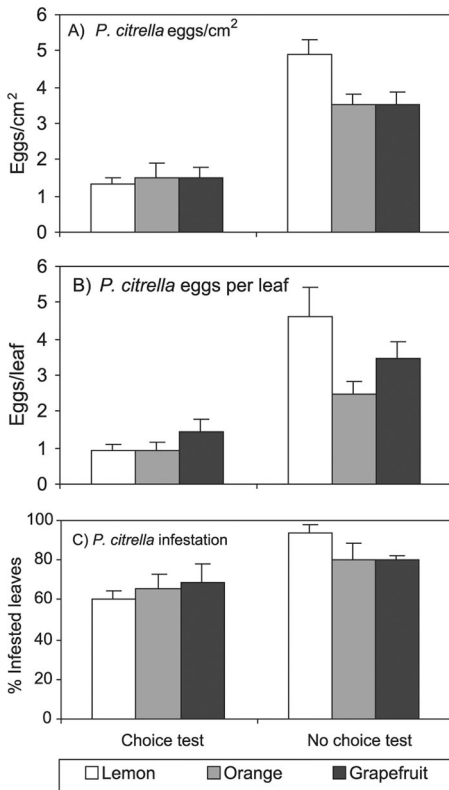
Fig. 1. Average (±SE) number of *P. citrella* eggs per leaf (A), eggs per square centimeter (B), and percentage of infested leaves (C) in lemon (white bars), orange (gray bars), and grapefruit (black bars) potted plants. Results from choice (left) and no-choice (right) tests in enclosed outdoor experiments. In all cases, differences were not significant (ANOVA, $P > 0.05$).

ence for lemon was not found (Fig. 3): at Las Salinas, there were no significant differences among the three citrus species, either considering the average number of eggs per leaf (ANOVA, $F_{2,27} = 0.34$, $P = 0.71$) or the percentage of infested leaves (ANOVA, $F_{2,27} = 1.08$, $P = 0.35$). At El Ojo, grapefruit plants received more eggs than lemon and orange (ANOVA, $F_2 = 8.15$, $P = 0.002$) and showed a higher percentage of infested leaves (ANOVA, $F_{2,27} = 4.28$, $P = 0.02$).

Field samples taken during three sampling seasons, between 2002 and 2005, showed statistically similar average density of *P. citrella* eggs (ANOVA, $F_{2,6} = 1.52$, $P = 0.29$) and percentage of infested leaves (ANOVA, $F_{2,6} = 0.82$, $P = 0.49$) for all three host plant species (Table 1). Infestation of *P. citrella* on each citrus host, as expressed by percentage of infested leaves, showed the same pattern throughout the sampling period (Table 1). Examination of the seasonal cycles on the three host species also showed a strong similarity, although it must be noticed that *P. citrella* infestation continued during winter months on lemon plants, when no infestation was recorded on orange or grapefruit plants (Fig. 4).

Citrus Leafminer Performance. Most performance indicators did not seem to be affected by the plant species on which *P. citrella* larvae were reared in our greenhouse experiments (Table 2). Pupal length, considered as one of those indicators, was independent of the number of pupae per leaf (lemon: $r = -0.17$, $P = 0.10$; orange: $r = 0.05$, $P = 0.73$; grapefruit: $r = 0.09$, $P = 0.53$); therefore, values for all female pupae were included regardless of their growing alone or sharing a leaf. There were no significant differences in larval development time (ANOVA, $F_{2,12} = 0.16$, $P = 0.85$), overall development time from egg to adult (ANOVA, $F_{2,12} = 1.50$, $P = 0.26$), sex ratio (ANOVA, $F_{2,12} = 0.03$, $P = 0.97$), or pupal length (ANOVA, $F_{2,9} = 3.16$, $P = 0.09$) of *P. citrella* reared on lemon, orange, and grapefruit. However, *P. citrella* individuals reared on orange plants suffered significantly higher mortality rates (ANOVA, $F_{2,9} = 5.18$, $P = 0.03$) than those reared on lemon or grapefruit.

The uniform performance of the leafminer, regardless of plant origin, was also noticed in data from field samplings at the EEAOC research farm. There were no significant differences (ANOVA, $F_{2,224} = 2.03$, $P = 0.13$) in pupal size between individuals growing on lemon (2.68 ± 0.02 mm), orange (2.65 ± 0.02 mm), or grapefruit (2.70 ± 0.02 mm). Moreover, *P. citrella* mortality by unknown causes (i.e., not directly attributable to predation or parasitism, and thus probably related to plant factors, although some host-feeding by parasitoids and death by predators might also be involved) did not differ among plant species in field samples (ANOVA, $F_{2,6} = 1.49$, $P = 0.30$), reaching average values of $25.0 \pm 3.8\%$ on lemon, $30.1 \pm 2.9\%$ on orange, and $31.9 \pm 2.2\%$ on grapefruit plants.

Parasitism and Predation. No differences were observed regarding total parasitism (ANOVA, $F_{2,6} = 3.40$, $P = 0.10$) and predation rates (ANOVA, $F_{2,6} = 2.80$, $P = 0.14$) for *P. citrella* growing on lemon, orange, or grapefruit, according to samples taken during three seasons at the EEAOC research farm (Table 1).

The impact of individual parasitoid species on *P. citrella* was also independent of plant host, for the introduced parasitoid *A. citricola* (ANOVA, $F_{2,6} = 0.26$, $P = 0.78$), as well as for the native *C. neotropicus* (ANOVA, $F_{2,9} = 0.85$, $P = 0.48$). Parasitism rates by other parasitoids, *Elasmus phyllocnistoides* Diez, Torréns and Fidalgo and *Galeopsomya fausta* LaSalle, was extremely low and did not differ (ANOVA, $F_{2,9} = 0.23$, $P = 0.80$) among hosts (Table 1).

Greenhouse experiments supported the apparent lack of effect of citrus species on the parasitoid *A. citricola* (ANOVA, $F_{2,9} = 0.78$, $P = 0.49$), because similar rates of parasitism were shown by *P. citrella* individuals reared on lemon (58.8 ± 8.0), orange (69.5 ± 15.1), and grapefruit (47.7 ± 14.5).

Discussion

Oviposition preferences of *P. citrella* among citrus host species varied depending on how such preferences were tested. The most significant differences were obtained from field experiments, in which ovi-
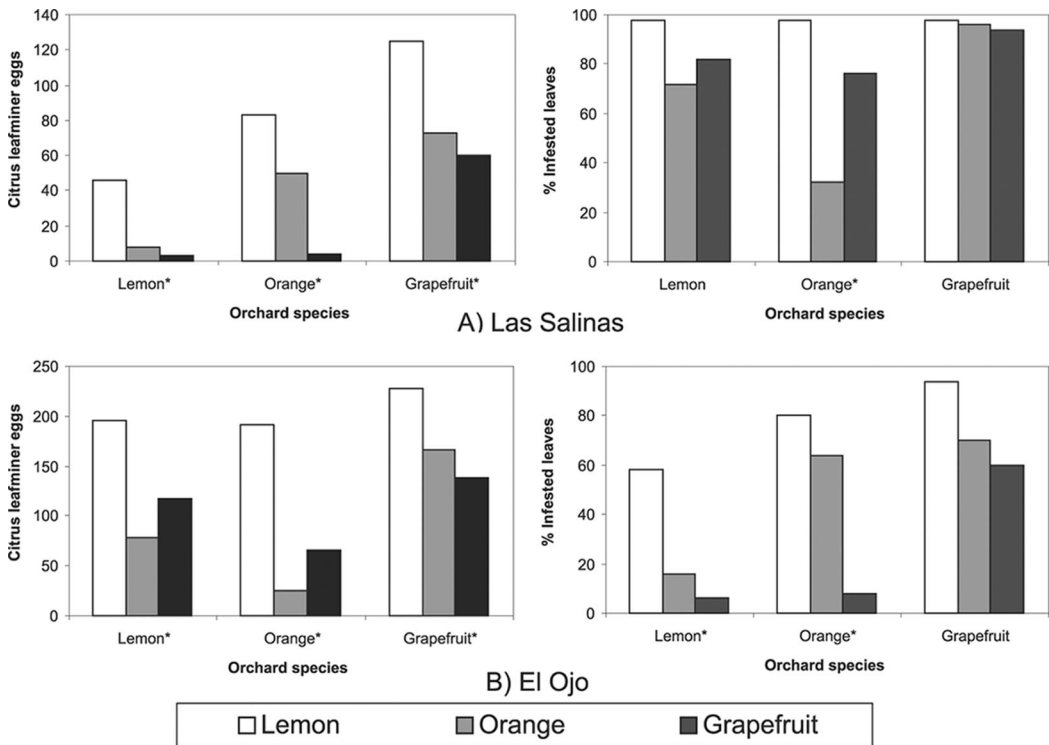
Fig. 2. Total number of *P. citrella* eggs (left) and frequency of mined leaves (right) in lemon (white bars), orange (gray bars), and grapefruit (black bars) potted plants simultaneously offered within lemon, orange, and grapefruit orchards in two locations: Las Salinas (A) and El Ojo (B), Tucumán, Argentina. Results from open field experiments, December 2002. Sample size = 50 leaves/species/orchard. *Data significantly depart from random distribution (χ^2 test, $P < 0.05$).

position was significantly higher on potted lemon than on potted orange or grapefruit plants. Because the same response was observed in orchards of all of the studied citrus species, the apparent preference for lemon should not be attributed to induction, i.e., increased fidelity to the species where the herbivores fed or developed (Bernays and Singer 2002). It must be noticed that infestation values were much higher on the established trees present in the orchards at Las Salinas than at El Ojo and that egg density in the experimental potted plants reflected those differences. The fact that even with such different *P. citrella* population levels, in both locations lemon potted plants received more eggs than the other hosts, increases the credibility of this preference trend.

However, a survey of the established trees in each orchard, coincident in time with the previous experiment, showed a uniform distribution of *P. citrella* eggs among the three citrus species. In one location, there was even higher egg density on grapefruit plants. Host plant choice in the field can be strongly influenced by ecological factors (Tinney et al. 1998), and the apparent preference for grapefruit at El Ojo plantation might be an indirect consequence of differences in environmental conditions, because this grapefruit orchard was the only one under sprinkle irrigation. Water irrigation can improve plant quality or otherwise create more attractive conditions for the leafminer

(Margaix and Garrido 2003). At the EEAOC plantation, under more homogeneous cultivation practices, average egg density from fortnightly samples along three seasons was similar for the three citrus species, although a tendency to lower values on lemon was observed.

The contradictory results from potted plants in field experiments and the surrounding orchard trees could be explained by variation in plant quality throughout phenological development (Smyth et al. 2003), so that only young lemon plants might be more attractive to *P. citrella,* whereas mature plants of all three species could represent more homogeneous resources. Our enclosed outdoor experiments did not support this hypothesis, because no significant preference for lemon was observed despite potted plants being also used in these trials, although a trend toward higher number of eggs on lemon potted plants was observed in no-choice situations. Because insects from the laboratory colony were reared mainly on lemon plants, they might have been conditioned to prefer this host. However, as mentioned for the field assays, *P. citrella* adult females did not show particularly strong affinities for the plant host in which they had developed.

Apparent host preferences have also been shown to differ among tests using different methodologies for other species (Mayhew 1998, Martin et al. 2005), emphasizing the need to use a variety of tests when
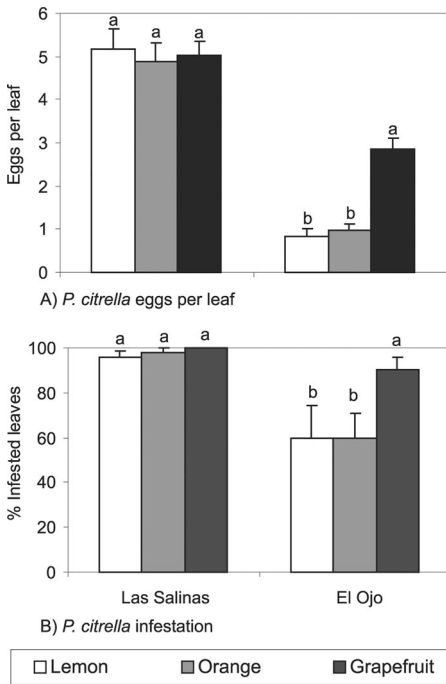
Fig. 3. Average (±SE) number of *P. citrella* eggs per leaf (A) and percentage of infested leaves (B) in established trees of lemon (white bars), orange (gray bars), and grapefruit (black bars) at each location where tests described in Fig. 2 were carried out. Within each group, different letters indicate significant differences (Tukey, *P* < 0.05).

assessing host plant preferences in phytophagous insects. For example, there was overall more oviposition in no-choice tests than in choice ones, which could be attributed to reduced efficiency in a more complex sensory environment (Bernays 1999) and exemplifies the context dependency of insect egg-laying behavior (Yang et al. 2008). By combining results from the different tests performed here, we conclude that females of *P. citrella* showed no sharp preference for any

Table 1. Percentage of infested leaves, no. of eggs/leaf, predation, and parasitism rates (mean ± SE in all cases) for *P. citrella* growing on lemon, orange, and grapefruit plants, from field samples at EEAOC, Las Talitas, Tucumán, Argentina. Values are the average of three seasons with 16 sample dates each (2002/03; 2003/04; 2004/05).

Species	Lemon	Orange	Grapefruit
P. citrella infestation			
Infested leaves (%)	38.4 (±2.3)	42.0 (±2.4)	44.1 (±4.4)
Eggs/100 leaves	28.2 (±9.5)	44.9 (±18.6)	38.8 (±21.7)
P. citrella predation			
Percent individual predated	20.3 (±2.9)	19.9 (±1.2)	24.2 (±2.6)
P. citrella parasitism			
Overall parasitism (%)	11.7 (±0.9)	8.6 (±1.9)	5.7 (±1.7)
By *A. citricola*	3.9 (±0.8)	5.0 (±2.1)	3.3 (±1.4)
By *C. neotropicus*	6.0 (±0.8)	3.0 (±1.1)	1.9 (±0.5)
By others	0.1 (±0.0)	0.1 (±0.0)	0.1 (±0.1)

Differences were not significant in all cases (ANOVA, *P* > 0.05).

of the citrus hosts tested or that preferences might be linked to ecological rather than host-specific factors. Factors that temporally alter plant quality, such as stress, phenology, or even environmental conditions (Cronin and Abrahamson 2001) could explain the inconsistent host ranking of *P. citrella* females. We have also included different measurements of egg distribution on plants, because they may provide different insights in the oviposition preferences. In general, percentage of infested leaves, which is the easiest measurement to obtain, showed very similar patterns to those obtained by counting eggs on individual leaves in our preference tests.

The availability or predictability of suitable age class foliage can be even more important than physical or chemical differences among host species in determining preferences of phytophagous insects (Steinbauer et al. 1998; Kursar et al. 2006). Citrus species show different flushing patterns throughout the year; thus, interspecific differences in temporal availability of young leaves could greatly affect host use by *P. citrella* (Jacas et al. 1997). In our system, although *P. citrella* infestation (as percentage of infested leaves) followed quite similar patterns on each citrus host throughout the year, infestation during winter months was restricted to lemon plants, with no leaves affected by *P. citrella* on either orange or grapefruit plants. Continuous *P. citrella* population development on lemon plants during winter months has also been observed in other temperate regions (Boulahia Kheder et al. 2002). Thus, some degree of preference for lemon trees could be explained by their greater temporal stability as a resource for *P. citrella*.

Resource use by herbivore insect populations is a result of complex interactions between detailed requirements of individual herbivores and biotic and abiotic variables affecting resource availability (Price 1992). Higher infestation levels on orange and grapefruit than on other citrus species have been recorded (Wilson 1991, Knapp et al. 1995, Bermudez et al. 2004), and a similar although nonsignificant trend was observed from our field samples. However, such trends indicate host use rather than preference, because many other factors (egg survival, host abundance, etc.) might be involved. Host preference, i.e., a decision made when resources are presented simultaneously or sequentially, is not necessarily equivalent to host use (Videla et al. 2006) and had not been tested until now for this species. Our tests do not support a *P. citrella* preference for orange or grapefruit as had been proposed on the basis of host use.

Overall, our analysis of fitness indicators suggest that *P. citrella* performs equally well on the three citrus species. Development time, pupal size, and sex ratio were remarkably homogeneous on all three host species in greenhouse experiments, the only exception being the higher *P. citrella* mortality recorded on orange plants. However, such differences must be considered cautiously, because this pattern was not supported by field mortality rates from unknown causes, which are usually considered a response to plant qual-
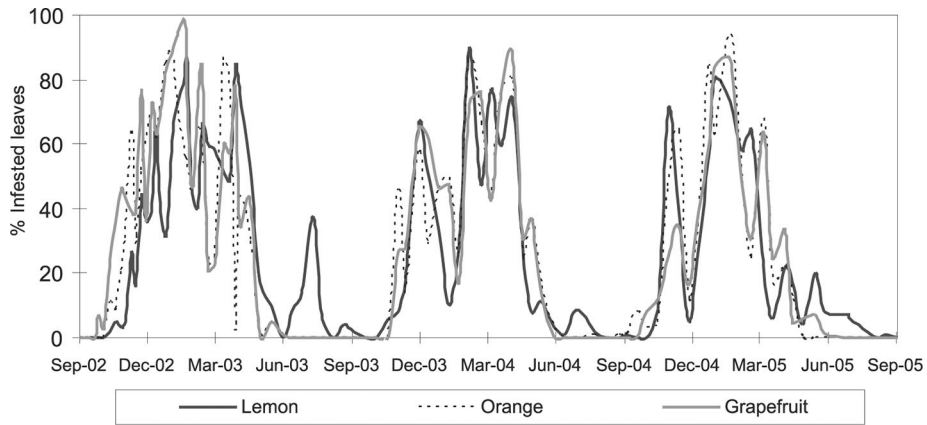
Fig. 4. *P. citrella* infestation levels (% mined leaves) on lemon (black solid line), orange (black dotted line), and grapefruit (gray solid line). Results from field sampling (every 2 wk) from September 2002 to September 2005, EEAOC, Las Talitas, Tucumán.

ity (Valladares and Lawton 1991) and would be the field equivalent to laboratory mortality data.

Herbivores must balance many selection pressures, among which ecological interactions such as predation or parasitism might rank as or even more strongly than intrinsic host plant attributes (Valladares and Lawton 1991, Kursar et al. 2006). However, no differential "enemy free space" (Stamp 2001) seems to be provided by any of the studied plant species, because predation and parasitism rates were similar for *P. citrella* growing on either lemon, orange, or grapefruit leaves.

Given that *P. citrella* is not native to Argentina, interactions with local natural enemies may be still developing, whereas a closer relationship and thus possibly coevolved effects seem more plausible for the introduced parasitoid *A. citricola.* However, no plant-related differences were observed for this parasitoid either from field samples or from laboratory choice tests. Interestingly, as *P. citrella* infestation continued on lemon plants during winter, *A. citricola* and *C. neotropicus* were also found on this plant species. Therefore, lemon plants would be an important resource to ensure population continuity for *P. citrella* and its parasitoids in the studied region.

From these results, lemon, orange, and grapefruit seem to represent intrinsically similar resources for *P. citrella* populations in northwest Argentina, a trend that was accompanied by a lack of consistent oviposition preferences in foraging females. Oviposition behavior and performance of *P. citrella* could be deter-mined by ecological conditions or resource availability rather than by physiological adaptation of larvae to each citrus species. Moreover, the inconsistent preferences shown by *P. citrella* females in our various assessments suggests an evolutionarily labile host order of preference (Carriére 1998, Smyth et al. 2003).

It must be acknowledged that other populations of *P. citrella* might be more selective, because there is evidence of geographic variations in oviposition preferences of phytophagous insects (Gotthard et al. 2004). Also, lability of host preference has been shown to vary among populations, which precludes generalization at the species level (Messina 2004). Finally, different preference–performance patterns might be detected if other citrus species are considered, because host ranking might vary depending on the options available (Martin et al. 2005). Further studies including other citrus species and different *P. citrella* populations are needed for a deeper understanding of host preference and performance in this pest species.

Acknowledgments

Table 2. Estimation of *P. citrella* performance on lemon, orange, and grapefruit in greenhouse experiments. Five replicates, each with ≥30 *P. citrella* eggs per plant. Data shown as mean (±SE).

Species	Larval development time (d)	Overall development time (d)	Mortality percentage (%)	Pupal size (mm)	Sex proportion (females)
Lemon	5.5 (±0.1) a	17.4 (±0.1) a	14.26 (±3.89) b	2.70 (±0.06) a	0.68 (±0.03) a
Orange	5.5 (±0.1) a	17.1 (±0.1) a	31.10 (±4.14) a	2.59 (±0.07) a	0.68 (±0.04) a
Grapefruit	5.6 (±0.0) a	17.6 (±0.1) a	12.27 (±1.73) b	2.77 (±0.02) a	0.70 (±0.02) a

Different letters within a column indicate significant differences (Tukey, $P < 0.05$).

References Cited

[ATC] Asociación Tucumana del Citrus. 2006. Tucumán: Datos del censo citrícola 2005. (http://www.atcitrus.com/noticia.asp?seccion=izquierda&id=106).

Bermudez, E. C., N. B. Martínez, J. V. Graziano, H.C.A. Bernal, and A. H. Paniagua. 2004. *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in citrus in Ecuador. Fla. Entomol. 87: 10–17.

Bernays, E. A. 1999. When host choice is a problem for a generalist herbivore: experiments with the whitefly *Bemisia tabaci.* Ecol. Entomol. 24: 260–267.

Bernays, E. A., and M. Singer. 2002. Contrasted foraging tactics in two species of polyphagous caterpillars. Acta Zool. Acad. Sci. Hung. 48: 117–135.

Boulahia Kheder, S., A. Jerraya, F. Jrad, and M. Fezzani. 2002. Étude de la mineuse des agrumes *Phyllocnistis citrella* Stainton (Lep. Gracillariidae) dans la région du Cap Bon (Tunisie). Fruits 57: 29–42.

Cáceres, S. 2000. The citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its native parasitoids in Corrientes (Argentina). International Society Citriculture Congress 2000, Orlando, FL.

Carriére, Y. 1998. Constraints on the evolution of host choice by phytophagous insects. Oikos 82: 401–406.

Courtney, S. P., and T. T. Kibota. 1990. Mother doesn't know best: selection of hosts by ovipositing insects, pp. 161–188. *In* E. A. Bernays (ed.), Plant-insect relationships vol. 2. CRC, Boca Raton, FL.

Craig, T. P., P. W. Price, and J. K. Itami. 1986. Resource regulation by a stem-galling sawfly on the arroyo willow. Ecology 67: 419–425.

Cronin, J. T., and W. G. Abrahamson. 2001. Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. Oecologia (Berl.) 127: 87–96.

Diez, P. A., J. E. Peña, and P. Fildalgo. 2006. Population dynamics *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in Tafí Viejo, Tucumán, Argentina. Fla. Entomol. 89(3): 328–335.

Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Biochem. Syst. Ecol. 28: 601–617.

Federcitrus (Argentine Citrus Federation). 2007. Argentina: the South America citrus land. (http://www.federcitrus.org/english/index.htm).

Fernandez, R., L. Chiggia, P. Fidalgo, A. Jaime De Herrero, P. Diez, and E. Willink. 1999. Parasitoids of *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae) and its distribution in citric ecosystems of Tucumán, Argentina. Resúmenes X Jornadas Fitosanitarias Argentinas, S. S. de Jujuy. 1999: 248.

Fritz, R. S., B. A. Crabb, and C. G. Hochwender. 2003. Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. Oikos 102: 601–613.

Gotthard, K., N. Margaçraf, and M. Rahier. 2004. Geographic variation in oviposition choice of a leaf beetle: the relationship between host plant ranking, specificity and motivation. Entomol. Exp. Appl. 110: 217–224.

Heppner, J. B. 1993. Citrus leafminer, *Phyllocnistis citrella,* in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). Trop. Lepid. 4: 49–64.

Hoy, M. A., and R. Nguyen. 1997. Classical biological control of the citrus leafminer *Phyllocnistis citrella* Stainton (Lep-

idoptera: Gracillariida): theory, practice, art and science. Trop. Lepid. 8: 1–19.

Jacas, J. A., and A. Garrido. 1996. Differences in the morphology of male and female pupae of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Fla. Entomol. 79: 603–606.

Jacas, J. A., A. Garrido, C. Margaix, J. Forner, A. Alcalde, and J. A. Pina. 1997. Screening of different citrus rootstocks and citrus-related species for resistance to *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Crop Prot. 16: 701–705.

Knapp, J. L., L. G. Albrigo, H. W. Browning, R. C. Bullock, J. B. Heppner, D. G. Hall, M. A. Hoy, R. Nguyen, J. E. Peña, and P. A. Stansly. 1995. Citrus leafminer, *Phyllocnistis citrella* Stainton: current status in Florida. Fla. Coop. Ext. Serv., IFAS, Univ. Florida, Gainesville, FL.

Kursar, T. A., B. T. Wolfe, M. J. Epps, and P. D. Coley. 2006. Food quality, competition and parasitism influence feeding preference in a neotropical lepidopteran. Ecology 87: 3058–3069.

Lewinsohn, T. M., V. Novotny, and Y. Basset. 2005. Insects on plants: diversity of herbivore assemblages revisited. Annu. Rev. Ecol. Evol. Syst. 36: 597–620.

Margaix, C., and A. Garrido. 2003. The effect of water irrigation on the *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)'s population dynamic. Bol. San. Veg. Plagas. 29: 149–158.

Martin, A. D., D. Stanley-Horn, and R. H. Hallett. 2005. Adult host preference and larval performance of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on selected hosts. Environ. Entomol. 34: 1170–1177.

Mayhew, P. J. 1998. Testing the preference-performance hypothesis in phytophagous insects: lessons from *Chrysanthemum* leafminer (Diptera: Agromyzidae). Environ. Entomol. 27: 45–52.

Messina, F. J. 2004. How labile are the egg-laying preferences of seed beetles? Ecol. Entomol. 29: 318–326.

Palacios, J. 2005. Origen de los agrios: producción mundial de cítricos. Citricultura, Tucumán, Argentina.

Parrella, M. P., K. L. Robb, and J. A. Bethke. 1983. Influence of selected host plants on the biology of *Liriomyza trifolii* (Diptera: Agromyzidae). Ann. Entomol. Soc. Am. 76: 112–115.

Peña, J. E., A. Hunsberger, and B. Schaffer. 2000. Citrus leafminer (Lepidoptera: Gracillariidae) density: effect on yield of "Tahiti" lime. J. Econ. Entomol. 93: 374–379.

Preszler, R. W., and P. W. Price. 1988. Host quality and sawfly populations: a new approach to life table analysis. Ecology 69: 2012–2020.

Price, P. W. 1992. Plant resources as the mechanistic basis for insect herbivore population dynamics, pp. 139–172. *In* M. Hunter, T. Ohgushi, and P. Price (eds.), Effects of resource distribution on animal plant interactions. Academic, New York.

Price, P. W. 1997. Insect ecology, 3rd ed. Wiley, New York.

Smyth, R. R., M. P. Hoffman, and A. M. Shelton. 2003. Larval performance in relation to labile oviposition preference of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) among phenological stages of cabbage. Environ. Entomol. 32: 765–770.

Sohi, G. C., and M. S. Sandhu. 1968. Relationship between citrus leafminer (*Phyllocnistis citrella* Stainton) injury and citrus canker (*Xanthomona citri* (Hassen) Dowson) incidence on citrus leaves. L. Res. Punjab Agri. Univ. 5: 66–69.

Stamp, N. 2001. Enemy-free space via host plant chemistry and dispersion: assessing the influence of tri-trophic interactions. Oecologia (Berl.) 128: 153–163.

Steinbauer, M. J., A. R. Clarke, and J. L. Madden. 1998. Oviposition preference of a Eucalyptus herbivore and the importance of leaf age on interspecific host choice. Ecol. Entomol. 23: 201–206.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. Exp. Appl. 47: 3–14.

Tinney, G. W., P. E. Hatcher, P. G. Ayres, N. D. Paul, and J. B. Whittaker. 1998. Inter- and intra- species differences in plants as hosts to *Tyria jacobeae.* Entomol. Exp. Appl. 88: 137–145.

Valladares, G., and J. H. Lawton. 1991. Host-plant selection in the holly leaf-miner: does mother know best?. J. Anim. Ecol. 60: 227–240.

Videla, M., G. Valladares, and A. Salvo. 2006. Tritrophic interactions of host preference and performance in a polyphagous leafminer. Entomol. Exp. Appl. 121: 105–114.

West, S. A., and J. P. Cunningham. 2002. A general model for host plant selection in phytophagous insects. J. Theor. Biol. 214: 499–513.

Willink, E., H. Salas, and M. A. Costilla. 1996. El minador de la hoja de los cítricos, *Phyllocnistis citrella* en el NOA. Avance Agroindustrial 16: 15–20.

Willink, E., D. Figueroa, P. Zamudio, and H. Salas. 1998. Control biológico del minador de la hoja de los cítricos. Avance Agroindustrial 18: 14–18.

Wilson, C. G. 1991. Notes on *Phyllocnistis citrella* Stainton (Lepidoptera : Phyllocnistidae) attacking four citrus varieties in Darwin. J. Aust. Entomol. Soc. 30: 77–78.

Yang, C., P. Belawat, E. Hafen, L. Jan, and Y. Jan. 2008. *Drosophila* egg-laying site selection as a system to study simple decision-making processes. Science 319: 1679–1683.

Zaia, G., E. Willink, and D. Figueroa. 2004. Metodología para la cría de *Ageniaspis citricola* y *Citrostichus phyllocnistoides*, parasitodes del "minador de hoja de los cítricos", *Phyllocnistis citrella.* Rev. Ind. Agríc. Tucumán 81: 39–46.