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Authors: Cañarte Bermúdez, Ernesto, Bautista Martínez, Néstor, Vera Graziano, Jorge, Arredondo Bernal, Hugo César, and Huerta Paniagua, Antonio

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PHYLLOCNISTIS CITRELLA (LEPIDOPTERA: GRACILLARIIDAE) AND ITS PARASITOIDS IN CITRUS IN ECUADOR

ERNESTO CAÑARTE BERMÚDEZ[1], NÉSTOR BAUTISTA MARTÍNEZ[2], JORGE VERA GRAZIANO[2], HUGO CÉSAR ARREDONDO BERNAL[3], AND ANTONIO HUERTA PANIAGUA[2]
[1]Instituto Nacional Autónomo de Investigación Agropecuaria, Casilla postal 100
Estación Experimental Portoviejo. Portoviejo-Ecuador

[2]Instituto de Fitosanidad. Colegio de Postgraduados. Montecillo, Texcoco, México 56230

[3]Centro Nacional de Referencia de Control Biológico. Km 1.5 Carr. Tecomán-Estación FFCC. Tecomán, Col. 28120

ABSTRACT

The objectives of this study were to determine the population fluctuations of the citrus leafminer, *Phyllocnistis citrella*, and its parasitoids in three locations of Ecuador, to identify and determine the geographic distribution of *P. citrella* parasitoids within Ecuador, and to establish which of eleven citrus species supported higher numbers of *P. citrella* and its parasitoids. The highest population density of *P. citrella* occurred during the dry season. The highest infestations in three localities were in Lodana (43.8%) in October, in Riochico (45.7%) in November, and in La Unión (17.3%) in December. The greatest percentages of parasitism occurred in Lodana in March (60%), in Riochico in January (18.9%), and La Unión in December (50%). The species *Ageniaspis citricola* Logvinovskaya, *Galeopsomyia* sp., and *Elasmus tischeriae* Howard were identified with 28.4, 2.2, and 0.07% parasitism, respectively. Although this is the first report of *A. citricola* in Ecuador, it is widely distributed in the main citrus producing zones of the country. Orange and grapefruit yielded higher numbers of citrus leafminers and their natural enemies than other citrus species.

Key Words: citrus leafminer, *Phyllocnistis citrella*, Gracillariidae, biological control

RESUMEN

Se determinó el porcentaje de infestación de el minador de hoja de los cítricos, *Phyllocnistis citrella* y, sus parasitoides; se identificó y documentó la distribución geográfica de los parasitoides de *P. citrella* y se estableció la preferencia de *P. citrella* y sus parasitoides a once especies de cítricos. La mayor densidad poblacional de *P. citrella* se presentó durante la época seca, observándose en Lodana la infestación más alta en octubre con 43.8%; en Riochico en noviembre (45.7%) y en La unión en diciembre con 17.4%. El parasitismo se presentó con mayor intensidad en Lodana en marzo con 60%, en Riochico en enero (18.9%) y La Unión con 50% en diciembre. Se identificaron las especies *Ageniaspis citricola* Logvinovskaya, *Galeopsomyia* sp. y *Elasmus tischeriae* con 28.4. 2.2 y 0.07% de parasitismo, respectivamente. Siendo este el primer reporte de *A. citricola* en Ecuador, ampliamente distribuido en las principales zonas citrícolas del país. Se denota cierta preferencia de *P. citrella* y sus parasitoides hacia las especies de naranja y pomelos.

Translation provided by author

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, is a pest of plants in the Rutaceae family. CLM mine leaves, surface tissue of young shoots and stems, and less frequently the fruit (Sponagel & Díaz 1994). The lamina of mined leaves dries and rolls, reducing leaf area and reducing photosynthetic activity of the plant.

CLM is native of Asia (Knapp et al. 1995). Today, it is found in nearly all citrus-growing regions of the world. In America it was reported for the first time in 1993 in Florida, U.S.A. (Heppner 1993), and later in different regions of the U.S.A. In Ecuador, it was reported in 1995 in the province of Manabí (INIAP 1995, 1996; Valarezo & Cañarte 1997). In a period of six months CLM invaded almost the entire Ecuadorian coast and later the interior regions. Infestations of 97.14% were observed in Manabí, and it was estimated that numbers of fruits of West Indies lime, (*Citrus aurantifolia* (Christmann) Swingle) decreased up to 45% and yield decreased 48% (Valarezo & Cañarte 1998).

Several different insecticides are used against this pest, but these may involve undesirable effects on the environment, including interference in control of the pest by natural enemies (Guerra et al. 1997). Biological control is the best option for controlling this pest (Peña 1997).

In many areas a reduction in the pest population has been observed because of the presence of a diversity of natural enemies. However, activity of

these natural enemies is variable and their value as a factor in the regulation of CLM populations differs in different geographical areas. This makes it necessary to determine the effect of climate and natural enemies on populations of the pest to establish their real value as CLM regulators.

Thirty-nine species of Hymenoptera have been observed attacking CLM in its native area (Heppner 1993). Most of them are Eulophidae, but also Encyrtidae, Elasmidae, Eurytomidae and Pteromalidae have been reported. In America, seven parasitoids have been reported in Honduras, seven species in Colombia, the same number in Cuba (González et al. 1995), and eight species have been reported each in Florida and Mexico (Perales et al. 1997).

The objectives of this study were to determine the population fluctuations of *P. citrella* and its parasitoids in three locations of Ecuador, to identify and determine the geographic distribution of *P. citrella* parasitoids within Ecuador, and to establish which of eleven citrus species *P. citrella* and its parasitoids prefer.

Materials and Methods

Phyllocnistis citrella and parasitoid population fluctuations were determined from September 2000 to March 2001 in Lodana, Riochico, and La Unión in the province of Manabí, Ecuador. Each location represents a different type of management system. In Lodana, leaves infested with CLM were collected every 10 days in a four-hectare West Indies lime orchard in which only neem (aqueous extract and oil) was applied. In Riochico, leaves were collected weekly in a 12-hectare West Indies lime orchard in which synthetic organic pesticides were used. In La Unión, leaf collection was done every 15 days in a five-hectare coffee (*Coffea arabica* L.) plantation in which sweet orange (*C. sinensis* (L.) Osbeck) and mandarin orange (*C. reticulata* Blanco), were planted at 50-80 trees/ha. For the samples, mandarin orange trees were selected. In this system no pesticides were applied.

Percent infestation of CLM in the three sites was determined by selecting 10 trees and collecting from them 60 lime shoots in Lodana, 20 in Riochico and 20 in La Unión. These shoots were no larger than 20 cm. Total number of leaves and mined leaves were counted on each shoot, and the percentage of infestation was calculated.

For percent parasitism of CLM, 50 leaves were collected from 10 trees (five leaves per tree) in each location. The leaves from the middle and lower thirds of the trees were selected from developed shoots (15 to 20 cm) with third instars and pupal chambers of CLM.

The evaluations of emergence of CLM or its parasitoids were carried out daily for 22 days. Leaves were checked to record parasitoids that did not emerge due to the effect of management, premature drying of leaves, or abiotic factors. The recovered parasitoid pupae were confined in trays with moistened cotton until emergence of the adults.

For the geographic distribution of the parasitoids of *P. citrella* in Ecuador, collections were done in 38 sites of 18 municipalities of the provinces of Manibí, Guayas, and Los Ríos (coastal region), Loja and Azuay (mountain region), and the Napo province (eastern region) (Fig. 1).

In each of the 38 sites, 100 leaves with CLM third instars or pupal chambers were collected once. The leaves were placed in transparent plastic bags 30.4 × 25.2 cm lined with absorbent paper to maintain the moisture necessary for the miner, or its parasitoids, to emerge. Twenty-five leaves were placed in each bag; bags were inflated, sealed with rubber bands, and hung with cords inside the greenhouse until the parasitoids were recovered. The parasitoids were identified with the keys of Schauff and La Salle (1996).

To determine the preference of *P. citrella* and its parasitoids for different species of citrus, 11 species of the national collection of citrus at the Portoviejo Experimental Station were evaluated. For each sample, 20 shoots per citrus species and around 100 leaves with CLM third instars and pupal chambers were collected; these data were used to calculate the percentage of parasitism.

Results and Discussion

Percent Infestation of *P. citrella* and its Parasitoids

On 6881 leaves examined, it was observed that in Lodana in October, December, and February,

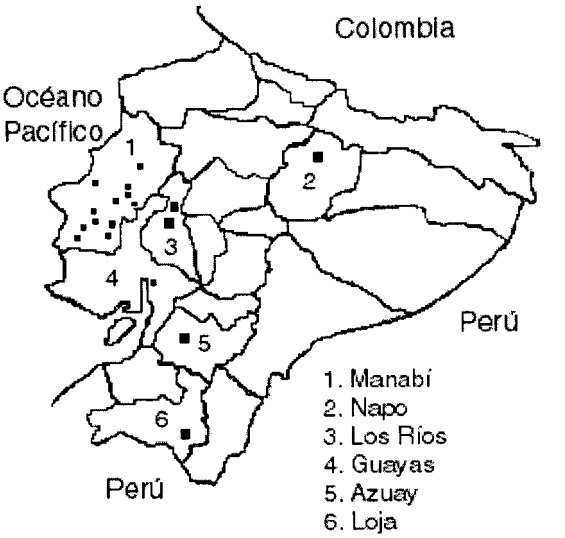

Fig. 1. Municipalities where collections were done to determine the presence of *P. citrella* parasitoids in Ecuador, 2000.

there were slightly higher percentages of infestation than on other dates (43.8%, 30.4%, and 38.5%, respectively). During these months there were periods when sprouting was 100% (Fig. 2), coinciding with the months of greatest *P. citrella* infestation. Similar results were found by Curt-Díaz et al. (1998) and Valarezo and Cañarte (1997). Parasitism of 58.7% was found in November, 53% in February, and 60% in March.

In Riochico, the highest values of infestation (45.7%, 40.8%, and 45.5%, respectively) were observed in September, October, and November. Flushing was quite uniform and not higher than 50% in any month. Flushing in the dry months of September, October, and November was 49.4%, 49.2%, and 47.2%, respectively, i.e., slightly high, while in the rainy season, (January to March) flushing decreased (Fig. 3). This reduction in flushing and infestation in the citrus of this location during the rainy season is possibly associated with the fact that during this period of precipitation diseases such as anthracnose (*Gloeosporium limetticola* R. E. Clausen) appear. These diseases cause shriveling of almost all of the tender shoots, or deform the tissue, interrupting normal development of the insect. As a result, the percentages of these variables fall substantially, and more so when growers do not use any type of control against the disease. This is consistent with the results of studies done by Robles and Medina (1997).

Parasitism was low, possibly due to the growers' frequent applications of pesticides. The highest percentage of parasitism, 18.9%, was observed in January. This is compatible with observations of Nuñez and Canales (1999) and Probst et al. (1999), in terms of the lower values of parasitism when insecticides are applied frequently.

In La Unión, in spite of the high increase in sprouting, no increase was observed in CLM from January to March; in fact, there was a decrease in population density, with the highest value in December with infestation of 17.4%. During the dry months, September to December, constant sprouting of about 25% was observed in the citrus. With the rainy season (January), sprouting intensity increased significantly, reaching 75% in February and 100% in March of the same year (Fig. 4). This is due to the fact that the citrus trees are grown in association with coffee, and physiologically have only one major sprouting period per year, which is activated with the first rainfalls in January. In December there was greater parasitism (50%), coinciding with the date of greatest infestation by CLM.

The low CLM population densities are possibly due to the combined action of biological regulators that exert natural control of the pest under this system of production (Mendoza 1995; Castaño 1996; Valarezo & Cañarte 1998; Nuñez y Canales 1999). It is clear that the action of beneficial organisms is favored by the fact that in this region no pest control measures are carried out in the coffee-citrus plantations.

Comparing the means, it can be seen that parasitism is different among the locations. The highest was in Lodana (47.6%), followed by La Unión (25.7%), and Riochico (11.6%). Pesticide

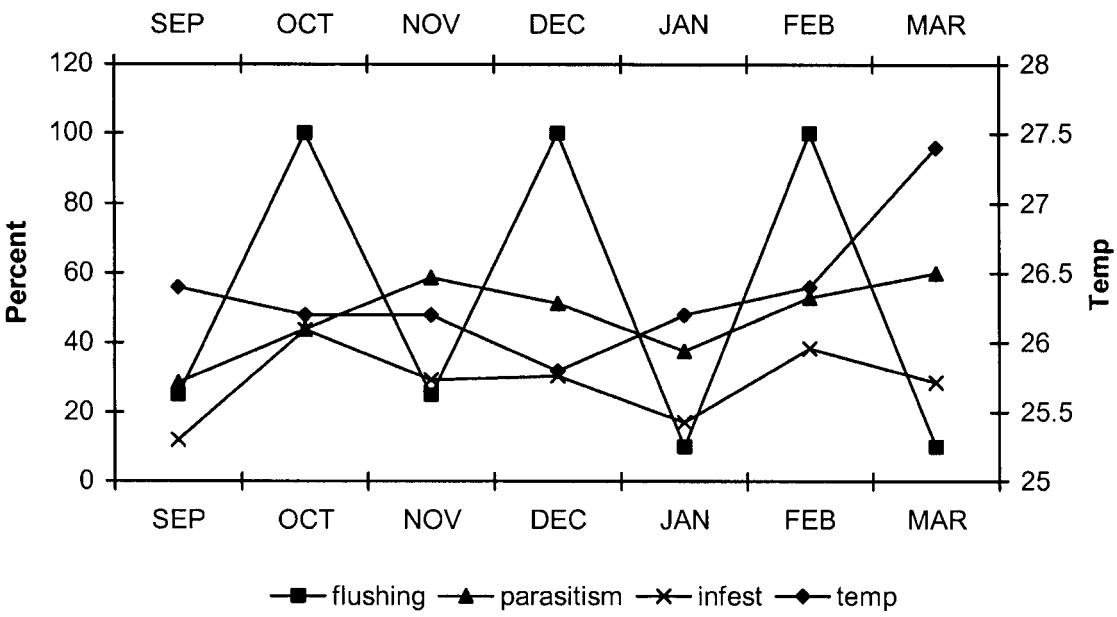

Fig. 2. Percent infestation of *P. citrella* and its parasitoids, and sprouting in lime in Lodana Ecuador. 2000-2001.

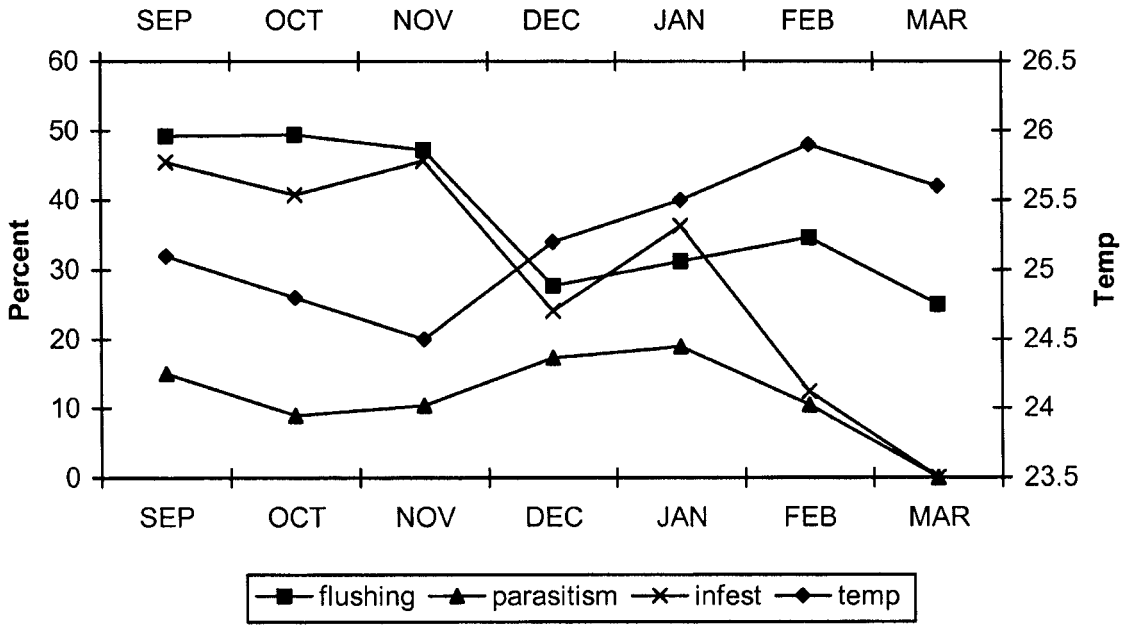

Fig. 3. Percent infestation of *P. citrella* and its parasitoids and sprouting in lime in Riochico, Ecuador. 2000-2001.

applications were different in each location. In Lodana, during the 2000-2001 period, insecticide (cypermethrin) was applied only once. In Riochico, insecticides were applied every two weeks or monthly, using diverse synthetic organic insecticides, natural substances, and mineral oils, containing at least 10 different active ingredients. In La Unión, no control measures were carried out. This suggests that pest management methods may influence the percentage of parasitism.

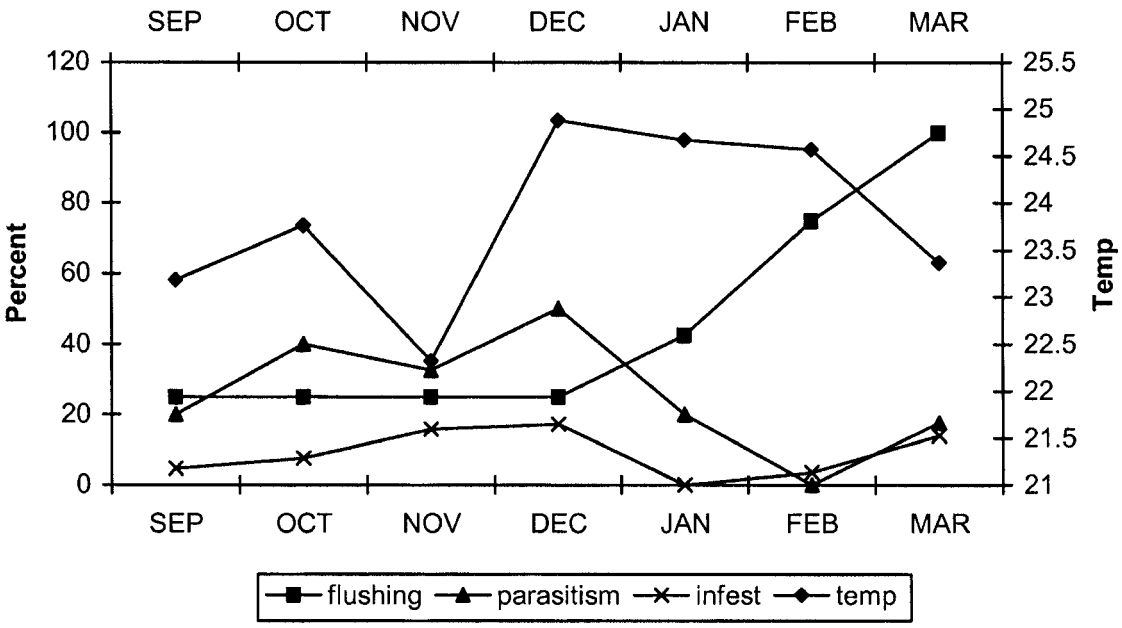

Fig. 4. Percent infestation of *P. citrella* and its parasitoids, and sprouting in mandarin orange in La Unión, Ecuador. 2000-2001.

It is assumed that the parasitoid *Ageniaspis citricola* Logvinovskaya arrived in Ecuador from Peru, where it was introduced in 1996 as part of a national program of classical biological control of CLM. Most of the parasitoids were released in Peruvian citrus-producing zones, including the border towns of Tumbes and Piura. Rates of parasitism here reached 98% (Nuñez & Canales 1999). Based on the presence of *P. citrella* in the 18 municipalities near the border (Fig. 1), a possible route of entry of the parasitoid was through the border province of Loja (Ecuador), and it advanced northwards through Azuay, Guayas, Manabí, Los Rios, and finally Napo.

Geographic Distribution of Parasitoid Species of *P. citrella* in Ecuador

A total of 4388 leaves infested with CLM were analyzed to determine the presence of parasitoids in the coastal, mountain and eastern regions. The following species were identified: *Ageniaspis citricola* (Hymenoptera: Encyrtidae), *Galeopsomyia* sp. (Hymenoptera: Eulophidae), and *Elasmus tischeriae* Howard (Hymenoptera: Elasmidae). The latter two also were reported in Colombia (Castaño 1996). The predominant species was *A. citricola* (Table 1). As this is the first record of this parasitoid in Ecuador, its discovery has important implication for the country's citrus production. Parasitism observed for this parasitoid varied between 13.3% and 79.3%. It is important to note the presence of *A. citricola* in the eastern region; since between the coast and this region there is a natural barrier, viz, the Andes Mountains. Movement of plant material apparently spreads the pest.

Ageniaspis citricola is a highly effective parasitoid of *P. citrella*, achieving 28.4% mean parasitism throughout Ecuador, while in one of the zones 79.3% of the leafminers were parasitized by this species (Table 1). Rates of parasitism of *P. citrella* have been reported as 80% in Florida, U.S.A. (Medina et al. 1997), and 100% in Australia (Peña 1997). In Ecuador, *A. citricola* is the predominant species, relative to other species, which had low incidences. Valarezo and Cañarte (1998) reported that in Ecuador *Elasmus* sp. was the most widely

TABLE 1. GEOGRAPHIC DISTRIBUTION OF PARASITOIDS OF *PHYLLOCNISTIS CITRELLA* IN ECUADOR AND PERCENTAGE OF PARASITISM.

Province/Municipality	Leaves analyzed	% parasitism			Total parasitism
		A. citricola	*Galeopsomyia sp.*	*E. tischeriae*	
Manibí Province (coastal region)					
Portoviejo	1825	18.31	2.45	0.09	20.85
Santa Ana	715	45.97	6.48	0.00	52.45
Jipijapa	490	18.08	3.15	0.00	21.23
Pichincha	187	29.56	0.87	0.00	30.43
Chone	154	27.89	0.53	0.00	28.42
Pajan	139	35.88	1.18	0.00	37.06
Junín	110	13.34	2.96	0.00	16.30
Flavio Alfaro	97	13.33	0.00	0.00	13.33
Olmedo	86	20.95	0.00	0.00	20.95
Bolívar	83	28.43	0.00	0.98	29.41
24 de Mayo	82	50.00	0.00	0.00	50.00
Sucre	70	79.31	6.90	0.00	86.21
Guayas Province (coastal region)					
Municipio Guayaquil	76	25.81	0.00	0.00	25.81
Los Ríos Province (coastal region)					
Quevedo	81	14.00	2.00	0.00	16.00
Buena Fé	85	17.14	2.86	0.00	20.00
Loja Province (mountain region)					
Municipio Malacatos	5	—	—	—	—
Azuay Province (mountain region)					
Santa Isabel	5	—	—	—	—
Napo Province (Eastern region)					
Coca	98	16.67	5.00	0.00	21.67
Mean		28.42	2.15	0.07	30.63

TABLE 2. SPECIES OF PARASITOIDS OF *P. CITRELLA* AND THE DEVELOPMENTAL STAGES THEY ATTACK.

Parasitoid	Biological stage attacked
Ageniaspis citricola Logvinovskaya	Eggs, larva I
Galeopsomyia sp1 Girault	Larva II, III, prepupa and pupa
Elasmus tischeriae Howard	Larva II, III and pupa

distributed species, followed by *Horismenus* and *Galeopsomyia*, which are present in five, four and two of the nine municipalities studied, respectively. Table 2 presents the developmental stages that are attacked by the parasitoids.

The success of *A. citricola* as a parasitoid of *P. citrella* can be explained by its specific and gregarious character, which makes it more efficient (Hoy & Nguyen 1994), and more competitive (Nuñez & Canales 1999), compared with the native generalist species. Native parasitoids survive on alternate hosts, such as leafminers of other cultivated plants or weeds, as in the case of *Leucoptera coffella* Guerin in coffee (Mendoza 1995; Bautista et al. 1997; Bautista et al. 1998; Valarezo & Cañarte 1998). This characteristic would explain the fact that, during this study, only two of the 11 species that had been reported up to 1998 were found (INIAP 1996a; Valarezo & Cañarte 1998).

Since, from the beginning of the study, the predominance of *A. citricola* in Londana, Ríochico, and La Unión, Manibí, was evident, the number of parasitoids present in each CLM pupa chamber was quantified. On 2503 infested leaves, it was determined that 69.3% of the chambers had three pupae of *A. citricola*, 23.14% had two pupae, and 7.6% had one, four or five pupae. Occasionally, up to six pupae of *A. citricola* per CLM chamber were observed, still within the range reported by Nuñez and Canales (1999), who found between two and nine pupae arranged like "sausages."

Preferences of *P. citrella* and its Parasitoids for Eleven Citrus Species

Between 50% and 75% of the plants in the National Citrus Collection at the time of evaluation were flushing. Infestation data seem to denote a certain preference of CLM for some species and varieties. It can be seen in Table 3 that Washington navel oranges (naranja 'Washington navel'), and red and white grapefruit (pomelo 'rojo' y 'blanco') have slightly higher rates of infestation (37.4%, 35.1%, and 33.1%, respectively). Several authors have mentioned a differential behavior of CLM toward certain species of citrus. In this regard, González et al. (1995) report that navel oranges are more susceptible than other types of citrus. This greater susceptibility of some varieties also could be related to leaf size (Zhang et al. 1994) as well as to their thickness and consistency (Latif & Yunnes 1951). Singh and Azam (1986) contend that miners prefer more succulent leaves with a thin cuticle. In this study, the difference in parasitism among the species was marked, with very high percentages of *A. citricola* (74.2%) in white grapefruit, compared with the low percentages in "tangor" and "chonera" mandarin orange with only 2.6 and 4.4% parasitism, respectively (Table 3).

CONCLUSIONS

1. The highest percent infestation of *P. citrella* occurred during the dry season. The highest

TABLE 3. INFESTATION AND NATURAL BIOLOGICAL CONTROL BY *PHYLLOCNISTIS CITRELLA* IN ELEVEN CITRUS SPECIES IN MANABÍ, ECUADOR.

Species	Common name	Flushing (%)	Infestation (%)	Parasitism (%)
C. aurantium	Naranja agria	50	14.37	21.43
C. reticulata	Mandarina Cleopatra	75	23.87	7.22
	Tangor	50	26.31	2.63
C. reticulata	Mandarina chonera	50	27.06	4.44
C. grandis	Toronja	50	27.56	45.45
C. sinensis	Naranja valencia	75	29.89	53.57
C. aurantifolia	Limón sutil	75	30.61	40.91
C. reticulata × *C. paradisi*	Tangüelo	50	32.76	13.51
C. paradisi	Pomelo blanco	75	33.07	74.19
C. paradisi	Pomelo rojo	50	35.10	52.83
C.sinensis	Naranja Wston. navel	50	37.43	56.38

infestations in three localities were as follows: in Lodana (43.8%) in October, in Riochico (45.7%) in November, and in La Unión (17.4%) in December.

2. The greatest intensity of parasitism occurred in Lodana in March (60%), in Riochico in January (18.9%), and in La Unión in December (50%).
3. The species *A. citricola*, *Galeopsomyia* sp., and *E. tischeriae* were identified at 28.4%, 2.2%, and 0.1% parasitism, respectively. Although this is the first report of *A. citricola* in Ecuador, it is widely distributed in the main citrus producing zones of the country.
4. A preference of *P. citrella* for varieties of orange and grapefruit over other citrus types was noted. The most preferred was Washington navel oranges.

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