

Population Dynamics of the Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), and Its Natural Enemies in Texas and Mexico

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The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was unreported in the western hemisphere until May 1993 when it was discovered in citrus nurseries in Florida. In Mexico, *P. citrella* was reported in 1994 in the state of Tamaulipas. Because of the potential threat to the citrus industries in both countries, a binational collaborative research project was initiated in 1997 to define and compare the status of *P. citrella* and levels of biological control between the two countries. In general, *P. citrella* populations increased in Mexico over the 3-year period from 1997 to 1999. In 1997, *P. citrella* peaked at ≈ 0.4 immatures/leaf in mid-October. In 1999, the pest began to increase in April, reaching peaks of ≈ 1.0 larva/leaf in early July. In both years, overall parasitism averaged $\approx 20\%$. The dominant parasitoid in Mexico was *Zagrammosoma multilineatum* (Ashmead) (Hymenoptera: Eulophidae), which constituted $>30\%$ of the parasitoid complex. Predators recovered were *Chrysoperla* spp. (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthracoridae), and *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae). In contrast, *P. citrella* generally declined in Texas over the same period. In 1997, the pest peaked at 0.8 immatures/leaf in August and did not exceed 0.4 immatures/leaf in 1998. The decline of *P. citrella* in south Texas is more evident when compared to a 1995 survey when pest densities exceeded 6.0 immatures/leaf. The monthly percentage parasitism of *P. citrella* in Texas increased from $<1\%$ in May to $\approx 10\%$ in November 1997. In contrast, parasitism in 1998 fluctuated from 0 to 20%. Numbers of parasites were always <0.05 individuals/leaf and often zero. Similar to Mexico, the dominant parasitoid was *Z. multilineatum*, which constituted 46.2% (128 of 277) of the parasitoids sampled. Differences in *P. citrella*

populations and those of its parasitoid complexes may be partially attributed to a hotter, drier climate in Texas than in Mexico. © 2001 Academic Press

INTRODUCTION

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), originated from south-east Asia and established itself as a major pest of citrus throughout Australia, the Middle East, and parts of Africa (Heppner and Dixon, 1995). *Phyllocnistis citrella* was unreported in the western hemisphere until May 1993 when the leafminer was discovered in citrus nurseries in Dade County, Florida (Heppner, 1995). Within 3 months, *P. citrella* had spread rapidly throughout the commercial citrus-growing areas of Florida, attaining the status of a major pest. The leafminer subsequently spread to other areas in Central, South, and North America, as well as the Caribbean (Peña *et al.*, 1996). The known geographical range of *P. citrella* in the continental United States currently includes Alabama, Louisiana, and Texas (Legaspi *et al.*, 1999), and most recently, California (L. Bezark, California Department of Food and Agriculture, pers. comm.). In Mexico, *P. citrella* was reported in 1994 in the state of Tamaulipas, with subsequent reports in Veracruz, Hidalgo, Nuevo Leon, and Colima (Perales-Gutierrez *et al.*, 1996; Bautista-Martinez *et al.*, 1998). The discovery of *P. citrella* in Mexico was viewed as a potential threat to the Mexican citrus industry, which ranks sixth internationally with a total annual production of $>3,845,000$ tons.

The leafminer attacks all cultivars of citrus, related species within the Rutaceae family, and several ornamentals (Legaspi and French, 1996). Plant damage is caused by the leafminer larvae as they bore through the leaf epidermis. Leaves become chlorotic, often deformed, and susceptible to infection by fungi or bacteria. Control measures include insecticides such as chlorfenapyr and tebufenozide (French *et al.*, 1997) and

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cultural controls to limit growth of the susceptible young flushes (Legaspi and French, 1996).

Biological control is a promising pest management option against *P. citrella*. Recent records indicate that ~80 species of parasitoids have been reared from the leafminer worldwide (Schauff *et al.*, 1998). Over 20 New World parasitoids were recorded on *P. citrella* as its geographical range increased, thereby resulting in fortuitous or "new association" biological control. These parasitoids are predominantly in the family Eulophidae, although others belong to the Elasmidae, Eupelmidae, and Pteromalidae (LaSalle and Peña, 1997). Efforts at classical biological control of *P. citrella* have focused on the introduction of *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) in Florida and Texas. Between May 1994 and September 1995, *A. citricola* (originally from Thailand) was imported from Australia and released into 52 sites in southwest Florida (Pomerinke and Stansly, 1998). *Ageniaspis citricola* has apparently established, attaining parasitism levels of 86% in October 1995 and surviving winter frosts. A similar program was undertaken in south Texas, but *A. citricola* has not established to date (Legaspi *et al.*, 1999). The eulophid *Cirrospilus ingenuus* Gahan [= *C. quadristriatus* (Subba Rao & Ramamani)] is another parasitoid from southeast Asia that has been released against *P. citrella* in many countries, including Australia, Cyprus, Israel, Morocco, Oman, Syria, Tunisia, and Turkey (Schauff *et al.*, 1998). *Cirrospilus ingenuus* was released in limited numbers in Florida in 1994 and recovered in field collections taken in November 1997 and January 1998, although the impact on the leafminer is unknown (LaSalle *et al.*, 1999).

The proximity of the citrus-producing areas along the border of Texas and Mexico suggests possible commonalities in pest species, as well as their solutions. A binational collaborative research project was initiated to define and compare the status of *P. citrella* and levels of biological control between the two countries. In this paper, we report the results of this project, i.e., the population dynamics of *P. citrella* and its natural enemies from 1997 to 1999 in south Texas and Nuevo Leon, Mexico.

MATERIALS AND METHODS

Population Dynamics of P. citrella and Its Natural Enemies in Nuevo Leon, Mexico (1997 and 1999)

Sampling for *P. citrella* and its natural enemies was conducted in the Mexican state of Nuevo Leon (located between 23° 10' to 27° 47' 30" N and 98° 24' 38" to 101° 12' 09" W; about 425 m above sea level) from February to December in 1997 and 1999. Five orchards were selected: three in Montemorelos and two in General Teran. The orchards in Montemorelos were planted to

'Valencia' orange [*Citrus sinensis* (L.) Osbeck var 'Valencia'], 'Ruby Red' grapefruit (*Citrus paradisi* Macf.), and 'Dancy' tangerine (*Citrus reticulata* Blanco). Orchards in General Teran were planted only to 'Valencia' oranges.

Population Dynamics of P. citrella and Its Natural Enemies in Texas (1997 and 1998)

Sampling in Texas was conducted in the citrus-growing areas of the Lower Rio Grande Valley (situated between 25° 50' to 26° 18' N and 97° 20' to 98° 15' W; ~11 m above sea level). In 1997, leaves were collected from May to November; in 1998, collections were performed from April to December. The orchards were planted to grapefruit, orange, and lemon [*C. limon* (L.) Burm.]. Unlike the collections in Mexico, leaf samples were not taken from predetermined sites; orchards were selected randomly to represent the geographical boundaries of the citrus-growing area of south Texas. Comparisons were made of historical temperature and rainfall data between citrus-growing areas of Texas (represented by Harlingen) and Mexico (represented by Montemorelos) to assess possible effects of climate on differences in population dynamics between the two countries.

Sampling Procedure

At about 2-week intervals, leaf samples were collected from selected citrus orchards in Texas and Mexico. About 100 expanded tender leaves were collected from each site, placed in plastic bags, and taken to the laboratory for examination. Numbers of leaves sampled and total numbers of *P. citrella* (sum of live and dead larvae, pupae, pupal cases) were recorded. Parasitized larvae were reared out and eclosing parasitoids were identified. In Mexico, numbers of predators were also recorded. Total numbers of parasitoids were calculated by adding numbers of parasitoids identified, together with those unidentified or that died prior to emergence. Percentage parasitism at a given time was calculated by dividing total numbers of parasitoids with the numbers of emergent parasitoids and leafminers.

Parasitoid identifications were performed by J. Woolley (Department of Entomology, Texas A&M University, College Station) and M. Reyes (Instituto Nacional de Investigaciones Forestales y Agropecuarias, Rio Bravo, Mexico). Voucher specimens of the parasitoid species collected from Texas are deposited at the USDA ARS Systematic Entomology Laboratory (Beltsville, MD).

Statistical Analysis

To test the effect of host density on percentage parasitism, regression analyses were performed on per-

centage parasitism (dependent variable) and numbers of *P. citrella* hosts per leaf (independent variable; samples with zero hosts were excluded). Data were pooled by country. Percentage data were transformed (arc-sine-square root method), prior to analyses, but are presented as untransformed data (Sokal and Rohlf, 1981). Mean monthly temperature and rainfall data of Texas and Mexico were compared using paired *t* tests (paired by month). Statistical analyses were performed using Systat (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Population Dynamics of P. citrella and Its Natural Enemies in Nuevo Leon, Mexico (1997 and 1999)

The total numbers of *P. citrella* and its natural enemies are shown in numbers per leaf (Fig. 1). Populations of *P. citrella* began to increase during the summer months of 1997, peaking at ≈ 0.4 immatures/leaf in mid-October (Fig. 1A). In 1999, *P. citrella* populations began to increase earlier (in April) and numbers per leaf were generally higher, reaching peaks of ≈ 1.0 /leaf in early July and ≈ 0.5 /leaf in late September (Fig. 1B). In both years, overall parasitism averaged $\approx 20\%$. However, parasites were recovered earlier in the year in 1999 (early May) compared to 1997 (mid-June) (Figs. 1C and 1D). The parasitoid *Z. multilineatum* (Ashmead) (Eulophidae) increased in dominance from 1997 to 1999 (Figs. 1E and 1F). In 1997, *Z. multilineatum* constituted 32% of parasitoids recorded (62 *Z. multilineatum*/194 total parasitoids), increasing slightly to 38% (297/782) in 1999. Predators recovered on citrus leaves were larvae of *Chrysoperla* spp. (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) (Figs. 1G and 1H). From a total predator sample of 2504 collected, *Chrysoperla* constituted 48.7% (1220), *O. insidiosus*, 28.1% (703), and *H. convergens*, 23.2% (581).

In summary, over the 3-year period from 1997 to 1999, *P. citrella* populations generally increased in the sample sites in Nuevo Leon, with *Z. multilineatum* being the dominant parasitoid. Although Perales-Gutierrez *et al.* (1996) reported *Z. multilineatum* as a parasitoid of *P. citrella* in the Mexican state of Colima, the relative dominance of the species was not reported. In contrast, Bautista-Martinez *et al.* (1998) did not report *Z. multilineatum* as a parasitoid of *P. citrella* in the state of Veracruz.

Population Dynamics of P. citrella and Its Natural Enemies in Texas (1997 and 1998)

When sampling was initiated in May 1997, numbers of *P. citrella* per leaf were already quite high, at about 0.6 total insects/leaf (Fig. 2A). The peak of 0.8/leaf was

reached in August, after which numbers declined in the fall. Populations of *P. citrella* were lower in the following year, never exceeding 0.4/leaf (Fig. 2B). These surveys indicate a general decline in levels of *P. citrella* populations in south Texas, especially when compared with 1995, when pest densities exceeded 6.0/leaf during the summer (Legaspi *et al.*, 1999).

The monthly percentage parasitism of *P. citrella* in Texas increased from $<1\%$ in May 1997 to $\approx 10\%$ in November (Fig. 2C). In contrast, parasitism in 1998 fluctuated from 0 to 20% with no definite trend apparent (Fig. 2D). Numbers of parasites were low for both 1997 and 1998, always <0.05 /leaf and often zero. A total of 277 parasitoids were recorded in 1997, of which 128 (46.2%) were *Z. multilineatum*. Other parasitoids reared were *Closterocerus* sp. (22 individuals; 7.9% of parasitoids) and *Pnigalio* sp. (4; 1.4%). In 1998, only 41 parasitoids were recorded, of which *Z. multilineatum* was most abundant (13; 31.7%). Also found were *Closterocerus* sp. (3; 7.3%) and *Pnigalio* sp. (1; 2.4%). A comparison with the surveys conducted in south Texas in 1995 and 1996 (Legaspi *et al.*, 1999) indicates declines in the native parasitoid complex attacking *P. citrella*. The maximum percentage parasitism in 1996 was almost 40%. The maximum level of 20% that we found in 1998 (Fig. 2D) is misleading because of the low host densities available (Fig. 2B). To compare parasitoid patterns from 1995 to 1998, we calculated totals of numbers of leaves sampled during each year and numbers of parasitoids reared from those leaves using data presented here and in Legaspi *et al.* (1999). From a total of 1650 leaves sampled in 1995, 111 total parasitoids were reared, yielding an overall count of 6.7 parasitoids/100 leaves. Corresponding counts in 1996 through 1998 were 8.6 (314 parasitoids from 3658 leaves), 9.4 (605/6426), and 0.81 parasitoids/100 leaves (42/5160), respectively. A large decline in parasitoids was thus recorded only in 1998, whereas *P. citrella* showed large declines by 1997. Similar to Legaspi *et al.* (1999), we were unable to recover *A. citricola* that was released in south Texas in early 1995.

Overall percentage parasitism was higher in Mexico (17.2%, SE = 1.8; $n = 25$) than in Texas (9.6%; SE = 1.5; $n = 39$) ($t = 2.7$; $df = 23$; $P < 0.05$). The numbers of *P. citrella* hosts per leaf and percentage parasitism yielded a significant regression in Mexico (Fig. 3A) ($F = 11.4$; $df = 1, 23$; $P < 0.01$; $R^2 = 0.33$) but not in Texas (Fig. 3B) ($F = 1.03$; $df = 1, 36$; $P = 0.32$; $R^2 < 0.01$). While this result is interesting, it is difficult to draw strong conclusions regarding the apparent density-dependent parasitism found in Mexico and its absence in Texas. In both countries, *Z. multilineatum* was the dominant parasitoid. Furthermore, parasitoids of *P. citrella* usually are not host specific but are generalists that attack other lepidopteran leafminers (Peña *et al.*, 1996). Therefore, strong host-parasitoid interactions, such as density-dependent

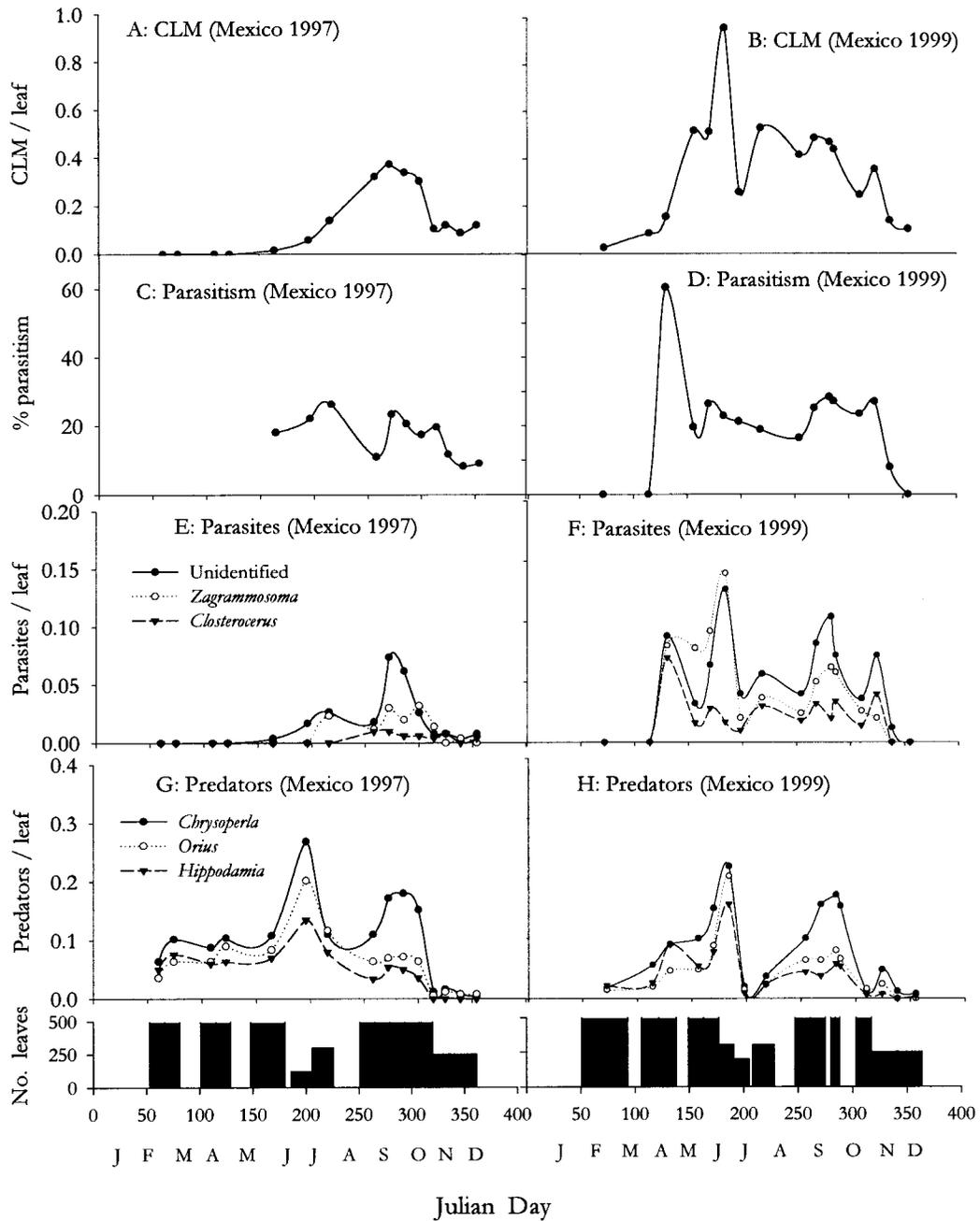


FIG. 1. Population dynamics of *P. citrella* and its natural enemies in Mexico (1997 and 1999). (A) Numbers of citrus leafminer (CLM) (sum of living and dead larvae, pupae, empty pupal cases divided by numbers of citrus leaves sampled) in 1997 by sampling date; (B) numbers of CLM/leaf in 1999; (C) monthly percentage parasitism of CLM in 1997; (D) Monthly percentage parasitism of CLM in 1999; (E) monthly numbers of parasites per leaf in 1997; (F) monthly numbers of parasites per leaf in 1999; (G) monthly numbers of predators per leaf in 1997; (H) monthly numbers of predators per leaf in 1999. Bar graphs below indicate corresponding months and numbers of leaves sampled.

parasitism, are not expected. Comparisons between parasitism patterns in Texas and Mexico may suggest overall higher levels of parasitism in Mexico and lower and more erratic parasitism in Texas due to lower population levels of *P. citrella* or lower availability of alternate hosts.

In general, *P. citrella* and its parasitoids increased in Mexico from 1997 to 1999 and decreased in Texas from

1997 to 1998. The decline becomes more apparent when compared with surveys taken in 1995 (Legaspi *et al.*, 1999). Many factors probably contributed to these differences, and one likely important determinant is climate. Mean monthly temperatures were higher in (Harlingen) Texas than in (Montemorelos) Mexico (Fig. 4) (paired $t = 2.74$; $df = 11$; $P < 0.05$). Mean monthly rainfall was not statistically different between

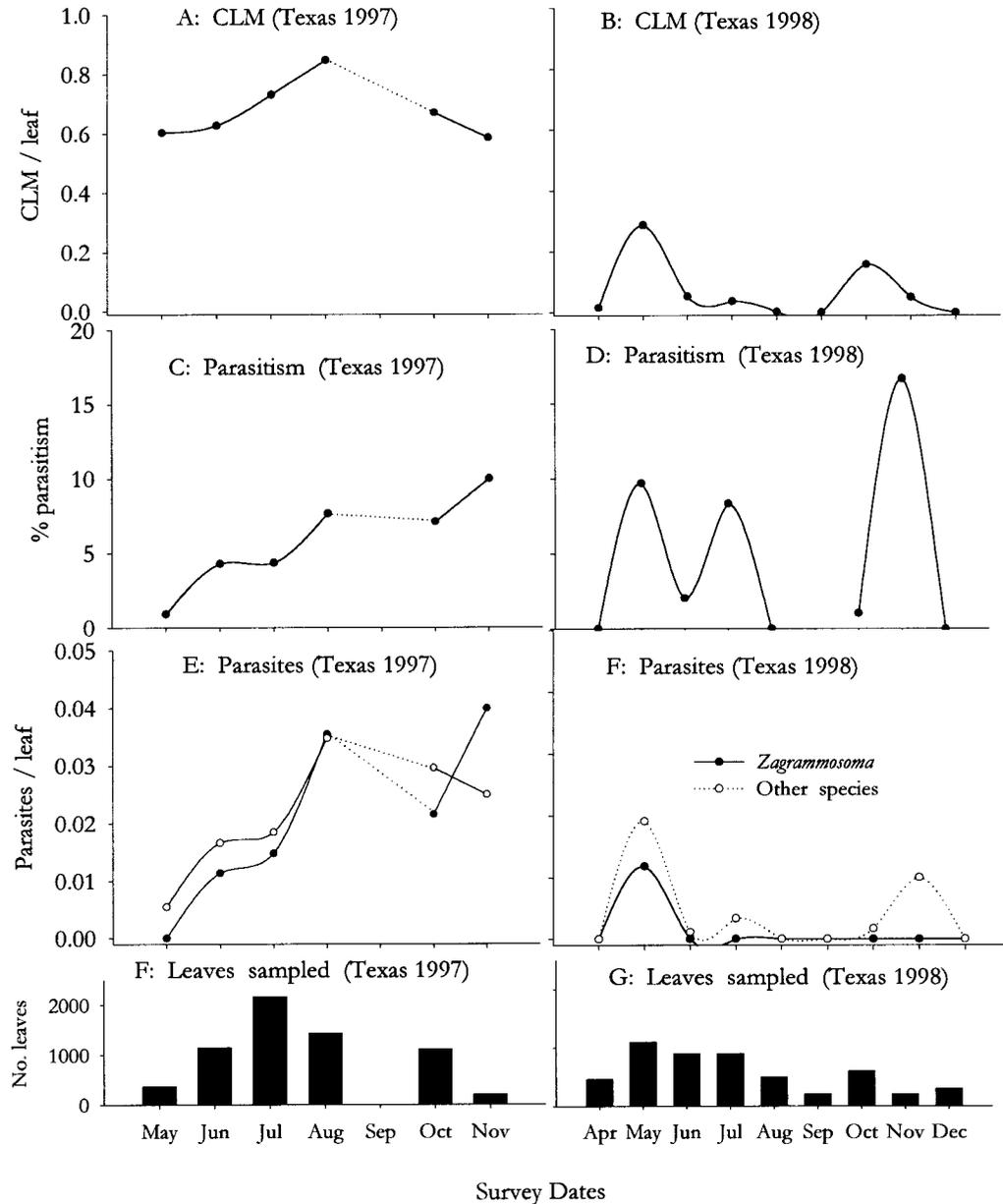


FIG. 2. Population dynamics of *P. citrella* and its natural enemies in Texas (1997 and 1998). (A) Monthly total of numbers of citrus leafminer (CLM) (sum of living and dead larvae, pupae, empty pupal cases divided by numbers of citrus leaves sampled) in 1997; (B) numbers of CLM/leaf in 1998; (C) monthly percentage parasitism of CLM in 1997; (D) monthly percentage parasitism of CLM in 1998; (E) monthly numbers of parasites per leaf in 1997; (F) monthly numbers of parasites per leaf in 1998. Dashed lines indicate samples not taken in September 1997. Bar graphs below indicate corresponding months and numbers of leaves sampled.

the two countries (paired $t = 2.087$; $df = 11$; $P = 0.06$). However, for the months (May to November) during which *P. citrella* field populations increase, rainfall was significantly higher in Mexico (paired $t = 3.45$; $df = 6$; $P < 0.05$). Although not conclusive, differences in *P. citrella* populations and, consequently, those of its parasitoid complexes may be at least partially attributed to a hotter, drier climate in Texas than in Mexico.

Comparisons of the native parasitoid complexes attacking *P. citrella* indicate interesting differences in

the dominant parasitoid. About 80% of the native parasitoid complex in Florida is composed of *Pnigalio minio* (Walker) (Hymenoptera: Eulophidae) (Peña *et al.*, 1996). Other species found were *Cirrospilus* sp. (7%), *Closterocerus* sp. (4%), *Z. multilineatum* (2%), and *Horismenus* sp. (5%) (all Eulophidae). Relatively high levels of parasitism, often 40–80%, indicated a potential need for conserving indigenous parasitoids in an integrated pest management system. In 1995 and 1996, the dominant native parasitoid in south Texas was *Z. multilineatum*, constituting 70–80% of all para-

sitoids recorded (Legaspi *et al.*, 1999). Minor parasitoids included species of *Pnigalio*, *Horismenus*, and *Closterocer*. In the state of Veracruz in Mexico, *P. citrella* is parasitized mostly by the eulophids *Galeopsomyia* sp. and *Cirrospilus* spp. Other parasitoids recorded were *Horismenus* sp. and *Elasmus tischeriae* Howard (Hymenoptera: Elasmidae) (Bautista-Martinez *et al.*, 1998). Total parasitism from November to March was high at about 70%. Surveys in the state of Colima, Mexico, also recorded parasitism of *P. citrella* by *Cirrospilus* spp., *Closterocer* spp., *Horismenus* sp., *Elasmus*, sp., *Z. multilineatum*, and an unidentified encyrtid (Perales-Gutierrez *et al.*, 1996) (relative abundances not indicated). *Zagrammosoma multilineatum* is the most important parasitoid in Nuevo Leon. While also true for south Texas, the importance of *Z. multilineatum* has declined together with populations of the host *P. citrella*. Although *P. citrella* is not currently a serious citrus pest in south Texas, migration from Mexico remains a risk. Furthermore, other citrus-growing areas of the United States are still vulnerable to *P. citrella*.

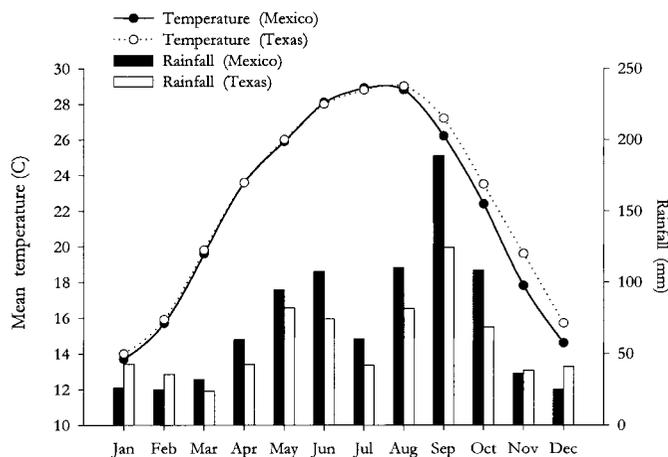


FIG. 4. Comparison of monthly mean temperature (°C) and rainfall (mm) between Mexico and Texas. Data for Mexico are historical records for Montemorelos: temperature from 1942 to 1983; rainfall from 1939 to 1986 (Global Historical Climatology Network, Arizona State University, Tempe, AZ and Oak Ridge National Laboratory, Oak Ridge TN). Data for Texas are historical records for Harlingen: temperature from 1961 to 1990; data for rainfall are from 1931 to 1995 (National Climatic Data Center, Asheville, NC).

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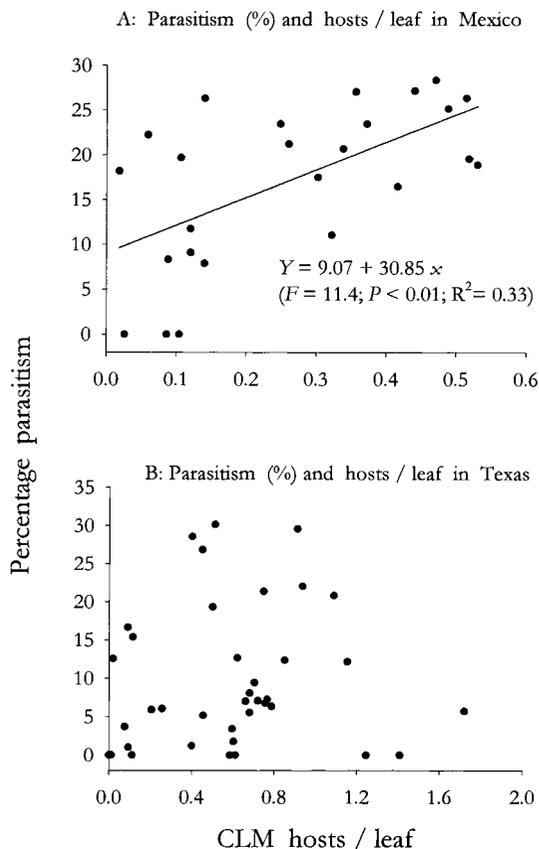


FIG. 3. (A) Regression of numbers of *P. citrella* hosts per leaf and percentage parasitism in Mexico. $Y = 9.07 + 30.85x$ (SE constant = 2.7; SE slope = 8.5; $F = 11.4$; $df = 1, 23$; $P < 0.01$; $R^2 = 0.33$). (B) Regression of numbers of *P. citrella* hosts per leaf and percentage parasitism in Texas ($F = 1.03$; $df = 1, 36$; $P = 0.32$; $R^2 < 0.01$).

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