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Authors: Lim, Un Taek, and Hoy, Marjorie A.

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OVERWINTERING OF THE CITRUS LEAFMINER, *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE), WITHOUT DIAPAUSE IN FLORIDA

UN TAEK LIM [1,2] AND MARJORIE A. HOY [1]
[1]Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Gainesville, FL 32611-0620 USA

[2]School of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea

ABSTRACT

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus that has spread around the world. The citrus leafminer invaded Florida in 1993 and rapidly colonized all citrus-growing areas. Despite the fact that the citrus leafminer has been studied extensively, gaps in our knowledge of its biology remain. One issue is how the citrus leafminer overwinters. Citrus leafminers have been observed to overwinter in many countries, but their capacity to diapause has not been confirmed. To investigate whether *P. citrella* overwinters in diapause, we evaluated the ability of eggs to develop to adulthood on potted citrus trees when reared in both outdoor and growth chamber conditions. No pupae were found to be in diapause in the outdoor assessment in which three populations of citrus leafminer were reared in a shade house in Gainesville, Florida beginning on Oct 15, Nov 11, or Nov 22. Larval and pupal mortality significantly increased in the population set out on Nov 22 compared to the other populations. Adult longevity significantly increased in the last two populations compared to the Oct population. However, similar degree days were accumulated during the adult lifespan among the three populations. In the growth chamber assessments, citrus leafminers showed no arrestment in development during the pupal stage by short-day treatments. Development (days) from egg to adult was not different between short- and long-day conditions in both sexes. No signs of reproductive diapause were found from dissections of adult females reared under short- or long-day conditions. We concluded that *P. citrella* may overwinter on the few small flushes available during winter in mid and central Florida, and the relevance of these results for citrus IPM programs is discussed.

Key Words: photoperiod, adult longevity, immature development, oogenesis

RESUMEN

El minador de la hoja de cítricos, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), es una plaga importante de los cítricos que ha sido esparecida alrededor del mundo. El minador de la hoja de cítricos invadio el estado de Florida en 1993 y rapidamente colonizó todas los áreas donde se siembran cítricos. Al pesar del hecho que el minador de la hoja de cítricos ha sido estudiado extensivamente, todavía hay brechas en nuestro entendimiento de su biología. Un asunto es como el minador de la hoja de cítricos inverna. La invernación del minador de la hoja de cítricos ha sido observada en muchas paises, pero su capacidad para pasar por el estado de diapausa no ha sido confirmado. Para investigar si *P. citrella* inverna en el estado de diapausa, nosotros evaluamos la capacidad de los huevos para desarrollarse al estado adulto al criados sobre árboles de cítricos sembrados en recipientes bajo condiciones exteriores y en una camara de crecimiento. Ninguna pupa fue encontrada en el estado de diapausa en la prueba de condiciones exteriores donde tres poblaciones del minador de la hoja de cítricos fueron criadas en condiciones de sombra en Gainesville, Florida empezando en el 15 de octubre, el 11 de noviembre, o el 22 de noviembre. La mortalidad de las larvas y pupas aumentó significativamente en la población del 22 de noviembre en comparación con las otras poblaciones. La longevidad de los adultos aumentó significativamente en las últimas dos poblaciones en comparación con la población de octubre. Sin embargo, los dias-grado acumulados fueron similares durante el período de vida del adulto entre las tres poblaciones. En las pruebas dentro de la camara de crecimiento, el minador de la hoja de cítricos no mostró ningún impedimento en su desarrollo durante los estadios de pupa en los tratamientos de dias cortos. El desarrollo (dias) del huevo hasta el adulto no fue diferente entre las condiciones de dias cortos y dias largos en ambos sexos. Ninguna señal de la diapausa reproductiva fue encontrada en las disecciones de las hembras adultas criadas bajo condiciones de dias cortos o dias largos. Nosotros concluimos que *P. citrella* podria estar invernando sobre unos pocos brotes pequeños de nuevas hojas disponibles durante el invierno en Florida, y se comenta sobre la importancia de estos resultados en los programas de MIP en la producción de cítricos.

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important citrus pest that is native to Southeast Asia. Adult females deposit eggs singly upon tender young citrus foliage and larvae immediately enter the leaf and start feeding on epidermal cells, producing broad serpentine mines (Hoy & Nguyen 1997). Mining of immature foliage reduces growth rates and yields, and the mines serve as foci for the establishment of the bacterium causing citrus canker (Gottwald et al. 2001; Graham et al. 1996; Liu et al. 1999; Ujiye 2000).

Various aspects of the biology of the citrus leafminer have been studied in the laboratory and field but questions remain, including how this pest survives the winter in Florida. Typically, this pest declines to very low densities during the winter and the first spring flush in Mar lacks significant populations of this pest (Knapp et al. 1995; Pena et al. 1996; Villanueva-Jimenez et al. 2000). The severe reduction in the number of overwintering hosts also reduces populations of the host-specific parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) in Florida, which means that populations of this parasitoid lag behind that of the leafminer until the second or third major flush cycle during the following growing season (Villanueva-Jimenez et al. 2000; Zappalà et al. unpublished). Understanding how the citrus leafminer overwinters could increase our ability to manipulate pest and natural enemy populations in Florida's citrus.

Many insects hibernate in diapause, a genetically determined state of arrested development that typically occurs prior to the onset of unfavorable conditions (Beck 1980; Danilevskii 1965; Denlinger 2002; Lees 1955; Tauber et al. 1986). Diapause can occur in eggs, larvae, pupae, or adults of insects, with adult diapause determined by comparing their longevity to nondiapausing adults or by examining the reproductive tracts of males and females for delays in the development of oocytes or spermatocytes. In most arthropods, diapause is induced by a combination of cues, including temperature, photoperiod, and host plant condition.

Winter diapause of the citrus leafminer has not been confirmed. Clausen (1931) and Ayoub (1960) indicated that the citrus leafminer passes the winter in the adult stage. Pandey & Pandey (1964) found no evidence of hibernation or aestivation in India, based on phenology. Barroga (1968) reported that in the Philippines citrus leafminer populations decreased to low levels during Jun, Oct, Nov and Apr, which correlated with the absence of flush. Citrus leafminer populations also occurred whenever the trees flushed, regardless of locality or season of the year in Darwin, Australia (Wilson 1991) and India (Batra & Sandhu 1981). Ujiye (2000) reported that because males of citrus leafminer are sexually active during winter, males do not appear to have a winter diapause, but he proposed reproductive diapause of females based on the findings by Hamamura (1980). Ovaries of female citrus leafminer emerging in autumn are absorbed while exposed to low temperatures in Japan (Hamamura 1980). Ali Mafi & Ohbayashi (2004) reported that "Infestation was recognized even in Dec, Jan, and Feb" in Japan on *Citrus iyo*. However, to our knowledge, no one has attempted to determine whether citrus leafminer has the genetic capability to enter hibernal diapause by rearing them under controlled temperatures and daylengths. We report here the results of rearing the citrus leafminer on potted citrus trees held in cages outdoors during the winter of 2003-2004 in Gainesville, Florida, and of rearing them in growth chambers under controlled temperatures and daylengths that could be expected to induce diapause if this species has the genetic capacity to do so.

Materials and Methods

Outdoor Assessment of Pupation and Adult Emergence

Ten potted rough lemon (*Citrus jambhiri* Lushington) trees were infested with 100 newly emerged unsexed citrus leafminers in a PVC pipe-framed cage (74.5 W × 46.5 L × 61.5 H cm) covered with a mesh bag for 3 d in a greenhouse maintained at 27°C, 80-100% RH, and photoperiod of 16:8 (L:D). Once oviposition was verified, each tree was covered with a plastic cylinder containing lateral holes covered with mesh fabric to prevent predation or parasitization and placed in an outdoor shade house. Once the first adults emerged, based on daily observations, the leaves containing citrus leafminers were pruned from the plants. Leaves containing pupal chambers were placed in inflated clear plastic bags with paper towels and held in the shade house. Adults that emerged in the bag were counted daily and, when adult emergence was discontinued, the leaves in the bag were examined to find any live larvae and pupae remaining. Adults that emerged were placed in diet cups in the shade house individually with a honey strip and a water-soaked cotton ball to evaluate longevity over the winter. The mortality of these unsexed adults was observed daily, and the diet cup, honey strip, or cotton ball was replaced with new ones as needed. Longevity was evaluated in two ways: number of days and number of degree days (DD). Daily 24-h mean temperatures were obtained from the Florida Automated Weather Network and used to calculate the number of DD per day, based upon a threshold of 12.1°C (Ujiye 2000; Vargas et al. 2001). These procedures were repeated three times by setting up lemon trees containing eggs of citrus leafminer on Oct 15, Nov 11, and Nov 22. Larval mortality, adult emergence, and longevity were obtained and analyzed with the Kruskal-

Wallis single factor analysis of variance by rank (SAS Institute 1995). The Dunn test was used for post hoc comparison (Zar 1996).

Evaluation of Diapause in Immature *P. citrella* in Growth Chambers

To verify the results of the outdoor experiments, the ability of citrus leafminers to diapause was assessed in a growth chamber under short-day conditions. Ten lemon trees with new shoots were exposed to 100 adult citrus leafminers for 1 d under the same conditions as in the outdoor assessment. Half of the trees were placed at 18°C with a photoperiod of 16:8 (L:D) in a growth chamber, and the other half were placed into a photoperiod of 8:16 (L:D) at 18°C in another growth chamber. Trees in both growth chambers were assessed daily for larval hatch. Leaves containing only one larva were marked (115 leaves in short day condition and 109 in long day condition) and larval development was assessed daily. Development (days) from egg to adult was analyzed with a *t*-test to find any effect of short daylength. Pupation rate, emergence rate, and sex ratio in both treatments and controls were compared by Fisher's exact test.

Evaluation of Reproductive Diapause in Adult *P. citrella* in Growth Chambers

The existence of a reproductive diapause in adult females was evaluated under short-day conditions. Ten lemon trees with new shoots were exposed to 100 adult citrus leafminers for one day under the same conditions as in the outdoor assessment. After being individually covered with a clear plastic cylinder, half of the trees were placed at 18°C and a photoperiod of 16:8 (L:D) in a growth chamber, and the other half at 18°C and a photoperiod of 8:16 (L:D) in another growth chamber. Emerging adults were collected daily and placed with water and honey in diet cups as described in the outdoor assessment. Six days after emergence, females were dissected under a microscope. Females that had mature eggs in the ovary when dissected were treated as non-diapausing females. This procedure was repeated for two additional photoperiods, i.e., 14:10 (L:D) and 10:14 (L:D) at 18°C.

Results and Discussion

Larval mortality significantly increased in the citrus leafminer population set out on Nov 22 compared to populations studied earlier (H_c = 15.949, df = 2, $P < 0.001$) (Table 1). This could have been due to a decline in ambient temperature (Fig. 1) and leaf hardening. Badawy (1967) indicated that *P. citrella* larvae develop during the winter months in Sudan, but that very few larvae successfully completed their development. None of the pupae were found to be in diapause during the outdoor tests (Table 1). Adults did emerge from the pupae of all the populations tested in Gainesville. However, fewer adults emerged in the population set out on Nov 22 than emerged from those that were set out on the earlier dates (H_c = 16.826, df = 2, $P < 0.001$) probably due to the increase in larval mortality (Table 1).

Adult longevity was significantly increased to 34 or 35 d in the populations set out on Nov compared to 18 d in the population set out during Oct (H_c = 50.811, df = 2, $P < 0.001$) (Table 1). However, the similar degree days accumulated by the adults among the three populations (H_c = 0.939, df = 2, P = 0.625) (Table 1) may indicate that citrus leafminers can survive the winter in Florida as adults without an increase in adult mortality or arrestment in immature development. Hamamura (1980) also found prolonged longevity of adults during the winter and proposed that citrus leafminers overwinter as adults in citrus groves in Hiroshima prefecture in Japan.

Photoperiod is a highly predictable component of both the tropical and temperate environment (Denlinger 1986; Pieloor & Seymour 2001) and has been shown to regulate diapause in several

Table 1. Assessment for diapause induction in citrus leafminers held outdoors in a shade house in Gainesville, Florida during winter of 2003-2004.

Date when trees were placed in shade house	Days to first adult emergence (degree days)	Mean no. of citrus leafminers per tree at pruning (SD)	Larval mortality (SD)	Adult emergence rate (SD	Proportion of pupae in diapause	Adult longevity (SD)	
						days	degree days
Oct. 15	18 (142.4)	69.3 (22.4)	0.00 (0.00) **a**	0.86 (0.06) **a**	0	18.4 (17.2) **a**	97.0 (61.6) **a**
Nov. 11	32 (116.5)	47.0 (18.4)	0.08 (0.06) **a**	0.65 (0.08) **a**	0	35.1 (19.8) **b**	91.0 (52.7) **a**
Nov. 22	44 (121.1)	63.9 (26.8)	0.24 (0.09) **b**	0.43 (0.11) **b**	0	34.4 (16.1) **b**	85.8 (48.9) **a**

See text for statistics.
Numbers with different letters were different based on Dunn test ($P < 0.05$).

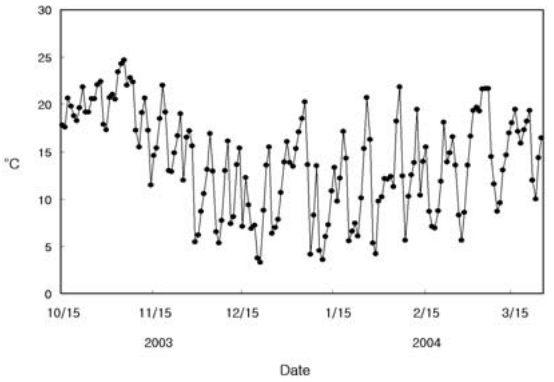

Fig. 1. Mean daily temperatures in Alachua County during 2003-2004 obtained from the Florida Automated Weather Network

tropical lepidopterans such as *Hypolimnas bolina* L. (Pieloor & Seymour 2001), *Diatraea grandiosella* Dyar (Kikukawa & Chippendale 1983), and *Heliothis armigera* Hubner (Hackett & Gatehouse 1982). However, immature citrus leafminers showed no arrestment in development induced by short-day conditions in the growth chamber experiments at 18°C (Table 2). No significant differences were found in pupation and emergence rates between the populations exposed to short-day or long-day conditions in growth chambers maintained at 18°C. Development time from egg to adult was also not affected by short-day conditions in both sexes (female $t = 0.967$, $df = 74$, $P = 0.337$; male $t = 0.141$, $df = 79$, $P = 0.888$) (Table 2). Finally, no signs of reproductive diapause were found from the dissections of adult females reared under short-day or long-day conditions (Table 3). Only one female citrus leafminer out of 52 that had been placed in 8L: 16D at 18°C lacked mature eggs and had ovarioles reduced in size. Hamamura (1980) found adult females absorbed mature eggs when adult females were exposed to 10°C and 12L:12D conditions and provided with honey solution (25%) for a long period of time (8% absorption from exposure of 10 d and 81% from 40 d). The effects of the parental moth's experiences also were assessed. Adult citrus leafminers that had been reared at 18°C with a photoperiod of 8:16 (L:D) in a growth chamber were allowed to oviposit on a lemon tree with flushes and their progeny were allowed to develop under these same conditions. No signs of developmental arrestment in eggs, larvae, pupae, or adults were found from two replications involving 15 and 20 parental moths, respectively, indicating that parents reared under potentially diapause-inducing conditions did not influence their progeny's ability to enter diapause (unpublished data).

In our studies, the Florida population of the citrus leafminer did not show any evidence of arrested development in immature or adult stages during the growth chamber or outdoor rearing experiments. Immatures probably overwinter in a few tender new shoots or leaves or as adults during the winter in mid and central Florida. Because citrus trees produce some flushes, even in winter, citrus leafminers may not need to diapause to synchronize with their food resources. Hence, as Knapp et al. (1995) suggested, suppression of winter flushing by limiting irrigation, in combination with cool temperatures, may help to reduce overwintering populations of leafminers in Florida.

Conclusions

These experiments provide no evidence that the Florida population of the citrus leafminer has a genetically determined diapause. It is always frustrating to obtain "negative" data because we can not prove a negative if we have not tested all possible combinations of diapause-inducing cues. Despite this, the weight of the evidence reported here, and the anecdotal evidence from several studies of the phenology of the citrus leafminer, indicate that *P. citrella* lacks the ability to overwinter in diapause.

Table 2. Assessment of immature diapause of citrus leafminers reared from egg to adult in a growth chamber at 18°C.

Photoperiod condition	*n*	Proportion of larvae pupated	Emergence rate of pupae	Proportion of females	Development in days (SD)	
					Females	Males
Short day 8L:16D	115	0.96	0.74	0.46	33.9 (2.2)	34.4 (2.4)
Long day 16L:8D	109	0.97	0.83	0.51	33.3 (2.1)	34.3 (2.9)
P		0.722[1]	0.137[1]	0.632[1]	0.337[2]	0.888[2]

[1]From Fisher's exact test.
[2]From *t*-test.

TABLE 3. DISSECTION OF CITRUS LEAFMINER FEMALES REARED UNDER DIFFERENT PHOTOPERIODIC CONDITIONS AT 18°C.

Photoperiod condition	Total number of adults emerged	Number of females dissected	Proportion of females with mature eggs	*P*
10L:14D	83	47	1.00	1.000[1]
14L:10D	85	44	1.00	
8L:16D	114	52	0.98	1.000[1]
16L:8D	68	32	1.00	

[1]From Fisher's exact test.

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