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DESCRIBING SEASONAL PHENOLOGY OF THE LEAFMINER *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) WITH PHEROMONE LURES: CONTROLLING FOR LURE DEGRADATION

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ABSTRACT

Traps baited with pheromone lures were deployed in a Florida citrus grove at various dates over the course of 1 year to describe the seasonal flight phenology of the leafminer *Phyllocnistis citrella* Stainton. To compensate for lure degradation, a correction factor was applied based on a regression model of relative lure efficiency, expressed as a percent of the catch of a freshly deployed lure as a function of the number of days each set of lures was deployed. The regression of percent trap catch vs. number of days deployed yielded a quadratic expression that predicts 50% loss of lure attractiveness at 50 d after lure deployment and 90% loss at 137 d. The data transformed for lure degradation revealed 4 apparent population density peaks including 2 minor peaks with highest mean trap catch in early Apr and late Oct, and 2 major peaks with highest mean trap catch on 31 May and 1 Aug. A very small number of moths were collected on control traps without a lure. However, the pattern of trap catch on unbaited sticky cards closely paralleled that of the pheromone-baited traps.

Key Words: citrus, sex pheromone, leafminer, relative abundance, lure degradation

RESUMEN

Trampas fueron desplegadas en una huerta de cítricos en el estado de Florida durante el curso de un año para describir la fenologia de vuelo del minador de hojas *Phyllocnistic citrella* Stainton. Para compensar por la degradación del cebo (feromona sexual), se utilizó un factor de corrección derivado de un modelo de regresión de eficiencia relativa del cebo, expresado como un porcentaje del número de insectos atrapados en un cebo recién desplegado, y el número de dias desplegados. La regresión del porcentaje de minadores atrapados sobre dias de desplego produjo una ecuación cuadrática que estima una perdida de atracción de 50% despues de 50 dias y de 90% despues de 137 dias. Los datos transformados por la degradación predicha revelaron cuatro picos de densidad del minador incluyendo dos picos menores en abril y octubre, y dos picos mayores en mayo y agosto. Un número pequeño de minadores fueron atrapados en trampas sin cebo. Sin embargo, el patrón del número de minadores atrapados fue muy similar a las trampas con el cebo fermonal.

Translation provided by the authors.

The leafmining moth, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in southern Florida in 1993 (Heppner 1993) and has since spread to all Florida citrus-growing counties, the states of Alabama, Louisiana, Texas, California (Gil 1999), and Hawaii (Nagamine & Heu 2003). Damage from larval mining causes loss of photosynthetic capacity (Peña et al. 2000), stunting and malformation of leaves, and increased susceptibility of leaves to the Asiatic citrus canker pathogen, *Xanthomonas axonopodis* pv. *citri* (Bergamin-Filho et al. 2000; Cook 1988). Ando et al. (1985) found attraction in Japanese populations of *P. citrella* to traps baited with (*Z,Z*)-7,11-hexadecadienal. Attempts to show attraction of this material to populations in other countries were not successful (Sant'ana et al. 2003). Leal et al. (2006) detected 3 active compounds from female pheromone gland extracts of a Brazilian population of *P. citrella* by gas chromatography-coupled electroantennogram (GC-EAG): (*Z,Z,E*)-7,11,13-hexadecatrienal [*Z*7*Z*11*E*13-16Ald], (*Z,Z*)-7,11-hexadecadienal [*Z*7*Z*11-16Ald], and (*Z*)-7-hexadecenal [*Z*7-16Ald] in a ratio of 30:10:1, respectively. They also demonstrated that traps baited with a mixture of the 2 major constituents caught more males than traps baited with virgin female *P. citrella*. Simultaneously, Moreira et al. (2006) identified *Z*7*Z*11-16Ald and *Z*7*Z*11*E*13-16Ald by GC-EAG as critical components of the pheromone and reported that the isomeric (*Z,Z,Z*)-7,11,13-hexadecatrienal inhibited attraction of the binary blend. Lapointe et al. (2006) showed that a binary mixture in the ratio of 30:10 of *Z*7*Z*11*E*13-16Ald and *Z*7*Z*11-16Ald attracted significantly more moths of *P. citrella* compared with unbaited traps in a Florida citrus grove. The addition of *Z*7-16Ald did not increase trap catch.

Insect sex pheromone traps can be used for several purposes including monitoring relative abundance and seasonal phenology of pest species. One difficulty associated with the use of pheromone lures to estimate relative abundance is the conflation of pheromone loss through emission and degradation, and fluctuations in pest density. For purposes of pest management, trap catch could be corrected based on knowledge of the rate of pheromone loss from the emitter. Many factors influence the rate at which sex pheromones are degraded or change their effectiveness, including weather, temperature, wind velocity and direction, lure substrate composition, and, in some cases, the attractiveness of pheromone breakdown products. As Mayer & Mitchell (1999) pointed out, pheromone lure emissions in the field are difficult to predict over time.

We deployed pheromone-baited traps over a period of 14 months to determine the phenology of adult abundance of *P. citrella*. We developed a mathematical method to standardize lure catch, described the seasonal phenology of *P. citrella* in Florida citrus, and estimated lure longevity based on relative trap catch.

Material and Methods

*Z*7*Z*11*E*13-16Ald (96% purity) and *Z*7*Z*11-16Ald (98% purity) were synthesized as previously described (Leal et al. 2006). These two constituents were incorporated in grey halobutyl rubber septa in a 3:1 ratio by loading a 100 µL of hexane solution containing 50 µg of the triene, 17 µg of the diene, and 16 µg of a stabilizer, butylated hydroxytoluene (BHT). Lures were maintained in sealed plastic bags at -80°C until use. Pherocon 1C Wing Traps (Trecé, Inc., Adair, OK) baited with septa were deployed in a citrus grove infested with *P. citrella* at the experiment farm of the U.S. Horticultural Research Laboratory, Ft. Pierce, FL over 14 months from Nov 2005 through Jan 2007. Traps without lures (unbaited controls, $n = 3$) were deployed beginning in Jan 2006. Traps were randomly assigned to orange trees (approximately 2 m tall) within a section of 6 rows of 29 trees/row naturally infested with citrus leafminer. In a study at the same location, Lapointe et al. (2006) showed that trap height (1.3, 1.7, or 2.0 m) did not affect the yield of traps baited with the *P. citrella* sex phermone. Therefore, traps were placed at 1.7 m above the ground and at a distance of at least 25 m from other traps. The adhesive cards were removed approximately weekly and examined in the laboratory for the presence of *P. citrella*. Moths were counted and the total number of moths on each card was divided by the number of days deployed to calculate the mean number of moths/d during the deployment period. The mean number of *P. citrella* captured on unbaited traps on each sample date was subtracted from the trap catch data to compensate for random catch of moths. Treatments consisted of various deployment dates of baited traps. Fresh lures (3 replications per deployment date) were warmed to room temperature and then deployed in the field on eight arbitrarily selected dates in Nov 2005 and Feb, Mar, Apr, May, Jul, Sep, and Dec 2006 for a total of 24 lures. Lures deployed on a given date were left in the field until the mean trap catch declined to <10% of that of the most recently deployed set of lures. Initial lure locations were randomly assigned and rotated at each sample date. There were 12 traps with lures corresponding to 4 deployment dates, and 3 traps without lures (controls) deployed during the period of Mar through Dec 2006 (Fig. 1).

Analysis. The entire surface of the sticky card traps was counted on each of 63 sample dates over 14 months. The mean ($n = 3$) number of insects per card on each sample date was corrected by subtracting the mean number of moths caught on traps with no lure (control). The corrected data were converted to the number of moths trapped per day by dividing total count by the number of days the trap was deployed for each lure. Trap catch (moths/d) was expressed as a percentage of the trap catch of the highest-yielding lure on the respective sample day (usually the most recently deployed lure) and then regressed on the total number of days deployed for each lure. Sample dates when mean daily catch was <20 moths/d/trap were excluded to avoid excessive variability introduced by including dates with low trap catches and therefore with low signal/noise ratios. The resulting regression equations described the rate of decay of attraction by the deployed lures. A quadratic equation was compared with an exponential function based on correlation coefficient and subjective fit to the observed data. The quadratic decay equation was applied as a correction factor to all data points (moths/d) to adjust the individual counts for the loss of attraction of lures due to pheromone loss or degradation. Corrected data for Fig. 3A were minimally truncated by removing trap catch data of lures deployed for >147 d, corresponding to the lower inflection point of the quadratic decay equation. The resulting data were plotted to estimate relative density of moths over the sampling period. Area under the curve was calculated for each of the 4 population peaks discernible over the sampling period to estimate relative peak size.

Results and Discussion

Trap catches on individual sample dates ranged from zero to over 1,700 *P. citrella* moths per sticky card deployed for 9 d (192 moths/d) beginning on 1 Aug 2006. The uncorrected trap

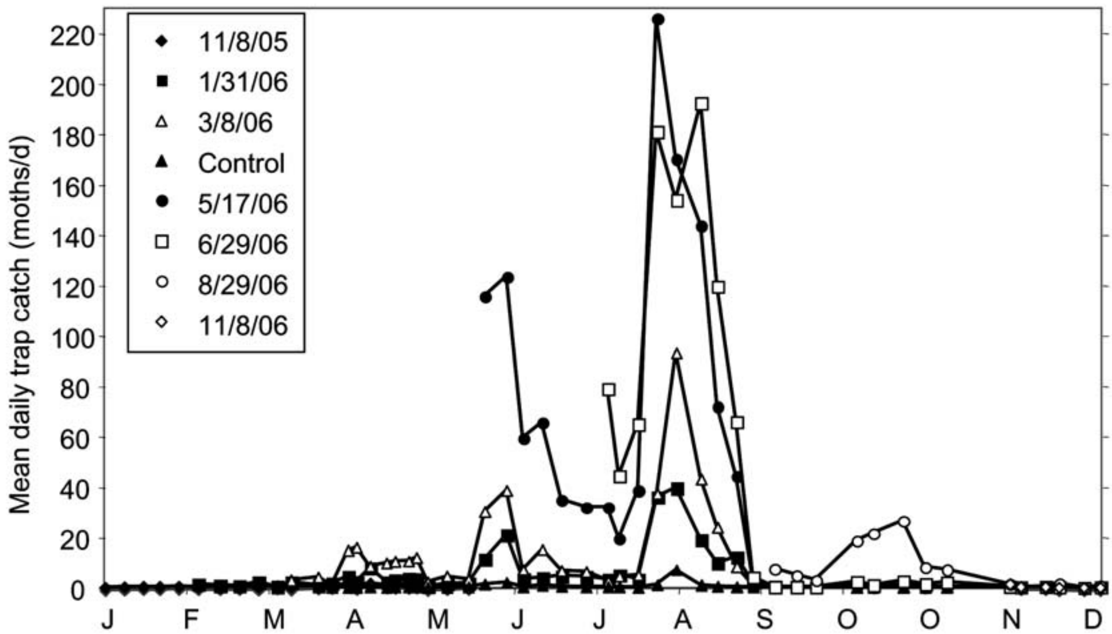

Fig. 1. Mean daily catch of *P. citrella* in pheromone-baited traps at Ft. Pierce, FL deployed in a citrus grove at various dates during 2006. Each set of points represents the mean daily catch of 3 traps deployed on the date shown in the legend over the period shown, until catch decayed below 10%.

catches (Fig. 1) suggest minor density peaks in Apr and Oct with major peaks occurring in May and Aug. The regression of percent trap catch on days deployed yielded a quadratic expression with a high correlation coefficient (Fig. 2) that best described the observed decline in trap catch over time. While the quadratic expression is clearly inaccurate at x > 147 d, it more closely reflects the rate of loss in trap catch observed during the first 100 d of lure deployment, the period of greatest interest in this study, compared with the exponential fit. The quadratic equation predicts 50% loss of trap attractiveness at 50 d after lure deployment and 90% loss at 137 d. This information will be of great value for estimating lure degradation and signal longevity for future efforts to design a mating disruption strategy for *P. citrella*.

The data allowed us to estimate the relative size of adult generations by correcting for lure emission and degradation that occur during lure deployment and to estimate pheromone longevity under field conditions in a Florida citrus grove. Lures of varying ages were deployed throughout the year and we calculated a correction for lure age based on relative trap catch during periods when trap catch was above a threshold of 20 moths/d. The raw data were then resolved into a single curve describing relative abundance of flighted *P. citrella* males. After applying the correction for lure degradation, based on regression of lure age on relative trap catch, the data showed a small first peak in Apr, followed by a second larger one in May-Jun, a large peak in Jul-Aug, and a small peak in Oct (Fig. 3A). The highest mean trap catches occurred on 31 May and 1 Aug. The absolute size of the peaks has no meaning, but the timing of the peaks and their relative size do reflect actual population dynamics. By setting the area under the curve for the first population peak (April) to 1, the remaining populations peaks in May, Jul-Aug, and Oct-Nov) were 4, 23, and 2 times as large, respectively (Fig. 3A). A very small number of moths were collected on control traps without a lure. However, the pattern of trap catch on unbaited sticky cards closely paralleled that of the pheromone-baited traps (Fig. 3B).

Our calculations are based on trap catch and not pheromone emission that presumably follows first-order kinetics of evaporation loss, i.e., the change in pheromone content of the lure is a constant function of the concentration of pheromone remaining in the lure. Our quadratic expression of loss of lure attraction assumes that pheromone emission and therefore trap catch efficiency begins to decline immediately upon deployment. However, for an initial period following lure deployment, Mayer & Mitchell (1999) showed that the emission rate of the pheromone of the diamondback moth, *Plutella xylostella*, was proportional to the amount of pheromone loaded, resulting in an initial plateau of constant pheromone emission before the rate of emission began to de-

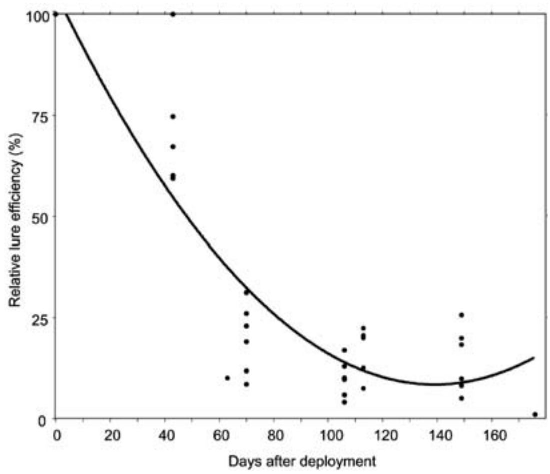

Fig. 2. Regression of the relative trap catch of lures deployed at varying dates expressed as a percentage of the most recently deployed lure against the number of days the lures were deployed in a citrus grove at Ft. Pierce, FL. $Y = 5.1E\text{-}5x^2 - 0.014x + 1.063$; $R^2 = 0.82$; $P < 0.01$.

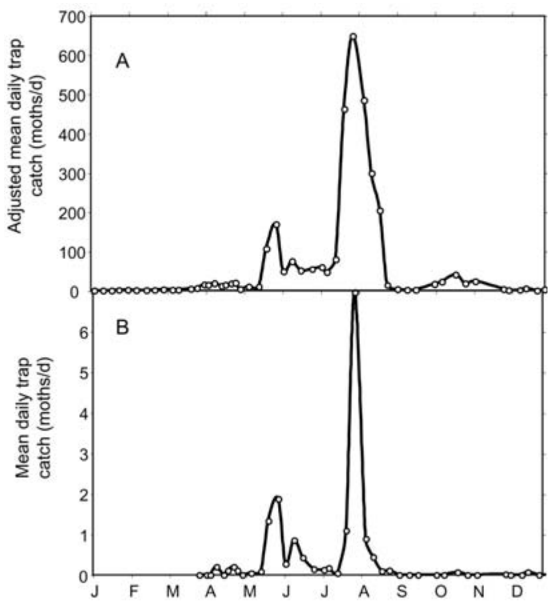

Fig. 3. Mean daily trap catch of *P. citrella* in pheromone-baited traps adjusted for duration of deployment (A) and mean daily trap catch of *P. citrella* in unbaited (control) traps (B) at Ft. Pierce, FL during 2006.

cline at a constant rate. Relative trap catch at 43 d of deployment in our test was high compared to the control lures, suggesting an initial period of constant pheromone emission followed by a more rapid decline in emission as measured by trap catch compared with the curve presented in Fig. 2. The expression of trap catch loss was also calculated after eliminating the 0 d (100%) points, yielding a quadratic expression ($y = 8.9E\text{-}5x^2 - 0.022x + 1.459$) with $R^2 = 0.70$. The poorer fit is attributable to elimination of initial data points and the resulting line produced only a minor improvement of apparent fit to the data. Application of this expression to the raw data yielded a curve substantially similar to that shown in Fig. 3A (data not shown).

The binary pheromone for *P. citrella* has been shown to be an effective lure under Florida grove conditions (Lapointe et al. 2006). While low numbers of flighted males were present throughout the winter months, the first noticeable increase in males occurred in Apr. If this pattern is representative, application of pesticides for control of the first larval population may be most effective if initiated during or subsequent to this early peak of flight and presumably mating activity. Additional sampling will be necessary to establish the validity of the phenology suggested here. The mathematical process for correcting for pheromone lure degradation can be used in many situations to control for declining lure attraction during the deployment period.

P. citrella has been shown to be a poor vector of the bacterial pathogen responsible for citrus canker, *Xanthomonas axonopodis* pv. *citri* (Belasque et al. 2005). However, mining of citrus leaves, particularly young flush, creates highly susceptible infection courts for prolonged periods of time resulting in increased and more dispersed spread of citrus canker (Gottwald et al. 2007). Chemical control of larval *P. citrella* by applications of insecticides or horticultural oils is complicated by the protection afforded by the leaf cuticle to the larvae in mines within the leaf (Mafi & Ohbayashi 2006). Hoy et al. (2007) reported no reduction in *P. citrella* larval feeding damage in trees sprayed with horticultural oil or the systemic insecticide imidacloprid compared with untreated trees. The data presented here on lure longevity suggest that deployment of the *P. citrella* sex pheromone to disrupt mating may be a highly effective control option for this pest.

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