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Placement density and longevity of pheromone traps for monitoring of the citrus leafminer (Lepidoptera: Gracillariidae)

Pilar Vanaclocha[1,], Moneen M. Jones[1,2], César Monzó[1,3], and Philip A. Stansly[1]*

Abstract

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of all citrus varieties. Larvae damage young leaves, thereby reducing photosynthesis and tree vigor, and may impact yield. Wounds opened by *P. citrella* larvae may also increase susceptibility to citrus canker disease caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (Xanthomonadales: Xanthomonadaceae). Sex pheromones coupled with appropriate traps are used as monitoring tools for this and other lepidopteran pests. Information compiled from trap captures is indicative of seasonal population fluctuations and may be used to guide management practices. Trap density and pheromone dispenser longevity are factors affecting the accuracy of trapping data. Our objectives were to evaluate capture of *P. citrella* in relation to trap density and duration under field conditions. Almost 2 yr of citrus leafminer monitoring demonstrated that a density of 1 trap per approximately 2 ha yielded similar results to the higher recommended density of 1 trap per 0.4 to 1.6 ha. Trap catch with the 2 pheromone brands tested declined by 25% after 3 to 6 wk and 50% after 6 to 10 wk during the spring through fall growing season in Florida. Therefore, correction factors are required if traps are replaced at 8 to 13 wk intervals. Results of the present study will help optimize monitoring programs that can serve as early warning of potential damaging populations of *P. citrella*.

Key Words: lure degradation; trap density; citrus canker; IPM; *Phyllocnistis citrella*

Resumen

El minador de los cítricos, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), es una plaga importante en todas las variedades de este cultivo. El daño asociado a sus larvas al alimentarse sobre hojas tiernas reduce la capacidad fotosintética del árbol así como su vigor, y puede afectar al rendimiento productivo. Además, las heridas producidas por este minador incrementan la susceptibilidad de la planta al canker de los cítricos, enfermedad causada por la bacteria *Xanthomonas axonopodis* pv. *citri* (Xanthomonadales: Xanthomonadaceae). La utilización de atrayentes sexuales para trampas de captura es ampliamente utilizada en el monitoreo de esta y otras especies plaga de lepidópteros. La información recogida de esta manera permite conocer las fluctuaciones poblacionales de la especie monitoreada y por lo tanto, puede ser de gran utilidad para su gestión. La densidad de trampas y la longevidad del dispensador son factores que afectan a la precisión de los datos obtenidos. Nuestros objetivos fueron evaluar la captura de adultos de *P. citrella* en relación a la densidad de trampas y duración de dispensadores en condiciones de campo. Prácticamente dos años de monitoreo demostraron que densidades de 1 trampa cada 2 ha produjo resultados similares a los obtenidos a la densidad recomendada de 1 trampa cada 0.4-1.6 ha. El número de capturas para las dos marcas de dispensadores evaluadas se redujo en un 25% después de 3-6 semanas y en un 50% entre las 6 y 10 semanas entre primavera y otoño. Por lo tanto se necesita de factores de corrección si las trampas son reemplazadas en un intervalo de 8-13 semanas. Los resultados del presente estudio ayudarán a optimizar los programas de monitoreo que mediante esta técnica pretenden reducir los daños potenciales asociados a *P. citrella*.

Palabras Clave: degradación de atrayente; densidad de trampas; el canker de los cítricos; MIP; *Phyllocnistis citrella*

Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae), the citrus leafminer, is an important pest of citrus (Sapindales: Rutaceae) (Heppner & Fasulo 1998; Ujiye 2000). The citrus leafminer oviposits on new growth shoots (flush). After eclosion, 1st instar larvae penetrate directly into the leaf and begin feeding on sap through the 3rd instar (Ujiye 2000). The resulting serpentine mines reduce photosynthesis and ultimately tree vigor with a possible impact on yields (Peña et al. 2000). Leafmining also increases susceptibility to citrus canker caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (Xanthomonadales: Xanthomonadaceae) (Christiano et al. 2007). Citrus leafminer damage is thus especially important in canker-susceptible varieties such as grapefruit and early-season oranges (Dewdney & Graham 2014).

Sex pheromones are used as monitoring tools for many lepidopteran pests worldwide (Witzgall et al. 2010). Ando et al. (1985) were first to report attraction of the citrus leafminer to a single pheromone component (*Z*,*Z*)-7,11-hexadecadienal in Japan. However, the single pheromone component was ineffective outside Japan until combined in a 2-component system with (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal resulting in the 3:1 triene:diene mixture used today (LaPointe et al. 2006; Leal et al. 2006; Moreira et al. 2006).

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Table 1. Plot characteristics, number of traps, distance between traps, and grid size (trap density) used in the optimal trap density study.

Plot name/treatment	Cultivar	Plot size (ha)	Traps (No.)	Distance between traps (m)	Trap density (ha per trap)
C-13W/High	'Flame'	2.70	6	74.0	0.43 ha
8-6W/High	'Ray Ruby'	2.91	7	60.0	0.28 ha
C-15/High	'Flame'	2.13	5	58.0	0.26 ha
3-4E/Medium	'Flame'	9.26	7	113.0	0.99 ha
C-11E/Medium	'Flame'	5.25	5	126.0	1.24 ha
7-1E/Medium	'Ray Ruby'	8.81	7	116.0	1.05 ha
C-1/Low	'Flame'	10.66	4	170.0	2.27 ha
6-5/Low	'Flame'	12.66	6	146.5	1.69 ha
6-3W/Low	'Flame'	15.42	6	153.0	1.85 ha

The potential impact of leafmining on citrus trees depends on both the citrus leafminer population and the amount of susceptible young flush present at any particular time. Insecticidal control of the citrus leafminer in Florida on bearing trees is based on the presence of susceptible young flush and mostly ignores pest demography (Rogers et al. 2015). Including information about pest density and its seasonal fluctuations through monitoring with pheromone traps could provide early warning of impending damage to young flush and therefore could help optimize the management of this pest.

Several studies with other insects showed that efficacy and longevity of pheromone lure attractants depend on the compound blend, the type of dispenser used, and environmental factors such as temperature and wind speed (Witzgall et al. 2010; Vacas et al. 2012; Vanaclocha et al. 2012; Williams et al. 2013). In addition, captures are influenced by the trap density (Thwaite & Madsen 1983; Buckman & Campbell 2013). Therefore, the cost-effective implementation of trap monitoring with a particular pheromone blend requires knowledge of optimal trap densities and lure degradation rates under varying environmental conditions (LaPointe & Leal 2007).

The present study seeks to help optimize the implementation of citrus leafminer pheromone trapping in citrus groves by (i) determining whether commercial recommended trap densities are optimal for accurately monitoring seasonal flight activity and (ii) evaluating the rate of pheromone deactivation for 2 commercial lures under field conditions during peak flight periods.

Materials and Methods

TRAP DENSITY STUDY

The experiment was conducted at a commercial citrus orchard in 9 plots all located within 5 km of 26.295°N, 81.416°W near Immokalee, Collier County, Florida. Trees were 10- to 37-yr-old grapefruit ("*Citrus paradisi*") 'Flame' and 'Ray Ruby' varieties bud-grafted to 'Swingle' citrumelo rootstock planted at a density of 355 trees per ha. Plots were of varying shape, separated by 597 to 8,343 m, and ranged from 2.13 to 15.42 ha in size (Table 1). All were micro-sprinkler irrigated and managed using standard commercial practices (Davies & Jackson 2009).

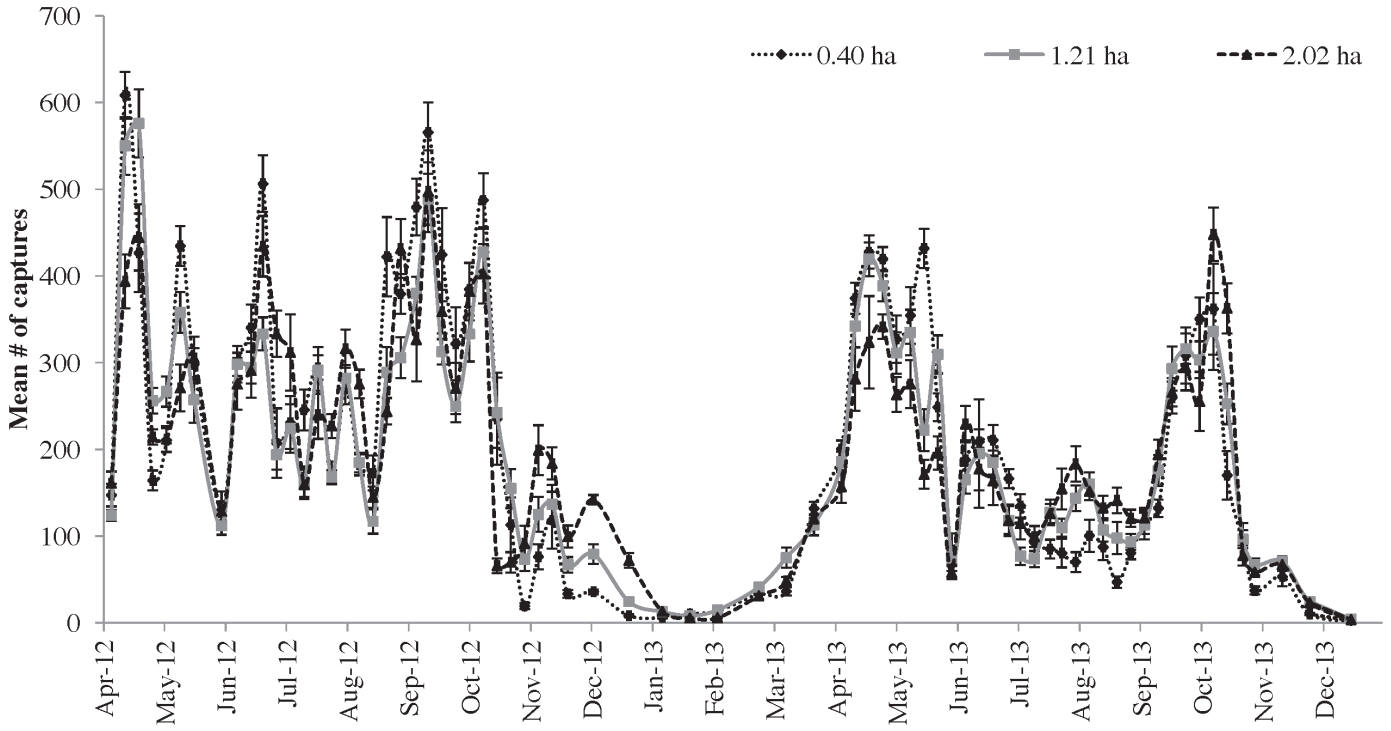


Fig. 1. Mean number of *Phyllocnistis citrella* adult male captures per trap and day (± standard error) from Apr 2012 to Dec 2013, at the 3 trap densities tested: high: approximately 1 trap per 0.40 ha (1 acre), medium: approximately 1 trap per 1.21 ha (3 acres), and low: approximately 1 trap per 2.02 ha (5 acres).

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Optimal trap density for citrus leafminer monitoring was studied in a completely randomized design with 3 trap densities determined according to recommendations by manufacturers (0.83 traps per ha for AlphaScents, Inc., West Linn, Oregon, and between 2.5 and 5 traps per ha for ISCA Technologies, Inc., Riverside, California): low (<0.45 traps per ha), medium (0.45 to 1.65 traps per ha), and high (>1.65 traps per ha). Three replicates per density (plots) were conducted (Table 1). *Phyllocnistis citrella* male adult populations were monitored in each plot from Apr 2012 to Dec 2013 using white plastic delta traps (28 × 20 × 15 cm) provided with white, sticky liners (19.5 × 17.5 cm) and baited with IT203 citrus leafminer pheromone lures (ISCA Technologies, Inc.) (ISCA).

Traps were deployed approximately equidistantly within each plot to create a grid corresponding to the tested density of the treatment. Each trap was hung in the middle of the tree canopy at about 2 m height and left in the same location for the duration of the experiment. Lures were replaced every 6 wk, which is 2 wk less than the replacement time recommended by the manufacturer (8 wk for ISCA lures). Trap liners were replaced and brought to the laboratory every 2 wk from Nov to Mar (low citrus leafminer activity) and weekly from Apr to Nov (high citrus leafminer activity). All male adult moths on a liner were counted under a magnifying lamp in the laboratory if approximately 300 male moths were found. Counts at higher densities were made on 20 of the 72, 3.6 cm^2 liner squares, and these values were multiplied by 4.5 to estimate the entire trap capture. For each trap density, the total number of male adult moth captures per trap and season was obtained by summing in each trap all moth captures from the beginning to the end of the study. The number of male adult captures per trap and day was also calculated for each sampling date.

LURE LONGEVITY STUDY

Lure decay rates were determined using lures from 2 commercial sources: IT203 (ISCA lure) (ISCA Technologies, Inc.) and PHYCIT (AlphaScents lure) (AlphaScents, Inc.). Both lures were composed of a 3:1 triene:diene blend consisting of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal and (*Z*,*Z*)-7,11-hexadecadienal absorbed on rubber septa.

The study was conducted at a commercial citrus grove in a 15.8 ha block of 11-yr-old 'Pineapple' sweet orange trees (*Citrus sinensis* [L.] Osbeck) on 'Carrizo' citrange rootstock planted at a density of 458 trees per ha in Hendry County near LaBelle, Florida (26.295°N, 81.617°W). Trees were irrigated with micro-sprinklers and grown using standard commercial practices (Davies & Jackson 2009). Two experiments were conducted in 2013 during 2 periods of major *P. citrella* activity: spring (20 May to 21 Jun) and late summer/mid-fall (9 Sep to 30 Oct). The grove was divided into 24 plots, each approximately 0.66 ha, and assigned in a completely randomized design to 3 replications of 8 treatments: 3 lure-aging treatments plus a control (not aged) for each lure source.

Treatment locations within the grove were the same throughout each experiment. One ISCA white plastic delta trap was deployed in mid-canopy of a centrally located tree in each plot. For the 3 lure-aging treatments, lures were aged for 8, 6, and 4 wk before deployment, whereas fresh lures were used and replaced weekly for the control treatment. Lures designated for aging were placed in delta traps hung in poles at approximately 2 m height under varying sun and shade conditions, depending on the time of the day, in an open field at the Southwest Florida Research and Education Center in Immokalee, Florida, for the requisite weathering time (4, 6, or 8 wk). This process was necessary so that all the lures had the required age just before starting the field experiment. On day 0 of the experiment, all lures (aged and fresh) were placed in the delta traps of their designated plots. Aged lures were not replaced during the course of the experiment so that the aging process could continue through the end of the study. Moth captures were counted weekly as described for the trap density study.

DATA ANALYSES

All statistical analyses were done with SAS Version 9.3 software (SAS Institute 2010). Data for total captures per trap and season and total captures per trap and day were tested for normality and homoscedasticity using the Univariate procedure before model selection. A general mixed model was used to analyze the effect of trap density on the total number of citrus leafminer captures per season. Trap density was treated as a fixed factor and grapefruit variety as a random factor. Seasonal variability in treatment effects on the number of captures per trap and day was evaluated using Spearman's correlation analysis.

Differences in male adult moth catches per trap and day between fresh lures of the 2 manufacturers (AlphaScents and ISCA) and the 2 study periods (spring and late summer/mid-fall) in the lure longevity study were tested using the General Mixed Model procedure. Brand, season, and the interaction between both variables were treated as fixed factors. An autoregressive covariance structure between measures of each trap at various times was selected based on the Akaike and Bayesian information criteria (AIC and BIC, respectively). Post hoc *t*-test (least significant difference, LSD) comparisons were made in case of any significant effect ($P < 0.05$).

Decay of efficacy for each lure was calculated weekly as the ratio of the citrus leafminers captured per trap to the mean number of captures in traps containing fresh lures (i.e., the controls). The relative number of captures with respect to fresh lures was then related to lure age by repeated measures analysis using the General Mixed Model procedure assuming that all measures from the same lure were correlated throughout the experiment. Using these data, the number of weeks until 25 and 50% reduction of captures with respect to that of fresh lures was estimated for each brand and season. Differences in lure degradation pattern between brands and seasons were analyzed by repeated measures analysis using the General Mixed Model procedure. Two categorical variables, "brand" and "season," plus their interactions with "lure age" were included in the models. A significant ($P = 0.05$) effect of either of the 2 categorical variables was indicated by significantly different intercepts, whereas significant interaction between either of these 2 variables and "lure age" was indicated by significantly different slopes. In the event of no differences between brands or seasons, data were combined to derive a single equation.

Results

TRAP DENSITY STUDY

No significant differences were found among the 3 trap densities tested (Table 1) in total number of *P. citrella* male adult captures per trap from the beginning to the end of the experiment ($F = 1.11$; df = 2,49; $P = 0.336$). Seasonal variations in flight activity over the 2 yr study period were correlated among the 3 trap densities tested (high and medium: $\rho = 0.95$, $P < 0.0001$; high and low: $\rho = 0.86$, $P < 0.0001$; medium and low: $\rho = 0.91$, $P < 0.0001$). A spring and a late summer/

Table 2. Male adult moth catches (mean ± standard error) per trap and day attracted by fresh lures of the 2 brands tested (ISCA and AlphaScents) during the periods of major *Phyllocnistis citrella* flight activity (spring and late summer/mid-fall) in the lure longevity study. Means followed by the same letter are not significantly different (LSD means: $P > 0.05$).

Lure brand and season	Male adult moth catches per trap and day
ISCA spring	262.07 ± 41.88 a
ISCA summer/fall	260.69 ± 36.62 a
AlphaScents spring	164.56 ± 24.43 ab
AlphaScents summer/fall	94.63 ± 15.30 b

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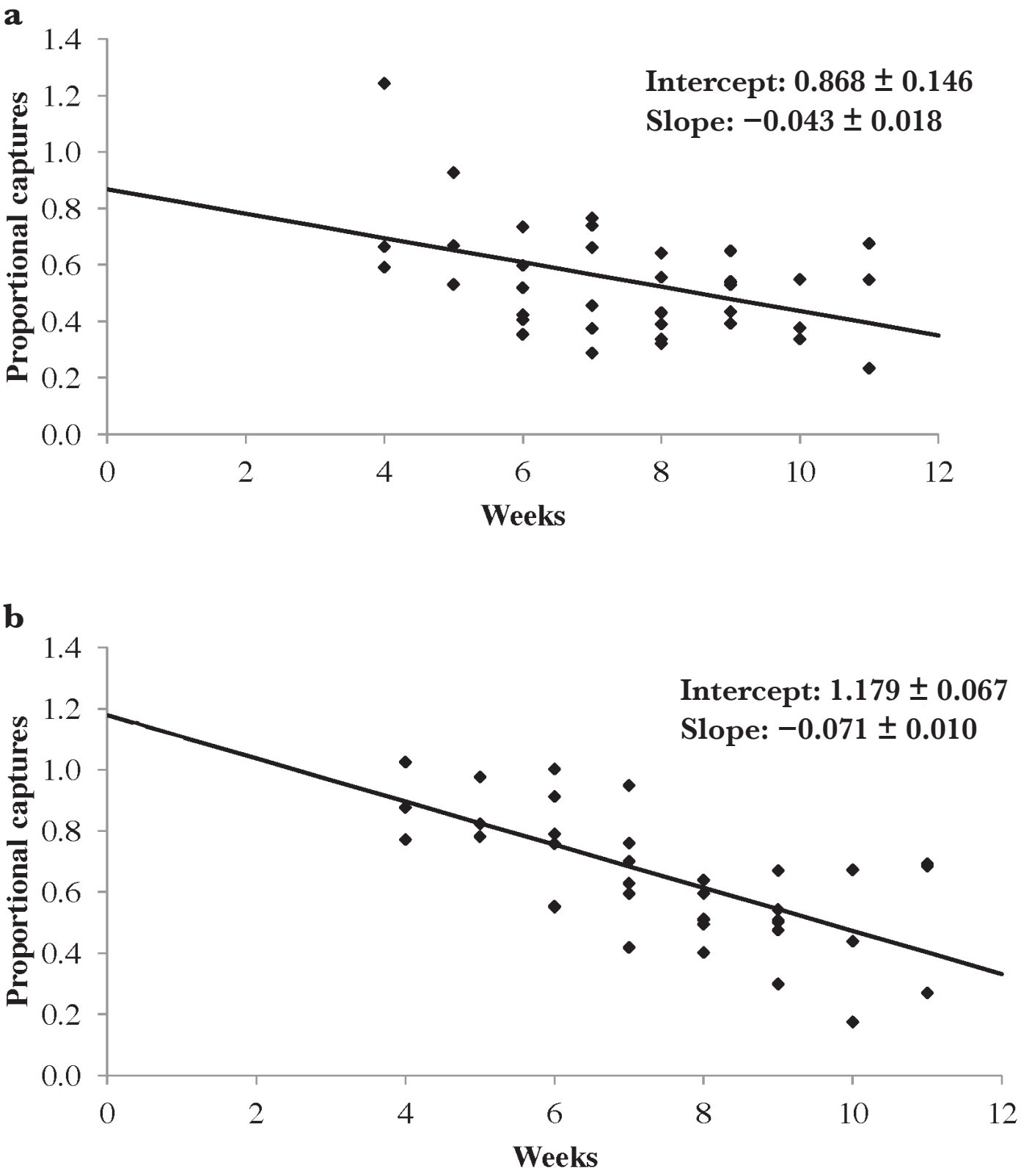


Fig. 2. Relationship between the proportional number of *Phyllocnistis citrella* captures per trap and day of aged lures with respect to unaged lures, and the number of weeks that each lure was exposed to field environmental conditions during spring 2013, for the 2 commercial brands of lures a) ISCA and b) AlphaScents.

mid-fall peak in flight activity were evident both years, with the latter peak coming 1 mo earlier in 2012 than in 2013. Secondary peaks in late spring and mid-summer were more evident in 2012 than in 2013 (Fig. 1).

LURE LONGEVITY STUDY

ISCA fresh lures caught significantly more adult moths than AlphaScents lures (F = 7.98; df = 1,24; P = 0.0094). No differences between

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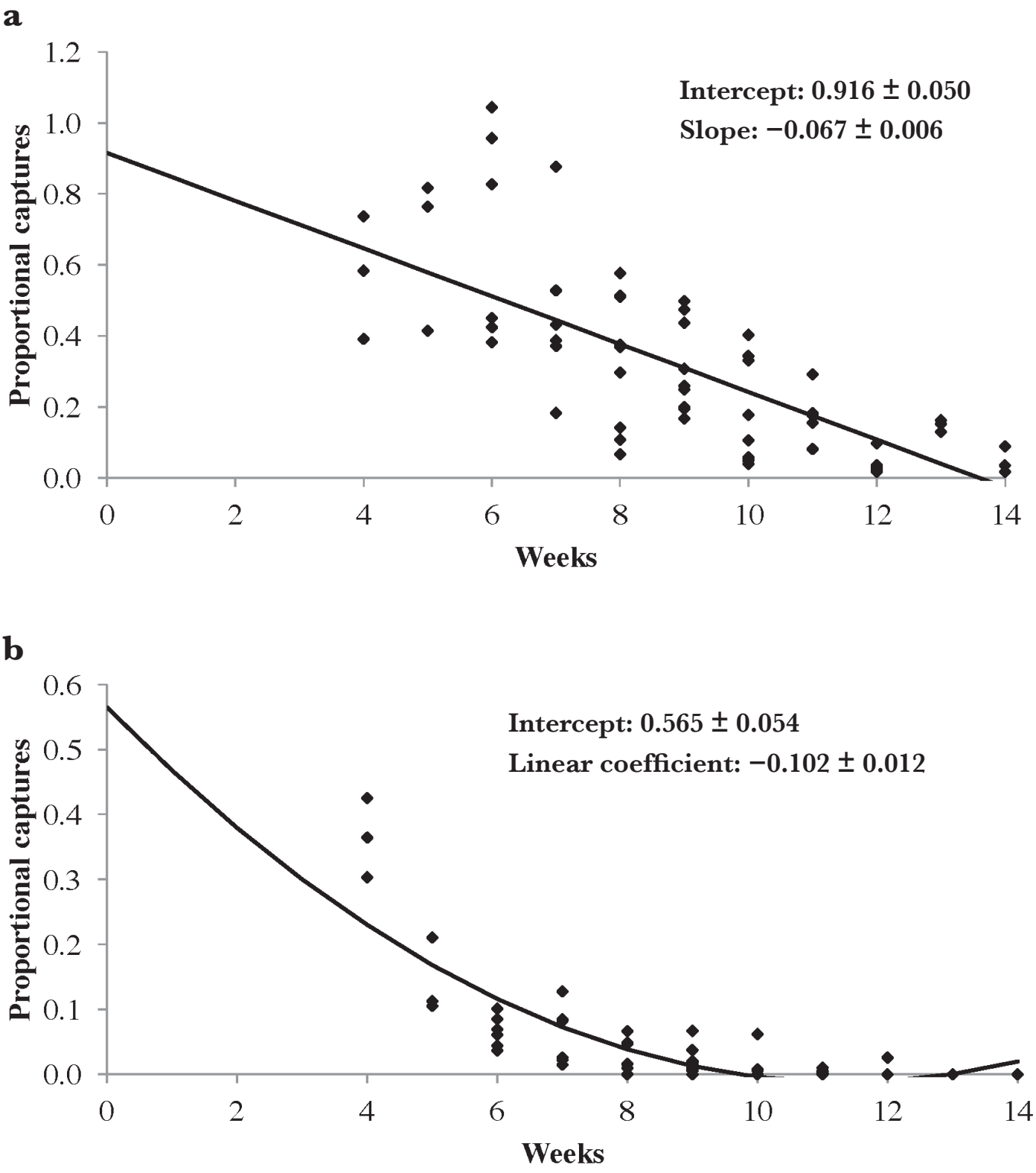


Fig. 3. Relationship between the proportional number of *Phyllocnistis citrella* captures per trap and day of aged lures with respect to unaged lures, and the number of weeks that each lure was exposed to field environmental conditions during summer/fall 2013, for the 2 commercial brands of lures a) ISCA and b) AlphaScents.

seasons or interaction effect was found (season: $F = 0.71$; df = 1,16; $P = 0.407$; interaction: $F = 0.46$; df = 1,24; $P = 0.5019$) (Table 2).

Efficacy of both lures decayed linearly in spring (ISCA: $F = 5.97$; df = 1,8; $P = 0.0404$, AlphaScents: $F = 54.91$; df = 1,8; $P < 0.0001$). No significant differences in lure degradation rate between brands were found ($F = 2.70$; df = 1,16; $P = 0.1197$). Estimated half-life of the ISCA lure was 8.5 wk (CL95: 6.30–10.72) with 25% reduction in less than 4 wk. Half-life of the AlphaScents lure was estimated at 9.6 wk (CL95: 8.63–10.60) with a 25% reduction at 6.1 wk (CL95: 5.45–6.68) (Fig. 2).

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Table 3. Predicted lure degradation rates, expressed as proportional captures with respect to fresh lures, for ISCA and AlphaScents pheromone dispensers in the spring and summer/fall experiments. Equations obtained from repeated measures analysis were used. Temperatures are averages ± standard errors for the study site from the beginning of the lure aging process to the end of each experiment.

	Spring T = 23.83 ± 0.33 °C		Fall T = 26.30 ± 0.15 °C	
Lure age (wk)	ISCA	AlphaScents	ISCA	AlphaScents
4	0.69	0.90	0.65	0.23
5	0.65	0.82	0.58	0.17
6	0.61	0.75	0.51	0.12
7	0.57	0.68	0.44	0.08
8	0.52	0.61	0.38	0.04
9	0.48	0.54	0.31	0.01
10	0.44	0.47	0.24	0.00
11	0.39	0.40	0.18	0.00
12	0.35	0.33	0.11	0.00

Efficacy of the ISCA lure also decayed linearly during late summer/early fall (F = 139.25; df = 1,8; P < 0.0001). Estimated ISCA lure half-life was 6.2 wk (CL95: 5.61–6.75) with a 25% reduction in less than 4 wk. However, the AlphaScents lure decay during this interval was better explained by a 2nd-order linear model (age: F = 74.10; df = 1,8; P < 0.0001; age × age: F = 54.74; df = 1,8; P < 0.0001). In this case, lure efficacy decayed so rapidly that almost no captures were obtained after 8 wk. Estimated lure half-life was 0.7 wk (CL95: −0.28–1.66) (Fig. 3).

In contrast with the spring experiment, we found significant differences in lure degradation rate between brands in the summer/fall experiment (F = 38.67; df = 1,16; P < 0.0001). Average mean temperature for the spring experiment (T = 23.8 ± 0.33 °C, from 25 Mar to 21 Jun) was significantly colder than during the summer/fall experiment (T = 26.3 ± 0.15 °C from 15 Jul to 31 Oct) (F = 52.42; df = 1,196; P < 0.0001). Nevertheless, no differences in lure degradation were found between the spring and summer/fall experiments for the ISCA lure (F = 1.80; df = 1,8; P = 0.2166). In contrast, efficacy of the AlphaScents lure was significantly less in the summer/fall experiment compared with the spring experiment (F = 160.76; df = 1,8; P < 0.0001). Predicted lure degradation rates for each brand and season are displayed in Table 3.

Pooling data from ISCA spring, ISCA summer/fall, and AlphaScents spring resulted in a linear decay with time (F = 137.08; df = 1,26; P < 0.0001). The estimated lure half-life was 7.8 wk (CL95: 7.24–8.44) with a 25% reduction after 4.2 wk (CL95: 3.28–5.10) (Fig. 4).

Discussion

We observed 2 major and approximately equal flight peaks in early spring and late summer/early fall, interspersed with at least 2 summer secondary peaks (between mid-Jul and mid-Aug). These trends correspond to flushing patterns of citrus trees in Florida (Hall & Albrigo 2007). LaPointe & Leal (2007) also noted 2 peaks of flight activity in a 1 yr study on the generally wetter and warmer east coast of Florida (Windsberg 2003), the 1st peak in late May and the 2nd and larger peak in early Aug. Similar differences between the east coast and southwest citrus growing regions in seasonal abundance of another flush-dependent pest, the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), were noted in a study conducted during 2006–07 and attributed to differing weather patterns in the 2 regions (Qureshi et al. 2009).

The manufacturers recommend deploying traps at densities of 1 trap per 0.2 to 1.2 ha (0.5–3.0 acres) (AlphaScents 2014; ISCA 2014). We found no differences in magnitude or pattern of moth captures up to 1 trap per 2.5 ha (5 acres). Therefore, the lower recommended trap density could be used to provide more cost-effective monitoring without compromising accuracy.

Trap efficacy is influenced by the pheromone release rate. With the exception of the AlphaScents lure in fall, we found similar decay rates between lure types and seasons. The greater decay rates in AlphaScents lures during the summer/fall experiment were associated with lower moth catches in fresh lures compared with catches in fresh ISCA lures (Table 2). AlphaScents lures in spring showed a similar trend.

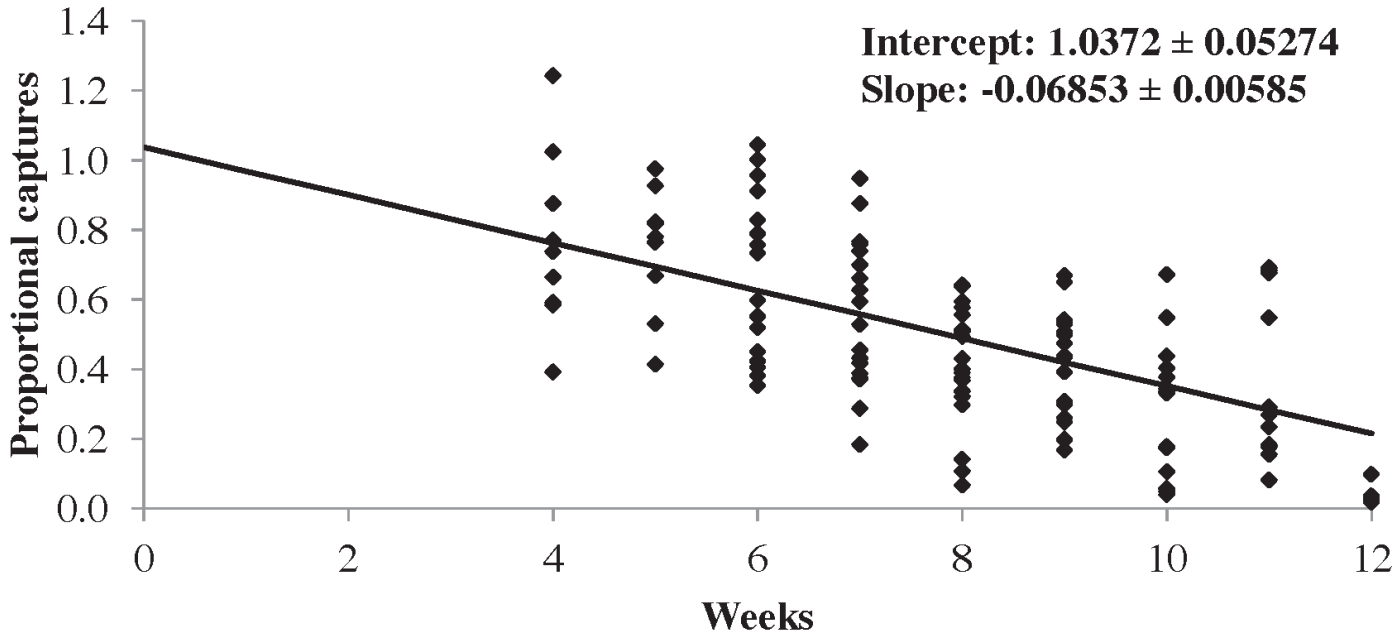


Fig. 4. Relationship between the proportional number of *Phyllocnistis citrella* captures per trap and day of aged lures with respect to unaged lures, and the number of weeks that each lure was exposed to field environmental conditions, with data combined for ISCA and AlphaScents during spring and ISCA during summer/fall 2013.

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These facts lead us to believe that lures provided by AlphaScents for the summer/fall experiment were defective although we cannot say why. The cause could be a different loading rate of the two blend pheromone components or a defect in rubber septa.

Linear models adequately described decay rates over an interval of from 4 to 12 wk for ISCA lures and AlphaScents lures in spring (Figs. 2 and 3a). LaPointe & Leal (2007) derived a quadratic regression equation to predict the degradation rate of noncommercial lures of the same diene:triene ratio for up to 21 wk and predicting a half-life of 7 wk. We observed half-lives of 6 to 10 wk in 3 of the 4 evaluations, independent of the season (spring or summer/fall). Combining the data from Figs. 2 and 3a, we derived a linear equation that predicts a half-life of 7.8 wk and a 25% decay at 4.2 wk (Fig. 4), which agrees well with the linear portion of the quadratic equation of LaPointe & Leal (2007).

An accurate estimate of emission decay rates will help minimize errors in the evaluation of flight activity (Riedl 1980; Lapointe & Leal 2007). Unaccounted reductions of 25% or more in lure efficacy may lead to incorrect conclusions regarding moth phenology. In the absence of correction factors, commercial lures would have to be replaced at least every 4 wk in order to avoid errors greater than 25%. Accurate monitoring of *P. citrella* flight, together with citrus flushing patterns (Hall & Albrigo 2007), should be helpful in predicting and avoiding undue loss due to leafmining and associated citrus canker, especially on young trees and for canker-susceptible varieties such as grapefruit and early-season oranges.

Pest demography information obtained through monitoring might eventually be a key component for development of treatment thresholds. Further studies are needed to evaluate the economic benefits of combining plant and insect phenology data for making decisions on citrus leafminer management (Grafton-Cardwell et al. 2012).

In conclusion, optimal trap density and accurate degradation rates will help researchers and producers to better evaluate and respond to changes in *P. citrella* flight activity. We showed no gain in accuracy with trap densities greater than 2 per ha, which is more economical than previously recommended densities. Corrections based on degradation curves herein provided can also reduce costs by postponing the need for replacing lures. The information thus obtained on citrus leafminer flight coupled with observation of foliage growth could provide early warning in time to deter pending damage to new flush. Realistic trap density and lure replacement will help producers to save money by reducing the cost of monitoring material and wage labor without compromising accuracy of information obtained.

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