

DEVELOPMENT OF TRAPPING METHODS WITH A SYNTHETIC SEX PHEROMONE OF THE PINK HIBISCUS MEALYBUG, *MACONELLICOCCUS HIRSUTUS* (HEMIPTERA: PSEUDOCOCCIDAE)

Authors: Francis, Antonio, Bloem, Kenneth A., Roda, Amy L., Lapointe, Stephen L., Zhang, Aijun, et al.

Source: Florida Entomologist, 90(3) : 440-446

Published By: Florida Entomological Society

URL: [https://doi.org/10.1653/0015-4040\(2007\)90\[440:DOTMWA\]2.0.CO;2](https://doi.org/10.1653/0015-4040(2007)90[440:DOTMWA]2.0.CO;2)

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

DEVELOPMENT OF TRAPPING METHODS WITH A SYNTHETIC SEX PHEROMONE OF THE PINK HIBISCUS MEALYBUG, *MACONELlicoccus hirsutus* (HEMIPTERA: PSEUDOCOCCIDAE)

ANTONIO FRANCIS¹, KENNETH A. BLOEM², AMY L. RODA³, STEPHEN L. LAPOINTE⁴,
AIJUN ZHANG⁵ AND OGHENEKOME ONOKPISE⁶

¹Florida A&M University, College of Engineering Sciences, Technology, and Agriculture,
Center for Biological Control, Tallahassee, FL

²USDA-APHIS-PPQ-Center for Plant Health Science and Technology, Raleigh, NC

³USDA-APHIS-PPQ-Center for Plant Health Science and Technology, Miami, FL

⁴USDA-ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL

⁵USDA-ARS-BARC-PSI-Chemicals Affecting Insect Behavior Laboratory, Beltsville, MD

⁶Florida A&M University, College of Engineering Sciences, Technology, and Agriculture, Tallahassee, FL

ABSTRACT

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), threatens numerous crops of economic importance and could spread from populations in California and Florida to 33 other states. Field experiments conducted in Florida evaluated 3 commercially available trap designs baited with synthetic female sex pheromone for efficiency in trapping adult male *M. hirsutus* as well as ease in processing. Delta traps and double-sided sticky cards captured more males than Jackson traps. The Delta and Jackson traps were more effective at minimizing the capture of non-target insects. The effect of lure age on males captured was also evaluated by pre-aging lures outdoors for 0 to 8 months before testing. Fewer males were caught in Delta traps as the age of the lure increased, with significantly fewer caught in traps that had been pre-aged for 2 months. Monitoring of male flight activity throughout diel cycle with baited Delta traps indicated that males were most active around dusk. The field experiments also showed that the pheromone traps often capture males in areas where no visual indication of an infestation is evident. The pheromone trap may serve as a valuable tool to detect new infestations of pink hibiscus mealybug.

Key Words: *Maconellicoccus hirsutus*, sex pheromone, monitoring, trap design, lure longevity, male flight activity

RESUMEN

La cochinilla rosada del hibiscus, *Maconellicoccus hirsutus* (Green) es una amenaza para varios cultivos de importancia económica y puede esparcirse de poblaciones presentes en California y Florida a otros 33 estados. Experimentos de campo realizados en Florida evaluaron 3 diseños de trampas comerciales disponibles con cebos de una feromona sexual sintética de hembras para atrapar machos adultos de *M. hirsutus* con eficiencia y facilitar su proceso de elaboración. Las trampas de tipo "Delta" y las tarjetas con los dos lados pegajosos capturaron mas machos que las trampas de tipo "Jackson". Las trampas Delta y Jackson fueron mas efectivas en minimizar la cantidad capturada de insectos que no se busca controlar. El efecto de la edad del señuelo sobre la cantidad de machos capturados también fue evaluado por señuelos pre-añejados en el exterior por cero a 8 meses antes de la prueba. La cantidad de machos capturados en las trampas Delta fue menor con el aumento en la edad del señuelo, con significativamente menos capturados en trampas que han sido pre-añejadas por 2 meses. El monitoreo de la actividad de vuelo de los machos a través del ciclo de "diel" [un ciclo biológico de 24 horas] con trampas Delta cebadas indicó que los machos fueron mas activos por el tiempo alrededor del atardecer. Los experimentos de campo también muestrearon que las trampas de feromonas a menudo capturaron machos en áreas donde no hubo una indicación visual evidente de una infestación. La trampa de feromona puede servir como una herramienta valiosa para detectar nuevas infestaciones de la cochinilla rosada del hibiscus.

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), has a reported host range of >125 plant species (Ghose 1972; Williams 1986; Mani

1989), attacking many agricultural crops, forest trees, and ornamental plants (Persad 1995; Kairo et al. 2000). Heavy infestations of this mealybug

can eventually kill the host plant (Stibick 1997; Kairo et al. 2000). In the absence of effective control measures, the economic risk to U.S. agriculture has been estimated at \$750 million per year (Moffitt 1999). Therefore, early detection is important for the timely application of control measures to suppress new outbreaks of *M. hirsutus* and prevent significant economic and environmental losses.

Serrano et al. (2001) first reported attraction of adult male *M. hirsutus* to virgin females, suggesting the action of an unidentified sex pheromone. The active components of this female sex pheromone were recently identified (Zhang et al. 2004a) and synthesized (Zhang et al. 2004b; Zhang & Nie 2005). Its effectiveness in sticky traps under field conditions as a male attractant has been demonstrated (Zhang & Amalin 2005). The synthesized pheromone was found to be highly specific and lure activity remained for up to 21 weeks in the field.

Prior to deploying pheromone-based trapping systems, the factors that may influence trap capture need to be assessed and trapping protocols standardized. We evaluated 3 commercially available trap designs for effectiveness in capturing the target species and selectivity for non-target captures, and more closely evaluated the longevity of the sex pheromone lure in the field at different sites. We also used pheromone-baited traps to determine the diurnal pattern of flight by male *M. hirsutus*. An effective monitoring program with *M. hirsutus* sex pheromone should include recommendations on frequency of lure replacement and an effective trap design that would allow detection at low population levels.

MATERIALS AND METHODS

Lures

Lures for all of the field trials were prepared at the USDA-ARS-BARC facility, Beltsville, MD, with gray halo-butyl rubber septa (5 mm, West Pharmaceutical Services, Kearney, NE) impregnated with 1 µg of *M. hirsutus* synthetic sex pheromone (Zhang & Amalin 2005). They were stored in air-tight plastic bags and shipped by express carrier on the same day. Lures for all experiments were from the same batch and were stored in a deep freezer at $-25 \pm 2^\circ\text{C}$ until needed. The blank control septa used in these field trials were not loaded with solvent of any type. Chemical and optical purities of synthetic pheromone components used in current study were the same as previously reported (Zhang & Amalin 2005).

Trap Design Study

Field trials were conducted from April 13 to May 18, 2005 at 2 sites on the USDA-ARS Subtropical Horticulture Research Station, Miami, FL. Prior sampling with lure-baited traps re-

vealed high numbers of adult male *M. hirsutus* in the area despite the lack of visual signs of infestation (e.g., mealybug colonies, 'bunchy top', etc.) on station property. The 2 sites were separated by >300 m and were >15,000 m² each. The first site had a mixture of large non-fruit bearing trees such as *Ficus* spp., while the second site had plots of mature mango and avocado trees.

The traps tested were Delta traps (Scentry, Inc., Buckeye, AZ), Jackson traps (Scentry Inc., Buckeye, AZ), and Pherocon V, scale-monitoring, two-sided sticky cards (Trécé, Inc., Salinas, CA). All traps were white and constructed from weather-resistant paperboard. These traps are commercially available, relatively inexpensive, and widely used in other insect monitoring programs. The Delta trap has a triangular-shaped design with perforated ends that can be folded inwards to create a narrower opening when assembled and has a total sticky surface area of 575.25 cm² on its 3 interior surfaces. The Jackson trap is also triangular-shaped, but the 2 ends are completely open and only the removable bottom insert has a sticky surface with an area of 120.97 cm². The Pherocon V sticky card is a 2-dimensional, rectangular trap with total sticky surface area of 193.6 cm² (96.8 cm² on each side).

A randomized complete block design was used with 2 replicates at each site. Each replicate consisted of 6 treatments—the 3 trap designs baited with pheromone lures or blank septa. Lures and blank control septa were placed inside plastic baskets and secured with wire holders to the top interior of the Delta and Jackson traps. For the sticky cards, the baskets/lures were suspended from the top of 1 of the 2 exposed sides. The traps were then mounted to stakes buried in the ground throughout both sites at a height of 0.45 m (Zhang & Amalin 2005) and were arranged in a 3 × 4 grid with the traps spaced 30 m apart. Traps were replaced and re-randomized weekly for 4 weeks and the lures were transferred to the new traps. Collected traps were placed into separate transparent plastic bags and brought to the laboratory to count trapped *M. hirsutus* males and non-target insects. Under ≥5× magnification, adult *M. hirsutus* males generally range from 2.5-3 mm in length (excluding wings and caudal filaments). The 2 caudal filaments are about as long as the body. However, because previous work had indicated that >99% of male mealybugs captured in traps baited with pink hibiscus mealybug pheromone were *M. hirsutus* (Zhang et al. 2004a), and initial identifications in these experiments provided by G. Hodges (FL Department of Agriculture and Consumer Services, Gainesville, FL) showed similar findings, no efforts were made to determine if other mealybug species were found on traps. At the end of the trial, used lures were collected and stored in individual plastic bags held in a freezer as previously described until analyses.

Lure Longevity Study

Fresh to 8 Months Aged Lures: Field trials were conducted from Jun 2 to Jun 30, 2005 at 3 sites on the USDA-ARS research station. The 2 sites from the trap design study were again used, as well as a third site that had similar characteristics to the site with avocado and mango trees and was separated from the other 2 sites by approximately 300 m. As previously described, male *M. hirsutus* were captured in monitoring traps prior to running the experiment at each of these sites, although no visible signs of infestation or colonies could be found. Lures were aged outdoors in Delta traps (Ecogen, Inc., Billings, MT) that were suspended in tree canopies for periods ranging from 1-8 months. This 'aging' process commenced on Oct 1, 2004 and ended on Jun 1, 2005. Aged lures of 1, 2, 4, 6, and 8 months, a 'fresh' (not aged) lure and a blank control septum, were then tested in white Delta traps to determine the influence of age on their attractiveness to male *M. hirsutus*. The Delta trap was chosen based on the results from the trap design study.

Procedures for preparation and field placement of the 7 treatments were the same as those described for the trap design study. Traps were arranged in a randomized complete block design with 2 replicates at each of the 3 sites. All traps and the lures of the 'fresh lure' treatment were replaced weekly for 4 weeks. The lures in the 6 aged-lure treatments were transferred to new traps each week for the duration of the study. Collected traps were put into separate transparent plastic bags and brought to the laboratory to count only trapped males. Used lures were collected at the end of the trial and stored in a freezer as previously described. These were later sent to the USDA-ARS-BARC for analysis to determine the amount of sex pheromone remaining in each septum.

To determine pheromone residue, 3 lures from each of the 6 different time periods were placed individually into 3 mL hexane in a 4-mL vial and soaked for 8 h. Extracts (20 μ L each) were diluted with hexane to an approximate volume (ca. 10 ng per μ L) for gas chromatography-mass spectrometry (GC-MS) analyses. Electronic impact GC-MS analyses of pheromone lures were conducted on a Hewlett-Packard 6890 GC coupled to a HP 5973 Mass Selective Detector with a DB-WAXETR capillary column (J&W Scientific, Inc., Folsom, CA, 60 m \times 0.25-mm ID, 0.25- μ m film-thickness, 50°C for 2 min, then programmed to 230°C at 15°C per min and held for 15 min) with helium as carrier gas. A 70 eV electron beam was employed for sample ionization. The ions, m/z 93, 121, and 136 were selected as the monitor ions and remaining pheromone concentrations were obtained by comparison with synthetic *RS* pheromone standards at the same conditions.

Fresh to 7 Weeks Aged Lures: To further verify that lures <2 months old were as effective as fresh lures at attracting male *M. hirsutus*, a second field experiment was conducted from Oct 12 to Oct 19, 2006 at one site on the USDA-ARS research station. As previously described, lures were aged outdoors in Delta traps that were placed in tree canopies for periods ranging from 1 week to 7 weeks. Aged lures of 1, 2, 4, and 7 weeks and a 'fresh' (not aged) lure were tested in white Delta traps to determine the influence of short term ageing on their attractiveness to male *M. hirsutus*. Traps were arranged in a randomized complete block design with 5 replicates of each lure age. After 1 week, traps were processed and all male *M. hirsutus* were counted as previously described.

Male Flight Activity Study

Field studies of male flight activity over a 24-h period were conducted from Jun 2 to Jun 5, 2005 at 2 residential properties, Miami, FL. The 2 sites had hibiscus hedgerows of >50 m infested with low to moderate levels of *M. hirsutus*. Sample periods were defined as 1 sample covering 0800 to 1600, hourly samples taken from 1600 to 2400, and a single sample covering 0000 to 0800. Three white Delta traps baited with sex pheromone lures and mounted to 0.45 m high stakes were used at each site, spaced 15 m apart and 1 m from the hedge. Traps were initially placed at 0800 on the first day and replaced at the start of each sample period with lures being transferred to the new traps. Traps and lures were processed as previously described.

Statistical Analyses

Data from all males captured during the field studies were analyzed by analysis of variance (ANOVA) by Standard Least Squares (JMP Statistical Discovery 6.0.2, SAS Institute 2006). Factors of variation in the statistical models were trap design, baiting of traps, site, and week for the trap design study; and lure age, site, and week for the lure longevity study. The numbers of male *M. hirsutus* and non-target insects caught on traps were the dependent variables. When the models indicated significant treatment effects and/or significant interactions, differences among means were separated by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$ for multiple comparisons.

Residual amounts of pheromone recovered from lures were analyzed by regression analyses (SPSS 10.0 for Windows, George & Mallery 2002) with time as the factor of variation in the statistical model and amount of pheromone residue as the dependent variable. The numbers of male *M. hirsutus* caught by traps baited with the differ-

ent lure-treatments also were analyzed by regression analyses (SPSS 13.0 for Windows Student Version, SPSS, Inc. 2005) with time as the factor of variation in the statistical model and numbers of males caught as the dependent variable.

Sample periods (time intervals in hours) and site were the factors of variation for the male flight activity study. The number of male *M. hirsutus* caught on traps was the dependent variable. When the models indicated significant treatment effects and/or significant interactions, differences among means were again separated by Tukey's HSD test.

RESULTS

Trap Design

Analysis of the data for males captured with the different trap designs showed that the model was significant ($df = 9, 84$; $F = 20.86$; $P < 0.0001$) and that there were significant differences in male trap catch among the different baited traps ($df = 2, 84$; $F = 5.06$; $P = 0.008$). The only significant interaction was between trap baiting status (baited versus unbaited) and site ($df = 1, 84$; $F = 26.42$; $P < 0.0001$). Unbaited traps caught a total of only 3 male *M. hirsutus* during the course of the experiment. Males captured in baited traps were higher at site 1 than they were at site 2. Delta traps generally caught about 1.5 \times as many males as did sticky cards, although the difference was not significant, and they caught significantly (approximately 2.5 \times) more males than Jackson traps (Fig. 1). Captures with Jackson traps and sticky cards were not significantly different (Fig. 1).

Analysis of data for non-target insects captured by the different trap designs showed that the model was significant ($df = 8, 93$; $F = 12.61$; $P < 0.001$) and that there were no significant interactions. There was no significant difference in the number of non-target insects caught between baited and non-baited traps ($df = 1, 85$; $F = 0.36$; $P = 0.55$); however, there were significant differences among the 3 trap designs ($df = 2, 85$; $F = 37.27$; $P < 0.0001$). Sticky cards caught significantly more ($\geq 2\times$) non-target insects than either Jackson traps or Delta traps (Fig. 1). Captures with Jackson traps and Delta traps were not significantly different (Fig. 1). Non-target catches for both baited and unbaited traps were primarily hymenopteran and dipteran species, but noticeably not the mealybug parasitoids *Anagyrus kamali* Moursi and *Gyranoidea indica* Shafee, Alam and Agarwal.

Lure Longevity

Fresh to 8 Months Aged Lures: The amount of pheromone in the septa decreased as the lures aged (Fig. 2). The greatest decrease in residual

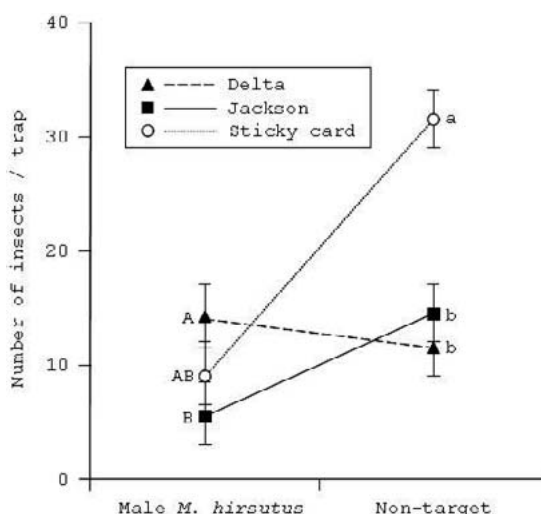


Fig. 1. Mean number of male *M. hirsutus* and non-target insects captured per week in different types of pheromone baited traps located at the USDA ARS Subtropical Horticulture Research Station, Miami, FL. Significant differences between means of male *M. hirsutus* and non-target insects captured in the different traps are indicated by different letters ($\alpha = 0.05$).

pheromone occurred during the first 2 months, dropping from an initial load of 1.00 $\mu\text{g/septum}$ (± 0.01 SE) to 0.30 μg (± 0.03 SE). Thereafter, the decrease was more gradual, dropping from 0.30 μg to 0.07 μg after 9 months. This decrease of pheromone over time was best described by the equation $\ln y = -0.2794x - 0.4477$; $r^2 = 0.85$. Based on this equation, the half-life of the lure was approximately 0.9 months (25 d).

Analysis of data for male *M. hirsutus* captured in traps with fresh and aged lures by site by week showed that the model was significant ($df = 83, 84$; $F = 8.18$; $P < 0.0001$), and that all primary and secondary interactions also were significant. This indicates that there were factors other than the different lure ages at each site and from week-to-week that were influencing trap captures. To allow for a simpler interpretation of the treatment effects, males captured for site 1, week 1 were analyzed separately. Site 1 was selected because this site had the highest overall number of males trapped. Week 1 was selected because any influence of the aging of lures over the 4-week duration of the experiment was excluded. This analysis indicated that lure age did significantly affect males captured ($df = 7, 6$; $F = 13.07$; $P = 0.003$). Based on Tukey's HSD separation of means ($\alpha = 0.05$), traps baited with fresh lures did not capture significantly greater numbers of males than traps baited with lures aged 1 month; however, they did capture significantly more males than traps baited with lures aged 2 months or longer (Fig. 2). Traps baited with lures aged 2, 4, 6, and

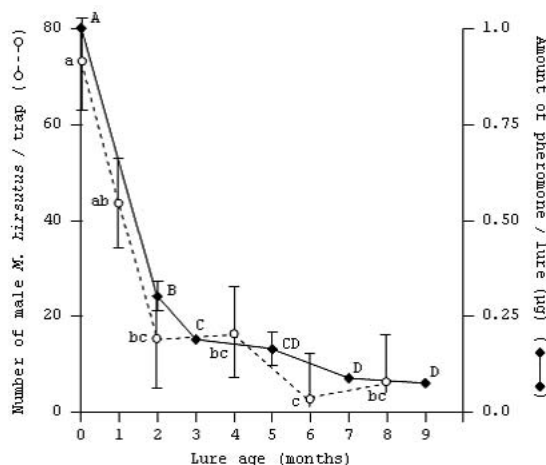


Fig. 2. Relationships of lure age to number of male *M. hirsutus* caught in pheromone-baited traps during a week-long field trial at one site on the USDA-ARS Subtropical Horticulture Research station, Miami, FL; as well as the relationship of lure age to amount of *M. hirsutus* pheromone residue in the lures. Septa were loaded with 1 µg of pheromone and aged for 1, 2, 4, 6, and 8 months prior to use. Significant differences between means of male *M. hirsutus* and pheromone residue are indicated by different letters ($\alpha = 0.05$).

8 months in the field prior to the experiment did not capture significantly greater numbers of males than traps baited with blank septa. Regression analysis of the full model data for the number of males caught by different aged lures was best described by the equation $y = 16.346e^{-17.45x}$; $r^2 = 0.94$. Based on this equation, the half-life of the fresh lure in terms of males captured was about 3.5 months.

Fresh to 7 Weeks Aged Lures: No differences in male *M. hirsutus* captures were found among traps baited with fresh lures and lures aged for 1, 2, 3, 4 and 7 weeks ($df = 2,24$; $F = 0.39$; $P = 0.85$). The average number of males trapped (\pm SE) was found to be 16 (4.9), 31.8 (14.1), 17.4 (3.2), 20.6 (8.8), 25.4 (9.8), and 21.4 (10.3) for fresh, 1, 2, 3, 4, and 7 weeks aged lures, respectively. The pheromone residue remaining in the lures used for this experiment was not determined.

Timing of Male Flight Activity

Significantly different numbers of male *M. hirsutus* were captured at different times of the day ($df = 9,179$; $F = 5.91$; $P < 0.0001$), with the highest male captures of 6.5/trap (± 2.0 SE) occurring from 1600 h to 2400 h. No males were captured during the sample period 0000 h to 0800 h, and only 1.0 male/trap (± 0.5 SE) was captured from 0800 h to 1600 h. During the sample period 1600 hours to 2400 hours, hourly changes of traps were made. This revealed that male activity was greatest be-

tween 1700 h and 2000 h, with 1.5 (± 0.8 SE), 1.6 (± 0.6 SE), and 3.4 (± 1.1 SE) males captured/trap during the sample periods 1700 to 1800, 1800 to 1900, and 1900 to 2000 h, respectively. No males were captured after 2000 h.

DISCUSSION

The sex pheromone developed by Zhang et al. (2004a) was an effective attractant for adult male *M. hirsutus* as shown previously by Zhang & Amalin (2005). As suggested by its unique chemistry (Zhang et al. 2004a), the pheromone appears to be specific to this mealybug based on observational data from males captured in traps, although this was not specifically tested and surveys to determine what other mealybug species were in the area were not conducted. Differences in trap design affected total trap capture of both target and non-target insects, with Delta traps being the most selective to male *M. hirsutus*. There appeared to be a correlation between the number of males captured and the area of sticky trapping surface; i.e., male catch decreased as a function of decreasing area of sticky surface among trap designs.

There was no significant difference in the capture of non-target insects between baited and unbaited traps, suggesting that they were the result of insects randomly flying into the sticky surfaces. As such, the degree of exposure of the sticky surface influenced the number of non-target insects captured to a greater extent than the area of sticky surface. Sticky cards with fully exposed sticky surfaces caught significantly greater numbers of non-target insects than either Delta or Jackson traps. Delta traps, which had the largest sticky surface area but the smallest trap opening, caught the fewest non-target insects. Sticky cards also had the most debris (e.g., leaves, sticks, sand) stuck to them (author, unpublished data). Because adult male *M. hirsutus* are small, large amounts of debris and non-target insects greatly increase the time to process a trap and the probability of missing individual males. This is a particularly important consideration for a monitoring program if early detection of a new infestation is the goal.

Our findings support previous work on trap designs for other mealybugs. Millar et al. (2002) reported that Delta traps were more effective at trapping male *Planococcus ficus* Signoret than sticky cards. Although Zada et al. (2004) reported that plate traps caught more male *P. citri* (Risso) than Delta traps, they did find that larger traps (900 cm² of trapping surface) caught more males than smaller traps (225 cm² of trapping surface). Vincent & Simard (1986) similarly found that sticky traps accumulated large amounts of non-target insects and debris. Adams et al. (1989) reported that this was particularly problematic when processing traps for very small insects such

as mealybug males, because non-target species increased the time required to service traps. An area of concern for pest monitoring programs is attraction of natural enemies to baited traps; however, Zhang & Amalin (2005) reported that there was no statistical difference between sticky traps baited with lures of the *M. hirsutus* sex pheromone and blank control traps for mealybug parasitoids caught. No parasitoids (*A. kamali* and *G. indica*) were found in pheromone-baited traps in this study. These findings suggest that large-scale monitoring programs would not adversely affect biological control efforts.

Characteristics of the lure dispenser can affect the longevity of the pheromone release, the uniformity of the release rate over time, pheromone stability, and the pheromone release rate (Sanders 1989). Release rates also can be affected by environmental conditions such as temperature, relative humidity, and wind speed (Bierl-Leonhardt et al. 1979; Walton et al. 2004; Zhang & Amalin 2005). Therefore, the effectiveness of a lure over time needs to be determined in order to maintain maximum trap capture, especially when monitoring in areas with very low pest numbers.

Preliminary field experiments conducted in 2004 suggested that males captured were not significantly different when traps were baited with lures that were 0-4 weeks of age (author, unpublished data). In the 2005 study, males captured also were not significantly different between Delta traps baited with fresh lures changed weekly and traps with lures aged for one month. Similarly, the Oct 2006 field experiments showed that the effectiveness of fresh lures was not different from lures aged 1, 2, 3, 4, and 7 weeks. However, males captured were significantly lower when traps were baited with lures aged for 2 months; effectiveness continued to decline numerically thereafter with increasing lure age. Traps baited with lures aged for 2 months caught about 32% of the number of males that were captured in traps baited with fresh lures; traps baited with lures aged for 8 months only captured about 4% of what was caught in traps baited with fresh lures. In fact, traps containing lures that were ≥ 2 months old did not catch significantly more insects than traps with blank septa, i.e., they often caught no insects even though traps with fresh and 1-month old lures indicated that males were present. Based on these findings, we recommend that the lures be replaced every 1-2 months when using these traps for early detection of new (low level) infestations.

Lures used in our field longevity studies appeared to maintain their attractiveness to male *M. hirsutus* for a shorter period of time relative to those of equal loading dose used by Zhang & Amalin (2005). They found the lures attracted males for up to 21 weeks. A possible explanation for the difference might be that these authors used locations in south Florida with high infestation levels

of *M. hirsutus* and the baited traps were placed close to infested hibiscus plants. Typically 100-800 males were captured per trap per week. In contrast, in our experiments the baited traps were placed in fields with no visible signs of infestation in the immediate vicinity and only 10-80 males were captured per trap per week. Thus, although the residual pheromone in used lures in both studies was similar ($\approx 0.18 \mu\text{g}$ after 21 weeks in Zhang & Amalin (2005) and $\approx 0.17 \mu\text{g}$ after 20 weeks in our studies), it is not surprising they caught significant numbers of males for a longer time period. Also, in our lure longevity studies trap captures of males with 2 and 4 month-old lures were significantly different from zero at $\alpha = 0.1$).

Studies have shown a similar duration of lure activity in the field for other mealybug species. For example, lures with 100 μg of the sex pheromone for *P. ficus* remained attractive for >12 weeks in vineyards in California (Millar et al. 2002). Walton et al. (2004) also reported that lures of the same loading dose as those in California continued to attract *P. ficus* males for up to 10 weeks in South African vineyards. Zada et al. (2004) reported that lures with 200 μg of *P. citri* sex pheromone could remain attractive for up to 16 weeks in Israel.

The greatest decrease in pheromone residue occurred during the first 2 months with a more gradual decrease after that period. A lower pheromone release rate may explain why traps baited with older lures caught fewer male *M. hirsutus*. Septa loaded with a higher initial amount of pheromone might prolong the period of high release rate and increase the length of time that the lures are maximally attractive (i.e., as attractive as fresh lures with 1 μg of pheromone). However, Zhang & Amalin (2005) reported that high levels of pheromone could have an inhibitory effect on males and suggested using high release rates of the pheromone for mating disruption. Additional studies to evaluate the impact of pheromone release rate on males captured and applications of the pheromone to disrupt mating seem warranted.

Many mealybug species fly mainly around sunset while others are early-morning flyers (Aldrich 1996). Male *M. hirsutus* were active in the late afternoon to early evening hours with almost no flight activity at other times. This study was conducted over 3 consecutive days with heavy showers in the late afternoons to early evenings. Consequently, male activity may have been disrupted to some degree. However, because the crepuscular diel activity pattern emerged despite the inclement weather at this time of day, we are confident of the findings. Our own unpublished data suggest that oriented flight by male mealybugs to pheromone plumes occurs at <100 m. For small insects like *M. hirsutus* males, environmental factors like rain and wind above, landscape features, and landscape management such as mowing probably

have significant impacts on dispersal and therefore their capture in baited traps. These factors may help explain the interactions we found among treatments, sites, and sampling dates. Further work is needed to better understand the dynamics of male dispersal and the influence of environmental factors and cultural practices. Understanding male flight activity could provide information critical to locating an infestation once males have been detected in a trap.

ACKNOWLEDGMENTS

We gratefully acknowledge the following individuals of the University of Florida/USDA-APHIS at USDA-ARS SHRS, Miami, FL for field assistance: Divina Amalin, Lizandra Nieves, Jason Bravo, Roger Coe, Luis Bradshaw, Shawron Weingarten, and Juang-Horng Chong. We thank Kathy Moulton and Carol Wyatt-Evens at the USHRL, Fort Pierce, FL for field assistance. Dr. Greg Hodges of FL-DPI, Gainesville helped with initial mealybug identifications and commented on pheromone specificity. Edward Jones, USDA-APHIS, and Gilbert Queeley, Florida A&M University, provided statistical assistance. USDA-APHIS and Florida A&M University funded this research and we acknowledge both institutions. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES CITED

- ADAMS, R. G., K. D. MURRAY, AND L. M. LOS. 1989. Effectiveness and selectivity of sex pheromone lures and traps for monitoring fall armyworm (Lepidoptera: Noctuidae) adults in Connecticut sweet corn. *J. Econ. Entomol.* 82: 285-290.
- ALDRICH, J. R. 1996. Sex pheromones in Homoptera and Heteroptera, pp. 199-233 *In* C. W. Schaefer [ed.], *Proceedings, Symposium: Studies on Hemipteran Phylogeny*. XVIII International Congress of Entomology, Vancouver, Canada. 1988. Thomas Say Publications in Entomology: Proceedings. Entomological Society of America, Lanham, MD.
- BIERL-LEONHARDT, B. A., E. D. DEVILBISS, AND J. R. PLIMMER. 1979. Rate of release of disparlure from laminated plastic dispensers. *J. Econ. Entomol.* 72: 319-321.
- GEORGE, D., AND P. MALLERY. 2002. *SPSS for Windows Step by Step: A Simple Guide and Reference*. 4th ed. Allyn & Bacon, Boston.
- GHOSE, S. K. 1972. Biology of the mealybug *Maconellicoccus hirsutus* (Green) (Pseudococcidae). *Indian Agric.* 16: 323-332.
- SPSS, INC. 2005. *SPSS 13.0 for Windows Student Version*. 1st ed. Prentice Hall, NJ.
- KAIRO, M. T. K., G. V. POLLARD, D. D. PETERKIN, AND V. F. LOPEZ. 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) in the Caribbean. *Int. Pest Manage. Rev.* 5: 241-254.
- MANI, M. 1989. A review of the Pink mealybug—*Maconellicoccus hirsutus* (Green). *Insect Sci. Applic.* 10: 157-167.
- MILLAR, J. G., K. M. DAANE, J. S. MCELFRESH, J. A. MOREIRA, R. MALAKAR-KUENEN, M. GUILLEN, AND W. J. BENTLEY. 2002. Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California vineyards. *J. Econ. Entomol.* 95: 706-714.
- MOFFITT, L. J. 1999. Economic Risk to United States Agriculture of Pink Hibiscus Mealybug Invasion. A report to the United States Department of Agriculture, Animal and Plant Health Inspection Service.
- PERSAD, C. 1995. Preliminary List of Host Plants of *Maconellicoccus hirsutus* Green—Hibiscus or Pink Mealybug in Grenada. *Carib. Agric. Res. Dev. Inst., Grenada*.
- SANDERS, C. J. 1989. The Further Understanding of Pheromones: Biological and Chemical Research for the Future, pp. 323-351 *In* A. R. Jutsum and R. F. S. Gordon [eds.], *Insect Pheromones in Plant Protection*. John Wiley & Sons Ltd., UK.
- SAS INSTITUTE. 2006. *JMP Statistical Discovery* 6.0.2, Cary, NC.
- SERRANO, M. S., S. L. LAPOINTE, AND D. E. MEYERDIRK. 2001. Attraction of males by virgin females of the mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). *Environ. Entomol.* 30: 339-345.
- STIBICK, J. N. L. 1997. New Pest Response Guidelines—Pink hibiscus Mealybug, *Maconellicoccus hirsutus*. USDA, Marketing and Regulatory Programs, APHIS, PPQ.
- VINCENT, C., AND L. G. SIMARD. 1986. Monitoring *Orthosia hibisci* (Lepidoptera: Noctuidae) with Pherocon 1C and Hara traps. *J. Econ. Entomol.* 79: 1497-1500.
- WALTON, V. M., K. M. DAANE, AND K. L. PRINGLE. 2004. Monitoring *Planococcus ficus* in South African vineyards with sex pheromone-baited traps. *Crop Protect.* 23: 1089-1096.
- WILLIAMS, D. J. 1986. The identity and distribution of the genus *Maconellicoccus* Ezzat (Hemiptera: Pseudococcidae) in Africa. *Bull. Entomol. Res.* 76: 351-357.
- ZADA, A., E. DUNKELBLUM, M. HAREL, F. ASSAEL, S. GROSS, AND Z. MENDEL. 2004. Sex pheromone of the citrus mealybug *Planococcus citri*: Synthesis and optimization of trap parameters. *J. Econ. Entomol.* 97: 361-368.
- ZHANG, A., D. AMALIN, S. SHIRALI, M. S. SERRANO, R. A. FRANQUI, J. E. OLIVER, J. A. KLUN, J. R. ALDRICH, D. E. MEYERDIRK, AND S. L. LAPOINTE. 2004a. Sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*, contains an unusual cyclobutanoid monoterpene. *Proc. Natl. Acad. Sci. USA*. 101: 9601-9606.
- ZHANG, A., J. NIE, AND A. KHRIMIAN. 2004b. Chiral synthesis of maconelliol: A novel cyclobutanoid terpene alcohol from pink hibiscus mealybug, *Maconellicoccus hirsutus*. *Tetrahedron Lett.* 45: 9401-9403.
- ZHANG, A., AND D. AMALIN. 2005. Sex pheromone of the female pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae): Biological activity evaluation. *Environ. Entomol.* 34: 264-270.
- ZHANG, A., AND J. NIE. 2005. Enantioselective synthesis of the female sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*. *J. Agric. Food Chem.* 53: 2451-2455.