IMPROVED PHEROMONE-BASED TRAPPING SYSTEMS TO MONITOR TOXOTRYPANA CURVICAUDA (DIPTERA: TEPHRITIDAE)

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ABSTRACT

A membrane-based formulation method that provided a constant release rate of synthetic pheromone for the papaya fruit fly, Toxotrypana curvicauda Gerstaecker, was developed. Release rate measurements over 23 days indicated that lures loaded with 5, 15, 25, and 50 μl of synthetic pheromone released an average of 120, 360, 580 and 1120 ng per hr and the half-life of the lures was estimated to be 67, 184, 300 and 48 days, respectively. Field tests conducted in Mexico compared efficacy of blank and pheromone-baited sticky green spheres, cylindrical traps made from green opaque plastic that either contained a toxicant or were coated with sticky material, and cylindrical traps prepared from green sticky paper. Green opaque traps containing a toxicant and sticky paper traps captured approximately five times more papaya fruit flies than either the sticky-coated green opaque traps or the sticky-coated green spheres, and the presence of pheromone did not affect numbers of flies captured. Thus, the combination of the green color and the cylindrical shape provided a visual cue sufficient for papaya fruit fly capture. The pheromone lure significantly increased trap capture in similar tests conducted in Guatemala. Capture was highest in the sticky paper traps and in sticky-coated spheres. Use of the membrane-based synthetic pheromone in a cylindrical trap may provide an effective tool for monitoring papaya fruit flies.

Key Words: Insecta, papaya fruit fly, pheromone formulation, trap.
Fue desarrollado un método de formulación basado en una membrana que provee una velocidad constante de liberación de la feromona sintética para la mosca frutera de la papaya, Toxotrypana curvicauda Gerstaecker. Las medidas de la velocidad de liberación durante 23 días indicaron que los cebos cargados con 5, 15, 25 y 50 µl de la feromona sintética liberaron un promedio de 120, 360, 580 y 1120 ng por hora y la vida media de los cebos fue estimada en 67, 184, 300 y 48 días, respectivamente. En experimentos de campo llevados a cabo en México se comparó la eficacia de esferas verdes adhesivas con y sin feromona, trampas cilíndricas hechas con plástico verde opaco que contenían un tóxico o estaban cubiertas con material adhesivo, y trampas cilíndricas hechas de papel adhesivo verde. Las trampas verdes opacas que contenían un tóxico y las trampas de papel adhesivo verde capturaron aproximadamente cinco veces más moscas de la papaya que las trampas verdes opacas cubiertas de goma o las esferas verdes cubiertas de goma. La presencia de la feromona no afectó el número de moscas capturado. La combinación del color verde y la forma cilíndrica proveyeron una pista visual suficiente para la captura de las moscas fruteras. El cebo de feromona aumentó significativamente la captura en pruebas similares conducidas en Guatemala. La captura fue más alta en las trampas de papel adhesivo y en las esferas cubiertas de goma. El uso de la feromona sintética con el método de la membrana en la trampa cilíndrica podría ser una herramienta efectiva para el monitoreo de las moscas fruteras de la papaya.

**Resumen**

Toxotrypana curvicauda Gerstaecker es el insecto principal que ataca la papaya (*Carica papaya* L.); es de distribución mundial. Los estudios de comportamiento en el campo y en el laboratorio indicaron la existencia de un pheromone producido por los machos que mediaba la interacción con las hembras (Landolt & Hendrichs 1983, Landolt et al. 1985). Este pheromone masculino fue identificado por Chuman et al. (1987) como 2-methyl-6-vinylpyrazine (2,6-MVP). El pheromone sintético 2,6-MVP provocó el mismo comportamiento de las moscas frutera madres en el campo (Landolt & Heath 1990). Sin embargo, el uso generalizado de este sistema de atracción ha sido limitado por la fragilidad de los tubos de vidrio en los que se formulan las altas concentraciones de pheromones, lo que dificultaba su mantenimiento. Las trampas cilíndricas con señales visuales adecuadas han demostrado ser una alternativa a las esferas para la captura de moscas fruteras (Heath et al. 1995).}

Herein, we report the application of a membrane-based formulation system that provides a variety of release rates depending on the amount of chemical used. Field tests were conducted to determine if the membrane-release formulation and green cylindrical traps could be used to overcome the shortcomings of the earlier trapping system.
Membrane-Based Formulation System

A 3 by 5 cm lure was prepared by folding a 6 by 5 cm piece of 6 mill impermeable polyethylene (backing) in half. A 1.17 cm diam hole was cut in the center of the front of the lure and a piece of 1 mill high density polyethylene (membrane) film (Consep Inc., Bend, OR) was placed inside the lure. The bottom and sides were heat sealed to form an envelope and to secure the membrane. The release area of the membrane was reduced to a 5 mm diam circle by placing a piece of aluminum tape (United Tape Company, Cumming, GA) over the 1.17 cm hole in the front of the lure. A piece of rectangular filter paper and a plastic grid that were slightly less than 3 by 5 cm were placed in the envelope to provide stability; synthetic pheromone was added and the top of the lure was heat sealed. Lures were loaded with 5, 15, 25 and 50 μl of >99% pure 2,6-MVP (Fuji Flavour Co. LTD., Tokyo, Japan). Lures were placed in a hood with a 0.25 cm per sec air flow and, beginning two days after filling, the release rates from at least three lures of each load were measured every three to four days over a 23-day period. Volatiles were collected and analyzed using collection systems and gas chromatographic conditions described previously (Heath & Manukian 1992, Heath et al. 1993). Mean release rates for each pheromone load were used in linear regression analysis to determine the change in release rate over time and the half-life of each lure.

A field-test comparison of the membrane-based lures and the capillary lures used by Landolt & Heath (1990) was conducted in Homestead, Florida, using sticky-coated sphere traps. Both lures released approximately 1 μg 2,6-MVP per h. Solid styrofoam spheres (12.7 cm diam) painted dark green were used (Great Lakes IPM, Vestaburg, MI). Capillary lures were mounted in holes drilled in the spheres and lures were positioned so that lure openings were 2-3 cm from the top of the sphere. A 4 by 7 cm piece of 6 mill plastic (Faulkner Plastics, Gainesville, FL) was folded in half to form a tent. The membrane-based lure was attached to the inside of the tent to protect the lures from rain, and this assembly was placed on the top of the sphere (Fig. 1a). Paintable sticky coating (Tangle Trap, The Tanglefoot Co., Grand Rapids, MI) was applied to the outside of the sphere to retain responding flies. Ten pairs of sticky spheres baited with either a capillary lure or a membrane-based lure were hung by wires from papaya leaf petioles near fruit clusters located about 1.5-2 m above ground in trees along the outside edge of a papaya grove. Traps were placed in the border rows because the papaya fruit fly activity tends to be the highest and fruit damage the greatest along the borders of a grove (Landolt & Heath 1988). The experiment was replicated over time; traps were checked every 7-10 days per replicate. There were four consecutive replicates. Total numbers of males and females captured on the ten traps per lure type were summed separately to give the number of each sex captured per replicate. Separate t-test comparisons were conducted for numbers of males and females captured using Proc TTEST (SAS Institute 1985).

Cylindrical Traps

Cylindrical traps (Fig. 1b,c) consisted of three major components; the main trap body, which was a cylindrical container (9 cm diam by 15 cm long); two removable end caps for quick access into the trap for bait replacement; and a wire hanger for holding the trap together and supporting the complete assembly on a tree (Heath et al. 1995). The trap bodies for the green opaque traps (Fig. 1b) were prepared from a rectangular
piece (15.0 cm wide × 30.0 cm long) of green opaque plastic (0.025 cm thick, Faulkner Plastics, Gainesville, FL), which was rolled to form a 9.0 cm diam cylinder. The green opaque traps used one of two methods for catching insects that responded to the trap and/or bait. One type used paintable sticky coating, as was used for the sphere traps, applied to the outside of the trap. The second type used internally-placed toxicant panels (Heath et al. 1995), which contained a feeding stimulant and a toxin, to kill insects after they entered the trap. The toxicant panels are coated with a solution (1.0: 0.5: 0.01) of paint [Hunter Green 100% acrylic latex paint (Glidden, Cleveland, OH)], sugar [American Chemical Society grade sucrose (Mallinkrodt, Paris, KY)], and pesticide [technical grade methomyl (DuPont, Newark, DE); 98%(AI)]. Panels were air dried for at least 48 h before use and were placed on the inside of both the top and bottom end caps using double-sided tape.

Sticky-paper traps were made from dark green fruit fly adhesive paper (FFAP) supplied by the Atlantic Paste and Glue Co., Inc. (Brooklyn, NY). The efficacy of the adhesive paper was determined in laboratory tests of the retention of alighting flies. Flies were obtained as mature larvae from field-collected papaya fruit in Dade County, Florida. Larvae exited the fruit and pupated in vermiculite (Landolt & Heath 1988). In tests conducted in a greenhouse, a piece of FFAP (12 by 12 cm) was placed in a screen cage (30 by 30 by 30 cm) containing 30 sexually mature females. Males were not tested because females are the primary target of the traps. An observer counted the number of fly landings on the sticky paper over a one-h time period. Total

Figure 1. Illustration of three types of papaya fruit fly traps used in field studies conducted in North and Central America. A) Solid styrofoam spheres were painted with dark green paint and coated with paintable adhesive to retain flies. B) Opaque cylindrical traps were constructed using green plastic. This trap either contained toxicant panels or was coated with paintable adhesive to retain attracted flies. C) Sticky-paper cylindrical traps were similar to the opaque cylindrical traps, but the trap body was made with dark green fruit fly adhesive paper. The adhesive paper was supplied with protective paper (shown as white overlay) that was removed when traps were placed in the field to expose the sticky trap surface.
numbers of flies remaining on the paper after the one-h time period were counted, and ratio of landed to captured flies was determined. The test was replicated three times over a period of three days.

The sticky-paper traps (Fig. 1c) were prepared similarly to the green opaque traps. A rain guard was used with this trap to protect the paper trap body; it was made from the top half of a 150 x 15 mm petri dish (P/N #1058, Becton Dickinson, Lincoln Park, NJ). A 5 cm length of polyvinyl chloride tubing (9.0 cm outside diam, Hughes Supply, Gainesville, FL) was glued to the center underside of the petri dish, which provided a holder for the trap body. The protective paper supplied with the adhesive paper was removed when traps were placed in the field to expose the sticky trap surface.

Field Tests

A study was conducted at a papaya plantation on the grounds of the Centro de Desarrollo de Productos Bióticos (CEPROBI) of the Instituto Politécnico Nacional (IPN), Morelos, Mexico, from November 1994 through January 1995, the dry season during the coolest time of the year. The native vegetation adjacent to the study site is classified as “selva baja caducifolia” or lowland deciduous forest (Soria 1985). Gonolobus soridius (Asclepiadaceae) and Jacaratia mexicana (Caricaceae), which are native hosts for the papaya fruit fly (Castrejon-Ayala & Camino-Lavin 1991), occur in the area. The papaya plantation had two rows of papaya that serve as a trap crop and are separated by 10 m from the main plot. The experimental plot was 177 by 63 m, with papaya trees planted every 3 m. Treatments consisted of four trap types: 1) sticky-coated green spheres, 2) sticky-coated green opaque cylinders, 3) green opaque cylinders with internally-placed toxicant panels, and 4) sticky-paper cylinders made with the dark green FFAP material. These traps were either baited with membrane-based pheromone lures, which released about 1 µg 2,6-MVP per h, or were unbaited. Lures were taped to the inside of the opaque cylinders or attached to a plastic rain tent of sticky-paper traps using double-sided sticky tape. Traps were placed near the immature fruit within the papaya tree because papaya fruit fly females successfully oviposit in those fruit (Landolt 1985). The eight treatments (four trap types times two bait treatments) were placed randomly within a block and treatments were moved sequentially at time of sampling. There were six blocks placed in the trap crop, i.e., around the periphery of the papaya orchard. Traps were placed in every other tree within a block, and there were 8-19 papaya trees without traps between the experimental blocks. The sticky-paper trap bodies were replaced weekly, the sticky-coated traps were cleaned and recoated as needed. Pheromone lures were replaced after four weeks of field use. The numbers of males and females captured per trap were recorded weekly for six weeks (replicates) and then after two weeks for the final sample, for a total of seven replicates.

Similar field tests were conducted in a papaya orchard at finca Cuilapa, Guatemala. However, liquid protein-baited McPhail traps, bell-shaped glass traps with a water reservoir (Newell 1936), were added because of local interest in using this trap for papaya fruit flies. McPhail traps were baited with five torula yeast-borax pellets (ERA Int., Freeport, NY) in 300 ml of water (Gilbert et al. 1984). Traps were placed in papaya trees located around the periphery of the papaya orchard, with individual traps placed near the smaller fruit within the tree, as above. All treatments (four trap types, two bait treatments, plus McPhail traps) were placed randomly within a block and treatments were moved sequentially at time of sampling. There were four blocks placed in the periphery of the papaya orchard. There were at least ten papaya trees without traps that separated the experimental blocks, and traps within a block
were placed in every third tree. Traps were sampled weekly. Sticky-coated traps were cleaned and recoated as needed, sticky-paper trap bodies were replaced weekly. Pheromone lures were replaced after four weeks of field use. McPhail traps were cleaned and protein solutions were replaced every two weeks. The numbers of males and females captured per trap were recorded weekly for eight weeks, for a total of eight replicates.

Statistical Analysis. The total number of trapped flies per treatment was determined from the sum of each sex collected per replicate. Thus, one replicate consisted of the sum total captured in six traps (Mexico) or four traps (Guatemala) per trap type. Sum total was converted to percentage trapped within replicate for statistical analysis. Data were analyzed with two-way analysis of variance (ANOVA) with interaction using Proc GLM (SAS Institute 1985) followed by LSD mean separation tests \( (P = 0.05) \). Factors used in the ANOVA were bait (2 levels: pheromone baited or no pheromone; data from McPhail traps were not included in this analysis) and trap type (4 levels: sticky sphere, sticky green opaque, green opaque with toxicant or sticky-paper trap). One-way ANOVAs were conducted to test all nine trap type/bait combinations in the Guatemalan tests. Data were log-transformed \( (x + 1) \) to stabilize the variance prior to analysis (Box et al. 1978). Sex of the trapped flies was considered to be a response variable, not a treatment factor, therefore separate analyses were conducted for females, males and total (females plus males) papaya fruit flies captured in each country.

RESULTS

Lures loaded with 5, 15, 25, and 50 ml released an average \( (\pm \text{std}) \) of 0.12 ± 0.01, 0.36 ± 0.02, 0.58 ± 0.02 and 1.12 ± 0.11 \( \mu \text{g} \) per hr during the 23 days that release rates were obtained \( (n = 16, 16, 16 \text{ and } 15, \text{ respectively}) \). The projected half-lives of the lures were 67, 184, 300 and 48 days, respectively. Lures prepared with 5, 15, or 25 \( \mu \text{l} \) of material showed very little decrease during the time that release rates were measured (Fig. 2). To cover the optimum range of pheromone release, we selected the 50 \( \mu \text{l} \) loaded pheromone lure, which released an average of about 1 \( \mu \text{g} \) per hr, for subsequent field experiments. There were no significant differences in catch \( (\text{average}\pm \text{std}) \) on sticky spheres baited with capillaries or the membrane-based lure of either males \( (2.0 \pm 3.4 \text{ versus } 2.5 \pm 0.6) \) or females \( (7.8 \pm 4.5 \text{ versus } 8.3 \pm 3.3) \).

Number of papaya fruit flies captured in Mexico was low, with an average of 15 flies captured among all six blocks \( (48 \text{ traps total}) \) per replicate. Trap type significantly affected capture of females \( (F = 7.41; \text{df} = 3, 48; P = 0.0004) \) and total flies \( (F = 3.79; \text{df} = 3, 48; P = 0.0162) \), but not males \( (F = 1.39; \text{df} = 3, 48; P = 0.26) \). A higher percentage of females was captured on the green opaque traps with toxicant and on the sticky-paper traps than on the traps that used paintable sticky coating \( \text{(sphere-sticky, opaque-sticky)} \). Presence of the pheromone lure did not affect capture of the papaya fruit flies, although pheromone-baited traps usually captured slightly more flies than their unbaited counterparts (Fig. 3).

Capture of papaya fruit flies was also low in the tests conducted in Guatemala, with an average of 17 flies captured among all four blocks \( (36 \text{ traps total}) \) for the first 5 weeks of the study. Numbers trapped dropped to 0.3 for the last 3 weeks, therefore these data were deleted and only the first five replicates were included in the analyses. Presence of pheromone lure and trap type significantly affected capture of females, males and totals, and there was no interaction between these factors. Percentage of papaya fruit flies captured in pheromone-baited traps was always higher than capture in the same traps without pheromone (Fig. 4). Percentage of pa-
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Papaya fruit flies captured in the liquid protein-baited McPhail traps was no higher than that in the least effective traps tested. No females were captured in the sticky-coated green opaque traps, and percentage captured in these traps was lower than in the other three trap types. For both males and total flies, percentage captures in the sticky-paper traps and the sticky-coated spheres were higher than in either of the green opaque traps.

In laboratory trials, the FFAP material used for the sticky-paper traps was highly effective in retaining flies that landed on the surface. In the three tests, nine, six and four flies landed on the paper and all (19 out of 19) were captured on the sticky surface.

Discussion

These studies demonstrated that a membrane-based lure can be used to formulate synthetic papaya fruit fly sex pheromone for field use, and that cylindrical traps may be used to replace the sphere traps used previously. The cylindrical traps apparently provided a sufficient visual cue because the unbaited cylindrical traps also captured papaya fruit flies. Landolt & Heath (1990) found that although mated females responded to the visual aspects of the spheres alone, virgin females represented most of the females captured in response to pheromone. Number of males also increased directly with pheromone dose, indicating that males responded to the pheromone presence. It is not known why there was no effect of pheromone in the trials in Mexico as

Figure 2. Average release rates (µg per h) of papaya fruit fly synthetic pheromone which was formulated in membrane-based lures, over time. Regressions were determined from lures (n=3) containing 5, 15, 25 and 50 µl of 2-methyl-6-vinylpyrazine.
Figure 3. Results of trap capture of female (top), male (middle) and total (bottom) papaya fruit flies captured in field trials conducted in Mexico (n = 7). Traps tested (left to right) were green sticky spheres, green opaque cylinders with sticky exterior, green opaque cylinders with internally-placed toxicant, and green sticky-paper cylinders. Traps were either baited with papaya fruit fly pheromone membrane-based lures (shaded bars) or left unbaited (open bars). Pairs of bars within a graph headed by the same letter are not significantly different (LSD mean separation test on log (x + 1) transformed data, P = 0.05; non-transformed means presented).
Figure 4. Results of trap capture of female (top), male (middle) and total (bottom) papaya fruit flies captured in field trials conducted in Guatemala (n = 5). Traps tested (left to right) were green sticky spheres, green opaque cylinders with sticky exterior, McPhail traps, green opaque cylinders with internally-placed toxicant, and green sticky-paper cylinders. Except for the McPhail traps, which were baited with aqueous torula yeast plus borax solution, the traps were either baited with papaya fruit fly pheromone membrane-based lures (shaded bars) or left unbaited (open bars). Bars within a graph headed by the same letter are not significantly different [LSD mean separation test on log (x + 1) transformed data, P = 0.05; non-transformed means presented].
was observed in the trials in Guatemala. A number of factors, including the demographics of the fly population (e.g. presence or absence of virgin females in the population) and environmental parameters at each site, may have affected the results. The mated status of the females captured was not determined in our studies.

The membrane-based lure can be formulated to provide a multitude of release rates, and release rates were directly related to amount of 2,6-MVP added to the lure. The lure used in trials reported herein was based on optimal release rate for sticky-coated spheres (Landolt & Heath 1990). It is not known if this release rate is optimal for traps other than sticky spheres. A lower release rate may improve catch in the opaque cylindrical traps containing the toxicant panel because this trap requires that the flies enter the trap to be captured. Additional studies are needed to examine a range of pheromone doses for use in cylindrical traps that use toxicant or sticky materials.

The use of the FFAP material provided a facile method to prepare cylindrical traps. This material was easily fabricated and the sticky material did not adhere to personnel who contacted the adhesive. Exposure over time indicated that it was impervious to rain when the material was used as described. The adhesive material did not drip or run as was observed when paintable sticky coating was used. It should be noted, however, that in experiments conducted with sticky-paper traps for other tephritid pests in southern Florida, we observed that on occasion small birds and lizards were either blown into the trap or came in contact with the FFAP material. While the sticky-paper trap caught non-target insects in studies in both Mexico and Guatemala, we did not observe the capture of small animals such as was observed in Florida. The captured insects and animals can be remove from the FFAP material with the use of mineral oil. This process had no observed detrimental effect on the animals if the animals were removed shortly after capture. Another problem with this, as well as other sticky-coated traps, is that wind-borne dust or debris may coat the trap and limit the longevity of the adhesive in the field. The green opaque trap used with the toxicant provided an alternative to a sticky trap, although it may not be as sensitive under all environmental conditions.

Papaya fruit flies were captured in protein-baited McPhail traps in this study in Guatemala, but in low numbers. This suggests that volatiles from protein baits may be attractive to papaya fruit flies. If so, addition of protein bait volatiles may provide synergists that could further improve trap capture of the pheromone-baited traps. It is envisioned that the information presented here may provide a facile method to monitor papaya fruit fly infestations. This would result in decreased pesticide application and, potentially, offer a method of control through trapping alone.

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