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Source: Florida Entomologist, 90(2) : 304-308

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2007)90[304:MBAFPU]2.0.CO;2

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MATING BEHAVIOR AND FEMALE-PRODUCED PHEROMONE USE IN TROPICAL SOD WEBWORM (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Research was initiated to develop a pheromone-based monitoring system for the tropical sod webworm, *Herpetogramma phaeopteralis* (Guenée). A laboratory rearing procedure was developed to produce individuals for field tests and behavioral bioassays. Virgin females placed in Unitraps in the field attracted and captured males for 8 d, while no males were captured in unbaited traps. Total male capture ranged from 1 to 24, and there was a slight decrease in capture as females aged. Laboratory mating behavior studies suggested that mating occurs later in the scotophase. Males responded to virgin females in a linear olfactometer throughout the dark period (scotophase), although there was a trend for higher male activity late in scotophase. There was no observed calling behavior, and adults exhibit simple mating behavior. Lack of both calling posture among virgin females and periodicity of male response will make it difficult to determine the optimal time periods for pheromone production, which would facilitate the collection and subsequent identification of pheromone components.

Key Words: *Herpetogramma phaeopteralis*, female calling, male attraction, turf

RESUMEN

Se inicio una investigación para desarrollar un sistema de monitoreo en base de feromonas contra el gusano tropical de césped *Herpetogramma phaeopteralis* (Guenée). Se desarrolló un procedimiento de cría en el laboratorio para producir individuos para pruebas del campo y de bioessayos de comportamiento. Hembras vírgenes puestas en "Unitraps" en el campo atrayeron y capturaron machos por 8 días, mientras que ningún macho fue capturado en trampas sin cebo. El número total de machos capturados fue de 1 a 24, y hubo una menor disminución en el número de machos capturados cuando las hembras envejecieron. Los estudios de comportamiento del apareamiento en el laboratorio sugerieron que el apareamiento ocurre más tarde en la scótofase. Los machos respondieron a las hembras vírgenes en un olfactometro linear a través del periodo oscuro (scótofase), aunque hubo una tendencia para un aumento en la actividad del macho en la parte final de la scótofase. No fue observado el comportamiento de llamar, y los adultos exhibieron un comportamiento del apareamiento sencillo. La falta del la postura de llamar entre las hembras vírgenes y la periodicidad de la respuesta de los machos hace difícil el poder determinar el periodo del tiempo optimo para la producción de feromonas, con la cual facilitaría la identificación y recolección de los componentes de la feromona.

Tropical sod webworm (TSW), *Herpetogramma phaeopteralis* (Guenée) (Lepidoptera: Crambidae), is a pest of Florida turfgrasses. Species within *Herpetogramma* attack grasses in the continental United States, Australia, Hawaii, and Guam, and *H. licarsisalis* (Walker) is the most serious turfgrass pest in Hawaii (Davis 1969; Murdoch & Tashiro 1976; Tashiro 1976). TSW also has a wide tropical distribution and occurs throughout the Caribbean (Wolcott 1936). In the U.S., TSW occurs across the Gulf Coast from Texas to Florida (Kerr 1955).

TSW feeds on a variety of grasses including bermudagrass *Cynodon dactylon* (L.) Persoon, centipedegrass *Eremochloa ophiuroides* (Munro) Hackel, seashore paspalum *Paspalum vaginitium* Swartz, St. Auginegrass *Stenotaphrum secundatum* (Walter) Kuntze, and zoysiagrass *Zoysia japonica* Steudel (Kerr 1955; Reinert 1983; Korndorfer et al. 2004). TSW populations are managed by using primarily chemical and cultural controls (Reinert 1973, 1974, 1976, 1983; Buss & Meagher 2005), although resistant cultivars have been reported (Reinert & Busey 1983).

Few studies have been conducted on the general biology of TSW (Kerr 1955; Reinert & Busey 1983). More recently, Cherry & Wilson (2005) determined that more adults of both sexes rested in

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unmowed grass versus mowed grass, and that when disturbed, adults flew only a short distance. Seasonally, more TSW adults were attracted to light traps in the fall (Sep-Nov) than summer, spring, or winter in southern Florida. TSW larvae are sampled by locating damaged turf and conducting soap flushes (Buss & Meagher 2005). Adult monitoring is accomplished by either sweep nets or light traps (Cherry & Wilson 2005), both of which have limitations for turfgrass consultants or homeowners. Studies were initiated in 2004 to develop a pheromone-based monitoring system for this pest. Pheromone monitoring could allow turfgrass managers to predict future damaging populations and prescribe insecticide applications based on action levels. Field studies were conducted to confirm production of female-produced volatile chemicals that could be used for attraction of male moths. Laboratory studies were conducted to evaluate the mating behavior of TSW and periodicity of pheromone release. This study provides information on the biology of this important pest, which will be needed for identification and formulation of a synthetic pheromone lure for field use.

Materials and Methods

TSW Colonies

TSW were colonized at 2 locations from larvae collected from the Everglades Research and Education Center (EREC), Belle Glade, FL. At the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL, adults were placed in screen cages ($24 \times 24 \times 24$ cm) and supplied with distilled water and 2% sugar-honey solution for nourishment. After 2 d, a 237-mL plastic cup containing greenhouse-grown bermudagrass ('NuMex Sahara', Pennington Seeds, Madison, GA) was placed in a cage for oviposition. Cups were left for 2 d, removed, and replaced with new cups of bermudagrass. This oviposition cycle was repeated until adults died. Cups containing grass with TSW eggs were placed on top of a plastic grate within plastic tubs, 35 (l) × 24 (w) × 13 (h) cm, lined with paper towels (Sparkle™, Georgia-Pacific, Atlanta, GA). When egg hatch was complete, the grass in the cups was cut and the soil and cup were removed from the tub. Bermudagrass was added daily for 7 d. After 7 d field-grown 'Florona' stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) was added by placing it on a metal screen that was placed on top of the grate. Each day new grass was placed under the screen while the old grass was placed on top of the screen. In this way, larvae feeding on the old grass could move down to the new grass. The old grass was removed the next day. This technique slowed mold development in the rearing tubs. Pupae were harvested from the grass and paper toweling, and the rearing procedure repeated. Larvae and adults were reared in incubators or large rearing units at ≈26°C, 70% RH, and 14:10 (L:D) photoperiod. Rearing procedures were similar at EREC except that larvae were reared on St. Auginegrass and adults were fed 0.25 M sucrose solution.

Field Study

The attraction of male moths to females was tested with Standard Universal Moth Traps, 'Unitraps' (Great Lakes IPM, Vestaburg, MI) baited with virgin females obtained from the EREC colonies. Unitraps are comprised of a green top with a 2.0-cm hole, yellow funnel, and white collecting bucket. The trap is designed to have the attractant placed within an insert (1.8 cm W × 5.0 cm L) that is put in the hole in the top. Moths that are attracted to the lure become excited and fall through the funnel into the collecting bucket. For our experiments, the traps were modified by placing fine-meshed window screen around the insert and attaching a small vial (2 dram, 1.5 cm W × 5.5 cm L) with a cotton dental wick to the bottom of the insert. A virgin female (<24 h old) was placed in the insert and had access to the vial, which contained 0.25 M sucrose solution.

The experiment was conducted at the EREC during Jul and Aug 2004. Wild TSW adults were observed at the EREC during this time and were present for testing pheromone attraction in the field. Pairs of baited and unbaited traps (10 m apart) were attached to metal poles (1.5 m) placed in mowed grass of different species. Traps were placed in the shade to avoid exposure to the sun. Two to 5 pairs of traps were deployed per week 9 Jul through 25 Aug. Each trap pair was >10 m apart and there were 11 trap pairs. Survival of the females and number of males captured was observed daily until females died. Baited trap capture numbers were compared against number of males captured in unbaited traps (Paired *t*-test, SigmaStat, Systat Software, Richmond, CA). Additionally, the length of time that females continued to attract males was tested 2 to 3 d, 4 to 6 d, or 7 to 8 d later (One-way analysis of variance, ANOVA, SigmaStat).

Laboratory Mating Behavior

Pupae from CMAVE were shipped to the USDA-ARS Subtropical Horticulture Research Station, Miami, FL (SHRS) for laboratory tests. Newly emerged adults were collected each day and maintained in single-sex cages in separate holding rooms at 25°C and 70% RH until time of testing. The holding rooms had windows to provide natural lighting and were supplemented with room lights set to a photoperiod of 12:12 (L:D) h, with lights off at 2000 h and lights on at

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0800 h. Adults were provided with water and a sucrose solution.

Tests were conducted to document aspects of mating behavior under laboratory conditions. All tests were conducted in rooms with windows so that natural light was available and room lights were set to same photoperiod so that bioassay rooms had the same light conditions as the adult holding rooms. Observations on adult mating behavior (e.g., female "calling" or pheromone release posture, periodicity of calling, and time of mating) were made by filming adults under an infra-red light with a low light CCTV camera (BP330, Panasonic Corp., Secaucus, NJ). Moths were placed in clear plastic 140-mL vials (8.6 cm length × 4.8 cm ID) with removable snap-top lids (Thornton Plastics, Salt Lake City, UT). A piece of aluminum window screen (4 cm diam) was attached to the bottom of the clear vial with hot glue, and a piece of filter paper (Whatman #1, 7.62 cm × 7.62 cm) was placed along the back wall of the vial to provide foot-holds for the moths. The vial was inverted with the snap-lid becoming the floor and a moistened piece of cotton wick (~2 cm long) was added to provide water to the adults. Moths were placed in the vials prior to the start of the dark period (scotophase), video output was recorded on a VCR throughout scotophase, and the tapes were reviewed for observations of mating behavior. All moths were dissected at the end of the observation period to confirm sex, mating status, and presence of mature eggs in females. Initial studies evaluated behavior of 10 sets of male and female adults that were 0-5 d old at time of testing.

Linear olfactometers (Analytical Research Systems, Inc., Gainesville, FL) were used to evaluate female behavior during time periods of male response. Individual virgin females were placed in small glass chambers (~12.7 cm × 2.2 cm ID) with a downwind screen that was attached to a glass tube (35.5 cm total length × 2.54 cm ID). A moistened piece of cotton wick (~1.5 cm long) was added to provide water to the females and to add humidity to the air. Purified air was delivered to the chamber containing the female and then into the olfactometer through connectors made from Teflon tubing. A single male was released at the downwind end of the tube prior to the start of scotophase, and there were 4 linear olfactometers used per test. Tests were conducted on 5 different nights, for a total of 20 samples. Activity of females in the small glass chambers and of males in ~5 cm of the upwind ends of the glass tubes (response window) during 0000-0800 h (ET) was recorded by video capture on a VCR. Video tapes were reviewed, entrance and exit times for males into the response windows were recorded, and the difference in entrance and exit time was used to determine number of min that males spent per individual visit. Min per visit were summed for each h to quantify sum total time per h per male. Sum total time per h per male was used as the response variable and effect of time period was analyzed by one-way ANOVA in Proc GLM (SAS Institute 2001). Tapes also were reviewed to evaluate posture and activity of the females during time periods of male response as indications of female calling behavior.

RESULTS

Field Study

Females survived in the traps for up to 8 d. Significantly more males were collected in virgin female-baited traps than in traps with no females (baited traps, mean ± SEM = 9.3 ± 2.5 males per female; unbaited, 0.0 ± 0, t = 3.76 with 10 df, P = 0.004). Total male capture per trap ranged from 1 to 24, with a total of 102 males collected. Males were collected in baited traps up to 8 d after females were placed in traps. Females aged 2-3 d attracted 5.7 ± 1.9 males, those aged 4-6 d attracted 3.6 ± 1.5 males, and those aged 7-8 d attracted 3.3 ± 1.7 males to the traps. Although there was a decline in the number of males captured as females aged, this difference was not significant (F = 0.6; df = 2, 21; P = 0.555).

Laboratory Mating Behavior

Mating occurred in only 3 of the 10 pairs videotaped. Of these, mating occurred at 0218, 0234, and 0310 h, which was 3-4 h before sunrise. Two of these 3 pairs completed mating during the videotaped time period and they remained paired for 78 and 98 min. Eight of the 10 females videotaped were sexually mature, as indicated by presence of mature eggs, and all 3 females that mated were sexually mature. There was no obvious calling posture observed among females that either did or did not eventually mate, and there were no obvious differences in behaviors of successful versus unsuccessful males. TSW used a simple courtship pattern, with behavioral steps most similar to those described for *Amyelois transitella* (Walker) and *Laetilia coccidivora* (Comstock) (Phelan & Baker 1990), although females for both of those species displayed calling behavior. TSW females that eventually mated tended to be positioned close to the bottom of the vial, remained stationary or moved a short distance away when approached by the male but then remained stationary until copulation was successful. As described for *A. transitella* and *L. coccidivora*, the male approached the female from behind, with rapid wing-fanning and walking. The male faced the same direction as the female and attempted copulation with a ventrolateral thrust. If the initial attempt was unsuccessful and the female moved away, the male would follow and make additional attempts. After a successful copulation attempt, the pair moved to a tail-to-tail

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position and remained stationary for the duration of the copulation. This behavioral sequence was observed for all 3 successful matings.

Sixteen of the 20 males tested in the linear olfactometer were observed in the upwind end of the glass tube of the linear olfactometer at some time during the sample period. Total time spent in the upwind end of the glass tube over the 8-h test period ranged from 7.4 min to 368 min, with the overall average (± SEM) of 125.2 ± 27.0 min. The highest sum total number of min per h that males were observed in the upwind end of the glass tube occurred from 0600-0700 h (Fig. 1), but males were observed during all time periods and there were no significant differences among time periods ($F = 1.15$; $df = 7, 120$; $P = 0.3352$; square-root $x + 0.5$ transformed data). Females were active periodically throughout scotophase and again there were no obvious calling postures observed throughout scotophase. Females tended to be quiescent during the time periods of greatest male response, but quiescence and male response were not always concurrent.

Discussion

Sex attractants have been identified for other crambid (Crambini) sod webworms including the bluegrass webworm, *Parapediasia teterrella* (Zincken) (Clark & Haynes 1990), the cranberry girdler, *Chrysoteuchia topiaria* (Zeller) (Kamm & McDonough 1979; McDonough & Kamm 1979; Kamm & McDonough 1980), and the western lawn moth, *Tehama bonifatella* Hulst (McDonough et al. 1982). Pheromones also have been identified for several moth species in the same tribe (Spilomelini) as TSW, including *Cnaphalocrocis medinalis* Guenée (Ramachandran et al. 1990; Ganeswara Rao et al. 1995; Kawazu et al. 2000) and 3 species of *Diaphania*. One chemical, (*E*)-11-hexadecenal, was a major component in the pheromone blends of *D. indica* (Saunders) (Wakamura et al. 1998), melonworm *D. hyalinata* (L.) (Raina et al. 1986), and pickleworm *D. nitidalis* (Stoll) (Klun et al. 1986).

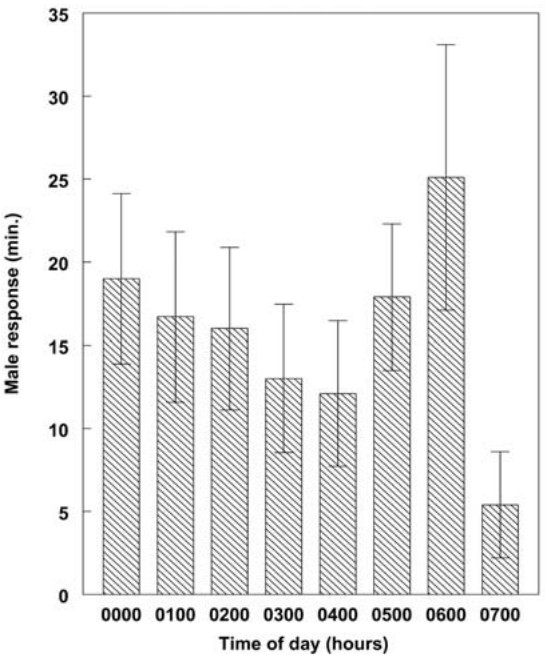


Fig. 1. Number of min (mean ± SE) that virgin males appeared in response to volatile chemicals from virgin female tropical sod webworms ($n = 16$). Number of min per h was determined from videotape recordings of male activity in linear olfactometers 4 h after the start of scotophase.

Our linear olfactometer results suggested that both males and females are active throughout scotophase, with a trend for higher male response 4 to 5 h after the onset of scotophase and at the end of scotophase. Research with other crambids/pyralids has shown variable results relating calling behavior, pheromone production, and mating. In some species, the relationship between female calling and pheromone production was weak, where pheromones appeared to be produced without apparent calling behaviors (Coffelt et al. 1978; Kawazu & Tatsuki 2002). In other species, however, females initiated calling, males responded and mating occurred within a relatively short time period either in early scotophase (Elsey 1982; Valles et al. 1992) or in late scotophase (Hight et al. 2003).

In summary, these results show that female moths release pheromone that is attractive to males and that Unitraps are a suitable trapping system for this species under field conditions. Our field and laboratory studies confirm that female tropical sod webworms use a sex pheromone for chemical communication and if available, a synthetic pheromone lure could be used for trapping males. However, preliminary tests have found that TSW females release very small amounts of pheromone (P.E.A. Teal, personal communication). The lack of calling posture among virgin females and flexibility in the periodicity in the time period of male response to volatile chemicals make it difficult to determine if there is a specific calling period for optimal chemical collection, which would facilitate pheromone chemical identification.

Acknowledgments

We thank C. Dillard and B. Dueben (USDA-ARS, Gainesville), A. Wilson (Univ. of Florida, EREC, Belle Glade), P. Anderson and N. Theresias (USDA-ARS SHRS, Miami) for technical support. We thank P.E.A. Teal (USDA-ARS, Gainesville) and E. Buss (Univ. of Florida) for review of an earlier manuscript.

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