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CABBAGE LOOPER MOTHS (LEPIDOPTERA: NOCTUIDAE) TRAPPED WITH MALE PHEROMONE

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ABSTRACT

Traps in field plots assessed attraction of the cabbage looper moth, *Trichoplusia ni* (Hübner), to lures emitting synthetic chemicals identified as the pheromone of the male; linalool, *p*-cresol and *m*-cresol. Male and female cabbage looper moths were captured in traps baited with racemic linalool, but significantly greater numbers of both sexes were captured in traps baited with the 3-component blend. Virgin and mated female cabbage looper moths were captured, with up to 5 spermatophores per female in mated ones. Pheromone was dispensed from polypropylene vials, and numbers of moths captured in traps increased with the size of the hole in the vial lid, up to the maximum 25-mm diameter hole tested. Rates of release of pheromone from vials with 25-mm diameter holes in the laboratory decreased from 4 to 3 milligrams per h over a four-week duration. This is the first evidence in the field of cabbage looper response to the chemicals identified as pheromones of the male.

Key Words: pheromone, attraction, trap, behavior, cabbage looper, linalool, *p*-cresol, *m*-cresol.

RESUMEN

Mediante trampas de campo se estimó la atracción de la palomilla del falso medidor, *Trichoplusia ni* (Hübner), a cebos que emiten linalool, *p*-cresol y *m*-cresol, químicos sintéticos identificados como la feromona del macho. Se atraparon machos y hembras de la palomilla del falso medidor en trampas cebadas con sólo linalool racémico, pero se atraparon números significativamente mayores de ambos sexos con la mezcla de los tres componentes. Se capturaron palomillas vírgenes y apareadas con hasta cinco espermatóforos por hembra. La feromona se expuso en viales de polipropileno y el número de palomillas capturadas por trampa se incrementó con el tamaño del orificio en la tapa del vial, hasta el máximo probado de 25 mm de diámetro. La tasa de liberación de la feromona de los viales con orificio de 25 mm en laboratorio disminuyó de 4 a 3 miligramos por hora en un periodo de cuatro semanas. Esta es la primera evidencia de campo de la respuesta de la palomilla del falso medidor a los químicos identificados como la feromona del macho.

Translation provided by the authors.

The cabbage looper moth, *Trichoplusia ni* (Hübner), uses two mate-finding strategies (Landolt & Heath 1990; Lenczewski & Landolt 1991). One strategy involves male attraction to the female-produced sex pheromone which includes the major component *Z*-7-dodecenyl acetate (Berger 1966), and several other structurally related compounds (Bjostad et al. 1984). The other strategy involves female attraction to the male pheromone composed of the major component *S*-(+)-linalool, as well as *p*-cresol and *m*-cresol (Heath et al. 1992a; Landolt & Heath 1989; Landolt 1995). *S*-(+)- or racemic linalool alone and the 3-component male pheromone blend of linalool, *p*-cresol, and *m*-cresol attracted females in a laboratory flight tunnel assay (Heath et al. 1992a). However, these chemicals have not been tested in the field for their attractiveness to cabbage looper moths.

A synthetic lure for females could be developed for use in monitoring the activities of females and also for reducing reproduction of this pest in agricultural crops. To date, however, there have been no demonstrations in the field of female cabbage looper moth attraction to synthetic male pheromone, although female and male attraction to chemicals from flowers is well documented (Cantelo & Jacobson 1979; Haynes et al. 1991; Heath et al. 1992b). We report here the results of trapping experiments that tested the hypothesis that cabbage looper moths are attracted to the male pheromone compounds. Because pure *S*-(+)-linalool was not available in amounts sufficient for these experiments, we used racemic linalool, both alone and in combination with *p*- and *m*-cresol.

MATERIALS AND METHODS

Universal moth traps, (UniTraps, IPM Technologies, Portland, OR) were used in all tests. These traps had white buckets, yellow cones, and green tops, and included a 6.5-cm^2 piece of Vaportape (Hercon Environmental Inc., Emigsville, PA) stapled to the inside of the bucket wall to kill captured insects. Traps were hung from fences or stakes in or adjacent to irrigated fields of alfalfa, *Medicago sativum*, or corn, *Zea mays*, at a height of 0.5 m, and were 10 to 15 m apart. Pheromone chemicals were dispensed from polypropylene narrow mouth bottles (vials) (#2006 9125 for 4 ml vials, #2118 9050 for 15 ml vials, Nalge Nunc International, Rochester, NY). These pheromone dispensers (vials) were suspended vertically with wire inside the UniTrap buckets.

The first experiment tested attractiveness of the 3-component blend of racemic linalool, *p*-cresol and *m*-cresol (Aldrich Chemical Co., Milwaukee, WI) to cabbage looper moths. Two ml of a 90:5:5 mixture of linalool, *p*-cresol, and *m*-cresol were added to a 2.5-cm diam cotton ball inside of a 4-ml vial. Vials had a 3-mm diameter hole in the lid for pheromone emission. Control traps had no lures. Five pairs of treated and control traps were maintained from 20 to 28 August 2001. Traps were checked for moths three times (every 2 to 3 days), providing 15 samples. Treatment and control traps were alternated in position each time that traps were checked.

The second experiment tested for a role of the cresols in cabbage looper moth attraction to the 3-component pheromone blend of linalool, *p*-cresol, and *m*-cresol. The 3 treatments were (1) a trap with no lure as a control, (2) a trap with a 4-ml vial containing 2 ml of racemic linalool on a cotton ball, and (3) a trap with a 4-ml vial containing 2 ml of a 90:5:5 mixture of racemic linalool, *p*-cresol, and *m*-cresol on a cotton ball. Each vial had a 3-mm diameter hole in the lid for pheromone emission. A randomized complete block experimental design was used, and the ten replicate blocks were maintained from 17 July to 4 September 2003. Traps were checked and treatments randomized each week for 7 weeks, providing 70 samples. Lures were replaced every two weeks.

The third experiment evaluated a range of release rates of the 3-component blend of racemic linalool, *p*-cresol, and *m*-cresol. The objective was to determine if attractiveness of the pheromone to cabbage looper moths increased with increasing amounts of pheromone released and to determine an optimum lure for trapping cabbage looper moths with male pheromone. Lure release rate was altered by changing the diameter of the hole in the vial lid. Treatments were 15-ml vials, each with 2 ml of an 90:5:5 mixture of racemic linalool, *p*-cresol, and *m*-cresol, and with holes 1.5, 3, 6, 12.5, and 25 mm in diameter. The larger vials were used to accommodate holes of a greater diameter in the vial lid. A randomized complete block design was used, and the 5 blocks were maintained from 4 to 25 September 2003. Traps were checked and treatments randomized each week, providing 15 samples. Lures were replaced every week.

Female moths captured in traps baited with the 3-component blend in experiments two and three were stored in 70% ethanol and then dissected under a binocular microscope for determination of their reproductive status. The presence of fat in the abdomen was noted, the numbers of mature eggs in the ovaries were counted, and the number of spermatophores in the bursa copulatrix was recorded. This information was used to categorize the reproductive state of female moths captured in the system of Hitchcox (2000). Moths in category I were unmated and immature, with no spermatophore, abundant fat, and fewer than 10 mature eggs present. Moths in category II were mated and immature, with one or more spermatophores, fat in the abdomen, and fewer than 10 mature eggs present. Moths in category III were mated and mature, with one or more spermatophores, 10 or more mature eggs and with some fat present. Moths in category IV were senescent, with one or more spermatophores present, no fat, and fewer than 10 mature eggs.

Release rates of male pheromone from vials with 25-mm diameter holes were determined as weight lost over time. Ten 15-ml polypropylene vials were each loaded with 5 ml of male pheromone (90:5:5 ratio of racemic linalool, *p*-cresol, and *m*-cresol) on 3 cotton balls. Dispensers were then weighed one day after loading, then daily until 28 days after loading. Daily weight loss was determined by subtracting the vial weight from the weight of the vial the day before. Hourly weight loss was calculated by dividing daily weight loss by 24. The weight lost was then attributed to emission of male pheromone from the dispensers.

Trap catch data for treatments in experiments number 1 and 2 above were compared with a paired *t*-test. Data for experiment 3 were subjected to a quasilinear (with a square root transformation) regression analysis to determine if numbers of moths captured varied with pheromone release rate (vial hole size). Daily weight loss data were subjected to a regression analysis to determine if dispenser weight loss per day changed with time. All statistical analyses were performed with the Statmost software (DataMost 1995).

Results

In the first experiment, cabbage looper moths were captured in traps baited with the mixture of racemic linalool, *p*-cresol, and *m*-cresol, while no moths were captured in unbaited traps (Table 1). Numbers of moths in baited traps were significantly greater than in unbaited traps ($t = 3.45$, $df = 14$, $P = 0.002$). These moths were not sorted by sex, and were not dissected to determine reproductive status. A total of 143 cabbage looper moths were captured in traps in this experiment.

TABLE 1. MEAN (± SE) NUMBERS OF CABBAGE LOOPER MOTHS CAPTURED IN TRAPS BAITED WITH VIALS LOADED WITH THE RACEMIC LINALOOL, *P*-CRESOL, AND *M*-CRESOL.

Test 1.		Moths/trap	
	Control	0.0 ± 0.00 a	
	Linalool, *p*-cresol, *m*-cresol	9.5 ± 2.80 b	
Test 2.		Females/trap	Males/trap
	Control	0.0 ± 0.00 a	0.0 ± 0.00 a
	Linalool	0.2 ± 0.08 b	0.5 ± 0.13 b
	Linalool, *p*-cresol, *m*-cresol	0.5 ± 0.14 c	0.9 ± 0.15 c

Means followed by the same letter are not significantly different by paired *t*-test at $P < 0.05$.

In the second experiment (Table 1), numbers of both sexes of the cabbage looper moth were greater in traps baited with racemic linalool alone ($t = 3.65$, $df = 84$, $P < 0.001$ for females, $t = 4.75$, $df = 84$, $P < 0.001$ males) or with the combination of linalool and cresols ($t = 4.33$, $df = 84$, $p < 0.001$ for females; $t = 5.53$, $df = 84$, $P < 0.001$ for males) compared to unbaited traps. Numbers of both sexes captured in traps baited with racemic linalool, *p*-cresol, and *m*-cresol were significantly greater than the numbers of both sexes trapped with racemic linalool alone ($t = 2.18$, $df = 84$, $P = 0.016$ for females; $t = 1.62$, $df = 84$, $P = 0.05$ for males). Totals of 79 female and 141 male cabbage looper moths were captured in this test.

In the third experiment, numbers of both sexes of the cabbage looper moth increased with the increases in diameter of hole in the vial lid (Fig. 1), with greatest numbers of males and greatest numbers of females in traps baited with pheromone in vials with a 25-mm hole in the lid. For females, there was a significant regression of numbers of moths captured by vial hole diameter ($r^2 = 0.80$, $P = 0.01$, Y = -01.36 + 16.6 * SQRT X, where Y is vial hole diameter, and X is numbers of moths per trap). For males there was also a significant regression of numbers of moths captured by vial hole diameter ($r^2 = 0.97$, $P = 0.0004$, Y = 1.35 + 13.0 * SQRT X). Totals of 48 females and 61 males were captured in this test.

Seventy-one female cabbage looper moths that were captured in traps baited with linalool, *p*-cresol and *m*-cresol were dissected in order to categorize their reproductive condition. The greatest number of females were in category III, mated and mature (n = 31). The smallest number of females dissected were in category II, mated and immature (n = 3). Category I, unmated and immature, and category IV, senescent, were represented by 19 and 18 moths respectively. Fifty-one female moths were mated (71.8%), and those that were mated, possessing from one to 5 spermatophores (mean = 2.5 ± 0.15 spermatophores per female). Twenty female moths of the 71 dissected (28.2%) were unmated.

There was a significant negative regression of daily vial weight loss in relation to the age of vials ($r^2 = 0.14$, $P = 0.03$, Y = 3.87 - 0.0386X, where Y is weight lost, and X is days in age). Daily weight loss of vial dispensers loaded with cabbage looper male pheromone was near 4 milligrams per h during the first several days after loading of the vials with pheromone, dropping to near 3 milligrams per h near the end of the 4 week evaluation (Fig. 2)

DISCUSSION

Previous studies have demonstrated attraction of female and male cabbage looper moths to male pheromone. Females were attracted to male cabbage looper moths in a flight tunnel (Landolt & Heath 1989) and a field cage (Lenczewski & Landolt 1991), and both sexes were attracted to males in cotton fields (Landolt 1995). Female cabbage looper moths in a flight tunnel were attracted to the combination of *S*-(+)- or racemic linalool and *p*-cresol and *m*-cresol, 3 compounds isolated from hairpencils of male cabbage looper moths (Heath et al. 1992a). The flight tunnel attraction response was significantly reduced with the omission of the cresols from the blend or the omission of *S*-(+)-linalool from the blend. These studies did not address moth attraction to male pheromone compounds under field conditions or the possible use of male pheromone as a lure for trapping female cabbage looper moths.

This is the first demonstration in the field of male and female cabbage looper moth attraction to the chemicals identified as the male pheromone of *T. ni* by Heath et al. (1992a). We interpret captures of the moths in traps as evidence of orientation responses to the chemicals used as lures in the trap. Males and females were attracted then to racemic linalool and to the 3-component blend of linalool and the two cresols. Numbers of male or female cabbage looper moths in traps were higher when the cresols were present in the lure, indicating some importance of these compounds in male attractiveness to females. Additional testing in the field will be necessary to

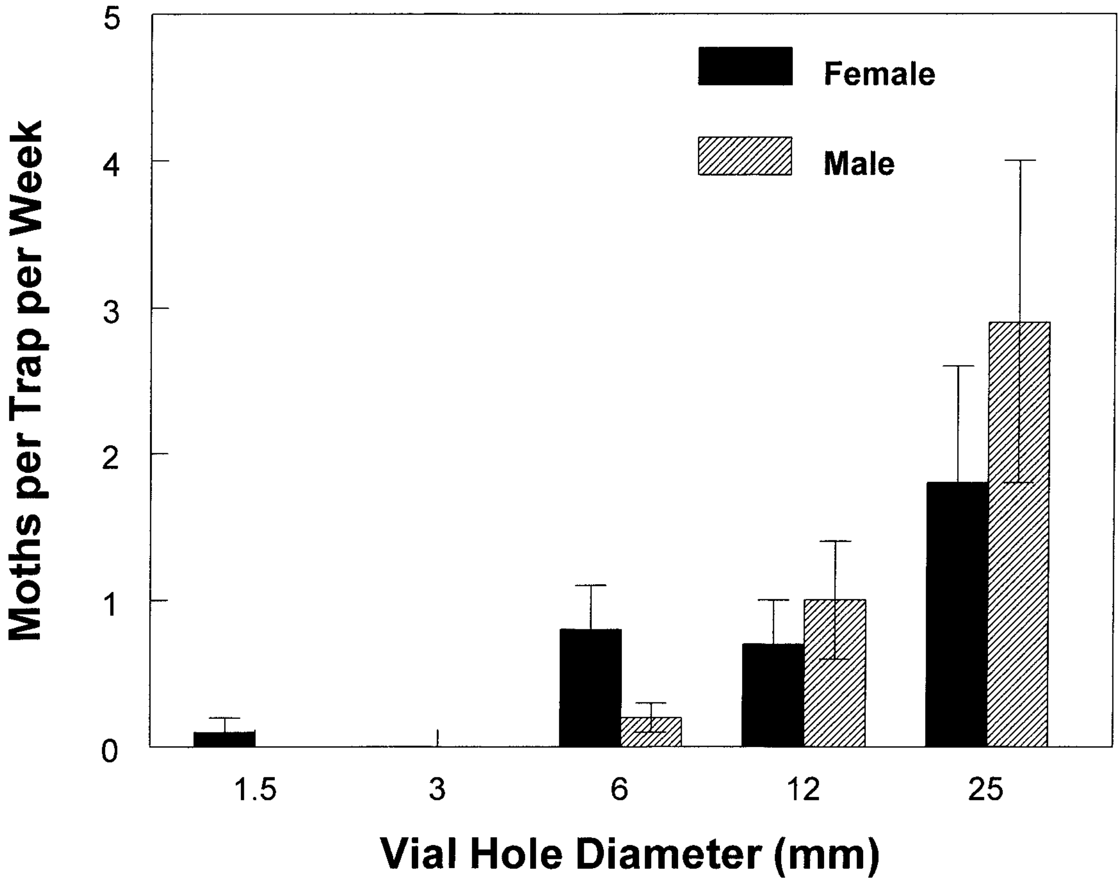

Fig. 1. Mean (± SE) numbers of female and male cabbage looper moths captured in traps baited with linalool, *p*-cresol, and *m*-cresol in a polypropylene vial with a hole in the lid for pheromone release. Vials had different hole diameters (1.5, 3, 6, 12, and 25 mm) to alter the rate of release of pheromone.

determine if attraction of moths to pheromone is stronger when *S*-(+)-linalool is used instead of racemic linalool, and to determine if both *p*-cresol and *m*-cresol increase attractiveness of the pheromone to cabbage looper moths.

Other species of moths were not captured in traps in this study, and the cabbage looper male pheromone chemicals are not reported as attractants for other species of insects. Linalool was tested previously as a possible floral lure for alfalfa looper moths, with no indication of attractiveness to either alfalfa looper or cabbage looper moths (Landolt et al. 2001). At that time (summer 2000) there were many alfalfa looper moths but few cabbage looper moths in the area; thus, the lack of cabbage looper moths captured did not indicate a lack of attractiveness of chemicals tested. Specificity of this lure in attracting only or primarily cabbage looper moths would be desirable for monitoring applications, because responses of other species of moths might be interpreted as false positives for cabbage looper moths.

The attraction of cabbage looper moths to male pheromone in the field may be a mate-finding or a food-finding response, or both. In addition to its presence in, and release by, male cabbage looper moths (Heath et al. 1992a), linalool is present in the odor of honeysuckle flowers which are visited by cabbage looper and other moths in search of nectar (Pair 1994; Schlotzhauer et al. 1996). Cabbage looper females that are deprived of sugar are more strongly attracted to males, suggesting responses to male pheromone may be based in part on food-finding needs (Landolt et al. 1996). Females attracted to males (Landolt 1995) and synthetic male pheromone (herein) include both mated and unmated individuals. Some possessed 5 spermatophores, indicating mating up to 5 times before responding to the male pheromone in the study. Perhaps male cabbage looper moths contribute nutritional material in the spermatophore and mimic flowers by releasing chemicals characteristic of certain moth-visited flowers, as a strategy of luring females.

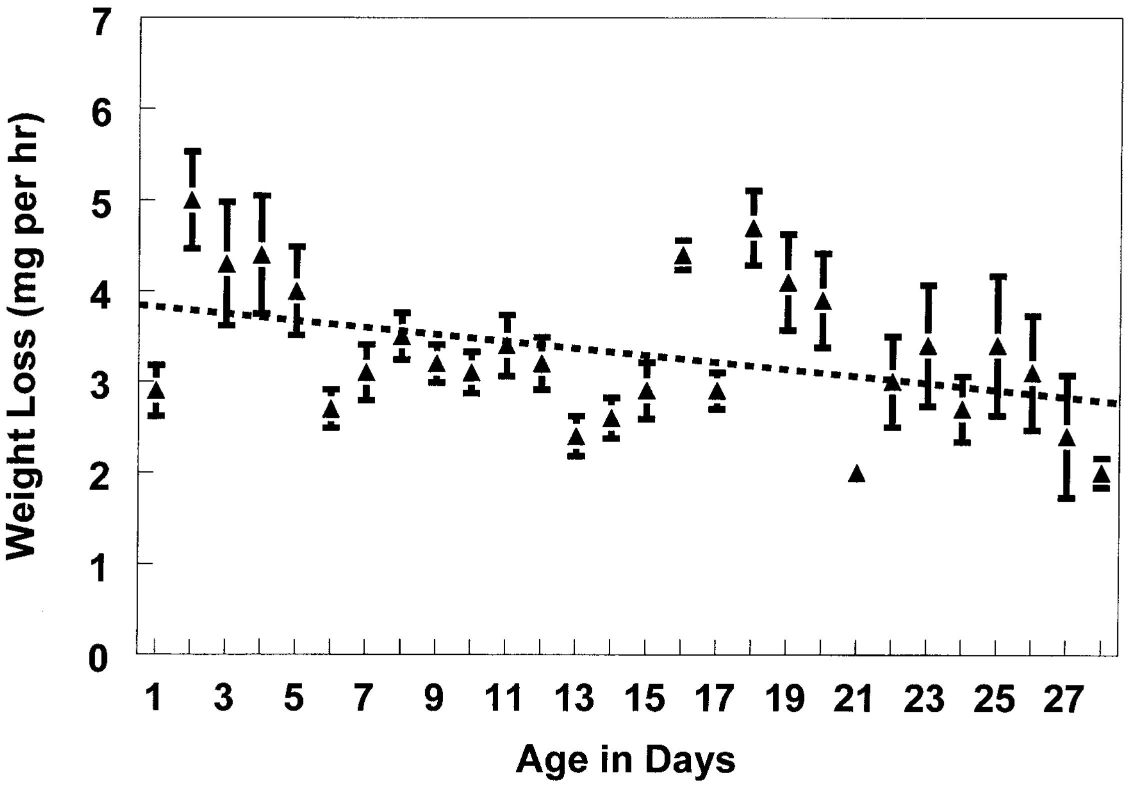

Fig. 2. Mean (± SE) loss of weight per hour over time, for 15-ml polypropylene vials loaded with 5 ml of male cabbage looper pheromone, with a 25-mm diameter hole in each vial lid.

Males of other noctuid moths produce chemicals in hairpencils that are also present in the odors of flowers (Birch & Hefetz 1987), but are not known to be attractive to conspecific moths. For example, 2-phenylethanol is present in the volatiles of the moth-visited flowers *Abelia grandiflora* (Haynes et al. 1991) and *Gaura drummondii* (Shaver et al. 1997). This compound is also found in the hairpencils of *Mamestra configurata* (Walker) (Clearwater 1975), *Polia nebulosa* (Hufnagel), and *Peridroma saucia* (Hübner) (Birch 1972; Birch et al. 1976). Birch & Hefetz (1987) suggested that the tendency for male scents to resemble plant odors is because females likely already have receptors for these chemicals and have behavioral responses to those food plant odors.

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