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SEX PHEROMONE SPECIFICITY IN *Heliothis*[1,2]

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Abstract—Electric grid traps baited with *Heliothis subflexa* (Guenée), *H. virescens* (F.), or *H. zea* (Boddie) females captured conspecific males with few exceptions. *Heliothis subflexa* females reduced the attraction of *H. virescens* and *H. zea* males when used as bait simultaneously with females of either of these two species. Backcrosses were made with *H. virescens* males and female hybrids from a cross between *H. subflexa* females and *H. virescens* males. The backcross (BC) females and *H. virescens* females attracted approximately equal numbers of *H. virescens* males in field traps. BC males released in field cages were attracted to *H. virescens* females and to the synthetic pheromone of *H. virescens*. When laboratory-reared male *H. virescens*, BC males, *H. subflexa* males, and F_1 hybrid males were exposed to the synthetic pheromone of *H.virescens* in Plexiglas wind tunnels, *H. virescens* males and BC males responded to the pheromone, but *H. subflexa* and F_1 hybrid males did not. The peak activity of both *H. subflexa* and *H. zea* males occurred approx. 4 hr after sunset. Male *H. zea* were active throughout most of the night; male *H. virescens* were most active approx. 6 hr after sunset.

Key Words—Sex pheromone, *Heliothis subflexa*, *Heliothis virescens*, *Heliothis zea*, hybrid sterility.

INTRODUCTION

Recent studies suggested that it might be possible to utilize the sterile hybrid

[1] This paper reports the results of research only. Mention of a pesticide or of a commercial or proprietary product in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended.

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males (Laster 1972) resulting from matings between female *Heliothis subflexa* (Guenée) and male *H. virescens* (F.) in a sterile-release program to control *H. virescens*. Laster et al. (1976) therefore presented a population model illustrating the decline of *H. virescens* populations that could follow release of various ratios of hybrid moths capable of transmitting genetic sterility to the population in this way. However, populations of *H. subflexa*, *H. virescens*, and *H. zea* (Boddie) may or may not occur in a particular area at the same time. Although both *H. virescens* and *H. zea* have many of the same host plants, *H. subflexa* is found almost exclusively on ground cherry, *Physalis* spp., and is not known to be a pest of any economic importance (Laster 1972, Kimball 1965). Also, little is known about how these species and introduced hybrids might interact in the field.

We therefore made field, field cage, and laboratory studies to determine whether there was cross-attraction or inhibition between *H. zea*, *H. subflexa*, *H. virescens*, the F_1 hybrid, and selected backcross (BC) moths.

METHODS AND RESULTS

Insects used for bait in traps in the field or released in cages were reared in the laboratory on artificial diet. The *H. subflexa* were taken from a culture started from larvae collected from the wild host, ground cherry, at Gainesville, Florida, in September 1975. The *H. virescens* were obtained as pupae from Oxford, North Carolina, and the *H. zea* pupae were obtained from laboratory cultures at Oxford, North Carolina, and Gainesville, Florida. The hybrid females from *H. subflexa* females and *H. virescens* males were backcrossed to normal *H. virescens* males and subsequent backcross (BC) generations were produced at the Gainesville laboratory. Male progeny from these matings were sterile and female progeny were fertile.

Field Studies

For one field study, one or two electric grid traps (Mitchell et al., 1972) were baited with 3 females each of either *H. subflexa*, *H. virescens*, or *H. zea* to determine attraction of these species to the pheromone (female-baited) traps. These traps were located in farming areas where host plants were available at either Hastings or Gainesville, Florida, and were operated from June 28 through July 24, 1975 (total of 37 trap nights for each species). Also, females from BC_8, BC_9, or BC_{10} were used in one trap and *H. virescens* in another as bait (3 females/trap) for *H. virescens* males (total of 49 trap

nights) at Gainesville. In a similar study at Gainesville, either *H. subflexa* plus *H. virescens* females (17 trap nights) or *H. subflexa* plus *H. zea* females (10 trap nights) were used in combination as bait (3 females of each species/trap) in grid traps to determine whether any inhibition existed between these species. Captured insects were collected and counted every 1 or 2 days. The paired *t* test at the 5% level of probability was used for mean separation.

Traps baited with *H. subflexa*, *H. virescens*, or *H. zea* females almost always captured males of the respective species. The trap baited with *H. subflexa* females captured 689 *H. subflexa*, 9 *H. virescens*, and 6 *H. zea*; *H. virescens* females attracted no *H. subflexa*, 766 *H. virescens*, and 2 *H. zea*; and *H. zea* attracted only *H. zea* (1028). BC females (from BC_8, BC_9, and BC_{10}) and *H. virescens* females attracted statistically equal numbers of *H. virescens* males (392 and 402, respectively), and only a few *H. subflexa* were captured by these baits.

In the test in which combinations of species were used as bait, the trap baited with *H. subflexa* captured 61% and the trap baited with *H. subflexa* plus *H. virescens* captured 39% of the total (456) *H. subflexa* collected. However, the catches were not significantly different at the 5% level under the conditions of the test. The trap baited with *H. virescens* captured significantly more *H. virescens* (71% of the 303 total) than the trap baited with *H. subflexa* plus *H. virescens*. Thus, the *H. subflexa* females apparently reduced the attraction of *H. virescens* females for *H. virescens* males when both species of females were present in the same trap.

Likewise, traps baited with *H. subflexa* captured 46% of the total (250) *H. subflexa*, and the trap baited with *H. subflexa* plus *H. zea* captured 54%. However, the trap baited with *H. zea* captured 73% of the total (370) *H. zea*, and the trap baited with *H. subflexa* plus *H. zea* captured only 27%. Thus, the presence of *H. subflexa* females in the same trap with *H. zea* females significantly reduced the attraction of *H. zea* males, although the presence of *H. subflexa* females in the same trap with *H. zea* females had no apparent effect on the attraction of *H. subflexa* males. Haile et al. (1973) found that when *H. virescens* and *H. zea* females were used as bait in the same trap, the number of males of both species that were captured was reduced. (We have obtained similar results in unpublished field tests.) However, they reported that the reduction of *H. virescens*, unlike the reduction in *H. zea*, was apparent only at a high density.

The field data therefore gave no indication of the interspecific sex attraction among *H. subflexa*, *H. virescens*, and *H. zea*, and there was some apparent inhibition. Mitchell et al. (1976) reported results indicating that individual components of a pheromone can be highly effective as mating

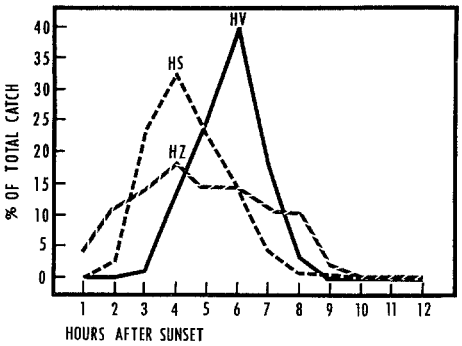

FIG. 1. Nocturnal activity of *Heliothis subflexa* (HS), *H. virescens* (HV) (Goodenough and Snow 1973), and *H. zea* (HZ) as determined with electric grid traps baited with virgin females.

inhibitors. For example, in small field tests, (*Z*)-11-hexadecenal, a component of the *H. virescens* pheromone, reduced the mating of *H. zea* females. Nevertheless, (*Z*)-9-tetradecenal, another component of the *H. virescens* pheromone, was ineffective against *H. zea* but this component reduced the mating of *H. virescens* females by 95%. (The pheromones of *H. subflexa* and *H. zea* have not yet been identified.)

The nocturnal activity of adult *H. subflexa* males was determined by operating a female-baited cylindrical electric grid equipped with an automatic sample changing device (Smith et al., 1973) from July 3 to 24, 1975, at Hastings in an area where host plants were available. Also, seasonal populations of *H. subflexa* and *H. virescens* were surveyed with female-baited grid traps at Gainesville from September 1975 to December 1976. In this case, traps (one for each species) were placed along the edges of fields in which host plants were present. (Trapping studies in process in this farming area guided us in location of these survey traps.)

The peak response of *H. subflexa* males to females occurred 3–5 hr after sunset (Figure 1); 78% were captured during this period, and an additional 14% were caught the following hour. The peak response of *H. zea* males to females occurred at the same time (approx. 4 hr after sunset), but activity remained relatively high throughout most of the night. Goodenough and Snow (1973) determined that *H. virescens* males were most active approx. 6 hr after sunset, and our data are in agreement.

When we subsequently surveyed populations of *H. subflexa* and *H.*

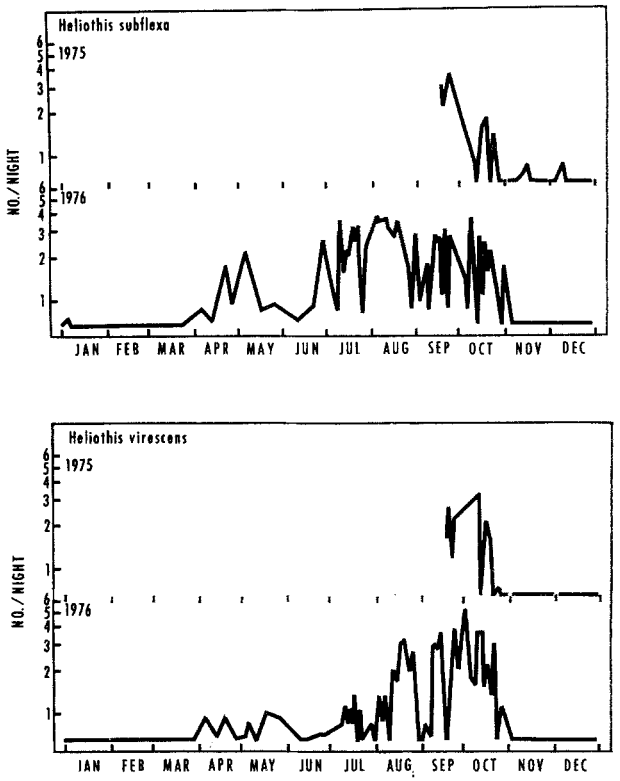

FIG. 2. *Heliothis subflexa* and *H. virescens* captured in female-baited electric grid traps, number [as $\log_e (n + 1)$]/night, Sept. 1975–Dec. 1976, Gainesville, Florida.

virescens at Gainesville, Florida, in an area where host plants were available to both species, we observed that population trends for these two species were similar (Figure 2). Diel and seasonal activities did not appear to be sufficient for reproductive isolation.

Cage Studies

Six cage studies were made (Table 1) in which *H. subflexa*, *H. virescens*, F_1 hybrids, or BC males were released in a 29 × 10-m arc-shaped cage with a maximum height of 3.5 m. Two electric grid traps were placed in the cage and baited either with females (3/trap) or with the synthetic pheromone of *H. virescens*, a 16:1 ratio of (*Z*)-11-hexadecenal and (*Z*)-9-tetradecenal (Tumlinson et al., 1975) dispensed in a Hercon® plastic strip (6.5 cm^2 of surface

TABLE 1. MEAN PERCENTAGE OF TOTAL MALES CAPTURED IN ELECTRIC GRID TRAPS (2/TEST) LOCATED IN A 29 × 10-m FIELD CAGE IN WHICH MALE MOTHS WERE RELEASED. TRAPS BAITED WITH *H. virescens* PHEROMONE OR *H. virescens*, F_1 HYBRID, OR BACKCROSS FEMALES, 3/TRAP, SIX TESTS)

Test	Released males	Bait females	$\bar{X}$ % of total males captured in indicated test
1A[a]	*H. virescens*	*H. virescens*	93[e]
		F_1 hybrid	7
B[b]	*H. virescens*	*H. virescens*	51
		BC_1	49
2[c]	F_1 hybrid	*H. virescens*	87[e]
		H. subflexa	13
3[d]	BC_1	*H. virescens*	72
		Pheromone	28
4A	BC_6	*H. virescens*	98[e]
		H. subflexa	2
B[b]	BC_6	*H. virescens*	64
		Pheromone	36
5	BC_7	*H. virescens*	67[e]
		BC_7	33
6A	BC_8	*H. virescens*	70
		BC_8	30
B	BC_8	*H. virescens*	59
		Pheromone	42

[a] Each treatment replicated three times unless otherwise noted.
[b] Four replicates per treatment.
[c] Six replicates per treatment.
[d] Two replicates per treatment.
[e] Means in the same test differ significantly at $P = 0.05$ level, Student's t test.

area on one side). Treatments were rotated daily between the two traps. Each treatment was replicated 2–6 times; a replication consisted of the catch for one night. Of the males released (200–400/test), approx. 40% were captured by the traps. The paired t test at the 5% level of probability was used for mean separation.

The results, although extremely variable, showed that the F_1 hybrid females attracted few *H. virescens* males, but the F_1 hybrid males and BC_6 males were more attracted to *H. virescens* females than to *H. subflexa* females. BC_1 and *H. virescens* females attracted approximately equal numbers of *H. virescens*; BC males were more attracted to *H. virescens* than to BC females.

Laboratory Studies

In the laboratory studies, the responses of *H. subflexa*, *H. virescens*, F_1 hybrid, BC_{1-5}, and BC_{10} males to the synthetic pheromone were compared in olfactometer tests. Laboratory-reared males that had been held in reverse photoperiod were released into three 30 × 30 × 350-cm Plexiglas wind tunnels (10–12/tunnel) used by Mayer (1973) and McLaughlin et al. (1974). The temperature and relative humidity in the wind tunnels were approx. 24–26 °C and 60%, respectively, and a light intensity of 0.5 lux was maintained. Moths were held in the downwind compartment at the beginning of each test. The pheromone (500 ng) was coated on the inside of glass tubes (Mayer 1973) and dispensed into the upwind compartment of each tunnel with filtered air at an airflow rate of 50 ml/min. Meanwhile, filtered air was passed through the tunnels at a rate of 0.25 m/sec. After the chemical had time to reach the holding compartment (calculated from air velocity and distance to holding compartment), the males were released and allowed 30 sec of free flight. Then dividers were inserted in the tunnels, and the

TABLE 2. MEAN CORRECTED PERCENTAGE RESPONSE (+SE) OF MALE *Heliothis virescens* (HV), *H. subflexa* (HS), AND CROSSES (F_1 AND BC) TO *H. virescens* PHEROMONE IN OLFACTOMETERS[a]

Insect species released in tunnel[b]	Mean % (±SE) in upwind compartment[c]
HV	32.8±5.0a
HS	1.1±1.1c
F_1	4.7±2.0c
BC_1	27.6±4.4ab
BC_2	36.7±5.9a
BC_3	30.8±8.0ab
BC_4	25.9±5.7b
BC_5	30.0±8.4ab
BC_{10}	38.2±2.6a

[a] Means followed by the same letter do not differ significantly at $P = 0.05$ level (Duncan's multiple range test).

[b] Pheromone = (*Z*)-11-hexadecenal + (*Z*)-9-tetradecenal (16:1), 500 ng of chemical dispensed into the tunnel with an airflow of 50 ml/min.

[c] Plexiglas tunnel 30 × 30 × 350 cm. Airflow 0.25 m/sec, 24–26°C, approx. 60% RH. Light intensity 0.5 lux.

number of moths in each compartment was recorded. Moths flying to the upwind compartment were considered to be responding to the pheromone. A control (no chemical released) was run each day that tests were conducted. All treatments were replicated 10 times.

Because the tests were conducted at three different times, the response of *H. virescens* to the synthetic pheromone (500 ng) was used as the standard in each test. Data are shown in Table 2 as corrected percentages (actual–control). The *H. virescens*, BC_{1-5}, and BC_{10} males responded to the *H. virescens* pheromone. However, the *H. subflexa* males did not respond, and only a few F_1 hybrid males (less than 5%) responded. This result was similar to that of the cage studies: more F_1 males were attracted to the trap baited with *H. virescens* than to the trap baited with *H. subflexa*, but few moths were caught in either trap.

The results of the olfactometer tests were therefore comparable to the results obtained in the cage and field studies, although all treatments were not made in each study. There was no evidence of cross-attraction among *H. subflexa*, *H. virescens*, or *H. zea*, and there was some evidence of inhibition. The F_1 hybrid males did not respond to the *H. virescens* pheromone, but BC males were attracted to both the pheromone and *H. virescens* females. Also, BC females and *H. virescens* females attracted equal numbers of *H. virescens* males. Thus, our results show that the sterile BC males will respond to *H. virescens* females and might be used, as Laster (1972) suggested, to suppress populations of *H. virescens*.

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