SPECIALIA

Response of *Heliothis virescens* to pheromonal components and an inhibitor in olfactometers

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Summary. Laboratory-reared males of *Heliothis virescens* (F.) that were released in olfactometers in the laboratory were attracted to the *H. virescens* synthetic pheromone, but not to (Z)-9-tetradecen-1-ol formate (Z-9-TDF), or to either pheromonal component, (Z)-11-hexadecenal (Z-11-HDAL) or (Z)-9-tetradecenal (Z-9-TDAL). Also, they did not respond to the pheromone when it was dispersed simultaneously with Z-9-TDF. The proximity of the test chemicals in the olfactometer made little, if any, difference in the response of *H. virescens* males to the pheromone source. Preexposure to the synthetic pheromone, Z-9-TDF, Z-11-HDAL, or Z-9-TDAL greatly reduced the number of *H. virescens* males responding to the pheromone. This reduction was probably caused by habituation of the moths to these chemicals.

The sex pheromone produced by female *Heliothis virescens* (F.) consists of 2 components, (Z)-11-hexadecenal (Z-11-HDAL) and (Z)-9-tetradecenal (Z-9-TDAL), in a 16:1 ratio[2]. However, both Z-9-TDAL and (Z)-9-tedradecen-1-ol formate (Z-9-TDF), a chemical of non-biological origin, are highly effective in inhibiting mating between males and females of *H. virescens*[3]. We therefore conducted olfactometer tests to observe the response of *H. virescens* males to the pheromone mixture, the individual pheromonal components and Z-9-TDF. We also observed the response of moths that were preexposed to the chemicals before they were released in the olfactometers. Disruption of pheromonal communications has been reported with a number of insects species[4]. The purpose of this research was to determine whether the response of *H. virescens* males obtained in the olfactometers were comparable to those obtained in field tests[3] and to establish the validity of evaluating potential mating inhibitors in the olfactometers.

Materials and methods. Laboratory-reared 2- or 3-day-old *H. virescens* males that had been held in reverse photoperiod were released into 3 plexiglass wind tunnels[5,6] measuring $30 \times 30 \times 350$ cm (10–12 moths/tunnel) ca. 6 h after the beginning of the dark period, the time when these moths are most active[7]. The temperature and relative humidity in the wind tunnels were maintained at 24–26 °C and 60%, respectively, and the light intensity was ca. 0.5 lux. Each tunnel could be separated into consecutive compartments of 27, 238 and 85 cm in length (beginning at the downwind end) by inserting 6-mm mesh hardware cloth dividers. Moths were held in the downwind compartment at the beginning of each test. An equal volume of each chemical was coated on the inside of glass tubes[5] and dispensed into the upwind compartment(s) 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 we allowed time for the chemical to reach the holding compartment, the divider was removed and the moths were released for 30 sec of free flight. Then dividers were inserted in the tunnels, and the number of moths in each compartment was recorded. Moths flying to the extreme upwind compartment were considered to be responding to the chemical. A control (no chemical released) was run each day that tests were conducted. All treatments were replicated 10 times.

The experiments were conducted in 2 groups, in the first of which each chemical was introduced in all treatments at a release point (A) located at the upwind end of each tunnel 280 cm from the holding compartment. The *H. virescens* pheromone, its components, Z-9-TDF, and the pheromone plus Z-9-TDF were released separately into the tunnels. Purity of the pheromone and its components was > 99%, and that of Z-9-TDF was > 95%. 3 doses of the pheromone were tested: 5, 50 and 500 ng. The other chemicals were tested at the 500-ng dose which was considered the standard. For some treatments the male moths were held in closed 3.8-l glass containers and exposed for 1 h to 25 mg of pheromone, Z-11-HDAL, Z-9-TDAL, or Z-9-TDF and then flown in the tunnels within 5 min of their removal from this pretreatment.

In the 2nd group of tests the pheromone and Z-9-TDF were introduced from either point A or a point B located midway between point A and the insect-release point (ca. 140 cm upwind of the holding compartment). The chemicals (500 ng) were dispensed either separately and simultaneously from both A and B or together from point A. In 1 treatment the insects were preexposed to 25 mg of Z-9-TDF for 1 h in the 3.8-l containers. As in the 1st group, a 500-ng dose of the pheromone was tested against the *H. virescens* males and was considered

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Table 1. Mean corrected percentage of response (± SE) of male *H. virescens* to (Z)-11-hexadecenal (Z-11-HDAL), (Z)-9-tetradecenal (Z-9-TDAL) and (Z)-9-tetradecen-1-ol formate (Z-9-TDF) in olfactometers

Chemical released in tunnel[a]	Preexposure chemical	Mean % response (± SE) in upwind compartment[c]
Pheromone[b] (5 ng)	–	4.6 ± 2.4[c]
Pheromone (50 ng)	–	33.6 ± 6.4[a]
Pheromone	–	32.2 ± 4.0[a]
Z-11-HDAL	–	5.2 ± 2.7[c]
Z-9-TDAL	–	3.1 ± 1.5[c]
Z-9-TDF	–	1.9 ± 1.3[c]
Pheromone + Z-9-TDF	–	2.4 ± 1.0[c]
Pheromone	Pheromone	17.8 ± 3.2[b]
Pheromone	Z-11-HDAL	12.1 ± 3.1[bc]
Pheromone	Z-9-TDAL	12.2 ± 4.3[bc]
Pheromone	Z-9-TDF	9.1 ± 2.8[bc]
Z-11-HDAL	Z-9-TDAL	9.2 ± 3.1[bc]
Z-9-TDAL	Z-11-HDAL	5.3 ± 2.5[c]

[a] 500 ng of chemical unless otherwise noted. When insects were preexposed to a chemical, 25 mg of pure chemical were used. [b] Pheromone = (Z)-11-hexadecenal + (Z)-9-tetradecenal (16:1). [c] Means followed by the same letter do not differ significantly at p = 0.05 level (Duncan's multiple range test).

a standard (or treated control). This treatment, as well as a control in which no chemical was released was run each day that tests were conducted.

Results and discussion. The tests were conducted at 3 different times, and the response of *H. virescens* to the pheromone mixture was used as the standard in each test to establish the fact that the wind tunnels were operating uniformly. Data are shown in table 1 as corrected percentages (actual-control).

About equal numbers of *H. virescens* males were attracted to the 50-ng and 500-ng dosages of pheromone, but significantly fewer males (< 5%) responded to the 5-ng dosage. They were not attracted to either pheromonal component alone, Z-9-TDF, or to the pheromone (500 ng) when it was dispensed from the same point simultaneously with Z-9-TDF.

Preexposure to the pheromone, to Z-9-TDAL or Z-11-HDAL, or to Z-9-TDF significantly reduced the number of males responding to the pheromone. Also, whether preexposed or not to either pheromonal component, when the moths were tested for responsiveness to the other component, they showed little response.

Results obtained in the 2nd group of tests (table 2) were similar to those obtained in the 1st group. As expected, the *H. virescens* males were attracted to the pheromone except when it was dispensed simultaneously with Z-9-TDF. The proximity of the test chemicals (introduced at points A and B) in the olfactometer made little, if any, difference in the response of *H. virescens* males to the pheromone. Also, preexposure of the moths to Z-9-TDF did not change the results obtained when the pheromone and Z-9-TDF were released simultaneously in the tunnels. When pheromone and the formate were evaporated from the same locus in the wind tunnels, males were observed flying upwind, but they did not gather about the mouth of the pheromone dispenser and did tend to drop back down the tunnel.

We do not know why the response of *H. virescens* males to the pheromone was diminished in these cases. Since the chemical structures of Z-11-HDAL and Z-9-TDF are very similar, the formate may be acting as a pheromone mimic of the aldehyde and may interfere in some way with the antennal receptor sites that perceive the true pheromone[8].

In conclusion, we demonstrated in the olfactometer tests that the pheromonal components and the formate inhibited the response of *H. virescens* males to the *H. virescens* pheromone. Furthermore, moth response was less when moths were preexposed to the pheromone, probably because of habituation of the male moths. Our study indicates that the olfactometers can be useful in evaluating potential mating inhibitors of *H. virescens*. However, the mechanisms by which disruption is accomplished should be better understood before the initiation of control programs in which atmospheric permeation is used for insect control.

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Table 2. Mean percentage of response (± SE) of male *H. virescens* to the synthetic pheromone, (Z)-11-hexadecenal and (Z)-9-tetradecenal (16:1), and (Z)-9-tetradecen-1-ol formate in olfactometers

Treatment	500 ng chemical Point A	Point B	Preexposed (1 h)	Mean % response (± SE) in compartment[a] 1 (release)	2	3 (upwind)
Control	–	–	–	51 ± 6 a	42 ± 5 a	7 ± 3 b
1	Pheromone	–	–	24 ± 5 b	36 ± 4 a	40 ± 4 a
2	Pheromone	Formate	–	51 ± 5 a	41 ± 4 a	8 ± 3 b
3	Formate	Pheromone	–	45 ± 5 a	45 ± 5 a	10 ± 3 b
4	Pheromone + formate	–	–	52 ± 5 a	34 ± 5 a	14 ± 3 b
5	Pheromone + formate	–	Formate	59 ± 5 a	32 ± 5 a	9 ± 2 b

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