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Antagonistic Effect of (Z)-11-Hexadecen-1-ol on the Pheromone-Mediated Flight of *Helicoverpa zea* (Boddie) (Lepidoptera:Noctuidae)

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Flight-tunnel experiments were conducted using Helicoverpa zea *males to determine whether or not* (Z)*-11-hexadecen-1-ol (Z11-16:OH), a compound emitted by another heliothine moth species,* Heliothis subflexa, *is a behavioral antagonist when admixed with the two-component pheromone blend of* H. zea. *Males were less likely to fly upwind all the way to the source when 0.3% Z11-16:OH was present in the blend. Even 0.1% Z11-16:OH caused differences in the flight behavior of* H. zea *males; they steered more off the windline than males responding to the pheromone blend alone, resulting in more oblique track angles. Thus Z11-16:OH appears to act antagonistically, along with another compound,* (Z)*-11-hexadecen-1-ol acetate (Z11-16:Ac), when it is added to the* H. zea *pheromone blend.*

KEY WORDS: *Helicoverpa zea*; upwind flight; sex pheromone; antagonist; wind tunnel.

INTRODUCTION

Sex pheromone communication in four species of North American heliothine moths involves blends of components for each species that act agonistically to evoke upwind flight to the source of pheromone by conspecific males. Some of these components also cause antagonistic behavioral responses in males of other heliothine species. Two components that are part of the sex pheromone blend of *Heliothis subflexa* are (Z)-11-hexadecen-1-ol (Z11-16:OH) and (Z)-11-hexadecen-1-ol acetate (Z11-16:Ac) (Teal *et al.*, 1981). As in two other species, *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie), *H. subflexa* uses the corresponding aldehyde (Z)-11-hexadecenal (Z11-16:Ald) as its major component

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(Teal *et al.*, 1981), and cross-attraction of *H. zea* and *H. virescens* males to *H. subflexa* emissions is known to be prevented by the antagonistic effect of Z11-16:Ac (Vickers and Baker, 1996, 1997; Fadamiro and Baker, 1997; Baker *et al.*, 1998) when it is added to blends containing Z11-16:Ald plus the secondary components of these two species, either (*Z*)-9-tetradecenal (*H. virescens*) or (*Z*)-9-hexadecenal (*H. zea*).

Recently, a type of receptor neuron on the antennae of *H. zea* males was discovered that responds to both Z11-16:Ac and Z11-16:OH (Cossé *et al.*, 1998). This neuron is the only type on *H. zea* antennae known to be tuned to Z11-16:Ac, and this compound's only known effect on behavior is antagonistic. This led us to hypothesize that because Z11-16:Ac is a known *H. zea* behavioral antagonist, then Z11-16:OH should be an antagonist, too, since it stimulates the same neuron that is tuned to Z11-16:Ac at approximately the same dosage. We therefore conducted a series of experiments to determine whether or not Z11-16:OH antagonizes *H. zea* sex pheromone-mediated upwind flight.

MATERIALS AND METHODS

Moths

H. zea larvae were reared on a modified pinto bean diet (Shorey, 1965). Following pupation the adults were separated according to sex and placed in separate environmental chambers on a 14:10 (L:D) photoperiod, 25°C and 60 ± 10% RH. Adults were supplied with a 10% sugar solution. Males were used in flight tunnel assays when they were between 1 and 3 days old. Before initiating the experiment, the males were placed individually into small wire-screen cages (6-cm-diameter cylinder × 7 cm in height). These cages were placed on plastic trays (15 individual cages per tray). The trays were introduced into the wind tunnel 1 h before beginning the experiment to acclimate the males to the ambient conditions within the wind tunnel. Flight tunnel assays were conducted between the 5th and the 8th h of scotophase. A male was scored only once and then discarded.

Chemicals

Only two of the four components that constitute the pheromone of *H. zea*, Z11-16:Ald and Z9-16:Ald (Klun, 1980), are necessary to attract males (Vetter and Baker, 1984). Ten microliters of a hexane solution containing 10 μg of the major component Z11-16:Ald and Z9-16:Ald at a 20:1 ratio was used as the attractive pheromone blend and was loaded onto a piece of filter paper (Whatman No. 1) attached by an alligator clip to a cork. The other treatments contained the same binary blend with either 0.1, 0.3, or 1% Z11-16:OH added relative to the major component. All the chemicals were at least 98% pure by capillary gas chromatographic methods.

Wind Tunnel

The wind tunnel was from a design modified from that of Miller and Roelofs (1978). Its dimensions were 2.4 × 1 × 1 m. The wind speed was held constant at 40 cm/s and the light intensity inside the tunnel was 0.5 lux (mixture of red and white light). The temperature and relative humidity were 25°C and 65%, respectively. The floor of the tunnel was scattered with red dots (David, 1982) to provide cues for visual feedback used by a moth in monitoring upwind progress (Marsh *et al.*, 1978). The pheromone source was placed on a platform 30 cm from the upwind end of the tunnel, such that the plume of pheromone emanating from the filter paper was 23 cm above the tunnel floor. Males were released individually by hand at a height of 23 cm above the floor and 170 cm downwind from the pheromone source. Each male was hand-held in its screen cage at the level of the plume for at least 30 s before opening the cage for release. Each male was allowed 2 min to take off from the cage.

A Sony RSC 1050 camera positioned above the wind tunnel (1 m × 0.75-m field of view) was used to record flights of the males on a Toshiba videocassette recorder. The field of view for the recordings encompassed an area beginning 20 cm downwind of the platform and ending 120 cm downwind, with a width of 75 cm. The audio channel on the videotape was used to record the sequence of behaviors that occurred out of the camera's field of view.

Five to 10 males were tested for each treatment per day in a randomized complete-block design, with a total of 160 males tested for all the treatments. Only tracks of males that flew through the entire field of view of the camera and contacted the source were later analyzed and compared. In addition, the only tracks of pheromone-alone control males that were analyzed were those that were recorded in the same block (day) in which at least one male successfully located the 0.1% Z11-16:OH source. These criteria reduced the numbers of tracks available for analysis.

Tracks were played back using a Toshiba time-lapse video analyzer (KV-6300A) and viewed on a Panasonic monitor. The male's position every 1/30th s was marked on a sheet of acetate placed over the screen. Tracks were digitized on a Hitachi digitizing pad and the data were subjected to a triangle of velocities program (Marsh *et al.*, 1978) that calculated the male's course angle, track angle, airspeed, and groundspeed every 1/30th s. Averages of the flight parameters for each male were calculated, and then grand means for the moths' flight behaviors were used for each treatment. Course and track angles values can be either positive or negative, depending on which side of the wind-line the moth is flying, and so absolute values were used to calculate these means. Otherwise, the mean values of reversal (track leg) would always average near-zero. The counterturning frequency was calculated from the average duration of interreversal track legs in seconds, and these were measured directly from the flight tracks (Vickers and Baker, 1996). A reversal was considered to have occurred when a change in sign of the track angle greater than 30° occurred and persisted for at least three vectors (1/10 s). Interreversal legs were considered to be those track segments occurring between the two consecutive reversals (Marsh *et al.*, 1978).

Statistical Analysis

Differences between behavioral categories for each treatment were compared using a χ^2 2 × 2 test of independence with Yates' correction (Sokal and Rohlf, 1969). Statistical analysis of the triangle of velocity data was performed using a two-way analysis of variance (ANOVA); means were compared using a least significant difference test (LSD) (Statistica, 1984).

RESULTS AND DISCUSSION

The addition of 0.3% Z11-16:OH to the binary pheromone blend of *H. zea* significantly reduced the percentage of males flying close to and locating the filter paper source (Fig. 1). Adding 1% Z11-16:OH caused significantly fewer males to fly even halfway to the source (Fig. 1) as well as fewer making it to the source. When the lowest percentage, 0.1% Z11-16:OH, was admixed; only 23% of the males reached the source. However, this success rate was not significantly lower than the levels of source contact evoked by the pheromone blend alone (47%).

Although 0.1% Z11-16:OH had insignificant effects on the percentage of males reaching the source, there were discernable differences in the flight tracks

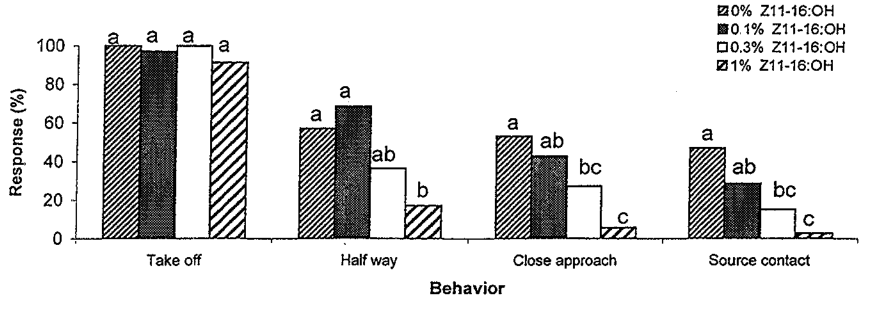

Fig. 1. Wind tunnel behavioral responses of male *H. zea* to a binary blend (10 μg Z11-16:Ald + 500 ng Z9-16:Ald) alone or containing either 0.1, 0.3 or 1% Z11-16:OH. Bars in the same behavioral category with no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P < 0.05$; N = 49, 35, 33, and 31 males for the 0, 0.1, 0.3, and 1% Z11-16:OH treatments, respectively).

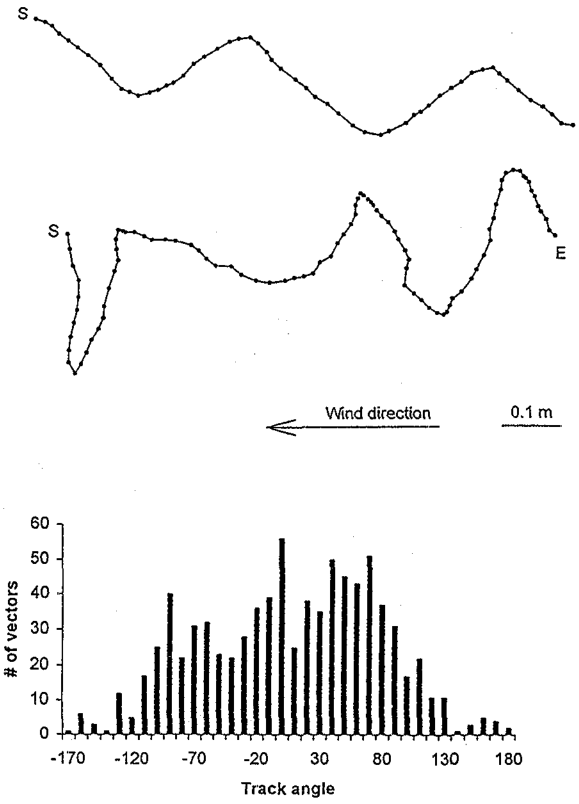

Fig. 2. Flight tracks of *H. zea* males in response to different treatments. All tracks start to the left of the figure and move upwind to the right. The interval between dots along a track is 1/30th s. In the absence of the antagonist, the tracks were more directly upwind (A) than the tracks in response to pheromone containing 0.1% Z11-16:OH (B). The frequency distributions of track angle vectors for male flights in response to each treatment are also shown.

of males flying in response to this blend (Fig. 2B) compared with those flying to the binary pheromone blend alone (Fig. 2A). The tracks appeared to have more side-to-side excursions, with more track legs appearing to be of the casting type (ca. 90° or more crosswind) than for those males flying to the binary pheromone component blend (Fig. 2A). This difference was apparent even when considering only the tracks of those males that reached the source. In response to the blend containing 0.3% Z11-16:OH there were too few males that flew entirely through the camera's field of view to analyze.

Analysis of the flight tracks showed that males flying in response to the binary pheromone component blend (Fig. 2A) steered more directly upwind than

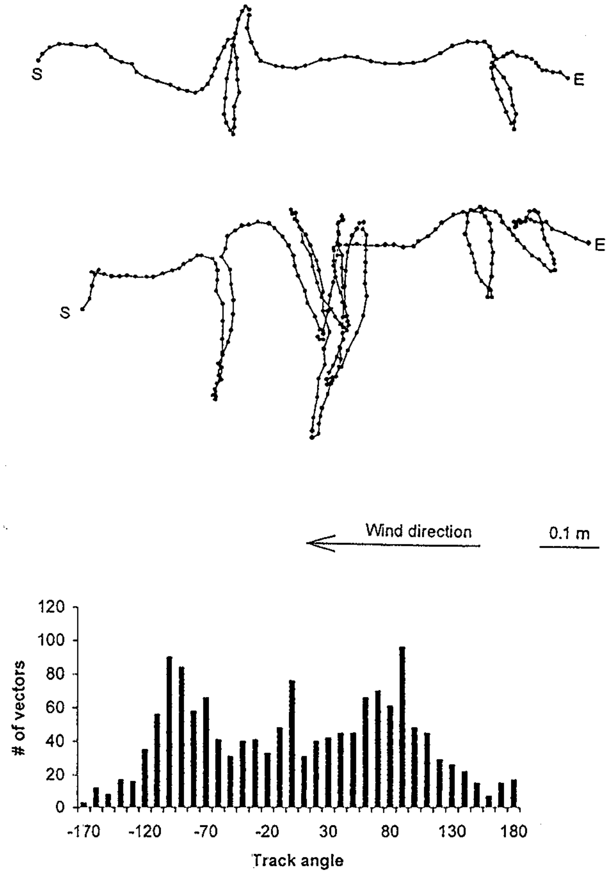

Fig. 2. Continued.

males flying in response to the blend containing 0.1% Z11-16:OH (Table I, Fig. 2B). The track angles of the latter males (Fig. 2B) appeared to exhibit slightly more of a bimodal distribution of angles at either plus or minus 90° than did the tracks of males flying in plumes of pheromone alone (Fig. 2A). This was substantiated in that the mean track angle in the response to 0.1% Z11-16:OH was significantly greater than to pheromone alone (Table I). A bimodal distribution of track angles is characteristic of tracks in which a lot of time is spent in casting flight rather than in upwind flight toward the source. Many of the tracks in response to the 0.1% Z11-16:OH treatment contained long sections of casting and other convoluted flight track sections.

The more oblique, off-windline track angle of males flying in response to the blend containing 0.1% Z11-16:OH (Table I) was caused by moths steering course angles that were significantly greater (off the windline) than the course angles of males flying in response to pheromone alone (Table I) while maintaining similar airspeeds. However, no significant reduction in groundspeed occurred, and no difference in counterturning frequency was observed in response to 0.1% Z11-16:OH. Males counterturned, on average, at a rate of 2.6 ± 0.07 turns/s (SD) when 0.1% alcohol was present in the blend and 2.6 ± 0.08/s in response to pheromone alone.

There was, therefore, an antagonistic effect of Z11-16:OH on the upwind flight of *H. zea* males when added to the blend of two pheromone components. Fewer males flew close to and located the source when more than 0.1% of Z11-16:OH was added to the blend. No agonism was observed, even at the lowest proportion of 0.1% Z11-16:OH tested, and even to this treatment the trend was toward antagonism of upwind flight behavior of *H. zea* males. These behavioral observations correspond well to the reductions in trap capture in the field reported by Teal *et al.* (1984) when as little as 1% Z11-16:OH was added to the *H. zea* synthetic pheromone blend.

The antagonism observed with the addition of Z11-16:OH also correlates well with the existence of a type of neuron on *H. zea* antennae that is tuned to both Z11-16:OH and the known antagonist, Z11-16:Ac (Cossé *et al.*, 1998). It should be noted that the emission rates of Z11-16:OH from the pheromone cartridges used in the neurophysiological recordings were consistently more than 10 times lower than the amounts issuing from cartridges loaded with corresponding amounts of Z11-16:Ald (Cossé *et al.*, 1998). Therefore, the 0.3% Z11-16:OH that caused a significant reduction in source contact in our study was likely even lower than the 0.3% that we loaded onto the filter paper.

Antagonism by Z11-16:Ac (Fadamiro and Baker, 1997), is known to begin occurring at a loading of 10% in combination with the binary pheromone blend, but in those experiments the filter paper was enclosed in a glass cartridge, where, again, the emitted amounts of Z11-16:Ac are known to be more than 10 times lower than for the same loadings of Z11-16:Ald (Cossé *et al.*, 1998). The emitted proportion of Z11-16:Ac at which antagonism was observed was therefore ca. 1% Z11-16:Ac. In addition, the plume was mechanically generated by a puffing device at a filament-generating frequency of from 1 to 5 Hz. The antagonism in response to the addition of Z11-16:Ac became significant only at the faster 5-Hz generation rate with 10% Z11-16:Ac added to the pheromone (Fadamiro and Baker, 1997). It is possible that more severe antagonism would have occurred with the addition of lower proportions of Z11-16:Ac had the plume been from a filter paper exposed to an open air stream. A greater antagonistic effect in *H. zea* males was seen with continuous compared with pulsed filaments of Z11-16:Ac admixed with pheromone generated from glass cartridges (Baker *et al.*, 1998).

The increase in course angles steered by males in response to 0.1% Z11-

Table I. Mean of Track Parameter ± SD of Male *H. zea* Responding to Two Treatments, Either Pheromone Alone or Pheromone Plus 0.1% Z11-16:OH

		Length (cm)	Airspeed (cm/s)	Ground speed (cm/s)	Track angle (deg)	Course angle (deg)	Drift angle (deg)	Counterturning interval (s)
Binary	Mean	1.81	85.27	59.81	57.25	32.91	24.34	0.38
($n = 10$)	±SD	0.38	12.09	12.83	16.17	11.07	7.23	0.08
Binary + 0.1% Z11-16:OH	Mean	1.75 (NS)	74.08 (NS)	57.13 (NS)	71.40*	42.22*	29.17 (NS)	0.39 (NS)
($n = 7$)	±SD	0.56	27.25	19.78	15.85	9.51	4.62	0.07

*For each parameter, means for the Z11-16:OH treatments are statistically different at $P < 0.05$.

16:OH is a response similar to what was found when changes were made in the pheromone blend quality of *Grapholita molesta* (Busck) (Willis and Baker, 1988). *G. molesta* males flew with slower airspeeds and steered more crosswind when the proportion of the secondary sex pheromone component in the blend exceeded an optimal one, thereby beginning to act antagonistically, which reduced location of the pheromone source (Willis and Baker, 1988).

The *H. zea* upwind flight response to the addition of an antagonist such as Z11-16:Ac (Quero *et al.*, unpublished) appears to be similar to that of *H. virescens* (Vickers and Baker, 1996). The single upwind surge by *H. zea* in response to a single filament appears to be shorter in duration when Z11-16:Ac is present, with both a reduced airspeed and a more oblique course angle contributing to the truncated surge (Quero *et al.*, unpublished). The reiterative performance of these truncated surges in a normal, filamentous plume would result in reduced upwind progress and a poorer ability to intersect and respond to oncoming filaments. Although analysis of single surges in response to blends containing the Z11-16:OH antagonist has not been performed, one would expect there to be a similar effect, based on the current analysis of male flights in filamentous plumes containing this antagonist.

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