Contextual Chemical Ecology: Male to Male Interactions Influence European Corn Borer[1] Male Behavioral Response to Female Sex Pheromone in a Flight Tunnel[2]

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ABSTRACT The responses of European corn borer, *Ostrina nubilalis* (Hübner), males in a flight tunnel to sex pheromone, [11-tetradecenyl acetate (97:3, Z:E)] was dependent upon the context in which the males were exposed to the stimulus. Males, held individually in isolation before being exposed to pheromone, flew upwind in the pheromone plume and landed on the pheromone source significantly more often than males caged with other males before exposure to the pheromone. When groups of males were simultaneously exposed to female sex pheromone, they responded, on a per-male basis, with significantly more upwind flights to pheromone and intense behavior near the pheromone source than did males exposed to the pheromone individually. Heightened intensity of male response in group flight was independent of whether the males were individually isolated or caged with other males before being exposed to the pheromone. The enhanced behavioral output of males responding to pheromone in groups may represent an evolutionary adaptive advantage in instances where several males are simultaneously pursuing a single calling female.

Key Words *Ostrinia nubilalis,* Lepidoptera, Pyralidae, 11-tetradecenyl acetate

Insect chemical ecology is a field of study that places emphasis upon chemical and behavioral characterization of compounds that influence insect behavior. Robertson et al. (1995) proposed that much could be learned from an ecological and evolutionary perspective, if contextual aspects of an insect's encounter with a chemical signal were studied. They called this approach contextual chemical ecology. The idea was to evaluate the behavioral responses of an insect to a specific chemical stimulus using different contextual conditions. Within this conceptual framework, we tested the response of the European corn borer males, *Ostrinia, nubilalis* (Hübner), to female sex pheromone.

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Adults of the European corn borer are known to aggregate and mate in headland vegetation of cornfields (Showers et al. 1974, Showers et al. 1976, Showers et al. 1980). Because moth density is high in this setting, it is likely that males interact and actively compete for males in the aggregations. We thought that varying male moth density might influence the way males respond to female sex pheromone. It was hypothesized that if adult European corn borer males were confined with other males before being exposed to sex pheromone in a flight tunnel or if they were exposed to the pheromone in groups, their response to the pheromone might be more competitive. If this effect could be observed in a flight tunnel, it would provide circumstantial evidence that such male interactions could occur in natural aggregations of European corn borer moths. Thus, the experiment involved studying the effects of varying the context in which males were exposed to a constant pheromone stimulus.

We report results that show European corn borer male competitive behavioral responses to an upwind pheromone source in a flight tunnel is influenced by male to male interactions. The effect provides insight into what may be an evolutionarily-adaptive competitive behavior that males might exhibit when several males simultaneously pursue a female in moth aggregations in the field. To our knowledge, no similar studies of European corn borer male-male mate seeking competition have been conducted heretofore. A search of the literature showed four examples of conspecific competition among male moths for mates. Males of the North American saltmarsh caterpillar moth, *Estigmene acrea* (Drury), and swift moths, *Hepialus* spp., form leks, display coremata to attract females, and compete for optimal sites within a lek (Willis and Birch 1982, Turner 1988, Birch and Hefetz 1987). Two other examples of conspecific male competition included the armyworm, *Pseudaletia unipuncta* (Haworth), and the tobacco budworm, *Heliothis virescens* (F.). Using a flight tunnel, Hirai et al. (1978) found that armyworm males compete by releasing a scent near calling females that causes inhibition of precopulatory behavior in other downwind males. The effect was confirmed later in field-caged experiments. In the case of the tobacco budworm, Hendricks and Shaver (1975) reported evidence of another form of chemical competition in which the male produced a pheromone that caused cessation of pheromonal calling by females near the male.

Materials and Methods

The European corn borer was reared on an agar-based diet (Reed et al. 1972) and pupae were isolated individually in 30-ml clear plastic creamer cups. The insects were of the *cis* pheromonal type (Klun and Huettel 1988) isolated from cornfields near Beltsville, MD. The culture was established in the laboratory in 1988 and was infused every other year with new genetic *cis*-female stock collected from the field. Upon emergence, adult males were divided into two groups of equal numbers. In one group, males were combined (15 to 36 males) in a cage measuring 20 cm^2. These were called group M males. In the second group, males were isolated and were designated group M_{is} males. Each M_{is} male was held at 5 cm × 5 cm (O.D) cylindrical wire-mesh cage contained in a water-sprayed clear plastic food-storage bag (16.5 cm × 9.9 cm). Separate incubators for both groups provided conditions near 24°C and 70% RH. The reverse photoperiod was 16:8 (L:D) h. Group M cages were sprayed with water daily, and individual cages were watered when no visible condensation existed inside the bag.

Conditions in the wind tunnel (Raina et al. 1986) ranged from 19°C to 24°C and from 40% to 72% RH. A laminar air flow was maintained at 50 cm/sec. Overhead illumination in the tunnel was provided by red incandescent lights regulated to 2.5 lux. The males were randomly selected and were exposed to a source of synthetic European corn borer female sex pheromone (Klun and Huettel 1988). The pheromone source was 149.6 µg 11-tetradecenyl acetate obtained from our chemical library and placed on a rubber septum in 10 µL heptane. The compound was chemically pure and had a geometric composition of 97:3 (Z:E) according to gas chromatographic analysis. Tests were conducted at 1 to 6 h into the scotophase, and males ranged in age from 3 to 5 d old. The distance in the tunnel between the male-release tube and the upwind pheromone was 220 cm. Each male was tested only once. Four treatments were as follows: M1 = single male retrieved from a group of caged males and exposed to pheromone, M4 = four males retrieved from a group of caged males and exposed to pheromone as a group, $M_{is}1$ = single isolated male exposed to pheromone, and $M_{is}4$ = four previously isolated males placed together and exposed to pheromone. Behavioral events were recorded for a maximum of 3 min after an individual moth or sets of four moths were released in the tunnel. The frequency of events in three behavioral categories was recorded: Partial UpW = upwind flight tracking in pheromone plume and losing track of plume at 15 to 30 cm from source of pheromone; Near = number of times a male displayed upwind flight tracking in pheromone plume followed by hovering within 3 cm of the pheromone source; Land = number of upwind flight trackings in the pheromone plume with landing on the pheromone source for a minimum of one sec. Moths that failed to leave the release tube or failed to fly upwind in the pheromone plume for at least one-fourth of the length of the tunnel were recorded as being nonresponders.

The study involved 767 male moths. The number of males tested against each treatment was 201 M1, 188 M4, 202 $M_{is}1$, and 176 $M_{is}4$. Tests were conducted using a complete randomized block design with replication over time. Data were analyzed statistically using the MIXED model procedure of SAS, version 6.11 (SAS Institute 1996). The fixed sources of variation were male containment (M versus M_{is}), flight test method (single moth versus four moths), and all possible interactions. The dependent variables were percentage males responding, the number of partial flights per responder, the number of near source occurrences per responder and the number of landings on the pheromone source per responder. The residuals from the analysis of variance were examined for possible outliers and to see if assumptions of normality and homogeneity of variances were adequately met. The latter three dependent variables required data transformations (Log_{10} [partial + 0.8]; Log_{10} [land + 0.5]; and $[near)^{1/2}$). Tests for statistical differences were made by using the *F*-test. The inverse transformations were applied to the transformed means, and the means and statistical inferences were presented in tabular form.

Results and Discussion

The percentage of moths responding positively to the pheromone was not significantly different between the M and M_{is} males and for single-male and grouped-male pheromone exposures (Table 1). Similarly, the frequency of partial upwind flights (UpW) made by males among the four treatments was not significantly different. The frequency of Near responses show that when groups of males were exposed to pheromone, they always responded, on a per-male basis, with a higher frequency than males exposed individually. This increase was independent of whether the males had been preconditioned in groups or as single males before the bioassay. We consider this heightened behavioral output in groups to be an expression of increased mate-seeking competitiveness by individuals in the group. Males flown in groups apparently detected the presence of competitors as they simultaneously flew toward the pheromone source and were stimulated to a level of competitiveness that was higher than the level expressed by males flown individually. Determination of how males sense one another while flying in groups will require further study. Preliminary observations indicate that the heightened frequency of Near behavior is due to general heightening of activity among all moths in a group, and it is not due to select "hot shot" males (Turner 1988) within the group. The observations in the Land category showed that males, preconditioned in isolation and flown individually or in groups, flew to and landed on the pheromone source significantly more often than males preconditioned in groups. We surmise that the isolated males performed better in the Land behavioral category because they may have been more physically fit having spent 3 to 5 days in solitude as compared to grouped males that were observed to repeatedly agitate one another in the group cage before exposure to pheromone.

Table 1. Behavioral responses of European corn borer males caged in isolation or in groups prior to being exposed to female sex pheromone individually or in groups of four with a wind tunnel.

			Mean events/responding male		
Treatments	n males	% Response	UpW	Near	Land
M1	201	68 a	1.12 a	3.20 b	1.34 c
M4	188	70 a	1.14 a	4.33 a	1.20 c
$M_{is}1$	202	77 a	1.09 a	3.02 b	1.68 b
$M_{is}4$	176	66 a	1.18 a	5.11 a	1.88 a

According to the *F*-Test, means followed by the same letter in the Near and Land columns are not significantly different at $P = 0.0001$ and at $P = 0.05$, respectively. n = number of males tested.

It is reasonable to hypothesize that the enhanced aggressiveness seen among males flying groups in the flight tunnel might also be expressed by European corn borer males in nature. Because of the tendency of the adults to aggregate, it is probably that many males could find themselves simultaneously pursuing an individual calling female and heightened competitive behavior would be an adaptive advantage in this setting. When many males are pursuing a limited number of females, it would make sense, from an evolutionary viewpoint, to be as competitive as possible. Testing of this hypothesis will require additional observations in the field.

The results of this study also provide evidence of the usefulness of the contextual chemical ecology approach to the study of behavior-inducing stimuli. As predicted by Robertson et al. (1995), the contextual approach provided us with a new perspective and additional information about the manner in which European corn borer behavior toward pheromone can be influenced by contextual conditions. The study revealed enhanced intraspecific male competitiveness among males flying in groups. Observation of this heightened behavioral output provides fundamental insight into what may be an evolutionary aspect of the European corn borer sex pheromone communication system.

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