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COMPARING THE EFFECTIVENESS OF SEXUAL COMMUNICATION DISRUPTION IN THE ORIENTAL FRUIT MOTH (*Grapholitha molesta*)[1] USING DIFFERENT COMBINATIONS AND DOSAGES OF ITS PHEROMONE BLEND[2]

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Abstract—The relative efficacy of disruptant blends comprised of different combinations of the Oriental fruit moth's pheromone components was determined in field tests. Disruption was evaluated by comparing male moth catch at synthetic and female-baited traps in disruptant and non-treatment areas. Three atmospheric dosages of a 8-dodecenyl acetate (93.5% *Z*:6.5% *E*) blend, representing two successive 10-fold decreases in concentration (2.5×10^{-2} g/hectare/day to 2.5×10^{-4} g/hectare/day) were tested alone and in combination with an additional percentage of (*Z*)-8-dodecen-1-ol. Male moth orientation to traps was eliminated in plots exposed to the two highest binary acetate dosages. However, significantly more males were captured in synthetic-baited traps in the lowest acetate-alone treatment, indicating a diminution of disruption efficiency. In contrast, inclusion of (*Z*)-8-dodecen-1-ol in the disruptant blend effected essentially complete disruption of orientation at all concentrations tested. Mating success of *G. molesta* pairs confined in small cages apparently was not affected by the presence of relatively high concentrations of the binary acetate and the acetate-alcohol blends. This suggests that habituation and/or adaptation of male response, at least for comparatively "close-range" behaviors, did not occur.

Key Words—Disruption of communication, Oriental fruit moth, *Grapholitha molesta*, Lepidoptera, Tortricidae, sex pheromone, sex attractant, (*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecen-1-ol.

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INTRODUCTION

Disruption of Oriental fruit moth, *Grapholitha molesta* (Busck), communication by atmospheric permeation with pheromone has been demonstrated in several field trials (Gentry et al., 1974, 1975; Rothschild, 1975, 1979; Cardé et al., 1977). These tests generally involved the emission of (*Z*)-8-dodecenyl acetate (*Z*8–12:Ac) plus a small percentage of its geometrical isomer (*E*)-8-dodecenyl acetate (*E*8–12:Ac) from different dispensing systems. However, these two acetates alone comprise only a portion of the Oriental fruit moth's natural pheromone blend, which has since been characterized (Cardé et al., 1979 and references therein) as a 4-component mixture: *Z*8–12:Ac and *E*8–12:Ac in a 100:7 ratio, (*Z*)-8-dodecen-1-ol (*Z*8–12:OH) and dodecanol (*n*-12:OH).

Additional studies (Baker and Cardé, 1979b) have demonstrated the behavioral effect that each component contributes to orientation and close-range behavior. The two acetates alone elicited upwind flight and other behaviors such as flight initiation. However, concomitant emission of a small percentage of *Z*8–12:OH resulted in an increase in these behaviors, and especially in those behaviors occurring "late" in the sequence, such as close-range orientation and hairpencil display. Only these three components substantially affect male behavior when emitted in a blend approximating that of a *G. molesta* female; dodecanol elicits significant increases in hairpencil display only when *Z*8–12:OH is emitted at suboptimal levels.

Given the behavioral differences elicited by these various combinations of compounds, we initiated field experiments designed to compare the efficacy of disruptant blends comprised of the two acetates vs. blends containing these acetates in combination with *Z*8–12:OH.

METHODS AND MATERIALS

Experimental Design. Field tests to determine the relative effectiveness of different formulations and concentrations of disruptants were conducted during September 9–26, 1979 in an experimental orchard near Fennville, Michigan. The experiment utilized a randomized complete block design with three replicates. All blocks were located in an orchard of standard, mature apple trees planted on a 12-m spacing. These had received fungicide spray only for the previous 10 years. Two of the blocks adjoined each other, whereas the third was separated from the nearest block by 200 m of semi-dwarf apple plantings and bordered a block of peaches.

Twenty-one 0.065 hectare replicate plots were established in the test area. Each plot consisted of a 5-tree array arranged as a central trap tree surrounded by 4 trees which formed a square with a 17-m separation between adjacent corner trees. Center trees of adjoining plots were separated by at least 40 m.

Disruptant Treatments. Seven treatments were evaluated. In addition to untreated controls, three atmospheric dosages, representing two successive decade decreases in concentration of the $Z8-12:Ac$ (6.5% E) blend were tested alone and in combination with an additional percentage of $Z8-12:OH$. The chemicals used in these tests when placed in the field were >99% pure as determined on OV-1 (100–120 mesh Gas-Chrom Q) and OV-275 (100–120 mesh Gas-Chrom R) GLC columns; the acetate mixture contained <0.2% of any 12-carbon chain-length alcohol. Chemicals were dispensed from 0.20-mm-ID hollow fibers supplied by the Controlled Release Division of Albany International Corp. These release dispensers offer long-term, zero-order release kinetics of compounds from numerous point sources.

To control precisely their number and arrangement, the fibers were stapled to 3-m wooden poles at 0.6 m intervals (from 1.2 to 3 m). Four of these poles were positioned equidistant (1.5–2.5 m) from each other in the canopy of each tree of appropriate replicate plots. This arrangement should have resulted in a relatively even atmospheric permeation of the plots.

At the highest concentration tested in $Z8-12:Ac$ (6.5% E) plots, 50 fibers were deployed per staple point; 5 fibers per staple point were used at the intermediate level. At the lowest dosage, 8 fibers were individually stapled at a height of ca. 2 m throughout the foliage of each tree in the plot. The acetate-alcohol treatments used an identical number and distribution of acetate fibers but either 5, 1, or 1 $Z8-12:OH$ fibers were stapled alongside the acetate fiber tapes at the highest, intermediate, and lowest concentrations, respectively. Laboratory determinations of meniscus recession indicated that the emission rate of the acetate mixture was 0.4×10^{-6} cm^3/day/fiber at 21° C. The $\bar{X}$ of the daily maximum temperature recorded during this experiment was 21° C; the maximum daily temperature occurs during midafternoon, corresponding closely to the time of sexual activity in *G. molesta*. A summary of the total number of fibers deployed and the estimated concentration of disruptant in different treatment areas is provided in Table 1.

Male attraction was assessed with Pherocon 1C sticky traps (Zoecon Corporation); three were deployed at a height of ca. 2 m in the canopy of the center tree in each replicate plot. One trap was baited with a 5 × 7-mm rubber septum (Arthur H. Thomas Co.) impregnated with 100 μg $Z8-12:Ac$ (5% E) plus 1 μg $Z8-12:OH$ and 300 μg $n-12:OH$. Another trap held a 2.5-cm-diam. × 3-cm-high cylindrical screen cage containing three 1 to 2-day-old virgin *G. molesta* females, while a third trap served as an unbaited control. Traps were monitored and females replaced every 1–3 days and sticky bottoms replaced as necessary to maintain trapping efficiency.

Efficacy of disruption was evaluated according to two different methods. The first involved calculation of percent disruption as the reduction in trap capture in plots exposed to disruptant treatments relative to trap catch in untreated plots. Trap data were analyzed with a 2-way ANOVA following

TABLE 1. DISRUPTION OF *Grapholitha molesta* ATTRACTION TO BAITED TRAPS USING DIFFERENT ATMOSPHERIC CONCENTRATIONS OF Z8-12:Ac (6.5% *E*) ALONE AND IN COMBINATION WITH Z8-12:OH DISPENSED FROM HOLLOW FIBER SOURCES.

	Trap bait[a]					
	Synthetic dispenser[b]			3 Virgin ♀♀		
Disruptant treatment	♂ trap catch[c]	Percent disruption[d]	Index of source location[e]	♂ trap catch[c]	Percent disruption[d]	Index of source location[e]
Z8-12:Ac (6.5% *E*)						
4000 fibers/0.065 ha (2.5×10^{-2} g/ha/day)	1a	100	3ab	0a	100	0a
400 fibers/0.065 ha (2.5×10^{-3} g/ha/day)	7a	98	18cd	0a	100	0a
40 fibers/0.065 ha (2.5×10^{-4} g/ha/day)	39b	90	57e	3a	98	12bcd
Z8-12:Ac (6.5% *E*) + Z8-12:OH						
4400 fibers/0.065 ha (2.5×10^{-2} g/ha/day)	1a	100	3ab	2a	99	3ab
480 fibers/0.065 ha (2.5×10^{-3} g/ha/day)	2a	99	6abc	1a	99	3ab
80 fibers/0.065 ha (2.5×10^{-4} g/ha/day)	18a	95	24d	8a	94	6abc
Check (no disruptant)	398c	—	88f	139c	—	57e

[a] Catch in unbaited traps is not included due to the negligible (<1%) male capture.

[b] Septa loaded with 100 μg Z8-12:Ac (5% *E*), 1 μg Z8-12:OH, and 300 μg *n*-12:OH.

[c] Values represented by the same letter are not significantly different ($P < 0.05$) according to Student-Newman-Keul's test of means transformed to $\sqrt{X + 0.5}$.

[d] Percent disruption for a specific treatment was calculated as:

$$\frac{\text{(catch in untreated areas)} - \text{(catch in disruptant area)}}{\text{catch in untreated area}} \times 100\%$$

[e] Index of source location = percentage of traps catching ≥ 1 male per trapping interval. Percentages in same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence ($P < 0.05$).

transformation to $\sqrt{X + 0.5}$. Treatment mean differences based on daily trap catch were tested for significance using the Student-Newman-Keul's multiple-range test ($P < 0.05$).

The alternative method entailed scoring traps either as empty ($X = 0$) or containing males ($X = 1$) for each trap-monitoring interval. These values were then summed over the entire test period, and results from replicates of a particular treatment were combined. The ratio of the resultant value to the total number of observations constituted a measure of the proportion of successful male orientations over time to a specific treatment (Rothschild, 1981). We suggest the term index of source location for this coefficient.

Confined Mating of Moths. To compare the effect of different disruptant blends on the mating propensity of moths confined in small cages, moths were exposed to one of three treatments: the acetate only and the acetate–alcohol blend, both at their highest atmospheric concentration (2.5×10^{-2} g/hectare/day), and a disruptant-free control. This test was conducted from September 8 to 12 using the same experimental plots as in the previous investigation.

One-day-old virgin *G. molesta* adults came from a laboratory colony maintained on a 16:8 light–dark photoperiod regime at 25°C. Males were confined, 15 per replicate, with access to water in 15-cm-diam. × 30-cm-long cylindrical screen cages. Six replicates ($N = 90$ moths), were positioned ca. 2 m high in the shaded canopy of center trees in the experimental plots. After 24 hr, 15 females were added to each cage. Following an additional 24 hr, the females were individually removed to small plastic cups and returned to the laboratory to ascertain the proportion producing fertile ova.

RESULTS AND DISCUSSION

Disruption of Orientation to Baited Traps. Evaluation of the efficacy of disruption to pheromone-baited or female-baited traps is usually measured by comparison of the total catch in the treated and comparable check areas. However, the percent reduction in catch may not reflect the probability of a female being located by an individual male over a given observation period. For example, the percent reduction in trap catch may exceed 90%, but all sources (synthetic or female) may have been located by at least one male. Thus, the index of source location may be more indicative of the probability of mating than the percent reduction in trap catch. However, the index of source location measure will be more influenced by the sampling interval and the density of the population than the percent reduction in trap catch.

Male trap catch in each treatment plot is summarized in Table 1. In plots exposed to the two highest concentrations of the binary acetate blend, there was a virtually complete reduction of male moth orientation to traps, as measured by both percent disruption and the index of source location. However, significantly more males were caught in synthetic-baited traps located in plots treated with the lowest acetate dosage, indicating that at this level, disruption was less efficacious than at the two higher dosages. Elevation of successful orientation to traps at the lowest dosage (8 fibers/tree) of binary acetate disruptant may have been due in part to the existence of layers or windows of disruptant-free air, resulting from a less uniform dispersal of disruptant at this concentration. Alternatively, the critical concentration of pheromone may have been below the level necessary to disrupt attraction. These results parallel those obtained in a field test conducted during 1977 (Baker and Cardé, unpublished) using a similar experimental setup; emission of $Z8-12$:Ac (7% E) at disruptant levels comparable to those reported here resulted in 100%, 99%, and 87% disruption of male orientation to synthetic-baited traps at the highest, intermediate, and lowest dosages, respectively.

In the acetate–alcohol treatment areas, male orientation to baited traps again was essentially eliminated at the two highest concentrations. However, in contrast to results obtained with the acetates alone, inclusion of $Z8-12$:OH in the disruptant blend resulted in a significant increase in disruption at the lowest dosage. The suppression of male captures could be attributable to a $Z8-12$:OH-mediated modulation of pheromone response, either resulting in a diminution of behaviors in the orientation sequence, particularly those associated with comparatively "late" orientation and precopulatory behaviors, or causing these behaviors to be displaced temporally or spatially from their position in a normal sequence.

Confined Mating of Moths. Mating success of caged pairs of males and females apparently was not affected by the presence of pheromone components. Females mated with a mean frequency of 50% in the acetate-only plot and 56% in the acetate–alcohol plot compared to 56% in the check area (not significant at the 5% level by χ^2).

Results of similar field tests with *Argyrotaenia velutinana* (Walker) (Cardé et al., 1975) and *G. molesta* (Cardé et al., 1977) also indicated no measurable reduction of mating under confined conditions. However, in both of these tests, the disruptant blend emitted did not include the pheromone components which are most important in eliciting comparatively late or close-range behaviors. Notwithstanding, our results indicated that inclusion of $Z8-12$:OH in the disruptant mixture did not measurably interfere with male mating initiative. In fact, on several occasions, males were observed to hairpencil toward and copulate with females shortly after they were introduced into cages exposed to the alcohol-containing blend. Thus, although preexposure to the pheromone may have altered the male response threshold, elimination of precopulatory behaviors clearly did not occur.

Several factors may have contributed to the failure of the disruptant to suppress mating. The relatively high density of moths (ca. 3.3 moth pairs/m^2 of surface area) in these cages presumably enhanced the likelihood of random intersexual encounters. Behavioral observations on the Indian meal moth, *Plodia interpunctella* (Hübner) (Sower et al., 1975) under conditions of high moth density revealed that males habituated by exposure to pheromone would nonetheless copulate with females encountered during random movement. The close proximity of individuals also may have accorded more importance to other mating stimuli such as the tactile and visual components of precopulatory behavior known to be of importance in this species (Baker and Cardé, 1979a). Additionally, over these relatively short distances, the concentration of the female-produced pheromone plume may have been sufficiently above the background concentration of disruptant, thereby allowing successful male orientation.

CONCLUSION

Our results indicate that even very low levels of the binary acetate disruptant blend, on the order of 2×10^{-3} g/hectare/day (or a mere 200 mg/hectare over an entire season), can effect virtually complete disruption of long-range orientation in this species. Moreover, the addition of a third component, *Z*8–12:OH to the disruptant mixture potentially will achieve the same level of disruption with a reduction in the overall amount of pheromone applied. Inclusion of the *Z*8–12:OH in the blend may result in an alteration of the pheromone response, causing a truncation or displacement of certain behaviors, especially close-range ones, in the orientation sequence. Use of the complete pheromone blend may also confer some protection against evolution of resistance to pheromone management; blends composed of only a portion of the natural pheromone could allow selection for resistance by accentuating the contribution of the missing component (Cardé, 1976).

Notwithstanding, it is plausible that the *Z*8–12:Ac and *E*8–12:Ac combination could achieve effective disruption of long-range communication without causing a large proportion of males to land and engage in close-range or late behaviors, as could occur with the three-component blend. This phenomenon would be most apt to take place if the atmospheric concentration of the acetate–alcohol blend was above threshold for these behaviors (Cardé, 1981). Thus, both the two-acetate and the two-acetate plus alcohol systems should be evaluated further in a large-scale operational test where actual mating and reduction of damage are measured, although the present test suggests that the three-component blend would be the most efficacious disruptant system.

When pairs were confined under conditions that simulated very high population densities, the frequency of mating apparently was not affected by preexposure of males to the pheromone, although chance intersexual encounters may have influenced the results. The suppression of the male's ability to locate baited traps from a distance indicates that, in this species, elimination of the mating response should not be a prerequisite to successful disruption of sexual communication. Together these findings suggest that the mechanism whereby atmospheric permeation with pheromone effects mating disruption may be by camouflaging the natural aerial pheromone plumes (Cardé, 1981) rather than by CNS habituation of pheromone response or adaptation of peripheral pheromone perception.

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