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# BEHAVIORAL RESPONSES OF MALE *Argyrotaenia velutinana* (LEPIDOPTERA: TORTRICIDAE) TO COMPONENTS OF ITS SEX PHEROMONE

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**Abstract**—Males of the redbanded leafroller, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), were studied for their behavioral responses in laboratory olfactometers and in the field to the 3 components of the female-produced sex pheromone: *cis*-11-tetradecenyl acetate (c11-14:Ac), *trans*-11-tetradecenyl acetate (t11-14:Ac), and dodecyl acetate (12:Ac). Dodecyl acetate, when evaporated with c11-14:Ac (8% *trans*) in the field, modified the behavior of feral males nearby the chemical source, causing an increase in the frequency of landing and close approach to the pheromone dispenser. Apparently, an in-flight behavioral modification concerning landing or not landing occurs within 60 cm of the source and is mediated by 12:Ac. In laboratory olfactometers, c11-14:Ac (8% *trans*) demonstrated a lower threshold for male activation than pure c11- and t11-14:Ac and blends of the two isomers. Additionally, over a wide range of dosages, males responded with optimum wing-fanning response to c11-14:Ac (8% *trans*) compared to pure c11-14:Ac, c11-14:Ac (30% *trans*), and pure t11-14:Ac, suggesting that the *cis*:*trans* ratio rather than absolute amounts of either isomer, is a crucial factor in eliciting male response. When presented with c11-14:Ac (8% *trans*) (1:1), dodecyl acetate caused a significant prolongation of wing-fanning over c11-14:Ac (8% *trans*) alone and resulted in a greater percentage of males moving upwind to the source. Since the increase in wing-fanning and orientation occurred at higher concentrations of the 3-component mixture, the effect of 12:Ac in the laboratory may reflect the close-range role of 12:Ac in the field.

**Key Words**—*Argyrotaenia velutinana*, redbanded leafroller, sex pheromone, attractant, *cis*-11-tetradecenyl acetate, *trans*-11-tetradecenyl acetate, dodecyl acetate, male behavior, synergist, inhibitor.

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## INTRODUCTION

The redbanded leafroller moth, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), uses a female-produced sex pheromone comprised of 3 active components (Roelofs et al. 1975). The primary sex pheromone component of *A. velutinana* was identified as *cis*-11-tetradecenyl acetate (c11-14:Ac) by Roelofs and Arn (1968). Two other pheromone components, *trans*-11-tetradecenyl acetate (t11-14:Ac) and dodecyl acetate (12:Ac), were shown to be instrumental in increasing the trap catch of male *A. velutinana* in the field. The former was shown to be necessary for attractancy when present in low ratios to the *cis* isomer, and gave optimum attractancy when mixed in a *trans*:*cis* ratio of approximately 7:93 (Klun et al. 1973, Roelofs et al. 1975). At ratios higher than 7:93, trap catch of males was reduced. In field screening tests, dodecyl acetate was found to increase trap catch of *A. velutinana* males when evaporated with the other two components (Roelofs and Comeau 1968, 1971). Further tests showed that optimum attractancy was obtained with the addition of 12:Ac at ratios greater than 3:2 to the *trans*:*cis* (8:92) blend (Roelofs et al. 1975).

Recently, both t11-14:Ac and 12:Ac were isolated and identified from female *A. velutinana* abdominal tip extract and calling female effluvia (Roelofs et al. 1975). The former was found to be present in a ratio of about 9:91 to the *cis* isomer, whereas in airborne collection of calling female effluvia, 12:Ac was found to be emitted at a 5:4 ratio of 12:Ac to △11-14:Ac.

The chemical requirements for optimum attractancy of males in the field had thus been defined, and the chemicals identified from female tip extract and effluvia, but the role of the 3 pheromone components in eliciting the appropriate behavioral responses culminating in trap catch—or, in the case of live females, copulation—remained undefined. In this paper, we describe some of the differences in male behavioral responses that can account for the dramatic increase in trap catch when all 3 components are present in their correct ratios.

## METHODS AND MATERIALS

### *General*

Adult redbanded leafrollers were reared on a pinto bean diet modified from Shorey and Hale (1965). Wax-coated drinking cups (5.2 cm diameter bottom, 12.5 cm long), containing pinto bean diet and covered with plastic snap-on lids, served as containers for developing larvae. Approximately 75 larvae were reared in each cup until pupation, when pupae were removed and sexed. Male pupae were placed in a screened cage (38 × 38 × 47 cm)

containing a 5% sucrose solution on cotton in a petri dish and held in a walk-in growth chamber. All stages of larval growth and the holding of adults occurred at 24°C on a 16 h:8 h light:dark photoperiod regime, with photophase at 1,400 lx.

Eggs for each generation of diet-reared larvae were produced by adults from a greenhouse colony maintained on fava bean plants (Glass and Hervey 1962). Rearing on diet in growth chambers allowed strict control over photoperiod and temperature and the development of large numbers of adults in a small space.

Solutions of synthetic pheromone components (various ratios of c11- and t11-14:Ac) were prepared by making dilutions in 2 ml Skellysolve B in 10-fold increments, starting with neat material. Ratios of c11- and t11-14:Ac (Farchan Corp.) were analyzed by gas-liquid chromatography (GLC). The following mixtures of the 2 isomers were used: thin-layer chromatography (TLC)–pure c11-14:Ac, 3% t11-14:Ac, 8% t11-14:Ac, 15% t11-14:Ac, 30% t11-14:Ac, 50% t11-14:Ac, 80% t11-14:Ac, and TLC-pure t11-14:Ac. Dodecyl acetate (Eastman Kodak) was purified by preparative GLC. All solutions were stored at −10°C in 1-dr screw-cap vials with Teflon-lined lids and were used within 18 m. Laboratory bioassay treatments were prepared by dispensing 20 μl solution onto a filter-paper tab for presentation to males. For field experiments, the neat pheromone components were loaded directly into polyethylene caps (Glass et al. 1970).

### *Laboratory Observations of Behavior*

*Laboratory Courtship Behavior.* Cardé et al. (1975*c*) demonstrated in *A. velutinana* that at 24°C, male responsiveness to female abdominal tip extract occurs only during scotophase. However, responsiveness can be advanced as much as 6 h prior to onset of darkness by dropping the ambient temperature from 24° to 16°C 15 min before assay. This feature of the *A. velutinana* chemical communication system was exploited in all bioassays so that the viewing of male behavior could be accomplished during photophase at 1,400 lx.

About 2 h before light-off, 1 virgin female and 1 unmated male were placed in each of a number of clear plastic boxes (12.4 × 9.0 × 7.0 cm). The box to be observed was transferred to a 16°C room on an identical photoperiod regime. Once the female commenced "calling", usually within a matter of minutes (Cardé et al. 1975*c*), the male's behavior was observed and recorded on a portable casette recorder and later transcribed. A second male was sometimes added to a box with a calling female and nonresponding male. Twenty courtship sequences ending with copulation were observed.

*Box Olfactometers.* Approximately 1 h before bioassay, males were

drawn from their screened holding cage in the 24°C chamber and placed in clear plastic olfactometer boxes (12.4 × 9.0 × 7.0 cm) (Roelofs and Feng 1967), 10 males/box. Care was taken to sample all areas of the cage equally, so that a uniform distribution of floor-dwelling and ceiling-active males was obtained for each box. One hour later, during the last 2 h of photophase, boxes were transferred to an adjoining chamber of equal light intensity, but with a temperature of 16°C. The moths were allowed to chill for 15–45 min, and during this time, chemical treatments were prepared outside the chamber.

Preparation of chemical treatments involved cutting 2-cm$^2$ filter-paper tabs with crescent-shaped handles (the tabs resembled mushrooms) from Whatman No. 1 filter paper (9.0 cm diam) and impregnating them with 20 μl test solution. The solvent was allowed to evaporate before the tabs with their nonimpregnated handles were placed in numbered glass shell vials stoppered with corks. A randomized complete block design was employed. Treatments were tested double-blind.

Wing-fanning was the "key" response recorded, except in the two earliest series, in which activation (either rapid walking, flight, or wing-fanning) was the response scored. The males in each box were assessed for background activity for 60 sec immediately before presentation of the treatment, and males exhibiting a prestimulus key response were eliminated from further consideration. Each tab was inserted through a slit in the side 2 cm long and 1 cm from the floor of each box, and the number of males exhibiting wing-fanning response during the next 60 sec was recorded. The nontreated filter-paper "handle" attached to each tab remained outside the box. Percentage response was calculated by the formula:

$$\frac{\text{stimulus response} - \text{background response}}{10 - \text{background response}} \times 100 = \text{percentage response}$$

At the end of each block, males were returned to the 24°C room and released into the emergence cage before lights-off. Boxes were soaked in strong detergent solution overnight, rinsed thoroughly, and allowed to air-dry after each assay.

*Orientation Tube Olfactometers.* Orientation tube olfactometers, glass tubes 2 cm in diameter and 99 cm long including a ground-glass joint on one end, were used to monitor orientation toward the chemical source (Sower et al. 1973) as well as percentage response of *A. velutinana* males. Each tube was fitted internally with a screen barrier 8 cm from the upwind end. The downwind end was plugged with a cheese cloth–covered plastic ring that permitted air to flow out. Laboratory air from an outside source was filtered through charcoal. Twelve plastic tubes linked to a spherical glass manifold were connected to glass connecting tubes (105°) that delivered air at 0.36 m/sec to the orientation tubes. The third arm of each connecting tube was

used for the introduction of the chemical treatments and was closed with a ground-glass stopper. The apparatus for delivery of chemically impregnated filter paper into the airstream was as follows: A piece of copper wire 7.5 cm long with a loop clip at one end was inserted into the end of a cork stopper, and a filter paper disc (Whatman, 2.4 cm diameter), folded in half, was lodged in the loop. After being impregnated with chemical, the filter paper was stored in a glass shell vial with the cork end forming a stopper until time of assay.

One hour or more before assay time, males were drawn from their emergence cage and distributed (10 males/tube) in glass orientation tubes, with care taken to sample uniformly among all areas of the emergence cage. Males were held in these tubes at 24°C until approximately 2 h before lights-off, and then transferred by block in staggered fashion to the 16°C room, where 15 min elapsed before commencement of the assay. Males were allowed to acclimate to the air flow for at least 5 min. A randomized complete block design was employed, and treatments were tested double-blind. Background activity of the key response, wing-fanning, was monitored for 60 sec; then, after introduction of a treatment, wing-fanning was recorded at 10, 30, and 60 sec. The most active treatments also commonly evoked higher-level responses, such as clasper extension and copulatory attempts with other males. Upwind orientation was scored by assessment of the number of males occupying the upwind 10 cm of the tube before assay, and then after 30 and 60 sec of treatment exposure. In calculating percentage orientation, pre-stimulus upwind males were not scored.

After assays, males were returned to the emergence cage, and all tubes were washed with a strong detergent solution and rinsed with hot water followed by redistilled acetone.

### *Field Observations of Behavior*

*Sticky Tables and Pherocon® 1C Traps.* Sheet-metal tables of 60 cm *r* were coated with Stikem Special®. In the center of each table was placed a polyethylene dispenser (Glass et al. 1970) containing either 10 mg c11-14:Ac (8% *trans*) or 10 mg c11-14:Ac (8% *trans*) plus 15 mg 12:Ac. The tables were stationed 1 m off the ground in an abandoned orchard in Lakemont, New York, from July 23 to July 26, 1974. Captured males' distances from the lure were measured, the males removed, and the tables rerandomized daily.

Pherocon® 1C (Zoecon Corp., Palo Alto, California) insect traps using the same 2 treatments were deployed at Lakemont, New York, July 5–15, 1974. Males were removed and the traps rerandomized every 2 days.

*Nonsticky Tables and Nonsticky Pherocon Traps.* Circular sheet-metal tables of 60 cm *r* with concentric circles engraved every 10 cm from the center

were employed to observe behavior of feral males close to 2 pheromone sources in the field: (1) 10 mg c11-14:Ac (8% *trans*), and (2) 10 mg c11-14:Ac (8% *trans*) plus 15 mg 12:Ac. Both treatments were loaded in polyethylene caps. The tables were located 1 m off the ground in the grassy aisles between rows of apple trees in an experimental orchard in Geneva, New York, during May 5–15, 1975. This period coincided with the spring flight of *A. velutinana*, and males responded to the pheromone during the afternoon (Comeau et al. 1976), thus making detailed observations of these small moths possible. Observers, one for each treatment, were stationed about 3 m downwind and slightly crosswind of the pheromone source. Portable cassette recorders were used to record observations, which later were transcribed.

Pherocon 1C insect traps were assembled using a nonsticky top as the floor instead of the normal sticky floor. The 2 pheromone treatments used above were again employed. Observations took place from May 5 to May 15, 1975, in an orchard in Sodus, New York, abandoned for 10 yr. Traps were hung on the outer branches of apple trees at a height of about 1.5 m.

Trap locations were changed about every 10 min for tables and every 5 min for Pherocon traps to minimize the possibility of multiple observations of a single individual.

## RESULTS

### *Laboratory Courtship Behavior*

For a quiescent male (antennae pointing posteriorly and parallel to the substrate), a sequence of behavior in response to a calling female involved the following steps:

(1) Antennae were elevated, perpendicular to the substrate,
(2) Male preened antennae, drawing first one foretarsus, then the other, along the full length, sometimes bringing it into contact with the mouthparts and face in between wiping an antenna.
(3) Male walked rapidly or flew a short distance toward the female.
(4) Male fanned its wings while walking rapidly toward the female, approaching either posteriorly or laterally.
(5) Male touched the costal tip of the female's slightly raised forewing with its antennae and head, while facing at a 45° angle from behind and continuing to fan its wings (sometimes a male's head would become concealed beneath the wing).
(6) Male's abdomen, claspers extended, curled toward the female's abdomen and probed until successful in grasping the tip.

(7) Male's wings stopped fanning and were folded back once more to a resting position, but beneath the female's wings—the two moths now faced in opposite directions.

Steps 5–7 usually occurred within 1 or 2 sec. The durations of the earlier steps were quite variable.

Some steps in this sequence of behavior either were omitted sometimes or perhaps occurred so quickly that they could not always be observed. For example, some males commenced wing-fanning without first either flying or walking rapidly. Some responses, however, occurred in all 20 successful courtships, these being steps 1, 4, and 5–7. No contact between any part of the male's body and the female's antennae was observed; the male's abdomen and genitalia always curled toward the tip of the female's abdomen during copulatory attempts.

*Box Olfactometer Experiments*

TLC-pure c11-14:Ac alone at $2 \times 10^{-3}$ $\mu$g elicited 27% activation, but the 3% *trans* treatment at this dosage raised the response significantly, to 49% (Fig. 1). Furthermore, 8% *trans* elevated the response to a significantly higher level of 76%. Raising the percentage of *trans* to 15% caused a significant decrease in the response to 37%, while treatments with higher ratios of *trans*:*cis* continued to elicit even lower responses. The addition of $2 \times 10^{-3}$ $\mu$g 12:Ac to each of the *cis*:*trans* ratios resulted in a significant increase of percentage response for the TLC-pure c11-14:Ac and 15% *trans* treatments only ($\chi^2$ $2 \times 2$ test of independence, $P < 0.05$). It can be seen, however, that

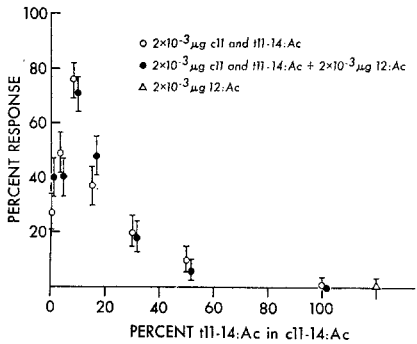


FIG. 1. Percentage activation response of *A. velutinana* males to mixtures of c11- and t11-14:Ac, both with and without the addition of 12:Ac. Brackets above and below the means denote the 95% binomial confidence limits ($n$ = 160).

8% *trans* both with and without addition of 12:Ac gave a percentage activation response significantly higher than any of the other 13 treatments.

Since the decline in response with increasing percentages of *trans* could have been due to the presence of either more *trans* or less *cis*, a second series of treatments was assayed. The amount of *cis* presented remained absolute at $2 \times 10^{-3}$ μg, and *trans* was added to *cis* to make 30% *trans* and 50% *trans*, 2 treatments in which the large amounts of *trans* would have had the greatest effects. There was no statistical difference between responses to 30% and 50% *trans* treatments prepared either way using the $\chi^2$ $2 \times 2$ test of independence ($P > 0.05$).

In order to observe responses to various ratios of *cis* and *trans* over a large range of concentrations, a dosage series of TLC-pure *cis*, pure *trans*, 8% *trans*, and 30% *trans* was tested in box olfactometers. The key response recorded was wing-fanning.

One unexpected result was the occurrence of wing-fanning response when males were exposed to $10^1$ μg and $10^2$ μg pure t11-14:Ac (Fig. 2). At lower concentrations, pure *trans* had elicited almost no response, but at these higher concentrations, the male response appeared to be similar to that elicited by the pure *cis* or 8% *trans* treatments in terms of wing-fanning persistence or movement toward the filter paper. Additionally, over 4 orders of magnitude of dosage ($10^{-2}$–$10^1$ μg), pure *cis*, pure *trans*, and 8% *trans* elicited levels of wing-fanning response significantly different from each other, using the binomial confidence interval (C.I.) at 95%, and the $\chi^2$ $2 \times 2$ test of independence for $10^{-2}$ μg (Fig. 2).

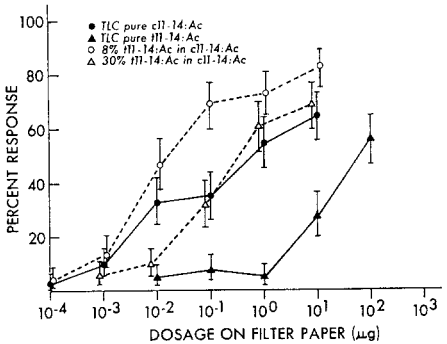


FIG. 2. Percentage wing-fanning response by *A. velutinana* males in box olfactometers to various concentrations of c11- and t11-14: Ac alone and in mixtures. Brackets above and below the means denote the 95% binomial confidence limits ($n$ = 130).

The data in Fig. 2 also reveal that in box olfactometers, about 100 times more pure *cis* and 10,000 times more pure *trans* than 8% *trans* was required to elicit wing-fanning response in 50% of the males. Thus, the threshold for 50% wing-fanning appears to be about 100 times lower for 8% *trans* than for pure *cis*, and about 10,000 times lower than pure *trans*.

*Orientation Tube Olfactometer Experiments*

Differences in the amount of orientation to the pheromone source could not be easily elucidated in box olfactometers, so experiments in long glass tubes with a directional airflow were conducted (Sower et al. 1973).

Testing was initiated with a dosage level for each treatment that would elicit about 50% wing-fanning response. The dosage-response box olfactometer series showed that $10^2$ μg pure t11-14:Ac, 1 μg TLC-pure c11-14:Ac, and $10^{-2}$ μg c11-14:Ac (8% *trans*) each elicited approximately 50% wing-fanning response, and these 3 treatments were used both with and without an equivalent amount of dodecyl acetate (Fig. 3).

It is apparent that the addition of $10^2$ μg 12:Ac to $10^2$ μg pure *trans* did not alter significantly the percentage of either wing-fanning or orientation

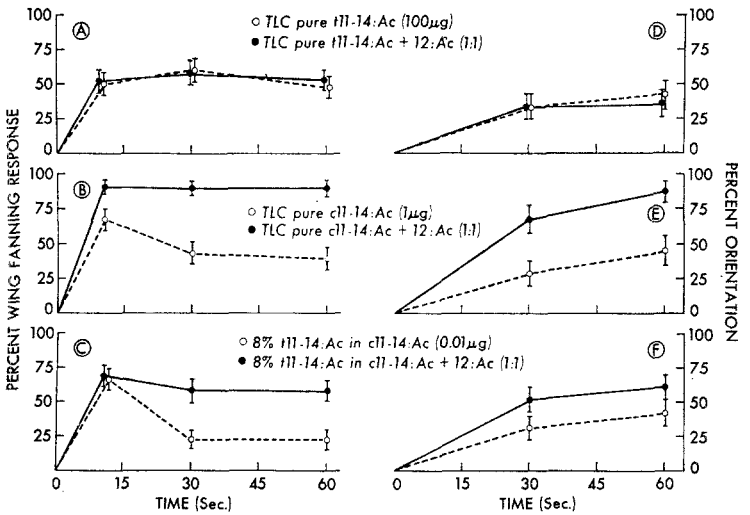


FIG. 3. Percentage wing-fanning response and orientation by *A. velutinana* males in glass tube olfactometers to mixtures of c11- and t11-14:Ac and 12:Ac at dose levels eliciting 50% response in box olfactometers. Brackets above and below the means denote the 95% binomial confidence limits ($n$ = 160).

during the 60-sec period (Figs. 3A and D). However, 1 $\mu$g 12:Ac evaporated with 1 $\mu$g pure *cis* increased the percentage wing-fanning response during the initial 10 sec and caused it to persist at a significantly higher level throughout the entire 60 sec (Fig. 3B). Dodecyl acetate also increased the duration of wing-fanning in response to $10^{-2}$ $\mu$g 8% *trans*, but did not elicit a higher response during the first 10 sec (Fig. 3C).

For all 3 treatments not containing 12:Ac, the percentage of males orienting to the upwind 10 cm of the tube was approximately 45% after 60 sec. The addition of 12:Ac to pure *trans* produced no increase in percentage orientation (Fig. 3D). However, adding 1 $\mu$g 12:Ac to 1 $\mu$g pure *cis* (Fig. 3E) resulted in a significant increase in orientation to nearly 90%, after 60 sec. The effect of 12:Ac on the 8% *trans* treatment was not as dramatic, yet 12:Ac elicited a significant increase in orientation after both 30 and 60 sec (95% C.I.).

In order to test the effect of 12:Ac at lower dosages, treatments of $10^{-1}$ $\mu$g pure *trans*, $10^{-3}$ $\mu$g pure *cis*, and $10^{-3}$ $\mu$g 8% *trans* were chosen from the box bioassay dosage-response experiment to approximate a 10% wing-fanning response level. An equivalent amount of 12:Ac was added to each treatment. No significant increase in wing-fanning response was noted with the addition

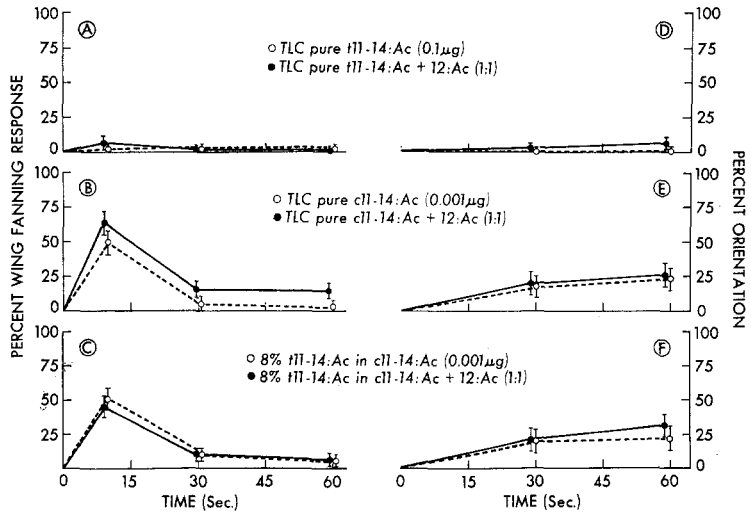


FIG. 4. Percentage wing-fanning response and orientation by *A. velutinana* males in glass tube olfactometers to mixtures of c11- and t11-14:Ac and 12:Ac at dose levels eliciting 10% response in box olfactometers. Brackets above and below the means denote the 95% binomial confidence limits ($n$ = 160).

TABLE 1. PERCENTAGE WING-FANNING RESPONSE AND ORIENTATION BY *A. velutinana* MALES TO 12:Ac

| | Percentage wing-fanning response[a] | | | Percentage orientation[b] | | |
|---|---|---|---|---|---|---|
| Dosage 12:Ac | 10 sec | 30 sec | 60 sec | 0 sec | 30 sec | 60 sec |
| $10^{-3}$ μg | 1.7* | 0.8* | 0.8* | 0 | 4.9* | 4.9* |
| $10^{0}$ μg | 1.7* | 2.5* | 0* | 0 | 3.4* | 4.5* |
| $10^{3}$ μg | 19.2† | 20.8† | 12.5† | 0 | 9.4* | 16.5† |
| Control[c] | 85.0‡ | 10.0‡ | 11.3† | 0 | 13.4* | 30.3† |

[a] Percentages in same column with different superscript symbols are significantly different using 95% binomial confidence limits ($n = 120$).
[b] Percentages in same column with different superscript symbols are significantly different using a $\chi^2$ $2 \times 2$ test of independence with Yates' correction ($P < 0.01$).
[c] c11-14:Ac (8% *trans*), $10^{-2}$ μg.

of 12:Ac (Fig. 4), except in the case of pure *cis* (Fig. 4B), where $10^{-3}$ μg pure *cis* plus $10^{-3}$ μg 12:Ac elicited significantly higher levels of wing-fanning at 30 and 60 sec than $10^{-3}$ μg pure *cis* alone. No differences in percentage orientation occurred at this dosage level.

Dodecyl acetate was then tested by itself at 3 different dosages—$10^{-3}$ μg, 1 μg and $10^{3}$ μg—to examine its possible effects, since it seemed to enhance the response to the treatments mentioned above. Surprisingly, at $10^{3}$ μg, the highest level tested, 12:Ac elicited 19%, 21%, and 13% wing-fanning response at 10, 30 and 60 sec (Table 1). However, percentage response to the 2 lower concentrations was near 0. Hence, at the levels used in previous bioassays, 12:Ac alone was at subthreshold levels for wing-fanning response. Its enhancement of response to pure *cis* and 8% *trans* could not have resulted from any separate elicitation of wing-fanning, but instead was due to some other effect that occurred in combination with the other sex pheromone components.

### *Field Observations of Behavior*

*Sticky Tables and Pherocon Traps.* Only 2.0 times as many males were caught on table traps with the treatment containing 12:Ac as on traps lacking this component, whereas in the smaller Pherocon traps, the 12:Ac-containing treatment caught 5.4 times more males (Table 2). Males were caught at a mean distance of 49.0 cm on the tables lacking 12:Ac, and at 46.7 cm on tables containing 12:Ac (no significant difference).

*Nonsticky Tables.* Feral males were observed orienting upwind toward

TABLE 2. EFFECT OF TRAP SIZE ON RATIOS OF MALES CAUGHT IN TRAPS LACKING AND CONTAINING 12:AC IN DISPENSERS WITH C11-14:AC (8% *trans*)

| Type of trap | Mean males captured per trap | | Ratio of males caught in traps lacking and containing 12:Ac |
|---|---|---|---|
| | 10 mg c11-14:Ac (8% *trans*) | 10 mg c11-14:Ac (8% *trans*) +15 mg 12:Ac | |
| Sheet-metal tables, 60 cm *r*, Summer 1974[a] | 9.7 | 19.7 | 1:2.0 |
| Pherocon 1C traps, 14 cm *r*, Summer 1974[b] | 2.7 | 14.7 | 1:5.4 |
| Sectar traps, 7.6 cm *r*, Spring 1973[c] | 2.0 | 24.6 | 1:12.3 |

[a] 3 replicates. [b] 18 replicates. [c] 15 replicates (from Roelofs et al. 1975).

the tables from as far away as 15 m downwind. Many males flew very low to the ground and landed in the grass periodically before proceeding onward. This seemed to occur more often when the breeze was strong or increased suddenly in velocity. Males that landed in the grass sometimes exhibited wing-fanning behavior as they walked up to the top of a blade of grass, and then proceeded upwind toward the table. Antennal preening, consisting of dragging each foretarsus over the entire length of an antenna, occurred both in the grass and on the table surface while males were stationary. Many times, the foretarsi would be brought into contact with the mouthparts before being dragged over the antennae. Antennal preening was almost always followed immediately by renewed wing-fanning, walking, or flight. When a male finally reached the vicinity of the table, he would usually spend many seconds casting (oscillating vertical or horizontal flight with little forward progress) at the edge of the metal surface before landing. The mean landing distance from the dispenser was 50.6 cm for both treatments.

Many males made more than one approach toward the table. After spending some time on the surface, males would often fly downwind 0.5 to 10 m and begin approaching the trap again. Of the males landing on the table in response to the treatment lacking 12:Ac, 43% made 2 or more upwind approaches, compared with 51% (not significantly different, $\chi^2$ $2 \times 2$ test of independence) of those responding to the treatment containing 12:Ac. As many as 6 approaches were observed for single males in response to both treatments. All males approaching closer than 10 cm from the dispenser on

their initial approach exhibited wing-fanning behavior either near the dispenser or while walking on the dispenser surface. No copulatory attempts with the dispenser were observed. Departure for the final time usually occurred after an extended stationary period, and involved vertical flight up to 5 m in height immediately on leaving the surface, followed by rapid downwind flight to more than 20 m away.

The addition of 15 mg 12:Ac to 10 mg c11-14:Ac (8% *trans*) resulted in a significant increase in the number of males landing on the table surface, and in the mean closest approach to the attractant dispenser among all males scored (Table 3). When considering only landers, there was no significant difference between the 2 treatments in mean closest approach, although there was a trend toward a closer approach to the 12:Ac-containing treatment.

TABLE 3. BEHAVIOR OF *A. velutinana* MALES NEAR DISPENSERS BAITED WITH c11-14.AC (8% *trans*) LACKING OR CONTAINING 12:AC AND LOCATED AT THE CENTER OF CIRCULAR SHEET-METAL TABLES

| Male behavior | 10 mg c11-14:Ac (8% *trans*) | 10 mg c11-14:Ac (8% *trans*) +15 mg 12:Ac |
|---|---|---|
| No. of males observed | 41 | 67 |
| Percentage approaching to <0.5 m from table[a,b] | 70.7 | 82.1‡ |
| Percentage landing on table[a,b] | 39.0 | 71.6* |
| Percentage fanning while walking on table:[a,b] | | |
| all males | 26.8 | 56.7* |
| landers only | 78.6 | 81.0‡ |
| Percentage approaching <10 cm from dispenser:[a,b] | | |
| all males | 17.1 | 52.2† |
| landers only | 50.0 | 74.5‡ |
| Mean closest approach to dispenser ±SD:[b,c] | | |
| all males[d] | 40.9±25.18 cm | 18.2±24.77 cm† |
| landers only | 20.4±22.14 cm | 11.5±19.19 cm‡ |
| Mean time spent fanning while walking by landers ±SD[b,c] | 20.0±23.72 sec | 18.6±21.95 sec‡ |

[a] Percentages in the same row tested for significance by a $\chi^2$ 2×2 test of independence with Yates' correction.

[b] *$P<0.01$; †$P<0.001$; ‡$P>0.05$.

[c] Means in the same row tested for significance using the *t*-test.

[d] Males flying within 0.5 m of the table edge were scored as approaching to 60 cm; males not approaching to within 0.5 m were not scored. The remaining approaches were by males walking on the table surface.

The percentage of all males approaching to within 10 cm of the dispenser was significantly higher for the treatment containing 12:Ac than for the treatment lacking 12:Ac, but among landers, there was no significant difference between the 2 treatments (Table 3). (The approximate radius of the sticky surface of a Pherocon 1C trap is 10 cm.) The percentage of males approaching to within 0.5 m of the table was higher for the treatment containing 12:Ac, but not significantly different from the treatment lacking 12:Ac. The percentage of males exhibiting wing-fanning behavior was directly proportional to the percentage landing on the table surface. While the percentage of all males exhibiting wing-fanning behavior was significantly different for the treatments containing and lacking 12:Ac, the percentage of landers fanning their wings was similar.

*Nonsticky Pherocon Traps.* Males were observed orienting toward the Pherocon 1C traps from as far away as 10 m downwind. Flight sometimes appeared to be erratic and of high velocity at first, but as males neared the trap edge, their forward progress slowed and the flight pattern was refined into small (10–20 cm) vertical or horizontal casting motions, which lasted as

TABLE 4. BEHAVIOR OF *A. velutinana* MALES NEAR DISPENSERS BAITED WITH c11-14:Ac (8% *trans*) LACKING OR CONTAINING 12:Ac AND LOCATED IN NONSTICKY PHEROCON 1C TRAPS

| Male behavior | 10 mg c11-14:Ac (8% *trans*) | 10 mg c11-14:Ac (8% *trans*) +15 mg 12:Ac |
|---|---|---|
| No. of males observed | 49 | 40 |
| Percentage approaching to 0.5 m from trap[a,b] | 85.7 | 87.5† |
| Percentage landing on trap[a–c] | 26.5 | 87.5* |
| Percentage fanning while walking on trap[a,b] | 26.5 | 87.5* |
| Percentage "caught"[a,b,d] | 20.4 | 87.5* |
| Percentage touching dispenser:[a,b] | | |
| all males | 14.3 | 62.5* |
| landers only | 54.0 | 71.4† |
| Mean time fanning while walking by landers ±SD[b,e] | 42.0±39.68 sec | 51.5±38.41 sec† |

[a] Percentages in the same row tested for significance by a $\chi^2$ 2×2 test of independence with Yates' correction.

[b] * $P<0.001$; † $P>0.05$.

[c] Males alighting anywhere on the trap surface for more than 1 sec were scored as "landing."

[d] Males walking on the area on the trap floor normally coated with Stickem were judged to have been "caught."

[e] Means in the same row tested for significance using the *t*-test.

long as 17 sec before landing was attempted. Some males approached at grass level and were well below trap altitude, but proceeded to rise to trap level and land. Many males fanned their wings while walking either on both sides of the roof of the trap, on the underside of the floor, or on the connecting wires before walking onto what would have been the sticky floor of a normal Pherocon trap. Only rarely would a male fly directly into the trap onto the floor without first fanning while walking on the edge of the trap. Males always approached the attractant dispenser by walking while fanning their wings on the initial approach.

The simultaneous evaporation of 12:Ac from the dispenser resulted in a significantly greater percentage of males landing on the trap surface compared with the treatment lacking 12:Ac, although both treatments lured an equivalent percentage of males to within 0.5 m of the trap (Table 4). All males that approached to within 0.5 m of the trap emitting 12:Ac landed, whereas only 31% of the males approaching to within 0.5 m of the trap lacking 12:Ac landed. Of the males observed orienting toward the trap containing 12:Ac, 87.5% would have been ensnared had there been a sticky floor, whereas only 20.4% of observed males would have been caught in the trap lacking 12:Ac. The percentage of males observed fanning their wings while walking was the same as the percentage landing on the trap in both treatments: All males that landed were observed to fan. Of males that landed in response to both treatments, similar percentages oriented to and touched the dispenser, although the percentages were very different if the total number of males observed orienting in both groups is considered.

## DISCUSSION

### *Laboratory Observations of Behavior*

In a response sequence to calling virgin females, *A. velutinana* males exhibited certain behavioral characteristics that were useful for assaying synthetic chemicals. The most evident response during courtship was wing-fanning while walking, and not one male approached and touched a calling female without fanning his wings simultaneously. This behavior was easily discernible from steps occurring earlier in the sequence that in some contexts may have no relationship to sexual behavior. Except for the first two series of tests, then, wing-fanning behavior was selected as the "key response" to be observed and reflected a naturally occurring behavior requisite to successful courtship.

In laboratory activation assays, males responded optimally to c11-14:Ac when it contained 8% of the *trans* isomer (see Fig. 1). Interestingly, Bartell and Roelofs (1973) postulated a missing component or components in addi-

tion to *cis*, as detected by laboratory bioassays of their female tip extract. Extrapolating from their data, $2 \times 10^{-3}$ μg pure *cis* elicited about 25% male activation response, while $2 \times 10^{-3}$ μg female tip extract elicited about 65% response. Figure 1 shows that TLC-pure c11-14:Ac elicited 27% activation response in our series, while 8% *trans* in c11-14:Ac gave 75% response. The difference in behavior in response to crude extract and pure *cis* observed by Bartell and Roelofs (1973) can be explained by the addition of 8% *trans* to *cis*. Although the addition of 12:Ac to pure *cis* significantly increased the percentage response to 40%, this level was not sufficient to account for their observed difference.

The 8% *trans* mixture proved to be more potent in eliciting wing-fanning response than pure *cis*, pure *trans*, and 30% *trans* over a wide range of dosages covering 4 orders of magnitude (see Fig. 2). It can be concluded that in responding preferentially to treatments containing 8% *trans*, male *A. velutinana* were not determining the absolute quantity present of either isomer. At any one dosage level from $10^{-2}$ μg to $10^{1}$ μg, the reduced response to 30% *trans* compared to 8% *trans* can be attributed to either too much *trans* present or too little *cis*. However, when one considers the reduced response to pure *cis* compared to 8% *trans* at each dosage, the argument for too little *cis* is eliminated. Additionally, the increase in response elicited by progressively higher dosages of 30% and 8% *trans* (with the amount of *trans* increasing 10-fold each step) nullifies the argument that reduced response is a result of too much *trans*. The insect must be detecting the *cis*:*trans* ratio and responding accordingly. In these dosage-response series, then, it can be argued that the 2 isomers are at various times "excitants," "synergists," and "inhibitors," depending on their dosage and proportion. These terms thus become inappropriate for describing the behavior elicited by these pheromone components and may be misleading.

The wing-fanning response elicited by high concentrations of *trans* was unexpected. Roelofs and Comeau (1971) reported a "short-lived buzzing response" when *trans* was tested at 1 μg, which was 1,000 times the observed threshold for *cis*. Our data also show very little wing-fanning response at 1 μg, but at $10^{1}$ μg and $10^{2}$ μg, the wing-fanning response to *trans* was qualitatively indistinguishable from that to *cis* or 8% *trans*.

Likewise, the wing-fanning response to 1 mg (detectable by the human nose) 12:Ac in orientation tube olfactometers was unexpected, and was qualitatively indistinguishable from that to 8% *trans*. The higher dosages required for response to these 2 components may represent the relative degree of affinity and intrinsic activity that these compounds have for the *cis* antennal receptor sites (O'Connell 1972, 1975) to produce the intensity and quality of sensory neuron impulse generation required for wing-fanning response.

The 2 assays in orientation tube olfactometers (see Figs. 3 and 4) can be viewed as support for the role of 12:Ac as a modifier of behavior close to the source. At low dosages in the laboratory (i.e., long-range in the field), 12:Ac had no effect on wing-fanning response to 8% *trans*, the most active *cis*:*trans* mixture (Figs. 4C and F). However, at a higher dosage of 8% *trans* (close-range in the field), 12:Ac became effective in prolonging the wing-fanning response and increasing percentage orientation toward the source (Figs. 3C and F). Since wing-fanning behavior always occurs as a male approaches a female before attempting to copulate, these assays are important in explaining a close-range role for 12:Ac. The enhancement of response by 12:Ac (Fig. 3) to pure *cis* and 8% *trans*, but not pure *trans*, indicates that 12:Ac might be acting in conjunction with *cis* rather than *trans* receptor sites in modulating wing-fanning response.

*Field Observations of Behavior*

Observations of male *A. velutinana* in the field indicate that 12:Ac modified the behavior of males close to the c11-14:Ac (8% *trans*) source. This modification is evidenced by the greater percentage of landing and closer mean approach to the dispenser on large nonsticky tables (see Table 3) and the greater percentage of landing in nonsticky Pherocon 1C traps by males that approached to within 0.5 m of 12:Ac-containing traps (see Table 4). A large percentage (69%) of the males that approached to within 0.5 m of the Pherocon traps in response to the lure lacking 12:Ac turned away and flew rapidly downwind, whereas all males that approached within 0.5 m of the trap containing 12:Ac landed.

Interestingly, for nonsticky tables and traps, no significant differences in frequency or duration of wing-fanning or mean closest approach to the dispenser could be discerned between the 2 treatments once males had landed on their surfaces. For both surface sizes, however, the significant differences in the percentage of males landing after flight close to the surface edge suggest that an in-flight behavioral step involving landing (or not landing) is mediated by 12:Ac.

Further evidence to support the role of 12:Ac as a close-range modifier of behavior resulted from the relative numbers of males caught in traps having progressively smaller sticky surfaces. Large sticky table traps with a radius of 60 cm gave a 2-fold increase in catch with the addition of 12:Ac to the 8% *trans* in *cis* mixture, whereas the 12:Ac-added treatment in Pherocon 1C traps with a sticky radius of about 14 cm resulted in a 5.4-fold increase of males trapped compared to the treatment lacking 12:Ac. In addition, Roelofs et al. (1975) reported a 12-fold increase in males caught using still smaller Sectar traps with a radius of 7.6 cm. All these findings suggest that

the presence of 12:Ac becomes increasingly critical for trap catch as trap size is reduced.

The near-maximal mean distance from the dispenser (49 and 47 cm) of males caught on the surface of 60 cm *r* sticky tables and observed landing (50.6 cm) on the surface of nonsticky tables for both treatments indicate that male *A. velutinana* will take the first opportunity to land and complete the approach when a suitable surface is presented. This, along with their casting flight at the table's edge before landing, suggests that visual cues play an important role in their approach to a pheromone source. Likewise, males were observed to slow their forward progress and cast sideways and vertically a few centimeters from the edge of Pherocon traps before either landing or departing. A visual response to the trap surface is again suggested, because a purely chemical response to odor concentration should have caused a cessation of forward progress and casting to occur at the same distance from the dispenser for the Pherocon traps as for the tables. The phenomenon of multiple approaches to tables by a large percentage of the males observed indicates that male *A. velutinana* may become dishabituated to the pheromone by flying downwind and again beginning the behavioral sequence with upwind anemotaxis. This behavioral pattern occurred even after coming in direct contact with the dispenser for uninterrupted periods as long as 1 or 2 min. Thus, males do not appear to become habituated easily to a single source of pheromone at optimum concentration for attractancy. Another explanation could involve the more transient phenomenon of sensory adaptation, allowing the insect to recover its odor-perceiving ability relatively quickly after returning to an area of lower odor concentration.

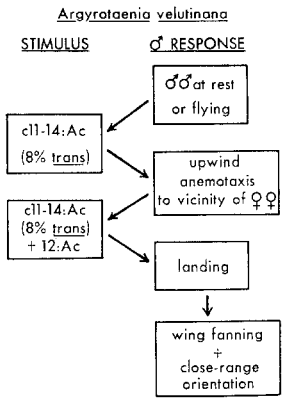


FIG. 5. Stimulus–response reaction chain diagramming possible steps involved in the location of an *A. velutinana* female by conspecific males.

The laboratory and field results from this study can be used to construct a stimulus–response action chain diagramming the possible steps involved in the location process (Fig. 5). In the field, a greater percentage of males at rest or flying would become activated and fly upwind to a blend containing 8% t11-14:Ac in c11-14:Ac than to other mixtures or to c11-14:Ac alone (see Fig. 1). After the males had flown to the vicinity of the female, the higher concentration of pheromone and the presence of 12:Ac would cause a greater frequency of landing, wing-fanning while walking, and close-range orientation by males. The smaller the available landing surface, the more pronounced becomes the effect of 12:Ac, so a female calling from a small twig or leaf might receive few male visitors unless she were emitting 12:Ac along with c11-14:Ac (8% *trans*).

Although both laboratory bioassays and field observations were employed to try to ascertain the behavioral function of the *A. velutinana* pheromone components, the latter were the key to elucidating the role of 12:Ac. Laboratory assays demonstrated the enhancement of activity by this component only at certain discrete concentrations, but a behavioral function mediated by 12:Ac could be described only after observing males during response in the field.

The finding of a close-range chemical modifier of behavior in *A. velutinana* is similar to the communication system of the Oriental fruit moth, *Grapholitha molesta* (Cardé et al. 1975*a,b*). Dodecyl alcohol (12:OH), when presented with *cis*-8-dodecenyl acetate (c8-12:Ac) with 7% of the *trans* isomer present, elicited increases in the percentage of males landing near the source, fanning while walking, and exhibiting hairpencil display behavior near the dispenser. In addition, the mean approach to the dispenser was significantly closer with treatments containing 12:OH. However, 12:OH has not been identified from Oriental fruit moth female tip extract. For *A. velutinana*, however, all 3 compounds investigated in this study are known to be either emitted by female *A. velutinana* or present in the pheromone gland. Thus, the close-range mediation of behavior by 12:Ac appears to represent a naturally occurring phenomenon in the location of a female by a male redbanded leafroller moth.

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