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# SEX PHEROMONE EVIDENCE FOR TWO DISTINCT TAXA WITHIN *Graphania mutans* (WALKER)[1]

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**Abstract**—The sex pheromones of two populations of *Graphania mutans* (Walker) were analyzed. Females from an Auckland population produced (*Z*)-9-tetradecenol (*Z*9–14 : OH), (*Z*)-9-tetradecenyl acetate (*Z*9–14 : OAc), (*Z*)-7-tetradecenol (*Z*7–14 : OH) and (*Z*)-7-tetradecenyl acetate (*Z*7–14 : OAc), while females from a Lincoln population produced these four compounds and a large amount of (*Z*)-9-tetradecenal (*Z*9–14 : Ald). Significant differences, paralleling the difference between females, were observed when the responses of males of both populations to the above and other related compounds were tested by electroantennogram, field-trapping, and wind-tunnel bioassays. The most distinct difference was observed in the wind tunnel. Males from both taxa flew upwind and touched pheromone sources containing sex pheromone extract of females of their own taxon, but either did not initiate upwind flight or arrested upwind flight shortly after taking flight in response to extract from females of the other taxon. The difference between the pheromone systems of the two populations is probably due to the presence and importance of *Z*9–14 : Ald in the pheromone blend of the Lincoln population. Thus the addition of a relatively large amount of *Z*9–14 : Ald to a four-component pheromone blend (i.e., *Z*9–14 : OH, *Z*9–14 : OAc, *Z*7–14 : OH, and *Z*7–14 : OAc) attractive to Auckland males completely suppressed trap catches of male *G. mutans* in Auckland but large numbers of males were caught at both Lincoln and Nelson in traps baited with this five-component blend. In wind-tunnel studies, the addition of even small (1% of amount of *Z*9–14 : OH) amounts of *Z*9–14 : Ald to the four-component blend resulted in a significantly greater proportion of Auckland males arresting upwind flight than to the four-com-

[1] Lepidoptera: Noctuidae: Hadeninae.
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ponent blend. It is suggested that these two populations of *G. mutans* represent distinct sibling species within the described concept.

**Key Words**—*Graphania mutans*, Lepidoptera, Noctuidae, sex pheromones, (Z)-9-tetradecenyl acetate, (Z)-9-tetradecenol, (Z)-9-tetradecenal, sibling species, identification, wind tunnel.

## INTRODUCTION

*Graphania mutans* (Walker), (Noctuidae: Hadeninae), an endemic species distributed throughout New Zealand, is a pest of some horticultural crops such as apples. The larvae of this species feed on and scar the surface of the fruit, thereby making it unsuitable for export (Suckling et al., 1990). This damage often occurs early in the season and is additive to the damage caused by other lepidopterous pests such as leafroller moths and codling moth (Wearing et al., 1991).

In New Zealand, the identification of sex pheromones of all the other major lepidopterous pests found on apples, and the more accurate monitoring of populations of these pests that this has allowed, has facilitated the development of reduced insecticidal spray programs (Suckling, 1989). In particular, a number of insecticidal sprays can be omitted in many apple-growing areas during the early part of the season. During this period, damage to fruit by other pests such as *Graphania mutans* can occur, and hence the ability to specifically monitor populations of this pest, using sex pheromones, would be of great value in reduced spray programs.

The genus *Graphania* contains a number of species morphologically very similar to, and sympatric with, *G. mutans*. The adults of *G. mutans* are highly variable in color pattern; seven names have been synonymized with *mutans* (Dugdale, 1988). With this variability in mind, we used the approach previously used in the analysis of the sex pheromones of sibling species complexes of various tortricid species in the genera *Planotortrix* and *Ctenopseustis*, i.e., analysis of the sex pheromones of distinct geographic populations and determination of whether differences exist between the sex pheromones of these populations (Foster et al., 1991). We report here the results of this study on *G. mutans*.

## METHODS AND MATERIALS

*Insects.* Two laboratory cultures of *G. mutans* were established by collecting gravid females in light traps situated in Auckland and Lincoln (near Christchurch) and allowing these females to oviposit inside small glass vials (2.5 cm diam. × 10 cm).

Neonate larvae were allowed to feed on a semisynthetic corn diet (Poitout

and Bues, 1970) until they had reached the second instar, whereupon they were transferred into a small waxed cardboard ice cream cup (Lily Inc., No. 134, Auckland) (three larvae per container), containing more of the corn diet. The larvae were maintained inside the cup at 18–20°C, 16:8 hr light–dark photoperiod until they had pupated. The sexes were separated as pupae and placed in moist vermiculite. Adults were used either in the various experiments (see below) or to perpetuate the culture. For the latter, groups of males and females (one to three each) were placed inside a plastic bag, supported by an inner wire frame (35 × 25 × 13 cm), and allowed to mate. The resultant eggs were removed and placed inside a small, humid container until they hatched.

*Determination of Sexual Activity.* The beginning of sexual activity by female *G. mutans* was defined as when the female extruded the terminal segments of her abdomen, thereby exposing her pheromone gland (i.e., "calling"). In order to observe the periodicity of calling behavior, females from the Auckland colony were placed in small transparent containers (4 cm high × 40 cm diam.) that allowed observation of, but did not interfere with, this behavior. Females 1–5 days old were observed every 15 min, commencing from 2 hr preceding the scotophase through to 1 hr into the photophase.

Mating of single pairs of 3-day-old Auckland males and females was observed under the above conditions.

*Pheromone Extraction and Chemical Analysis.* The tip of the abdomen of a 3- to 4-day-old female was everted and dissected just prior to the commencement of the calling period, placed in 10–50 μl of distilled pentane, and extracted for 0.5–12 hr prior to analysis by gas chromatography (GC) or gas chromatography–mass spectrometry (GC-MS). One to 10 glands were extracted and analyzed at a time.

Extracts were analyzed by GC using two columns: a 50-m × 0.32-mm-ID Carbowax 20 M (Quadrex Corp., New Haven, Connecticut), temperature-programmed from 100 to 210°C at 4°C/min (initial delay of 1 min), and a 60-m × 0.25-mm-ID SP2340 (Supelco Inc., Bellefonte, Pennsylvania), temperature-programmed from 60 to 140°C at 8°C/min (initial delay of 1 min), then to 180°C at 2°C/min. The gas chromatographs used were, respectively, a Philips 4500 and a Varian 3500. Splitless injection, and nitrogen at a linear flow velocity of 10 cm/sec as carrier gas, were used for both columns. This combination of columns and GC conditions allowed the separation of all monounsaturated tetradecenyl acetate isomers and virtually all tetradecenal isomers from each other (Heath et al., 1980). Alcohols could not be analyzed adequately using the SP2340 column as they were degraded on this column. Therefore they were converted to acetates by reaction with acetyl chloride for further confirmation of their structure. Tridecanyl acetate was used as an internal standard.

The mass spectra of compounds in extracts of pheromone glands were

recorded using a Nermag 10-10C equipped with either a 25-m × 0.22-mm-ID CPSIL 5CB or a 25-m × 0.22-mm-ID CPSIL 88CB (Chrompack, Middelburg, Netherlands). The mass spectra of the various compounds were compared with the mass spectra of synthetic samples.

*Electroantennograms.* The electroantennogram (EAG) responses of isolated male antennae were recorded according to the method of Roelofs (1984). The various chemicals (monounsaturated, 12-, 14-, and 16-carbon acetates, alcohols, and aldehydes; 1-$\mu$g dose on filter paper) were presented into the airstream using a 5-ml syringe. Data were corrected according to Renou (1979). The responses of at least five different antennae were tested to each of the series of chemicals.

*Field-Trapping.* Field trapping was carried out at Mt Albert Research Centre (Auckland, north North Island), Appleby Research Orchard (Nelson, northern South Island), and at Lincoln (near Christchurch, mid-coastal South Island). The appropriate amount of chemical was loaded onto a rubber septum (Thomas Scientific) and placed inside a Pherocon 1C sticky trap (Zoecon Corp., Palo Alto, California). The traps were hung approximately 1.5 m above the ground on various fruit trees (apple, nectarine, peach) or on monkey apple (*Acmena smithii*) hedges surrounding the orchards. Traps were placed at least 10 m apart. Unless stated otherwise, traps were checked and rerandomized every one to two days.

For analysis of the field-trapping experiments, data were transformed by $\sqrt{(X + 0.5)}$, an ANOVA conducted, and the means of the treatments compared using Duncan's multiple range test. Comparisons between treatment means were made at the $P = 0.05$ level.

*Wind Tunnel.* Male moths were flown inside a 1.3-m-long wind tunnel based on the design of Miller and Roelofs (1978). The tunnel was illuminated using six 15-W incandescent red light bulbs (Osram Co., Auckland) situated above the tunnel. The temperature throughout the various experiments was controlled at 20 ± 1°C. The chemicals or pheromone gland extract were placed on a piece of filter paper (1.5 × 1.5 cm) seated on a thin metal rod stand approximately 15 cm above the floor of the tunnel. Just prior to the start of the scotophase, individual males were placed inside capped wire mesh cylinders (2.5 cm diam. × 5 cm) and transferred into the wind tunnel room. In a preliminary experiment, males were found to respond best between 5.5 hr and 6.5 hr after the onset of the scotophase; all subsequent tests were carried out during this period. Individual 3-day-old male moths were introduced into the mouth of the tunnel (approximately 1.2 m downwind of the chemical source), and their behavioral responses to the source recorded. The following behaviors were recorded: raising of the antennae and wing movement (activation), initiation of upwind flight (take flight), flight to beyond the midpoint of the tunnel (midpoint), flight to within 15 cm of the source (approach), touching of the source

(touch), and landing on the source (land). Males from the Auckland colony were used in all the wind-tunnel experiments testing the responses of males to synthetic chemicals. Each wind-tunnel experiment was run as a complete randomized block design. The distributions of the separate behavioral responses to the various blends tested within an experiment were compared using Kruskall and Wallis tests. Differences are reported at $P = 0.05$.

## RESULTS

*Sexual Activity.* Virtually all of the insects used in these observations emerged towards the end of the scotophase. In the first night following emergence, only 4/18 of the females called. However, on the second night following emergence, 80% of the females called. In subsequent nights (up to the fifth night following emergence), the percentage of females that called declined gradually. Females of all ages began calling during the fourth hour of the scotophase through to the onset of the photophase; the greatest percentage of females called during the fifth and sixth hours of the scotophase. Following the commencement of calling by females, males became highly activated and copulation quickly followed.

*Chemical Analysis.* Extracts of the pheromone glands of females from the Auckland *G. mutans* colony were initially analyzed by GC using the Carbowax 20 M column. From these extracts, the following compounds were tentatively identified on the basis of retention times (relative amounts): tetradecyl acetate (14:OAc) (0.9), (Z)-7-tetradecenyl acetate (Z7–14:OAc) (2.7), (Z)-9-tetradecenyl acetate (Z9–14:OAc) (23.1), tetradecanol (14:OH) (8.6), (Z)-7-tetradecenol (Z7–14:OH) (14.5), and (Z)-9-tetradecenol (Z9–14:OH) (100). Analysis of the crude extract using the SP2340 column was consistent with the assignments of the above acetates. When the extract was acetylated and the reaction product mixture analyzed by GC (using the Carbowax 20 M column), the chromatogram showed the almost complete disappearance of the peaks tentatively identified as alcohols and much greater amounts (relative to the internal standard) of the three acetates. Mass spectral analysis of gland extract from the Auckland females, and comparison with spectra of authentic samples, confirmed the assignments of these compounds. Females contained on average, 3.3 ng of Z9–14:OH.

*G. mutans* from Lincoln were analyzed similarly. GC analysis using the Carbowax 20 M column yielded chromatograms that were very different from those obtained from the analysis of Auckland *G. mutans* females. The following tentatively identified compounds (relative amounts) were observed: (Z)-9-tetradecenal (Z9–14:Ald) (100), Z7–14:OAc (0.5), Z9–14:OAc (34.9), 14:OH (3.0), Z7–14:OH (15.4), and Z9–14:OH (40.9). The tentative identification of

these acetates and aldehyde was supported by GC analysis using the SP2340 column. Acetylation of the gland extract and subsequent analysis using the Carbowax 20 M column, as above, was also consistent with the assignment of the three alcohols. GC-MS analysis of gland extract confirmed the assignments of the above compounds. Females contained on average 3.5 ng of Z9–14 : Ald.

*Electroantennograms.* For both the Auckland and Lincoln *G. mutans* males, the greatest EAG response in each of the series (i.e., 12-, 14-, and 16-carbon monounsaturated acetates, alcohols, or aldehydes) tested, was always to the isomer with a (*Z*)-9 double bond. Between the series, the largest amplitude response for both types of males was to the 14-carbon compounds; for the Auckland males the largest response amplitudes were to the acetates, whereas for the Lincoln males they were to the aldehydes. Between the 14-carbon series the converse was true for the lowest amplitude responses, i.e., the aldehydes and the acetates elicited the lowest amplitude response from the Auckland and Lincoln males, respectively. Except for the relative amplitudes of response, the EAG profiles to each of the series tested were very similar for both populations of males.

*Field-Trapping.* Field trials 1–5 (Table 1) tested the effects of the various chemicals identified in females of the Auckland and Lincoln populations on trap catches of males of the Auckland population.

In the first field trial (Table 1), *G. mutans* males were caught in significant numbers in traps baited with the four monounsaturated chemicals found in the Auckland females (Table 1); traps baited with the higher dosage (1000 μg of Z9–14 : OH) of the four-component blend caught significantly greater numbers of males than traps baited with the lower dosage (100 μg). At the higher dosage (1000 μg of Z9–14 : OH), the addition of 1% of Z9–14 : Ald to the four-component blend resulted in a significant decrease in the number of males caught.

In trial 2 (Table 1), blends that lacked Z7–14 : OAc did not elicit trap catches of male *G. mutans* significantly greater than the number caught in the blank traps. Similarly, in trial 3 (Table 1), traps baited with the blend that lacked Z9–14 : OAc did not catch any males. Increasing the amount of Z9–14 : OAc in the blend from 0 to 500 μg increased trap capture. However, increasing the amount of Z9–14 : OAc in the blend still further to 1000 μg significantly decreased (relative to the blend containing 500 μg) the catch (trial 3).

Changing the amount of Z7–14 : OH in the blend over the range 0–1000 μg resulted in slight, but not significant changes in the numbers of males caught, relative to the standard blend containing 130 μg of Z7–14 : OH (trial 4, Table 1). In contrast, the removal of Z7–14 : OAc from the three-component blend tested in this trial (trial 4) significantly reduced the numbers of males caught to a level not significantly different from the blank, thereby confirming the result from trial 2.

In trial 5, traps baited with blends containing less than 1000 μg of Z9–

TABLE 1. TRAP CATCHES OF MALE *Graphania mutans* TO VARIOUS BLENDS

| Dosages of chemicals ($\mu$g) | | | | | Trap catch |
|---|---|---|---|---|---|
| Z9–14 : OH | Z9–14 : OAc | Z7–14 : OH | Z7–14 : OAc | Z9–14 : Ald | (males/trap)[a] |
| Trial 1[b] | | | | | |
| 100 | 23 | 13 | 3 | | 5.5 c |
| 100 | 23 | 13 | 3 | 1 | 3.3 cd |
| 100 | 23 | 13 | | | 1.8 cd |
| 100 | 23 | | | | 2.5 cd |
| 1000 | 230 | 130 | 30 | | 35.5 a |
| 1000 | 230 | 130 | 30 | 10 | 23.8 b |
| | | | | | 0.0 d |
| Trial 2[c] | | | | | |
| 1000 | 230 | 130 | 30 | | 16.7 a |
| 1000 | 230 | 130 | | | 0.3 b |
| 1000 | 230 | | | | 0.3 b |
| | | | | | 0.0 b |
| Trial 3[d] | | | | | |
| 1000 | 230 | 130 | 30 | | 7.3 b |
| 1000 | | 130 | 30 | | 0.0 c |
| 1000 | 50 | 130 | 30 | | 2.3 c |
| 1000 | 100 | 130 | 30 | | 4.7 c |
| 1000 | 500 | 130 | 30 | | 11.7 a |
| 1000 | 1000 | 130 | 30 | | 2.5 c |
| | | | | | 0.0 c |
| Trial 4[e] | | | | | |
| 1000 | 500 | 130 | 30 | | 13.3 a |
| 1000 | 500 | | 30 | | 10.0 a |
| 1000 | 500 | 50 | 30 | | 8.7 a |
| 1000 | 500 | 500 | 30 | | 8.0 a |
| 1000 | 500 | 1000 | 30 | | 5.7 a |
| 1000 | 500 | | | | 2.0 b |
| | | | | | 0.0 b |
| Trial 5[f] | | | | | |
| 1000 | 500 | 130 | 30 | | 20.8 a |
| | 500 | 130 | 30 | | 7.8 c |
| 100 | 500 | 130 | 30 | | 14.8 abc |
| 500 | 500 | 130 | 30 | | 10.5 b |
| 1000 | 500 | 130 | 100 | | 17.2 ab |
| 100 | 1000 | 130 | 30 | | 13.0 abc |
| 500 | 1000 | 130 | 30 | | 13.8 ab |
| | | | | | 0.0 d |

[a] Means accompanied by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

[b] Conducted at Mt Albert Research Centre (MARC), Auckland, between January 28 and February 15, 1988; four replicates of each treatment were tested.

[c] Conducted at MARC and Kumeu Research Orchard, between February 2 and 19, 1988; four replicates per treatment.

[d] Conducted at MARC between February 16 and March 6, 1988; three replicates per treatment.

[e] Conducted at MARC between April 18 and June 1, 1988; three replicates per treatment.

[f] Conducted at MARC between September 7 and November 14 , 1988; four replicates per treatment. Traps were examined and rerandomized every week.

14 : OH caught fewer males than traps baited with the blend containing 1000 μg of Z9–14 : OH; with the exception of the blend that contained 100 μg of Z9–14 : OH, these catches were significantly different from the number of males caught in the 1000 μg (of Z9–14 : OH) blend. Also in this trial, the amount of Z7–14 : OAc in the four-component blend was increased from 30 μg to 100 μg. There was no significant difference between the number of *G. mutans* caught in traps baited with either of these two blends.

The field trials conducted at Lincoln and Nelson tested various blends containing chemicals identified in the females from the Lincoln population against the four-component blend that was attractive to male *G. mutans* in Auckland (Table 2). In both Lincoln and Nelson, traps baited with the Auckland-type blend caught *G. mutans* males. However, the greatest catch (not significantly different from the number caught in traps baited with the Auckland-type blend) of males was in traps baited with the five-component blend containing 1000 μg of Z9–14 : Ald. Traps baited with the four-component blend lacking Z7–14 : OAc but containing Z9–14 : Ald caught significantly less males than traps baited with the five-component blend, but significantly more males than the blank traps.

A trial comparing the four-component blend developed for the Auckland population of *G. mutans* with the five-component blend used in the Lincoln and Nelson trial was conducted in Auckland. Another five-component blend, consisting of the Auckland-type blend plus 1000 μg of Z9–14 : Ald, was also included (Table 3). Over two months, male *G. mutans* were caught only in traps containing the Auckland-type blend; the blank traps and traps baited with either the Lincoln-type blend or the other five-component blend did not catch any males.

TABLE 2. TRAP CATCHES OF MALE *Graphania mutans* AT LINCOLN AND NELSON TO VARIOUS BLENDS[a]

| Dosages of chemicals (μg) | | | | | Trap catch (males/trap) | | |
|---|---|---|---|---|---|---|---|
| Z9–14 : OH | Z9–14 : OAc | Z7–14 : OH | Z7–14 : OAc | Z9–14 : Ald | L | N | Total |
| 1000 | 230 | 130 | 30 | | 8.5 | 103 | 40.0 a |
| 150 | 350 | | | 1000 | 2.5 | 15 | 6.3 bc |
| 150 | 350 | 140 | | 1000 | 7.0 | 17 | 10.3 b |
| 150 | 350 | 140 | 50 | 1000 | 19.5 | 112 | 46.3 a<br>0.3 c |

[a]Trial conducted from February 1 to May 11, 1988, at Lincoln (L; two replicates) and Appleby Research Orchard, Nelson (N; one replicate). Traps were checked and rerandomized weekly. Means accompanied by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

TABLE 3. TRAP CATCHES OF MALE *Graphania mutans* TO VARIOUS BLENDS[a]

| Dosages of chemicals | | | | | Trap catch |
|---|---|---|---|---|---|
| Z9–14:OH | Z9–14:OAc | Z7–14:OH | Z7–14:OAc | Z9–14:Ald | (males/trap) |
| 1000 | 230 | 130 | 30 | | 7.4 a |
| 150 | 350 | 140 | 50 | 1000 | 0.0 b |
| 1000 | 230 | 130 | 30 | 1000 | 0.0 b |

[a]Trial conducted from March 30 to June 1, 1988 at MARC, using five replicates. Traps were checked and rerandomized weekly. Means accompanied by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

*Wind Tunnel.* Male *G. mutans* from the Auckland colony were flown initially to extract of sex pheromone glands of their own females. Males responded to as little as 0.1 female equivalents (FE) of extract, with 33% (6/18) of the males tested touching the source. The percentage of males touching the source containing 1 FE of extract was greater (53%; 15/28), but not significantly ($P > 0.05$) so. After being placed in the plume of the 1 FE source, males initially remained stationary, with a mean latency to activation of 54 sec (range 6–217.8 sec). Activation consisted of males raising their antennae and fanning their wings, with a mean duration from activation to initiation of flight of 88.8 sec (range 6.6–93.6 sec). Once males took flight, the time of flight (to landing on the source) was relatively short, with a mean of 58.8 sec (range, 42–108 sec).

Three wind-tunnel experiments investigating the effects on male flight responses of varying the amount or ratios of Z9–14:Ald, Z9–14:OH:Z7–14:OH, and Z9–14:OAc:Z7–14:OAc, respectively, were conducted. In each of these experiments, males were flown to a standard reference blend consisting of 10 ng Z9–14:OH, 3 ng Z9–14:OAc, 1.5 ng Z7–14:OH, and 0.5 ng Z7–14:OAc, in addition to the various other blends tested.

An increase in the amount of Z9–14:Ald in the blend had a significant effect on male responses (Figure 1). The addition of 1% (of amount of Z9–14:OH) of Z9–14:Ald to the four-component blend resulted in a significant ($P < 0.05$) decrease in the percentage of males touching the source (74% of males for the four-component blend as opposed to 33% of males for the blend with 1% Z9–14:Ald). Rather than being due solely to a decrease in the percentage of males activating to the respective sources, this difference was the result of arrestment of flight occurring throughout each stage of upwind flight. However, greater amounts of Z9–14:Ald added to the blend reduced the number of males activating and significantly ($P < 0.05$) increased the percentage of arrestment of flight before males had reached the midpoint of the tunnel. Only 5% (1/20)

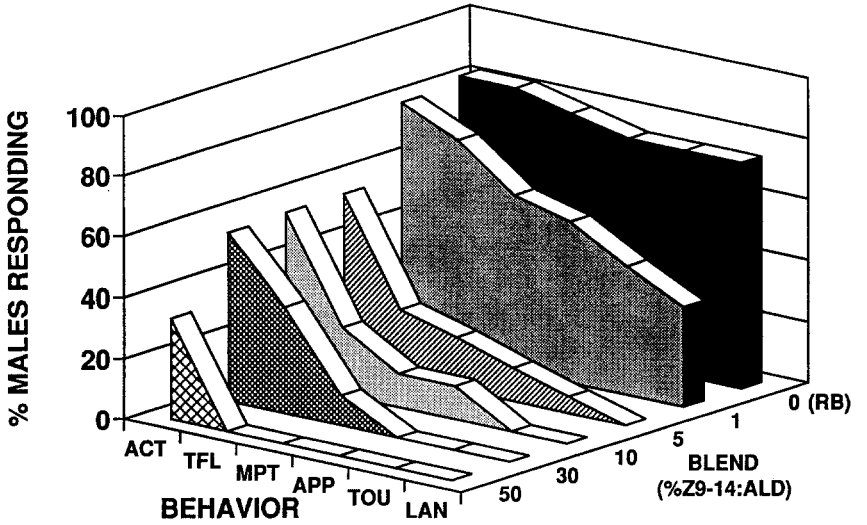


FIG. 1. Percentage of male *Graphania mutans* (Auckland population) responding to a four-component reference blend (RB) plus varying amounts of (*Z*)-9-tetradecenal (Z9–14:Ald), in a wind tunnel. The RB consisted of 10 ng (*Z*)-9-tetradecenol, 3 ng (*Z*)-9-tetradecenyl acetate, 1.5 ng (*Z*)-7-tetradecenol, and 0.5 ng (*Z*)-7-tetradecenyl acetate on filter paper. The responses measured were: activation (ACT), taking flight (TFL), midpoint (MPT), approach (APP), touch (TOU), and landing (LAN); for a detailed description of these responses refer to text.

of the males touched the source containing 5% Z9–14:Ald, and no males touched the sources with blends containing greater amounts of Z9–14:Ald.

Of all the ratios of Z9–14:OH:Z7–14:OH tested (Figure 2) the greatest percentage of males that touched the source did so to the reference blend; this percentage was significantly greater ($P < 0.05$) than the percentages of males that touched the sources containing any of the other blends tested (i.e., with higher or lower ratios of Z9–14:OH:Z7–14:OH). Blends with lower ratios of Z9–14:OH:Z7–14:OH (i.e., with lower amounts of Z9–14:OH) were characterized by greater numbers of males having arrested flight by each of the successive positions in the tunnel. However, to the blend lacking Z7–14:OH, once males had reached the midpoint of the tunnel they continued on to the source without any arrestment of flight.

To the different ratios of Z9–14:OAc:Z7–14:OAc, the greatest percentage of males that landed on the source was to the reference blend ratio (i.e., 3:0.5) (Figure 3). The percentage of males touching the source (64%) containing this blend was significantly greater ($P < 0.05$) than the percentages of males touching the source for all other blends except the 3:1.5 blend (41% of

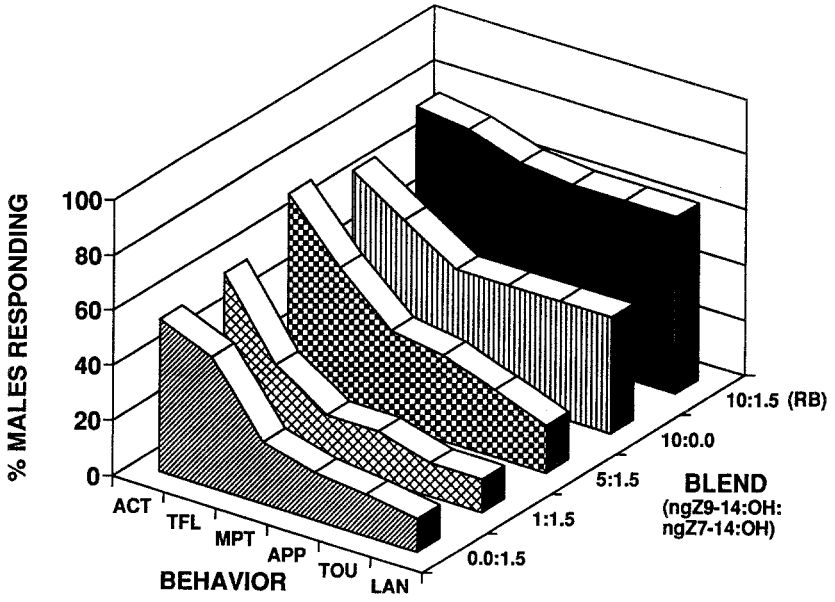


FIG. 2. Percentage of male *Graphania mutans* (Auckland population) responding to a four-component reference blend (RB) and four component blends with varying amounts of (Z)-9-tetradecenol (Z9–14 : OH) and (Z)-7-tetradecenol (Z7–14 : OH), in a wind tunnel. The RB and acronyms are as in Figure 1.

males). Consistent with the lack of catches in the field-trapping trials, blends lacking either Z9–14 : OAc or Z7–14 : OAc failed to elicit any males to touch the source. However, there was a significant ($P < 0.05$) difference between the responses of males to these two blends; the blend lacking Z9–14 : OAc did not activate males, but 50% of the males were activated by the blend lacking Z7–14 : OAc. Arrestment of flight to all blends (except the one lacking Z9–14 : OAc) tended to occur after males had activated and before they had reached the midpoint of the tunnel.

Males of the Auckland and Lincoln cultures were flown to pheromone extract from their own or the other females (Figure 4). Approximately 80% of both Auckland males and Lincoln males flew upwind and landed on the sources containing 1 FE of pheromone extract from their respective females. However, none of the Auckland or Lincoln males flew upwind and touched the source containing pheromone extract of the Lincoln or Auckland females, respectively. This failure to fly upwind and touch the source was generally characterized by low percentages of males activating (25–40%; c.f., 76% and 100% of males of both cultures were activated by extract from their own females). None of the nine (of 21) Lincoln males that were activated by Auckland female pheromone

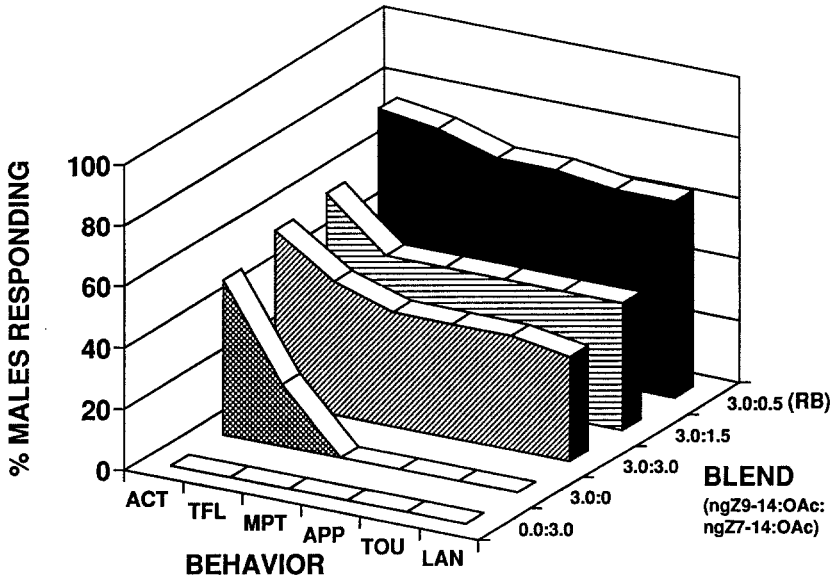


FIG. 3. Percentage of male *Graphania mutans* (Auckland population) responding to a four-component reference blend (RB) and four-component blends with varying amounts of (Z)-9-tetradecenyl acetate (Z9–14 : OAc) and (Z)-7-tetradecenyl acetate (Z7–14 : OAc), in a wind tunnel. The RB and acronyms are as in Figure 1.

extract flew upwind for greater than 20–30 cm, but all five (of 19) of the Auckland males that were activated by Lincoln pheromone extract flew upwind for approximately 30 cm; all of these males arrested upwind flight before the midpoint of the tunnel.

## DISCUSSION

By analyzing the sex pheromones of two distinct populations of *G. mutans*, we have shown that this morphologically described species consists of (at least) two taxa that differ in their sex pheromones. In their respective pheromone blends, females of both taxa produce four components in common: Z9–14 : OH,Z7–14 : OH,Z9–14 : OAc, and Z7–14 : OAc, although in somewhat different ratios. However, females of the taxon from Lincoln also produce relatively large amounts of Z9–14 : Ald while females of the taxon from Auckland do not produce this compound. Paralleling pheromone production by the females, males of both taxa also show similarities and differences in responses to the chemicals found in females. Profiles of EAG responses to series of monounsaturated chemicals are quite similar for males of both taxa, suggesting

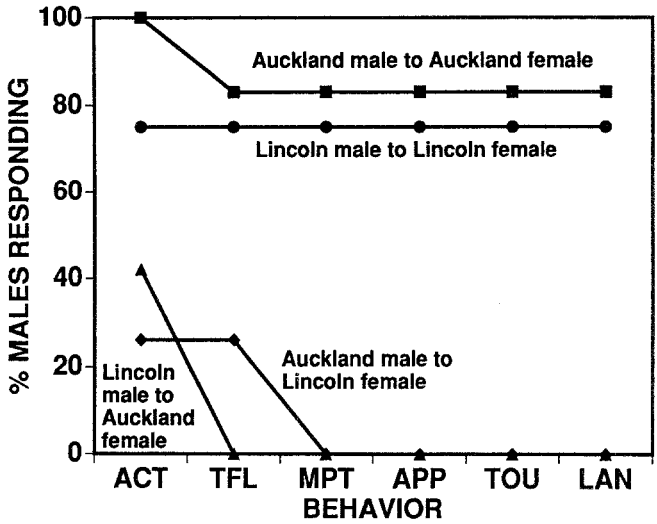


FIG. 4. The flight responses of male *Graphania mutans* from Auckland and Lincoln populations to pheromone extract from females of their own and the other population. The response acronyms are as in Figure 1.

that they share a similar olfactory receptor complement. However, the relative amplitudes of EAG responses to the different series by males of the two taxa suggest that the relative abundances of the different olfactory cells differ between the two types of males. These differences are further matched by the behavioral responses of males in a wind tunnel to pheromone extract from the respective females. Males of either taxon only respond by touching the filter paper source containing pheromone extract of their own females. In response to pheromone extract of the other females, males either did not activate or they arrested flight shortly after take off.

While there are probably other differences, such as the importance of ratios of the various compounds, we believe that a key difference between the sex pheromone systems of the two taxa is the presence and importance of Z9–14 : Ald in the blend of the Lincoln taxon. Although, due to a shortage of insects, we have not fully defined the sex pheromone blend of the Lincoln taxon and, in particular, unequivocally established the role of Z9–14 : Ald, we nevertheless find the evidence for Z9–14 : Ald being a critical element in the sexual communication system of this taxon to be compelling. This compound is the most abundant of the compounds identified in the gland of females of this taxon. Males of this taxon show a high-amplitude EAG response to this compound. In the field trials at Lincoln and Nelson, males were caught in high numbers in traps baited with the four common components plus 1000 $\mu$g of Z9–14 : Ald, as

well as in traps containing Z9–14 : Ald and lacking Z7–14 : OAc (in contrast, males of the Auckland taxon were not caught in significant numbers in traps baited with blends lacking Z7–14 : OAc). Finally, in the wind-tunnel experiment using extracts, the difference in response between males of the two taxa to their respective females' extract correlated with the major observed difference in extract from these females being Z9–14 : Ald content (i.e., either none or a relatively large amount). Therefore, the circumstantial evidence suggests that Z9–14 : Ald is a critical component in the sex pheromone of this taxon. If this is accepted, then it follows that the field-trapping results at Nelson and Lincoln, where males were caught in high numbers in traps baited with either the Lincoln-type blend or the Auckland-type blend is the result of both taxa being present in sympatry in these two locations. Further evidence in support of this comes from the field trial in Lincoln where traps baited with the Lincoln-type blend caught more than twice the number of males than traps baited with the Auckland type blend; unfortunately, only two replicates were used at Lincoln and so this difference was not statistically significant.

In contrast to the proposed effect of Z9–14 : Ald in the blend to Lincoln males, the presence of even small amounts of Z9–14 : Ald in the common four-component mix appears to inhibit the flight responses or field catches of *G. mutans* males from Auckland. Likewise, in field-trapping trials, the five-component blend that gave good catches of males in both Lincoln and Nelson did not catch any males in Auckland. Clearly, the very high relative proportion of Z9–14 : Ald produced by Lincoln females would result in the complete inhibition of upwind flight responses of the males of the Auckland taxon, as was observed in the wind tunnel using female extracts.

The effect of Z9–14 : Ald in blends on males of the two *G. mutans* taxa is contrasting; in one taxon (the Lincoln) it serves as a component (''major'') of the pheromone, in the other taxon (Auckland) it results in the arrestment of upwind flight responses when added to the pheromone blend. Compounds that are observed to have the latter function are usually viewed as ''reproductive isolating mechanisms'' between closely related species (in the sense outlined by Cardé 1986) and more recently as ''behavioral antagonists'' (Linn et al., 1988; Glover et al., 1989; Linn and Roelofs, 1989), i.e., their use in pheromone systems has evolved as a direct adaptation to ensure that so-called ''mating mistakes'' are reduced or eliminated. An alternative, more parsimonious explanation for the evolution of such compounds, based on the ''specific mate recognition'' theory of Paterson (1985), would be that Z9–14 : Ald has evolved in mate recognition in the Lincoln taxon. Therefore, the second function of this compound, as an intertaxon isolating mechanism or behavioral antagonist, would arise as an incident of recognition in the Lincoln taxon. Appealing as these possibilities are, neither parsimony nor the coincidence of the compound apparently eliciting different and exclusive behavioral responses from males of the

two taxa are evolutionary proof. Nevertheless, the two *G. mutans* taxa may offer an excellent example to study, and therefore test, specific mate recognition, reproductive isolation, or behavioral antagonism.

Given these differences between the pheromone systems of these two taxa, it appears that the two *G. mutans* taxa may in fact be distinct sibling species. This phenomenon in *G. mutans* is similar to that observed during similar studies on endemic New Zealand tortricid pests. These studies, led by sex pheromone analyses of different populations, showed that the previously described *Planotortrix excessana* and *Ctenopseustis obliquana* actually consist of two and three sibling species, respectively (Dugdale, 1990; Foster et al., 1991). These sibling species differ somewhat in their allozyme patterns and are morphologically very similar. However, there are major differences between their pheromone systems. The difference in the pheromonal communication systems of the two taxa of *G. mutans* is similarly striking, although, so far, no morphological difference between these two taxa has been detected (Dugdale, personal communication). More complete characterization of the sex pheromone of the Lincoln taxon, and further examination of the morphology, ecology, and allozyme patterns of these two taxa of *G. mutans* is planned and, it is hoped, will establish whether they should be considered as distinct sibling species.

In addition to elucidating the distinct pheromone taxa within *G. mutans*, we have identified and chemically and behaviorally characterized the sex pheromone of a *G. mutans* population from Auckland. The sex pheromone of this taxon consists of (at least) a four-component blend of two alcohols and two acetates. The most abundant compound identified in the gland was *Z*9–14 : OH; however, somewhat surprisingly, in our bioassays we found that this component could be removed from the blend without the complete suppression of either field catches or the upwind flight responses of males. In contrast, two of the less abundant components in the gland (*Z*9–14 : OAc and *Z*7–14 : OAc) could not be removed from the blend without complete suppression of field catches or arrestment of flight before the midpoint of the wind tunnel. At this time we do not have any information regarding the relative ratios of the compounds released in the effluvia of female *G. mutans*. However, it is interesting that the relative abundance of *Z*9–14 : OH in the gland does not correlate with a "major" behavioral function in the sense usually assigned to the most abundant component in a blend in pheromone studies. Rather this function is apparently served by *Z*7–14 : OAc and *Z*9–14 : OAc.

The fourth component in the blend of this taxon, *Z*7–14 : OH, when added in large amounts to the three-component blend significantly reduced trap catches. However, when *Z*7–14 : OH was completely removed from the blend, trap catches decreased slightly but not significantly. The effect of this component in the blend at relative ratios close to that found in the female was established in the wind tunnel. A comparative study using field-trapping techniques generally

observes differences in the end results of behavioral responses rather than differences in the actual behavioral responses themselves (Kennedy, 1977). Consequently, subtle effects in behavior elicited by chemicals are less likely to be detected by field-trapping techniques than by more direct, discriminating bioassays such as wind tunnels (Baker and Linn, 1984). By use of the wind tunnel we were able to establish the importance of *Z*7–14 : OH in the pheromone blend of *G. mutans*; the absence of this compound from the reference blend (i.e., four components) increased the percentage of males arresting flight before the midpoint of the tunnel and therefore resulted in a decrease in the percentage of males touching the source. The wind tunnel study also confirmed that the other three components elicited behavioral activity. Thus the absence of either *Z*9–14 : OAc or *Z*7–14 : OAc from the blend significantly decreased the percentages of males activating and taking flight relative to the four component blend, while significantly fewer males reached the midpoint of the tunnel when presented with the blend lacking *Z*9–14 : OH.

In many species of moths the ratios of pheromone components produced by females are regulated within fairly tight ranges that usually correspond with the ratios of these components to which males respond optimally (see for example Miller and Roelofs, 1980; Roelofs et al., 1975). Our data suggest that this is the case with *G. mutans*. Our initial formulation of a synthetic blend was based on the ratios observed in females. When various ratios in this blend were changed, generally the responses of males, as measured by trap catches and flight-tunnel behavior, decreased. In the flight tunnel, changes in the relative amounts of any of the four components were characterized by males arresting flight after activation and before reaching the midpoint of the tunnel rather than by arrestment at later stages (near the source) of upwind flight. A further study looking at more subtle changes in the various ratios along with examining the variability in production of the four components between individual females is planned.

The pheromone blends for both taxa of *G. mutans* can now be used for monitoring this pest in orchards. This should allow a better understanding of the pest status of both taxa and hence control programs can be modified for more effective control of these pests as necessary. The use of these two pheromone blends should also facilitate work examining the distribution of these two taxa around New Zealand.

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