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POST-LANDING BEHAVIOR OF ALATE MYZUS PERSICAE
AS ALTERED BY CHEMICAL REPELLENTS

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James R. Miller
Major professor

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POST-LANDING BEHAVIOR OF ALATE *MYZUS PERSICAE* AS ALTERED BY CHEMICAL REPELLENTS

by

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ABSTRACT

POST-LANDING BEHAVIOR OF ALATE *MYZUS PERSICAE* AS ALTERED BY CHEMICAL REPELLENTS

by

Paul Larry Phelan

Potential chemical disruption of *Myzus persicae* host-selction was investigated in an aphid flight chamber using (*E*)-β-farnesene (EBF) (an aphid alarm pheromone) and 3 fatty acids: undecanoic, dodecanoic and heptadecanoic acids. EBF reduced probe durations and increased wandering, however, aphids eventually settled on this treatment. Undecanoic and dodecanoic acids reduced all host-selection behaviors and aphids never settled on either fatty acid, even after several landings. Heptadecanoic acid had no significant effect on behavior.

To determine if fatty acid repellency was limited to aphids, the Pharaoh ant, *Monomorium pharaonis*, was observed on a randomized checkerboard distribution of fatty acid and control squares. Ants clearly avoided undecanoic and dodecanoic acids while heptadecanoic did not alter behavior.

Finally, a chemical separation technique was adapted for the separation of geometrical isomers of farnesene and other unsaturated insect pheromonal compounds. The method used reverse phase high pressure liquid chromatography with a mobile phase containing $AgNO_3$.

This work is dedicated to Paul and LaVerne,
for 26 years of sacrifice and boundless love.

ACKNOWLEDGEMENTS

To James R. Miller, my major professor, goes my deepest appreciation. Jim was not only a teacher, a friend, and a constant source of encouragement, but also proved to be a first rate auto mechanic and cook.

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INTRODUCTION

Aphids cause serious economic damage through several avenues. First, because aphids are capable of very high rates of reproduction, infestations can rapidly become heavy, leading to severe stress and even collapse of the plant. Secondly, damage may be caused by the copious amounts of honeydew which are excreted by aphid colonies. This very sugary substance causes mold to develop which interferes with the photosynthetic process. The third and probably most economically important damage caused by aphids is the transmission of plant viruses. Control of virus spread by killing aphids with pesticides has had mixed results. Systemic insecticides, for example, have been effective in controlling the spread of some persistent viruses. However, attempts to control non-persistent viruses by insecticides have failed. The use of oil sprays, on the other hand, have been successful in reducing non-persistent virus spread, although the mechanism involved here is not fully understood (Vanderveken 1972).

Finding a control measure which will act quickly, before test probes are made by the aphid is obviously very important. The discovery of a trans-specific aphid alarm pheromone, (*E*)-β-fanesene (EBF) (Dahl 1971, Kislow and Edwards 1972, Bowers *et al*. 1972, Nault *et al*. 1973, Wientjens *et al*. 1973) raised hopes that this might be used as a means of controlling virus spread, including non-persistent viruses. EBF looked particularly promising since non-feeding alates were the most sensitive aphid form, responding to 0.03ng applied to small filter paper triangles (Montgomery and Nault 1977).

For the present work, I tested EBF for potential virus control. Rather than take the material directly to the field, I chose to perform laboratory behavioral studies which allowed observation of individual alate responses when landing in the presence of EBF. To satisfy the alate requirement for migratory flight before settling, these studies were carried out in an insect flight chamber which allowed free flight and free landing of alates with a minimum of handling. Also tested for effects on alate landing behavior were 3 carboxylic acids: undecanoic, dodecanoic, and heptadecanoic acids. The first two compounds were reportedly repellent to apterous _Myzus persicae_ (Sulzer) and the third was said to enhance settling (Greenway _et al_. 1978).

Although EBF is easily synthesized by the dehydration of farnesol or nerolidol, commonly used methods (_eg_. Brieger _et al_. 1979) give rise to a mixture of farnesene isomers. These polyunsaturated hydrocarbons are very difficult to separate and require either gas chromatography or open-bed $AgNO_3$-impregnated column chromatography (Bowers _et al_. 1977), both of which are very tedious when large amounts of material are required. Before starting the behavioral studies, it therefore was necessary to find a method for purifying large quantities of EBF. The technique which proved most useful entailed reverse-phase high pressure liquid chromatography with a mobile phase containing silver nitrate. Because the need for large quantities of highly pure material is a common problem for others working in the insect pheromone field, application of the mobile Ag^{+}/RP HPLC technique was expanded to include a wide range of isomeric insect pheromonal compounds.

CHAPTER 1

POST-LANDING BEHAVIOR OF ALATE *MYZUS PERSICAE* AS ALTERED BY (*E*)-β-FARNESENE AND THREE CARBOXYLIC ACIDS

INTRODUCTION

Aphids constitute a major agricultural threat because of damage from direct feeding by a large number of individuals and, more importantly, from transmission of a large number of plant viruses. Thus, when aphids were reported to emit a broadly trans-specific alarm pheromone (Dahl 1971, Kislow and Edwards 1972, Nault _et al_. 1973) identified as (_E_)-β-farnesene (Bowers _et al_. 1972, Edwards _et al_. 1973, Wientjens _et al_. 1973), it was hoped that this compound might provide a novel means of control for aphids. Three primary methods for control have been suggested: 1) dispersal of established aphid colonies by broadcasting alarm pheromone (Bowers _et al_. 1972, Nault 1973), 2) use of alarm pheromone in combination with insecticides to increase the probability of aphids contacting the insecticide (Edwards _et al_. 1973, Nault 1973), and 3) prevention of alighting and/or probing by immigrant alates (Nault and Montgomery 1977, Nault and Montgomery 1979). This latter method would be particularly important for reducing virus transmission.

Phelan _et al_. (1976) showed that the first method was probably untenable, at least in a low growing, herbaceous crop. Aphids dispersed by alarm pheromone had a greater tendency to relocate on nearby hosts, thus increasing the overall level of infestation. Discussion of the second method will be deferred until later. The third control possibility was investigated by Hille Ris Lambers and Schepers (1978); they scattered PVC bars impregnated with (_E_)-ß-farnsene (EBF) throughout potato plots, and then tested for infection by potato virus Y^N, a non-persistent virus. No reduction in virus transmission was realized over control plots. However, it is difficult to know if failure was due to the ineffectiveness of EBF or the method of dispensing and distributing the chemical. For example, since no release rates were determined, it is not known whether enough material was being released to elicit behavioral responses over the relatively long distances between the pheromone sources (placed on the ground) and potential feeding sites on the plant. In all previous behavioral experiments, this alarm pheromone was dispensed within 3cm of aphid clusters. In addition, it is difficult to know whether the test plants were completely and constantly enveloped by pheromone plumes.

In light of these problems, I investigated the behavioral effects of EBF on alighting aphids under more controlled laboratory conditions. Also included in this study were 3 carboxylic acids: undecanoic, dodecanoic, and heptadecanoic acids. The first 2 were judged by Greenway _et al_. (1978) to deter settling and the third was reported to increase settling in apterous aphids. To investigate the action of the carboxylic acids as either species-specific or more broadly active, I tested them for repellency against the non-aphid-attending ant, _Monomorium pharaonis_ (L.).

MATERIALS

Rearing

Myzus persicae (Sulzer), the green peach aphid, was chosen for this study because it is ubiquitous, it is a transmitter of a large number of plant viruses, and it shows a strong alarm response to EBF (Montgomery and Nault 1977). Aphids were reared on radish, *Raphanus sativus* (L.) in a greenhouse under natural light supplemented by a 1:1 mixture of cool white™ and warm white™ fluorescent lighting. The lighting regime was 16L:8D, and the temperature 20-25°C. Aphids were contained by a saran™ plastic screen cage, the top of which formed a sharply sloped Plexiglas™ pyramid with a small hole at the apex. A 8.5cm diam cardboard cylinder, topped by a glass petri dish, was mounted over the pyramid. Alates ready for flight moved upward toward the light through the top of the pyramid to the petri dish. Test aphids were collected by removing the petri dish and covering it with a glass top. This collection method proved very convenient and required a minimum of handling, shown by Kennedy and Booth (1963) to affect aphid behavior.

Chemical treatments

Three carboxylic acids were tested for behavioral activity: undecanoic, dodecanoic, and heptadecanoic acids (Sigma Chemical, St. Louis, MO; ca 99% pure). Because of difficulties in purifying (*E*)-ß-farnesene, a synthetic mixture of 6 farnesene isomers containing 32% EBF was tested in the first experiment. This material was synthesized by farnesol dehydration (Brieger et al. 1979), and then purified via a Fluorisil column. A second experiment compared the activity of the farnesene isomer mixture and pure EBF (>99.9%). (E)-β-Farnesene was purified either by preparative GC or by the preferred technique of mobile Ag+/reverse phase high performance liquid chromatography (HPLC) (Phelan and Miller 1981). Purity of the material was determined by gas chromatography using a 2mm x 1.8mm column packed with 10% GE XF-1150 (50% cyanoethyl, methyl silicone) on 100/120 GasChrom Q.

Insect flight chamber

The main chamber was a 1x1x1m flat black box with a sliding Plexiglas™ front which used a counterweight system for easy manipulation. Above the main chamber was an upper compartment which supported a 400W metal halide lamp (General Electric, #MV400/BU/I) and ballast. The upper and main chambers were separated by 2 layers of cotton cloth (15 threads/cm) painted black except for a central 37cm diam light window.

Aphids released in the chamber flew upward to the light window. This vertical displacement of the aphid was countered by a fan which forced air through the top screen of the chamber. The vertical fan (Fig 1) was a variable speed ¼HP motor with a 40cm diam blade, capable of displacing 71CMM at a maximum speed of 1500RPM. Fan speed was controlled by a variable voltage transformer. The vertical position of the aphid could be maintained within an arbitrarily established 10cm flight zone by adjusting the fan speed in response to the aphid's continuously changing rate of climb. A hot wire anemometer (Hastings-Raydist, Hampton, VA) was mounted in the

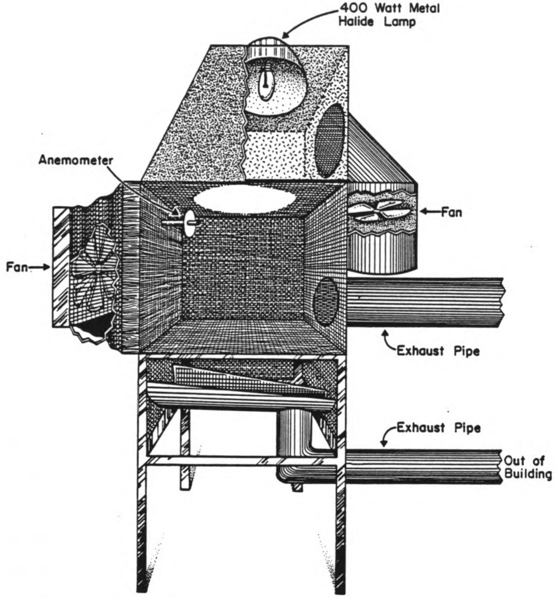

Figure 1. Insect flight chamber

chamber to measure changes in downward airflow which reflected changes in the photokinetic response of the aphid. The anemometer was wired to a strip chart recorder which provided a permanent record of flight behavior for each aphid. Due to a positive phototaxis, the lateral displacement of the aphid was restricted by the boundary of the light window throughout most of the flight.

Since the exhaust system was not capable of evacuating the entire chamber, I selectively eliminated only the central region of the chamber where the chemical sources were positioned during testing. When the vertical airflow was operating, chemically laden air was caught by a trough under the floor and directed out of the building via a 20 cm diam exhaust pipe. "Smoke tests" using titanium tetrachloride demonstrated that this system effectively removed contaminants from the flight chamber. Further, temporal spacing of experiments allowed the chamber to "air out."

A significant modification over previous flight chambers (Kennedy and Booth 1963, Kring 1966, Halgren and Rettenmeyer 1967, Kennedy and Ludlow 1974) was the use of a horizontal airflow in addition to the vertical. The horizontal airflow, important for the delivery of chemical treatments, was generated by a 50 cm diam 3-speed window fan (Fig 1) which forced air through 4 layers of black cheesecloth into the chamber. The opposite wall of the chamber supported a 20 cm diam exhaust pipe and was covered by an adjustable black cheesecloth screen (4 layers). By adjusting the distance between the screen and the exhaust, one could regulate the distribution of negative pressure across the plane of the screen. Because aphids have limited control over horizontal movement (Kennedy and Thomas 1974), a relatively slow airspeed (20 cm/s) was used on this axis. Although the lateral fan was always on, at this speed the horizontal airflow was not significant until the vertical airflow was greatly reduced.

<u>Artificial leaf plates and chemical delivery system</u>

A landing surface was provided for the aphids by artificial leaf plates (ALPs). ALPs were 75 x 75 mm tiles painted fluorescent yellow to provide a strong landing stimulus. A ridge of silicone sealant (<u>ca</u>. 4 mm high) was formed around the top edge of the tile to contain 5 ml of a 20% sucrose/ 0.1% L-methionine solution (Mittler 1967); this was covered by a stretched Parafilm™ membrane. Aphids readily probed through the membrane and fed on the solution. Chemical treatments were applied to the membrane as an acetone solution which contained 1.25% (w:w) Carboset 525 (B.F. Goodrich, Cleveland OH), a film-forming slow release agent. One mg of test compound (100 µl formulated solution) was applied to the ALP and was spread evenly with a small brush. The resulting concentration of active ingredient was 20 $\mu g/cm^2$; this level evoked an alarm response when brought near to a colony of <u>M</u>. <u>persicae</u>. In the case of the farnesene isomer mixture, application was based on EBF content not the total mixture.

Treated ALPs were placed on a stand consisting of a small wooden base and a vertical tube which held the ALP 31 cm above the floor of the chamber. A 10 x 10 cm wiremesh screen

(6 mm hole) positioned 15 cm to the upwind side (with respect to horizontal flow) of the ALP and an alligator clip between the screen and the ALP were attached to the vertical tube by a horizontal wire. The clip held a black cotton dental wick, which in addition to the chemical on the ALP, was impregnated with 1 mg of test chemical (no Carboset added). Titanium tetrachloride "smoke" demonstrated that the screen formed a low pressure area behind it when the vertical airflow was off. Thus as chemical was emitted from the wick, a "cloud" was created which surrounded the ALP. This secondary chemical source was necessary since smoke tests suggested that volatiles coming from the ALP itself formed a chemical plume which rose only _ca._ 1 cm above the plate.

METHODS

Exp 1- _Relative effects of 3 carboxylic acids and farnesene isomer mixture on alighting aphids_

In the first experiment, 6 treatments were tested: undecanoic acid, dodecanoic acid, heptadecanoic acid, the mixture of farnesene isomers, a Carboset control, and an untreated control. Aphids were held in the petri dish collector and allowed to acclimate for _ca._ 0.5 hr in the flight chamber. After the ALP, stand, and cotton wick were placed in the chamber, an aphid was removed from the dish with a small brush and brought up to the light window. After take-off, the vertical airflow was continuously adjusted to maintain the aphid within a flight zone 10 to 20 cm below the light window. The distance between the flight zone and the ALP was _ca._ 25 cm. Aphids were allowed to fly as long as they would do so; aphids landing on anything other than the ALP were brought back to the light with a brush. When an aphid landed on the ALP, its path was recorded on a grid representing the 49 cm^2 grid (1 sq/cm^2) on the ALP. Locations and durations of probes were also recorded. I considered the aphid to be probing when it remained motionless with the antennae laid back and the rostrum held perpendicular against the ALP surface. An aphid was allowed to land and take-off as many times as it would do so. An experiment was terminated by: 1) the aphid not flying when brought to the light, 2) losing the aphid in the chamber, or 3) a probe lasting longer than 10 min. I found that aphids probing for 10 min usually continued probing much longer, therefore, these aphids were designated as settled. The experimental design was a randomized complete block with 11 replications; each aphid was exposed to only one treatment. The length of aphid flights ranged from 0.25 to 4.0 hr, and the number of flights before settling ranged from 1 to 8.

Exp II- Relative effects of farnesene isomer mixture and (E)-β-farnesene on alighting aphids

To determine the possible effects of other farnesene isomers on EBF activity, pure EBF and the isomer mixture were compared in the flight chamber. Procedures were the same as in Experiment I except that the ALPs were replaced by radish leaves. Small radish plants were transferred to 50 ml beakers with soil, and all leaves were removed except one. The leaf, with an area of ca 12-15 cm^2 (1 side), was positioned with the stem vertical. A larger version of the chemical cloud-generating stand described earlier was used to accomodate the beaker. In this experiment, no treatment was applied to the leaf. Applications were made only to the cotton wick which was either impregnated with 700 μg EBF or 2100 μg farnesene mixture (containing 700 μg EBF) or left untreated. While no release rates were determined for cotton wicks, the rates were high enough to give a near 100% response when a colony of apterous aphids was placed in the chemical plume. This response could still be effected 24 hr after impregnation of the wick. As in Experiment I, aphid paths were recorded along with appropriate times. Occasionally, aphids in the colony would not respond to either synthetic EBF or crushed aphids. I have no explanation for this phenomenon and avoided testing on those days. The experimental design was a randomized complete block (n=12).

Exp III- Relative repellency of 3 carboxylic acids against the Pharoah ant

Undecanoic, dodecanoic, and heptadecanoic acids were tested for repellency against *Monomorium pharaonis* (L.), the Pharoah ant. Treatments were applied to a 25 x 25 cm Plexiglas^{TM} plate which was divided into twenty-five 5 x 5 cm squares. The carboxylic acids were combined with Carboset 525 and each acid was tested individually against Carboset-treated and untreated squares, using the same concentration (20 μg/cm^2) as in the aphid experiment. Treatments were randomly assigned to an equal number of squares (8), with the middle square remaining blank to serve as the site for ant release. Ants were placed in the release square with a small brush. The path of each ant was traced on a grid and times that the ant crossed from one square to the next were recorded. Observations ended when the ant left the board. Behavior in the release square was not included in the data presented. Thirty-six ants were tested for each carboxylic acid, and the treatments were re-randomized after every 6 ants.

RESULTS AND DISCUSSION

Action of farnesene mixture and EBF on host-selection behavior (Exps I & II)

The characteristic host-selection behavior of most species of aphids is to make one or more brief ($\leq$30 s) probes followed by a long probe. Nault and Gyrisco (1966) called the long probes, phloem-seeking probes, during which the stylets penetrated beyond the plant epidermis; the brief probes were termed test probes and were presumed to provide information about the quality of the host. Test probes by alighting aphids are important for the transmission of non-persistent plant viruses; Pirone and Harris (1977) have speculated that the uniqueness of this behavior is the reason that aphids and not other insects act as vectors of this group of viruses. Acquisition and inoculation of non-persistent viruses can result from probes as short as 5 s with the optimal range being 15-30 s (Pirone and Harris 1977). It is obvious then that for aphid host-selection disruption to be an effective control of non-persistent viruses, the disruption must occur before the test probe is made.

In the present study, neither the farnesene isomers nor the pure EBF significantly reduced the probability of a test probe (Table I). The farnesene mixture and EBF almost never completely prevented a test probe, and only in a few cases was flight resumed after making only probes which lasted <10 s (suboptimal for non-persistent virus transmission). If such were the case in the field, it is doubtful that any significant reduction of non-persistent virus spread would result.

The host-selection behavior of alighting green peach aphid was not, however, unaffected by EBF. Figure 2 illustrates the distribution of probes $\leq$60 s made during first landings on untreated, EBF-treated, and farnesene mixture-treated radish leaves. The propriety of the term "test probe" to encompass all probes $\leq$30 s is questionable. Nevertheless, I employ the term simply as a matter of convenience to refer to those probes in which the stylets of the aphid do not go beyond the plant epidermis (Nault and Gyrisco 1966). EBF significantly increased ($p<0.05$) the number of test probes made (Fig 2); 49 test probes were made on first landings with EBF present and 24 test probes were made on the control leaves. The increase caused by the farnesene mixture (35 probes) was not significant. Although EBF increased the number of test probes, the total number of probes (including both test probes and phloem-seeking probes) on first landing was not significantly different from the control: EBF, 61 probes; farnesene mixture, 49; and control, 51. This reflects a shift in mean probe duration in that probes $\leq$30 s constitute a greater pro-

Table I. Effect of 3 fatty acids, a farnesene isomer mixture and EBF on disruption of test probes by M. persicae alighting on ALPs (Exp I) and radish leaves (Exp II). No sign. diff.

Treatment	# landings without probes	# landings with <10 s probes only
Experiment I		
Control	0(26)[1]	0(26)
Carboset	0(25)	0(25)
Farn. mix	0(32)	0(32)
Undecanoic	3(33)	5(33)
Dodecanoic	2(45)	6(45)
Heptadecanoic	0(19)	0(19)
Experiment II		
Control	0(12)	0(12)
EBF	1(25)	3(25)
Farn. mix	0(21)	6(21)

[1]Number in parentheses represents total number of landings.

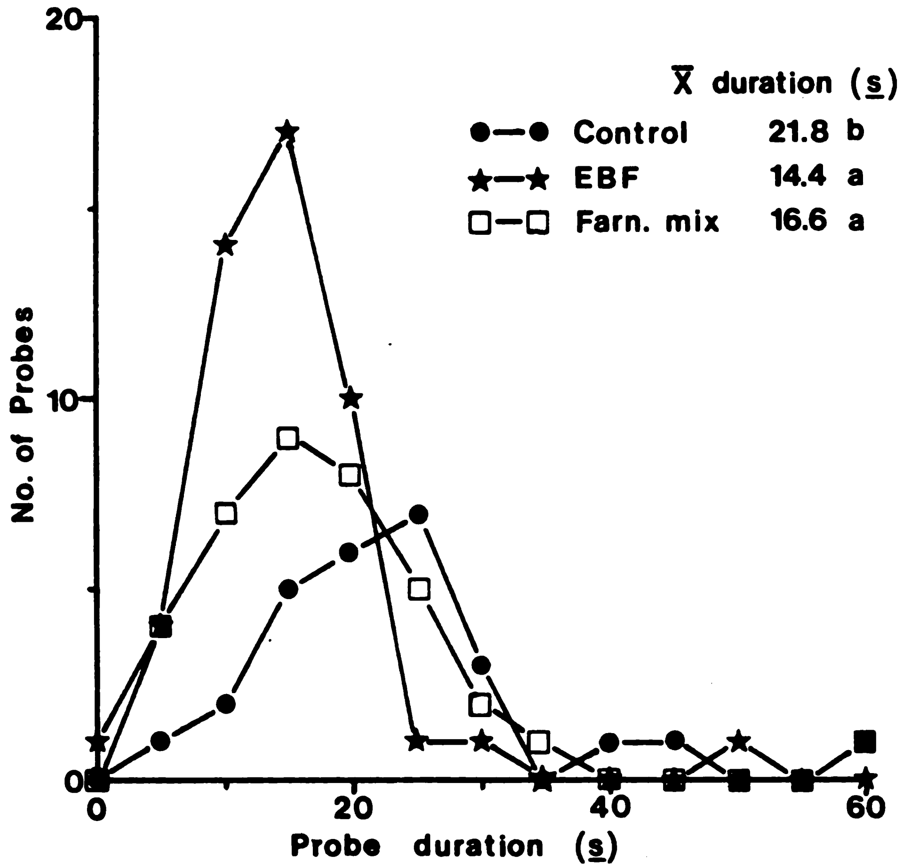

Figure 2. Distribution of test probe durations (in 5 s intervals) made by *M. persicae* on radish leaves treated with a mixture of farnesene isomers, pure (*E*)-β-farnesene, or left untreated. Numbers at upper right are mean test probe durations. Values followed by different letters differ significantly ($p<0.05$, planned F-test).

portion of the total EBF and farnesene probes: 80% for EBF, 71% for the farnesene mixture, but only 47% for the control. In addition, the distribution of test probes with EBF and farnesene mixture are shifted somewhat to the left of the control curve, with the mean test probe duration being significantly reduced by both chemical treatments ($p<0.05$) (Fig 2). The overall reduction in mean probe duration by EBF and the farnesenes is shown in Figure 3a, and the same result was observed for farnesene-treated ALPs (Fig 4a).

EBF and the farnesene mixture also significantly reduced settling, defined earlier as an uninterrupted probe lasting longer than 10 min. All 12 aphids settled on control leaves on the first landing, whereas only 6 settled on the first landing on farnesene-treated leaves, and only 4 on EBF. Both of these were significantly lower than the control (Chi square, $p<0.02$). Settling on farnesene-treated ALPs was lower, but not significantly so.

Alterations of other post-alighting behaviors on ALPs due to the farnesene mixture are illustrated in Figure 4(b-f) and farnesene and EBF effects on leaf landings are presented in Figure 3(b-d). The effects of the farnesene isomers on radish leaf landing behavior were: 1) a significant reduction in the total time spent probing per landing (Fig 3b), 2) no significant effect on the mean walk duration (Fig 3c), and 3) no significant effect on the total time spent walking per landing (Fig 3d). Beside the reduction in mean probe duration already mentioned (Fig 4a), the farnesene mixture had no significant effect on post-ALP-landing behavior.

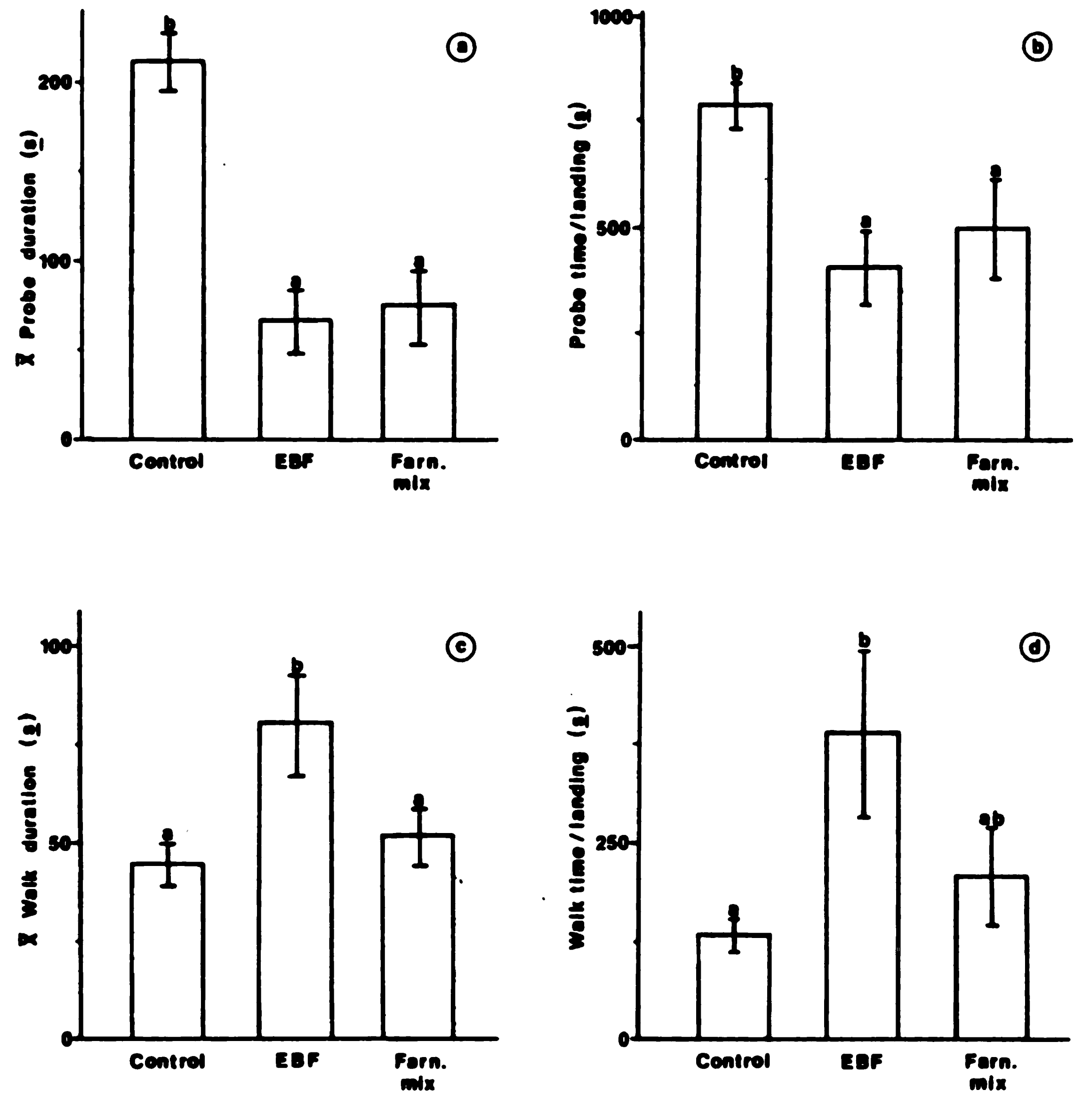

Figure 3. Effects of pure ($\underline{E}$)-β-farnesene (EBF) and a mixture of farnesene isomers containing EBF on various aspects of post-landing behavior of M. persicae. T-bars denote standard errors. Means marked by different letters are significantly different ($p<0.05$, planned F-test).

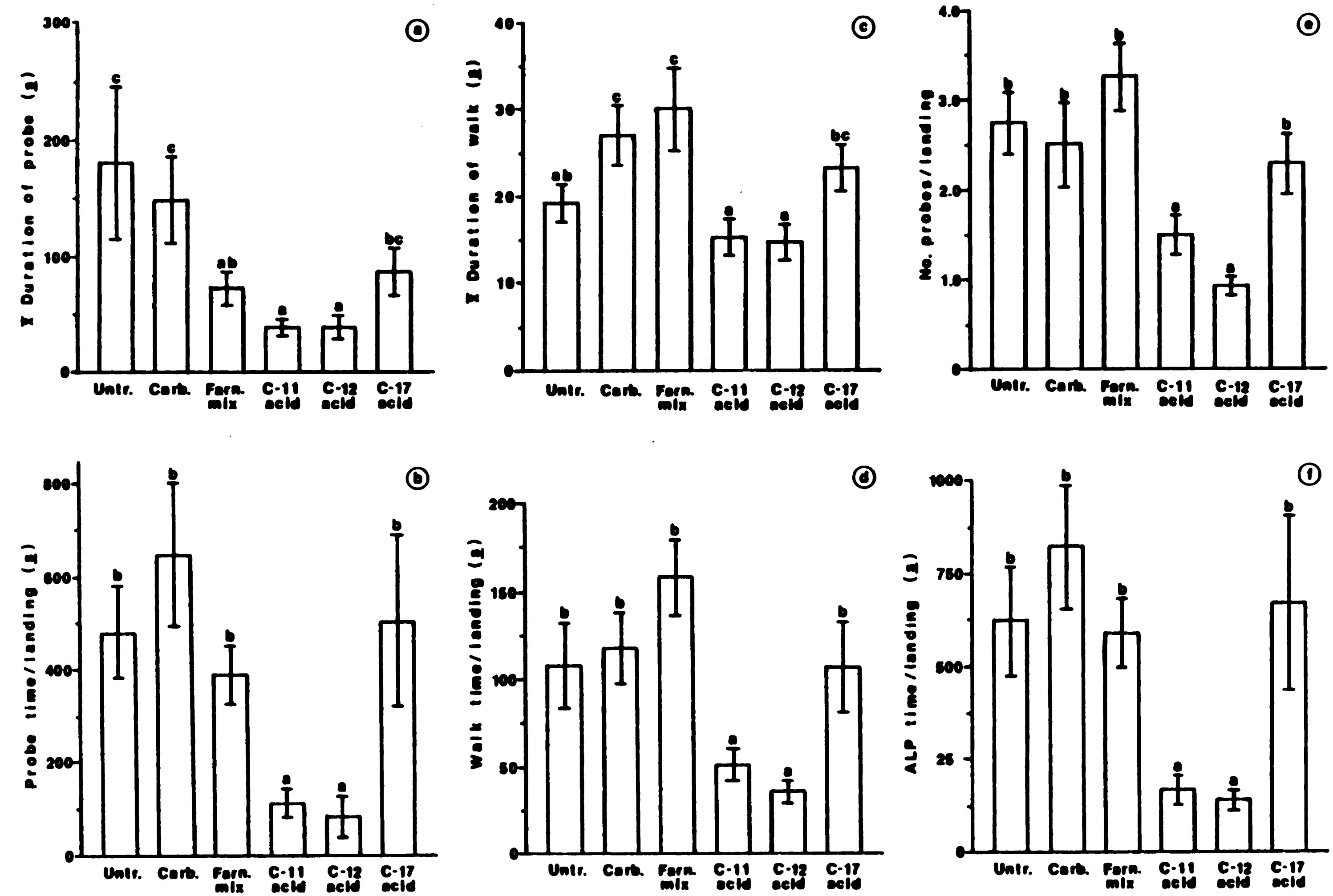

Figure 4. Various aspects of M. persicae post-landing behavior on ALPs as affected by a farnesene isomer mixture containing (E)-β-farnesene and by 3 fatty acids. T-bars denote standard errors. Means marked by different letters are significantly different ($p<0.05$, planned F-test).

The effect of EBF on probe duration (Fig 3a) and total time spent probing per landing (Fig 3b) were very similar to that of the farnesenes; both parameters were significantly reduced compared to the control. However, EBF also caused a significant increase (relative to the control) in the mean length of walks (Fig 3c) and time spent walking per landing (Fig 3d), both of which were unaffected by the farnesene mixture. It appears that the addition of the other farnesene isomers suppresses this behavioral effect. However, since no release rate studies were performed, I cannot say that the differences between pure EBF and EBF with the other isomers was not due to differential release rates from the cotton wick, although both treatments contained an equal amount of EBF.

It is interesting that the alate response to EBF was more frequently an increase in the amount of walking, rather than an increase in take-offs. If the field response is to remain on the plant and spend significantly more time wandering, combining EBF with insecticides as suggested in the Introduction might have potential for success. In a recent study, Griffiths and Pickett (1980) were able to bring about a 2.4-fold increase in the effectiveness of permethrin under lab conditions by first exposing aphid colonies to a mixture of farnesene isomers containing 16% EBF. Although their tests were carried out on established colonies of apterous aphids, my results suggest that such a treatment might also be effective against immigrating alate aphids. My results also suggest that combining pure EBF with insecticides might provide a greater synergistic effect than a farnesene mix/insecticide combination.

Effect of carboxylic acids on aphid settling behavior

As shown in Figure 4(a-f), the behavior of alate *Myzus persicae* on heptadecanoic acid-treated ALPs was not significantly different from either the untreated or Carboset control. Thus, no enhancement of settling was observed for this compound. Since each aphid was exposed to only 1 treatment, these results are consistent with the earlier work of Greenway *et al*.(1978). Although they concluded that heptadecanoic acid enhanced settling by apterous *M. persicae* in a choice test, they found it had little activity in a no-choice situation.

The effects of undecanoic and dodecanoic acids were very different from heptadecanoic acid, both clearly disrupting the host-selection behavior of alighting aphids (Fig 4a-f). Like the farnesene isomers, undecanoic and dodecanoic acids failed to reduce significantly the probability of a test probe (Table I). However, unlike the farnesene isomers, undecanoic and dodecanoic acids effected a significant reduction in all host-selection behaviors recorded. The average length of a probe was 148 s on the Carboset control (Fig 4a); on undecanoic and dodecanoic acids, it was only 37 and 39 s, respectively. The number of probes made per landing was also reduced by the 2 acids (Fig 4e). Thus, the end result was a reduction in the total time spent probing per landing (Fig 4b), 655 s for the Carboset control, 113 s for the undecanoic acid and 96 s for dodecanoic acid. Similar reductions were also effected in the mean walk time and walk time/ landing (Fig 4c&d). The most prominent effect of the 2 acids was the absolute disruption of settling (> 10 mim probe); in a total of 33 landings on undecanoic and 44 landings on dodecanoic, no aphids probed for longer than 3.5 min.

Acknowledging that the extrapolation of lab results to field conditions should be done with caution, it would appear that as with EBF, undecanoic and dodecanoic acids have little promise in controlling non-persistent virus spread. Based on the observation that these 2 treatments did not inhibit test probes and increased the mobility of alate aphids, it is conceivable that these compounds would increase non-persistent virus spread. In the case of persistent viruses which require longer probes for transmission, these compounds might provide some measure of control. The use of undecanoic and dodecanoic acids as feeding deterrents, however, appears to hold greater promise. Even after several landings on these treatments, aphids refused to settle. This is significant because there appears to be no sensory adaptation or habituation by the aphid to these chemicals. Further testing needs to be done to determine the minimum dosages needed for aphid response. Also since treatments have only been applied to Parafilm™ membranes, future experiments should include host plants.

<u>Repellent activity of carboxylic acids against *M. pharaonis*</u> (Exp III)

The pattern of carboxylic acid activity was the same for Pharoah ants as it was for the aphids; undecanoic and dodecanoic acids were repellent to the ants and heptadecanoic acid had no apparent effect. The average distance traveled by an ant on undecanoic acid-treated squares (1.6 cm, Table II) was almost 10 times less than that covered on Carboset alone (15.4 cm). In an average 69.9 s perambulation, less than 2.0 s (3%) was spent on undecanoic acid as compared to 38.5 s (55%) on Carboset and 29.4 s (42%) on untreated squares (Table II). The comparison between dodecanoic acid and the Carboset control of that experiment is similar; ants spent ca. 1/5 as much time and traveled ca. 1/4 the distance on dodecanoic acid as on the Carboset control. The times spent and distances traveled on heptadecanoic acid-treated squares were similar to those on the Carboset control. Likewise, Carboset values were never different from the untreated control. Values for the 2 controls, however, did vary between fatty acid experiments for some parameters (eg. distance, rate of movement, and encounters). Unfortunately, the source of these differences cannot be determined since the three experiments were executed separately. Thus the compounds could be differentially affecting the behavior of the ants in the control squares or the differences may simply be due to differences in ant populations.

Two representative ant tracks are presented from each carboxylic acid experiment in Figure 5. One track was randomly chosen for each acid from all the ants observed. For convenience of presentation, a second track was randomly chosen from the same block as the first track so that the treatment pattern would be the same. No trail-following was observed in any of the ants tested.

In addition to locomotory behavior, grooming behavior was also tabulated. Grooming primarily consisted of cleaning the

Table II. Locomotory behavior of *M. pharaonis* on fatty acid-treated, Carboset-treated, and untreated squares in a randomized checkerboard distribution.

Treatment	Distance (cm)	Time (s) w/groom[1]	Time (s) no groom[2]	Linear Velocity (cm/s)	Rate of Turning (t/cm)
Exp. 1 (n=34)					
Untreated	14.7a[3]	29.4a	16.2a	0.9a	0.4a
Carboset	15.4a	38.5a	22.0a	0.7a	0.5a
Undecanoic	1.6b	2.0b	2.0b	0.8a	0.6a
Exp. 2 (n=31)					
Untreated	29.9a	74.8a	59.3a	0.5a	0.5a
Carboset	24.5a	61.3a	48.2a	0.5a	0.5a
Dodecanoic	6.0b	12.0b	12.0b	0.5a	0.5a
Exp. 3 (n=36)					
Untreated	14.9a	29.8a	25.8a	0.6a	0.4a
Carboset	11.5a	28.8a	22.6a	0.5a	0.4a
Heptadec.	13.5a	27.0a	24.8a	0.5a	0.4a

[1] includes time spent in grooming

[2] does not include grooming time. This value used for determining rate of locomotion.

[3] Values within the same experiment and followed by different letters differ significantly ($p<0.05$, planned F-test).

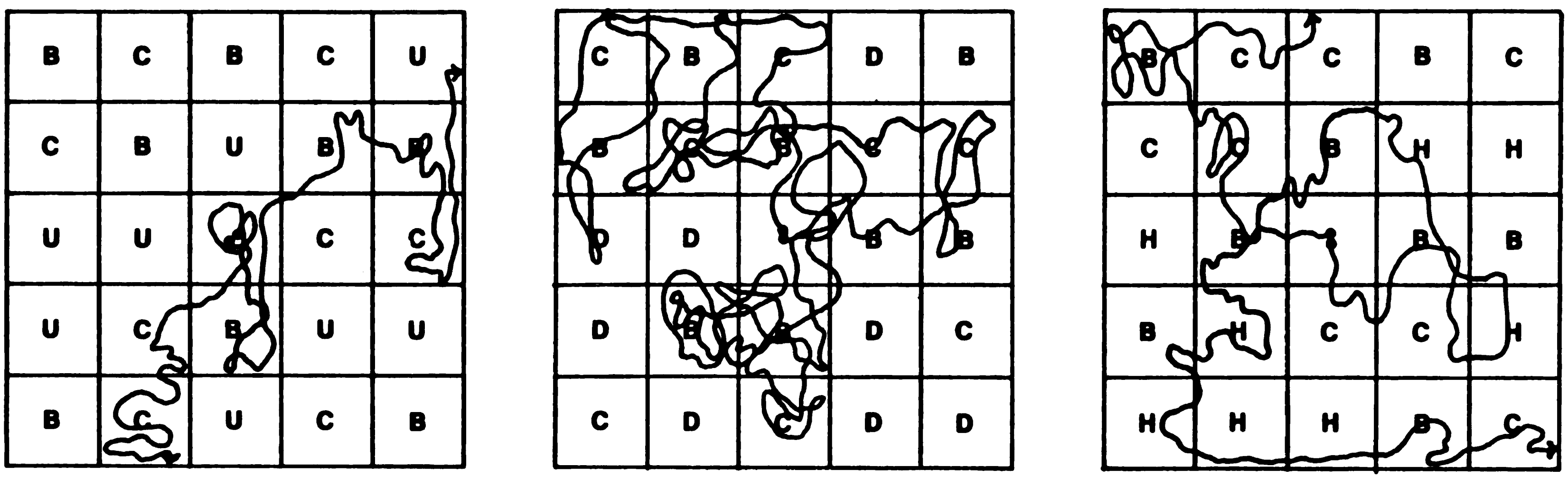

Figure 5. Randomly chosen tracks (2 per experiment) made by Pharoah ants on a distribution of squares left untreated (B), treated with Carboset 525 (C), or treated with a fatty acid and Carboset (U=undecanoic, D=dodecanoic, and H=heptadecanoic).

head and antennae with front tarsi and spurs and rubbing the abdomen with the hind legs. Grooming behavior was categorized as: 1) within treatment grooming, the length of grooming while in a treatment, expressed as a ratio of the number of visits to that square, and 2) post-encounter grooming, a measure of grooming after contacting a treatment, expressed as a ratio of the number of encounters with that treatment. An encounter occurred when an ant came within 0.5 cm of a treatment border, whether or not the border was crossed. Grooming never occurred within either undecanoic or dodecanoic acid-treated squares (Table III); there was, however, a significant increase in grooming immediately following encounters with either of these compounds, relative to the controls. Heptadecanoic acid once again had no effect.

Four orientation mechanisms have been described by Fraenkel and Gunn (1961) which allow organisms to reduce contact with maladaptive conditions: orthokinesis, klinokinesis, klinotaxis, and tropotaxis. Orthokinesis is a change in linear velocity which is roughly proportional to the magnitude of the stimulus. Thus, by increasing the linear velocity, an organism reduces the time spent on a repellent material, but not the distance. In the present experiment, this mechanism appeared not to be operating; when the time which the ants spent grooming was not included, the rate of locomotion remained unchanged between the controls and any of the fatty acids (Table II). Klinokinesis is a change in either the rate of random turning or angular velocity. In determining rates of turning, a turn was defined as a deviation of $> 30°$ from a straight couse. The

Table III. Length of grooming by M. pharaonis presented with respect to the treatment on which the behavior occurred (within treatment grooming) and the treatment encountered immediately prior to grooming (post-encounter grooming).

Treatment	Within treatment grooming (s)/visit[1]	Post-encounter grooming (s)/encounter[1]
Exp. 1 (n=34)		
Untreated	5.42(455)[2] a[3]	1.12(113)a
Carboset	5.78(549) a	0.88(114)a
Undecanoic	0.00 (0) b	8.84(898)b
Exp. 2 (n=31)		
Untreated	2.84(449) a	1.02(213)a
Carboset	2.07(283) a	0.92(162)a
Dodecanoic	0.00 (0) b	6.19(354)b
Exp. 3 (n=36)		
Untreated	1.34(149) a	1.81(214)a
Carboset	2.07(180) a	1.31(140)ab
Heptadec.	0.84 (81) a	0.39 (46)b

[1]See text for further explanation of terms.

[2]Totals are given in parentheses.

[3]Values within the same experiment and followed by different letters differ significantly ($p<0.05$).

rates of turning by the ants (Table II) were very similar on all treatments encountered, although the severity of turns (ie. degrees/turn) was not calculated and could have contributed to the avoidance mechanism.

Taxes, unlike kineses, represent directed non-random movements toward or away from a stimulus, and require a steep gradient of the stimulus, a condition found within a short distance of a chemical source. Tropotaxis is that behavior in which a simultaneous comparison of stimulation intensity between 2 receptor organs is made. Movement occurs either in the direction of the less stimulated (negative tropotaxis) or more stimulated (positive tropotaxis) receptor. Ant interaction with the treatment squares was classified according to 3 categories (Table IV): 1) treatment encounters, previously defined as that situation in which the ant came within 0.5 cm of the treatment border; 2) treatment visits, in which the ant actually entered the treatment square; 3) shallow penetrations, where after entering a square, the ant reversed direction before penetrating >0.5 cm.

The number of treatment encounters did not significantly differ for undecanoic or heptadecanoic acids relative to the controls. Dodecanoic acid encounters, however, were significantly lower than both controls. The number of visits to undecanoic acid-treated squares expressed as a ratio of treatment encounters was significantly lower than either control. The same was true for dodecanoic acid. Heptadecanoic acid, once again, had no effect. In the category of shallow penetrations, 57% of undecanoic acid square visits were aborted

Table IV. Behaviors[1] of *M. pharaonis* at the borders of treatment squares in a randomized checkerboard distribution.

Treatment	Treatment encounters	% encounters that resulted in visits	% visits that resulted in only shallow penetrations
Exp. 1 (n=34)			
Untreated	101a[2]	0.84(84)[3]a	0.05 (4)a
Carboset	115a	0.83(95) a	0.04 (4)a
Undecanoic	102a	o.23(23) b	0.57(13)b
Exp. 2 (n=31)			
Untreated	178a	0.90(160)a	0.15(23)a
Carboset	163a	0.84(137)a	0.16(22)a
Dodecanoic	69b	0.59 (41)b	0.66(27)b
Exp. 3 (n=36)			
Untreated	126a	0.88(111)a	0.15(17)a
Carboset	102a	0.85 (87)a	0.16(14)a
Heptadec.	106a	0.92 (97)a	0.15(15)a

[1]Table III, footnote 1

[2]Table II, footnote 3

[3]Table III, footnote 2

before the ants traveled 0.5 cm into this treatment, a significantly greater proportion than for either control. Similarly, 66% of dodecanoic acid visits resulted in a <0.5 cm penetration, as compared to 15% and 16% for the untreated and Carboset squares, respectively. Heptadecanoic acid had no effect on shallow penetrations.

These behavioral observations suggest that the avoidance mechanism used by the ants was tropotaxis. However, without appropriate experiments on unilateral extirpation of paired receptors, klinotaxis cannot be eliminated. Hangartner (1967) was able to demonstrate tropotaxis in Lasius fuliginosus in relation to trail following. Upon removal of one antenna, workers "veered" in the direction of the intact antenna along an artificial trail. The extent of the veering increased with increased concentration of the trail. When antennae were crossed, ants consistently turned away from chemical trails.

The site of fatty acid reception either for the ants or aphids was not addressed by the present study. Presumably, reception is either on the antennae or the tarsi, or both. Whether contact is needed for repellency is also unclear for all cases. Since significantly fewer ants came within 0.5 cm of the dodecanoic acid squares than either control, olfactory perception must occur for this compound. However, avoidance of undecanoic acid was only observed when the ants came within 0.5 cm of the treatment. Once again experimental design does not allow comparisons between acids, therefore it is not possible to deduce if this represents a difference in fatty acid action.

The repellent activity of certain fatty acids appears not to be limited to *Myzus persicae* and *Monomorium pharaonis*. Hwang *et al*. (1980) report ovipositional deterrency by 10^{-4}M to 10^{-3}M solutions of octanoic, nonanoic, decanoic acids in 3 species of mosquitoes. Oviposition in *Aedes aegypti* was significantly deterred by 10^{-5}M solutions of nonanoic acid. Fatty acid toxicity is also reported in some insects. LaLonde *et al*. (1979) found significant larvicidal activity in *Aedes triseriatus* with dodecanoic, tetradecanoic, and *cis*-9-hexadecenoic acid (LD_{50} values= 7, 4, and 3 ppm respectively). Decanoic, octanoic, and hexanoic acids are toxic to larvae of the sarcophagid fly *Psuedosarcophaga affinis* (House 1967). Toxicity of various fatty acids is also reported for a number of aphid species, including *Myzus persicae* (O'Kane *et al*. 1930, Siegler and Popenoe 1924, Tattersfield and Gimingham 1927, Puritch 1975). For *M. persicae*, mortality was greatest for dodecanoic and decanoic acids. Thus peak toxicity correlates with peak repellency as determined by Greenway *et al*. (1978).

It is of interest to note that the cornicle secretions of aphids are composed primarily of medium chain fatty acids. Although Strong (1967) and Callow *et al*. (1973) report that the fatty acids are present only in the triacylglycerol form, the aphid extraction procedure of Greenway *et al*.(1978) suggests that free fatty acids are present. If the latter case is true, one might speculate that aphids use the broadly repellent and/or toxic qualities of these fatty acids for defense. The defensive function of the non-volatile portion of the cornicle droplets has been documented (Dixon 1958, Dixon and Stewart

1975), however, the defensive nature has been attributed to "gumming up" the predator's mouthparts. The presence of repellent fatty acids in the secretions may supplement this role.

CONCLUSIONS

Behavioral tests of (_E_)-β-farnesene, the aphid alarm pheromone, and 3 fatty acids produced no candidates for control of non-persistent plant viruses. While EBF, undecanoic, and dodecanoic acids disrupted normal host-selection behavior, they did not prevent probing by alighting _Myzus persicae_. In fact, due to the stereotypic nature of probing, which usually occurred immediately upon landing, the potential of other compounds eliminating probing behavior by aphids seems doubtful. EBF was not a powerful repellent since the majority of alates eventually settled on EBF-treated artificial leaf plates and radish leaves. EBF did significantly increase the time spent walking before settling. This suggests that combining EBF with insecticides could enhance the effectiveness of the latter through increased contact.

Undecanoic and dodecanoic acids appear to have some potential for reducing infestation by aphids. At the concentration tested, I found these compounds were highly deterrent to the feeding and settling of alighting _M. persicae_. In addition, the action of medium chain fatty acids is broad spectrum, with repellency or toxicity being demonstrated in such diverse groups as aphids, ants, flies, and mosquitoes. It is hoped that further work on medium chain fatty acids and possible analogs will help determine their potential for insect control.

CHAPTER 2

SEPARATION OF ISOMERIC INSECT PHEROMONAL COMPOUNDS USING REVERSE PHASE HPLC WITH $AgNO_3$ IN THE MOBILE PHASE

INTRODUCTION

The separation of geometrical isomers of insect pheromones has been achieved utilizing the π-electron complexing abilities of silver nitrate in column chromatography, silver resin cation-exchange chromatography (Warthen 1976, Houx *et al*. 1974), and $AgNO_3$-impregnated silica gels in high performance liquid chromatography (HPLC) (Heath *et al*. 1975, Heath *et al*. 1977). Gas chromatography (GC) is capable of effecting a good separation of most of these compounds, which are primarily open chain unsaturated acetates, alcohols, aldehydes, and hydrocarbons. However, the requirement for large quantities of highly pure material (>99.9%) (Hill and Roelofs 1975) for field studies makes GC collections impractical. In this study, the application of a new HPLC technique to pheromonal isomer separation is presented. This approach, first reported by Janák *et al*. (1970) for column chromatography and Schomburg and Zegarsky (1975) for HPLC, entails reversed-phase (RP) HPLC with a polar mobile phase which contains $AgNO_3$. In the past, the mobile Ag^+/RP method has separated a wide range of unsaturated compounds: short chain 2-alkenes and polyunsaturated cyclic hydrocarbons (Schomburg and Zegarsky 1975), straight chain and cyclic mono- and polyunsaturated hydrocarbons (Schomburg and Zegarsky 1975, Vonach and Schomburg 1978), unsaturated fatty acids, triglycerides, heterocyclic hydrocarbons (Vonach and Schomburg 1978), and pharmaceutical compounds (Tscherne and Capitano 1977). The use of this technique to separate positional isomers and geometrical isomers of mono-, di-, and polyunsaturated insect pheromones is reported.

EXPERIMENTAL

Instruments and chemicals

The analytical liquid chromatographic system (Waters Assoc., Milford MA) used in this work consisted of a U6K injector with a 2 ml sample loop, a Model M6000A solvent delivery system, a Model 440 UV absorbance detector with a fixed wavelength (254 nm), and a Series R-400 differential refractometer. Separations were carried out on a 4 mm x 30 cm Waters' μBondapak™ C_{18} column or a 4.6 mm x 24 cm DuPont (Wilmington DE) Zorbax™ ODS column. Two preparative systems were also utilized; the first consisted of a Milton-Roy (State College PA) pump with a maximum flow of 40 ml/min and a 2.5 cm x 30 cm glass column packed with E. Merck (Darmstadt, West Germany) LiChroprep™ RP-18 (25-40 μm). The Waters' R-400 refractometer was adapted to this system via a flow splitter. The second preparative system was a Waters PrepLC™/System 500 with a maximum flow of 500 ml/min. This system required a PrepPak™ 500/C_{18} cartridge (5.7 cm x 30 cm) which is held under radial compression to increase chromatographic bed uniformity.

Mobile phase solvents consisted of "distilled in glass" methanol (Burdick and Jackson Labs, Muskegon MI), distilled water, and Mallinckrodt (St. Louis MO) Analytical Reagent™ silver nitrate. Solvents were filtered, degassed, and continuously stirred during separation runs.

RESULTS AND DISCUSSION

Mono-unsaturated geometrical isomers

Several straight chain mono-unsaturated geometrical pairs represent 3 different functional groups and 3 chain lengths were tested. Using a mobile phase of 20% H_2O/80% MeOH with 100 mM $AgNO_3$ and the μBondapak analytical column, very high resolutions were achieved; however, 50 mM was sufficient for baseline separation of the isomeric pairs. Results using this concentration are reported in Table V and Figure 6 shows 3 representative chromatograms. For all compounds tested, separation was easily achieved in less than 10 min, usually less than 5 min. Although the functional group and chain length affected the retention volume, separations were effected irrespective of these factors.

Mono-unsaturated positional isomers

Separations of compounds differing only in the position of the double bond was attempted using (Z)-isomers of 12-carbon chain acetates. Using the Waters' μBondapak C_{18} column, 30% H_2O/70% MeOH with 100 mM $AgNO_3$ was the best mobile phase. However, the DuPont Zorbax ODS column, used with a mobile phase of 20% H_2O/80% MeOH/100 mM $AgNO_3$ provided better separation than the μBondapak column. Separation of adjacent positional isomers are given in Table VI. Figure 7 shows the retention volumes of the various isomers, demonstrating the relative order of elution and the effect of double bond position on the degree of Ag^+-complexation. Starting from the

Table V. Separation of mono-unsaturated geometrical isomers using 20% H_2O/80% MeOH/ 50mM mM $AgNO_3$ and μBondapak column.

Compound	Resolution	V_{R_Z}/V_{R_E}	Flow/Press. ml/min/psi
(Z)/(E)-7:12 Ac	2.1	10.4/13.9	3.0/2000
(Z)/(E)-7:16 Ac	2.3	34.3/48.4	5.3/3500
(Z)/(E)-11:16 Ac	2.6	35.5/50.4	5.3/3500
(Z)/(E)-7:12 OH	2.0	6.4/8.0	0.9/550
(Z)/(E)-8:12 OH	2.0	6.6/8.2	1.6/1000
(Z)/(E)-9:12 OH	2.0	6.2/7.8	0.9/550
(Z)/(E)-11:14 OH	2.1	9.7/13.1	3.0/2000

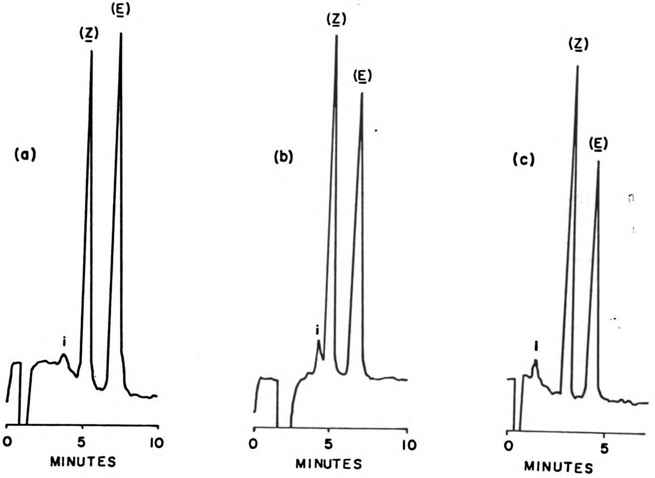

Figure 6. Separation of mono-unsaturated geometrical isomers.

a) (Z) and (E)-7-hexadecenyl alcohol, 3.5 ml/min (2500 psi);

b) (Z) and (E)-11-tetradecenyl aldehyde, 1.9 ml/min (1300 psi);

c) (Z) and (E)-11-tetradecenyl acetate, 6.0 ml/min (4000 psi).

Mobile phase: 20% H_2O/80% MeOH/50 mM $AgNO_3$. Column: DuPont Zorbax ODS. Detector: RI = 4X. i = impurity.

Table IV. Separation of adjacent positional ($\underline{Z}$)-isomers of dodecenyl acetates using 20% H_2O/80% MeOH/100 mM $AgNO_3$ and DuPont Zorbax ODS column.

Compound	Resolution	Flow/Press. (ml/min)/(psi)
($\underline{Z}$)-3:12 Ac		
	3.9	3.0/3400
($\underline{Z}$)-4:12 Ac		
	2.5	2.7/3100
($\underline{Z}$)-5:12 Ac		
	2.5	2.2/2500
($\underline{Z}$)-6:12 Ac		
	0.6	1.6/1900
($\underline{Z}$)-7:12 Ac		
	0.5	1.9/2200
($\underline{Z}$)-8:12 Ac		
	1.0	1.9/2200
($\underline{Z}$)-9:12 Ac		
	2.9	1.6/1900
($\underline{Z}$)-10:12 Ac		
	1.7	1.6/1900
($\underline{Z}$)-11:12 Ac		

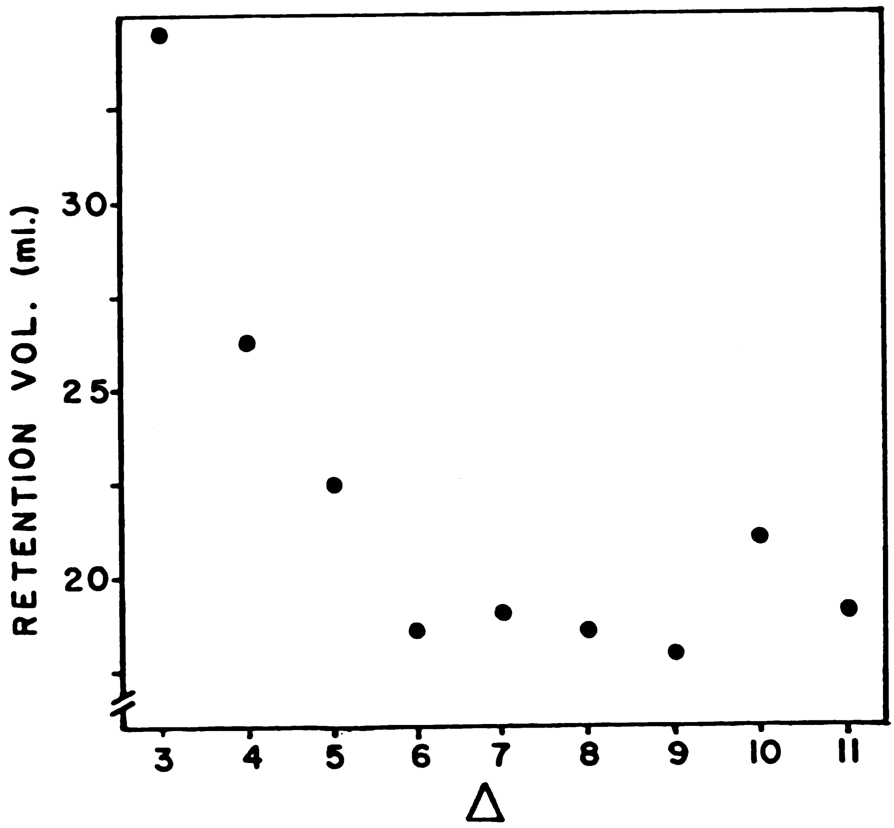

Figure 7. Retention of volumes of various positional (Z)-isomers of dodecenyl acetates. Mobile phase: 20% H_2O/80% MeOH/100 mM $AgNO_3$. Column: DuPont Zorbax ODS. Note: double bond at the 11-position does not show geometrical isomerism.

6-position, the retention volume increased dramatically as the position of the double bond approached the acetate functional group. This might be explained by a reduction in Ag^+-complexation due to: 1) a steric hindrance of the double bond by the acetate group, 2) delocalization of the π-electrons by the functional group, as suggested by Morris et al. (1967), or more likely, 3) a combination of the two.

The effect of varying position of the double bond through the remainder of the molecule is somewhat more difficult to explain. Morris et al. (1967) found a sinusoidal pattern in the migration of positional isomers of octadecenoates on Ag^+-impregnated thin layer plates. However, no recognizable pattern in the elution of isomers was found in the present study as the double bond moved from the 7- to the 11-position.

Di- and polyunsaturated compounds

Isomers of the doubly-unsaturated compounds, 7,11-hexadecadienyl acetate and 3,13-octadecadienyl acetate, were separated on the DuPont Zorbax ODS column. Although all 4 isomers of the 18-carbon acetate were used, only the (Z,Z)- and (Z,E)-isomers of hexadecadienyl acetate were available. The results of these separations are given in Figure 8. As expected, the doubly-unsaturated compounds, which engage in greater Ag^+-complexation, had a lower optimal silver ion concentration in the mobile phase than was found for mono-unsaturates.

The polyunsaturated compounds tested in this system were farnesols and farnesenes. Farnesol has 3 double bonds, 2 of which exhibit geometrical isomerism. Farnesene has 4 double

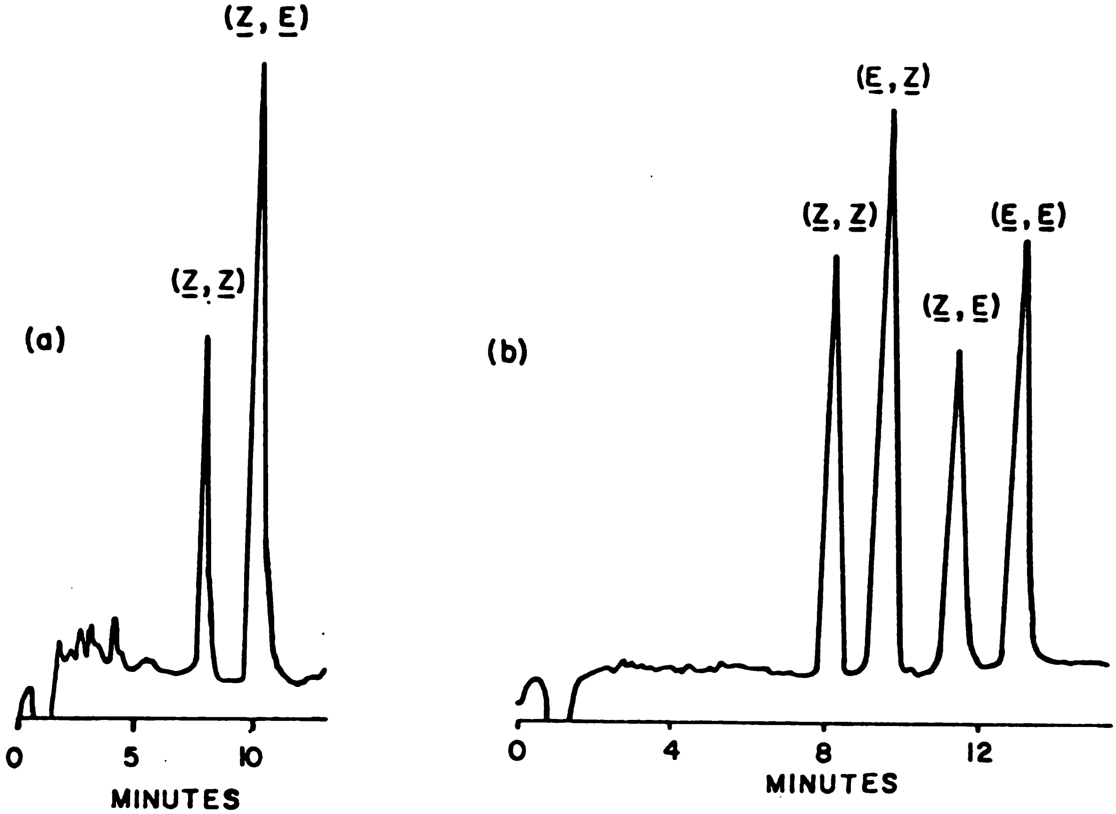

Figure 8. Separation of di-unsaturated geometrical isomers.

a) (Z,Z) and (Z,E)-7,11-hexadecadienyl acetate, 3.5 ml/min (4000 psi), 20% H_2O/80% MeOH/50 mM $AgNO_3$. Zorbax™ ODS, RI = 4X;

b) (Z,Z), (E,Z), (Z,E), and (E,E)-3,13-octadecadienyl acetate, 3.5 ml/min (3000 psi), 10% H_2O/90% MeOH/50 mM $AgNO_3$, Zorbax ODS, RI = 4X.

bonds, and an attempt was made to separate the (*E*)-β-isomer, an aphid alarm pheromone (Bowers *et al*. 1972), from (*Z*)-β- and the α-farnesenes. In addition, both the farnesols and the α-farnesenes have methyl substitution at the isomeric 3- and 7-double bonds. Substitution at the double bond is reported to hinder strongly Ag^+-complexation (Vonach and Schomburg 1978). Furthermore, in farnesenes, double bonds at the 1- and 3-positions form a conjugated diene system; conjugation of the double bonds also reduces complexation (Vonach and Schomburg 1978). Figure 9 shows the separation of farnesol isomers on the C_{18} column. Although I expected this separation to improve by using the more effective Zorbax ODS column, in fact, resolution was poorer. This was possibly due to a lower water concentration in the mobile phase, which was necessary to maintain a reasonable pressure in the ODS column. An unexpected result of this separation was a reversal of the normal elution order. While the (*Z*)-configuration normally provides greater exposure of the double bond and thus shorter retention times, (*Z*,*Z*)-farnesol eluted last. I feel this is due to steric hindrance by methyl substitution at the double bond, which possibly causes the greatest exposure of the double bond when in the (*E*)-configuration. This separation was repeated on a preparative scale using first a 2.5 cm x 30 cm LiChroprep RP-18 column and then the Waters' PrepLC/System 500. For the synthesis of (*E*)-β-farnesene, interest centered only on the (*E*,*E*)-isomer of farnesol, which fortunately eluted first. Using a sample size of 420 mg on the RP-18 column, 95% of the (*E*,*E*)-isomer was retrieved with 98% isomeric purity, as deter-

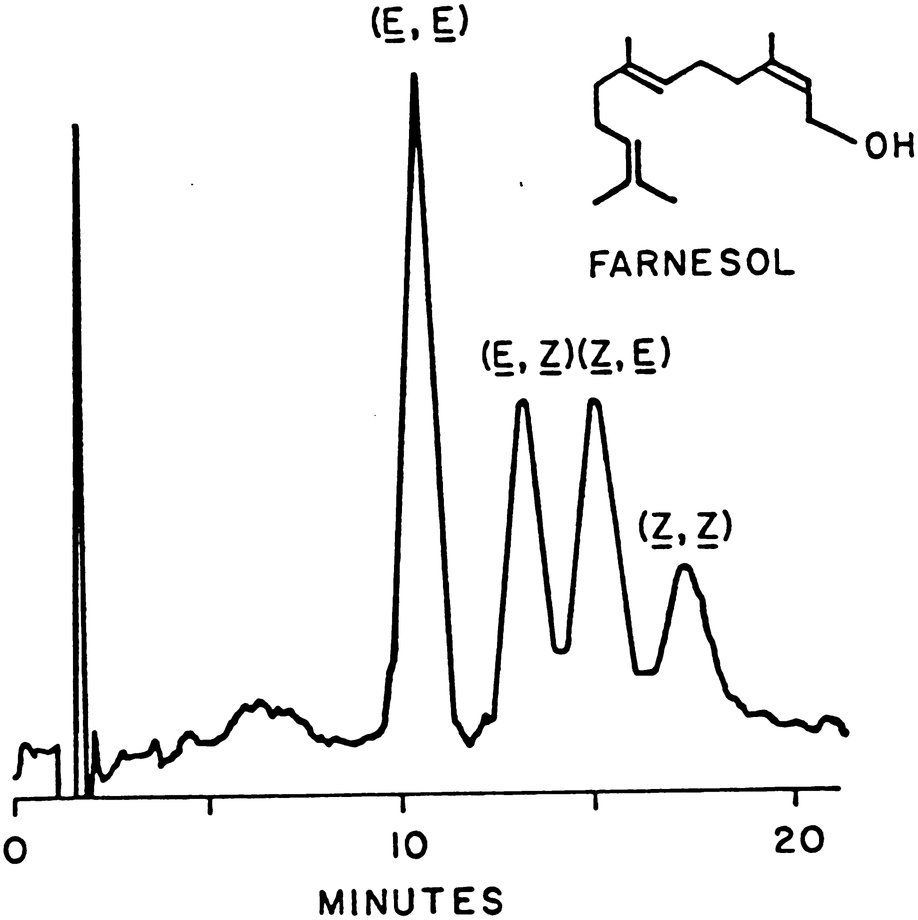

Figure 9. Separation of farnesol isomers. Mobile phase: 40% H_2O/60% MeOH/12 mM $AgNO_3$. Column: μBondapak C_{18}. Flow rate: 2.4 ml/min (2200 psi). Detector: RI = 2X.

mined by GC. The capacity of the Prep-500 system was considerably greater, achieving 98% purity with a 1 gram sample size.

The synthetic farnesene mixture contained a large number of extraneous compounds, and since interest was primarily in (*E*)-β-farnesene, only one α-isomer, (*E*,*E*)-, was identified. This peak was identified by comparison of the retention volume with that of the pure isomer extracted from apple cuticle (Anet 1970). The separation of (*E*)- and (*Z*)-β-farnesene is shown in Figure 10. (*E*,*E*)-α-Farnesene eluted much later than the β-isomers, with a retention volume of 217 ml (33.4 min), compared to 99 ml (15.2 min) for (*E*)-β-farnesene. This separation is due to the unhindered β-double bond which engages in stronger Ag^+-complexation than the internal α-double bond.

General discussion

In most of the separations attempted, baseline separation was achieved with short (<15 min) retention times, thus allowing the performance of semipreparative runs on analytical columns. Complete resolution was not realized in separations of some positional isomers and some of the farnesol and α-farnesene isomers. These separations might be improved on other reversed-phase columns. While all separations were carried out at room temperature, subambient temperatures have been used to increase Ag^+-complexation (Warthen 1976, Morris *et al*. 1967). This also might improve some problem separations, and would probably reduce necessary silver concentrations. However, higher pressures due to increased viscosity of cold solvents (especially aqueous systems) would be a lim-

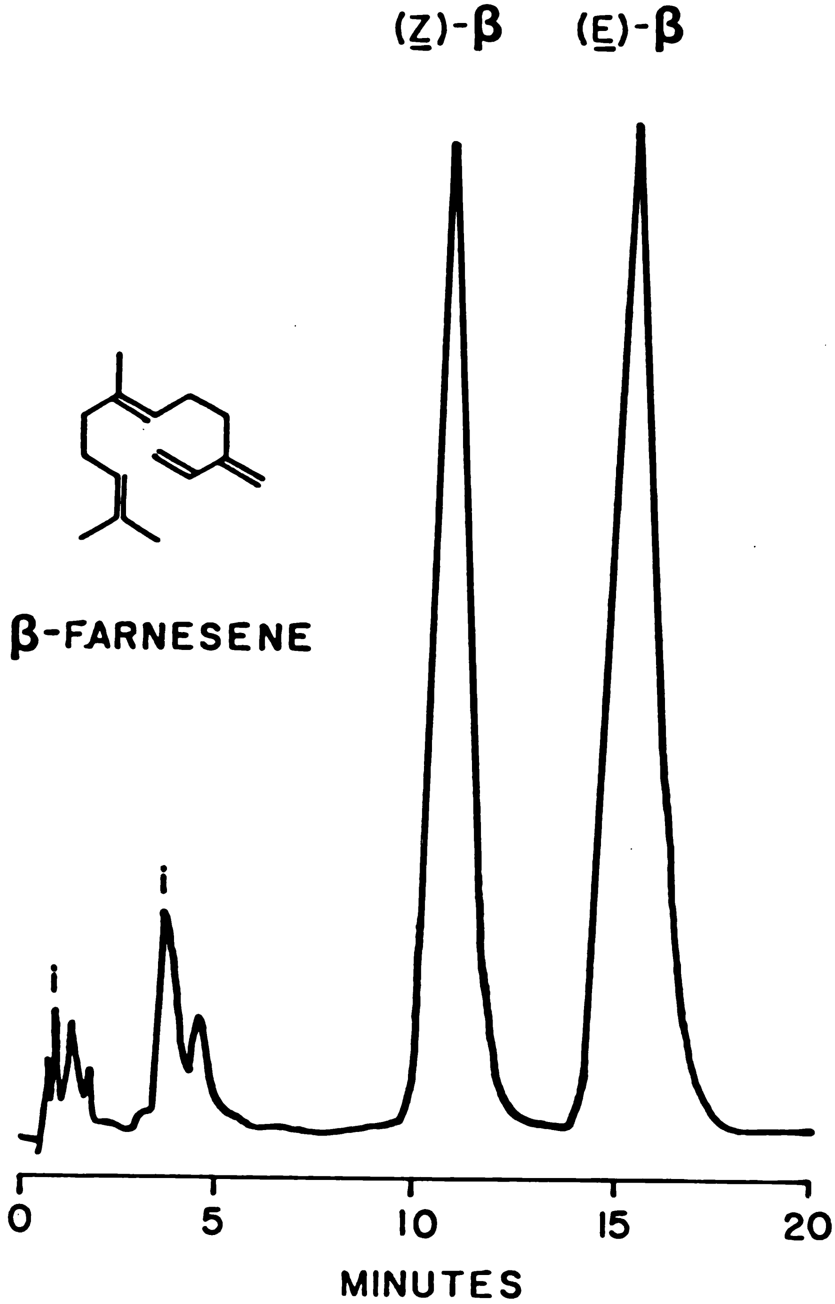

Figure 10. Separation of (Z) and (E)-β-farnesene. Mobile phase: 40% H_2O/ 60% MeOH/15 mM $AgNO_3$. Column: μBondapak C_{18}. Flow rate: 6.5 ml/min (5800 psi). Detector: UV = 0.1. i = impurities.

itation.

Optimal silver concentrations were dictated by the complexing ability of the compounds and thus by the number of double bonds. Of the concentrations tested (15 mM, 50 mM, 100 mM, and 150 mM), greatest separation of mono-unsaturated geometrical and positional isomers was achieved with 100 mM $AgNO_3$, di-unsaturaes separated best with 50 mM $AgNO_3$, and 15 mM $AgNO_3$ was the most selective for the multi-unsaturated compounds.

The DuPont Zorbax ODS column was found to be considerably more efficient than the Waters' μBondapak C_{18}, probably due to the smaller particle size of 5-6 μm of the Zorbax ODS as compared to the μBondapak C_{18} 10 μm particle. Also, the C_{18} column was older than the ODS column at the time of these separations. The smaller particle size, however, had the drawback of higher back pressures, thus limiting the concentration of water that could be used without exceeding the column pressure limit. Some separations requiring high solvent polarity could, therefore, not be achieved on the Zorbax ODS.

Of the silver-complexing methods used for pheromone isomer separation to date, the most successful has been the use of silver-impregnated silica gels. Using $AgNO_3$-coated microparticulate silica HPLC columns, Heath _et al_. (1975, 1977) were able to achieve impressive resolution of insect pheromone geometrical isomers. In addition, these separations were carried out in relatively short times, usually less than 10 min. The level of these separations was, at least in part, due to highly efficient columns utilizing small particle sizes and a high pressure packing technique. They reported a 50-

fold increase in efficiency in these columns over slurry-packed columns (Heath et al. 1977). Although the stationary Ag^+ technique has proved very successful, I feel the mobile Ag^+/RP technique is an alternate method which holds several advantages:

1) This method uses commercially available reversed-phase columns, thus relieving the experimenter of the necessity to pack columns using high pressure packing pumps.
2) Since the Ag^+-complexation takes place in the mobile phase, normal interaction with the stationary phase can still take place. Thus, the polarity of the column can be changed within the range of available bonded phase columns.
3) In $AgNO_3$-coated silica gels, dry nonpolar solvents are necessary to avoid leaching of silver from the column. However, with mobile Ag^+/RP chromatography, a wide range of solvent polarities can be utilized to effect separations in a minimum of time.
4) Since different compounds have different Ag^+-complexing abilities, this technique gives the experimenter more flexibility in determining optimal silver content.

While I do not see this technique as being the optimal one under all circumstances, the above characteristics make it more flexible in terms of chemical applications, effecting rapid separation regardless of the polarity of the solute. Refinement of this technique will come with the utilization of columns with different bonded phases and different compound selectivities. In addition, the use of reversed-phase chromatography with other complexing metal ions (Guha and Janák 1972) in the mobile phase may also prove useful.

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