ALMOND BOARD PROGRESS REPORT - 1992

Project No. 92-BA1: Two-Spotted Spider Mite Pheromones

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Objectives

1. identify isolated components of adult female two-spotted spider mite sex pheromone
2. demonstrate laboratory disruption of mite mating behavior

Interpretive Summary

The primary objective of this project was originally to characterize compounds produced by quiescent deutonymph (QD) females of the twospotted spider mite. These compounds function as a sex pheromone for males that are searching for mates. It is our hope that we can develop either a natural mite-control strategy through direct behavioral disruption or a material that increases the effectiveness of acaricides by increasing contact with droplets of the toxin. Although there is a product presently on the market reputed to be based on the twospotted spider mite pheromone with similar intent, its effectiveness in the field has been questioned. Furthermore, in laboratory studies, we have not found these putative pheromone compounds to elicit any mite response. In contrast, we have isolated other compounds from QD females that elicit male response >15 times longer than a blend of the previously identified compounds. Analytical studies on the identity of the new compounds are proceeding. In a leaf-disk bioassay, we found both males and females to be repelled by areas treated uniformly with QD pheromone. The pheromone also acts to inhibit egglaying by females.

In the course of our studies on the QD pheromone this year, we discovered a second pheromone produced by adult females that is attractive to adult females. We refer to this pheromone as an "aggregation" pheromone since it appears to cause migrating females to cluster. Thus far, we have isolated at least two active components of the pheromone by liquid chromatography. The two active fractions, when recombined, cause females to remain on a treated spot as long as on a spot treated with female extract. A number of compounds unique to one fraction have been identified and a single compound unique to the second fraction has been partially identified. Further analytical work is needed to complete the identification of these compounds. In contrast to the arrestment response of females to a point source of the pheromone, when applied to a large area, females showed nearly 100% avoidance even 24 hrs after application. Furthermore, female egglaying was inhibited 100% on pheromone-treated areas for at least 24 hrs. A mite-control strategy based on the aggregation pheromone could complement or surpass the use of QD sex pheromone since the aggregation pheromone shows greater activity against adult females.

Experimental Procedures

The primary goal of this project is to develop an improved means of control for spider mites based on pheromones. However, to justify grower support for detailed chemical identifications of the active components of this pheromone, we felt it necessary to determine if there indeed was any potential for manipulating mite behavior in a manner beneficial to their control. Without knowing the actual components involved, we are restricted to using mite extract for this demonstration, which requires the work be conducted on a small scale. Three bioassays were employed to demonstrate behavioral activity and disruption. The first we termed the arrestment bioassay. In this bioassay, the test material (5 mite equivalents) was applied as a 3-mm-diameter circle on a glass microscope slide. The test individuals were then carefully transferred from their host plant to the edge of the treated circle with a camel-hair brush. We then recorded the duration of time the mite remained in contact with the circle before leaving. The time period ended when the mite did not return within 5 sec. After replication, treatments were compared for mean duration of mite arrestment using ANOVA and a protected LSD.

The second bioassay, which was used only for the adult female pheromone, employed a 0.35-cm-diameter Y-tube olfactometer with each arm 4.5 cm long and the stem 3.5 cm long. The extract was placed in one of the arms and the other arm treated with the appropriate solvent. The treatments were randomly assigned to the two arms and were allowed 30 min for solvent to evaporate. Female mites were released individually into the stem of the Y-tube and observed for 20 min or until one arm was chosen. The tube was solvent-washed and extract reapplied after every five mites.

The third bioassay was used to measure behavioral disruption by mite extracts. The bioassay was conducted either on a 1.6-cm-diameter leaf disk or a 1.8-cm-diameter glass cover slip. Half of the leaf disk, which was bisected by the midrib of the leaf, was treated with extract, while the other half was treated with hexane (the extract solvent). Glass disks were treated similarly. Five quiescent deutonymph equivalents (QDE) of QD extract was applied to the treated half. For the adult female pheromone, we initially used an amount of extract comparable to that used in the arrestment bioassays on a per-area basis. However, after seeing a very strong female response, we reduced this concentration eight-fold to 1.9ME/cm^2. In all disk bioassays, five mites were placed on each half of the disk, which was held on wet cotton in a closed petri dish. The cotton confined the mites to the disk and maintained leaf tissue. Mites were observed at intervals and the distribution of mites, eggs, and fecal pellets recorded. Disk halves were compared using Chi-square.

Results

Quiescent Deutonymph Pheromone. Using the leaf-disk bioassay, we measured the potential for disruption by the QD pheromone on male behavior. As seen in Figure 1, guarding males avoided the leaf-disk half that was treated with pheromone. At 1 hr after pheromone application and male release, 91% of males were located on the solvent-treated side ($X^2=36.2$, $p<.005$). At 4 hrs, 81% of males were on the solvent-treated side ($X^2=18.5$, $p<.005$), and at 24 hrs, there was still a significant bias in distribution, with 70% of males located on the control side ($X^2=6.6$, $p=.01$). These results suggest that at least on a small scale, male mate-finding behavior is disrupted by a uniform application of the QD pheromone with greater than 90% of males avoiding treated areas. Although there was a decline in response after 24 hrs, it is expected that field longevity of QD pheromone

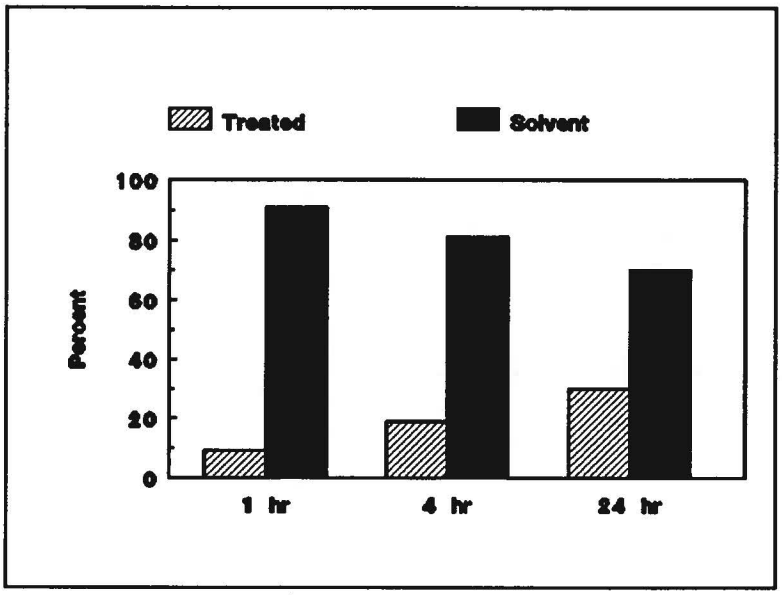

Figure 1. Percent distribution of male TSSM on two halves of a 1.6-cm-diam leaf disk treated with QD extract vs. hexane (n=6 reps of 10 males).

components could be extended by formulation additives.

To determine the potential of QD pheromone for applied behavioral disruption, it was also important to measure the response of adult females to uniform applications of the pheromone. To do so, we repeated the leaf-disk bioassay using adult females. Female response was similar to that of males, although with less intensity (Figure 2). After 1 hr, 84% of females were located off the pheromone-treated side ($X^2=26.2$, $p<.005$). Similarly, at 4 hrs, this percentage was 82% ($X^2=12.1$, $p<.005$) and 73% at 6 hrs ($X^2=9.9$, $p<.005$). However, by 24 hrs, there was no significant difference in the distribution of females on the two disk halves.

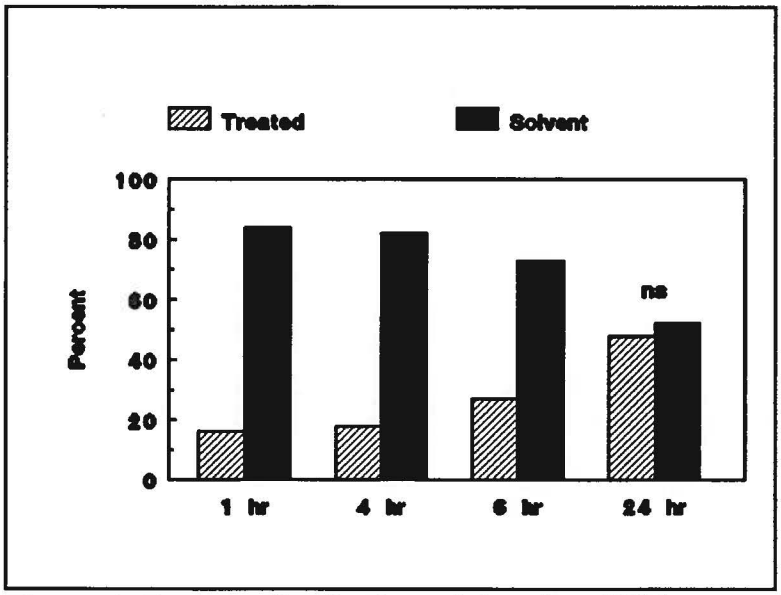

Figure 2. Percent distribution of female TSSM on two halves of a 1.6-cm-diam leaf disk treated with female extract vs. hexane (n=6 reps of 10 females).

Looking only at female distribution may underestimate the practical impact of the QD pheromone on females. There was a very strong asymmetry in the distribution of eggs after 24 hrs laid by the females, with 88% of eggs on solvent-treated side ($X^2=72.4$, $p<.005$, Figure 3). There was only a small asymmetry in the distribution of female fecal pellets, with 63% on the control side of the disk ($X^2=5.6$, $p<.025$). Fecal pellets are a measure of feeding, so that in total the results suggest that the bias in time off the pheromone-treated half of the disk was short lived relative to that of males. However, females continued to discriminate against the pheromone-treated area with regard to egglaying. This effect would be of great practical significance if it also occurred in a treated orchard.

Adult Female Pheromone. Although the intended objectives of this project focused solely on

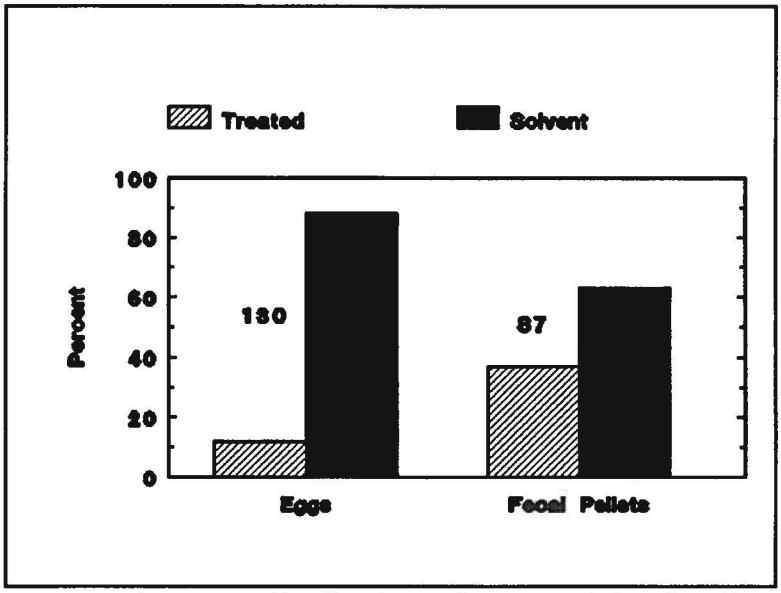

Figure 3. Percent distribution of eggs and fecal pellets produced by females on halves of a leaf disk treated with QD pheromone vs. hexane (See Figure 2).

the sex pheromone produced by the quiescent deutonymph, in the course of these studies, we made an important discovery for application of TSSM pheromones in control programs. This breakthrough was in the discovery of an additional pheromone produced by the adult female. Based on results thus far, we believe this pheromone functions in communication between females to assess population levels, and holds substantial promise for developing a practical control measure. As such, the pheromone appears to elicit arrestment in adult females at low concentrations and/or small point sources, whereas when encountered as a large zone, females exhibit an avoidance response.

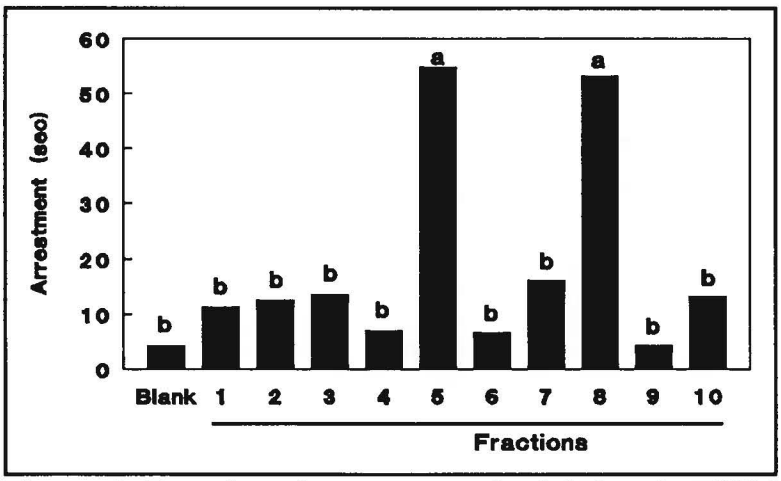

Figure 4. Duration of arrestment of adult female TSSM on a 3-mm diameter circle treated with HPLC fractions of adult female extract. Bars marked with the same letter are not significantly different.

At low population densities, TSSM shows a strong tendency to form clusters, especially during early stages of colonization (Gerson, 1985). Previously, there had been no attempts to determine the mechanism underlying this behavior. It is possible, for example, that these aggregations on the leaf simply represent locations of favorable conditions, such as microclimates or plant-nutritional sinks. However, our work has revealed this aggregation of female mites to be chemically mediated. Using the Y-tube olfactometer, extract from adult females (5 mite equiv.) was placed in one of the Y-tube arms and individual females were released in the stem. Of 39 females responding within one minute, 35 (90%) entered the extract-treated arm and only 4 (10%) entered the untreated arm. These results suggest that: 1) adult females possess a pheromone that elicits an aggregation response in other females, and 2) this pheromone is volatile such that females are attracted to it.

We next fractionated the female extract using high-performance liquid chromatography (HPLC). The resulting fractions were then tested for behavioral activity using the arrestment bioassay. The mean durations of time spent within the treated circle by females were compared. In our initial experiment, we presented the first 10 fractions individually, and found fractions 5 and 8 to elicit significantly longer arrestment than the solvent control or the other eight fractions (Figure 4). A blend of the two active fractions, alone and in combination with all the late fractions (10-15), was then compared to the unfractionated extract and to a solvent control. The two-component blend was significantly more active than the solvent control and not significantly different in activity from the unfractionated female extract (Figure 5). Combining Fractions 5 and 8 with the six late fractions actually appeared to reduce activity, although the reduction was not statistically significant. These results suggest that the active components are confined to the two fractions and that the addition of other fractions does not increase activity.

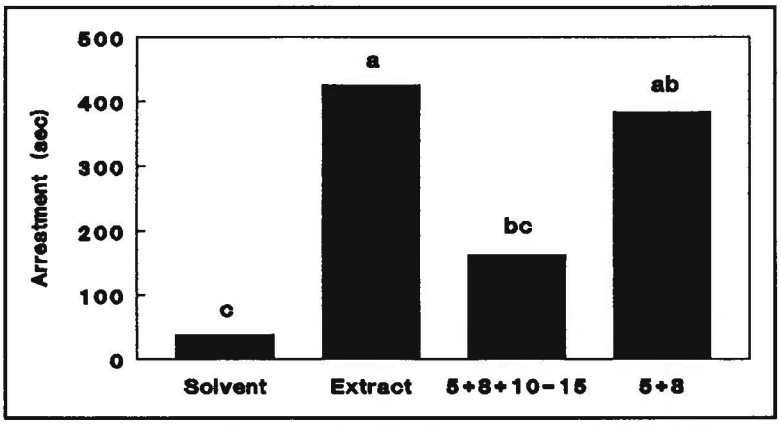

Figure 5. Arrestment of adult female TSSM on a 3-mm diam circle treated with unfractionated female extract or combined HPLC fractions of that extract. Bars marked by the same letter are not different.

This inference was further supported by a third bioassay that compared Fractions 5 and 8, individually and in combination, against the unfractionated female extract and a solvent control. In this case, either fraction elicited arrestment as long as did the unfractionated extract (Figure 6). Each of these fractions was significantly more active than the solvent control when presented individually; however, when the two fractions were combined, arrestment was significantly shorter than for the extract and not different than the solvent control. Although it not clear why this would be the case, it is possibly related to the fact that female response to this extract is concentration dependent (see below) and that combining the two fractions in their purified form may produce a stronger signal.

Having demonstrated arrestment in female TSSM to a point source of the female pheromone, we further hypothesized that females would avoid larger areas treated uniformly with the pheromone. This hypothesis was based on the assumption that there is an optimal size for clusters of females. While small clusters may be advantageous for colonizing females, large concentrations of egglaying females would rapidly deplete a host resource, resulting in intense competition for offspring. Thus, the female-produced pheromone might represent a context-dependent signal, which when encountered as point source and/or at low concentrations elicits arrestment, but when at high concentrations and/or wide distributions causes females to leave the area. This hypothesis was tested using the two-choice leaf-disk bioassay. Even after lowering the concentration of pheromone, as discussed in the

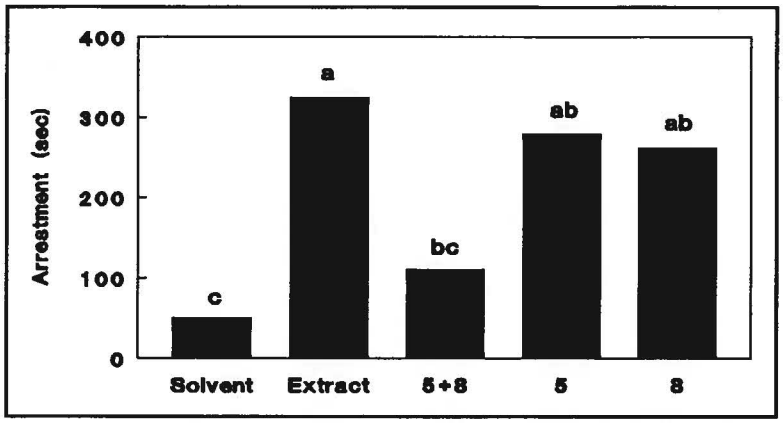

Figure 6. Arrestment of adult female TSSM on a 3-mm-diameter circle treated with unfractionated female extract or fractions of that extract. Bars marked by the same letter are not different.

Experimental Procedures, females still immediately showed an avoidance of the extract-treated area. We did not attempt to reduce this concentration further to determine the lower limits of the avoidance response. After 1 hr, 90% of the females (n=7 replicates of 10 mites) were located on the solvent-treated area (Figure 7). In a parallel experiment determining the effect of hexane on the mites, females showed a preference for the untreated half of the leaf over the hexane-treated half (76% vs. 24%). However, by 24 hrs, there was no significant difference in the distribution of mites between the two disk halves. To be clear, the bias in distribution due to the solvent does not explain any of the effect seen with the female extract since that experiment compared the extract to a solvent-treated leaf area. Furthermore, in contrast to the solvent experiment, after 24 hrs, the avoidance of the extract-treated area not only continued, but became even more pronouced, with only 3% of mites found on the extract-treated half (Figure 8). Other measures of activity also confirm this avoidance response. Of the 251 eggs laid by those females, 100% were found on the solvent-treated half of the disk, as were 97% of the fecal pellets, a measure of feeding activity. A similar distribution pattern was seen even after 48 hrs.

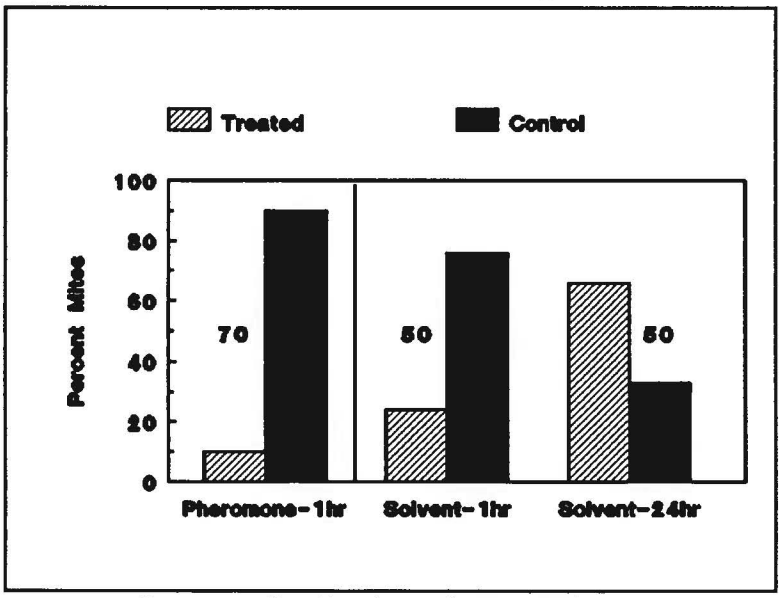

Figure 7. Percent distribution of TSSM females on two halves of a leaf disk treated with: a) female extract vs. solvent (70 mites), or b) solvent vs. blank (50 mites).

There clearly was a very strong and lasting avoidance of areas treated with adult-female extract by the female mites; however, it was possible that the effect was due to damage of the leaf tissue by the extract but not by the solvent. To test this possibility, our final bioassay repeated the comparison of extract vs. solvent on a glass disk with a diameter similar to the leaf disk. The results of this bioassay match those on the leaf disk

(Figure 9). After 1 hr, 70% of females were located on the solvent-treated side (n=3 replicates of 10 mites), and at 24 hrs, this had risen to more than 90%. Rather few eggs were laid on the glass substrate, but none of these were found on the extract-treated side. Since the mites could not feed, no fecal pellets were found. This bioassay is more severe than the leaf-disk bioassay since confinement on a glass surface represents a very unfavorable environment, and we would expect much greater host-seeking movements. These results further indicate the strength of the response to the pheromone in that even after 24 hrs of starvation and ovipositional deprivation, the females still showed avoidance.

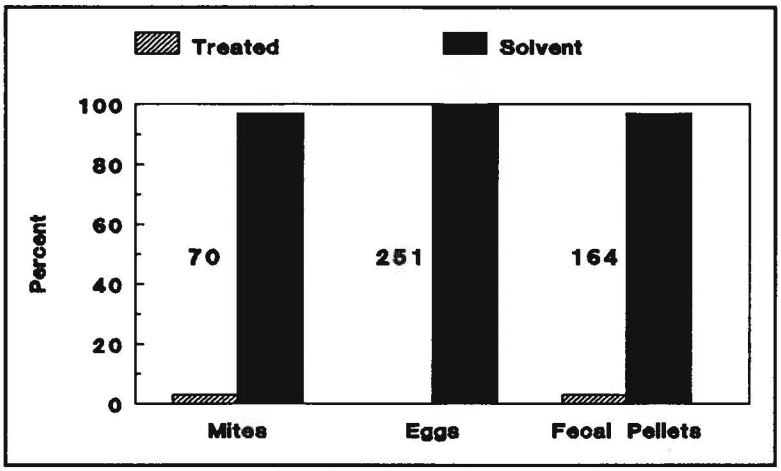

Figure 8. Percent distribution of TSSM females, eggs laid, and fecal pellets produced after 24 hrs on two halves of a leaf disk treated with female extract vs. hexane.

Discussion:

The ultimate goal of the TSSM project is to derive a control strategy based on behavioral manipulation. With the QD sex pheromone, a blend of active components could be used either in combination with acaricides or used alone for disruption of mating. By combining pesticides with an attractant or an arrestant, one can overcome the common tendency of a pest to avoid pesticide droplets; by increasing the contact time with the toxicant, its efficacy is increased. Mating disruption is envisioned to occur by males either becoming habituated to the sex pheromone and/or by spending time at pheromone sources instead of actual females. Disruption of mating could significantly reduce the rate of population increase without the use of pesticides. Our work this past year with the QD pheromone suggests that a uniform application would not only disrupt male behavior, but also elicits female avoidance and reduces egglaying. The combined impact of these responses on mite populations could be profound if they can be reproduced on the scale of an orchard.

This year's findings with the adult female pheromone open up an alternative approach that has even greater potential for control. First, although both pheromones affect females, response to the adult female pheromone appears greater and thus its impact on the population could be greater. Secondly, the context- and/or concentration-dependency of the female response suggests two seemingly contradictory possibilities for control depending on how the material is formulated for application. Thus, since when encountered as a point source, females become arrested by the pheromone, it could be blended with an acaricide and adjuvants that would produce discrete spray droplets. As with male response to QD pheromone, this might be used to increase contact with the acaricide; however, the important difference is that this strategy would increase mortality of females. Alternatively, if the

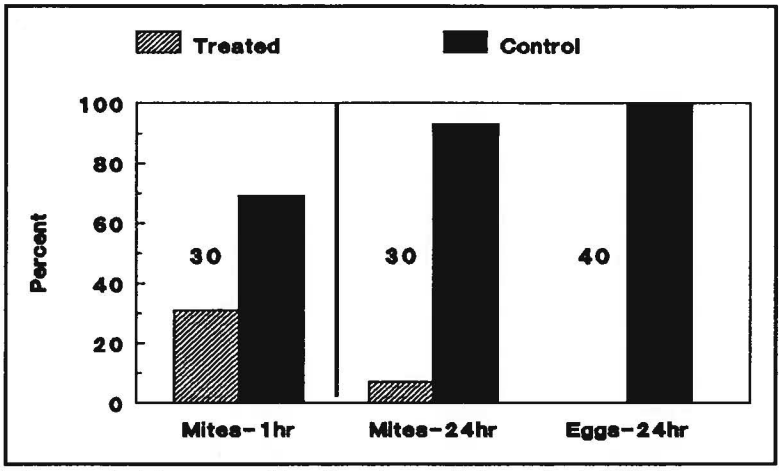

Figure 9. Percent distribution of female TSSM on two halves of a 1.8-cm-diam glass disk treated with female extract vs. hexane.

pheromone components were formulated without acaricide but with a wetting\speading agent that would provide more uniform coverage of the leaf, females might be repelled from treated orchards. Our work thus far has only been with colonizing females, which show an extraordinarily strong avoidance of the material. More work obviously is necessary to determine if the response on a small scale is indicative of what would happen on the scale of an orchard; however, these results are extremely encouraging and suggest that colonization by migrating females could be inhibited. We do not yet know the response of other TSSM lifestages to the pheromone, and it would be useful to know the effect on established colonies; however, in addition to repelling colonizing females, other beneficial effects are suggested by our initial studies. First, even if females do not leave a treated orchard, application of the pheromone may reduce rates of oviposition. Females in those leaf-disk bioassays comparing pheromone-treated areas with hexane-treated ones produced only about half the number of eggs (3.59 eggs/female) as did females in the bioassays comparing hexane-treated areas with untreated ones (6.92 eggs/female). A reduction in spider-mite egglaying due to crowding alone has been previously reported (Wrensch and Young, 1978). Furthermore, Wrensch and Young (1978) found that when female densities were increased from 3 to 12/leaf, the ratio of female-to-male offspring dropped dramatically from 2.7 to 1.2, even when both groups of females were reared on and allowed to oviposit on high-quality leaves. Reducing the sex ratio will result in a lower rate of population increase. For example, Wrensch (1992) determined that reducing the proportion of females by 25% would result in a 50% decline in the intrinsive rate of increase of spider mites.

This year we have demonstrated that both the female QD and adult pheromones elicit a variety of strong behavioral responses in males and females that could be manipulated for control of TSSM in almond orchards. Particularly encouraging is the level and duration of female response to adult-pheromone-treated areas (near 100% avoidance by females even under adverse conditions) at very low concentrations (<2 ME/cm^2), in combination with the potential secondary effects of reduced egglaying and proportion of female offspring. In all, these results suggest a high probability of success for a nonpesticidal control strategy being developed based on either or both of these pheromones. In addition, the attraction/arrestment of females to the adult pheromone and males to the QD pheromone when presented as a point source suggest their utility as additive to enhance acaricide efficacy.

References

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