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INTERFERENCE OF SONIC COMMUNICATION AND MATING IN LEAFHOPPER *Amrasca devastans* (DISTANT)[1] BY CERTAIN VOLATILES

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Abstract—Mating between the two sexes in the leafhopper *Amrasca devastans* was inhibited by cineole vapors surrounding host plant leaves bearing the insects. There was a decline in the percentage of pairs mating and mated females fertilized. The vapors were not toxic and did not prevent the insects' arrival on the leaves. The cineole vapors inhibited the mating chiefly by interfering with the surface-mediated sonic communication between the sexes. The inhibition of mating was chemical specific since other volatiles, e.g., citral, were not as effective as cineole. Exposure to cineole vapors resulted in a decline in the sexual response of the females to the sonic signals of the male

Key Words—Volatile mating inhibitor, cineole, sonic communication inhibition, leafhopper, *Amrasca devastans,* Homoptera, Cicadellidae.

INTRODUCTION

In recent years, several reports have shown that appropriate chemicals can interfere with chemical communication in various insects and their consequent behavior (Shorey and McKelvey, 1977; Ritter, 1979; Mitchell, 1981). However, many insect species use mainly sonic communication for attracting mates. Such insects include many species of auchenorrhynchous homopterans (Ossiannilsson, 1949; Claridge and Howse, 1968; Claridge and Reynolds, 1973; Strübing, 1965; Shaw et al., 1974; Ichikawa, 1977; Traue,

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1978) in which the two sexes communicate by means of substrate-borne rather than airborne vibrations. Such communication and mating can be interfered with by certain artificially generated sonic signals as reported by us (Saxena and Kumar, 1980) for the leafhopper *Amrasca devastans* (Distant) which is a serious pest of cotton, okra, eggplant, etc. But, it is not known whether such communication and mating in these homopterans can be interfered with by the vapors of a volatile chemical even though there is no chemical communication between the sexes (Kumar, 1980). We are therefore screening various volatiles for such effects on *A. devastans* in the laboratory. Our observations on the effects of these chemicals are given in this paper.

METHODS AND MATERIALS

The nymphs of *Amrasca devastans* (Distant) were collected from fields of okra (*Abelmoschus esculentus* (Linnaeus) Moench) and reared one each in a vial (5 × 2.5 cm) containing a piece of okra fruit wrapped in a twofold stretched parafilm M_R membrane at 28 ± 1°C under a 13-hr photophase. The emerging adults also were kept singly on the same fruit under the same conditions. Upon attaining the age of 5–7 days when they show maximum mating (Kumar and Saxena, 1978), the females and males were tested for acoustic communication and sexual responses in the presence or absence of cineole vapors, as described below.

For most tests, we used, 30–50 pairs of males and females, arranged in 3–5 replicates of 10 each. However, for recording the number of sound phrases emitted by each individual, 10 pairs of males and females were used. The data on the responses of these insects in the presence or absence of cineole vapors were compared statistically using Student's *t* test (Snedecor and Cochran, 1967).

The effects of the vapors of a test chemical on the acoustic communication and sexual behavior of the leafhopper were studied in a cylindrical chamber (250 ml capacity, 7 cm high) of clear plastic. The chamber had a detachable lid of nylon net (40 mesh/cm) and a fixed nylon net bottom which rested on the top open end of another plastic chamber of the same size. Each chamber was newly constructed, washed thoroughly with the detergent teepol, and dried.

A freshly excised leaf of the host plant cotton (*Gossypium hirsutum* cv. PS-10) was trimmed to form a 5 × 3-cm rectangle with a 2.5-cm petiole which was immersed in water in a stoppered vial (2.5 cm high, 3.5 cm wide). The leaf and the vial were placed in an upper chamber. The required volume of the test chemical in 0.1 ml of liquid paraffin in a glass cup (1 cm high, 1 cm diameter) was placed in the lower chamber so that the chemical's vapors could diffuse into the test chamber above. The chemicals and their doses, selected on the basis of preliminary trials, were: carvacrol, clove oil, cineole, citral, citronellol, farnesol, geraniol, and linalool. Each of these was tested in a dose of 0.4 ml per test chamber. However, a few found to have a marked effect were subsequently tested at a lower dose of 0.05 ml per test chamber. A control had liquid paraffin in the lower chamber without the chemical. For each test, one or five pairs of males and females were released in the upper chamber for 30 or 60 min. The substrate-borne sounds of the insects on the leaf were picked up by a gramophone cartridge, amplified, and recorded by methods described before (Saxena and Kumar, 1980, 1984). The number of sound phrases emitted by each individual as well as the percentages of insects emitting them and showing different sexual responses also were recorded as described before (Saxena and Kumar, 1984).

The effects of test chemicals were further studied by exposing the insects to their vapors around the host leaves in cylindrical chambers of glass or wire net (40 mesh/cm) having a capacity of 500 ml (10 cm high) or 1000 ml (20 cm high). The required amount of the test chemical in 0.1 ml of liquid paraffin in a glass cup (1 × 1 cm) with open top covered with wire net (40 mesh/cm) having a capacity of 500 ml (10 cm high) or 1000 ml (20 cm high). The required amount of the test chemical in 0.1 ml of liquid paraffin in a glass cup (1 × 1 cm) with open top covered with wire net (40 mesh/cm) was placed at the bottom of the jar. A 5 × 3-cm rectangle of a freshly excised cotton leaf, with its 2.5-cm petiole wrapped in wet cotton wool covered with alumunium foil, was suspended vertically on a string in the center of each chamber, with the base of the petiole almost touching the glass cup top. The vapors of the chemical were allowed 30 min to diffuse through the jar and surround the leaf.

Five pairs of unmated males and females were introduced into the jar but not directly on the leaf. During the tests, the insects sometimes visited the walls, including the top cover of the test chamber. If the cover was of a material like nylon net or muslin cloth, which transmitted the sonic signals of the insects (Kumar, 1980), they would readily mate on it. In order to examine the effects of the vapors of the test chemical surrounding a mating site, i.e., host leaf, it was important that the insects arriving on any other site did not mate so that their responses after their arrival on the leaf could be observed. Therefore, wire net which would not permit sonic communication and mating in the insects was used for the top of the chamber or the entire test chamber. The test jar was kept in the experimental room at 28–30° C under an illumination of 150–160 lux from an overhead fluorescent light (40 W). On the basis of two such tests using 10 pairs of males and females, the percentage of those which mated during successive 60-min intervals over a 4-hr observation period was recorded.

Thereafter, the females were removed from the jar and kept one each in a separate vial (7.5 cm high, 2.5 cm diameter) containing an unripe okra fruit for feeding and egg-laying. The fruit was replenished on alternate days, and the insects were maintained at 28 ± 1°C under a 13-hr photophase until they were 15 days old, after which egg-laying sharply declines (Saxena and Saxena, 1971). Each okra fruit removed from the vials was retained for 9 days by which time all the fertile eggs laid therein would hatch. This allowed us to determine the percentage of mated females that were fertilized.

The effects of cineole vapors on the leafhopper's arrival, length of stay on the cotton leaf, and the survival of the insects were tested by methods described previously (Saxena and Basit, 1982).

RESULTS

As reported before (Saxena and Kumar, 1980, 1984), when a male and a female are present together on a leaf of their host plant cotton, the male emits its "croaking" sounds. The latter travel through the substrate to the female to render it stationary and stimulate it to emit its "cooing" sounds. These coos also travel through the substrate to the male to stimulate it to: (1) increase the number of its croaks, (2) start "dancing" movements and approach the female, (3) get "arrested" by the side of the female, (4) extend its genitalia towards those of the female, and, finally, (5) copulate. The effects of the test chemical's vapors surrounding the plant leaves on these responses of the leafhoppers were examined.

Effects of Cineole Vapors on Sexual Behavior. The results of our preliminary trials with the vapors of different chemicals on the sexual behavior of the leafhopper are given in Table 1. Since the vapors of cineole inhibited the mating to a maximum degree, this chemical was chosen for detailed study. Carvacrol and clove oil killed the insects within the observation period and thus were not tested further. Of the remaining volatiles, which inhibited mating to almost an equal degree, we took citral as their representative for testing the effect of its vapors on the sexual behavior of the leafhopper.

When single pairs of males and females were released directly on a leaf in the 250-ml control chamber for 60 min, 100% showed the above-mentioned sexual responses leading to mating, as described before (Saxena and Kumar, 1980, 1984). On surrounding the insects and the leaves with the vapors of the test chemicals, the percentage of pairs mating was significantly reduced to 43 ± 3 with citral and to 13 ± 3 with cineole. The remaining test were performed using cineole since it was much more effective than citral in inhibiting mating in the leafhopper. The percentage of individuals of each sex emitting sexually stimulating sounds was not significantly different in

TABLE 1. EFFECTS OF VAPORS OF CERTAIN VOLATILES[a] ON MATING IN LEAFHOPPER *Amrasca devastans*

Test chemical	Source of chemicals[b]	Pairs of males and females mating (Mean % ± SD)[c]
Nil		77 ± 15
Carvacrol	KK	toxic[d]
Clove oil	VMF	toxic[d]
Cineole	GS	13 ± 11.5*[e]
Citral	KK	33 ± 11.5*
Citronellol	KK	37 ± 9.0*
Farnesol	KK	40 ± 0*
Geraniol	KK	43 ± 6.0*
Linalool	KK	43 ± 6.0*
1 SD at $P = 0.05$		19.7

[a]0.4 ml of test chemical in 0.1 of liquid paraffin per test.
[b]GS: Goldensun Manufacturing Co., Bombay, India; KK: K & K Labs Division, ICN Pharmaceuticals, Inc., U.S.A.; VMR: V. Mane Fils, Grasse, France.
[c]For each test, five pairs of males and females were released in the upper compartment of 250-ml cylindrical chamber for 60 min. Two such tests comprised one replicate. Data based on three replicates of 10 pairs each.
[d]All the insects died within the observation period.
[e]Asterisks indicate that the difference in the percent pairs mated in presence of a test volatile and that in the control is significant at $P = 0.05$.

the presence of cineole vapors as in their absence (Figure 1A, B). However, the number of sound phrases emitted by each sex in the presence of the chemical declined sharply to less than one fourth that in the control (Figure 1G, H). Also, the dancing of the males, their arrest by the females, and extension of their genitalia was 23–25% less than that in the absence of cineole (Figure 1C, D, E). Finally, the percentage of pairs which mated was only about one fourth of that in the absence of the chemical (Figure 1F).

The effects of cineole were further studied by exposing the insects to its vapors around the host leaves for durations longer than 60 min in different types of chambers, releasing the insects within the latter anywhere except on the leaves. The insects would then have to arrive on the leaves in response to their visual (color), olfactory, and hygro-stimuli (Saxena and Saxena, 1974) before substrate-borne sonic communication and mating between the sexes could occur. In each type of test chamber, the percentage of pairs mating in the presence of cineole vapors increased with the duration of exposure but remained significantly less than that in the absence of the chemical (Table 2). The interference by cineole of mating in the leafhopper was maximum during the first 60 min and declined with the increase in the time interval. By

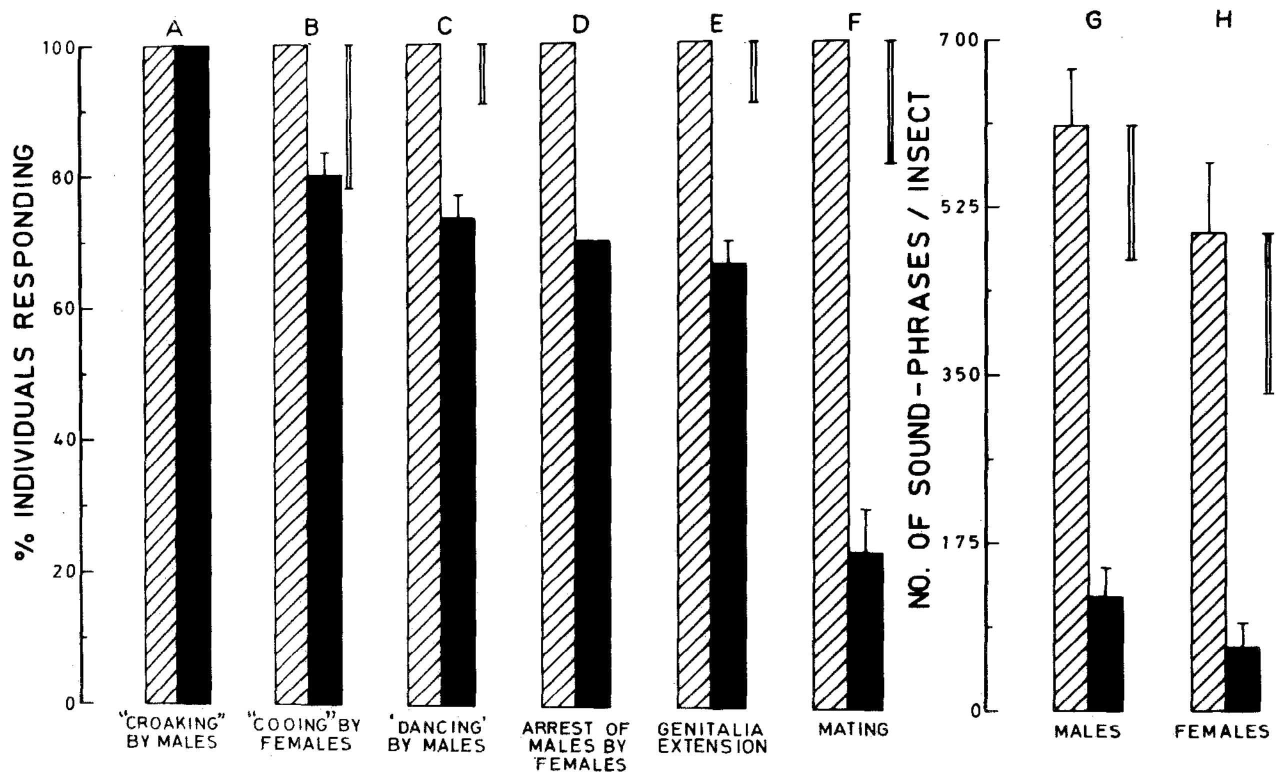

FIG. 1. Effects of cineole vapors on acoustic communication and mating in *Amrasca devastans*. For each experiment, 30 pairs of 5-day-old males and females were tested in three replicates of 10 pairs each for 30 min in the 250-ml test chamber permeated with vapors from 0.05 ml cineole in 0.1 ml liquid paraffin. Solid columns: responses in the presence of cineole; hatched columns: responses in the absence of cineole; single vertical bars: standard errors; double vertical bars: LSD at $P = 0.05$ for the two columns immediately on the left.

TABLE 2. EFFECT OF CINEOLE[a] VAPORS ON MATING IN LEAFHOPPER *Amrasca devastans*

Observation period (min)	Pairs of males and females mating (Mean % ± SD)[b]					
	Glass chamber, 500 ml		Glass chamber, 1000 ml		Wire net chamber, 1000 ml	
	Cineole present	Cineole absent	Cineole present	Cineole absent	Cineole present	Cineole absent
60	26 ± 9.0	62 ± 11.0*[c]	14 ± 9.0	50 ± 20.0*	27 ± 15.0	59 ± 14.0
120	38 ± 13.0	78 ± 15.0*	28 ± 8.5	57 ± 11.5*	39 ± 14.0	71 ± 8.0*
180	54 ± 5.5	92 ± 11.0*	42 ± 13.0	60 ± 10.0*	48 ± 18.0	79 ± 13.0*
240	64 ± 11.0	96 ± 9.0*	50 ± 12.0	70 ± 10.0*	51 ± 18.5	87 ± 9.0*

[a]0.05 ml in 0.1 ml liquid paraffin per chamber.
[b]For each experiment, 50 pairs of 5-day-old males and females in five replicates of 10 pairs each were tested in each type of chamber.
[c]Asterisk indicates significantly different at $P = 0.05$ from that in the presence of cineole.

the end of 4 hr, the percentage of pairs mating in the presence of cineole was reduced by about one third compared to the percentage mating in the absence of the chemical. Even in the wire net chamber of the same size (1000 ml), which allowed the cineole vapors to diffuse out through the side walls, the percentage of pairs mating was reduced by about two fifths as compared with that in the absence of the chemical.

Of the females which mated with the males in the presence of cineole, the percentage of those which laid fertile eggs was significantly less than of those mating in the absence of the chemical vapor (Figure 2). These results suggest that cineole vapors interfere with fertilization.

Effects of Cineole Vapors on Arrival and Length of Stay of Insects on Leaf. As propagation of the sounds of the two sexes occurred through the leaf but not through the glass or wire net wall of the test chambers, it was essential for the insects to arrive and stay on the leaf before they could communicate and mate (Saxena and Kumar, 1980, 1984). These insects have been reported to arrive and stay on the cotton leaf in response to the latter's visual, olefactory, and hygro-stimuli (Saxena and Saxena, 1974). In the presence of cineole vapors, the percentage of insects present on the leaf at the end of 1, 2, 3, and 4 hr was almost the same as in the absence of the chemical (Figure 3). Hence, the decline in mating in the presence of cineole vapors would not be due to the failure of insects to arrive/stay on the leaf and, thereby, to communicate with one another.

Effect of Cineole Vapors on Survival. The survival of insects in the presence or absence of cineole vapors was observed for 6 days after they

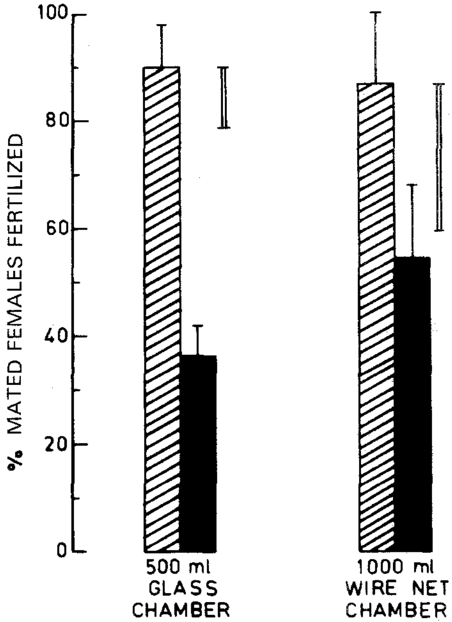

FIG.2. Effects of cineole vapors on the fertilization of mated females by males of *Amrasca devastans* For each experiment, 40 5-day-old females which mated in the presence of 0.05 ml cineole vapors in 0.1 ml liquid paraffin and 30 females mated in the absence of the chemical over a period of 240 min were arranged in replicates of 10 each to calculate the percentages of those laying fertile eggs. Hatched column = control; solid column = treatment Single and double vertical bars: as in Figure 1.

begin oviposition (Saxena and Saxena, 1971). The percentage of insects surviving in the presence of cineole vapors during this period was statistically as high (90% ± 3 SE) as in the absence of the vapors (92% ± 2 SE). Thus, these vapors did not have any acute toxicity to interfere with mating in the leafhopper.

DISCUSSION

There are very few reports on the interaction between sonic and chemical stimuli influencing distance communication between insects for their orientation, mating, or other behavioral responses. The most notable examples include certain species of bark beetles of the genus *Dendroctonus* whose aggregation behavior is inhibited by certain antiaggregative pheromones secreted by the females on stimulation by certain sonic signals from the males (Rudinsky and Michael, 1972). On the other hand, the sonic

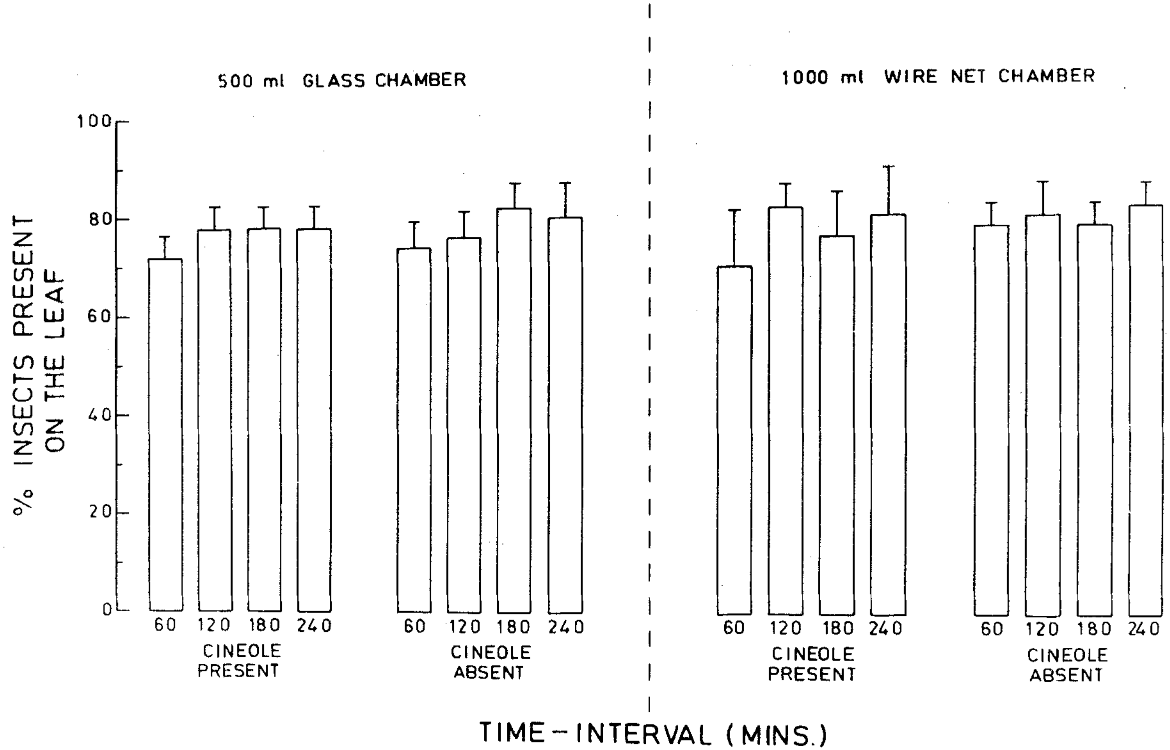

FIG. 3. Effects of cineole vapors on the arrival and stay of *Amrasca devastans* on the host leaves. Fifty pairs of 5-day-old males and females were tested in five replicates of 10 pairs each in the 500-ml glass or 1000-ml wire-net chamber in the absence or presence of 0.05 ml cineole in 0.1 ml liquid paraffin. Single vertical bars: as in Figure 1, the mean responses in the presence of cineole not significantly different at $P = 0.05$ from those in the control for any given time interval as shown by Student's t test.

signals of the males may themselves be evoked by the pheromones of both sexes, those of females eliciting "attractant" chirps and those of males "rivalry" chirps from other males (Rudinsky and Michael, 1974; Rudinsky et al., 1974). Some other important examples are those of certain species of ground crickets of the genera *Allenemobius* and *Pictonemobius* whose males emit sonic signals to attract females in response to chemical stimulation from the latter (Paul, 1976).

The above-mentioned insects are those which emit and use both sonic and chemical signals for distance communication between individuals. However, there is no information on the interaction of these two types of signals in an insect species like *A. devastans,* in which distance communication is mediated by sonic but not by chemical signals (Kumar, 1980). The present work is significant in demonstrating that vapors of a chemical, namely cineole, surrounding the two sexes of this leafhopper on plants/leaves can interfere with their acoustic communication and mating, although this interference declines with the increase in the time interval under the conditions tested. Such an interference by cineole vapors is reflected in: (1) a decline in the proportion of males and females mating, and, (2) a decline in successful matings and, hence, in the proportion of mated females fertilized to lay viable eggs.

Considering the mode of interference by cineole vapors with mating in the leafhopper, the present work shows that the numbers of coos of the female and croaks of the male are reduced in the presence of the chemical, the proportion of each sex emitting these sounds remaining unaffected. Since the mutual acoustic stimulation causes the male and female to increase the emission of their respective sounds (Saxena and Kumar, 1980, 1984), the reduction in the emission of sounds by both the sexes in the presence of the chemical suggests its interference with the acoustic stimulation of the two sexes. Also, the coos of the female stimulate the males to commence their dancing and subsequent sexual responses, leading them to the former and mating (Saxena and Kumar, 1980, 1984). In view of this, the decline in the emission of coos by the females in the presence of cineole vapors would be responsible for the fall in the sexual responses of the males and for the eventual sharp decline in mating, as observed in this work.

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