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# ABSORPTION AND RELEASE OF PHEROMONE OF *Epiphyas postvittana* (LEPIDOPTERA: TORTRICIDAE) BY APPLE LEAVES

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**Abstract**—The absorption and release of the pheromone of *Epiphyas postvittana* (Lepidoptera: Tortricidae), *E* 11-14:OAc and *E,E* 9,11-14:OAc (95:5) by apple leaves was studied using electroantennograms (EAG) and sticky traps baited with pheromone-treated leaves. Leaves exposed to an airstream containing pheromone reached a constant level of pheromone release within 3 min. Release occurred over a period greater than 24 hr, following removal of leaves from the pheromone-saturated environment. Pheromone-treated leaves were effective as lures in sticky traps for at least three nights, although the average catch per night decrease logarithmically with time. In the field, pheromone was detected by EAG on leaves harvested from up to 25 cm away from a central point source of pheromone. The shape of a surface representing equal pheromone re-release from leaves around a central point source was defined by interpolation from a three-dimensional transect. Leaves harvested from 5 cm under the dispensers showed the highest pheromone release rate. Leaves downwind of the dispensers also had higher releases of pheromone. In a treated orchard, significantly higher EAG measurements were recorded in the rows of trees that contained dispensers, compared to grass interrows or untreated trees. The implications of foliar pheromone adsorption and release on atmospheric concentrations and insect behavior require further investigation.

**Key Words**—*Epiphyas postvittana*, Lepidoptera, Tortricidae, electroantennogram, pheromone, dispenser, apple, mating disruption, atmospheric concentration.

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## INTRODUCTION

Considerable progress has been made in using lepidopterous sex pheromones for pest control by disrupting male mating behavior (Jutsum and Gordon, 1989; Ridgway et al., 1990; Suckling, 1993). Successful use of this method requires the release of large amounts of pheromones over the target area. The mechanism of disruption is still the subject of conjecture (Cardé, 1990). Information about the actual pheromone concentration required and its spacial and temporal distribution should help to understand how disruption operates.

Earlier models describing pheromone distribution suggested the presence of a slow-moving pheromone cloud with the average pheromone concentration decreasing with distance from the pheromone source (Wright, 1958; Bossert and Wilson, 1963; Wilson et al., 1969). In such an environment, pheromone molecules reaching male moth antennae elicit upwind flight towards the odor source, e.g., a calling female, or a synthetic pheromone lure (Kennedy et al., 1980). However, higher concentrations have been shown to result in arrestment (Kennedy, 1978) according to the "threshold hypothesis," which defined a middle range of behaviorally active pheromone concentrations (Roelofs, 1978). In addition, there is evidence that fluctuating rather than continuous pheromone stimulation is necessary for sustained upwind flight, providing that the peak-to-trough amplitudes reaching the antennae are sufficiently high (Kennedy et al., 1980, 1987; Kennedy, 1982; Willis and Baker, 1984; Baker et al., 1985; Baker and Haynes, 1989). Baker et al., (1985) also showed that the lack of upwind flight in a volume of uniform permeation was correlated to high, continuous antennal activity, measured by electroantennogram (EAG), which contrasts with the turbulent cloud in which upwind flight is achieved.

The notion of a uniform pheromone cloud was challenged by the measurements of Murlis and Jones (1981), using negative ions to simulate the distribution of pheromone. The discontinuous and filamentous structure of plumes was confirmed by additional experiments by Murlis (1986) and Murlis et al. (1992). However, these experiments were performed using ions as a model for pheromones. Furthermore, the absence of the natural habitat such as plant foliage, may influence the distribution of pheromone in several ways. Buffering effect of foliage in shelter belts is clearly important in reducing wind, thereby decreasing the dilution of the released pheromone. Within the boundaries of the treated area, adsorption and release of the pheromone released from leaves could contribute to a uniform distribution of the pheromone (Wall et al., 1981; Noldus et al., 1991). Adsorption and release of pheromone by leaves could affect the atmospheric concentration of pheromones and enhance its effect on mating disruption.

One technique showing promise for assisting in the determination of atmospheric pheromone in real time and at low concentration is the EAG (Baker and

Haynes, 1989; Sauer et al., 1992; Bengtsson *et al.*, 1994; Suckling et al., 1994). We wondered whether apple foliage could function as a pheromone buffer system for *Epiphyas postvittana* (Walker). This paper reports laboratory and field experiments to elucidate the nature of the possible relationship between pheromone and apple foliage over time and space. We investigated whether the EAG technique could show pheromone uptake and release from apple leaves, as a function of time of exposure, time since exposure, and distance from the point of exposure, as well as a behavioral corroboration of the effect on male moths.

## METHODS AND MATERIALS

### *Insects*

All experiments were carried out with antennae from laboratory-reared male light-brown apple moth, *E. postvittana*. After eclosion, males were kept in a refrigerator at 12°C for a maximum of 2 to 4 days before antennae were used.

### *Pheromones*

The natural blend of pheromone for this species is a 95:5 ratio of (*E*)-11-tetradecenyl acetate (*E*11-14:OAc) and 5 μg (*E*,*E*)-9,11-tetradecadienyl acetate (*E*,*E*9,11-14:OAc) (Bellas et al., 1983). Rubber septa (Arthur H. Thomas) were used as lures in pheromone traps and as calibration sources for the EAG, and contained 100 μg *E*11-14:OAc and 5 μg *E*,*E*9,11-14:OAc (Bellas et al., 1983).

Pheromone dispensers were obtained from Shin-Etsu Chemical Co., Tokyo, and contained 54.9 mg of (*E*11-14:OAc), 2.5 mg of (*E*,*E*9,11-tetradecadienyl acetate (*E*,*E*9,11-14:OAc), and 19.7 mg of (*Z*)-11-tetradecenyl acetate (*Z*11-14:OAc), as well as 16.8 mg of other substances, such as stabilizers. These dispensers were used here as they have been used extensively in over 350 ha of trials of mating disruption of this species (Suckling and Shaw, 1991; Suckling et al., 1990, 1991; unpublished data) and are the most likely dispenser to be of commercial significance. The disadvantage of this blend, containing *Z*11-14:OAc, relates to its role as an inhibitor of trap catch when present with the pheromone (Rumbo et al., 1993). These dispensers release the pheromone at 7–15 μg/hr at 12–20°C, after ten weeks in the field (unpublished data).

### *Electroantennogram apparatus*

The EAG apparatus consists of a Perspex antenna holder placed inside a glass chamber, which isolates the antenna from ambient air (Sauer et al., 1992). The ends of the antenna are held in wells filled with Ringer's solution, containing silver–silver chloride electrodes. A suction pump generated a steady airstream

through the chamber past the antenna. The EAG elicited by odors reaching the antenna was amplified, filtered, and stored on a 386SX portable computer operating with an A-D card, sampling at 18.2 Hz. The glassware used in these experiments was acetone-washed, heated to 170°C for at least 16 hr, and cooled before reuse.

The ground potential of the antenna (the electrical potential of the antenna in clean air) was measured with a charcoal filter attached to the inlet of the glass chamber. The EAG in ambient air was measured after removing the charcoal filter. Calibration was achieved using a rubber septa standard (above), with a defined volume of air (15 ml) blown across the septa and added to the main air stream. The measurement of atmospheric pheromone was always preceded by three calibration pulses from the rubber septa standard. EAG peak heights were measured with computer software (Sauer, 1991).

The antennal response to pheromones was always normalized to the response to the rubber septa standard (calibration pulse) by dividing the measured response by the mean voltage generated by accompanying calibration pulses. This unitless value is defined as the normalized EAG amplitude. For some experiments, the EAG response to leaf volatiles from untreated leaves had to be subtracted from the total response to treated leaves. This value was defined as the differential EAG amplitude and has been presented on a log scale to better represent real concentrations, since the dose–response is log-linear. There was a 10-fold increase in pheromone concentration for a change in differential EAG of ca. 0.25 (unpublished data). Preliminary experiments showed that antennae varied in the degree of baseline sensitivity to host-plant or environmental volatiles and pheromone in orchard air. Single antennae were therefore used for all related measurements, but absolute concentrations could not be calculated without knowing the intercept of the dose–response for an individual antenna.

*Laboratory Experiments*

*Uptake of Pheromone by Leaves.* Apple leaves of approximately uniform surface area (ca. 42 $cm^2$, cv. Granny Smith) were picked from an orchard where there were no pheromone dispensers (untreated area). The EAG elicited by each leaf was measured to determine the antennal response to the leaf volatiles. The petioles were then placed in a vase of water and the leaves held 15 cm downwind from two Shin Etsu pheromone dispensers mounted on the outlet side of a small fan, simulating field conditions of leaves in pheromone-treated environments. Single leaves were exposed in this way for either 3 sec, 10 sec, 30 sec, 1 min, 3 min, 10 min, or 30 min, and then put in a clean glass "cold trap" (95 ml). The cold trap was connected to the EAG apparatus, and five to seven EAG measurements of the pheromone release rate were taken using 1-sec air pulses into the cold trap within 30 sec of removal from the airstream. These EAGs

were normalized to the related calibration pulses (see above). A normalized pretreatment measurement (on the same leaf) was subtracted giving the differential EAG amplitude. Preliminary experiments indicated little difference between untreated leaves; therefore, only one leaf was measured on each occasion as a reference standard.

*Release of Pheromone from Apple Leaves.* Branches (20 cm length) with at least four leaves (as above) were exposed in a pheromone-saturated environment for at least 24 hr. The branches were held in a plastic bag (6-liter volume) containing two pheromone dispensers and a small fan, to ensure an even pheromone concentration in the entire bag. After removal from the pheromone-saturated bag, the branch stems were placed in water, with the leaves in a clean airstream (a fume hood with lowered shield, giving an airspeed of ca. 0.5 m/sec). Pheromone rerelease rate was measured with the EAG after time intervals of 0.25, 10, 30, 135, 360, and 1440 min.

*Field Experiments*

*Trapping with Saturated Leaves.* This experiment aimed to test whether trap catches could be made in an untreated area, with pheromone-treated leaves as lures. Traps were placed in apple trees at the Lincoln University Biological Husbandry Unit, Canterbury, New Zealand, between April 2 and April 22, 1993. Standard delta traps (Suckling and Shaw, 1990) with sticky bases were baited with leaves that had received pheromone exposure in a plastic bag, with a fan circulating the air around two Shin Etsu dispensers and around a branch with the base in a vase. Leaves were exposed in this manner to pheromone for $> 3$ days before use in traps, and catch was monitored nightly for the same leaves for three nights ($N = 10$ traps/treatment). Traps baited with clean leaves were used as controls ($N = 5$), while traps baited with standard rubber septa were also operated ($N = 5$) to indicate flight activity. Traps were prepared about 3 hr before dusk and hung at ca. 1.5 m height with a minimum distance of 5 m between traps. They were inspected and rerandomized daily. After three days in the traps, the leaves were replaced by new, freshly treated leaves, and the cycle repeated. All treatments were present simultaneously. The mean trap catch per trap and night for each leaf age (1, 2, or 3 days after pheromone exposure) was calculated.

*Dispersion of Pheromone from a Point Source.* This experiment was designed to determine the dissemination of pheromone released from dispensers, into nearby apple foliage in the field. Two weeks before the EAG measurements commenced, two dispensers, which had been aged for 10 weeks in the field, were placed in a tree (cv. Red Delicious) grown on the Lincoln canopy system (Dunn and Stolp, 1987). In this canopy, branches are trained horizontally and upright shoots from the base of lateral branches provide a uniform array of leaves in all dimensions.

Leaves of similar size (ca. 20 $cm^2$) were picked after two weeks on $x$, $y$, and $z$ axes (50, 25, 12, 6, and 0 cm) from the two central dispensers, and immediately placed individually in a glass cold trap. The differential EAG amplitude was measured as described above, in which the EAG response elicited by an untreated leaf alone was subtracted from the EAG measured for a leaf post-treatment. An isosphere showing the location of equal response around the two Shin Etsu dispensers (indicated by a differential EAG amplitude of 0.2) was generated by mathematical interpolation of the measured data based on the inverse square root of distance, the Pythagorean function expected to explain changes in concentration with distance.

*Measurement of EAG in an Orchard.* EAG measurements were made on transects across an untreated and a pheromone-treated apple orchard. The trees (cv. Royal Gala) had been planted in 1983 (3.0 × 4.5 m), in north–south oriented rows. Trees were ca. 4.5 m high. Four Shin Etsu pheromone dispensers were placed in a cluster on every second tree, in every second row, resulting in 185 point sources/ha. Dispensers were placed at 1.2–1.6 m above ground, 1 m to the east of the trunk, 11 weeks prior to EAG measurement. The EAG apparatus was placed on a tripod at 1.4 m above ground and was moved in a transect across rows, with measurements taken at four types of sites (shown in Figure 6, p. 1838): (A) 1 m from the trunk of a treated tree, near the dispensers; (B) grass rows, 1 m east of a dispenser; (C) 4.5 m east of A in a row of trees without dispensers; and (D) grass rows, 1 m east of C.

Relative EAG amplitude (mean of three orchard air measurements) was determined at each site ($N$ = 12–22), in a transect across sites A–D. In the untreated block (without dispensers), transects measurements were made in the corresponding positions ($N$ = 13–16). Additional measurements ($N$ = 6–12 per cultivar) were made in untreated blocks containing cv. Royal Gala, Braeburn, Cox's Orange Pippin, and Red Delicious trees, in order to determine the effect of fruit cultivar on EAG response. The subscripts $t$ and $u$ refer to treated and untreated, respectively, in the results.

## RESULTS

### *Laboratory Experiments*

*Uptake of Pheromone by Leaves.* The rate of uptake of pheromone in an airstream by apple leaves was very rapid, with the asymptote reached after ca. 3 min (Figure 1). The rate of uptake was described by the equation:

$$\text{EAG response} = a - a/e^{t}$$

where $a$ is the asymptote ($r^2$ = 94%, 5 $df$). These measurements are an indirect assessment of the process of pheromone uptake, given that our EAG results

reflect the rate of release of pheromone from the leaf, within 30 sec of removal of the leaf from the pheromone airstream. Additional time of exposure beyond 3 min did not lead to a higher pheromone release from the measured leaves, indicating that equilibrium for the application method was reached within 3 min.

The measurements of the EAG elicited by pheromone-treated leaves indicate the rate of rerelease of pheromone from the leaf surface, not the amount of pheromone actually absorbed into the leaf. Hence we do not know the degree of saturation of the leaves with pheromone, only that the leaf is able to release pheromone in proportion to the length of exposure. In our experiments we took the release as a measure of the uptake of pheromone by the treated leaves.

*Release of Pheromone from Leaves.* The release of pheromone from leaves was slower than the rate of uptake, with a declining rate of release detectable over several hours (Figure 2). Pheromone was still detected after 24 hr, at which

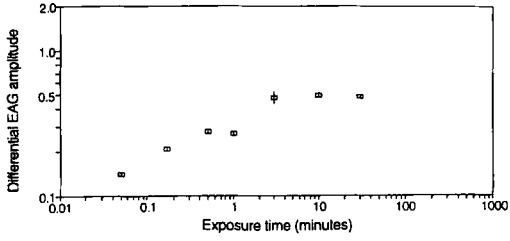


FIG. 1. Changes in differential EAG amplitude (±SE) over time measured with EAG, following increasing periods of exposure of leaves to a steady airstream containing pheromone of *Epiphyas postvittana*. The rate of uptake of pheromone by single apple leaf was very rapid, with the asymptote reached after about 3 min.

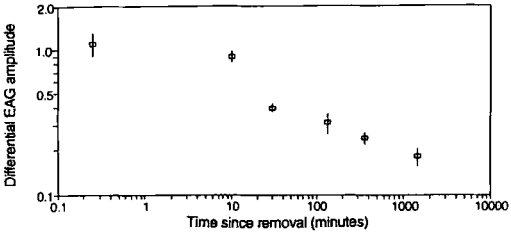


FIG. 2. Changes in differential EAG amplitude (±SE) over time measured with EAG, following removal of leaves from saturated environment of pheromone of *Epiphyas postvittana*. Measurement were made on four replicate leaves, using an untreated leaf as a reference for background plant volatile response. The release rate of pheromone initially declined rapidly, but pheromone was still detected after 24 h.

point the experiment was terminated. The differential EAG dropped from 1.1 to 0.2 in 24 hr, with half the EAG amplitude (0.65) reached after 20 min.

The different method of exposure in this experiment probably accounts partly for the high initial EAG amplitude reached by leaves kept in a pheromone saturated bag (Figure 2), compared to the amplitude reached by leaves exposed in a current of pheromone-treated air (Figure 1). The difference in the differential EAG amplitude between 30 min of exposure in an airstream, and after 24 hr in the bag was approximately twofold. The initial rapid loss of pheromone (shown by the higher EAG response in Figure 2) led to differential EAGs after 20 min, which were similar to those of the first experiment (Figure 1). The most probable sites of pheromone adsorption with leaves are the surface waxes (Wall et al., 1981) due to their similar chemical attributes to pheromones (e.g., lipophilicity) (Fernandes et al., 1964; Baker, 1982; Jeffree, 1986). The waxes would function as pheromone reservoirs lacking a rate controlling mechanism, but probably following a rate of release proportional to $t^{-1/2}$ (Zeoli et al., 1981).

*Field Experiments*

*Trap Catch.* The catch of males in traps baited with leaves (Figure 3), gives behavioral corroboration of the EAG results. The mean catch per trap per night declined logarithmically over three nights, from 0.24 males per trap per night for traps baited with leaves removed from the saturated environment 3 hr before dusk, when flight and capture occurred. No moths were caught with untreated leaves, while the mean catch with rubber septa was 5.55 males per trap per night. Catch with freshly treated leaves releasing the pheromone plus

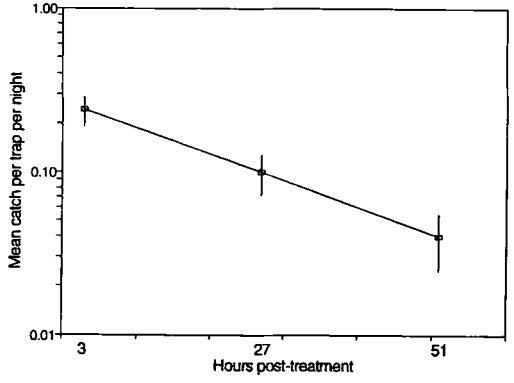


FIG. 3. Changes in trap catch of male *Epiphyas postvittana* (±SE) over time, following removal of leaves from a saturated environment of pheromone. Nearby traps baited with rubber septa caught a mean of 5.5 males per trap per night during this period.

inhibitor was therefore about 4% of catches with rubber septa lures releasing the pheromone. Since moth responses to traps baited with rubber septa are easily disrupted (Suckling and Shaw, 1992), this suggests that the attractiveness of leaves would be relatively insignificant in a mating disruption environment. This does not preclude their role in habituation or adaptation mechanisms of disruption. Traps were not replaced with new traps daily in this experiment, as it was considered that biologically significant quantities of pheromone released from the leaves would not be adsorbed and released from the traps, in contrast with leaves that had been exposed to pheromone in a saturated environment.

This experiment demonstrated that *E. postvittana* males were attracted to the pheromone blend rereleased from the leaves, despite the fact that a blend containing Z11-14:OAc has been reported to function as an inhibitor in Australia (Bartell, 1982; Rumbo et al., 1993) and New Zealand (Suckling and Rumbo, unpublished data), reducing catch by ca. three fold. Dispensers like those used in the current study (containing 30% Z11-14:OAc) were also successful at trapping moths, when placed in traps in untreated areas (Suckling, unpublished results).

*Dispersion of Pheromone from a Point Source.* This experiment shows the ability of apple leaves to function as a pheromone buffer system, under field conditions (Figure 4). The pheromone dispersed from the dispenser and was adsorbed onto the surrounding plants, which thus became pheromone dispensers of their own. The rate of pheromone release by leaves collected near two dispensers was a function of distance from the dispensers in the horizontal and vertical planes.

The differential normalized EAG amplitude (±SE) elicited by leaves was plotted against the different distances from a central pheromone point source.

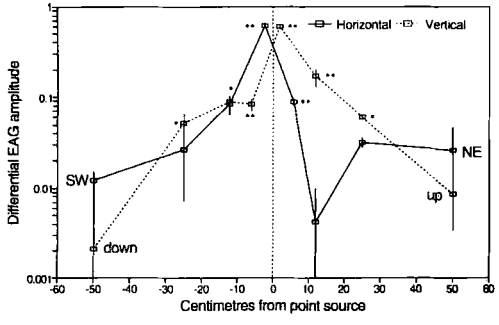


FIG. 4. Horizontal and vertical changes in differential EAG amplitude (±SE) elicited by leaves at different distances from a central pheromone point source of two *Epiphyas postvittana* pheromone dispensers placed in a three-dimensional array of apple leaves. (*$P < 0.05$; **$P < 0.01$).

The measurements used only one horizontal axis and the vertical axis, in order to sustain a good antennal response for the whole transect (Figure 4). Pheromone was detected above the background odor of untreated leaves (differential EAG) up to 25 cm away from the central point source. All differential EAG amplitudes were significantly higher than the untreated leaf ($P < 0.05$), except for SW 25 cm and NE 12.5 cm (Figure 4). Pheromone could not be detected on leaves 50 cm from the center. This is probably due to the interference of plant or environmental volatiles with the pheromone reception, which reduces the resolution of the EAG system. The leaves sampled varied in size (range 13–68 $cm^2$) and age, but we failed to find any consistent relation between leaf size or age and the level of EAG response to individual leaves.

Data from a second set of measurements (which covered a smaller range of distances, but across three dimensions) were used to estimate the three-dimensional shape of the surface of an isosphere of constant pheromone concentration surrounding the point source (Figure 5).

The isospheres of equal pheromone concentration (grey ribbons, differential EAG = 2) achieved by interpolation of the measured data deviate from the (ideal) sphere (white ribbon), which indicated equidistant points on the $x$, $y$, and $z$ axes around the central point source. Higher pheromone release was measured on the leaves beneath and to the south of the pheromone source.

Pheromone molecules presumably are dispersed downward once released from the dispensers, possibly due to the influence of downdrafts. Pheromone release rates are greater at warm temperatures, so the spatial pattern is probably more strongly influenced by warmer winds. Temperature may also affect the absorptivity of the leaves.

*EAG Measurements on a Transect through an Orchard.* There were significant differences in the EAG responses recorded at different sites. In the tree rows (site $A_t$, a treated site located 1 m from the trunk of a tree with dispenser), the mean EAG was 0.367 ($\pm$0.026). This normalized EAG amplitude was significantly different from those in all other sites ($P < 0.001$) and was higher than in the untreated orchard. The average pheromone concentration in the grass rows site, $B_t$ (1 m east of a dispenser) reached an average value of 0.252 ($\pm$0.009), which was not significantly different from sites $C_t$ and $D_t$. In the next tree row (site $C_t$ tree row with no dispenser, 4.5 m East of $A_t$), an average normalized EAG of 0.265 ($\pm$0.010) was measured. This was significantly different from site $D_t$ ($P < 0.05$), but not significantly different from site $B_t$. The lowest normalized EAG response of 0.228 ($\pm$0.009) was measured in site $D_t$, the grass row, 1 m East of $C_t$. Measurements made in the corresponding sites in an untreated block ($C_u$ and $D_u$), showed a significantly lower EAG amplitude than in the pheromone-treated sites. The normalized EAG amplitude in the grass row (site $D_u$) was 0.224 ($\pm$0.004), the average normalized EAG in the tree row (site $C_u$) 0.217 ($\pm$0.007). There was no significant difference between the

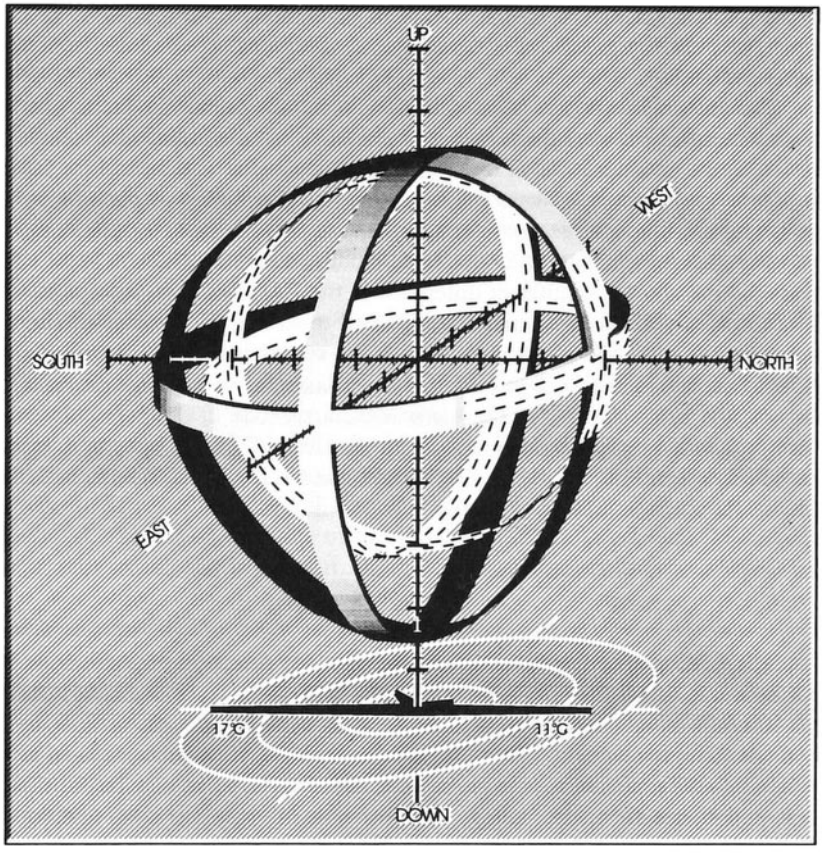


FIG. 5. Predicted isosphere of differential EAG response of 0.2 surrounding two *Epiphyas postvittana* pheromone dispensers placed in a volume of apple leaves. White bands represent a perfect sphere at 15 cm radius, while grey bands represent the derived EAG responses.

untreated tree (site $D_u$) and (1) untreated grass-row (site $C_u$) or (2) the treated grass row site $D_t$. The treated site $C_t$ was significantly higher than the corresponding untreated site, $C_u$. The measurements in the untreated block also showed much lower variance than sites in the pheromone-treated block.

With increasing distance, the pheromone concentration decreases, and the buffering effect of the plant surfaces become predominant. This can explain the increased EAG amplitude measured at site $C_t$ (tree row without dispenser). The EAG signal at this site was significantly higher than at site $B_t$ (grass row, $P < 0.05$), although further away from the dispenser. The increased response at site

$C_t$ was probably achieved by the release of pheromone from the leaves of the apple trees. In the grass rows, there was no additional pheromone from the leaves available.

The antennal responses elicited in these locations cannot be due to pheromone molecules but were the response to plant or other environmental volatiles, for which the antenna is much less sensitive. The smaller variance of the EAG amplitude in the untreated sites indicates that the distribution of plant or environmental volatiles was also less variable than the distribution of the pheromone.

The measurements made in untreated blocks containing different apple cultivars did not show a significant difference in the EAG baseline. Receptors for plant or other volatiles are generally described to have a wider range of stimulants than the pheromone receptors, which are very specific (Den Otter et al., 1978). Discrimination between different environmental volatiles in the field is not possible using the EAG. The same EAG amplitude in different cultivars, therefore, would not necessarily require an identical volatile composition, since the odorants contribution to the EAG could conceivably be different, but still achieve the same amplitude (although this seems unlikely).

## DISCUSSION

The EAG device has several disadvantages for detection of pheromone in low concentrations. It is probably the most sensitive detector available, being ca. 100-fold more sensitive to pheromone than standard gas chromatographic methods (Bengtsson et al., 1994). The measurements are instantaneous and give insight into the rapid changes in pheromone concentration that may be of behavioral importance for mate finding. The low cost of sampling is an additional advantage. Due to the fast response and the mobility of the device, results can be achieved that are not otherwise possible (e.g., transects).

The main disadvantage of the EAG is the difficulty in the estimation of absolute pheromone concentrations. Although the antenna is a sensitive pheromone detector, the antennogram is nonspecific due to the large number of receptors for plant volatiles on the antenna. For *Lobesia botrana* Hb. (Sauer et al., 1992) and *Cydia nigricana* F. (Bengtsson et al., 1994), the low level of baseline response presented much less a problem in estimating atmospheric pheromone concentrations. Individual light-brown apple moths vary in responsiveness to pheromone and environmental volatiles (unpublished data). Here, the use of differential EAG measurements circumvented this problem. However, the variation in sensitivity to environmental odor and pheromone stimuli between insects requires that all related measurements be taken with a single antenna. Since antennae only lasted up to ca. 2 hr, this limited the length of our experiments.

A second major disadvantage of the EAG for field measurements of pheromone is that the antennal response is essentially sigmoid. Depending on the pheromone concentration present in the odor "background" in the field, equal changes in the antennal electrical potential could indicate different changes in pheromone concentration. Therefore the baseline potential of the antenna in clean air and untreated orchards always needs to be measured (unless a reliable "environmental odor" calibration can be developed). Hence measurement of absolute pheromone concentrations is not possible with the EAG alone, although this can be achieved with additional EAG–gas chromatography measurements (Bengtsson et al., 1994).

Apple leaves can take up and release the pheromone of *E. postvittana*, according to both electroantennogram and behavioral studies. These findings agree with several previous studies. Wall et al. (1981) showed that pheromones rereleased from pea leaves are attractive to male *Cydia nigricana*. Noldus et al. (1991) reported that pheromone absorbed onto Brussels sprouts attracts *Mamestra brassicae* and their parasitoids. Karg et al. (1990) showed that the pheromone concentration and structure of the pheromone cloud in treated vineyards was highly dependent on the state of the vegetation in the target area. In summer, with fully developed vegetation, the pheromone concentration was higher and the pheromone cloud more evenly distributed. Pheromone concentration increased rapidly after application of pheromone dispensers. However, the removal of the dispensers was followed by a fast initial drop in the pheromone concentration due to the lack of pheromone release from the dispensers, followed by a rather slow process of decline of the remaining pheromone concentration due to the rerelease of the leaves. The more rapid uptake of *E. postvittana* pheromone, compared to rate of release from apple foliage shows the same pattern in grapes (Karg et al., 1990).

In this study, the portable EAG system enables us to measure the pheromone rerelease of single pre-exposed leaves as well as the pheromone concentration within a treated orchard, thus giving a description of the actual spatial distribution within these areas. The distribution of pheromone in the target areas is important in the identification of the mechanisms of mating disruption of *E. postvittana*.

The primary mechanism(s) of mating disruption are still unknown, but two are most likely in this case: false trail following and adaptation/habituation, although other mechanisms are possible (Bartell, 1982; Cardé, 1990). Polyethylene dispensers identical to those used here were found to be attractive in traps (Suckling, unpublished data), and false trails from these dispensers cannot be completely discounted. Peripheral adaptation seems unlikely to be a major factor, with the recovery of the receptors in clean air within seconds (Rumbo, 1981). In contrast, habituation at the CNS level (Bartell and Lawrence, 1973) is probably very important in *E. postvittana*. Disruption using only a partial

(nonattractive) blend (Suckling and Clearwater, 1990) speaks for adaptation, habituation, or combinations of these as the mechanism for successful mating disruption.

The comparatively low quantities of pheromone released from a single leaf in our experiments suggests that leaves probably cannot create false trails. However, leaves could function to multiply the number of plumes of varying strength (reducing with the inverse square of the distance from the dispenser), with the result of a complex pheromone cloud, rather than trails. The total contribution from leaves releasing pheromone in a treated orchard could be sufficient to assist adaptation of the sensory organs or habituation of the insects (Baker et al., 1988). In situations with a rather homogeneous pheromone cloud, upwind flight ceases to take place (Kennedy, 1978), so it is plausible that the foliage may have a role as a spatial buffer in influencing atmospheric pheromone concentrations, and hence disruption, in addition to providing wind resistance.

The pheromone quantity depended on location in the treated area (e.g., tree row or grass row, Figure 6.) Pheromone is present, at least intermittently, in the whole treated area, and a male moth in this area would perceive pheromone. The temporal resolution of the antenna becomes slower with higher concentration and sustained stimulation (Baker et al., 1985). The fusion of the antennal signal does not have to be complete, as Baker et al. (1985) originally stated, but smoothing of the signal can be sufficient (Baker and Haynes, 1989) to end upwind flight.

Pheromone release from foliage depends on the temperature and the pher-

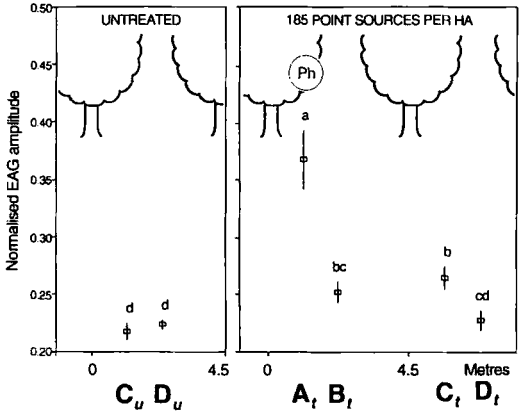


FIG. 6. Differences in mean normalized EAG amplitude (±SE) in treated and untreated blocks with samples taken in tree and grass rows. Sample sites (capital letters) are defined in the text. Ph = four pheromone dispensers. Data points separated by different lowercase letters were significantly different according to a $t$ test ($P < 0.05$).

omone concentration gradient between plant waxes and ambient air and can be described as a pheromone reservoir lacking a rate-controlling mechanism. It is most likely that such a leaf system has different release characteristics than the polyethylene dispensers, with greater influence from wind speed on release rate. These different release characteristics could conceivably have an averaging effect on the pheromone concentration, for example, by partly compensating for increased wind speed through increased pheromone release under higher winds.

Our results clearly show that adsorption and release of pheromone of *E. postvittana* and apple foliage occurs in the treated area. This effect may be a significant influence on the distribution over time and space of the pheromone and hence, on efficacy and potential mechanisms of disruption. Orchards that lack foliage canopy due to either seasonal effects or tree management, are more likely to experience difficulty in achieving successful disruption. Foliage can be important to atmospheric pheromone concentrations in orchards in either of two ways. Foliage can reduce wind speed from physical resistance or impact on concentrations through acting as a sink and source, due to the adsorption and release of pheromone from foliage described here. The effect of foliage on quantity of atmospheric pheromone has been demonstrated here, although it remains unclear whether this effect is large enough to have a significant impact on the efficacy of behavioral disruption. Foliage also contributes to reduced signal variance, but the role of release of pheromone from apple leaves on this effect also requires further elucidation.

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