

## MISLEADING THE COLORADO POTATO BEETLE WITH AN ODOR BLEND<sup>1</sup>

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**Abstract**—Walking tracks of Colorado potato beetles, *Leptinotarsa decemlineata* Say, were recorded on a locomotion-compensator in response to wind, odors of host plants *Solanum tuberosum* L. and nonhost plants *Lycopersicon hirsutum* f. *glabratum* C.H. Mull, and to mixtures of these plant species. Host-plant odor induced positive anemotactic responses in starved females, whereas odor of the nonhost *L. hirsutum* was neither repellent nor attractive. The attractiveness of host-plant odor, however, was neutralized in the odor blend of plant species. Masking the attractive host-plant odor will hinder the beetle's searching for host-plant patches, and this principle may be exploited in pest control by mixed cropping.

**Key Words**—Colorado potato beetle, *Leptinotarsa decemlineata*, Coleoptera, Chrysomelidae, *Solanum tuberosum*, *Lycopersicon hirsutum* f. *glabratum*, chemoattraction, masking, mixed cropping, olfactory orientation, searching behavior.

### INTRODUCTION

Insect pest levels are often lower in mixed cropping systems than in monocultures, and it has been suggested that the searching behavior of phytophagous insects is disrupted by the confusion of signals from intercropped plants (Cromartie, 1981; Kareiva, 1983; Stanton, 1983). There is, however, no direct evi-

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dence that when the odor of a nonhost plant is added to a plume of host-plant odor this will disrupt the insect's olfactory orientation. The purpose of the present study was to see whether odor blending affects the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, towards its host-plant potato *Solanum tuberosum* L.

The walking Colorado potato beetle responds to potato-plant odor by an odor-conditioned positive anemotaxis (Visser, 1976; Visser and Thiery, 1985). The composition of the potato leaf odor was previously studied (Visser et al., 1979; Visser, 1983), and electrophysiological experiments revealed the characteristics of the antennal olfactory receptors (Visser, 1979, 1983).

Although in laboratory studies experimental conditions are precisely controlled, which makes a comparison with field conditions difficult, this approach is essential to study the behavioral patterns that underlie the insect's colonization of host-plant patches in the field. This paper presents data on the odor-conditioned anemotaxes of Colorado potato beetles as released by host-plant odor, and the effects of nonhost-plant odor on these responses. We used as a source of nonhost-plant odor the strong-smelling wild tomato *Lycopersicon hirsutum* f. *glabratum* C.H. Mull.

#### METHODS AND MATERIALS

*Recordings of Behavior.* Experiments were previously described on the orientation of Colorado potato beetles towards a source of windborne potato-plant odor in a low-speed wind tunnel (Visser, 1976). In the present study, behavioral responses of beetles were recorded using a locomotion-compensator in front of this wind tunnel. The insect test section of the original tunnel was removed, and the flow leaving the contraction passed over the beetle walking on the locomotion-compensator. This instrument, which was designed by E. Kramer and P. Heinecke (Max-Planck-Institut fuer Verhaltensphysiologie, Seewiesen, FRG), has been used in a number of studies on insect orientation (Kramer, 1976; Weber et al., 1981).

In brief, the locomotion-compensator operates as follows: an insect walks freely on top of a large sphere while its positional change is monitored by means of a beam of light reflected by a small mirror which is glued to its elytra. Walking activity is continuously compensated by two motors rolling the sphere in the opposite direction. The rotations of the sphere are detected by two pulse generators in contact with the sphere, and pulses are recorded and analyzed by a computer. In this way, the beetle's position was recorded every second, and walking speed and direction were calculated. Full technical details of this equipment will be presented elsewhere (Visser and Thiery, in preparation).

*Insects and Plants.* Beetles were obtained from laboratory stock culture.

Newly emerged females were fed for 2 hr on potato foliage and then starved for at least 12 hr prior to the experiments. At the time of the experiments, the females were about one day old. Plants were reared in greenhouses: potatoes *Solanum tuberosum* cultivar Eigenheimer, and wild tomatoes *Lycopersicon hirsutum* f. *glabratum*.

*Experiments.* Individual females were exposed for 10 min to each of four subsequent stimuli: (1) a clean air flow of 80 cm/sec as control, and the same air flow carrying (2) the odor of wild tomato *L. hirsutum* f. *glabratum*, (3) the odor of potato *S. tuberosum*, and (4) the odor from a mixture of the two plant species. At least 1 hr elapsed between successive exposures of individual females. For each plant species, three pots of fully grown plants were placed in the dark upwind section of the wind tunnel, and contained approximately an equal volume of foliage. The experiments were conducted at 24°C. Light intensity on top of the sphere was set at 1750 lux by means of two high-frequency illumination units (2500 Hz). The air speed was measured at the outlet of the wind tunnel. Other conditions were previously described (Thiery and Visser, 1986).

*Analyses of Responses.* Five variables are used to describe the beetle's responses: (1) walking speed as the mean of 599 instantaneous speeds per individual, (2) vector length as the resultant displacement from the origin after 600 sec, (3) straightness of the track as the quotient of the vector length and the total length of the walking track, (4) time spent walking upwind corresponds to the proportion of angle observations with deviations of less than 60° from the wind direction, and (5) upwind length as the upwind displacement after 600 sec. Variables were compared between treatments using nonparametric statistics (Siegel, 1956): sign test (variable 3) and Wilcoxon's test (variable 1, 2, 4, and 5). In this way the variables from the treatments were tested as related samples which compensates for the large variations in walking speeds between individual beetles.

## RESULTS AND DISCUSSION

*Orientation Responses.* Representative walking tracks of an individual female in response to wind, nonhost-plant odor, host-plant odor, and odor of the mixture of host and nonhost plants are shown in Figure 1(a-d). Circular distributions of the beetles in the four treatments are depicted in Figure 2, and Table 1 shows the corresponding values of the variables that quantify orientation.

In a clean air flow, Colorado potato beetles walked with a weak preference for upwind directions which is manifested by their net upwind displacement (Table 1). In this stimulus condition the beetles did not walk very straight (straightness is 0.38). Odor of the wild tomato did not elicit an increase in

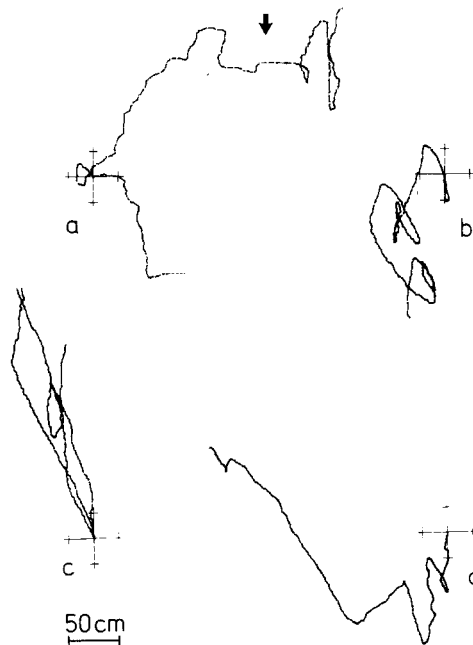


FIG. 1. Tracks of one female Colorado potato beetle in four successive treatments of 10 min each: (a) wind control, (b) *Lycopersicon hirsutum* odor, (c) *Solanum tuberosum* odor, and (d) odor blend of *L. hirsutum* and *S. tuberosum*. Arrow indicates wind direction. The plotter was reset to the origin automatically at reaching the edge of the paper.

upwind responses of the beetles. Vector length, straightness, upwind time, and upwind length were not changed as compared with clean air. However, walking speed decreased significantly relative to that in clean air.

When stimulated by potato-plant odor, all beetles orientated completely upwind (Figure 2, Table 1). They spent most of their time walking straight upwind towards the odor source, as can be seen in an increased straightness and upwind time. The resultant vector measured about twice the vector length from the previous experiments. The upwind length equaled approximately the vector length since the latter was pointed strictly upwind.

The odor blend consisting of *L. hirsutum* and *S. tuberosum* volatiles, however, caused a decrease of all variables. Vector length, straightness, upwind time, and upwind length of the track were comparable with those in the clean air treatment. The significant decrease of walking speed did not cause a further reduction in vector length or upwind length (Table 1). The presence of nonhost-plant odor in the air flow apparently inhibits the positive anemotactic responses which are observed with potato plant odor; the beetles do not walk upwind.

*Masking by Nonhost-Plant Odor.* When compared with the wind stimulus

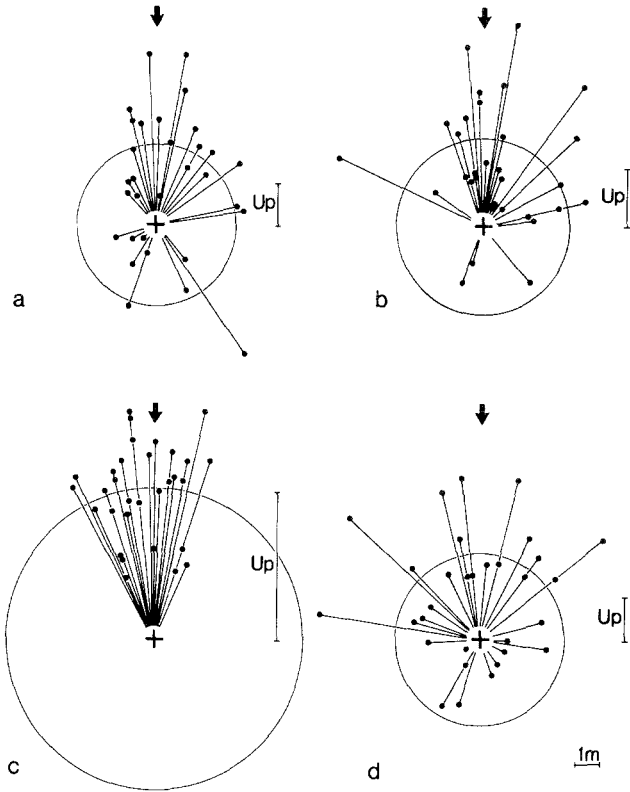


FIG. 2. Circular distribution of 31 female Colorado potato beetles in four successive treatments: (a) wind control, (b) *Lycopersicon hirsutum* odor, (c) *Solanum tuberosum* odor, and (d) odor blend of *L. hirsutum* and *S. tuberosum*. Each line represents the vector of displacement of one beetle after 10 min of walking. The mean vector length in each treatment is shown as the radius of the circle. Up: upwind component of the mean vector. Arrows indicate wind direction.

alone, wild tomato is neither repellent nor attractive. The beetle's walking speed is reduced on stimulation by both the odor of wild tomatoes, and the blend of odors. Nevertheless, in these conditions vector length and upwind length are comparable with those measured in pure wind. This implies that repellency is not involved since this term is defined as a movement being directed away from the odor source (Dethier et al., 1960), and would be directed downwind. The neutralization of orientation responses in the Colorado potato beetle is interpreted as the result of olfactory camouflage of the host plant by combining it with a different odor. An essential part of the attractive potato leaf odor consists of a complex of  $C_6$  alcohols, aldehydes, and corresponding derivatives generally distributed in green leaves (Visser et al., 1979). The specificity of this so-

TABLE 1. RESPONSES OF FEMALE COLORADO POTATO BEETLES TO ODORS OF *Lycopersicon hirsutum*, *Solanum tuberosum*, AND MIXTURES OF PLANT SPECIES<sup>a</sup>

| Stimulus            | Walking speed (mm/sec)   | Vector length (mm) | Straightness | Time walking upwind (%) | Upwind length (mm) |
|---------------------|--------------------------|--------------------|--------------|-------------------------|--------------------|
| Wind                | 14.2 ± 0.8a <sup>b</sup> | 3289 ± 626a        | 0.38 ± 0.06a | 57 ± 7a                 | 1675 ± 1054a       |
| <i>L. hirsutum</i>  | 11.8 ± 0.7b              | 3559 ± 733a        | 0.49 ± 0.09a | 65 ± 7a                 | 2442 ± 924a        |
| <i>S. tuberosum</i> | 13.8 ± 0.7a              | 6210 ± 682b        | 0.75 ± 0.06b | 86 ± 4b                 | 6014 ± 692b        |
| Odor blend          | 12.3 ± 0.6b              | 3420 ± 702a        | 0.46 ± 0.09a | 57 ± 7a                 | 1823 ± 918a        |

<sup>a</sup>Data represent means ±95% confidence intervals (two-tailed). Number of beetles is 31.

<sup>b</sup>Different letters in a column indicate statistical differences between treatments at  $P < 0.001$  (two-tailed).

called green odor is set by the ratios of its individual components, since changing their ratios, by addition of pure volatile chemicals, interrupts the upwind movements of the beetles (Visser and Avé, 1978). In the present study it is shown that addition of the odor of wild tomato plants to the potato-leaf odor, likewise blocks the release of positive anemotactic responses in this beetle.

It is noteworthy that an accession of *L. hirsutum* f. *glabratum* that contains high levels of 2-tridecanone, is resistant to the Colorado potato beetle (Kennedy and Sorenson, 1985). In other experiments, the camouflage of potato plants was also obtained by adding the odor of cabbage *Brassica oleracea* L. var. *gemmifera* DC. (Thiery and Visser, 1986). For that reason, masking of host-plant odor is thought to be caused by the change in green odor composition, although further experiments are needed in order to exclude 2-tridecanone as a masking component.

Potato plant odor releases odor-conditioned positive anemotactic responses in females as well as in males and postdiapause beetles (Visser, 1976; Visser and Nielsen, 1977). The disruption of olfactory orientation by the mixing of odors occurs independently of the beetle's feeding experience since its emergence; the same effects were found when "naive" females were used (Thiery and Visser, 1986).

*Mixed Cropping.* The translation of the present findings on odor blending to field conditions requires consideration of the relevance of the beetle's walking behavior to field conditions and the extent of odor blending in the atmosphere. In Western Europe host-plant finding by the Colorado potato beetle happens mainly while the beetle is walking (the late J. de Wilde, unpublished field observations 1979, 1981). Newly emerged and postdiapause adults are unable to fly until 8–10 days of age when flight muscles are fully developed (De Kort, 1969). In the field, wind turbulence will blend volatiles from a mixed stand of plants (Stanton, 1983; Visser, 1986). It is expected, therefore, that the

beetle's range of attraction to host-plant odor is reduced in mixed cropping systems. Masking the attractive host-plant odor will then hinder the beetle's searching for host plants.

Bach (1982) reported recaptures of Colorado potato beetles which were released between small field plots containing different arrangements of host and nonhost plants. In contrast with the conclusions of the present laboratory study, she emphasized that the colonization of these beetles did not appear to be negatively affected by the presence of nonhost plants, like melon. Apart from not mentioning sex, age, and prior starvation period of the beetles, information is lacking on relevant abiotic factors such as temperatures and the prevailing wind direction. Since Bach noted that statistics were not justified with the small sample size used, this study is insufficient to conclude that plant diversity does not hinder the colonization of host plants by Colorado potato beetles.

Panasiuk (1984) and Schearer (1984) reported volatiles of tansy *Tanacetum vulgare*, that repelled Colorado potato beetles. Their studies were undertaken in order to isolate components which cause the observed reductions of beetle populations when potatoes were interplanted with tansy. These two investigations applied nearly the same methodology; however, their results differed strikingly. For example, Panasiuk reported that  $\alpha$ -pinene attracted beetles, whereas Schearer's data suggested repellency of this compound. These observations contradict EAG data showing that the antennal receptors of the Colorado potato beetle are insensitive to this compound; small EAG responses were solely obtained to high doses that are not present in the headspace of plants (Visser, 1979). Moreover, the reports of Panasiuk and Schearer do not contain substantial data on beetle responses, and the applied doses exceeded many times their concentrations in natural plant-odor blends. The suggestion that the compounds which they identified as repellents are responsible for the reductions of beetle populations in interplantings is premature since an extreme dose of any volatile compound will be repellent (Visser, 1986).

*Conclusions.* Plant odors are composed of general as well as specific components (Visser, 1986). The present study shows evidence that the attractive host-plant odor is masked on blending with nonhost-plant odor. In heterogeneous ecosystems and in mixed croppings, it is expected, therefore, that host-plant odors are frequently camouflaged. This principle needs further experimentation to develop intelligent methods of intercropping for insect pest control.

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