TRAIL PHEROMONE-CONDITIONED ANEMOTAXIS BY THE ARGENTINE ANT, *IRIDOMYRMEX HUMILIS*

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Ent. exp. & appl. **32** (1982) 232—237. *Ned. Entomol. Ver. Amsterdam*

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Odor-conditioned anemotaxis was exhibited by Argentine ant workers, *Iridomyrmex humilis* (Mayr) to sources of the major trail pheromone component, (*Z*)-9-hexadecenal (Z9-16 : ALD). More ants oriented toward and successfully located point or permeated-field sources of Z9-16 : ALD in wind than in still air. Trail pheromone-conditioned anemotaxis may effectively increase the communication distance of the trial beyond that allowed by chemotaxis alone.

KEY WORDS: Trail pheromone — (*Z*)-9-hexadecenal — Odor-conditioned anemotaxis — Argentine ant — *Iridomyrmex humilis*.

In the trail-recruitment system of the Argentine ant, *Iridomyrmex humilis* (Mayr), (*Z*)-9-hexadecenal (Z9-16 : ALD) is important for leading workers to newly discovered food sources. It was identified from the (ventral) Pavan's gland (Cavill *et al.*, 1979, 1980) and shown to elicit prolonged and intense trail following behavior in newly recruited unfed workers (Van Vorhis Key & Baker, 1982). Trail following was highly specific to only this isomer, with other positional and geometric isomers, other aldehyde analogues, and formate "mimics" evoking no significient levels of trail following (Van Vorhis Key & Baker, 1982).

Argentine ant workers can accurately trail follow using only the vapor cloud of their trail chemicals (Van Vorhis Key *et al.*, 1981), and Z9-16 : ALD causes "aggregation and attraction" of workers at close range in a static air environment (Cavill *et al.*, 1979, 1980). We wondered whether the response of workers to volatilized Z9-16 : ALD would extend to even greater distances if wind were present as a directional cue. It would be of more than heuristic value to know whether these ants could use anemotaxis as well as chemotaxis to locate and maintain contact with trails of this compound. The speed with which toxic baits are located and picked up might be increased by the longer distance orientation of ants to the Z9-16 : ALD with which the baits could be impregnated. We report here that from several centimeters away, Argentine ants do move upwind in a vapor field permeated with Z9-16 : ALD and to point sources of this compound.

METHODS AND MATERIALS

Laboratory of colonies *I. humilis* collected locally in Riverside, California, were maintained as described by Van Vorhis Key *et al.* (1981). Ants used for bioassay had been recruited by nestmates to a newly introduced food source and were trail following inside flexible tubing towards the food. By diverting the tubing, ants were redirected without disturbance directly into the bioassay chamber. Chemical purity of Z9-16 : ALD used in "permeation" treatments was determined by gas liquid chromatography using 10% XF-1150 (50% cyano-ethyl, methyl silicone) on Chromosorb W, AW DMCS, 100/120 mesh on a 2.5 m × 2 mm column at 170° and 30 ml/min carrier flow rate. The Z9-16 : ALD was greater than 97% free of other volatile impurities.

The chamber (Fig. 1) was comprised of two 2 mm-thick horizontal glass plates, 29 × 33 cm, separated by 2 cm by plastic spacer bars along 2 sides. A piece of filter paper (Whatman #1, 24 cm diam) on the bottom plate covered the working area of the chamber floor. A smooth flow of air at 0.3—0.5 m/sec was created by placing one open end of the chamber inside and the other end outside a laboratory fume hood and closing the door of the hood until the desired velocity was created by the reduced pressure (Fig. 1).

The trail pheromone component, Z9-16 : ALD, was presented in 2 ways: as a wide, relatively uniform swath of permeated air, and

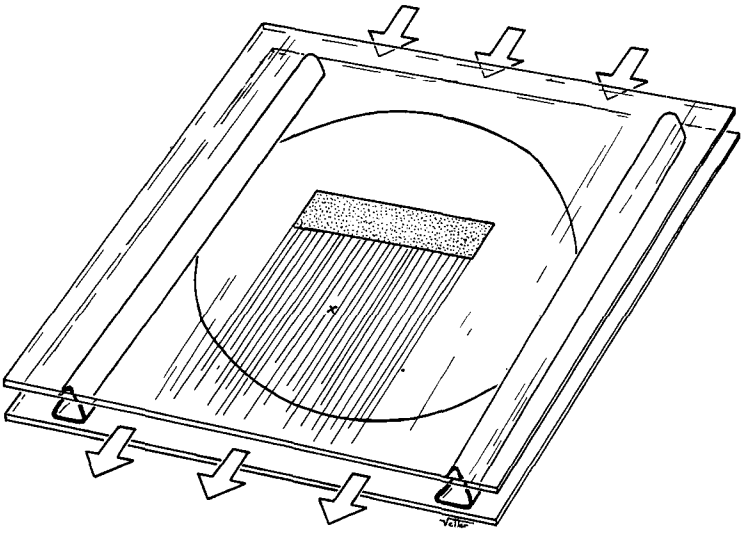

Fig. 1. Bioassay chamber: glass plates, plastic spacer bars and permeated field of pheromone (or solvent) on filter paper disc. Ant release site, "X", is 6 cm from applied source boundary. Air flow indicated by arrows.

as a point source creating a time-averaged plume ca 3.5 cm wide 6 cm downwind in the chamber. For the permeated swath, a 3 × 13 cm rectangular area of the filter paper floor upwind of the center of the chamber was impregnated evenly with 385 ng of Z9-16 : ALD in 1.75 ml dichloromethane applied by pipette. Chemical smoke ($TiCl_4$) visualization of the resulting emission verified that a relatively uniform 13 cm-wide field permeated down the length of the chamber. For still-air bioassays, the pheromone was first allowed to be carried across the chamber by the usual airflow, and then the upwind end was sealed abruptly to stop the flow. Ants were then introduced singly into the chamber center, 6 cm downwind from the impregnated section of paper by introducing recruited ants from the tubing into the chamber through the downwind end. Ants were placed similarly onto the equivalent spot in the chamber in moving air permeated with pheromone as well as in the solvent control treatments with and without moving air.

For point-source experiments, the same chamber and techniques for creating and stopping airflow were used as in the permeated swath experiments described above. However, Z9-16 : ALD (same purity as above) was emitted from an Albany International Controlled Release Division hollow fiber (1.7 cm × 0.2 mm i.d.) placed parallel to the wind flow in the chamber's upwind center so that its open (downwind) end was 6 cm directly upwind of the ant release site. An empty teflon tube (1.7 cm long; #30) placed in the same position was used as a control.

Video recordings were made of all tracks using a Sony RSC 1050 rotary shutter camera and SLO 340 video cassette recorder. Tapes were recorded onto a Sony SVM 1010 motion analyzer monitor and played back frame by frame. Ant positions were traced from the monitor onto acetate sheets each sec for 10 sec from the time of introduction or until they reached the source of pheromone (whichever came sooner) in the permeated airstream experiment. For the point-source experiment, ant positions were traced onto acetate every sec for 35 sec, or until they touched the fiber's tip, or left the scoring area, whichever occurred first.

Each ant's track was then transformed into a series of resultant vectors by connecting each 1 sec position with the next by straight lines (Fig. 2). For each vector, orientation (θ_i) relative to directly upwind (= 0°) and length (L_i) were calculated (the term "upwind" is used here to designate the same direction relative to the test chamber whether the air was moving or stationary). Then, for each ant an index of upwind orientation, UI, was calculated (Durand & Greenwood, 1958), being the upwind component of the mean vector or the net upwind movement. Our index differs from that of Durand & Greenwood (1958) in that we took account of the distance travelled as well as the

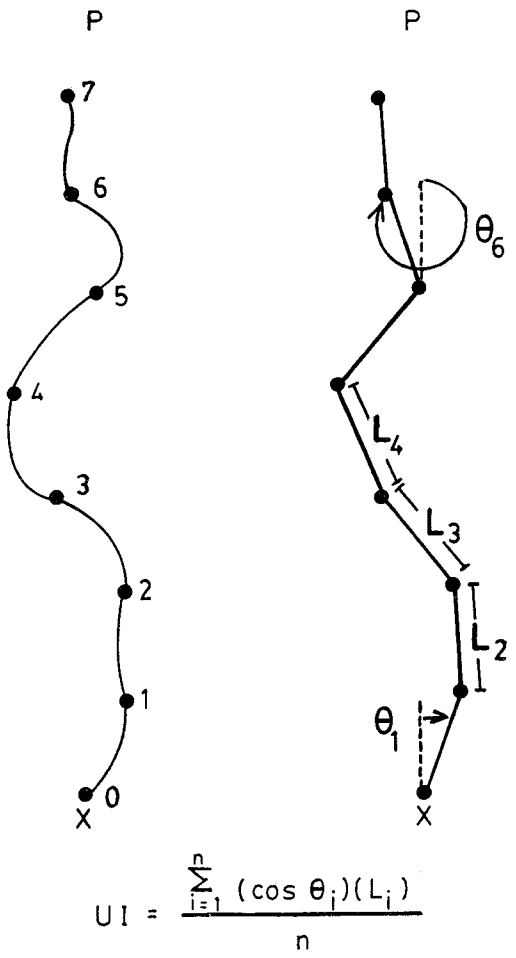

Fig. 2. Sample ant track to point source of Z9-16 : ALD θ_i; = orientation of i_{th} vector. P = source location. "X" = ant release site. L_i = length of i_{th} vector. (0° = directly towards source)

orientation. In effect, our index is a measure of the *speed* of upwind movement since it was calculated as follows:

$$UI = \frac{\sum_{i=1}^{n} (\cos \theta_i)(L_i)}{n} = \frac{\text{total upwind movement}}{\text{no. of seconds observed}}$$

The overall means of all the individual ants mean UI's in each treatment were compared using Duncan's New Multiple Range Test. The mean angle of orientation was also calculated, using, $\bar{X} = \frac{\Sigma \cos\theta}{n}$ and $\bar{Y} = \frac{\Sigma \sin\theta}{n}$ for each treatment according to the formulae:

$$\cos \bar{\theta} = \frac{\bar{X}}{r}; \sin \bar{\theta} = \frac{\bar{Y}}{r}; r = \sqrt{(\bar{X})^2 + (\bar{Y})^2}$$

The length of each mean vector, r, reflects the degree of dispersion about the mean orientation ($0 < r < 1$), and is based solely on the orientations of the individual vectors, and not on their lengths (Batschelet, 1965).

RESULTS AND DISCUSSION

Successful location of both Z9-16 : ALD pheromone source types was significantly greater in wind than in still air. The Z9-16 : ALD point source was located (touched) by 9 of 16 workers in wind, whereas 0 out of 16 located it without wind ($P < 0.005$; χ^2 2 × 2 test of independence). The mean "upwind" index, UI, of ants in wind to the point source was significantly greater than that of ants in the pheromone-free treatment and in pheromone without wind (Table I). Thus, ants travelled significantly farther toward the pheromone source in wind than without wind, or to an empty tube in wind. In wind, the mean angle of orientation to the point source (directly towards the source from the release point = 0°) was 26.5° (r = 0.69), whereas the mean angle of orientation to the pheromone source without wind was 162° (r = 0.49) (Fig. 3a,b). The distributions of orientation vectors for these treatments were nonrandom, and not significantly different from directly towards the source (0°), and directly away from the source (180°), respectively (V test; $P < 0.005$). The r values and mean angles for ants in blank tube treatments with and without wind were 0.80 (156°) and 0.39 (291°), respectively (Fig. 3c,d).

In the permeated field, 15 of 24 ants in wind reached the source, whereas none of the 19 ants located the source without wind ($P < 0.005$); χ^2 2 × 2 test of independence). In addition, only 2 of 18 ants walked to the solvent control source in wind and none of 23 did so in still air. The mean upwind index of ants in wind in the permeated field was significantly greater than for all other treatments (Duncan's New Multiple Range Test; $P < 0.01$). Thus, ants travelled significantly farther towards the permeated pheromone source in wind than without wind, or than to solvent controls in

TABLE I

Mean upwind indices, UI, of orientation responses to a permeated pheromone field and to point sources of the trail pheromone component, Z9-16 : ALD[1]

Permeated Pheromone Field	*Z9-16* : ALD	*CH_2Cl_2 (solvent control)*
Wind	0.546 ± 0.102[a]	0.017 ± 0.068[b]
No wind	−0.006 ± 0.046[b]	−0.054 ± 0.50[b]

Point Pheromone Source	Z9-16 : ALD	Empty Teflon Tube
Wind	0.261 ± 0.308[a]	−0.189 ± 0.041[b]
No wind	−0.192 ± 0.86[b]	0.067 ± 0.059[a]

[1] Means within same experiment not followed by same letter are significantly different ($P < 0.01$; Duncan's New Multiple Range Test).

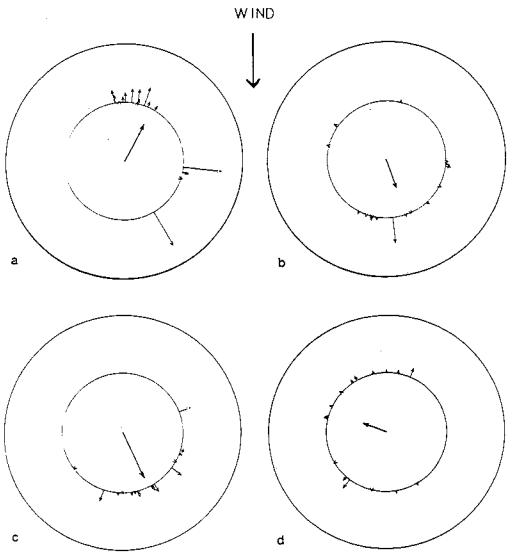

Fig. 3. Orientation responses to point sources of Z9-16 : ALD, and to empty fiber controls in still and moving air. Mean treatment orientations shown in center, individual ant means around periphery. a) Z9-16 : ALD-filled fiber in wind; b) Z9-16 : ALD-filled fiber in still air; c) empty fiber in wind: d) empty fiber in still air.

wind or not (Table I). The mean angle of orientation to the permeated field in wind was 1° ($r = .74$; Fig. 4a). In contrast, the r values and mean angles for ants in pheromone in still air and solvent with and without wind were 0.33 (264°), 0.09 (101°), and 0.19 (158°), respectively (Fig. 4b,c,d). Furthermore, the only distribution of vectors statistically different from random was for the pheromone plus wind (Raleigh test; $P < 0.01$), which was not significantly different from 0° (V test, $P < 0.001$; Durand & Greenwood, 1958).

All 15 ants successfully locating the source in wind travelled up the central portion of the field, and it is unlikely that there existed a longitudinal gradient from the source sufficiently steep to allow chemotaxis. The lack of significant directional displacement of ants in the windless pheromone field is evidence that no such gradient was present. Therefore, the movement of ants towards the source in wind must have been due to anemotaxis, not chemo-

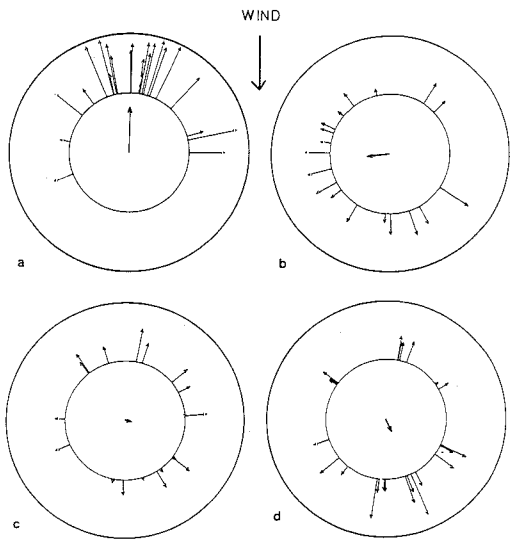

Fig. 4. Orientation responses to a permeated field of a) Z9-16 : ALD in wind; b) Z9-16 : ALD in still air; c) solvent in wind; d) solvent in still air. See Fig. 3 legend.

taxis. This does not mean that chemotaxis was not occurring where steeper gradients existed, such as a few mm from the source or at the lateral boundaries of the field. In fact, in the permeated pheromone field in wind, some ants which did not successfully locate the source were observed to travel both toward and away from the source along the field's "edges", a movement pattern characteristic of chemotaxis. To the point source, too, chemotaxis could have been employed to maintain plume contact. However, if only chemotaxis were operating, ants should have been displaced as frequently down as up this vapor "trail". Although they did go in both directions, the predominant direction in wind was towards the source, whereas without wind ants did not move towards the source. There was not a sufficiently steep longitudinal gradient to cause displacement toward the point source with no wind, and therefore as in the permeated field experiment, the preferential movement of workers to the fiber in wind must have been in part due to anemotaxis.

It appears, then, that *I. humilis* workers have the ability to use odor-conditioned anemotaxis (Kennedy, 1977, 1978) to locate sources of their trail pheromone, but a second mechanism, chemotaxis (probably tropotaxis; Hangartner, 1967), provides a dual system that is normally utilized during the usual windless trail following forays of workers (Van Vorhis Key, *et al.*, 1981; Van Vorhis Key & Baker, 1982). The terrestrial snail, *Helix aspersa* Müller, is capable of either chemotactically following streaked trails of food extract or orienting to airborne volatiles anemotactically (Farkas & Shorey, 1976), but in only one ant species, *Novomessor cockerelli* (André) has odor-conditioned anemotaxis been implicated as a contributing mechanism for the location of food sources. More *N. cockerelli* workers were recruited to a food source 3 m from their nest entrance when wind passed from the food towards the nest (Markl & Hölldobler, 1978). This effect was attributed to volatiles produced by recruiting ants which had discovered the food source.

In other insects, odor-conditioned anemotaxis may also be employed for the location of food as in *Drosophila* flying to rotting bananas (Kellogg *et al.*, 1962), or for mate finding as in *Plodia interpunctella* (Hübner) (Marsh, *et al.*, 1978). Starved locusts orient upwind in the presence of grass odor, and fed or starved hoppers orient upwind in the absence of odor when agitated (Kennedy & Moorhouse, 1969). In non-flying insects, anemotaxis is also used for mate finding as in *Periplaneta americana* (L.) (Rust & Bell, 1976; Rust *et al.*, 1976), and both positive and negative anemotaxis in the orientation movements of cockroaches were demonstrated to be odor concentration- and wind speed-dependent (Bell & Kramer, 1979).

Although trail pheromone-conditioned anemotaxis may not play a crucial or even usual role in the communication system of *I. humilis*, the fact that this mechanism exists suggests that it may be employed by displaced ants to locate trails from a distance. For a behavior-modification program using the synthetic trail pheromone, this extra distance of communication in wind could be very useful.

RÉSUMÉ

Anemotaxie induite par une phéromone de piste chez la fourmi d'Argentine, Iridomyrmex humilis.

Les ouvrières d'*Iridomyrmex humilis* (Mayr) présentaient une anemotaxie induite par l'odorat, à des sources émettant la principale phéromone de piste: (Z)-9-hexadecenal (Z9-16 : ALD). Dans la nature, un plus grand nombre de fourmis s'orientent et découvrent une source balayée par le vent de Z9-16 : ALD qu'en air calme. Une anemotaxie induite par la phéromone de piste peut effectivement augmenter la distance de communication de la piste au-delà de celle permise par le seul chimiotactisme.

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Accepted: April 21, 1982