Host Location by Apterous Aphids After Escape Dropping from the Plant

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In response to approaching enemies aphids may drop from their host plant to the ground. The risk of predation on the ground, desiccation and host loss, create the need for efficient host location by the dropping aphids. Most studies have focused on the factors that influence dropping behavior; only a few have addressed host location after dropping from the plant. We assessed post-dropping behavior in apterous Macrosiphoniella artemisiae *(Boyer de Fonscolombe) (Aphididae), which feed on* Artemisia arborescens L. *Vibration of the apical bud induced dropping in 36% of the colony members. Dropping rates were highest in mature aphids (63%). In the experimental arena (without ground predators), nearly all mature aphids that were dropped 13 cm from the plant, found their way back in ca. 40 s. The location process may be based on visual cues, as* M. artemisiae *is capable of visually discriminating between host and non-host targets and apparently does not react to volatiles emitted from the plant.*

KEY WORDS: escape response; host location; phototaxis; olfaction; orientation; vision.

INTRODUCTION

Sessile aphid colonies are an exposed and attractive food source for natural enemies, primarily carnivorous and parasitoid insects. Aphids are not defenseless when faced with these threats. They present an array

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of morphological, chemical and behavioral defense strategies, such as ant protection, chemical defense, kicking, walking away, and voluntarily dropping off the plant (Dixon, 1998). From an evolutionary perspective, the choice between defensive tactics, should be based on risk assessment and the tradeoff between cost and benefit (Dill *et al.*, 1990). Among the behavioral tactics, dropping provides the greatest advantage, immediately eliminating the danger of predation on the plant, although it exposes the aphid to the risks of desiccation, starvation and ground predation (Dill *et al.*, 1990; Losey and Denno, 1998a).

Considerable research effort has been devoted to factors that stimulate and promote dropping behavior while the aphids are still on the plant. Dropping can be triggered by tactile stimulation, sensing the chemical, visual and vibrational cues produced by enemies, detecting the alarm pheromone produced by conspecifics, and combinations of the above (Clegg and Barlow, 1982; Dill *et al.*, 1990; Stadler *et al.*, 1994; Losey and Denno, 1998a). Various factors may influence dropping behavior, including genotypic variation, temperature, humidity, host plant quality, alarm pheromone quality and quantity, age, disease infection, parasitism, and enemy-specific traits (e.g., McAllister and Roitberg, 1987; Dill *et al.*, 1990; Losey and Denno, 1998a; Braendle and Weisser, 2001). The frequent dropping in some species, despite the inherent risks, indicates that aphids should be able to efficiently locate a host after dropping. Nevertheless, host location by apterous aphids, has received less research attention.

Fast and accurate host location may require several mechanisms. In habitats that contain high densities of the host plant, random movement on the ground should eventually lead to a suitable host. In addition, aphids may sense and be attracted to an aggregation pheromone emitted by conspecifics remaining on the plant. Apterous aphids might also detect odors from fallen host plant matter or be attracted to odors emitted by the plant (Visser and Taanman, 1987; Quiroz and Niemeyer, 1998). Finally, apterous aphids may locate the plant by relying on visual cues. Niku (1975) found that apterous aphids climbed on plant stems and green sticks after being visually attracted to them. Phelan *et al.* (1976) has also shown visual attraction of apterous aphids to a vertical green dowel.

Few studies, most of which were conducted on the pea aphid, *Acyrthosiphon pisum* (Harris), have addressed post-dropping behavior. After dropping, aphids may exhibit thanatosis (feigning death), followed by one of two movement patterns described as "searching motivation" and "running motivation" (Niku, 1975; Roitberg *et al.*, 1979). "Searchers" show a high turning rate and slow running compared to the low turning rate and fast running of "runners." According to Roitberg *et al.* (1979), the ability of

apterous pea aphids to locate the host plant is age-dependent; older instars are able to locate host plants at greater distances than younger instars. The latter have difficulty finding the host plant at distances greater than 3 cm.

The poorly studied oligophagous aphid, *Macrosiphoniella artemisiae* (Boyer de Fonscolombe) (Aphididae) establishes large colonies on *Artemisia arborescens* L. bushes in Israel (Swirski and Amitai, 1999). In preliminary observations we found that the aphids frequently drop from the plant following vibration disturbance. The aim of this study was to track post-dropping behavior and the mechanisms involved in host location by apterous *M. artemisiae*. The host plant, *A. arborescens* is rich in aromatic compounds (e.g., Zarga *et al.*, 1995), and therefore special attention was paid to olfactory cues.

MATERIAL AND METHODS

The research was conducted outdoors in the botanical garden of Oranim College and in Neve-Ya'ar research center in the spring of 2003 and 2004. A stock of 50 *A. arborescens* plants, 30–40 cm high, was established in 35 cm diameter pots.

Dropping Behavior

No previous record of dropping behavior by *M. artemisiae* was available and therefore, as background for the host location study, the dropping behavior following vibration disturbance was examined. Three consecutive finger taps on the apical bud generated disturbance. Dropping aphids were collected in test tubes attached to a funnel, which was placed under the colonies ($n = 14$). The shoot containing the remaining aphids was cut and stored in a plastic bag. The fallen and the remaining aphids were counted separately according to age groups: mature, intermediate (3rd and 4th instars) and young nymphs (1st and 2nd instars).

Host Location Ability

The experiments were conducted outdoors under natural abiotic conditions. The aphids used in the host location experiments were collected from the stock plants. After dropping in response to tapping as described above, the apterous aphids, sorted by age groups, were kept in test tubes no longer than 10 min. A square cardboard arena (26×26 cm) was placed

adjacent to the base of a stem of an aphid-free plant (see chart in Fig. 3a), which was routinely switched between trials. Dropping aphids were re-dropped from a test tube onto the center of the arena, at a distance of 13 cm from the stem (the average radius of the stock plants). The plant's direction in relation to the arena was kept perpendicular to the direction of the sunlight, in order to ensure that aphid movement toward the plant could not be explained by phototaxis (see Hajong and Varman, 2002). The arena itself was randomly rotated between trials. Experiments were conducted outdoors, because *M. artemisiae* appeared to be less active and failed to locate the host plant under artificial lighting conditions. Aphid movement after dropping was visually observed. Aphids that reached the side of the arena adjacent to the plant were considered "returning aphids"; individuals that reached any other side were considered "lost aphids." When the aphids were dropped in groups, each group consisted of seven aphids, unless stated otherwise. Each group was treated as a single replication unit (n). Each aphid was used only once.

Location Efficiency

Location efficiency was calculated from host finding rates and the time needed for the process. Return rates of aphids ($n = 9$ groups containing 10 aphids for each age) that were dropped in the center of the arena were scored. The time needed for an aphid to locate its host, from contact with the surface until arrival at the plant, was measured individually ($n = 13$ mature, 14 intermediate and 16 young nymphs). We further focused on matures because they are the most likely to drop and can be easily observed. Efficiency of return was initially recorded in a cardboard arena covered with packed local soil. There was no difference in the matures' return efficiency between arenas with or without soil coverage (Mann-Whitney Test, $U = 30$, $p = 0.385$). Thus, for convenience, successive experiments were conducted in arenas without soil.

Host Plant Location

Recording Aphid Movement Tracks

A 0.5 cm^2 grid was drawn on the arena, and a digital camera (Canon PowerShot S200) positioned above it automatically took a picture approximately every 1.5 s. Eight mature aphids were dropped individually onto the center of the arena, which was adjacent to an *A. arborescens* branch

(33×8 cm). The location of an aphid in each frame was determined by the nearest grid coordinate. A similarly conducted experiment without the host plant served as the control ($n = 10$ aphids).

Attraction to Host Odor

The effect of host plant volatiles on the return process was examined using a dual choice Y-maze olfactometer (stem 12 cm, arms 12 cm, 45° angle), tilted at a 45° vertical angle. Air from a common source passed through distilled water and an active charcoal filter. The air (1 L min^{-1}) was then split into two streams; one passed through an empty control spherical flask and the second through an identical flask containing *A. arborescens* leaves. The flasks' positions were randomly switched between repetitions. Aphids were dropped in 17 small groups (5–7 aphids in a group for a total of 112 aphids) at the base of the stem tube and climbed separately on a thin, split wooden skewer. At the fork, the aphids could chose to continue climbing to the left or to the right. An aphid that reached one end of the skewer was documented as having chosen that side.

Return to Host Plant in the Dark

The importance of vision and olfaction in host location were examined under dark conditions. The experimental arena was darkened during the day, using cardboard screens, to the level of 0.01 μmols s^{-1} m^{-2}, so that it was still possible for the observer to see the movement of the aphids. The aphids were dropped in groups ($n = 15$) in the center of the arena; those that did not return to the plant within 3 min were considered lost. The control experiment was performed in full daylight.

The Importance of Vision in Host Location

A cardboard model (33×8 cm), the length and width of a typical branch of *A. arborescens*, was posted adjacent to the arena. The extent of return to the cardboard model was examined in 15 aphid groups. The ability of the aphids to discriminate between a cardboard model and an *A. arborescens* branch, was examined in a choice experiment. The cardboard model was placed on one side of the arena, facing a branch of *A. arborescens* positioned on the opposite side. The positions were randomly changed between trials. The aphids ($n = 13$

groups) were dropped in the center of the arena. The aphids' ability to discriminate between host and non-host plant cues was tested in a choice experiment, in which similarly sized branches of *A. arborescens* and the common plant *Inula viscosa* (L.) Aiton (Asteraceae) were placed on opposite sides of the arena. *Inula viscosa*, is a perennial pioneer plant (native to Mediterranean wetlands), widely distributed in disturbed habitats, especially along roadsides. (Zohary, 1962; A. Dafni, personal communication). Twelve groups of aphids were dropped in the center of the arena and the positions of the plant and the model were routinely changed. Data was analyzed using SPSS software. Means ± SE, and specific tests are listed in the Results.

RESULTS

Aphid Dropping in Response to a Disturbance

An average of 36.4 ± 3% of the individuals on the branch dropped in response to the disturbance. Dropping response was age-dependent; dropping rates were highest in mature aphids and lowest in young nymphs (Kruskal Wallis Test, $\chi^2 = 25.487$, df = 2, $P < 0.001$, Fig. 1).

Return Efficiency

When dropped in the experimental arena, nearly all aphids in all age groups returned to the plant (Kruskal Wallis Test, $\chi^2 = 0.535$, df = 2, NS,
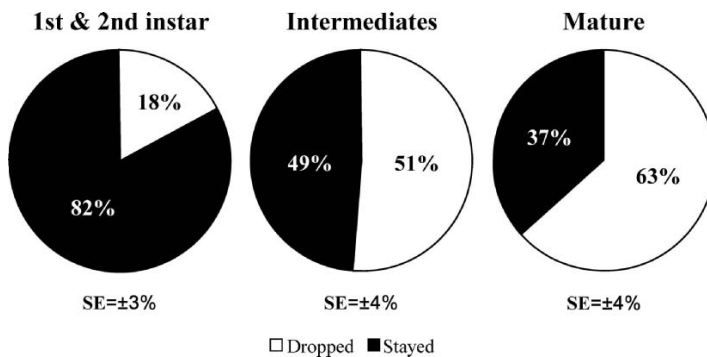
Fig. 1. Mean percentage of aphid dropping rates in the three age categories. A disturbance of three successive finger taps on the apical bud was applied to 14 colonies containing an average of 137 ± 20 individuals.
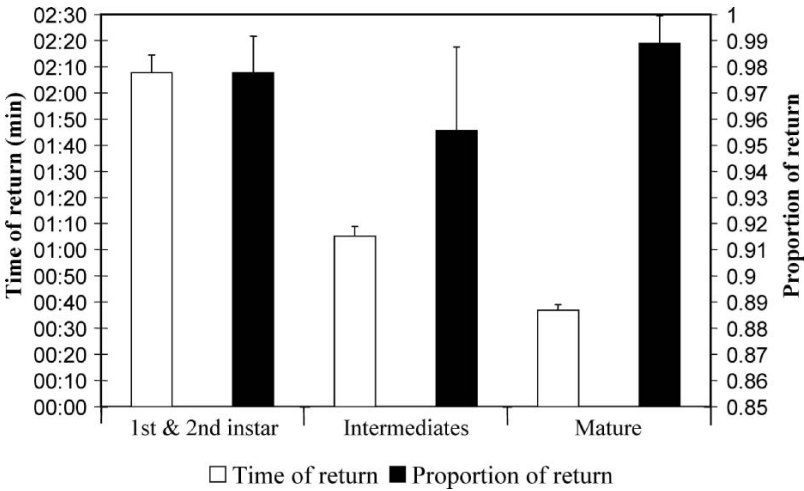
Fig. 2. Mean proportion of aphids dropping from the colony and mean return time to
the host plant according to age group.

Fig. 2). However, the return time was age-dependent. Matures returned
approximately three times faster than young nymphs (Kruskal Wallis Test,
$\chi^2 = 35.69$, df $= 2$, $p < 0.001$, Fig. 2).

Host Plant Location

Unlike other aphids (e.g., Niku, 1975), after dropping from the plant,
M. artemisiae shows no thanatosis. Faced with the target plant, the aphids
ran directly toward it with minimal turnings (Fig. 3a). In the absence of a
visual target, the "confused" aphids scattered randomly, making frequent
turns, with a clear element of negative phototaxis (Fig. 3b). In the darkened
arena, the aphids were still active but had difficulties locating the host plant.
Only 17% of the aphids returned to the plant and the rest were lost. In the
control group (sunlight), 96% of the aphids successfully returned to the host
(Sign Test, $n = 15$, $p < 0.001$).

When dropped onto the experimental arena (non-choice), 81% of the
aphids oriented toward the cardboard target and climbed it (Sign Test, $n =
15$, $p < 0.001$). However, visual attractant stimuli from the plant were more
appealing than the cardboard; in a choice experiment, 92% of the aphids
preferred the *A. aborescence* plant to the cardboard model (Sign Test, $n =
13$, $p < 0.001$, 2% of the aphids were lost and not included in the statistical

analysis). *Macrosiphoniella artemisiae* may be attracted specifically to cues emitted from its host plant, *A. arborescens*. In a choice experiment, 81% of the aphids moved toward the *A. arborescens* plant, whereas 12% moved toward the *I. viscosa* (7% did not reach either side). The preference for *A. arborescens* was statistically significant (Sign Test, $n = 12$, $p < 0.001$).

The importance of vision in host plant location was supported by the olfactometer test: 62 aphids preferred the *A. arborescens* side and 50 preferred the control side. These differences are not statistically significant ($\chi^2 = 1.286$, df = 2, NS), indicating that plant odors do not play a major role in host location.

DISCUSSION

Dropping off the host plant is constrained by the risk of ground predation, desiccation and starvation. To minimize the cost of dropping, aphids should be able to accurately and rapidly locate a suitable host plant. In the experimental arena, *M. artemisiae* (in particular mature aphids) located the host plant with remarkable efficiency (Fig. 2). Several conclusions concerning the mechanisms involved in efficient host finding by apterous *M. artemisiae* can be suggested. The aphids efficiently located the aphid-free plants in a clean experimental (cardboard) arena, so that neither aggregation pheromone nor plant material on the ground seems to be involved. Random movements can also be excluded, as the aphids exhibited oriented running toward the host (Fig. 3a). Although apterous aphids may respond to host volatiles (Pickett *et al.*, 1992; Quiroz and Niemeyer, 1998), our results show that olfactory cues emitted from the highly aromatic *A. arborescens* leaves had a negligible effect, if any, on host location by apterous *M. artemisiae*. Aphids also showed limited ability in locating the plant under darkened conditions.

In agreement with previous studies (Niku, 1975; Phelan *et al.*, 1976; Roitberg *et al.*, 1979), our data suggests that visual cues are the dominant (probably the only) factor used by apterous *M. artemisiae* to locate their host. When moving on the ground, the aphids must rely on easily recognizable cues. Odor may not fulfill this requirement, for aphids dropping off the plant upwind, would fail to detect its volatiles. Furthermore, it is not clear whether aphids walking on the ground can actually detect a volatile gradient emitted from the leaves. Interestingly, we observed alate morphs of *M. artemisiae* returning to the host plant by running rather than flying (Gish and Inbar unpublished observations).

The aphids recognized and were attracted to vertical objects on which they climbed. Niku (1972, cited in Phelan *et al.*, 1976) found that pea aphids
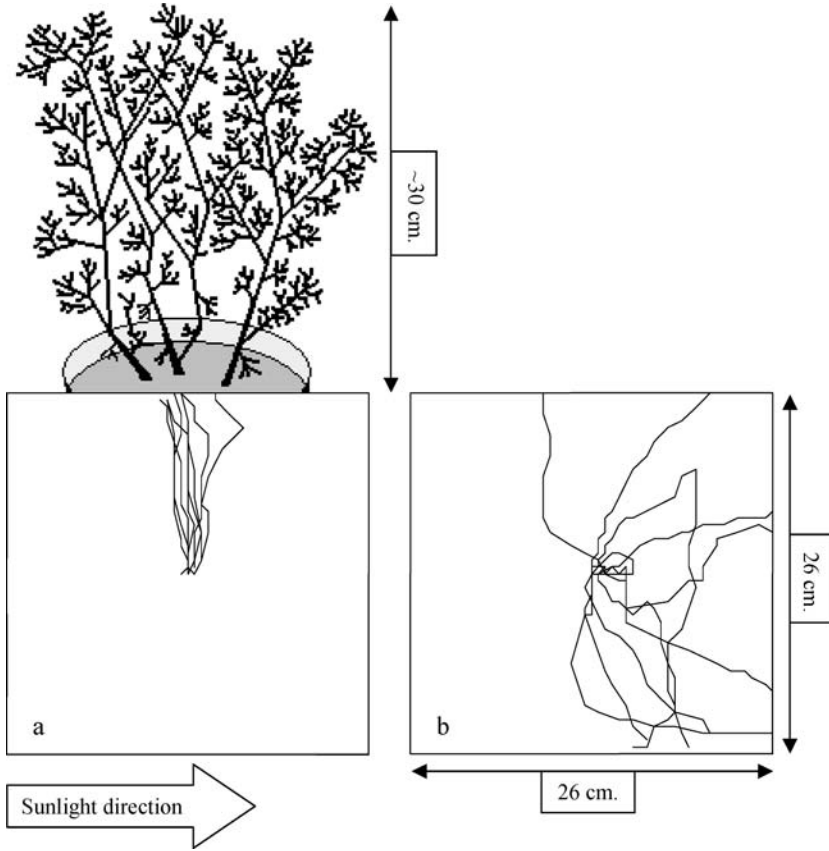
Fig. 3. Digitization of aphid movements after dropping. (a) aphid trails with an *A. arborescens* plant placed adjacent to the arena. (b) trails in the absence of a host plant. Note the negative phototaxis when visual host cues are absent.

were attracted to vertical objects (e.g. sticks and painted stripes). There was no difference between the attractance of these objects and the stems of the actual aphids' host. However, *M. artemisiae* can discriminate between vertical objects; cardboard objects are chosen only in the absence of a real host plant. Furthermore, it seems that this oligophagous aphid can visually discriminate between its host plant and the non-host *I. viscose*. It has been shown that the migrating (alate) aphid *Hyalopterus pruni* (Geoffroy) can discriminate between plant-specific colors (wave lengths) (Moericke, 1969). No doubt, the ability of *M. artemisiae* to discriminate visually between plant species needs further examination.

Mature aphids tend to drop more often than nymphs (Fig. 1), as found in other studies (Roitberg *et al.*, 1979; Losey and Denno, 1998b). Nearly all *M. artemisiae* individuals that drop, return quickly and efficiently to the host, with the nymphs returning at the slowest pace (Fig. 2). The slower return of the young nymphs might be attributed to slower running ability or to visual constraints. Under natural conditions, however, grains of dirt and stones could affect location efficiency (Roitberg *et al.*, 1979).

We described two post-dropping behaviors; fast and directional running toward a vertical object or plant (Fig. 3a), and "confused" behavior characterized by slow, non-directional movement with frequent turns (Fig. 3b). We did not find the "running motivation" behavior as described in pea aphids that ignored vertical objects (Niku, 1975; Phelan *et al.*, 1976; Roitberg *et al.*, 1979).

Harsh climatic conditions might affect the survival of dropping aphids (Dill *et al.*, 1990). In unfavorable hot and dry conditions, dropped aphids may quickly become paralyzed (Roitberg *et al.*, 1979). On the ground, in the absence of vertical objects, *M. artemisiae* shows a clear negative phototaxis (Fig. 3b). This appears to be shade-seeking behavior, which reduces the risk of desiccation. On hot spring days in the Mediterranean area (air temperature 32.9°C, and ground temperature 53.6°C at a depth of 2 mm), young nymphs and mature *M. artemisiae* became paralyzed within 1.1 ± 0.1 and 5.6 ± 0.3 s, respectively, after being dropped from a test tube onto the ground (Gish and Inbar unpublished).

Host location in apterous *M. artemisiae* was found to be highly efficient when tested in the experimental framework, but still needs to be tested under natural conditions. In addition, an interesting question arises: would aphids change dropping and returning tactics at night, when location ability is reduced? Future studies should also address the aphids' ability to discriminate between specific optical cues, such as size, shape, color and contrast.

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