Walking pattern of *Trichogramma nubilale* Ertle & Davis (Hymenoptera; Trichogrammatidae) on various surfaces

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Abstract

The leaf surfaces on which mass-reared *Trichogramma* spp. are released for augmentative biological control may be an important factor effecting the success of these releases. We observed and recorded the walking pattern of *Trichogramma nubilale* females on leaves of *Zea mays*, *Canna × generalis* (Canna lilly), *Silphium perfoliatum* (cup plant), *Abutilon theophrasti* (velvetleaf), *Schizachyrium scoparium* (little bluestem), a smooth and a fuzzy polyester material, and waxed paper. For each surface type, a total of 10 mated, 1–2 days old, naive and fed females were observed and their walking path was traced. Females walked fastest on waxed paper and leaves of *S. scoparium* and slowest on leaves of *A. theophrasti* and the fuzzy material. Turning rates were high on *Canna × generalis* and waxed paper. In general, walking speed was negatively affected by the presence and density of trichomes and possibly the lack of leaf veins. The effect of surface structures, such as trichomes is likely to scale to the body size of the searching parasitoid. Parasitoids can walk over short trichomes, but short directionally pointed trichomes (as on *S. scoparium*) can guide the search paths in certain directions. The effects of long trichomes may depend on trichome density relative to the parasitoid body length. When trichome density is on the same order of magnitude as $1/L^2$ (*Z. mays*), wasps will stand between trichomes, and will frequently run into trichomes, which will retard walking speeds and increase turning.

Keywords: *Trichogramma nubilale*; Trichomes; *Zea mays*; *Canna × generalis*; *Silphium perfoliatum*; *Abutilon theophrasti*; *Schizachyrium scoparium*

1. Introduction

*Trichogramma* species are minute parasitic wasps that develop inside the eggs of mostly Lepidopteran species. Mass-reared *Trichogramma* are utilized worldwide as augmentative biological control agents for Lepidopteran pests in forests, orchards, and row crops (Li, 1994; Smith, 1996) to kill pests before hatching larvae have the chance to inflict plant damage. That these species are well suited to biological control augmentation is seldom questioned. However, the need for large scale and repeated releases makes *Trichogramma* augmentation potentially expensive (Andow, 1997), and field experiences indicate mixed results in the effectiveness of the suppression of various crop pests (e.g. Ridgway and Morrison, 1985; Stinner, 1977; Andow et al., 1995).

Many researchers have ascribed failures in field releases to the intrinsic deficiencies of the *Trichogramma* used in the releases and focus on selecting the most appropriate species or strain against specific target pests (e.g. Pak, 1988) and the importance of quality control in mass rearing ( Olson and Andow, 2002; van Lenteren, 2003). Increasingly recognized is the importance of extrinsic factors (i.e., the suitability of the environment) on the behavior and performance of *Trichogramma* in the field (Romeis et al., 2005). Neither the selection of the most appropriate species or biotypes nor the advancement of mass-rearing technology will be of any consequence if the target environment prevents the *Trichogramma* from expressing their intrinsic life history attributes. Fecundity may be an important attribute of *Trichogramma* when used against high-density pests, but the ability to locate and accept hosts will be far more...
important when used against low-density pests (Stoutheamer and Luck, 1993).

There are many physical characteristics of the host plant that influence *Trichogramma* search. For example, often there is an inverse relationship between *Trichogramma* parasitism and foliage surface area of the host plant (Ables et al., 1980; Burbutis and Koepke, 1981; Need and Burbutis, 1979), and differential structure of the abaxial (lower) and adaxial (upper) surface of leaves and physical and chemical properties of trichomes and their densities can effect the walking speed, longevity, turning rates, propensity to fly, parasitism rates, and survival of foraging *Trichogramma* and other minute parasitoids (Headrick et al., 1996; McGregor et al., 2002; Obyrcky, 1986; Romeis et al., 2005; Sütterlin and van Lenteren, 1997). In addition, epicuticular waxes that cover all primary plant surfaces can reduce attachment by many predators and parasitoids (Eigenbrode, 2004), although they are also known to adsorb and re-release insect sex pheromones which may guide predators and parasitoids to their hosts (van Lenteren et al., 1995). The complexity of the host plant structure [size or surface area, heterogeneity (abundance and diversity of plant parts), and connectivity (abundance of connections between plant parts)] can also effect the search ability of *Trichogramma* females (Andow and Prokryn, 1990; Lukianchuk and Smith, 1997; Gingras and Boivin, 2002; Andow and Olson, 2003). However, the physical attributes of plants also often provide defense against the herbivore and can affect other members of the third trophic level (Obyrcky and Tauber, 1984; Hare, 1992), and the presences of trichomes are known to have many functions in plant physiology (Sütterlin and van Lenteren, 1997). Thus, successful augmentative releases by *Trichogramma* species of high quality may depend on the interaction of the species with the micro-environment surface created by the plant species on which the wasp will search.

The main objective of this study was to demonstrate how surface structures, such as leaf trichomes, influence the searching behavior of *Trichogramma nubilale* Ertle & Davis (Hymenoptera: Trichogrammatidae). To do this, we first describe how *T. nubilale* females search on various surfaces that differ in trichome presence, length, and associated chemistry. Second, we experimentally alter the surface structure by removing trichomes to show how the trichomes likely affected searching behavior. Finally, we provide *Trichogramma* with experimental surfaces to indicate how trichome density likely effected searching behavior. In total, these observations and experiments provide additional evidence that surface structure, such as trichomes, has a significant effect on *Trichogramma* searching behavior.

2. Materials and methods

*Trichogramma nubilale* were reared on eggs of European corn borer at the University of Minnesota Agricultural Experiment Station. The *T. nubilale* culture originated from field collections of European corn borer egg masses on maize plants in Delaware in 1991. As there were no hosts or host cues in the experiments, the wasps did not exhibit area restricted search.

2.1. Leaf surfaces

All leaf and non-leaf surfaces were chosen for their variability in the presence, length, associated resin, and density of trichomes. The leaf surfaces examined included those of *Zea mays* L. (maize; Poaceae), *Canna × generalis* Bailey (Canna lily; Cannaceae), *Silphium perfoliatum* L. (cup plant; Asteraceae), *Abutilon theophrasti* Medik. (velvetleaf; Malvaceae), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem; Poaceae). The non-leaf surfaces included waxed paper and a 100% synthetic polyester material with sides that differed in their fiber density without the potential confounding effects from chemicals and epicuticular waxes.

*Z. mays* is a host of the European corn borer (*Ostrinia nubilalis* (Hübner): Lepidoptera: Crambidae), which is itself a host species of *T. nubilale.* All other species except *Canna × generalis* can be found throughout the maize growing regions of southern Minnesota.

Surfaces were examined using scanning electron microscopy to describe gross surface morphology and determine trichome density. Specimens were fixed in 2% osmium tetroxide for 24 h, washed in double-distilled water, dehydrated in a series of acidulate EtOH concentrations, critical point dried, mounted on stubs, sputter-coated with platinum, and viewed with a Hitachi S-4000 field emission SEM.

The upper surface of *Z. mays* has long, slender, sparsely spaced trichomes (∼75 trichomes/cm²) running parallel to the leaf veins (Fig. 1A). The lower surface of *Z. mays* lacks trichomes but has veins and a surface grain that runs from the proximal to distal end of the leaf (Fig. 1B). The upper surface of *Z. mays* that had the trichomes removed had tiny pores in the center of a small mound where the trichomes had been (Fig. 1C). *Canna × generalis* leaves are smooth and waxy, lacking trichomes, veins, and any surface grain. The leaves of *S. perfoliatum* lack trichomes but they have veins that fan out from the mid-vein to the edges of the leaves. The leaves of *S. scoparium* have short, curved trichomes (∼1200 trichomes/cm²) that have their tips oriented in one direction (Fig. 1D). The leaves of *S. scoparium* that had the trichomes removed had tiny pores where the trichomes had been (Fig. 1E). The leaves of *A. theophrasti* have numerous (∼4800 trichomes/cm²), short trichomes which are not orientated in a particular direction and which have a sticky resin (Fig. 1F).

2.2. Searching on surfaces

Searching behavior on each surface type was observed for 10 mated, naïve, 1–2 days old and honey-fed *T. nubilale* females. To measure walking speed and searching pattern on excised leaves or non-leaf surfaces were placed in the center of a wood case (61.5 cm²) with a glass surface
suspended about 10 cm above the test surface which allowed for ventilation and enough vertical space for wasps to hop on and off surfaces without high hops being mistaken by the observer for flight. Chilled wasps were placed on each surface and allowed to warm up prior to collecting data. Walking patterns were traced onto plastic sheets attached to the glass surface, and the path and location every 10 s was recorded until the wasp left the surface or 2 min had passed. Behaviors were recorded for every trial using an event recorder, recording times when the wasps were moving (females that spent their time walking for \( \geq 1 \) min were classified as movers) or not moving (females that showed no visible movement for \( \geq 1 \) min were classified as stayers). This enabled us to calculate the total search time and time spent motionless. The total distance traveled by each female was calculated by digitizing the pathways using a digitizing tablet (time information was not preserved in the trace except for the net displacement positions). All trials were carried out from 1300 to 1500 in a greenhouse (ca 25 wasps per day were assayed over 9 days) at 25°C and 70 ± 10 RH to protect the wasps from wind and to provide as much sunlight as possible. Care was taken not to cast a shadow on the various surfaces while collecting data, but in any event the wasps did not appear to be affected by such shadows.

Total time walking (s), walking distance (cm), walking speed (cm/s walking), net displacement (cm from point of origin), speed of net displacement (cm/s), and the ratio of net displacement to total walking distance were calculated for each wasp for each surface. A ratio of net displacement to total walking distance near zero would indicate walking in tight circles and using intense area restricted search, and a ratio of one would indicate straight line movement.

The influence of plant species on ln-transformed time, un-transformed net displacement, and sqrt-transformed walking distance, walking speed and speed of net displacement and arcsine transformed ratio of net displacement to walking distance were tested with ANOVA (SAS Institute, Inc., 1998). Means were separated with Tukey’s HSD with \( P < 0.05 \) considered significant and un-transformed means are presented. The dependence of surface type on the proportion of stayers and movers was analyzed with a Chi-squared test (SAS Institute, Inc., 1998).

2.3. Experiments removing trichomes and experimental surfaces

Fig. 1. Scanning electron microscope images of (A) intact upper leaf surface of Z. mays, (B) lower leaf surface of Z. mays, (C) upper leaf surface of Z. mays with trichomes removed, (D) intact upper leaf surface of S. scoparium, (E) lower leaf surface of S. scoparium, (F) upper surface of A. theophrasti, (G) smooth surface of material, (H) fuzzy surface of material, and (I) image of Trichogramma wasp drawn approximately to scale. From 20 to 29 mated, naïve, 1–2 days old and honey-fed females were also tested on the upper and lower surfaces of intact leaves of Z. mays and S. scoparium. We removed the upper surface trichomes of both species using Scotch Transparent Magic™ tape. The tape removed all of the trichomes, did not adhere to the leaf surface waxes, and did not alter the leaf surface in any other way evident to the scanning electron microscope. In addition, we tore a strip of a Z. mays leaf to be the same width as S. scoparium to test if leaf width and not trichomes might have affected searching behavior on S. scoparium.
A final experiment was conducted to eliminate leaf chemistry and surface wax as factors and investigate how “trichome” density effected searching behaviors. A 100% synthetic polyester material was used. This material had both a fuzzy and smooth side, so that the effect of the surface could be compared while controlling for surface composition. The fiber density was approximately 700 and 4800 fibers/cm² for the ’smooth’ and ‘fuzzy’ surfaces, respectively. All other methods and statistical analysis were similar to the previous experiments.

3. Results

3.1. Searching on surfaces

There was a significant effect of surface type on the total time on the surface ($F=4.43$, $df=5$, $P<0.003$), net displacement on the various surfaces ($F=2.77$, $df=5$, $P<0.028$), walking distance ($F=8.01$, $df=5$, $P<0.001$) and walking speed ($F=17.84$, $df=5$, $P<0.001$). Females spent significantly more time on the waxed paper, *Z. mays* and *A. theophrasti* leaves than on *S. scoparium* leaves (Table 1). Net displacement, walking distance and walking speed were significantly higher on the waxed paper than on *Canna × generalis* leaves (Table 1). Walking distance was significantly longer on the waxed paper than on any of the other leaves (Table 1). Walking speed was significantly higher on the waxed paper, *S. perfoliatum* and *S. scoparium* leaves than on the other leaves, and was similarly low on *Z. mays*, *Canna × generalis* and *A. theophrasti* leaves (Table 1).

There was a significant effect of plant species on net displacement per second ($df=5$, $F=17.17$, $P<0.001$), and ratio of net displacement to walking distance ($df=5$, $F=19.56$, $P<0.001$). Net displacement per second gives an idea of which plants are likely to lead *Trichogramma* farthest from its point of origin in a given unit of time. *T. nubilale* had a significantly higher net displacement per second on leaves of *S. scoparium* than any of the other species and walked in a straight line when on this surface as indicated by the relatively high ratio of net displacement to walking distance (Table 2). Net displacement per second on the *Z. mays*, *Canna × generalis* *S. perfoliatum* and *A. theophrasti* leaves did not differ significantly from that on the waxed paper. Net displacement per second was significantly higher on *S. perfoliatum* than on *Z. mays*, and *A. theophrasti* (Table 2). Wasps on *S. scoparium*, *S. perfoliatum*, and *A. theophrasti* tended to walk in a straight line more so than when on waxed paper, and the *Z. mays* and *Canna × generalis* leaves (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Leaf</th>
<th>Time (s) Mean ± SEM</th>
<th>Net displacement (cm) Mean ± SEM</th>
<th>Walking distance (cm) Mean ± SEM</th>
<th>Walking speed (cm/s) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxed paper</td>
<td>78.40 ± 12.86a</td>
<td>7.20 ± 1.05a</td>
<td>27.06 ± 4.26a</td>
<td>0.357 ± 0.026ad</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>74.50 ± 12.26a</td>
<td>3.58 ± 0.54ab</td>
<td>11.24 ± 2.32b</td>
<td>0.153 ± 0.014d</td>
</tr>
<tr>
<td><em>Schizachyrium scoparium</em></td>
<td>19.60 ± 4.04a</td>
<td>5.91 ± 1.44ab</td>
<td>6.07 ± 1.49b</td>
<td>0.295 ± 0.003ab</td>
</tr>
<tr>
<td><em>Canna × generalis</em></td>
<td>56.3 ± 11.69ab</td>
<td>3.0 ± 0.81b</td>
<td>10.5 ± 3.31b</td>
<td>0.155 ± 0.032cd</td>
</tr>
<tr>
<td><em>Silphium perfoliatum</em></td>
<td>38.20 ± 8.83ab</td>
<td>4.23 ± 0.49ab</td>
<td>7.87 ± 1.80b</td>
<td>0.237 ± 0.035bc</td>
</tr>
<tr>
<td><em>Abutilon theophrasti</em></td>
<td>67.60 ± 15.78a</td>
<td>3.89 ± 1.00ab</td>
<td>4.80 ± 1.11b</td>
<td>0.081 ± 0.009ab</td>
</tr>
</tbody>
</table>

*N = 10. Different letters within columns are significantly different at *P* < 0.05.*
4. Discussion

Walking speed and walking pattern probably explains most of the variation in movement patterns by *Trichogramma nubilale* on the various surfaces we observed, because there was relatively little difference in the propensity of *T. nubilale* to move on the various surfaces. Females showed a variety of walking speeds and patterns on the various surfaces. Overall, they moved farthest from their point of origin in a given unit of time, and they spent relatively less time on leaves that lacked relatively long, sparse trichomes or dense, sticky trichomes.

Of the five leaf surfaces, the walking speed and straightness of path was highest on *S. scoparium*, in part because females were able to walk over the relatively short trichomes (<0.1 mm long, Fig. 1D). Interestingly, on these leaves the females all walked in the direction that the tips of the trichomes were facing. Although the trichomes were not long, they were highly curved which may have made it more difficult for females to walk in the opposite direction (Fig. 1D). Although *T. nubilale* also generally walked in a straight line over the trichomes on leaves of *A. theophrasti*, their walking speeds, net displacements, and speed of net displacement were very low probably because of the sticky resin on the trichomes (Fig. 1F). Females walked quickly on the upper surface of *Z. mays* leaves, but their walking speeds were much faster on the relatively smooth lower surface and on the upper surface with trichomes removed. On the upper surface with trichomes, they tended to turn frequently because they often walked into and detoured around the relatively sparse and long trichomes (>1.5 mm, Fig. 1A).

For *Trichogramma* spp., walking speed and pattern may strongly affect encounter rates with host species; females that are able walk rapidly in a straight line may search a greater area in a given unit of time and presumably encounter more hosts and host associated cues than those that have reduced speeds and higher turning rates (Romeis et al., 2005). The leaves of *S. perfoliatum* are large, relatively smooth and lack trichomes. Walking speeds of *T. nubilale* were fairly high on this surface and they mainly walked in a straight line. The leaves have numerous veins that fan out from the mid-rib to the leaf edges. Most of the females were observed to walk to the edge of the leaf on these leaf veins. It is not clear what properties of the veins led to their preferable use.

We evaluated *Canna × generalis* because it had large, waxy, smooth leaves, which we thought would be analogous to waxed paper. Although females spent about the same amount of time on the two surfaces, their net displacement and walking speed were much lower on *Canna × generalis*. Thus, some property(s), possibly the epicuticular waxes or chemicals, of the *Canna × generalis* leaf surface probably reduced the walking speed and net displacement of *T. nubilale*. In addition, even though walking speed was highest on the waxed paper, the females also had relatively high turning rates and low speeds of net displacement on this surface. The waxed paper lacked any chemical cues that could have stimulated a more straightened path. Therefore, even on simple surfaces *T. nubilale* loses some searching efficiency. It may be that leaf surfaces with some pattern (e.g. veins) are better suited to *Trichogramma* spp. in guiding their walking pattern.

<table>
<thead>
<tr>
<th>Material (cm/s)</th>
<th>Number below the means ± SEM are the number of females tested. Different letters within rows are significantly different at $P &lt; 0.05$.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zea mays</strong> (cm/s)</td>
<td>$0.089 ± 0.009^a$</td>
</tr>
<tr>
<td><strong>Schizachyrium scoparium</strong> (cm/s)</td>
<td>$0.144 ± 0.009^b$</td>
</tr>
<tr>
<td><strong>Material (cm/s)</strong></td>
<td>Fuzzy surface</td>
</tr>
<tr>
<td>$0.072 ± 0.006^b$</td>
<td>$0.131 ± 0.014^a$</td>
</tr>
<tr>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

Fig. 2. The proportion of movers and stayers on various surfaces: corn upper surface intact (cdi), corn lower surface intact (cbi), corn upper surface strip (cds), corn upper surface trichomes removed (cdt), bluestem upper surface intact (cdi), bluestem upper surface trichomes removed (bdi), fuzzy material (mfz), and smooth material (msm). Asterisks above the bars indicate significant deviation from expectation $p < 0.05$. Numbers above the bars are the number of females tested.
The presence of trichomes had a strong affect on the walking speed of *T. nubilale*. Removal of the trichomes from *Z. mays* (Fig. 1C) and *S. scoparium* (Fig. 1E) leaf surfaces increased walking speeds by a factor of about 3. Interestingly, *T. nubilale* walked faster and moved more on the upper surface of the *Z. mays* leaf strip than on the upper surface of intact *Z. mays* leaves. They also had high walking speeds on the narrow leaves of *S. scoparium*. These results suggest that they can recognize narrow versus wide leaves and will move faster on narrow leaves. More studies are needed to understand the mechanisms and consequences underlying this response.

Walking speed was nearly two times faster on the smooth side of the material with less dense fibers (Fig. 1H) than on the fuzzy side with more dense fibers (Fig. 1I), suggesting that trichome density in the absence of waxes and chemicals should have a large effect on the walking speed of *T. nubilale*. For many parasitoids and predators, there is an inverse relationship between trichome density and walking speed, turning rates, and or parasitism rates (reviewed in Obyrcyki, 1986). Breeding plants for an intermediate density of trichomes is often thought to be most amenable for increasing the foraging efficacy of many insect species on leaf surfaces. However, because of the variability in the responses to leaf surfaces by beneficial and pest species, both among leaf surface types and testing arenas (e.g. greenhouse versus field), experimental analyses within the field would be needed to gain an understanding of species specific responses for the development of appropriate management practices for the targeted species (Obyrcyki, 1986).

In summary, the presence of trichomes, associated resin, and trichome density and trichome length all had a large negative effect on the potential searching efficiency of *T. nubilale*. We suggest the following integrated model to account for these results. The effect of surface structures, such as trichomes is likely to scale to the body length, *L*, of the searching parasitoid (*T. nubilale* = 0.8 mm, Fig. 1I). Parasitoids can walk over short, <0.1mm trichomes (*S. scopa-rium*), but these can guide the walking paths in certain directions. The effects of longer trichomes depends on trichome density, *D*, relative to *L*. At low densities (*D* <1/*L* ²) the parasitoid will rarely encounter a trichome and walking pattern will be little affected. But when trichome density is on the same order of magnitude as 1/*L* ², (for *T. nubilale*, *D* ~1.5/mm²), the parasitoid will frequently run into trichomes, which will retard walking speeds and increase turning. As trichome density increases further (*D* >1/*L* ²), it will become increasingly difficult for a parasitoid to maneuver between the trichomes, and the surface may be avoided. Finally, trichome density can increase to a high enough density (*D* >1/*L* ²) that the parasitoid can walk on top of the trichomes (e.g. *A. theophrasti*; woolly mulelue, Keller, 1987). The structure of the top of trichomes may determine the extent that the leg of a searching parasitoid plunges between the trichomes or snaps them on, thereby retarding walking speeds (e.g. ‘fuzzy’ material). Although Obyrcyki and Tauber (1984) found substantial negative effects of glandular trichomes on several predator species in greenhouse studies, the negative effects did not transfer to the field populations. Dust, wind, and rain may mitigate the negative effect of the sticky chemicals on trichomes. We conclude that leaf surface structures will have varied effects on *Trichogramma* spp. and other members of the third trophic level, and these effects will need to be considered in developing desired cultivars for effective biological control.

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**References**


