Searching behaviour of *Trichogramma* wasps (Hymenoptera: Trichogrammatidae) on tomato and pepper leaves

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ABSTRACT

Walking speeds of both *Trichogramma brassicae* and *T. sibericum* were substantially lower on tomato than on pepper leaf disks. The difference may be due to the presence of glandular trichomes on tomato foliage. Total time spent on leaf disks during behavioural trials was lower on tomato than on pepper leaf disks for both species of wasps. This may indicate a higher propensity to disperse from tomato foliage than from pepper foliage. Lower walking speeds and shorter residence times on tomato leaves could result in a lower searching efficiency of wasps on tomato than on pepper. The subsequent efficacy of *Trichogramma* for biological control of cabbage loopers in greenhouses may be lower on tomato crops than on pepper crops.

Key words: *Trichogramma brassicae*, *Trichogramma sibericum*, *Trichoplusia ni*, searching behaviour, glandular trichomes, egg parasitoids, biological control, greenhouse vegetables

INTRODUCTION

Foliar pubescence on agricultural crops may interfere with the host location activities of small entomophagous insects and mites and reduce their subsequent effectiveness for biological control of arthropod pests (Obrycki 1986). Such effects have been documented for a variety of taxa. The predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseidae), was trapped by glandular trichomes more frequently on a tomato variety with a high trichome density than on a relatively hairless tomato variety (van Haren *et al.* 1987). Walking speed and parasitism of greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae)) by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was lower on cucumber varieties with higher levels of foliar pubescence (van Lenteren *et al.* 1995). Walking speeds of *E. formosa* recorded on pubescent tomato and gerbera foliage were slower than those recorded on hairless sweet pepper (Sutterlin & van Lenteren 1997). The estimated searching efficiency of *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) was lowest on pubescent tomato foliage compared to corn and bean foliage (Coll *et al.* 1997). Higher prey handling times were recorded for *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) on tomato plants compared to those recorded on eggplant or pepper plants (De Clerq *et al.* 2000). In this study, we compare the searching behaviour of *Trichogramma brassicae* Bezdenko and *T. sibericum* Sorokina (Hymenoptera: Trichogrammatidae) on tomato and pepper leaf disks in the laboratory.

Wasps of the genus *Trichogramma* are egg parasitoids that have been used widely for biological control of Lepidopteran pests (Li 1994). Searching behaviour and parasitism of hosts by a number of *Trichogramma* species are impeded by high levels of foliar pubescence. The time required to walk across leaf disks, the percentage of wasps initiating flight before crossing leaf disks, and the number trapped by trichomes were all higher for *T. pretiosum* Riley on tomato varieties with higher densities of glandular trichomes and containing either 2-tridecanone or 2-undecanone (Kashyap *et al.* 1991). In a field study, the highest levels of parasitism of *Heliothis* spp. eggs (Lepidoptera: Noctuidae) by *T. pretiosum* and *T. exiguum* Pinto & Platner were observed on tomato varieties with the lowest density of glandular trichomes (Kauffman & Kennedy 1989). Walking speeds of *T. exiguum* were lowest on highly-pubescent mullein, intermediate on intermediately-pubescent tomato, and highest on relatively hairless maize and soybean (Keller 1987). Parasitism of *Heliocoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) eggs by *T. chilonis* Ishii on pigeonpea varies widely across plant parts depending on the presence of trichomes. Parasitism is much lower on pods and calyxes that have a higher trichome density than on leaves (Romeis *et al.* 1998; 1999).

T. brassicae are released in British Columbia vegetable greenhouses for biological control of cabbage loopers (*Trichoplusia ni* (Huebner) (Lepidoptera: Noctuidae)) (Portree 1993). *T. sibericum* has been evaluated for management of cabbage loopers in vegetable greenhouses (E.S. Cropconsult Ltd., unpublished report). Although *Trichogramma* wasps are released in both tomato and pepper crops, no previous comparisons of searching behaviour on tomato and pepper foliage have been done. Previous work has shown that yellow sticky traps (that are used to monitor dispersal by *T. brassicae*) capture fewer wasps in tomato greenhouses than in pepper greenhouses (E.S. Cropconsult Ltd., unpublished data). In addition, numerous dead wasps have been found near release sites on tomato stems, presumably trapped by glandular trichomes (Prasad, personal observation). Both of these observations indicate that *Trichogramma* wasps may be less efficient for biological control of cabbage loopers on tomato than on pepper crops.

For the purposes of this paper, searching behaviour in *Trichogramma* is considered to be all activities that facilitate the location of suitable host eggs including walking on and examining host-free leaf surfaces. Here, we report measurements of walking speed on, time elapsed before exiting from, and proportion of time spent searching on excised tomato and pepper leaf disks in the laboratory by *T. brassicae* and *T. sibericum* females. Our objective was to identify differences in the behaviour of wasps between these two plant species caused by the presence of glandular trichomes on tomato leaves.

MATERIALS AND METHODS

Insects. *Trichogramma sibericum* were reared on sterilized *Ephestia kuehniella* eggs. *T. sibericum* females used in bioassays were between 1 and 3 d old. *T. brassicae* were obtained from Beneficial Insectary (Oak Run, California) and were 3 to 5 d old when used in trials. All wasps were provided continuously with dilute honey (50% by volume) applied as droplets to the inner surface of holding containers.

*Bioassay arenas.*Tomato and pepper leaves of unknown variety were collected from commercial greenhouses, wrapped in damp paper towelling, stored in Zip-lock® bags at 5° C, and used in trials within 5 d of collection. Arenas for measurement of searching behaviour consisted of leaf disks (2 cm in diameter) cut with a cork borer from tomato or pepper leaves and attached (underside up) to the bottom half of 9 cm plastic petri-dishes using double-sided tape. Behavioural observation and video recording were facilitated by confining wasps to this simple two-dimensional arena in the absence of host eggs. *Trichogramma* females search for hosts on both upper and lower leaf surfaces on entire plants (McGregor, personal observation). Here, we assume that the behaviour wasps display in this simple bioassay is analogous to behaviour displayed on whole plants in the field. The underside of leaves was used for trials because it has a higher density of trichomes on tomato leaves than the upper surface. Ten arenas (five tomato and five pepper) were prepared at a time for use in bioassays. After ten trials were completed, ten more arenas were prepared. This prevented leaf disks from deteriorating before use in bioassays.

General bioassay procedure. For each trial, a female wasp was released in the center of a tomato or pepper leaf disk from the tip of a fine paintbrush. The wasp was videotaped using a Panasonic WV-CD110 camera fitted with a Tamron 72mm camera lens and run by a Panasonic WV-PS10 drive unit., The position of the camera and zoom lens were adjusted until the two-cm leaf disk filled most of the screen on a video monitor. Wasps were videotaped for 5 min or until they flew or walked off the leaf disk. All trials were run under ambient conditions in the laboratory (temperature: 21-25° C; relative humidity: 37-65%). The trial area was illuminated from above with a single incandescent bulb (60 watt). Trials were alternated between plant species. Twenty trials were completed for each wasp species on each plant species (ie. total of 80 trials).

T. sibericum bioassays. All *T. sibericum* bioassays were run as above. However, trials that lasted less than 60 s were discarded. This was done to maximize the number of walking tracks that could be analysed for each wasp in order to increase the precision of estimates of walking speed. The first 20 trials for each plant species that lasted more than 60 seconds were retained for analysis.

T. brassicae bioassays. The majority of trials for this species lasted less than 60 seconds so none was discarded. The wasps walked or flew off the leaf shortly after release in most cases. The first 20 trials for each plant species were retained for further analysis no matter how short in duration.

Estimation of walking speed. An acetate sheet was taped to the screen of the video monitor. Walking tracks of *Trichogramma* females were traced with a felt pen on the acetate sheet. Tracks were only traced and analysed for walking on the surface of the leaf disk. Walking along the edge of the leaf disk was not recorded. One to five tracks of at least 1 cm were traced for each wasp. Tracing of a particular track was stopped when the wasp reached the edge of the leaf disk. The time a wasp spent walking along a traced path was recorded with a stopwatch. The length of the path was measured on the acetate sheet using a cartographer's odometer. The odometer consists of a rotating wheel (and associated accumulating scale) that is rolled along a curved line to measure its length in odometer units. Odometer units were calibrated to the image from the video display by measuring a tracing of a video clip of a centimeter ruler filmed at the same position that bioassays were conducted. Walking speed for each wasp was calculated by dividing the sum of the distance travelled in all measured tracks by the sum of the times recorded for walking those tracks.

Measurement of searching time and residence time. The videotape of each trial was viewed from the time of release of the wasp until the end of the trial. The total time each wasp spent on the leaf disk during the trial ("residence time") was recorded using a stopwatch (ie. the time from release until the time the wasp walked or flew off the leaf or until the trial was stopped at 5 m). As such, residence time ranged from zero to 300 s. Searching time was recorded on a second stopwatch as the time the wasp spent walking on the leaf disk during the trial. Time spent resting and cleaning wings or antennae was not included. The proportion of time spent searching during a trial was calculated by dividing searching time by residence time.

Data analysis. Data for all trials for both *Trichogramma* species and both leaf species were combined for analysis. The variables walking speed, total time spent on the leaf disk and proportion of time spent searching during the trial were analysed by two-factor analysis of variance (ANOVA) where the factors were *Trichogramma* species (WASP) and leaf species (LEAF). Data for proportion of time spent searching were arcsin-square root-transformed before analysis to normalize data. Untransformed means are reported for proportion of time spent searching. All analyses were conducted using Sigmastat version 2.0 (Fox *et al.*, 1995).

RESULTS

Walking speed. Walking speed was significantly higher (approximately twice as fast) on pepper leaves than on tomato leaves for both *Trichogramma* species (Table 1, Table 2). There was no significant difference in walking speed between the two *Trichogramma* species. There was also no difference in how variation in walking speed in the two wasp species was affected by the two plant species as indicated by the non-significant interaction term in the ANOVA.

Table 1

Mean walking speed, residence time and proportion of time spent searching (Mean ± SE) by female *Trichogramma* wasps on tomato and pepper leaf disks.

Variable	*T. sibericum*		*T. brassicae*	
	Tomato	Pepper	Tomato	Pepper
Walking speed (mm/sec)	0.9 ± 0.1	2.0 ± 0.2	1.1 ± 0.1	2.3 ± 0.2
Residence time (seconds)	219 ± 21	248 ± 19	48 ± 8	89 ± 20
Proportion of time spent searching	0.68 ± 0.05	0.63 ± 0.03	0.82 ± 0.06	0.73 ± 0.05

Table 2

Two-factor analysis of variance of walking speed, residence time and proportion of time spent searching by female *Trichogramma* wasps. Factors for analysis are wasp species (WASP; either *T. sibericum* or *T. brassicae*) and plant species (PLANT; either tomato or pepper leaf disks).

Variable	Factor	F	df	p
Walking speed	WASP	2.1	1	0.15
	LEAF	64.5	1	<0.001
	WASP*LEAF	0.3	1	0.62
Residence time	WASP	89.0	1	<0.001
	LEAF	4.0	1	0.048
	WASP*LEAF	0.1	1	0.73
Proportion of time spent searching	WASP	10.1	1	0.002
	LEAF	3.3	1	0.07
	WASP*LEAF	0.5	1	0.49

Residence time on leaf disk. Both *Trichogramma* species and leaf species affected the residence time of wasps on leaves (Table 1, Table 2). *T. brassicae* females spent significantly less time than *T. sibericum* on both tomato and pepper leaf disks. However, this reflects the manner in which trials were conducted for the two *Trichogramma* species. *T. sibericum* trials of less than 60 s were rejected and this artificially inflated the mean residence times for this species. Because *T. brassicae* females dispersed from leaf disks more readily than *T. sibericum* females, shorter trials were retained for analysis. For both wasp species, LEAF was a significant effect (ie. more time was spent on pepper than on tomato leaf disks). Wasps exited earlier from tomato foliage than from pepper foliage in trials. Variation in residence time in both wasp species was affected similarly on the two plant species as indicated by the non-significant interaction term in the ANOVA.

Proportion of time spent searching. Leaf species did not significantly affect the proportion of time that females spent searching while on leaf disks during trials (Table 1, Table 2). However, the proportion of time that females spent searching on leaf disks was significantly higher for *T. brassicae* than for *T. sibericum*. Again, this reflects the difference in the manner in which trials were conducted for the two species. *T. brassicae* trials often consisted of the wasp walking to the leaf edge and directly off the edge after release. This sort of trial has a proportion of time spent searching of 1 (or 100% of time searching), and was much more common for *T. brassicae* than for *T. sibericum*. Again, the interaction term was non-significant indicating that both wasp species react in a similar fashion to the two leaf species.

DISCUSSION

There are clear effects of leaf species on the behaviour of both *T. brassicae* and *T. sibericum*. First, wasps walk at approximately half the speed on tomato leaf disks as on pepper leaf disks. This difference was consistent in two *Trichogramma* species of different ages and physiological conditions. Such a reduction of walking speed on tomato is similar to what has been reported for other *Trichogramma* species and is probably caused by the presence of glandular trichomes which impair the walking behaviour of wasps (Keller 1987; Kauffman & Kennedy 1989; Kashyap *et al.* 1991). Second, wasps leave tomato foliage (with trichomes) sooner than they leave pepper foliage (without trichomes). This result may indicate a lower preference for tomato foliage than pepper foliage as a habitat for host search. A higher propensity to disperse from foliage with higher trichome densities has previously been reported for *T. pretiosum* (Kashyap *et al.* 1991). Because the wasps walked faster and stayed longer on pepper leaf disks, they likely examined more of the leaf surface available than wasps on tomato leaf disks.

The *T. brassicae* females used in this study were more behaviourally active and dispersed from leaf disks much more readily than *T. sibericum* females. This behavioural difference could be genetically based, caused by the difference in age between the groups of wasps (*T. brassicae* were 3-5 d old; *T. sibericum* were 1-3 d old), or caused by some other difference in physiological condition that influences dispersal. Whatever the explanation, the consistency of walking speed measurements for these two behaviourally-distinct groups of insects is remarkable. Also, despite differences in residence time between *T. brassicae* and *T. sibericum* caused by different bioassay methods, we still detected lower residence times on tomato leaf disks than on pepper for both species.

We have assumed that our measurements of behaviour on a two-dimensional excised leaf disk are analagous to behaviour on whole plants under field conditions. In addition, we have assumed that wasps are searching for hosts or evaluating leaf surfaces as host habitat when walking on leaf surfaces in the laboratory. Our method allowed the detection of behavioural differences between two plant species that are likely caused by the presence of trichomes on tomato. Measurement of walking speed in the laboratory has been recommended to evaluate performance quality for mass-reared *Trichogramma* wasps (Cerutti & Bigler 1994), although this measure of quality does not always correlate with parasitism success (van Hezewijk *et al.* 2000). In light of the results presented here, it seems critical to measure walking speed on the appropriate plant substrate. Prediction of performance on tomato crops using walking speed data from pepper leaves would overestimate searching efficiency of both species of wasps.

Our results show that both *T. brassicae* and *T. sibericum* behave differently on tomato leaf disks than on pepper leaf disks. If these behavioural differences also occur during searching behaviour in commercial greenhouses, the resulting level of biological control could be lower in tomato greenhouses vs. pepper greenhouses. Wasps might discover fewer hosts on tomato because lower walking speed and a higher tendency to exit tomato foliage would reduce the number of hosts encountered per unit time. This hypothesis has important implications for release rates of *Trichogramma* in the two crops (ie. higher release rates may be required in tomato greenhouses). However, our laboratory-derived results should be validated by conducting releases of *Trichogramma* into tomato and pepper crops under operational conditions.

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