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Movement and host finding of *Trichogramma brassicae* on maize plants

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Abstract

Direct observation of searching patterns and residence times of *Trichogramma brassicae* Bezdenko on maize plants were made at 18 °C and 25 °C. Temperature had a strong effect on the residence times: parasitoids spent an average of 44.9 minutes on the plant at 18 °C and 20.8 minutes at 25 °C. Observations on single plants showed that parasitoids mainly walked from one leaf to another. The leaf part closest to the stem was the most often visited and longest searched leaf area. At 18 °C, many parasitoids went to the lower leaf side and stopped searching. Leaf level, leaf side or size of the leaf where the parasitoid landed had no effect on residence time. Although about 20% of total searching time was spent in following the leaf edge and mid rib, *Ostrinia nubilalis* (Hubner) egg masses touching the mid rib were not found more often than those away from the mid rib. Host finding was also not influenced by the position of egg masses relative to the stem, or by the leaf height. Naturally laid egg masses were found twice as often as artificially placed egg masses. The scales that *O. nubilalis* adults left in small patches on the plant seemed to be as effective a cue as host cues artificially confined to a small area around the host egg mass. When maize plants were covered with fine netting and a number of *O. nubilalis* kept close to the plant for one night in such a way that the moths were unable to touch the plants, host finding and residence times of *T. brassicae* were significantly higher on these plants than on untreated plants. This indicates that volatile cues left by *O. nubilalis* had an arresting effect and were used in host finding by *T. brassicae*.

Key words: *Trichogramma brassicae*, *Ostrinia nubilalis*, host-patch finding, host finding, searching behaviour, searching pattern.

Introduction

Trichogramma brassicae Bezdenko is used in inundative releases for biological control of the European corn borer, *Ostrinia nubilalis* (Hubner), in maize fields (Li, 1994; van Lenteren, 2000; van Lenteren and Bueno, 2003; Bigler *et al.*, 2010; Parra *et al.*, 2010). Although extensive applied research has been carried out on *Trichogramma*, there is limited information on the behaviour of the individual parasitoids in the field (Smith, 1996; Thomson *et al.*, 2003; Suverkropp *et al.*, 2009). Parasitoids of the genus *Trichogramma* are generalist egg parasitoids that primarily parisitize Lepidoptera (Clausen 1940, Thomson and Stinner 1989, Pinto and Stouthamer 1994; Babendreier *et al.*, 2003; Kuske *et al.*, 2003). *T. brassicae* is a polyphagous parasitoid and has been found on a wide range of lepidopterous hosts (Fulmek, 1955; Orr *et al.*, 2000). Thus, it is reasonable to expect that *Trichogramma* spp. parasitoids will be adapted to the behaviour of lepidopterans in general (Fatouros *et al.*, 2008; Gingras *et al.*, 2008), but not necessarily to the behaviour of specific host species (Gardner and van Lenteren, 1986; Gardner *et al.*, 2007). The parasitoid will have to allocate an optimal amount of time to search on a patch that could contain hosts (Morrison and Lewis, 1981; Godfray, 1994; Gingras *et al.*, 2008). After having spent this optimal amount of time, behaviour that will make the parasitoid leave the patch (flight, walking straight instead of turning at the edge of the patch) is no longer inhibited. It depends on the type of search behaviour over the plant whether a leaf or a whole plant is defined as patch. If flight is the main mechanism for movement between leaves, and the parasitoid is just as likely to land on a leaf of neighbouring plant after leaving a leaf of the plant on which it was already searching, then each leaf is a separate patch. If the parasitoid walks from leaf to leaf while searching and only flies away after having searched a large proportion of the leaves, then the whole plant must be considered as one patch.

Many factors may influence searching efficiency of *T. brassicae*, such as habitat complexity, host type, host density and host distribution, and genetic and phenotypic diversity of *T. brassicae*, etc. Below, we shortly discuss three possible factors that may affect host foraging: temperature, host search patterns, and the influence host cues on host finding.

It is known that flight propensity and walking speed of *T. brassicae* depend on temperature: below 18 °C there will be no flight (Bigler *et al.*, 1982) and at low temperatures, walking is slower than at high temperatures (Suverkropp *et al.*, 2001). Thus, it is likely that residence time will increase as temperature decreases. The complexity of the host habitat and structure of the plant or leaf can also influence residence times (Romeis *et al.*, 2005; Gingras *et al.*, 2002; 2008; Grieshop *et al.*, 2008). Residence time of *Trichogramma nubilale* Ertle et Davis is higher on a single flat surface than on narrow ribbons with the same surface area (Andow and Prokrym, 1990). Babendreier *et al.* (2003) showed that architecture, size and leaf characters of different plants may have strong implications on host searching efficiency and parasitism of *T. brassicae*. For *Haeckeliania sperata* Pinto (Hymenoptera Trichogrammatidae) it was shown that presence of leaf trichomes decreased the total time travelled, increased the total search time, reduced searching speed and resulted in lower parasitism than on smooth leaves (Carrillo *et al.*, 2008).

A specific search pattern can make the parasitoid forage in those parts of the host habitat where hosts are most likely to be present (e.g. Gingras *et al.*, 2008; Grieshop *et al.*, 2008). On the leaf, *T. brassicae* walks in relatively straight lines in the absence of host cues (Gardner and van Lenteren, 1986; Gardner *et al.*, 2007). *Trichogramma pretiosum* Riley follows the edge of surfaces (Noldus *et al.*, 1991a), but *T. nubilale* does not (Andow and Prokrym, 1990). Information on movement patterns of *Trichogramma* on whole plants is lacking. Indirect information from field studies shows that there are no leaf layers and positions where *O. nubilalis* egg masses are not parasitized (Milani *et al.*, 1988). There was also no effect of leaf level on parasitism of *O. nubilalis* in maize by *T. brassicae* in a field experiment in France (Hawlitzky *et al.*, 1994). In Italian experiments in maize, clear differences were found between two *Trichogramma maidis* Pintureau et Voegele (= *brassicae*) strains, one mainly parasitizing above 140 cm, the other below 100 cm (Milani *et al.*, 1988). Experiments performed in Germany showed higher parasitism in the middle and upper leaf levels of maize plants for *Trichogramma evanescens* Westwood (Neuffer, 1987). *T. nubilale* shows a preference for *O. nubilalis* egg masses in the lower and middle third of maize plants (Burbutis *et al.*, 1977). But all these experiments about parasitism of egg masses in the field do not necessarily provide a reliable picture about movement of parasitoids on the plant, since only the end effect of the host searching process, i.e. in the form of parasitized host eggs, is represented.

Further, the parasitoid can react to host cues while searching for hosts, which may result in finding hosts faster than in case of random search. For an extensive recent review of the use of chemical information by egg parasitoids, we refer Fatouros *et al.*, 2008. *T. brassicae* reacts to several cues of *O. nubilalis*, such as scales (Kaiser *et al.*, 1989; Bieri *et al.*, 1990; Fatouros *et al.*, 2005), egg odour (Renou *et al.*, 1989; 1992) and sex pheromone (Frenoy *et al.*, 1992). The reaction to such cues has also been shown for several other egg parasitoids (Fatouros *et al.*, 2008). Work of Rani *et al.*, (2006) indicates that cuticular extracts - which mainly consists of wing scale extracts - of adult moths of the rice yellow stemborer, *Scripophaga incertulas* Walker, increase parasitization efficiency of *Trichogramma japonicum* Ashmead. The reaction to scales is in the form of increased klinokinesis (the searching path becomes more tortuous), reversed orthokinesis (the walking speed decreases) and flight inhibition (Gardner and van Lenteren, 1986; Bieri *et al.*, 1990; Gardner *et al.*, 2007). The klinokinetic and orthokinetic responses arrest the parasitoid in a very small area, while flight inhibition will arrest the parasitoid on the plant. Scales of *O. nubilalis* are found in small patches on the plant, but not necessarily within two or three centimetres of an egg mass (Suverkropp *et al.*, 2008). Bieri *et al.* (1990) predict in a model study that an area with scales around an egg mass has an optimum size: if the scale area becomes too large, the scale responses will arrest the parasitoid in a place where the eggs are not present. The same could be predicted for scale patches away from the egg mass. Scales have no attractive effect on *T. brassicae* (Noldus and van Lenteren, 1985b; Suverkropp, 1994). *Trichogramma* reacts to moth sex pheromones with upwind walking in olfactometers (Frenoy *et al.*, 1992) and reversed orthokinesis (Noldus *et al.*, 1991a). Volatiles of calling females of *Heliothis zea* (Boddie) inhibited flight of *T. pretiosum*. If the parasitoids were forced to fly, they made much shorter flights in the presence of the odours (Noldus *et al.*, 1991a). Levels of parasitism in petri-dish and olfactometer set-ups are increased in the presence of moth sex pheromones (Zaki, 1985; Frenoy *et al.*, 1991). Egg mass odour (Kaiser *et al.*, 1989; Renou *et al.*, 1992) and visual recognition (Laing, 1937; Pak *et al.*, 1990) of egg masses plays a role at very short range (2-3 mm) only.

All the above information on movement, foraging and parasitim of *T. brassicae* contribute to understanding of its host searching behaviour, but until now, a full description of searching behaviour on the plant is lacking. Therefore, we made direct observations of *T. brassicae* when searching on maize plants with the following objectives: (1) to determine residence times and how they are influenced by temperature, (2) to determine whether the position of host egg masses on the plant has affects host finding, and (3) to quantify the responses of *T. brassicae* to host cues in terms of searching success and residence time at the plant level.

Materials and methods

Plants

Maize plants (*Zea mays* L.) grown under greenhouse (cultivar LG11) and field conditions (cultivar Atlet) were used in the experiments. Field-grown plants were potted one day before use.

Parasitoids

T. brassicae was used in all experiments. The stock colony was obtained in 1975 from INRA, Antibes, France (strain 16). The strain originates from the former Soviet Republic of Moldavia. The stock colony is maintained on *O. nubilalis* eggs. The rearing system is described in Bigler (1994). The parasitoids used in the experiments were reared for one or two generations on *Ephestia kuehniella* Zeller eggs. Females used were one to three days old, had no oviposition experience and had not been exposed to leaves or leaf odours prior to the experiments. In experiments with individual parasitoids, each female was placed in a glass tube containing a droplet of water and honey one to eight hours before it was used in experiments.

Moths

The European corn borer, *O. nubilalis*, was used in the experiments. The rearing is established every year with overwintering larvae collected in the field in Switzerland.

Event recording

For detailed behavioural observations portable computers (Tandy 102® or Psion Organiser®) were used with an event recording program (The Observer®).

Experiment 1: Movement on plants without host cues with parasitoid releases at one leaf level

Single greenhouse grown maize plants with ten leaves (height: 85 cm, leaf area about 800 cm^2) were used for all observations. Plants were placed in a climate room set at 4 °C during night time hours to inhibit further growth. During the experiments the plants were placed in a climate room under fluorescent lamps (40 W) covered with white paper to obtain an even illumination of the set-up.

Both the location and behaviour of single female parasitoids were recorded during the experiment. The locations recorded were leaf one to ten, ear and stem. Leaf one was the lowest leaf. Leaf length was divided in three equal parts, close to the stem, middle and point. Location on these leaf sections and leaf side were recorded. Encounters of the parasitoid with the vein (mid rib) or the leaf edge were also recorded. Following the vein was defined as a direction change after encountering the vein in combination with walking along the vein. Following the edge was defined as walking along the edge while staying within one millimetre of the leaf edge. Three other types of behaviour were distinguished. 'Walking' was defined as all movement on the plant surface. 'Standing still' was defined as the time spent on the plant surface when the parasitoid was not moving. This included standing, grooming and feeding. 'Flight' was defined as any movement in the air.

Behavioural observations were begun immediately after placing a single female parasitoid on the upper side of the fifth leaf, about five centimetres from the stem. Parasitoids were placed on the plants by gently knocking the side of tube while it was positioned over a leaf. An observation was terminated when the parasitoid left the plant by flight without landing on another leaf, when it did not move for more than 20 minutes, or when it spent more than 150 minutes on the plant. Fifty five observations of unique female parasitoids were performed at 18 °C and 25 °C, respectively.

Experiment 2: Residence times on plants without host cues with parasitoid releases at all leaf levels

Female parasitoids were observed on greenhouse grown plants with six to seven leaves and an average leaf area of 825 cm^2 in a climate room set at either 20 or 25 °C. Half of the parasitoids were introduced on the upper leaf side, the other half on the lower side. Contrary to experiment 1, where parasitoids were only released at leaf layer 5, in this experiment the effect of parasitoid releases at all leaf levels were tested. An observation was terminated when the parasitoid left the plant or when it did not move for 20 minutes. For each temperature, 50 observations were made of the behaviour as described above, for each observation a new parasitoid was used.

Experiment 3: Effect of egg mass position on host finding

Potted plants were used with egg masses of *O. nubilalis* on various leaf levels and at different positions on the leaf. A total of 99 egg masses were offered to parasitoids during 7 replicates of this experiment: 47 touching the mid rib and 52 away (> 1 mm) from the mid rib. In each series of observations, three plants were used. The potted plants were placed on a row with the stem of the plants 30 cm apart. Two thousand female *T. brassicae* were released five cm away from the plant in the middle of this row. During two hours all *O. nubilalis* egg masses were continuously checked for *T. brassicae* females. Parasitoids found on an egg mass were recorded and removed from the plant using an aspirator. After two hours of observation, the plants were left unobserved for one hour and then a last count of *T. brassicae* on the egg masses was made. The experiments were done in a greenhouse at a temperature of 25 °C.

Experiment 4: Effect of oviposition host cues on host finding

Six field-grown maize plants were used. Three plants were exposed to *O. nubilalis* females in a field cage to obtain plants with host cues and egg masses, and to find out the location where egg masses were deposited by the females. For controls, the other three plants were not exposed to *O. nubilalis* females. Instead, egg masses were artificially placed on these plants in the same position as on the plant with naturally laid egg masses. The two sets of plants had approximately 16 egg masses so each plant had about 5 egg masses.

In the greenhouse, the plants with naturally and artificially laid eggs were placed opposite each other, with a space of two meters between them. A container with 2000 *T. brassicae* females was placed in the middle (one meter from each group of plants) and opened. During two hours, all *T. brassicae* females that reached the egg masses were counted and removed from the egg masses. The experiment was done twice. The experiment was done in a greenhouse at a temperature of 25 °C.

Experiment 5: Effect of localized host cues on host finding

A small container (3 cm diameter) of gauze with one *O. nubilalis* female was fastened overnight to the central lower side of the fourth or fifth leaf of a young maize plant with six leaves. The next day, one *O. nubilalis* egg mass was placed in the middle of the potential kairomone area. Another egg mass was placed in the same position (but without potential kairomone) on the next higher or lower leaf. The plant was placed in a greenhouse compartment and a container with 2000 *T. brassicae* females was opened at 140 cm from the plant. During 80 minutes, the females that found the egg masses were recorded. Eighteen replicates were made. The experiment was done in a greenhouse at a temperature of 25 °C.

Experiment 6: Effect of localized host cues on residence time

A small container (3cm diameter) of gauze with one *O. nubilalis* female was fastened overnight to the central lower side of a full size maize leaf. The next day, this leaf was fastened to a stand and an egg mass was stuck to the centre of the potential kairomone area. Three *T. brassicae* females were allowed to fly to the leaf. Their behaviour was recorded using a portable computer and event recording software. Moving, standing still, following edge and mid rib were defined as in the first experiment. Locations recorded were leaf upper side, leaf lower side, and inside or outside potential kairomone area. Observations were ended if the parasitoid left the leaf by flight or walking, found the egg mass, or after two hours. As a control, the same observations were made on a clean leaf with an egg mass placed in the same position as on the treated leaves. On the leaves with kairomones, 54 females were observed, and on the control leaves 36 females were observed. The experiments were done in a climate room at a temperature of 25 °C.

Experiment 7: Effect of volatile host cues on host finding

Six field-grown plants were used. Three of the plants were exposed in a greenhouse to ten *O. nubilalis* females (one to four days old) and five males. Physical contact between *O. nubilalis* and maize plants was prevented and contamination by *O. nubilalis* scales was avoided. Only volatile cues were allowed to reach the plants. This was done by caging the maize plants with netting (Monofiles Nylon netting 100 μm hole size, Scrynel). Previous work (Noldus, 1989) had shown that a mesh size of 340 μm was able to intercept 90% of scales so we assumed 100 μm netting was adequate to intercept all scales. The netting was kept as close to the plant as possible. *O. nubilalis* were introduced in the cage and left for 48 and 24 hours in the first and the second replicate, respectively. The other plants (non-exposed to *O. nubilalis*) were placed in another greenhouse in order to avoid any volatile contamination. Fifteen and 12 egg masses were placed on the plants of each group in the first and second replicate, respectively. The experimental procedure in the greenhouse was the same as in the previous experiment. Two replications were made. The experiments were done in a greenhouse at a temperature of 25 °C.

Experiment 8: Effect of volatile host cues on residence time

Field-grown maize plants were used in this experiment. Plants were treated in three ways. First, plants with natural host cues were prepared as described in the previous experiment: ten female and five male *O. nubilalis* were released in the cage, where they could move close to the plant but not contact it. Second, to obtain plants with artificial host cues, a rubber stopper with synthetic *O. nubilalis* sex pheromone [97% (*Z*)-11-Tetradecenylacetate, 3% (*E*)-11-Tetradecenylacetate] was placed on top of the middle leaf of the plant overnight. In both set-ups, moth sex pheromones could adhere to leaves during a 24-hour period (Noldus, 1989). Third, control plants were used which were kept in a separate compartment to avoid contamination by *O. nubilalis* volatiles.

Two plants from the two different treatments were placed two meters apart in a greenhouse compartment. A release container with 1500 females was placed between the plants and opened for three minutes. During two hours, the number of parasitoids on each of the plants was counted every five minutes. Six replicates were made for each combination of treatments (natural vs. artificial host cues, natural host cues vs. control, artificial host cues vs. control). The experiments were done in a greenhouse at a temperature of 25 °C.

Results

Experiment 1: Movement on plants without host cues with parasitoid releases at one leaf level

The behaviour on plants without host cues is summarized in table 1. Residence time is significantly longer at 18 °C than at 25 °C (throughout this paper, when we mention a signicant effect, we denote a statistically significant effect). Movement is strongly influenced by

Table 1. Experiment 1: Behaviour of *T. brassicae* on maize plants without host cues.

	18 °C	25 °C	p[1]
Number of observations	55	55	
Residence time (minutes)	44.9 ± 6.4[2]	20.8 ± 3.3	****[3]
Observations ended by:			
- Standing still (no movement > 20 min)	37 (67.3%)	7 (12.7%)	****[4]
- Flying away	11 (20.0%)	48 (87.3%)	
- Broken off (observation >150 min)	7 (12.7%)	0	
Mean number of leaf visits	1.8 ± 0.2[2]	1.2 ± 0.1	***[3]
Number of parasitoids that visited more than one leaf	23 (42%)	7 (13%)	**[4]
Walks between leaves	50	7	
Flights between leaves	2	3	
Downward movement	7	7	

[1] n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$

[2] Mean ± s.e.

[3] Mann-Whitney U-test

[4] Chi-square test

temperature. Significantly more parasitoids stood still at 18 °C than at 25 °C. Sixty-seven percent of the observations at 18 °C were terminated because parasitoids stopped moving for more than 20 minutes. Only seven of the parasitoids were still searching after 150 minutes at 18 °C. Flight is infrequent at this temperature, only 20% of the parasitoids left leaves by flight. At 25 °C residence times were significantly shorter and approximately half as long as at 18 °C. At 25 °C 87% of the parasitoids left leaves by flight. At both temperatures, the distribution of the residence times was approximately a negative exponential, which means that most of the parasitoids stayed on the plant for a short period of time only.

The mean number of leaf visits was significantly lower at 25 °C than at 18 °C. The majority of the parasitoids visit only one leaf after landing, especially at 25 °C. At 18 °C, movement between leaves was mostly by walking. The number of parasitoids visiting more than one leaf was significantly higher at 18 °C than at 25°C. The number of walks and flights between leaves at 25 °C was too small to statistically compare with that at 18 °C, but it seems that walking is important for between-leaf movement even at high temperatures where *T. brassicae* tends to fly more. At 18 °C movement was mostly upwards. Walking paths on the stem were usually very straight. Sometimes a 180° turn was made, and the parasitoid returned to the leaf it had just left. This occurred only if the parasitoid had not yet reached the next leaf. The maize leaf sheath encircles the stem, and on the side opposite to the leaf, the parasitoid has to cross a barrier of hairs if it continues following the stem instead of walking on the leaf. On the leaf side, there is an almost seamless connection between the stem and the mid rib on the leaf underside. Thus, parasitoids that walked upward at this side of the stem automatically walk on the leaf.

At 25 °C, 61.7% of the residence time was spent on the upper side of the leaf (figure 1). If the total time is split into walking and standing still, it is clear that the bias towards the upper leaf side is a result of the walking behaviour, because 71.8% of the walking took place on the upper side of the leaf. Time spent standing still was the same on the upper and lower leaf side. Parasitoids were released on the upper side, which might be the reason that more time was spent on the upper side. In fact, of the 55 parasitoids observed at 25 °C only 22 parasitoids visited the lower side. These 22 parasitoids spent more time walking on the upper than on the lower leaf side.

At 18 °C the results were very different (figure 1). Of the total residence time, only 43.5% was spent on the upper side of the leaf. The average walking time was also higher on the lower side than at 25 °C. Of the 55 parasitoids, 48 visited both leaf sides. At 18 °C, parasitoids spent more time walking on the under side of leaves. Standing still was generally observed (87.9%) on the lower side. Many of the observations at 18 °C were terminated because the parasitoid did not move for 20 minutes. In most cases, this took place on the lower side. Even when this final period of standing still was omitted, more time was spent standing still on the lower than on the upper leaf side.

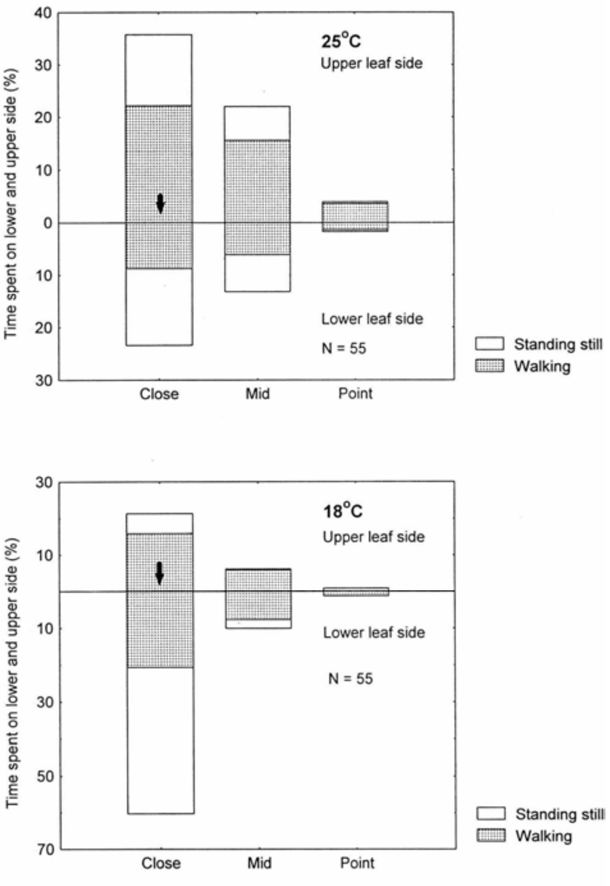

Figure 1. Experiment 1: Time spent by *T. brassicae* on the upper and lower leaf side of maize leaves and in the three leaf sections at two temperatures. Arrows indicate the release position of the parasitoids. Leaf length was divided in three sections: close to the stem, middle, and close to the leaf point.

To determine if an equal amount of time was spent on each section of the leaf, the leaf was divided in three sections, close to the stem, middle of the leaf and point of the leaf. At both temperatures, most time was spent close to the stem and least at the point (figure 1). Since parasitoids were released in the section close to the stem, many parasitoids only visited this section. At 25 °C, the parasitoids that visited all three sections spent more time in the middle part of the leaf than in the part close to the stem. At both temperatures, very little time was spent at the point section.

If a parasitoid encounters the leaf edge, four behaviours are possible. It can follow the edge, turn away from the edge, move to the other leaf side or follow the edge and move to the other leaf side. Table 2 shows that the most usual behaviour was following the edge without changing sides. The mean duration of following the edge was significantly longer at 18 °C than at 25 °C.

Changing sides was less common after the edge had been followed. The duration of edge following was usually about 10 to 15 seconds, although in some instances the edge was followed for 10 minutes or more. Standing still on the edge does occur, but is rare. The distribution of the edge following durations fits a log-normal distri-

Table 2. Experiment 1: Behaviour of *T. brassicae* at maize leaf edges.

	18 °C	25 °C	p[1]
Number of observations	55	55	
Total number of edge encounters	584	416	
Edge followed after encounter without changing side	273 (46.8%)	230 (55.2%)	
Edge followed and side changed	93 (15.9%)	56 (13.5%)	
Walked back on the leaf	127 (21.7%)	78 (18.8%)	
Side changed without following	91 (15.6%)	52 (12.5%)	
Total number of edge encounters	377	289	
Mean duration of following (seconds)	35.8 ± 5.1[2]	25.6 ± 3.2	***[3]
Median duration (seconds)	15.5	10.2	
Percentage of total walking time spent following the edge	12.5%	14.7%	
Edge as percentage of leaf area	5.0%	5.0%	

[1] ***: $P < 0.001$

[2] Mean±s.e.

[3] Mann-Whitney U-test

bution. The edge area of the leaf (< 1 millimetre from leaf edge) constitutes 5% of the total leaf area. If the percentage of time spent following the edge is compared with this, it is clear that the parasitoids spent more time following the edge than what would be expected from random movement.

When the mid rib is encountered, it is followed in about half of the cases (table 3). Close to the leaf point the mid rib is hardly recognisable and rarely followed. On the upper side of the leaf the mid rib is a shallow hairless depression, while on the lower side it forms a sharp crest which results in very straight movement by the parasitoids. The duration of mid rib following was usually short. At 25 °C, the mid rib was followed significantly longer on the lower than on the upper leaf side, at 18 °C there was no significant difference. The time spent following the mid rib is not higher than what would be expected from the surface area of the mid rib relative to the total leaf area.

Experiment 2. Residence times on plants without host cues with parasitoid releases at all leaf levels

In the previous experiment all parasitoids were released at the same position: on the fifth leaf and on the upper side, close to the stem. To measure residence times in a more natural situation, females were released on all leaf levels and on the upper and lower leaf sides.

There was a clear effect of temperature on residence times: at 20 °C parasitoids stayed significantly longer on the plants than at 25 °C (table 4). Residence times usu-

Table 3. Experiment 1: Behaviour of *T. brassicae* at maize leaf mid ribs.

	18 °C	25 °C	p[1]
Number of observations	55	55	
Total number of mid rib encounters	541	436	
Mid rib followed after encounter	289 (55.8%)	246 (56.4%)	
Mid rib encountered without following	252 (44.2%)	190 (43.6%)	
Mean duration of following mid rib (seconds)	15.6 ± 1.0[2]	17.4 ± 1.9	n.s.[3]
Mean duration (seconds)	8.7	7.9	
Mean duration of following mid rib upper side	13.0 ± 1.4[2]		n.s.[3]
Mean duration of following mid rib lower side	15.8 ± 1.3		
Mean duration of following mid rib upper side		15.8 ± 2.3[2]	*[3]
Mean duration of following mid rib lower side		16.9 ± 2.1	
Percentage of total walking time spent following mid rib	5.6%	9.5%	
Mid rib as percentage of leaf area	8.5%	8.5%	

[1] n.s.: $P > 0.05$; *: $P<0.05$

[2] Mean ± s.e.

[3] Mann-Whitney U-test

Table 4. Experiment 2: Residence times of *T. brassicae* on maize plants without host cues at two temperatures.

	18 °C	25 °C	p[1]
Number of observations	50	50	
Mean residence time (minutes)	37.9 ± 5.5[2]	16.8 ± 2.4	***[3]
Mean number of leaves visited	1.5 ± 0.1[2]	1.2 ± 0.1	n.s.[3]
Number of parasitoids that visited more than one leaf	12 (24%)	6 (12%)	n.s.[4]
Observations ended by:			
- Standing still (no movement > 20 min)	10 (20%)	2 (4%)	*[4]
- Flying away	40 (80%)	48 (96%)	

[1] n.s.: $P > 0.05$; *: $P < 0.05$; ***: $P < 0.001$

[2] Mean ± s.e.

[3] Mann-Whitney U-test

[4] Fisher's Exact Probability test

ally did not exceed 20 minutes at both temperatures (figure 2). Residence times and number of leaves visited at 25 °C were similar in this and in the previous experiment (figure 3). There is a considerable difference between residence times at 18 and 20 °C. At 20 °C, 80% of the parasitoids flew away (table 4), while at 18 °C only 20% flew away (table 1).

The average number of leaves visited and the number of parasitoids that visited more than one leaf was significantly higher at 20 °C. At both temperatures, most observations were ended by the parasitoids flying away. Significantly more observations were ended by standing still at 20 °C than at 25 °C. No significant correlations were found between leaf level on which the parasitoid was released, leaf side, leaf length, leaf width or leaf area, and residence times.

Experiment 3: Effect of egg mass position on host finding

No significant difference was found between the mean number of parasitoids that found egg masses touching the mid rib and the number of parasitoids that found egg masses away from the mid rib (table 5). Also, there were no significant differences either in the mean number of parasitoids finding egg masses in the leaf subsectors, close to the stem, middle leaf and close to the point (table 6). Further, there was no correlation either between leaf level of the egg mass and mean number of parasitoids that found the egg mass.

Experiment 4: Effect of oviposition host cues on host finding

T. brassicae found naturally laid host egg masses more than twice as often than artificially placed host egg masses (table 7). The average number of females found per egg mass was also significantly higher on the naturally laid eggs.

Experiment 5: Effect of localized host cues on host finding

A total of 376 parasitoids found the egg masses, but there was a large daily variation in the numbers. The

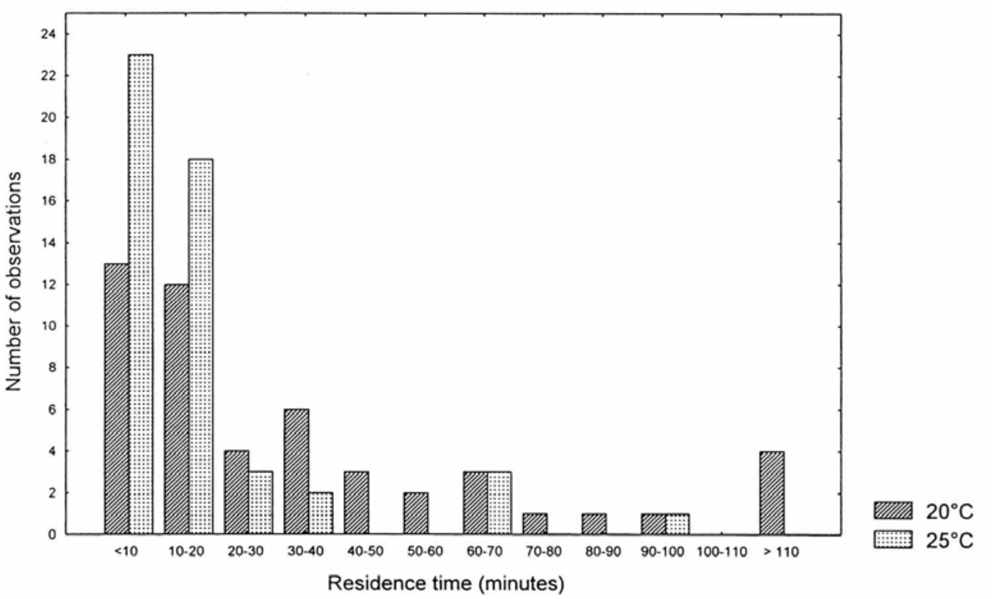

Figure 2. Experiment 2. Residence times of *T. brassicae* on maize plants without host cues at two temperatures.

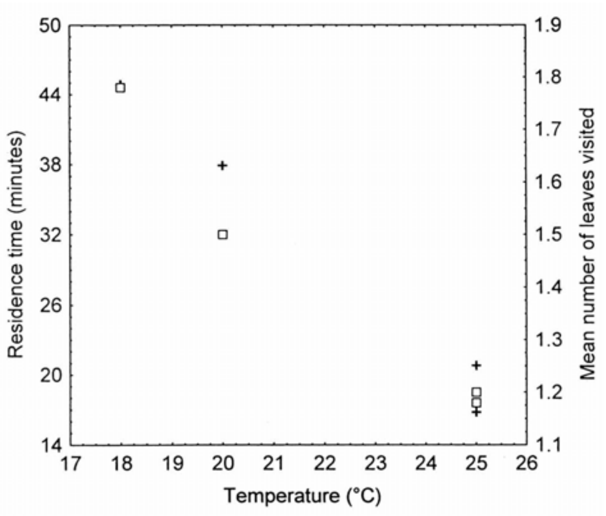

Figure 3. Experiment 1 and 2, combined data: Residence times (+) and mean number of leaves visited (□) by *T. brassicae* on maize plants at three temperatures.

results are shown in table 8. Scales close to an egg mass signifcantly increases the number of parasitoids that find an egg mass. The average time to find an egg mass was the same for egg masses with or without host cues.

Experiment 6: Effect of localized host cues on residence time

Confining an *O. nubilalis* female on a three centimetre spot on the leaf had no effect on either the residence time on this leaf or the activity of the parasitoid (table 9). Neither was the way in which the parasitoid left the leaf influenced by the presence of host scales. Although egg masses on the treated leaves were found more often, the difference was not significant. Significantly more parasitoids that came within 1.5 cm of the egg mass and entered the kairomone area on the treated leaves contacted host egg patches versus on plants that lacked host kairomones.

Table 5. Experiment 3: Effect of *O. nubilalis* egg mass position relative to the mid rib on number of *T. brassicae* finding egg masses on maize plants.

	Egg masses away from mid rib	Egg masses touching mid rib	p[1]
Number of replicates	7	7	
Total number of egg masses	49	50	***[3]
Total number of females finding egg masses	440	334	n.s.[3]
Average number of females per egg mass	8.9 ± 1.3[2]	7.4 ± 1.6	n.s.[3]

[1] n.s.: P > 0.05
[2] Mean ± s.e.
[3] Mann-Whitney U-test

Table 6. Experiment 3: Effect of egg mass position relative to the stem on number of *T. brassicae* finding *O. nubilalis* egg masses on maize plants.

Position	Close	Mid	Point	p[1]
Number of replicates	7	7	7	
Total number of egg masses	30	44	25	
Average number of females per egg mass	8.7 ± 1.9[2]	6.1 ± 1.2	11.2 ± 2.5	n.s.[3]

[1] n.s.: P > 0.05
[2] Mean ± s.e.
[3] Kruskall-Wallis test

Table 7. Experiment 4: Total number of *T. brassicae* females found on O. nubilalis egg masses on maize plants exposed to *O. nubilalis* (eggs naturally laid) and non-exposed maize plants with artificially placed egg masses.

	Natural	Artificial	p[1]
Number of replicates	2	2	
Number of egg masses offered	33	32	
Total number of females finding egg masses	183	80	
Average number of females per egg mass	5.5 ± 0.8[2]	2.5 ± 0.3	**[3]

[1] **: P < 0.01
[2] Mean ± s.e.
[3] Mann-Whitney U-test

Table 8. Experiment 5: Effect of localized *O. nubilalis* host cues on host finding of *T. brassicae*.

	Host cues	Control	p[1]
Number of replicates	18	18	
Number of parasitoids that found the eggs	14.6 ± 2.7[2]	6.3 ± 0.6	**[3]
Average time to find the eggs (minutes)	24.3 ± 0.9[2]	22.9 ± 1.4	n.s.[4]

[1] n.s.: $P > 0.05$; **: $P < 0.01$

[2] Mean ± s.e.

[3] Wilcoxon matched pairs test

[4] Mann-Whitney U-test

Table 9. Experiment 6. Effect of localized host cues on leaf residence times and walking activity (walking time/total time) of *T. brassicae* at 20 °C.

	Treated	Control	p[1]
Number of observations	54	36	
Mean residence time (minutes)	8.0 ± 1.22[2]	8.9 ± 1.5	n.s.[3]
Mean walking activity	0.78 ± 0.03[2]	0.76 ± 0.04	n.s.[3]
Observation ended by number of females:			
- Standing still	2 (3.7%)	4 (11.1%)	
- Flying away	37 (68.5%)	28 (77.8%)	
- Walking away	6 (11.1%)	2 (5.6%)	
- Finding egg	9 (16.7%)	2 (5.6%)	n.s.[4]
Number that came within 1.5 cm of egg mass	23	26	
Percentage of parasitoids that came within 1.5 cm of egg mass that found egg mass	39.1%	7.6%	**[4]

[1] n.s.: $P > 0.05$; **: $P < 0.01$

[2] Mean ± s.e.

[3] Mann-Whitney U-test

[4] Chi-square test

Experiment 7: Effect of volatile host cues on host finding

A significantly larger number of *T. brassicae* females were found on the egg masses on plants exposed to *O. nubilalis* than on the control plants (table 10).

Experiment 8: Effect of volatile host cues on residence time

A total of 374 parasitoids landed on the plants. Residence times were significantly longer on plants with natural *O. nubilalis* host cues than on plants without host cues or plants with artificial host cues (figure 4). Residence times on plants with artificial sex pheromone were not significantly longer than those on the control plants. After two hours, 18% of the parasitoids were still present on the plants with natural kairomones, against 7% on the plants with artificial sex pheromones and 4% on the control plants.

Discussion

Most visits of *T. brassicae* to plants without host cues were short (tables 1-4, figures 1-3), so only a small part of the surface of each plant was searched during a visit. Because most parasitoids stayed on the plant for a short period only and did not move very far over the plant's surface, the location of the area searched was strongly influenced by the landing site. Based on our observations of landing and searching, it would be expected that eggs on the middle and upper leaves, and close to the stem would be found most often. However, the experiment on the effect of egg mass position on host finding did not show significant effects of leaf level or distance from the stem on host finding (tables 5 and 6).

The effect of the distribution of landing sites and walking patterns was apparently not strong enough to cause significant differences in the probability that an egg mass is found at different positions on the plant. The effect of leaf level on parasitism found by Milani *et al.* (1988) and Burbutis *et al.* (1977) might be a result of the circumstances in the field, where there can be differences in light intensity, temperature, humidity and wind speed between the upper and lower part of the plant, effects which are weaker or missing in the greenhouse where the experiments were conducted.

Following the mid rib and the edge was common behaviour of *T. brassicae* (table 2), and this has also been reported by other authors (Gass, 1988; Noldus, 1989; Blanché, 1990; Noldus *et al.*, 1991a; Gingras *et al.*,

Table 10. Experiment 7. Total number of *T. brassicae* females that found artificially placed *O. nubilalis* egg masses on maize plants exposed to *O. nubilalis* and on maize plants not exposed to *O. nubilalis*.

	Exposed to *Ostrinia*	Not exposed to *Ostrinia*	p[1]
Number of experiments	2	2	
Number of egg masses	27	27	
Total number of females finding egg masses	127	46	
Average number of females per egg mass	4.7 ± 0.7[2]	1.7 ± 0.4	**[3]

[1] **: $P < 0.01$

[2] Mean ± s.e.

[3] Wilcoxon Matched Pairs test

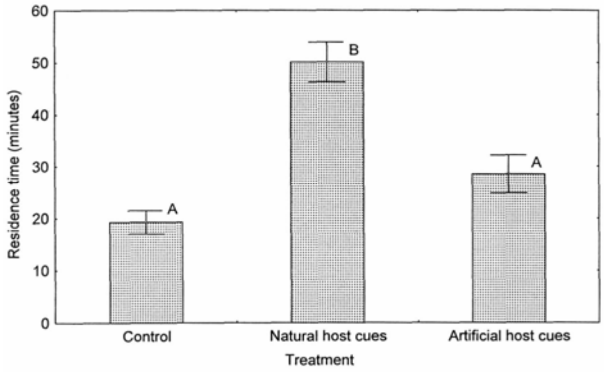

Figure 4. Experiment 8: Effect of *O. nubilalis* volatile host cues on average residence times of *T. brassicae* on maize. Bars represent mean ± standard error. Different letters indicate significant differences of means (LSD test of log-transformed data, $P < 0.05$)

2008). This behaviour is a thigmotactic response to linear structures encountered. Many insects are directed by such features if no host cues are present (Bell, 1990). It has been proposed that following the edge of leaves is an efficient way for *Trichogramma* to scan the leaf for scales left by moths that hang from the leaf edge (Noldus *et al.*, 1991b). *O. nubilalis* does not have any preference for the leaf edge when landing or ovipositing (Suverkropp *et al.*, 2008), so it is unlikely that following the edge will be advantageous for *T. brassicae* when searching for *O. nubilalis* host cues. Eggs of *O. nubilalis* are usually found touching the mid rib (Beck, 1987; Suverkropp *et al.*, 2008), so mid rib following, especially on the lower leaf side, could increase searching efficiency. *T. maidis* (=*brassicae*) spent 20% of total time on the lower leaf side following the midrib, and 10% of total time following the mid rib on the upper side (Gass, 1988). In our study, differences in mid rib following between upper and lower side were not so pronounced. Experiment 3 on the effect of the position of egg masses showed that following the mid rib had no effect on host finding: egg masses touching the mid rib were not found more often than those further away (tables 5 and 6).

Egg masses naturally deposited by a female on the plant were found twice as often as egg masses artificially placed on the plant (table 7). When *O. nubilalis* oviposits naturally on the plant, most of the egg masses have few or no scales in the vicinity. Further, there are many patches of scales on the plant that are far away from an egg mass (Suverkropp *et al.*, 2008). The fact that many scale patches are not immediately around an egg mass does not seem to have a detrimental effect on *T. brassicae* host finding, indicates that it is well adapted to patchwise distributed scales. Beevers *et al.* (1981) found that flight initiation of *T. pretiosum* is inhibited by contact with scale extract, even when the kairomone area has been left. The result is that the parasitoid will make an intensive search of the kairomone area, stays longer on the plant, and searches a larger area than on plants without kairomone. Lewis *et al.* (1979) found that uniform treatment with kairomone extracts reduced parasitism by *Trichogramma* compared to a situation where kairomones were distributed as discrete patches. A uniform treatment may cause parasitoids adapted to patchwise distributed kairomones to be to strongly arrested and this results in spending much time in areas without hosts (Gardner and van Lenteren, 1986; Gardner *et al.*, 2007).

Experiments 6-8 showed the effect of volatile host cues. Although netting with a fine mesh kept *O. nubilalis* and its scales away from the maize plants, more egg masses were found by *T. brassicae* on the treated plants than on the control plants (table 10). Since the moths could not touch the plants and their scales not reach the plants, the kairomones involved must be volatiles. In the experiments with volatile host cues, the kairomone is expected to be on the whole plant, not only where egg masses are present. In such a situation, a strong klinokinetic response would not lead to higher parasitism, since the cue is not connected with the egg mass positions. The mechanism involved seems to be flight inhibition, causing the parasitoid to spend more time on the plant where the volatile host cues were encountered. This is confirmed by the the results of experiment 8, which showed that the parasitoids stayed longer on plants with *O. nubilalis* volatile cues (figure 4).

Based on other work with *Trichogramma* (Noldus and van Lenteren, 1985a; Frenoy *et al.*, 1991), we hypothesize the host volatiles to be the sex pheromone of *O. nubilalis*. Moth sex pheromones can be absorbed by plants and released over at least 24 hours (Noldus *et al.*, 1991b). Synthetic sex pheromone did not increase residence times in experiment 8 (figure 4). This finding agrees with the results of Kaiser *et al.* (1989) and Frenoy *et al.* (1992). Thus, the compound of the sex pheromone mixture that attracts male *O. nubilalis* does not function as a kairomone for *T. brassicae*. Although several authors state that *Trichogramma* species generally search randomly (e.g. Smith, 1996; Chapman *et al.*, 2009) our findings reported above indicate that *T. brassicae* reacts to volatile and non-volatile cues and do not forage purely at random.

If host cues provide reliable information on the presence of host eggs, innate and fixed responses by the parasitoid are predicted (Vet and Dicke, 1992). Responses of *Trichogramma* species to host scales are indeed innate and fairly nonspecific (Thomson and Stinner, 1990). Fatouros *et al.* (2005) found arrestment responses for *T. brassicae* and *T. evanescens* to scales of *Pieris brassicae* L.. The response of *T. brassicae* to *Mamestra brassicae* (L.) scales is not reinforced by oviposition, but frequent contact with scales can lead to habituation (Gardner and van Lenteren, 1986; Gardner *et al.*, 2007). On the other hand, *T. brassicae* showed associative learning in its responses to sex pheromones of *O. nubilalis* (Kaiser *et al.*, 1989). Associative learning is a method by which host cues can be used in searching (Vet and Dicke, 1992). In common field situations where maize is grown in rotation with other crops, *O. nubilalis* adults emerge and mate outside the maize crop where they oviposit (Buechi *et al.*, 1981; Derridj *et al.*, 1986; Cordillot, 1989). In that situation, *O. nubilalis* sex pheromone does not very well indicate host presence, and scales will be a more reliable host cue to indicate egg mass presence for *T. brassicae*.

In conclusion, we found that residence times of *T. brassicae* on maize are short, and only a small part of the whole plant is searched per visit. Although there is some movement between leaves by walking, we conclude that a leaf can be considered to form a patch for *T. brassicae*. The position of *O. nubilalis* egg masses on the plant or on the leaf does not influence their chance of being found by *T. brassicae*. The presence of host cues does increase host finding.

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