

Oviposition-induced plant cues: do they arrest *Trichogramma* wasps during host location?

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Abstract

Plants can defend themselves against herbivorous insects before the larvae hatch from eggs and start feeding. One of these preventive defence strategies is to produce plant volatiles, in response to egg deposition, which attract egg parasitoids that subsequently kill the herbivore eggs. Here, we studied whether egg deposition by *Pieris brassicae* L. (Lepidoptera: Pieridae) induces Brussels sprouts plants to produce cues that attract or arrest *Trichogramma brassicae* Bezdekó (Hymenoptera: Trichogrammatidae). Olfactometer bioassays revealed that odours from plants with eggs did not attract or arrest parasitoids. However, contact bioassays showed that *T. brassicae* females were arrested on egg-free leaf squares excised from leaves with 72 h-old egg masses, which are highly suitable for parasitisation. We tested the hypothesis that this arresting activity is due to scales and chemicals deposited by the butterflies during oviposition and which are thus present on the leaf surface in the vicinity of the eggs. Indeed, leaf squares excised from egg-free leaves, but contaminated with butterfly deposits, arrested the wasps when the squares were tested 1 day after contamination. However, squares from egg-free leaves with 72 h-old butterfly deposits had no arresting activity. Thus, we exclude that the arresting activity of the leaf area near 72 h-old egg masses was elicited by cues from scales and other butterfly deposits. We suggest that egg deposition of *P. brassicae* induces a change in the leaf surface chemicals in leaves with egg masses. A systemic induction extending to an egg-free leaf neighbouring an egg-carrying leaf could not be detected. Our data suggest that a local, oviposition-induced change of leaf surface chemicals arrests *T. brassicae* in the vicinity of host eggs.

Introduction

Trichogramma wasps are minute egg parasitoids of lepidopteran species. Because of their widespread application as biological control agents, their host selection behaviour has been intensively investigated (reviews by Noldus, 1989a; Wajnberg & Hassan, 1994). Host selection is divided into three steps: host-habitat location, host location, and host acceptance (Nordlund et al., 1988). Infochemicals play a major role during host selection by parasitic wasps in general, including *Trichogramma* (Vinson, 1976; Lewis & Martin, 1990; Nordlund, 1994; Schmidt, 1994).

In the process of host location, chemical cues from the host itself (i.e., kairomones) are usually exploited by the parasitoids (overview in Wajnberg & Hassan, 1994). Egg parasitoids such as *Trichogramma* spp. must cope with the problem of searching for an immobile and inactive host stage. Therefore, they often rely on infochemicals from other stages, such as the host adult, that are easier to detect and in addition give a high probability for the presence of the host eggs (i.e., infochemical detour) (Vet & Dicke, 1992). Non-volatile kairomones, such as wing scales from lepidopteran adult hosts, affect the searching behaviour of *Trichogramma* spp., resulting in increased chances of encountering host eggs (see review by Noldus, 1989a). In addition, volatile infochemicals, e.g., sex pheromones from calling moths, can have a kairomonal effect (see Boo & Yang, 2000; Reddy et al., 2002; Schöller & Prozell, 2002

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and references therein). The eggs themselves can be a direct source of volatile or contact kairomones as well (see Boo & Yang, 2000, and references therein).

Moreover, plants produce chemical stimuli that can mediate host habitat location behaviour in parasitic wasps. Feeding-induced plant volatiles exploited by larval parasitoids have already been studied (reviewed by Dicke, 1999). However, plants may not only respond to feeding by herbivores, but earlier to the oviposition by herbivorous insects, thus activating a preventive anti-herbivore defence strategy. For three tritrophic systems, oviposition by herbivorous insects was shown to induce a change in the emission of plant volatiles resulting in the attraction of egg parasitoids such as *Oomyzus gallerucae*, *Chrysonotomyia ruforum*, or *Trissolcus basalus* (Colazza et al., 2004a; reviewed by Hilker & Meiners, 2002). It has not yet been tested whether generalist egg parasitoids such as *Trichogramma* species respond to oviposition-induced changes in the plant's chemistry.

In the present study we investigated a tritrophic system consisting of Brussels sprouts (*Brassica oleracea* var. *gemmifera* cv. Cyrus), the large cabbage white butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae), and the egg parasitoid *Trichogramma brassicae* Bezdeko (Hymenoptera: Trichogrammatidae). Egg deposition by *P. brassicae* has been suggested to induce changes in plant surface chemistry of Brussels sprout plants (Blaakmeer et al., 1994a).

We hypothesized that the egg deposition by *P. brassicae* induces changes in the emission of cabbage leaf volatiles or in chemicals on the plant leaf surface that are exploited by *T. brassicae* to locate host eggs. First, the arrestment time of *T. brassicae* on cabbage leaves with egg masses was compared to the time spent on leaves without eggs. Further

bioassays addressed the question of whether the longer arrestment period of oviposition-experienced *T. brassicae* on egg-laden leaves was due to cues from the eggs or from butterfly deposits such as scales or butterfly odour adsorbed onto the surface. When it appeared that neither cues from eggs nor from butterfly deposits were necessary to arrest the wasps, we studied whether egg deposition induced a change of plant cues: (a) in the vicinity of egg masses (local induction), and/or (b) in egg-free leaves adjacent to egg-laden ones (systemic induction). Furthermore, the response of the wasps to volatiles from egg-laden leaves was tested in both dynamic and static olfactometer bioassays.

Material and methods

Plants and herbivores

Brussels sprout plants were reared in a greenhouse (18 ± 2 °C, 70% r.h., L16:D8). Plants of 8–12 weeks age, having ca. 14–16 leaves, were used for the rearing of *Pieris brassicae* and for the experiments. *Pieris brassicae* was reared on Brussels sprout plants in a climate room (21 ± 1 °C, 50–70% r.h., L16:D8). Each day a plant was placed for approximately 24 h into a large cage ($80 \times 100 \times 80$ cm) with more than 100 adults to allow egg deposition.

Parasitoids

Trichogramma brassicae (strain Y175) was reared in the eggs of *P. brassicae* over more than 60 generations (25 °C, 50–70% r.h., L16:D8). For the rearing, 1–3-day-old *P. brassicae* eggs on leaves were used. Initially mated, naïve wasps (no oviposition experience in eggs of *P. brassicae*) were used (contact bioassay no. 1a, see Table 1). All subsequent bioassays were done with mated, oviposition-

Experiment no.		Duration of leaf treatment (h)	Treatment
Contact bioassays			
Contact 1	a	48	Eggs (naïve wasps)
	b	48	Eggs
Contact 2		72	Eggs removed
Contact 3	a	24	Deposits
	b	72	Deposits
Contact 4	a	<12, 24, 48, 72, 96	Locally induced (short range)
	b	72	Locally induced (medium range)
Contact 5		72	Systemically induced
Contact 6		96	Synergistic effect
Olfactometer bioassays			
Y-tube		24–72	Egg-leaf vs. clean air (long range)
Two-chamber 1		72	Egg-leaf vs. clean air (short range)
Two-chamber 2		72	Egg-leaf vs. clean leaf (short range)

Table 1 An overview of the experiments with *Trichogramma brassicae* and the eggs of *Pieris brassicae*

experienced female wasps, because naïve wasps were shown to have a low response level. However, naïve wasps were also used for the host-age suitability tests. An oviposition experience was given for a period of 18 h prior to the experiment with 2–3-day-old *P. brassicae* eggs deposited on Brussels sprout leaves. The wasps were about 2–5 days old when tested. They were always provided with honey solution prior to the experiment.

Contact bioassays with leaf squares

General. For the experiments, the test plants were placed into a cage with more than 100 *P. brassicae* adults to allow deposition of eggs, wing scales, and host odours onto the plants. After this exposure to the butterflies, which generally lasted 8 h, the treated plants were tested immediately or were kept in a climate chamber (21 ± 2 °C, 70% r.h., L16:D8), either only overnight, or for another 24–72 h after the exposure day. Thus, the period during which eggs or butterfly deposits could affect and induce the cabbage plant was in total either less than 12, about 24, 48, 72, or 96 h (Table 1). Control plants were never in contact with *P. brassicae* nor any other insect. However, they were grown under the same abiotic conditions as the treated plants.

For the experiments, only turgid leaves were used. Two leaves of corresponding size and position relative to the topmost leaves were always used. Corresponding sections were cut from the control and the treated leaves, so that their size and structure were as similar as possible.

A wasp was released into the centre of a small glass Petri dish (5.5 cm diameter) lined with filter paper. The wasps were simultaneously offered a test and a control leaf square of 2 cm² cut from an excised leaf. The total length of time spent on the leaves (with antennal drumming during most of the time) was observed for a period of 5 min using The Observer software, version 3.0 (Noldus Information Technology, 1993[®], Wageningen, The Netherlands). The time spent searching in the area outside the leaf squares was scored as ‘no response’. The number of wasps per experiment ranged from 30 to 64. Test and control squares were changed after testing three wasps. Each wasp was used only once and was then discarded.

Bioassays (see Table 1). In contact experiment 1, indicated as ‘eggs’, a leaf square with an egg clutch (≤ 50 eggs) was tested against a ‘clean’ leaf square taken from an egg-free control plant, with either naïve *T. brassicae* females, or oviposition-experienced ones. The test leaf squares carried eggs that had been laid about 48 h prior to the experiment. All subsequent experiments were conducted with oviposition-experienced *T. brassicae* females. In contact

experiment 2, denoted by ‘eggs removed’, the egg mass laid on a test square was carefully removed with a brush just prior to the bioassay. Such a test square was tested against the ‘clean’ leaf square of an egg-free control plant. In contact experiment 3, denoted by ‘deposits’, leaf squares were cut from a plant that had never received eggs, but had been explored by butterflies, as indicated by wing scales being deposited on the leaves. Egg deposition on to the plants was prevented by covering the plants with gauze (2 mm² mesh size). Either ca. 24 h or ca. 72 h after exposure to the butterflies, test leaf squares were tested against ‘clean’ control squares. Control plants were covered equally for the same period, but were kept separate from the butterflies so that no butterfly odour or scales could be adsorbed. In contact experiment 4, denoted by ‘locally induced’, the response of *T. brassicae* to a section of leaf area in the vicinity of an egg mass was tested. Test leaf squares were taken either in the very close vicinity of an egg mass (short range), or about 5 cm away from an egg mass (medium range). When the test leaf squares had been taken from short range, the egg masses had been deposited about 12, 24, 48, 72, or 96 h prior to the bioassay. These test squares were tested against ‘clean’ leaf squares. When the test leaf squares had been taken from medium range, the eggs had been deposited about 72 h prior to the bioassay. In contact experiment 5, denoted as ‘systemically induced’, a plant, of which one leaf was covered by gauze (about 0.5 mm² mesh size) was placed into a butterfly cage for 8 h. The gauze prevented egg deposition on the covered leaf, whereas eggs could be laid on all the other leaves of the plant. After being exposed, about 1–5 egg masses per leaf had been laid onto the test plants. Leaves from control plants were also covered for the same period, but these plants were kept apart from butterflies. About 72 h after egg deposition on the test plants, squares were cut from the covered test leaves and from leaves of a control plant and were used in the bioassay. In contact experiment 6, denoted by ‘synergistic effect’, test leaf squares were taken from a leaf carrying eggs and damaged by larval feeding. On an egg-laden plant with 72-h-old eggs, ca. 50 first instar larvae were placed and allowed to feed for another 48 h. Squares cut from a leaf area without feeding damage and about 3–5 cm away from the egg mass were tested against leaf squares from ‘clean’ control plants.

Dynamic Y-tube olfactometer test

General. To test whether the wasps use volatiles to locate leaves carrying egg batches, two-choice bioassays were conducted in a Y-tube olfactometer. The olfactometer has been described by Takabayashi & Dicke (1992). It was made of glass (4 cm inner diameter; stem 6 cm, arms 10 cm;

stem-arm angle 60°) with each arm connected to a 2 l glass container holding the odour source. Air was filtered through activated charcoal, humidified, and split into two air streams that were fed through the glass containers to the olfactometer at a flow of 1 l min⁻¹ in each arm. At the end of the stem it was sucked through, at a rate of 2 l min⁻¹. All experiments were conducted at 20–25 °C and 50–60% r.h., using a light bulb above the olfactometer (Philips, The Netherlands, HPL Comfort, 50 W). A cardboard box (15 cm high × 40 cm wide) surrounded the Y-tube olfactometer to prevent light entering from the side. Oviposition-experienced wasps were individually released at the down-wind end of the Y-tube and observed for a maximum period of 5 min. If the wasp walked to the end of one of the olfactometer arms, this was recorded as a choice. If the wasp did not reach the end of either arm within 5 min, it was scored as 'no response'. All wasps were used only once. After every second wasp, the odour sources and apparatus were exchanged to avoid any possible asymmetry effects.

Bioassay. For the Y-tube-olfactometer bioassay, two leaves carrying on average three egg masses per leaf were excised from an egg-carrying plant and placed with their petiole in a vial with water. The age of the egg masses on these test leaves ranged from 24 to 72 h (see the egg infestation process described for the contact bioassays). The vial with leaves was transferred to a glass odour container in the olfactometer. This odour source was tested against clean air. About 20 female wasps were tested per leaf pair per day, for a total number of 80 wasps.

Static two-chamber olfactometer tests

General. To test whether *T. brassicae* wasps are arrested by volatiles from Brussels sprouts plants carrying *P. brassicae* egg masses, bioassays were conducted in a two-chamber static air-flow olfactometer (Figure 1), a slightly modified version of the four-chamber olfactometer described by Steidle & Schoeller (1997). The olfactometer consisted of a cylinder of acrylic glass (18 cm high × 12 cm in diameter) divided into two chambers by a vertical plate. No airflow was generated. A removable walking arena (2 cm high, 9 cm diam.) was placed on top of the cylinder consisting of plastic gauze (mesh 0.1 mm) with a plastic rim and covered with a glass plate. The experiments were carried out in the laboratory at 21 ± 1 °C using a fibre optic light source (Euromex Coldlight Illuminator EK-I, The Netherlands) above the olfactometer. Each oviposition-experienced wasp was individually released into the middle of the two chambers. The time spent by the wasps walking in one of the two odour fields was observed

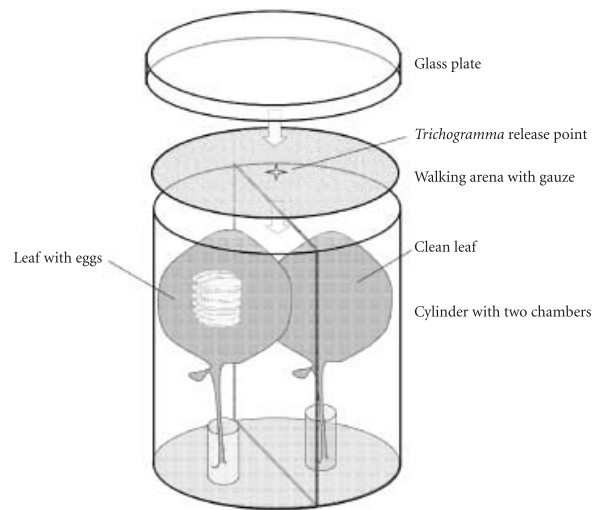


Figure 1 The static olfactometer with two odour fields.

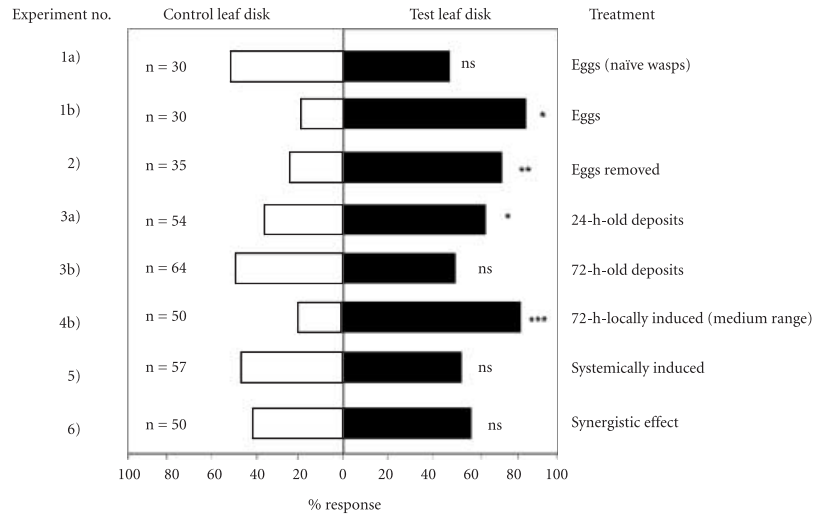
for 5 min. The entire cylinder was randomly rotated after every observation. Each leaf offered was kept with its petiole in a vial with water during the bioassay. Ten to 15 wasps were tested per day and plant. In total about 50 wasps were tested with 3–4 plants.

Bioassays. In static olfactometer bioassay no. 1, a leaf with 72-h-old egg masses of *P. brassicae* was placed in one chamber about 5 mm below the gauze, while the other chamber remained empty. Static olfactometer bioassay no. 2 was designed to examine whether *T. brassicae* was able to differentiate between close-range volatiles from egg-carrying leaves and those from egg-free leaves. Therefore, one chamber was supplied with an egg-carrying leaf (72 h-old eggs), while the other contained a leaf from a 'clean' egg-free plant. Both leaves were placed at a distance of about 5 mm below the gauze.

Host-age suitability tests

General. *Pieris brassicae* eggs of five different ages (<12, 24, 48, 72, and 96 h) were offered on a ca. 1 cm² excised piece of leaf from egg-carrying plants (see the egg infestation process described for the contact bioassays) to 1-day-old mated females of *T. brassicae* with no previous contact to host eggs. An egg clutch consisting of 15 eggs of the same age was offered for a period of 24 h to a female confined in a small glass vial. After that period, the wasp was removed from the vial. Eight females were tested per host age. When the eggs turned black (approximately 5 days after exposure to the parasitoids), the number of parasitized eggs was counted.

Figure 2 Response of *Trichogramma brassicae* females on differently treated cabbage leaf discs in a two-choice contact bioassay. The percentages of time spent on either the control or the test leaf disk is given. The number of tested females (n) is given on the left side of the bars. Experiment numbers correspond to the numbers given in Table 1. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant (Wilcoxon's matched pairs test).



Statistics

All contact bioassays and bioassays in the static two-chamber olfactometer were analysed using Wilcoxon's matched pairs signed rank test. A two-sided binomial test was used to analyse the choices in the Y-tube olfactometer. Parasitism rates for the different host ages were analysed with 5×2 contingency tables and individual χ^2 -tests were carried out, corrected by the sequential Bonferroni procedure for table-wide α levels (Rice, 1989). The response level of the wasps between the different treatments in contact bioassay no. 4a was analysed using Kruskal–Wallis ANOVA and subsequently compared with a Mann–Whitney U-test, corrected by the sequential Bonferroni procedure.

Results

Effects of host eggs and deposits

When leaf squares with or without eggs were offered, naïve wasps did not discriminate between them. Egg-laden leaf squares were not significantly more greatly explored by naïve wasps than 'clean' egg-free ones (Figure 2, no. 1a; $P = 0.98$, Wilcoxon's matched pairs signed rank test). The naïve wasps spent most of their time in the 'no choice' area (74%). However, oviposition-experienced wasps clearly preferred leaf squares with eggs deposited 48 h prior to the bioassay (Figure 2, no. 1b; $P = 0.04$, Wilcoxon's matched pairs test). Even when eggs had been removed from the leaf squares, experienced wasps remained significantly longer on such a square when compared to a leaf square which never had received eggs (Figure 2, no. 2; $P = 0.008$, Wilcoxon's matched pairs test). In order to elucidate whether cues from scales and other deposits of *P. brassicae* on the leaves affected *T. brassicae*, we tested leaf squares from plants that had been exposed to the butterflies, but were excluded from egg deposition. Leaf squares with fresh deposits (about

24 h old) significantly arrested the females of *T. brassicae* (Figure 2, no. 3a; $P = 0.03$, Wilcoxon's matched pairs test), whereas leaf squares with 72-h-old deposits did not (Figure 2, no. 3b; $P = 0.39$, Wilcoxon's matched pairs test).

Effect of contact plant cues from egg-carrying plants

In order to examine whether plant surface chemicals in the vicinity of an egg mass serve as cues that indicate the nearby host, leaf squares were excised right next to an egg mass and offered together with a leaf square from an egg-free 'clean' leaf (see Table 1, experiment no. 4a). The leaf square from a plant on which eggs had been deposited <12 h prior to the assay significantly arrested *T. brassicae* (Figure 3; $P < 0.001$, Wilcoxon's matched pairs test). The same behaviour was observed when leaf squares were offered from plants on which eggs had been deposited 24 h prior to the assay. The wasps were significantly arrested on the test square (Figure 3; $P < 0.001$, Wilcoxon's matched pairs test). Females of *T. brassicae* were not arrested on leaf squares cut from an egg-carrying leaf on which eggs had been deposited 48 h prior to the assay. However, they remained significantly longer on test leaf squares close to egg batches deposited 72 h prior to the bioassay (Figure 3; $P < 0.001$, Wilcoxon's matched pairs test). *Trichogramma brassicae* was not only arrested on a leaf area in close range of a 72-h-old egg mass, but also on a leaf area that had been excised about 5 cm away from 72-h-old egg batches (Figure 2, no. 4b; $P < 0.001$, Wilcoxon's matched pairs test). When squares from leaves with 4-day-old eggs were tested, *T. brassicae* still discriminated between the treated and the untreated leaf squares (Figure 3; $P < 0.012$, Wilcoxon's matched pairs test). Furthermore, when testing the leaf squares from leaves with about 72-h-old eggs against 'clean' squares, females of *T. brassicae* spent significantly less time in the 'no response' area than they did in the 24-h-old or

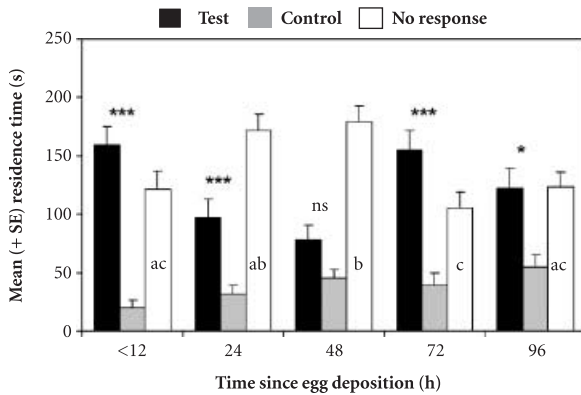


Figure 3 Response of *Trichogramma brassicae* females to ‘locally oviposition-induced’ leaf disks (test) from plants on which *Pieris brassicae* eggs had been deposited <12, 24, 48, 72, and 96 h prior to the bioassay. See Table 1, experiment 4a. Wasps were tested in a two-choice contact bioassay. Number of tested females per treatment, $n = 50$. Mean residence time and SE are shown. Asterisks indicate significant differences between test and control within the same treatment: * $P < 0.05$, *** $P < 0.001$, ns = not significant (Wilcoxon’s matched pairs signed rank test). Different letters indicate significant differences in the no response between the different treatments (Mann–Whitney U-test, Bonferroni corrected).

48-h-old treatments (Figure 3; $P < 0.001$, Mann–Whitney U-test, Bonferroni corrected). Squares taken from egg-free leaves neighbouring egg-laden ones did not arrest the wasps (Figure 2, no. 5; $P = 0.598$, Wilcoxon’s matched pairs test).

Effect of larval feeding in combination with egg masses

Leaves carrying eggs for 96 h and first instar larvae feeding for 48 h did not arrest females of *T. brassicae* (Figure 2, no. 6; $P = 0.097$, Wilcoxon’s matched pairs test).

Effect of volatile plant cues of egg-carrying plants

The wasps’ olfactory response to volatiles from leaves carrying eggs for 1–3 days was first tested in a dynamic Y-tube olfactometer. Oviposition-experienced females of *T. brassicae* were not attracted by the odour from an egg-carrying leaf, 46% of the 74 responding wasps chose the egg-carrying leaf ($P = 0.56$, binomial test). When testing the wasps’ response to volatiles from egg-carrying leaves at close range (<5 mm) in a static olfactometer, *T. brassicae* was not arrested by the odour from egg-carrying leaves. When offered against clean air, 45% of 48 tested wasps stayed longer above the egg-carrying leaf than above the control leaf ($P = 0.33$, Wilcoxon’s matched pairs test). When offered against ‘clean’, egg-free leaves, 49% of 51 tested wasps stayed longer above the egg-carrying leaf than above the control leaf ($P = 0.80$, Wilcoxon’s matched pairs test).

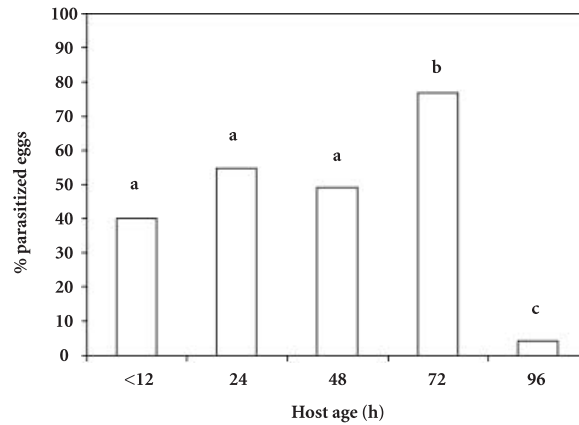


Figure 4 Host-age suitability of eggs from *Pieris brassicae* for *Trichogramma brassicae*. Fifteen eggs of different ages (<12 h, 24 h, 48 h, 72 h, or 96 h) were offered on leaf pieces to 1-day-old females for 24 h. Eight females was tested per egg age. Different letters in the columns indicate significant differences ($P < 0.05$) (5×2 contingency tables using χ^2).

Host-age suitability

The highest parasitism rate was found when *T. brassicae* was offered 72-h-old eggs. Host eggs of this age were significantly more frequently parasitized than the eggs of any other age tested (Figure 4; 5×2 contingency test, d.f. = 4, $P < 0.001$). Eggs that were 96 h old were unsuitable for the wasps, showing a low parasitization rate (Figure 4).

Discussion

Trichogramma brassicae was arrested on leaf areas excised from Brussels sprouts leaves in the vicinity of *P. brassicae* eggs. The factors causing this arrestment changed over the time after egg deposition had taken place. First, 24–48 h after egg deposition, butterfly deposits and eggs on the leaves were found to contribute to this arrestment. This was shown by the wasps’ response to egg-free leaves contaminated by butterfly deposits for 24 h and their response to leaves with eggs deposited for 48 h. Second, butterfly deposits were not arresting the wasps when tested 72 h after deposition. Nevertheless, a leaf area nearby 72-h-old host eggs had an arresting activity. Eggs of this age were found to be most suitable for parasitization (Figure 4). Taken together from these results, the following scenario can be deduced: (a) butterfly deposits and eggs were arresting the parasitoids up to 48 h after oviposition; (b) after 72 h, a different effect other than that of deposits or eggs operates, arresting the wasps in the vicinity of egg masses; (c) the parasitoids did not respond to volatiles from egg-laden leaves, suggesting an induction of arresting

leaf surface chemicals perceived after contact; (d) the induction of such leaf surface chemicals seems to be locally restricted, since the parasitoids were not arrested on leaf areas taken from egg-free leaves that were neighbouring 72-h-old egg-laden ones.

An alternative explanation for induction by 72-h-old egg masses could be that the substances associated with egg deposition have diffused into the leaf tissue or the leaf's wax layer. Yet another argument against the induction hypothesis could be that host females deposit a chemical trail around their egg masses. For example, females of *Ostrinia nubilalis* are known to release a secretion around the egg masses by sweeping their abdomen onto the leaf surface during egg deposition. *Trichogramma brassicae* was found to be arrested by this sweeping secretion of *O. nubilalis* (Garnier-Geoffroy et al., 1996). However, trail-marking behaviour by females of *Pieris brassicae* has never been observed.

Other studies have shown that oviposition by *P. brassicae* is capable of inducing defence responses in *Brassica* plants. Shapiro & DeVay (1987) showed that eggs of *P. brassicae* and *Pieris napi* (L.) laid on mustard leaves (*Brassica nigra*) may induce a hypersensitive response in the plant: 24 h after egg deposition, necrotic zones appeared around the eggs leading to their desiccation within 3 days. The plant's response was probably elicited by a substance in the glue attaching the eggs to the leaf. Rothschild & Schoonhoven (1977) demonstrated that *P. brassicae* avoids ovipositioning on Brussels sprout plants carrying conspecific eggs. This oviposition deterrence was shown to be due to compounds associated with the eggs (Klijnstra, 1986). Later studies by Blaakmeer and co-workers showed that none of the oviposition-deterrents, such as miriamides associated with the eggs of *P. brassicae* (Blaakmeer et al., 1994b), were detected on cabbage leaves from which eggs had been removed. Blaakmeer et al. (1994a) suggested that oviposition by *P. brassicae* induces changes at the level of individual leaves, resulting in a reduced acceptance of these leaves for further oviposition. Even egg-free leaves on a plant laden with eggs for 3–4 days on other leaves were observed to be less acceptable for oviposition by *P. brassicae* than leaves of similar age on a plant on which eggs had never been deposited (Blaakmeer et al., 1994a; J.J.A. van Loon, unpubl.), suggesting that changes in plant chemistry rather than cues produced by the ovipositing females or their eggs mediate the effects on conspecific *P. brassicae* females.

While other egg parasitoid species were shown to respond to oviposition-induced plant volatiles (reviewed by Hilker & Meiners, 2002), the *Trichogramma* species tested here did not. Egg deposition by *P. brassicae* on Brussels sprout plants might not have induced volatiles in those

quantities and qualities necessary to arrest the egg parasitoids for the particular cultivar studied and under the experimental conditions used. Headspace analyses could reveal whether egg-laden leaves emit volatile blends which are different from those emitted by egg-free leaves. Moreover, Noldus & van Lenteren (1983) demonstrated that *T. evanescens* was not attracted by volatiles from cabbage leaves infested with 24-h-old *P. brassicae* eggs and other deposits. Studies on the short distance dispersal ability of *Trichogramma* wasps have shown that the major means of movement of this minute organism are walking and short jumps, especially in low temperatures (Pak et al., 1985). A directed flight upwind towards a stimulus source over any significant distance is unlikely (Nordlund, 1994). Nevertheless, the responses of *Trichogramma* spp. to volatile plant infochemicals have often been reported (reviewed by Nordlund, 1994; Romeis et al., 1997). However, they were shown to arrest rather than attract the wasps. It is likely that *Trichogramma* spp. use plant cues after entering their habitat. They may reach the habitat/plant passively on wind currents. Another elegant solution to overcome this flight handicap is known for some *Telenomus* spp. These egg-parasitoids explore the sex pheromone of calling moths to locate and mount them and thereafter hitchhike along to the hosts' egg-laying sites (Arakaki et al., 1995, 1996). Recently, we were able to show that *T. brassicae* also uses this phoretic strategy to reach its hosts' eggs by detecting mated *P. brassicae* females via an anti-aphrodisiac produced by *Pieris* males (Fatouros et al., 2005). Such a strategy might render the use of oviposition-induced plant volatiles redundant and thus explain the parasitoids' inability to use induced plant volatiles during habitat location.

The arrestment response of *T. brassicae* to egg-induced leaf surface modifications contrasts with the behaviour of other egg parasitoids, which were shown to be attracted to local and systemic oviposition-induced plant volatiles (Meiners & Hilker, 2000; Hilker et al., 2002; Colazza et al., 2004b). When comparing the *Brassica*–*Pieris*–*Trichogramma* system with other tritrophic systems studied thus far with respect to the induction of plant volatiles by insect oviposition, two major differences are obvious (see Hilker & Meiners, 2002, for an overview of these systems). First, in contrast to the specialized egg parasitoids so far investigated, *Trichogramma* spp. are known to be fairly polyphagous, parasitizing a variety of mainly lepidopteran host eggs in association with different plant species (Noldus, 1989b; Babendreier et al., 2003). According to the concept of dietary specialization and infochemical use in natural enemies we expect them to respond to general plant volatiles rather than odours that are specific for a certain plant species (Steidle & van Loon, 2003; Vet & Dicke, 1992).

Indeed, Reddy et al. (2002) were able to show a positive response of *T. chilonis* to two green leaf volatiles, i.e., (Z)-3-hexenyl acetate and hexyl acetate, in an olfactometer. Since *T. brassicae* did not respond to any plant volatiles in our olfactometer test, we expect that egg deposition by *P. brassicae* on Brussels sprouts plants does not induce the release of general green leaf volatiles in such large amounts that *T. brassicae* becomes attracted. However, learning was shown for *Trichogramma* spp. Rearing the parasitoids on a particular host may be important in this respect (Kaiser et al., 1989). An early adult experience with the rearing host and an oviposition experience can influence the preference behaviour of *Trichogramma* wasps (Kaiser et al., 1989; Bjorksten & Hoffmann, 1995, 1998). Rearing *T. brassicae* for several generations in *P. brassicae* and giving them experience with *P. brassicae* eggs on cabbage leaves before each bioassay may have influenced them in their host location behaviour in a way that they begin using specific plant cues at close range.

Second, *P. brassicae* females do not damage the plant when laying eggs. The females just briefly drum on the leaf with their forelegs and tap on the leaf surface with their abdomen to determine its suitability for egg deposition (David & Gardiner, 1962). In other systems showing the attraction of egg-parasitoids to oviposition-induced plant volatiles, egg deposition was always associated with plant damage: (1) The elm leaf beetle removes the leaf epidermis prior to laying eggs at this site (Meiners & Hilker, 2000); (2) The pine sawfly slits a pine needle and lays its eggs into such slit needles (Hilker et al., 2002); and (3) *Nezara* bugs do not damage bean leaves before egg deposition, but egg parasitoids are only attracted to volatiles from leaves which have suffered both egg deposition and feeding damage (Colazza et al., 2004a). Our bioassays showed that even larval feeding associated with egg deposition does not induce the volatiles which attract *T. brassicae*.

In conclusion, our results suggest that the response of the generalist egg parasitoids to egg-laden plants differs from the reaction of the specialized wasps studied in other systems. While the latter are attracted by oviposition-induced plant volatiles (Wegener et al., 2001; Mumm et al., 2003; Colazza et al., 2004b), the generalist *T. brassicae* does not respond to volatiles from egg-laden leaves, but is instead arrested by their contact cues. The exact mechanism and the chemistry of these arresting infochemicals need to be elucidated in further studies.

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