Published in Agricultural and Forest Entomology 3, issue 3, 201-209, 2001
which should be used for any reference to this work

Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.)

Marco L. Bernasconi Ockroy†, Ted C. J. Turlings‡, Peter J. Edwards†, Maria Elena Fritzsche-Hoballah‡, Lara Ambrosetti, Paolo Bassetti§ & Silvia Dorn*

**Institute of Plant Sciences, Applied Entomology, Swiss Federal Institute of Technology (ETH), 8092 Zurich, †Institute of Geobotany, Swiss Federal Institute of Technology (ETH), 8044 Zurich, ‡Institute of Zoology, University of Neuchâtel, 2007 Neuchâtel, and §Swiss Federal Agricultural Research Station at Cadenazzo, 6593 Cadenazzo, Switzerland*

Abstract

1 In response to herbivore attack, maize plants (*Zea mays* L.) emit a specific blend of induced volatiles. Artificial damage and subsequent treatment of the damaged site with caterpillar regurgitant induces the same response. The induced volatile chemicals are known to be highly attractive to several parasitoids of herbivores in laboratory bioassays, but very limited information is available on how the plant odours affect entomophagous insects in the field.

2 Experiments were conducted to determine if induced maize volatiles attract parasitic and predatory insects under field conditions and whether they affect their spatial distribution.

3 In a preliminary field experiment with blue sticky traps near treated (damaged and treated with caterpillar regurgitant) and healthy plants, more entomophagous insects (total number of parasitic Hymenoptera, Anthocoridae and Syrphidae) were trapped near treated plants than near healthy plants.

4 In a second experiment, attraction to the induced volatiles was monitored with sticky traps placed next to treated and healthy maize plants in a regular maize field. No significant differences between the two treatments were found, but significantly more insects (parasitic wasps, thrips and anthocorid bugs) were trapped near to the top of plants than on traps placed near the mid-stem. Displacement of these insect groups within the field seemed to occur principally over the canopy, but under severe weather conditions they travelled lower in the canopy.

5 In a third experiment, the effect of induced maize odours on the spatial distribution of predators and parasitoids was investigated by placing sticky traps at different distances from healthy and treated plants. The higher catches of parasitoids near treated plants and the increased presence of these insects on the downwind side of treated plants support the notion that herbivore-induced maize odours attract natural enemies of maize pests in the field.

Keywords Anthocoridae, herbivore-induced volatiles, host location, parasitoids, synomones, Syrphidae, thrips, *Zea mays*.

Introduction

Price *et al.* (1980) and Vinson *et al.* (1987) were among the first to suggest that plants may actively recruit insect predators and parasitoids after they have been subjected to herbivore-attack. Since then, it has been found for several plants that herbivory induces the release of volatiles that are attractive to the natural enemies of the herbivores. This has been well studied for lima bean (Dicke *et al.*, 1990a, b; Takabayashi & Dicke, 1996), maize (Turlings *et al.*, 1990, 1998b), cotton (McCall *et al.*, 1993, 1994; Loughrin *et al.*, 1995b; Paré & Tumlinson, 1997a, b) and Brussel sprouts (Steinberg *et al.*, 1992; Mattiacci *et al.*, 1994, 1995; Geervliet *et al.*, 1996). In maize plants, caterpillar feeding induces the systemic release of volatile chemicals that are

Correspondence: T. C. J. Turlings, Institute of Zoology, University of Neuchâtel, Case postale 2, CH-2007 Neuchâtel, Switzerland. Tel.: + 41 32 7183158; fax: + 41 32 7183001; e-mail: ted.turlings@unine.ch

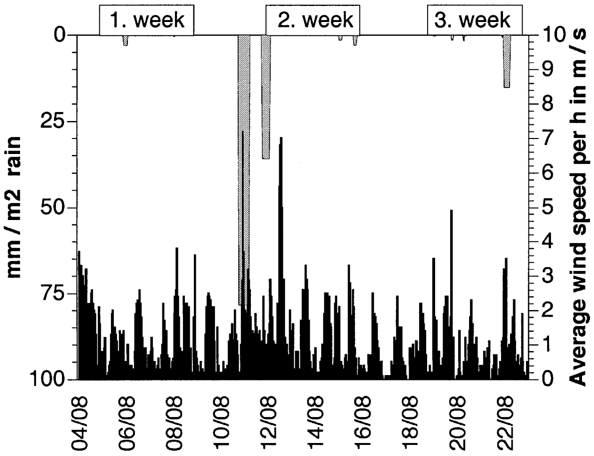

Figure 1 Weather conditions during the attraction experiment (unpublished data Swiss Meteorological Institute; SMI Zurich). Black bars at the bottom show the average wind speed per hour in m/s (scale at the right side). Grey bars from the top show the amount of rain per period of uninterrupted rainfall in m^{-2} (inverse scale at the left side). The three boxes at the top of the graph indicate the effective experimental periods. On the *x*-axis: 4–22 August 1996.

attractive to the parasitoids *Cotesia marginiventris* and *Microplitis croceipes* (Turlings *et al.*, 1990; Turlings & Tumlinson, 1992). Maize plants that are artificially damaged and subsequently treated with caterpillar regurgitant on the damaged sites produce the same blend of volatiles as plants damaged by the caterpillars themselves. Plants with only artificial damage (no regurgitant applied) do not emit these volatiles in significant amounts (Turlings et al., 1990). A factor in the regurgitant of *Spodoptera exigua* larvae that triggers the response in maize was isolated and identified as N-(17-hydroxylinolenoyl)-L-glutamine (Alborn *et al.*, 1997, 2000; Turlings *et al.*, 2000).

The fact that the volatiles are mainly produced in response to herbivore-specific factors appears to make them relatively reliable as foraging cues for predators and parasitoids (Vet & Dicke, 1992), but their significance in the attraction of natural enemies in the field is still poorly understood (Sabelis *et al.*, 1999). Drukker *et al.* (1995) found an increased presence of predators near cages with *Psylla*-infested pear trees. Similarly, Shimoda *et al.* (1997) found more predatory thrips on sticky traps near spider mite infested bean plants than on traps near healthy plants. However, in both these studies the herbivores were present on the experimental plants and an influence of signals originating directly from the herbivores could therefore not entirely be ruled out. On the other hand, laboratory studies have shown that the predators are readily attracted to herbivore-induced plant volatiles (Dicke *et al.*, 1990a, b; Takabayashi & Dicke, 1996; Scutareanu *et al.*, 1997; Sabelis *et al.*, 1999).

Qualitative variability in induced odour emissions seems to be largely determined by genetic variation among plants rather than the herbivores that feed on them (e.g. Takabayashi *et al.*, 1991; Turlings *et al.*, 1998a, b; Gouinguen *et al.*, 2001). Consequently, we speculate that most natural enemies of maize herbivores will be attracted to a common blend of induced volatiles. Such a general attraction may be reflected in a higher number of these natural enemies in the vicinity of plants emitting volatiles. The attraction may furthermore influence the general distribution of natural enemies in the field and cause large-scale spatial heterogeneity, which would be dependent on the prevailing wind direction.

This paper describes three different experiments designed to evaluate the effect of plant-born odours on common natural enemies of herbivores in maize fields. By artificially eliciting volatile production without the presence of the herbivore (Turlings *et al.*, 1998a), we ensured that there was no direct effect of the herbivores on natural enemy attraction and distribution.

Materials and methods

Study site

All experiments were carried out at the Swiss Federal Agricultural Research Station at Cadenazzo. The station is located in the southern part of Switzerland in the plain of Magadino (Canton of Ticino) at an altitude of 203 m above sea level. The plain of Magadino is one of the regions with the most intensive agriculture in Switzerland. The major crops are maize and tomatoes. The following herbivore pests in maize were observed during the experimental periods: the European corn borer *Ostrinia nubilalis* Hübner, aphid species *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabr.), and several thrips species. A preliminary experiment (circle experiment) was performed during summer 1995 from June until August. The main trials (attraction and distribution experiments) were carried out from August until the end of September of 1996. Insects were trapped on blue sticky traps measuring 25.7 × 10 cm with insect glue on both sides (Bio-pré, Langeraar, the Netherlands). Blue traps were chosen over yellow ones to avoid attracting yellow-sensitive insects to the treatments.

Recording of weather conditions

During the experimental periods of the attraction and the distribution experiment, weather conditions were recorded by the weather station ASTA Nr. 22 Locarno-Magadino (Swiss Meteorological Institute) (Figs 1 and 2). The station is located within 500 m of the experimental maize fields. Data for precipitation and wind-force were available in 1-h intervals.

Induction of volatile production

On the underside of three leaves, an area of 2 cm^2 was scratched with a scalpel on each side of the middle vein. Immediately after scratching the leaves, 10 µL of *Spodoptera littoralis* regurgitant was smeared on the damaged site of each leaf. In the case of multiple treatments, the same leaves, 2–3 cm down from old treatments were scratched again. Caterpillar regurgitant was collected from third- and fourth-instar larvae of *S. littoralis*, as described by Turlings *et al.* (1993), and kept at 5 °C for a maximum of 2 weeks. *Spodoptera littoralis* larvae were obtained weekly from CIBA Insect Control (Novartis, Basle,

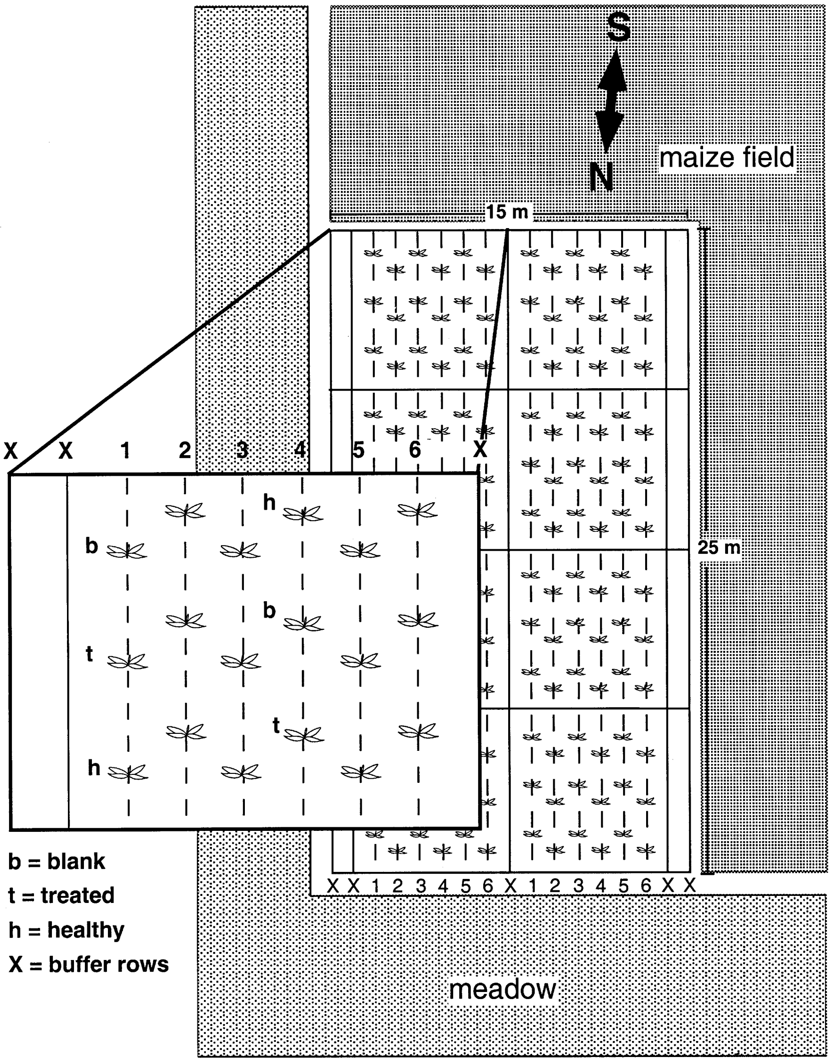

Figure 2 Situation plot of the field used for the attraction experiment with one enlarged block (for details see text).

Switzerland) and were maintained on an artificial diet based on wheat-shoots with added vitamins.

Preliminary 'circle' experiment

Zea mays L. cv. LG11 was grown in a greenhouse with natural light and no temperature or humidity control. Seeds were planted in pots (18 cm diameter) with local soil. The growing plants were fertilized weekly (1 L of 80.3 mg/L N, 34.4 mg/L P_2O_5 and 68.8 mg/K_2O). Three-week-old plants (nine-leaf stage) were used for experiments. Three circles (1.5 m diam), each with eight maize plants, were placed in the form of a triangle between two maize fields. In each circle, four treated plants were alternated with four healthy plants. Next to each plant, we placed a bamboo stick with a sticky trap at plant height.

Plants were treated at 09.00 hours every day for 3 days. Three leaves per plant were treated to induce volatile production (as described above). The traps were placed next to the plants on the first day of treatment and remained there until the day after the last treatment. We repeated the experiment seven times during seven subsequent weeks. Parallel to this experiment, using the same experimental set-up, we tested the repellent effect of the herbivore-induced volatiles on the corn leaf aphid *Rhopalosiphum maidis* (Fitch). In this experiment, on which we reported previously, laboratory-reared aphids were introduced into the three circles and were recovered in significantly higher numbers from healthy plants than from treated plants (Bernasconi *et al.*, 1998). After each treatment period, the traps were removed and placed into plastic freezer bags. the traps were stored at – 70 °C until the trapped insects could be identified. The numbers of insects trapped per week near healthy and treated plants were compared using Wilcoxon signed rank test ($\alpha = 0.05$).

Attraction experiment

A plot of 25 × 15 m^2 containing *Zea mays* L. cv. XR55 was selected. This plot was located in the corner of a larger maize field (Fig. 2). The northern and the eastern sides of the selected field were bordered by meadows, and no other maize fields were located within the next 100 m. The plants were distributed in 15 rows, each with approximately 130 plants of the same size. All the plants were between 2 m and 2.5 m tall and close to ear emergence.

The maize field was divided into eight blocks. Each block had six rows with approximately 30 plants per row. Two buffer rows were left at the edges of the field and one buffer row was left between the blocks (Fig. 2). All plants with recognizable *Ostrinia nubilalis* damage were removed before the experiment (about 5% of approximately 2000 plants in the field).

In each row of each block, three plants were selected and labelled (see Fig. 2 for the distribution of the selected plants). to ensure that the labelled plants were well separated from neighbouring plants, one plant on either side within the same row of each marked plant was removed. Next to each marked plant, we placed a bamboo stick of the same length as the plants. Three different treatments were chosen for this experiment: (1) 'healthy' plants were left unharmed; (2) 'treated' plants were scratched and treated with the regurgitant of *S. littoralis*; (3) control plants or 'blanks' were removed so that only the bamboo stick remained at its place. In each row of the eight blocks, the three treatments were distributed randomly over the three marked plants (Fig. 2). Three trials (each lasting 4 days) were carried out during three subsequent weeks from 5–22 August 1996. After each trial we removed the treated plants from the field.

For each of the three replicates, two rows per block were selected randomly. Beginning at 10.00 hours on the first day, selected plants were treated with regurgitant, 'healthy' plants were left unharmed, the plants that were selected as 'blanks' were removed from the field and bamboo sticks were placed near each of the three treatments.

At 14.00 hours on the same day, the traps were fixed to the sticks: one trap at the tip of the plants at ≈ 2 m (top) and one trap half-way along the length of the plants at ≈ 1.3 m (centre). We used two traps per plant to obtain information on the height at which the insects foraged. A total of 96 traps (32 per treatment) were placed in the field for each of the three trials. To ensure a consistently high level of volatile emissions, treatment of plants was repeated on the second and third day at 10.00 hours. At 14.00 hours on the fourth day, the traps were collected and placed in freezer bags. The traps were stored at – 70 °C until their evaluation.

Differences between treatments were analysed using ANOVA. The ANOVAs included the *treatment*, *position* of the traps (top or middle) and *week* as factors and all possible interactions between these factors. The log-transformed ($x' = \log_{10}(x + 1)$) data for numbers of thrips, parasitoids and anthocorid bugs were used as the dependent variables.

Distribution experiment

A maize field 140 × 60 m^2 with its long axis lying roughly north to south was selected (Fig. 3). The field was surrounded on three sides by meadows. On the southern side a small, seldom-used road separated the field from several plastic foil greenhouse tunnels. The field was planted with *Zea mays* L. cv. XR55. The entire field was divided into 14 blocks each 20 × 30 m. In the centre of each block, we selected and marked a group of four experimental plants of ≈ 1.5 m height. The blocks were arranged in a 2 × 2 design, with the two treatments ('healthy' and 'treated') in alternate positions (see Fig. 3). In the eastern and western directions (the prevailing wind directions in this location) of each marked group of four plants, bamboo sticks were placed at a distance of 1 m, 3 m and 10 m. In contrast to the previous experiment, no plants were removed from the field.

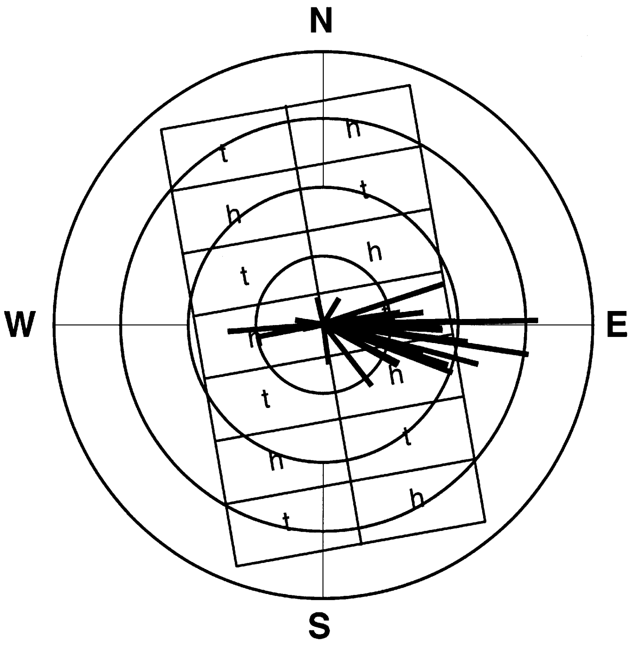

Figure 3 Plot of the wind conditions during the third (distribution) experiment. The lines indicate the average wind direction per hour within the wind rose. The overlaid square represents the maize field used in the third experiment with a 2 × 2 design alternating between 'healthy' (h) and 'treated' (t) blocks.

At 10.00 hours on the first day of the experiment, the four experimental plants in the centre of the 'treated' blocks were damaged on three leaves and *S. littoralis* regurgitant was applied to the damaged sites. Treatment of the plants was repeated in the morning of the second and third day to ensure a high, continuous level of volatile production. At 14.00 hours on the second day one sticky trap was fixed at each of the prepared bamboo sticks at approximately 1.5 m above ground level. A total of 84 traps were placed in the field. The traps were removed on the fifth day after initial treatment of the plants and handled in the same way as described for the previous experiments.

Because the predominant wind direction was east (Fig. 3), an attractive effect of the induced volatiles might yield more insects on the eastern traps of treated blocks. We therefore split the analysis of variance into east and west. The data were analysed by ANOVA, with *treatment* and *distance* as factors, and with all possible interactions between factors.

Exact species determination of trapped insects was not possible, because of the poor condition of the collected material. After a first assessment of the traps, we decided to distinguish between the most abundant insect groups. Traps were examined using a stereomicroscope and different insect groups were counted separately.

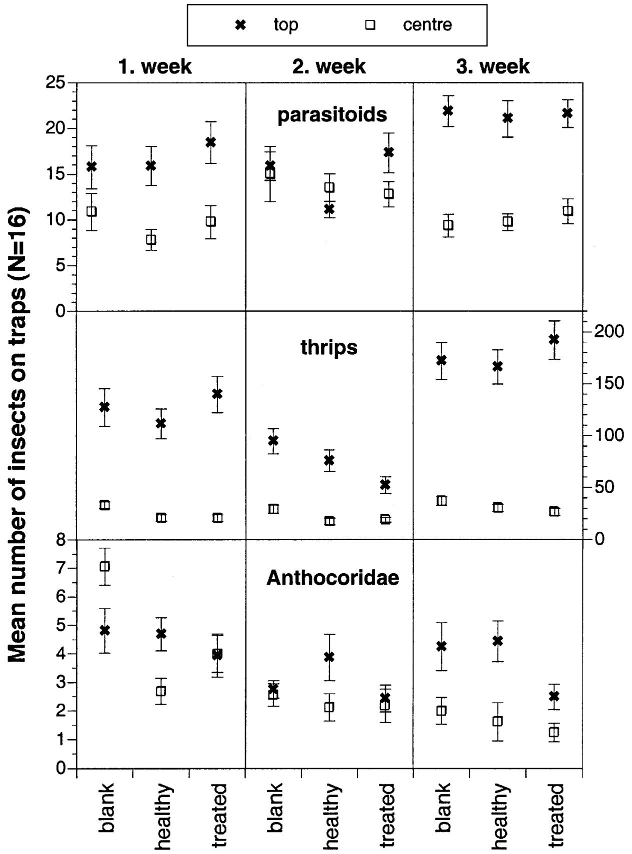

Figure 4 Mean number of insects recovered on traps in the attraction experiment. Symbols indicate mean number of insects on traps (blank, healthy, treated) per week separated by the position of the traps (top and centre) ± SEM. For statistical significant differences see Table 1.

Results

Preliminary circle experiment

In total, we recovered 586 (27.81 ± 7.33 SEM) entomophagous insects next to treated plants and 459 (21.85 ± 5.48 SEM) next to healthy plants. This difference was significant ($Z = 2.147$; $P = 0.0315$). The most abundant groups recovered on the traps were hymenopterous parasitoids (Hymenoptera: Terebrantes), hoverflies (Diptera: Syrphidae) and anthocorid bugs (Heteroptera: Anthocoridae). For each of these groups, more insects were found on traps near treated plants, but none of these differences were significant. For the parasitoids, 220 (31.4 ± 8.1 SEM) individuals were collected near treated plants and 169 (24.1 ± 6.5 SEM) near healthy plants ($Z = 1.775$; $P = 0.076$). For hoverflies, the numbers were 318 (45.4 ± 18.5 SEM) near treated plants and 263 (37.6 ± 12.7 SEM) near healthy plants ($Z = 0.93$; $P = 0.353$), and for anthocorid bugs 48 (6.6 ± 1.3 SEM) near treated plants and 27 (3.9 ± 1.3 SEM) near healthy plants ($Z = 1.363$; $P = 0.173$). Thrips (Thysanoptera) were recovered in relatively low numbers.

Attraction experiment

Over the three experimental periods the most abundant insects were: thrips with 21846 individuals, hymenopterous parasitoids with 4140 individuals, and anthocorid bugs with a total of 947 individuals. This time, hoverflies were recovered in low numbers.

Parasitoids

The total number of parasitoids recovered next to treated plants was higher than next to healthy plants, particularly in the second week (Fig. 4, Table 1), but again these differences were not statistically significant, neither for the top ($P = 0.13$) nor for the centre ($P = 0.75$) traps). We did find significant effects of week and position of the traps, as well as a highly significant interaction between these two parameters. For the first and third week (Fig. 4), the mean number of parasitoids was higher for all treatments on traps at the top of the plants than for those in the middle ($P < 0.0001$ and $P < 0.0001$, respectively). However, this was not obvious for the second week, where the mean was not significantly different for the two positions ($P = 0.397$). During that week, a decrease in catches on the top traps caused no overall decrease in the mean number of parasitoids, because it was accompanied by an increase of catches on the centre traps (Fig. 4). This may have been a direct consequence of the harsh weather conditions at the beginning of the second week (Fig. 1). The mean wind speed per hour and the number of events with high wind force were considerably higher during the second week. The increase of catches by the middle traps may have been caused by parasitoids moving into the shelter of the plants to avoid harsh wind conditions. The 10 h thunderstorm (110.4 L/m^2 rain; gusts of wind up to 15 m/s) the day before the start of the second-week experiment had no detectable effect on the population of parasitoids within the field.

Thrips

For thrips catches, we found a significant effect of treatment, week and position of the traps with a significant interaction between week and position (Fig. 4, Table 1). Treatment differences were due to significantly higher catches on the 'blanks' in the case of centre traps, but not on the top traps. For thrips, as for parasitoids (Fig. 4), there was a decrease in the mean number recovered on top traps during the second week (Fig. 4), which explains the effect of week and the interaction between week and position. This decrease was again probably caused by the harsher weather conditions in the beginning of the second week (Fig. 4).

Anthocorid bugs

Once more, a significant effect of the treatment, week and position was found, with significant interactions between treatment and position and position and week (Fig. 4, Table 1). In the first week, we recovered significantly more bugs on traps near 'blanks' than on traps near 'healthy' and 'treated' ($P = 0.017$ and $P = 0.041$, respectively). The significant effect of the week is due to a decrease of catches in the

Table 1 Insects of traps in the attraction experiment: analysis of variance of the log-transformed data (x = log10 (x + 1)) with the effects of treatment, week and position. SS = sum of squares

Source	d.f.	SS	*F*	*P*
Parasitoids				
Treatment	2	0.147	1.565	0.21
Week	2	0.549	5.857	0.003
Treatment*week	4	0.066	0.350	0.84
Position	1	2.944	62.780	< 0.0001
Treatment*position	2	0.054	0.571	0.57
Week*position	2	1.114	11.882	< 0.0001
Treatment*week*position	4	0.244	1.303	0.27
Residual	270	12.661		
Thrips				
Treatment	2	0.924	7.634	0.001
Week	2	3.913	32.321	< 0.001
Treatment*week	4	0.327	1.351	0.25
Position	1	30.518	504.140	< 0.0001
Treatment*position	2	0.281	2.320	0.10
Week*position	2	0.901	7.442	0.001
Treatment*week*position	4	0.570	2.354	0.054
Residual	270	16.345		
Anthocoridae				
Treatment	2	0.708	5.340	0.01
Week	2	2.555	19.284	< 0.0001
Treatment*week	4	0.302	1.138	0.34
Position	1	1.097	16.565	< 0.0001
Treatment*position	2	0.716	5.404	0.01
Week*position	2	0.951	7.178	0.001
Treatment*week*position	4	0.223	0.842	0.50
Residual	280	17.886		

second and the third week compared to the first week ($P < 0.0001$ for both comparisons). The anthocorids were almost equally distributed over the traps at the top and the centre of the plants, with an exception for the third week, where the catches on ‘top’ traps were significantly higher than on ‘centre’ traps ($P < 0.0001$) (Fig. 4).

Distribution experiment

The most abundant insect group recovered on the traps in this experiment were hymenopterous parasitoids, whereas thrips, hoverflies and anthocorid bugs occurred in rather low numbers. A total of 224 (5.62 ± 0.64 SEM) parasitic wasps were found on traps in blocks with treated plants and 186 (4.14 ± 0.43 SEM) were recovered on traps in blocks with healthy plants. The overall difference between treated and healthy was marginally non-significant (Table 2). No significant differences were observed between the traps at the three distances 1m, 3m and 10 m, neither in the blocks of healthy plants nor in the blocks of treated plants. However, we found a significant interaction between the treatment and direction parameters (Table 2). By distinguishing between east and west (Fig. 5), significantly higher numbers were found in treated blocks compared to healthy blocks on the downwind (eastern) side ($P = 0.0064$). On the upwind (western) side, the difference between treated blocks and healthy blocks was not significant ($P = 0.852$). As the wind was predominantly in eastern direction (Fig. 3), it is likely that the insects perceived the odours mostly on that side of the treated plants.

Discussion

An efficient ability to locate a host habitat and the host within the habitat is crucial for parasitoids. Various olfactory, visual and vibrational cues are utilized by foraging parasitoids to find a host (Vinson, 1981, 1998; Nordlund *et al*., 1988; Vet & Dicke, 1992; Wäckers & Lewis, 1994; Meyhöfer *et al*., 1994; Wäckers *et al*., 1998). In the case of phytophagous hosts, plants on which their hosts feed are often the source of volatile cues (reviewed by Vinson, 1981, 1984; Vinson *et al*., 1987; Nordlund *et al*., 1988; Whitman, 1988; Williams *et al*., 1988). In fact, plants appear to be actively involved in the process of host location. Under herbivore attack, several plants emit specific volatiles that are attractive to predators and parasitoids of the herbivore (Vet & Dicke, 1992; Turlings & Benrey, 1998). The effects of these plant-produced semiochemicals on natural enemies of herbivores have been demonstrated mostly in the artificial environments of olfactometers and wind tunnels, and remain to be confirmed under field conditions.

Few experiments support the evidence from the laboratory that entomophagous insects are attracted to odours emitted by herbivore-infested plants under field conditions. The predatory *Scolothrips takahashii* was attracted to lima bean infested by spider mites both under field and laboratory conditions (Shimoda *et al*., 1997). Similarly, Drukker *et al*. (1995) found that predatory anthocorid bugs were attracted to *Psylla*-infested pear trees under field conditions. A direct influence of the prey present on the attractive plants could not be excluded in these experiments. However, Scutareanu *et al*. (1997) confirmed in the laboratory that *Psylla*-infested pear trees produce significantly different volatiles compared to healthy uninfested plants and that the production of volatiles depends on the density of herbivores. De Moraes *et al*. (1998) directly observed attraction of the specialist parasitoid *Cardiochiles nigriceps* to plants damaged by its specific host, while Thaler (1999) found increased parasitization rates in tomato fields that had been sprayed with jasmonic acid (which induces odour emissions). Here, too, volatile emissions were induced artificially and therefore effects from the herbivores (e.g. frass, exuviae, pheromones) can be excluded.

Our circle and attraction experiments provide only limited evidence that parasitoids and predators are attracted to the herbivore-induced odours under field conditions. The total number of entomophagous insects recovered in the first experiment was significantly higher for traps near treated plants than for traps near healthy plants, but this difference was not significant for the separate insect groups. In the second experiment, a slight but not significant preference for treated plants was found for parasitoids. This was most pronounced for the second week, especially on traps at the top of the plants. Weather conditions during the experimental period may have affected the distribution of the insects. In the second week of the attraction experiment especially, a significant overall decrease of the populations of thrips and anthocorid bugs, but not for

Table 2 Spatial distribution of parasitoids in the distribution experiment: analysis of variance of the effects treatment, direction (west/east) and distance. SS = sum of squares

Source	d.f.	SS	*F*	*P*
Treatment	1	45.762	3.962	0.0503
Direction	1	17.190	1.488	0.23
Treatment*direction	1	58.333	5.050	0.028
Distance	2	52.310	2.264	0.11
Treatment*distance	2	11.452	0.496	0.61
Direction*distance	2	0.167	0.007	0.99
Treatment*direction*distance	2	43.881	1.899	0.16
Residual	72	831.714		

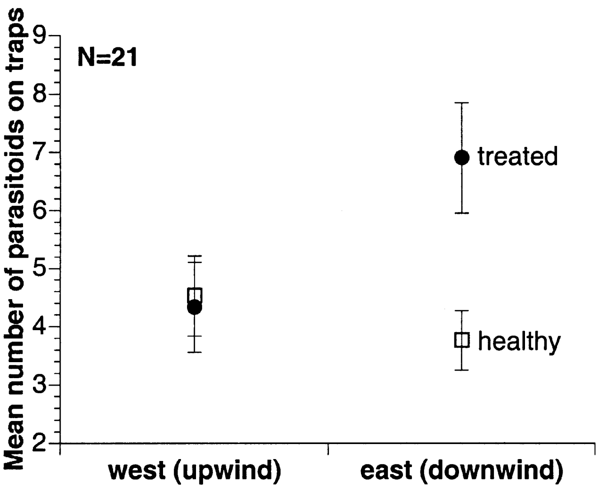

Figure 5 Mean number of parasitic wasps recovered on traps in the distribution experiment. Symbols indicate mean number of parasitoids on traps (healthy, treated) separated by east (downwind) and west (upwind) ± SEM. For statistically significant differences see Table 2.

parasitoids, was observed. This was probably caused by the heavy rain the day before the experiment and the wind conditions during the experiment.

Limited information is available on the effect of wind conditions on the foraging behaviour of parasitoids. Casas (1989) found that the eulophid wasp *Sympiesis sericeicornis* Nees stopped search flights around the canopy of apple trees when wind speeds exceeded 2m/s. We calculated for each week in the attraction experiment the number of hours with an average wind speed > 2m/s during the activity period of the parasitoids (19.00–21.00 hours). In the second week, we found more hours with high wind speed (23 h) than in the first (13 h) and the third (6 h) weeks. During the second week of our attraction experiment, the high wind speed may have hindered the parasitoids from searching and forced them into the shelter of the maize plants. This was reflected in the decrease of catches on traps on the top and an increase on centre traps.

For the anthocorid bugs there was no consistent difference between the treatments, although this insect group also seemed to be affected by the weather conditions. The foraging behaviour of the anthocorid bugs may have been influenced by two additional factors: the presence of high numbers of thrips and large quantities of pollen that fell from the panicles on the maize leaves during the experiment. Both thrips and pollen are major food sources for *Orius* species (Carnero *et al.*, 1993).

In the circle experiment, we found a deterrent effect of induced volatiles on the corn leaf aphid *Rhopalosiphum maidis* released in the centre of each circle, on which we reported previously (Bernasconi *et al.*, 1998). Significantly more aphids were recovered on healthy plants as compared to treated plants. Syrphidae and anthocorid bugs, as well as many parasitoids, are natural enemies of aphids. Possibly, the higher density of aphids on healthy plants caused a shift of part of the foraging predators and parasitoids away from treated plants to healthy ones. In the attraction experiment no aphids were released in the field, but large numbers of herbivores were recovered from traps during the entire experiment. During the first week, we recovered large numbers of aphids on the sticky traps, far more than during the second and the third weeks. The corn leaf aphid, which is native to the area, does not induce volatile production in maize (Turlings *et al.*, 1998b). However, studies with other aphid species and host plants do present evidence for aphid-induced plant volatiles (Guerrieri *et al.*, 1993; Micha & Wyss, 1995; Du *et al.*, 1996; Powell *et al.*, 1998). Furthermore, there is evidence that infestation by thrips leads to a production of induced-volatile chemicals in plants (Wien & Roesingh, 1980; Pallini *et al.*, 1997). The large numbers of thrips and aphids may therefore have 'diluted' the effect of artificially induced volatiles on their parasitoids and predators. In the distribution experiment, we recovered very low numbers of herbivores on the traps, which indicates low populations of these insects in the experimental field, and perhaps a less important effect on the distribution of their natural enemies.

In contrast to the circle and attraction experiments, a clear significant difference between catches on traps associated with treated plants and healthy plants was observed in the distribution experiment. Significantly more parasitic wasps were trapped on the downwind side of treated plants, which suggested that parasitoids are also able to detect odour plumes originating from treated plants in a field. Eller *et al.* (1988) describe how parasitoids following an odorous signal upwind in a flight tunnel fly back in a 'downwind loop' when they loose contact with the odour until they detect the signal again. Such behaviour should lead to retaining the insects downwind from the odour source (i.e. treated plants). Recently we found that older plants, such as the ones we used in the field experiments, release relatively little in comparison with young plants (Gouinguené & Turlings, unpublished data). It can therefore be expected that the distribution of natural enemies will be more affected by induced odours in fields with young maize.

The blue sticky traps may have been attractive to parasitoids and predators. The positive response to blue colour is believed to occur in the case of dispersal flight rather than being host-directed searching and may tend to guide the insects through open spaces in vegetation and upward towards the sky, as in dispersal flight of aphids (Kennedy *et al.*, 1961). There is no consistent information available on the effect of blue colour on parasitic wasps and predators because of a high variation between species. The examples range from a clear attractive effect (e.g. Weseloh, 1972; McClain *et al.*, 1990) to non-attractiveness (e.g. Dowell & Cherry, 1981; Capinera & Walmsley, 1978; Vargas *et al.*, 1991). However, we cannot rule out that some of the species in our experiment were actually attracted to the blue sticky traps. This is certainly true for thrips; blue sticky traps are specifically designed to monitor thrips populations.

In summary, more natural enemies were recovered near treated plants compared to healthy plants and herbivore-induced maize odours affected the distribution of parasitoids in the field. These results support the hypothesis that herbivore-induced volatiles attract a broad range of parasitoids and predators. The significantly higher catches of parasitoids on the downwind side of treated plants suggest that parasitoids foraging in a field are able to follow odour plumes that originate from a herbivore-infested plant. Therefore, wind direction should be taken into account in pest management strategies that involve the release of biological control agents.

Acknowledgements

We thank Kathrin Ockroy, Urs Lengwiler, Felix Wäckers and Tracey Webb for their constructive comments on earlier versions of the manuscript and the Swiss Meteorological Institute (SMI, Zurich) for providing unpublished data from the station Locarno-Magadino. We are grateful to P. Stoecklin, CIBA (NOVARTIS) Basle, for the weekly supply of *Spodoptera littoralis*. Financial support for this project was provided by CIBA (Novartis, Basle) and by the Swiss National Science Foundation (grant no. 31-044459.95 and 31-46237.95 to T.C.J.T.)

References

Alborn, H.T., Jones, T.H., Stenhagen, G. & Tumlinson, J.H. (2000) Identification and synthesis of volicitin and related components from beet armyworm oral secretions. *Journal of Chemical Ecology*, **26**, 203–220.

Alborn, H.T., Turlings, T.C.J., Jones, T.H., Stenhagen, G., Loughrin, J.H. & Tumlinson, J.H. (1997) An elicitor of plant volatiles from beet army worm oral secretion. *Science*, **276**, 945–949.

Bernasconi, M.L., Turlings, T.C.J., Ambrosetti, L., Bassetti, P. & Dorn, S. (1998) Herbivore-induced emissions of maize volatiles repel the corn-leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, **87**, 133–142.

Capinera, J.L. & Walmsley, M.R. (1978) Visual response of some sugarbeet insects to sticky traps and water pan traps of various colors. *Journal of Economic Entomology*, **71**, 926–927.

Carnero, A., Peña, M.A., Pérez-Padrón, F., Garrido, C. & Hernández, M.G. (1993) Bionomics of *Orius albipennis* and *Orius limbatus*. *IOBC WPRS Bulletin*, **16**, 26–30.

Casas, J. (1989) Foraging behaviour of a leafminer parasitoid in the field. *Ecological Entomology*, **14**, 257–267.

De Moraes, C.M., Lewis, W.J., Paré, P.W. & Tumlinson, J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature*, **393**, 570–573.

Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J. & Posthumus, M.A. (1990b) Plant strategies of manipulating predator prey interactions through allelochemicals: prospects for application in pest control. *Journal of Chemical Ecology*, **16**, 3091–3118.

Dicke, M., van Beek, T., Posthumus, M.A., Ben Dom, N., van Bokhoven, H. & de Groot, A.E. (1990a) Isolation and identification of volatile kairomone that affects acarine predator–prey interactions: Involvement of host plant in its production. *Journal of Chemical Ecology*, **16**, 381–396.

Dowell, R.V. & Cherry, R.H. (1981) Survey traps for parasitoids, and coccinellid predators of the citrus blackfly, *Aleurocanthus woglumi*. *Entomologia Experimentalis et Applicata*, **29**, 356–362.

Drukker, B., Scutareanu, P. & Sabelis, M.W. (1995) Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? *Entomologia Experimentalis et Applicata*, **77**, 193–203.

Du, Y.-J., Poppy, G.M. & Powell, W. (1996) Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. *Journal of Chemical Ecology*, **22**, 1591–1605.

Eller, F.J., Tumlinson, J.H. & Lewis, W.J. (1988) Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host-location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environmental Entomology*, **17**, 745–753.

Geervliet, J.B.F., Vet, L.E.M. & Dicke, M. (1996) Innate response of the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. *Journal of Insect Behavior*, **9**, 525–538.

Gouinguené, S., Degen, T. & Turlings, T.C.J. (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9–16.

Guerrieri, E., Pennacchio, F. & Tremblay, E. (1993) Flight behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in response to plant and host volatiles. *European Journal of Entomology*, **90**, 415–421.

Kennedy, J.S., Booth, C.O. & Kershaw, J.S. (1961) Host finding by aphids in the field. III. Visual attraction. *Annals of Applied Biology*, **49**, 1–21.

Loughrin, J.H., Manukian, A., Heath, R.R., Turlings, T.C.J. & Tumlinson, J.H. (1995b) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *Journal of Chemical Ecology*, **21**, 1217–1227.

Mattiacci, L., Dicke, M. & Posthumus, M.A. (1994) Induction of parasitoid attracting synomone in Brussel sprouts plants by feeding of *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. *Journal of Chemical Ecology*, **20**, 2229–2247.

Mattiacci, L., Dicke, M. & Posthumus, M.A. (1995) Beta-glucosidase: an elicitor of herbivore-induced plant odor that attracts host searching parasitic wasps. *Proceedings of the National Academy of Sciences of the U.S.A.*, **92**, 2036–2040.

McCall, P.J., Turlings, T.C.J., Lewis, W.J. & Tumlinson, J.H. (1993) Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera). *Journal of Insect Behavior*, **6**, 625–639.

McCall, P.J., Turlings, T.C.J., Loughrin, J.H., Proveaux, A.T. & Tumlinson, J.H. (1994) Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *Journal of Chemical Ecology*, **20**, 3039–3050.

McClain, D.C., Rock, G.C. & Woolley, J.B. (1990) Influence of trap color and san Jose scale (Homoptera: Diaspididae) pheromone on sticky trap catches of 10 aphelinid parasitoids (Hymenoptera). *Environmental Entomology*, **19**, 926–931.

Meyhöfer, R., Casas, J. & Dorn, S. (1994) Vibration mediated interactions in a host-parasitoid system. *Proceedings of the Royal Society London*, **264**, 261–266.

Micha, S.G. & Wyss, U. (1995) The importance of plant odours for the host search *of Aphidius uzbekistanus* (Hymenoptera, Aphidiidae), A parasitoid of the grain aphid (*Sitobion avenae*). *Gesunde Pflanzen*, **47**, 300–307(in German).

Nordlund, D.A., Lewis, W.J. & Altieri, M.A. (1988) Influence of plant-produced allelochemicals on the host/prey selection behavior of entomophagous insects. *Novel Aspects of Insect–Plant Interactions* (ed. by P. Barbosa and D. K. Letourneau), pp. 65–90. John Wiley, New York.

Pallini, A., Janssen, A. & Sabelis, M.W. (1997) Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. *Oecologia*, **110**, 179–185.

Paré, P.W. & Tumlinson, J.H. (1997a) Induced synthesis of plant volatiles. *Nature*, **385**, 30–31.

Paré, P.W. & Tumlinson, J.H. (1997b) De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology*, **114**, 1161–1167.

Powell, W., Pennacchio, F., Poppy, G.M. & Tremblay, E. (1998) Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control*, **11**, 104–112.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weiss, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.

Sabelis, M., Janssen, A., Pallini, A., Venzon, M., Bruin, J., Drukker, B. & Scutareanu, P. (1999) Behavioral responses of predatory and herbivorous arthropods to induced plant volatiles: from evolutionary ecology to agricultural application. *Induced Plant Defenses Against Pathogens and Herbivores* (ed. by A. A. Agrawal, S. Tuzun and E. Bent), pp. 269–296. APS Press, St. Paul.

Scutareanu, P., Drukker, B., Posthumus, B.J. & Sabelis, M.W. (1997) Volatiles from *Psylla* infested pear trees and their possible involvement in attraction of anthocorid predators. *Journal of Chemical Ecology*, **23**, 2241–2260.

Shimoda, T., Takabayashi, J., Ashira, W. & Takafuji, A. (1997) Response of predatory insect *Scolothrips takahashi* towards herbivore induced plant volatiles under laboratory and field conditions. *Journal of Chemical Ecology*, **23**, 2033–2048.

Steinberg, S., Dicke, M., Vet, L.E.M. & Wanningen, R. (1992) Response of the braconid parasitoid *Cotesia* (*=Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age, experience and barometric flux. *Entomologia Experimentalis et Applicata*, **63**, 163–175.

Takabayashi, J. & Dicke, M. (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Sciences*, **1**, 109–113.

Takabayashi. J., Dicke, M. & Posthumus, M. (1991) Variation in composition of predator- attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. *Chemoecology*, **2**, 1–6.

Thaler, J.S. (1999) Jasmonate-inducible plant defenses cause increased parasitism of herbivores. *Nature*, **399**, 686–688.

Turlings, T.C.J., Alborn, H.T., Loughrin, J.H. & Tumlinson, J.H. (2000) Volicitin, an elicitor of maize volatiles in the oral secretion of *Spodoptera exigua*: isolation and bio-activity. *Journal of Chemical Ecology*, **26**, 189–202.

Turlings, T.C.J. & Benrey, B. (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Écoscience*, **5**, 321–333.

Turlings, T.C.J., Bernasconi, M.L., Bertossa, R., Bigler, F., Caloz, G. & Dorn, S. (1998b) The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological Control*, **11**, 122–129.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M. & Wechsler, D. (1998a) Timing of induced volatile emissions in maize seedlings. *Planta*, **207**, 146–152.

Turlings, T.C.J., McCall, P.J., Alborn, H.T. & Tumlinson, J.H. (1993) An elicitor in caterpillar oral secretion that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology*, **19**, 411–425.

Turlings, T.C.J. & Tumlinson, J.H. (1992) Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Sciences, U.S.A.*, **89**, 8399–8402.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.

Vargas, R.I., Stark, J.D., Prokopy, R.J. & Green, T.A. (1991) Response of oriental fruit fly (Diptera: Tephritidae) and associated parasitoids (Hymenoptera: Braconidae) to different-color spheres. *Journal of Economic Entomology*, **84**, 1503–1507.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–172.

Vinson, S.B. (1981) Habitat location. *Semiochemicals: Their Role in Pest Control* (ed. by D. A. Nordlund, R. L. Jones and W. J. Lewis), pp. 51–77. John Wiley, New York.

Vinson, S.B. (1984) Parasitoid-host relationship. *Chemical Ecology of Insects* (ed. by W. J. Bell and R. T. Cardé), pp. 111–124. Sinauer Associates, Massachusetts.

Vinson, S.B. (1998) The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control*, **11**, 79–96.

Vinson, S.B., Elzen, G.W. & Williams, H.J. (1987) The influence of volatile plant allelochemicals on the third trophic level (parasitoids) and their herbivorous hosts. *Insects-Plants* (ed. by V. Labeyerie, G. Fabres and D. Lachaise), pp. 109–114. Junk Publishers, Dordrecht.

Wäckers, F.L. & Lewis, W.J. (1994) Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes*. *Biological Control*, **4**, 105–112.

Wäckers, F.L., Mitter, E. & Dorn, S. (1998) Vibrational sounding by the pupal parasitoid *Pimpla (Coccygominus) turionellae*: an additional solution to the reliability-detectability problem. *Biological Control*, **11**, 141–146.

Weseloh, R.M. (1972) Field response of gypsy moths and some parasitoids to colored surfaces. *Annals of the Entomological Society of America*, **65**, 742–746.

Whitman, D.W. (1988) Allelochemical interactions among plants, herbivores, and their predators. *Novel Aspects of Insect–Plant Interactions* (ed. by P. Barbosa and D. Letourneau), pp. 11–63. Wiley, New York.

Wien, H.C. & Roesingh, C. (1980) Ethylene evolution by thrips-infested cowpea provides a basis for thrips resistance screening with ethephon sprays. *Nature*, **283**, 192–194.

Williams, H.J., Elzen, G.W. & Vinson, S.B. (1988) Parasitoid host plant interactions, emphasizing cotton (Gossypium). *Novel Aspects of Insect–Plant Interactions* (ed. by P. Barbosa and D. Letourneau), pp. 171–200. Wiley, New York.