Strong Attraction of the Parasitoid *Cotesia marginiventris* Towards Minor Volatile Compounds of Maize

**Marco D'Alessandro · Virginie Brunner ·
Georg von Mérey · Ted C. J. Turlings**

Abstract Plants infested with herbivorous arthropods emit complex blends of volatile compounds, which are used by several natural enemies as foraging cues. Despite detailed knowledge on the composition and amount of the emitted volatiles in many plant-herbivore systems, it remains largely unknown which compounds are essential for the attraction of natural enemies. In this study, we used a combination of different fractionation methods and olfactometer bioassays in order to examine the attractiveness of different compositions of volatile blends to females of the parasitoid *Cotesia marginiventris*. In a first step, we passed a volatile blend emitted by *Spodoptera littoralis* infested maize seedlings over a silica-containing filter tube and subsequently desorbed the volatiles that were retained by the silica filter (silica extract). The volatiles that broke through the silica filter were collected on and subsequently desorbed from a SuperQ filter (breakthrough). The silica extract was highly attractive to the wasps, whereas the breakthrough volatiles were not attractive. The silica extract was even more attractive than the extract that contained all herbivore-induced maize volatiles. Subsequently, we fractioned the silica extract by preparative gas-chromatography (GC) and by separating more polar from less polar compounds. In general, *C. marginiventris* preferred polar over non-polar compounds, but several fractions were attractive to the wasp, including one that contained

compounds emitted in quantities below the detection threshold of the GC analysis. These results imply that the attractiveness of the volatile blend emitted by *Spodoptera*-infested maize seedlings to *C. marginiventris* females is determined by a specific combination of attractive and repellent/masking compounds, including some that are emitted in very small amounts. Manipulating the emission of such minor compounds has the potential to greatly improve the attraction of certain parasitoids and enhance biological control of specific insect pests.

Keywords Herbivore-induced plant volatiles (HIPV) · Parasitoids · Tritrophic interactions · Biological control · Preparative gas-chromatography

Introduction

Volatiles emitted by plants upon infestation with herbivorous arthropods are important foraging cues for natural enemies of these herbivores. Over the last two decades, numerous studies have provided evidence that these herbivore-induced plant volatiles (HIPV) indeed function as an indirect defense by which the plants purposefully recruit parasitoids and predators, although fully conclusive evidence for this function is still missing (for recent reviews: Turlings and Wäckers 2004; D'Alessandro and Turlings 2006; Heil 2008; Arimura et al. 2009; Dicke 2009; Dicke et al. 2009). Enormous progress has been made in terms of understanding the mechanisms of emission and biosynthesis of plant volatiles, including the emission of HIPV (Dudareva et al. 2006; Pichersky et al. 2006). For instance, some compounds are constitutively emitted by healthy, uninfested plants, whereas others are synthesized

M. D'Alessandro (✉) · V. Brunner · G. von Mérey ·
T. C. J. Turlings
Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), University of Neuchâtel, Institute of Biology,
Case Postale 158,
CH-2009 Neuchâtel, Switzerland
e-mail: marco.dalessandro@unine.ch

de-novo only after herbivore-damage (Paré and Tumlinson 1999). Among the HIPV, some compounds are emitted immediately upon herbivore damage, while the synthesis of others is truly induced, and it takes a certain time after initial infestation before they are emitted (Turlings et al. 1998). Several genes involved in the biosynthesis of HIPV now have been identified, and this opens the possibility to apply molecular tools to study their role both as attractants for natural enemies and as signaling compounds within and between plant species (Baldwin et al. 2001; Kappers et al. 2005; Paschold et al. 2006; Schnee et al. 2006; Ton et al. 2007; Frost et al. 2008).

Maize has been a model plant since the beginning of the studies on these chemically-mediated tritrophic interactions (e.g., Turlings et al. 1990), and the volatile blends emitted from the aboveground shoot, as well as from the below-ground roots, are well characterized (Degen et al. 2004; Köllner et al. 2004; Rasmann and Turlings 2008). Despite detailed knowledge on the chemical composition and the relevance of the entire blend as a host location cue to a range of parasitoid species (Tamò et al. 2006), we still lack an understanding of the relative importance of individual compounds or group of compound to specific parasitoids. Specifically, the importance of minor compounds that are emitted in quantities below the threshold level or compounds which cannot easily be detected by common analytical methods is unknown.

In this study, we used a combination of different fractionation methods (filtering tubes, preparative GC and different solvents) and olfactometer bioassays in order to study the attractiveness of different fractions of a blend of HIPV emitted by maize seedlings infested by *Spodoptera littoralis* (Lepidoptera: Noctuidae) to females of the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Cotesia marginiventris* is an important larval parasitoid of *Spodoptera* spp. larvae, which are major pest insects that cause substantial economic damage to maize throughout the Americas. The attraction of *C. marginiventris* females to *Spodoptera*-induced maize volatiles has been investigated in a series of previous studies, which showed clearly that this wasp strongly prefers volatiles emitted by caterpillar-infested maize seedlings over non-infested healthy seedlings (Turlings et al. 1991a, b; 2004). Recent studies, however, indicate that not all compounds emitted by infested seedlings are attractive, and some might even be repellent or mask attractiveness (D'Alessandro and Turlings 2005; D'Alessandro et al. 2006). This was evident from a series of experiments in which we tested attraction of *C. margin-iventris* females to volatiles emitted by *Spodoptera*-infested maize seedlings after passing the volatile blend through a selection of adsorbent-containing filter tubes (D'Alessandro and Turlings 2005). Surprisingly, the volatile blend that broke through a filter filled with silica had lost all

attractiveness to naïve females, although it still contained at least 70% of the volatiles of the original blend. By contrast, the volatile compounds that were adsorbed by the filter (silica extract) and subsequently extracted with a solvent and applied to filter paper were extremely attractive to *C. marginiventris* females.

Hence, the objective of the current study was to confirm and examine the high attractiveness of the volatiles in the silica extract, as a first step towards characterizing and identifying key volatile compounds or combinations of compounds that are used as foraging cues by *C. marginiventris*. Modifying the release of such key compounds in maize seedlings or applying synthetic versions of these compounds in a maize field could be part of a sustainable and environmentally sound strategy to control *Spodotera* larvae feeding on maize.

Methods and Materials

Insects and Plants

The caterpillar *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) and the solitary endoparasitoid, *Cotesia margin-iventris* (Cresson) (Hymenoptera: Braconidae) were reared as previously described (Turlings et al. 2004). Adult parasitoids were kept in plastic cages in incubators (25±1°C, 16:8 h L/D) and transferred to the laboratory 30 min before the bioassays. Two-4-d-old naive females were used in the bioassays. Plants (*Zea mays*, var. Delprim) were grown in plastic pots (10 cm high, 4 cm diam) with commercial potting soil (Ricoter Aussaaterde, Aarberg, Switzerland) in a climate chamber (25± 2°C, 60% r.h., 16:8 h L/D, and 50,000 lm/m2). Plants used for the volatile collection were 10-12-d-old and had 3 fully developed leaves. The evening before the volatile collection they were infested with 20 s instar *S. littoralis*, which were released in the whorl of the youngest leaf. After infestation, plants were kept under laboratory conditions (25±2°C, 40± 10% r.h., 16:8 h L/D, and 8000 lm/m2), and volatiles were collected on the following day, between 10 A.M. and 4 P.M.

Volatile Collection

Volatiles were collected by passing the herbivore induced volatile blend over a filter tube filled with 25 mg silica (63–200 mesh, 60 Å, Brunschwig, Basel, Switzerland) for 2.5 h and by adsorbing the breakthrough volatiles on a filter tube that contained 25 mg of the highly adsorbent SuperQ (25 mg, 80–100 mesh, Alltech Associates, Inc., Deerfield, IL, USA). Silica is a rather weak adsorbent and used mainly to adsorb polar compounds. Many volatiles break through the silica filters and can be recollected on SuperQ, which is a strong adsorbent and

commonly used to collect a broad range of different volatile compounds (D'Alessandro and Turlings 2005). Volatiles retained by the silica filters were desorbed with 300 µl dichloromethane (Suprasolve, GC-grade, Merck, Darmstadt, Germany) (silica extract). The volatiles adsorbed on the SuperQ filters were extracted with 150 µl dichloromethane (breakthrough). Further details on the volatile collection and volatile filtration are described by D'Alessandro and Turlings (2005). Several samples were pooled in order to obtain a standardized stock solution for each treatment (silica extract and breakthrough), which was stored at −80°C in small vials (Supelco, Amber Vial, 7 ml with solid cap w/PTFE Liner), and used throughout the experiments for all fractionation steps and bioassays.

Fractionation and Analyses of Volatile Blends

The silica extract was separated into several fractions by preparative gas-chromatography (preparative GC). For this purpose, a Hewlett Packard HP 6890 GC with an automated column injection system (HP G1513 A) was either equipped with a non-polar (HP-1 MS, 30 m, 0.25 mm ID, 0.25 µm film thickness; Alltech Associates, Inc, USA) or a polar column (HP-Innowax, 30 m, 0.25 mm ID, 0.25 µm film thickness; Alltech Associates, Inc, USA). Helium at constant pressure (non-polar column: 19.39 psi; polar column: 45.14 psi) was used as carrier gas, and 5 µl of the silica extract were injected in the "on column mode". After injection, the non-polar column temperature was maintained at 40°C for 3.5 min and then increased to 100°C at 8°C/min and subsequently to 200°C at 5°C/min followed by a post-run of 5 min at 250°C. The polar column was also maintained at 40°C for 3.5 min but then increased to 250°C at 8°C/min followed by a post-run of 5 min at 250°C. Fractions were recollected at specific retention times as indicated in Fig. 3 on Pasteur pipettes that contained 20 mg of SuperQ. The precise retention time for each fraction was calculated by installing the outlet of the GC column to a flame ionization detector (FID) prior to the fractionation steps verified by re-analyzing an aliquot of the recollected volatiles by GC-FID. For each fraction, a separate recollection pipette was attached at the outlet of the GC column and cooled to 4°C with ice in order to recollect all volatiles after GC-separation. Ten aliquots of 5 µl silica extract were recollected on one pipette in order to obtain sufficient material for one bioassay. All volatiles were desorbed from the SuperQ filters with 200 µl dichloromethane and applied to filter paper disks for the bioassay as described below. The recollection efficiency of the preparative GC procedure was calculated by injecting a mixture of synthetic volatile compounds with known

concentration and was ≥80% except for (Z)-3-hexenal that was recollected with approximately 66% efficiency (data not shown).

To separate non-polar from polar compounds in the silica extract we first desorbed the volatiles collected on the silica filters with 300 µl methanol (Suprasolve, GC-grade, Merck, Darmstadt, Germany). The silica extract in methanol similarly was attractive to the wasps just as the silica extract obtained with dichloromethane (data not shown), and it contained the same major compounds as found in the dichloromethane (Fig. 5). Subsequently, 1 ml of hexane was added to 1 ml of the silica extract in methanol, thoroughly shaken, and placed in the −80°C freezer in order to separate the non-polar hexane phase from the polar methanol phase. Each phase was transferred to a separate vial by a 1 ml GC-syringe and stored in the freezer until used for the bioassays as described below.

To identify the volatile compounds, at least one 2-µl aliquot of the silica extract and the breakthrough extract were injected in a gas chromatograph (Agilent 6890 Series GC system G1530 A) coupled to a mass spectrometer that operated in the electron impact mode (Agilent 5973 Network Mass Selective Detector; transfer line 230°C, source 230°C, ionization potential 70 eV, scan range 33–280 amu) in the pulsed splitless mode onto either the non-polar or the polar column with helium at constant flow (0.9 ml/min) as carrier gas. Oven temperature and ramp were similar to that described above for the preparative GC analyses. The identities of volatiles were confirmed by comparing their mass spectra with those of the NIST 02 library
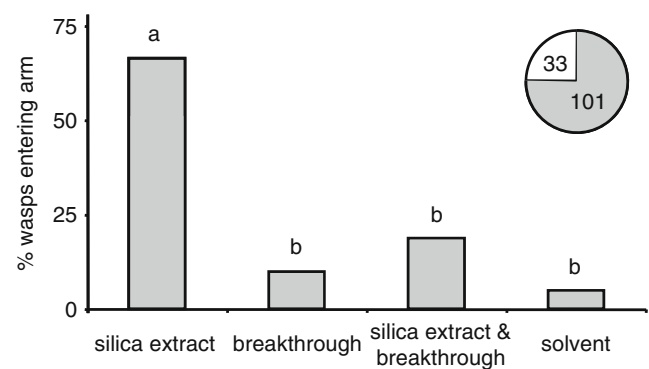

Fig. 1 Olfactometer responses of *Cotesia marginiventris* to different volatile extracts of a blend with volatile compounds emitted by *Spodoptera*-infested maize seedlings. Chemical composition of the extracts are given in Fig. 2. The pie-charts indicate the number of wasps that entered an olfactometer arm (*grey*) and the number of wasps that did not make any choice and remained in the center of the olfactometer (*white*). Different letters above the bars indicate significant differences among the various extracts (GLM: $P<0.05$)

and by comparing the retention times with those of previous analyses (D'Alessandro and Turlings 2005).

Olfactometer Bioassays

All extracts and fractions were tested for attractiveness to parasitoids in a four-arm olfactometer as described by D'Alessandro and Turlings (2005). Cleaned and humidified air entered the odor source vessels at 1.2 l/min (adjusted by a manifold with four flowmeters; Analytical Research System, Gainesville, FL, USA) via Teflon tubing and carried the volatiles through to the olfactometer compartment. Half of the air (0.6 l/min/olfactometer arm) was pulled out via a volatile collection trap that was attached to

the system above the odor source vessels. An aliquot of 100 µl of each extract or 50 µl of the fractions obtained by preparative GC was placed on a filter paper (1/2 disk, 55 mm diam, Schleicher & Schuell GmbH, Dassel, Germany) that was inserted in the glass tube connecting the odor source vessels to the olfactometer arms. After letting the solvent evaporate for 2 h, wasps were released in groups of 6 into the central part of the olfactometer. Wasps that had entered an arm of the olfactometer after 30 min were counted and removed. Wasps that did not enter an arm after this time were removed from the central part of the olfactometer and considered as "no choice". Bioassays were replicated at 4 to 8 d, and for each replicate a total of four groups of 6 wasps were tested as described before
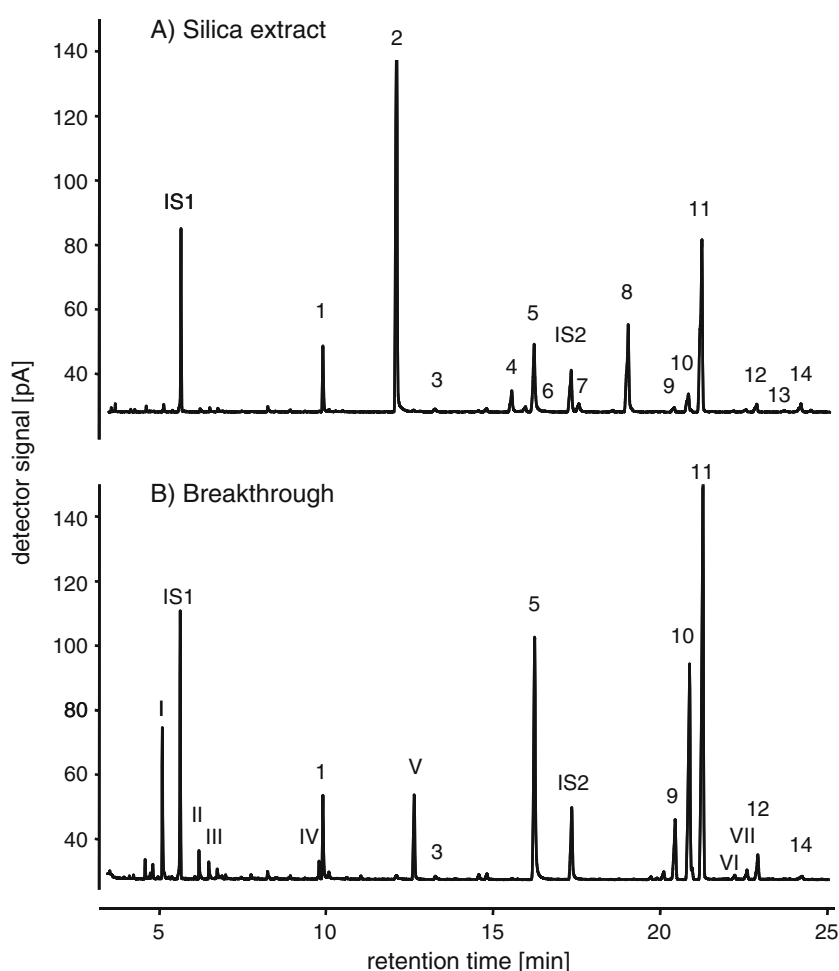
Fig. 2 GC-FID chromatograms of different extracts of a blend with volatile compounds emitted *Spodoptera*-infested maize seedlings that were obtained by passing the entire blend over a silica containing filter tube. Silica extract = volatiles retained in the silica filter. Breakthrough = volatiles that broke through the filter. The entire blend of *Spodptera*-infested maize seedlings contains the combination of both chromatograms. The compounds are: 1 = (Z)-3-hexenyl acetate, 2 = linalool, 3 = benzyl acetate, 4 = phenethyl acetate, 5 = indole, 6 = unknown compound, 7 = methyl anthranilate, 8 = geranyl acetate, 9 = (E)-β-

caryophyllene, 10 = (E)-α-bergamotene, 11 = (E)-β-farnesene, 12 = β-sesquiphellandrene, 13 = (E)-nerolidol, 14 = (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), I = (Z)-3-hexenal, II = (E)-2-hexenal , III = (Z)-3-hexen-1-ol, IV = β-myrcene, V = (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), VI = unknown sesquiterpene, VII = unknown sesquiterpene. Some compounds are not appearing well as a peak on this scaling, but their location is still indicated with a number. Compounds were identified by GC-MS analyses as indicated in Methods and Materials

(Turlings et al. 2004). All bioassays were carried out between 10 A.M. and 4 P.M.

Statistical Analyses

The functional relationship between parasitoids' behavioral responses and the different volatile extracts and fractions offered in the four-arm olfactometer was examined with a generalized linear model as described earlier by D'Alessandro and Turlings (2005). The model was fitted by maximum quasi-likelihood estimation in the software package R (R: A language and Environment for Statistical Computing, Version 1.9.1, Vienna, Austria, 2006, ISBN 3-900051-07-0, http://www.R-project.org), and its adequacy was assessed through likelihood ratio statistics and examination of residuals. Due to possible differences in the evaporation rate of individual compounds from filter papers, we included the time effect as

an explanatory variable in the statistics. However, in none of the experiments was a time effect found, so only statistical values of the treatment effects (volatile extract or fraction) are indicated in the results.

Results

Fractionation over Selective Filter Tubes

In a first four-arm olfactometer experiment, we compared the attraction of females of the parasitoid *C. marginiventris* to the volatile compounds emitted by *Spodoptera*-infested maize seedlings that were trapped on a silica containing filter tube (silica extract) to the ones that broke through the filter (breakthrough), as well as to a reconstitution of the whole volatile blend (silica extract and breakthrough)

Fig. 3 GC-MS chromatograms of the highly attractive silica extract in dichloromethane indicating different fractions that were isolated by preparative GC on two different columns. The identity of the compounds is given in Fig. 2
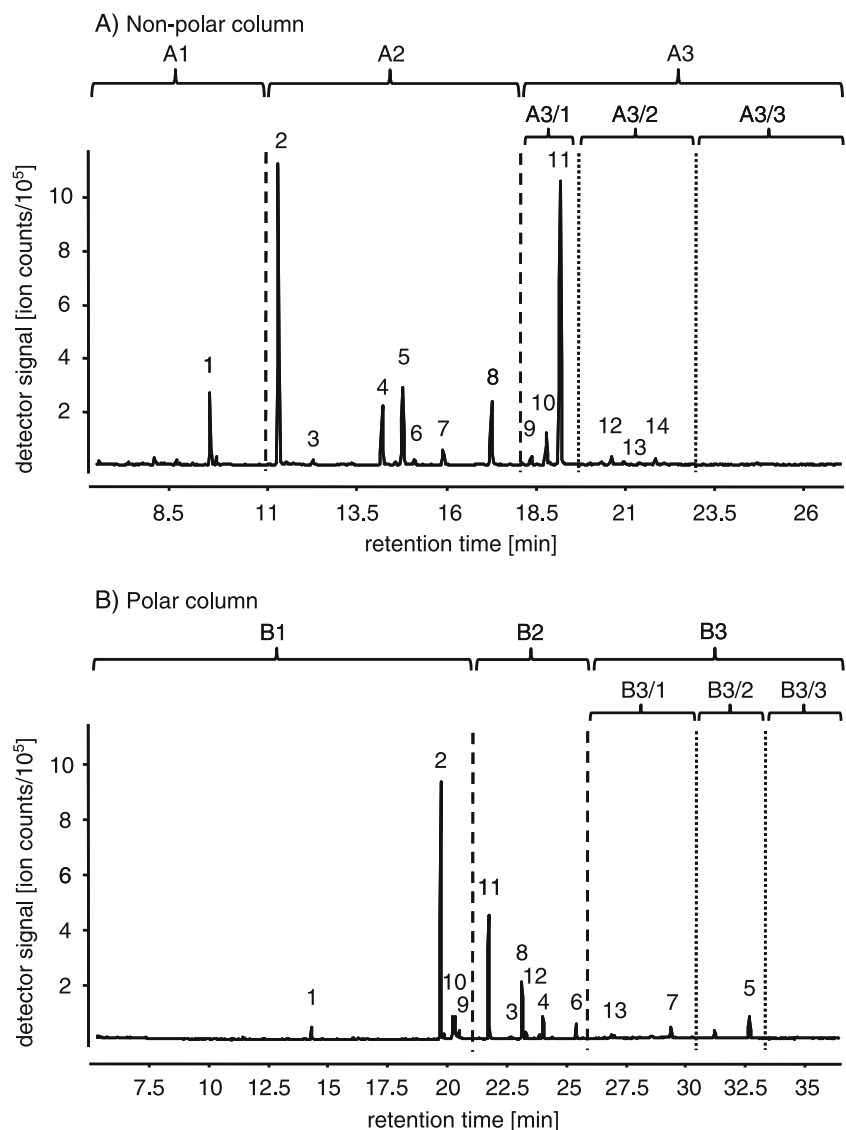
and to the solvent only. There was a significant difference in the attractiveness of these various odor sources (GLM: $F_{3,92} = 14.19$, $P<0.001$) (Fig. 1). As in the previous study (D'Alessandro and Turlings 2005), naïve *C. marginiventris* females were extremely attracted to the silica extract, but not to the volatile blend that broke through the filter (Figs. 1 and 2). Interestingly, the silica extract was also more than 3 times as attractive as the reconstitution of the whole blend. The precise composition of each volatile extract is shown in Fig. 2. The amounts of the individual compounds found in the different extracts were not quantified in this study, but the chromatographic analyses of the extracts indicated that they were similar to the previous study (D'Alessandro and Turlings 2005).
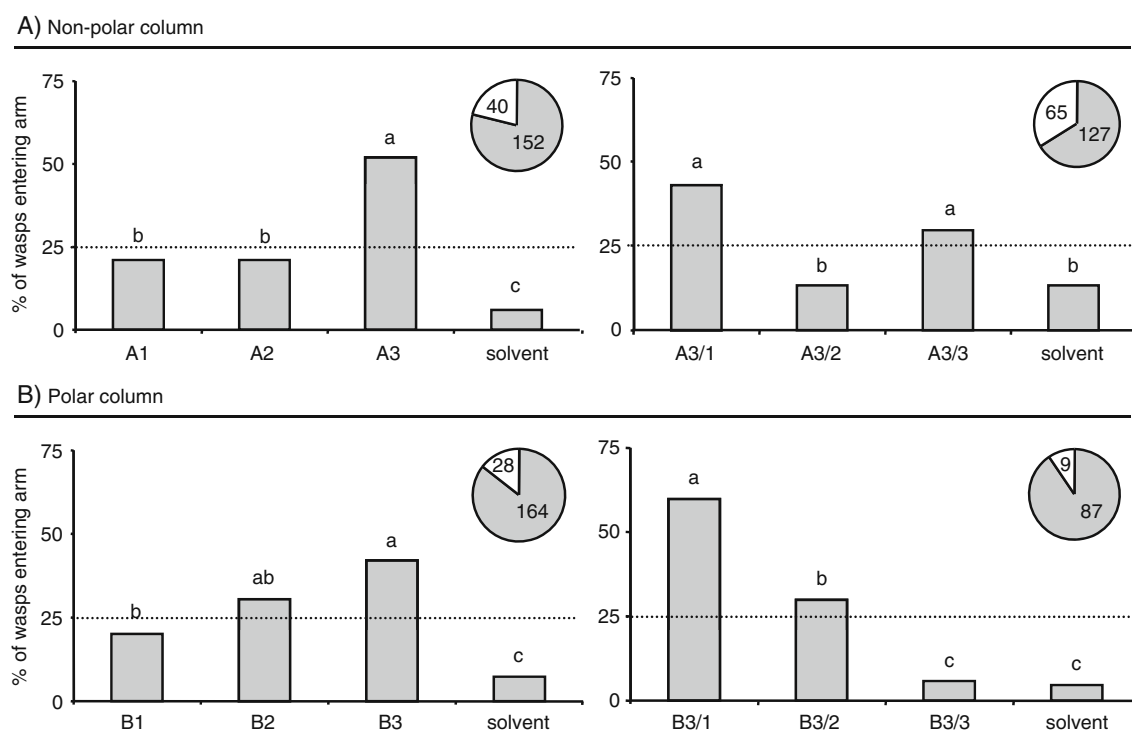
Fraction by Preparative GC

In order to determine the most attractive compounds in the attractive silica extract, the extract was further fractionated into three volatile extracts by preparative gas chromatography (preparative GC) on a non-polar GC-column (HP-1 MS) (Fig. 3A: A1, A2, A3) and tested for attraction in the olfactometer. Significantly more wasps entered the arm with fraction A3 than with fractions A1 and A2, but the latter were still more attractive than solvent only (Fig. 4A; GLM: $F_{3,124} = 14.19$, $P<0.001$). Subsequently, the most attractive fraction of the silica extract was again fractionated into three

extracts (Fig. 3A: A3/1, A3/2, A3/3) and tested for attraction. Fraction A3/2 was not attractive, while fractions A3/1 and A3/3 were similarly attractive to the wasps (Fig. 4A; GLM: $F_{3,116} = 4.07$, $P<0.01$).

A second fractionation of the silica extract was carried out on a polar column (HP-Innowax) (Fig. 3B). The most attractive fraction resulting from a first fractionation step was fraction B3, but all fractions with HIPV were significantly more attractive than solvent only (Fig. 4B; GLM: $F_{3,124} = 7.73$, $P<0.001$). Further fractionation of the most attractive fraction B3 resulted in two fraction, B3/1 and B3/2, that were more attractive than solvent only (Fig. 4B; GLM: $F_{3,60} = 14.60$, $P<0.001$).
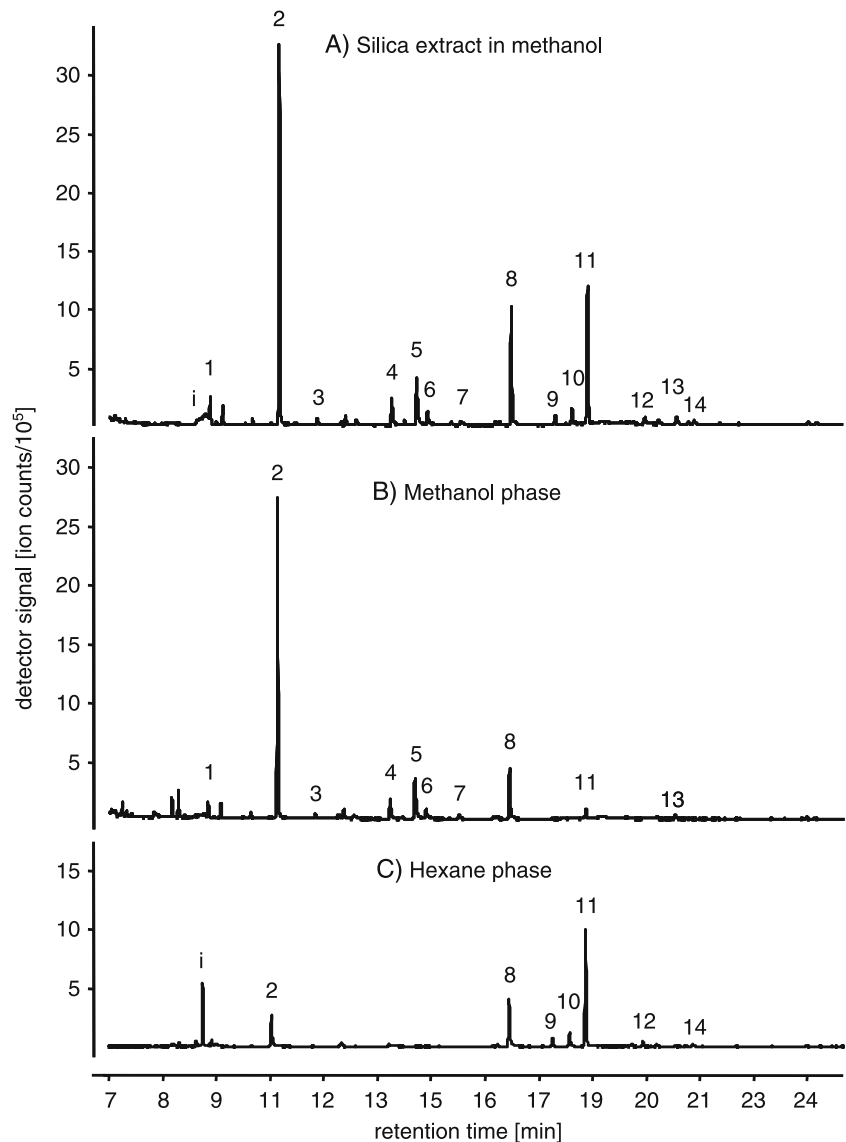
Fractionation with Different Solvents

In a subsequent experiment, we separated less polar from more polar compounds by the use of different solvents. Volatiles adsorbed on the silica filter were first desorbed with methanol. This methanol extract was similarly as attractive as the dichloromethane extract used in the previous experiments (data not shown), and both of these extracts contained the same major HIPV (Figs. 2 and 5). By adding a similar amount of hexane to the silica extract in methanol, we obtained a hexane phase that contained non-polar compounds and a methanol phase with polar compounds (Fig. 5). In olfactometer bioassays, we found that

Fig. 4 Olfactometer responses of *Cotesia marginiventris* to different fractions of the silica extract that were isolated by preparative GC as indicated in Fig. 3. Explanations to the pie-charts and to the statistics are given in Fig. 1

the polar methanol phase was significantly more attractive
than the less polar hexane phase, but the latter was more
attractive than the solvents only (Fig. 6A, GLM: $F_{3,92}$ =
65.60, $P<0.001$). Comparing the methanol phase to the
whole silica extract in methanol showed that the latter was
significantly more attractive than the extract without the
less polar compounds, indicating that some attractive
compounds are soluble in hexane and were either missing
or strongly reduced in the methanol phase (Fig. 6B, GLM:
$F_{1,46}$ = 6.68, $P<0.05$).

Discussion

In this study, we used a combination of fractionation
methods and olfactometer bioassays to characterize the
most attractive compounds of the volatile blend emitted by

Spodoptera-infested maize seedlings that are used by the
solitary endoparasitoid *C. marginiventris* as host-location
cue. Similarly, as in a previous study (D'Alessandro and
Turlings 2005), the extract that was obtained by passing the
entire HIPV-blend over a silica filter tube and contained
rather polar compounds and was highly attractive to naive
C. marginiventris females, whereas the breakthrough
extract was not attractive at all to this wasp even though
it also contained most measurable HIPV (Figs. 1 and 2).
Further separation of less polar from more polar com-
pounds in the silica extract proved that the wasp preferred
polar over non-polar compounds (Figs. 5 and 6). However,
the detectable polar compounds that were present in the
attractive silica extract but not in the non-attractive
breakthrough extract (compounds 2, 4, 6, 7, 8, and 13)
could not explain the high attractiveness of the silica
extract. First, a combination of synthetic versions of

Experiment A)

Experiment B)
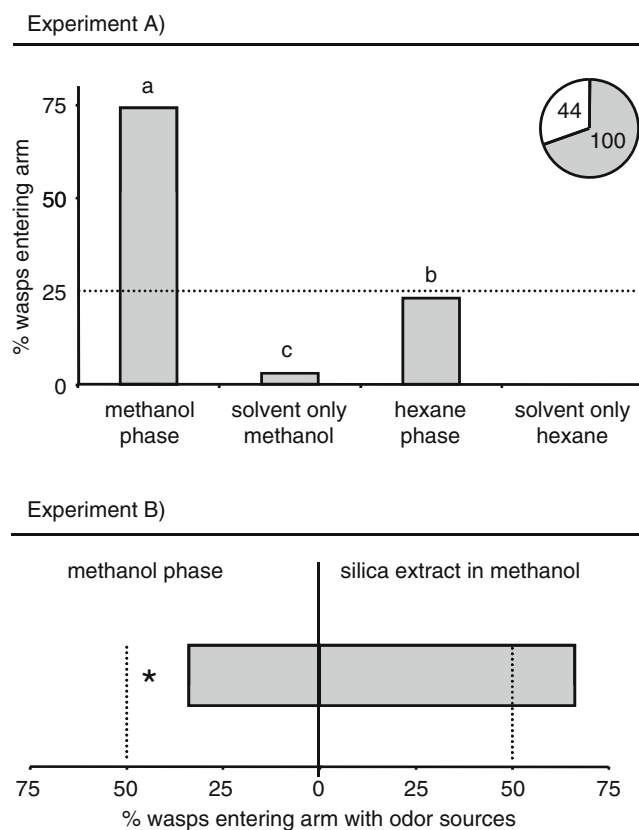
Fig. 6 Olfactometer responses of *Cotesia marginiventris* to different volatile fractions that were isolated by using solvents with different polarities as indicated in Fig. 5. A) Comparison of the polar and the non-polar fraction as well as the solvents as control. Explanations to the pie-charts and to the statistics are given in Fig. 1. B) Comparison of the polar fraction to the entire silica extract in methanol

compounds 2, 4, 7, 8, and 13 was not attractive to the wasps (data not shown). Second, further fractionation of the silica extract by preparative GC did not result in a clear allocation of these compounds to attractive and non-attractive fractions. For instance, although compound 13 was present in the most attractive fraction in some bioassays (fractions A3, B3, and in B3/1), in others it was present in the least attractive fraction (fraction A3/2) (Figs. 3 and 4). Similarly, compound 7 was present in the highly attractive fraction (B3/1), but fraction A2 was significantly less attractive than fraction A3 although it also contained this compound as well as most of other compounds that were present in the highly attractive silica extract. Finally, the unknown compound 6 was present in fractions with medium attractiveness. Overall, our experiments suggest that the compounds detected in the chemical analyses, are not likely to be the most attractive ones, nor the only ones that are needed by *C. marginiventris* as host location cues.

Another interesting observation was that the silica extract, which contained only a fraction of the whole

blend, was by far more attractive than the entire blend of herbivore-induced maize volatiles (Figs. 1 and 2). This suggests that certain compounds within a blend of HIPV have either repellent effects on the attraction of *C. marginiventris* or are masking the attractiveness of other attractive compounds. Similar findings have been found in earlier studies and with other insect species. For instance, naïve females of the parasitoid *Microplites rufiventris* clearly preferred volatile blends that did not contain indole, a major HIPV of maize, over blends that contained indole at normal concentration, and this difference was due to a masking effect of the volatile compound indole itself (D'Alessandro et al. 2006). Another common plant compound that recently has been claimed to interfere with the attraction of certain parasitic wasps is isoprene (Loivamaki et al. 2008). By contrast, other studies have shown synergistic effects of certain volatile compounds. For instance, neither nonanal nor geranylacetone alone was attractive to the parasitoid females of *Apanteles carpatus*, a parsitoid of the cloth moth *Tineae pennionella*, but a one-to-one blend of both compounds was as attractive as an extract of all volatile compounds from moth-infested beaver pelt (Takács et al. 1997). Similarly, the egg parasitoid *Chrysonotomyia ruforum* of the herbivorous sawfly *Diprion pini* did not respond to the sesquiterpene (E)-β-farnesene, an oviposition-induced pine twig volatile, if offered to the females as an individual compound at different doses (Mumm and Hilker 2005). However, the parasitoid was significantly attracted to this compound when tested together with the odor of pine twigs without eggs. These studies illustrate, that optimal attraction requires the presence of a specific combination of compounds. Unfortunately, so far no general pattern could have been recognized that would help to determine which compounds reduce or enhance the attractiveness of odor sources to a foraging insect (reviewed by Schröder and Hilker 2008).

One reason for the lack of a clear understanding of synergistic and antagonistic effects of individual compounds within complex blends might be the fact that insects respond to volatile compounds in a dose-dependent manner (De Boer and Dicke 2004; Ngumbi et al. 2005; Yan and Wang 2006). Such effects also have been reported in earlier studies with the parasitoid *C. marginiventris* (Fritzsche Hoballah et al. 2002; Turlings et al. 2004). The surprising finding in this study was the strong attraction of *C. marginiventris* to some fractions that contained volatile compounds in extremely low quantities (e.g., A3/3). In fact, the concentrations of these compounds were below the detection limit of the GC analyses, i.e., in the pico-gram range or lower, which has made it impossible to identify the compounds thus far. Previous electrophysiological analyses with *C. marginiventris* showed that some HIPV elicited strong electroantennographic responses in the antennae of the insects at concentrations

below the detection level of the GC analyses (Gouinguené et al. 2005). These observations strongly suggest that minor, yet unknown compounds, play a crucial role in the attraction of *C. marginiventris* to *Spodoptera*-induced maize volatiles. In fact, the olfactory sensitivity of insects is astonishing. For instance, by using the moth *Spodoptera littoralis,* it was nicely demonstrated that around five molecules of the sex pheromone and around ten molecules of a specific plant odor hitting the antenna during one second were sufficient to trigger a heartbeat frequency change (Angioy et al. 2003). It is likely that compounds emitted in such low quantities are likely to escape chemical analysis.

Another complicating factor in identifying minor compounds that are highly attractive to parasitoids is the specific chemical structure of some volatiles. The chemical diversity of volatiles compounds emitted by plants is enormous, ranging from alkanes, alkenes, alcohols, ketones, aldehydes, ethers, and esters to carboxylic acids and others (Niinemets et al. 2004). It is likely that a single volatile sampling and analyses method cannot provide the entire picture of the qualitative and quantitative composition of an herbivore-induced volatile blend. In this study, it is possible that key compounds did not chromatograph well and either did not elute or were eluted throughout the analyses by "bleeding" off the column. This latter possibility could also explain the attractiveness of multiple fractions that might all have contained elusive compound(s). Thus, alternative approaches to conventional volatile collection methods and GC analysis are needed to unravel the identity of highly attractive minor compounds and for a better understanding of the attractiveness HIPV-blends in general. Recent studies that have applied novel methods, such as PTR-MS, have revealed the presence of methanol in the volatile blend of a number of herbivore-infested plants, a compound, that has not been detected with conventional trapping and GC analysis methodologies (Penuelas et al. 2005; von Dahl et al. 2006). Other promising approaches might benefit from novel statistical tools (van Dam and Poppy 2008; Pareja et al. 2009) or also might consider that some plant volatiles are suppressed rather than induced upon insect infestation (Gaquerel et al. 2009). In conclusion, when new methods are used to analyze HIPV blends in a more comprehensive manner, the eventual identification of key attractants or repellents for parasitoids and predators may provide potential to improve biological control of insect pests. The transformation of maize plants with a gene responsible for the biosynthesis of a (E)-ß-caryophyllene, a key volatile attractant for entomopathogenic nematodes of the Western corn rootworm, is a recent example of how the HIPV-blend can be modified to successfully control this ferocious root pest of maize plants (Degenhardt et al. 2009).

References

ANGIOY, A. M., DESOGUS, A., BARBAROSSA, I. T., ANDERSON P., and HANSSON B. S. 2003. Extreme sensitivity in an olfactory system. *Chem. Sens.* 28:279–284.

ARIMURA, G., MATSUI, K., and TAKABAYASHI J. 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: Proximate factors and their ultimate functions. *Plant Cell Physiol.* 50:911–923.

BALDWIN, I. T., HALITSCHKE, R., KESSLER, A., and SCHITTKO, U. 2001. Merging molecular and ecological approaches in plant-insect interactions. *Curr. Opin. Plant Biol.* 4:351–358.

D'ALESSANDRO, M., HELD, M., TRIPONEZ, Y., and TURLINGS, T. C. J. 2006. The role of indole and other shikimic acid derived maize volatiles in the attraction of two parasitic wasps. *J. Chem. Ecol.* 32:2733–2748.

D'ALESSANDRO, M. and TURLINGS, T. C. J. 2005. In situ modification of herbivore-induced plant odors: A novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem. Sens.* 30:739–753.

D'ALESSANDRO, M. and TURLINGS, T. C. J. 2006. Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *Analyst* 131:24–32.

DE BOER, J. G., and DICKE M. 2004. The role of methyl salicylate in prey searching behavior of the predatory mite Phytoseiulus persimilis. *J. Chem. Ecol.* 30:255–271.

DEGEN, T., DILLMANN, C., MARION-POLL, F., and TURLINGS, T. C. J. 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol.* 135:1928–1938.

DEGENHARDT, J., HILTPOLD, I., KÖLLNER, T. G., FREY, M., GIERL, A., GERSHENZON, J., HIBBARD, B. E., ELLERSIECK, M. R., and TURLINGS, T. C. J. 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proc. Natl. Acad. Sci. USA* 106:13213–13218.

DICKE, M., 2009. Behavioural and community ecology of plants that cry for help. *Plant Cell Environ.* 32:654–665.

DICKE, M., VAN LOON, J. J. A., and SOLER, R. 2009. Chemical complexity of volatiles from plants induced by multiple attack. *Nat. Chem. Biol.* 5:317–324.

DUDAREVA, N., NEGRE, F., NAGEGOWDA, D. A., and ORLOVA, I. 2006. Plant volatiles: Recent advances and future perspectives. *Crit. Rev. Plant Sci.* 25:417–440.

FRITZSCHE HOBALLAH, M. E., TAMÒ, C., and TURLINGS, T. C. J. 2002. Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *J. Chem. Ecol.* 28:951–968.

FROST, C. J., MESCHER, M. C., DERVINIS, C., DAVIS, J. M., CARLSON, J. E., and DE MORAES, C. M. 2008. Priming defense genes and

metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytol.* 180:722–733.

GAQUEREL, E., WEINHOLD, A., and BALDWIN, I. T. 2009. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphigidae) and its natural host *Nicotiana attenuata*. VIII. An unbiased GCxGC-ToFMS analysis of the plant's elicited volatile emissions. *Plant Physiol.* 149:1408–1423.

GOUINGUENÉ, S., PICKETT, J. A., WADHAMS, L. J., BIRKETT, M. A., and TURLINGS T. C. J. 2005. Antennal electrophysiological responses of three parasitic wasps to caterpillar-induced volatiles from maize (*Zea mays mays*), cotton (*Gossypium herbaceum*), and cowpea (*Vigna unguiculata*). *J. Chem. Ecol.* 31:1023–1038.

HEIL, M. 2008. Indirect defence via tritrophic interactions. *New Phytol.* 178:41–61.

KAPPERS, I. F., AHARONI, A., VAN HERPEN, T., LUCKERHOFF, L. L. P., DICKE, M., and BOUWMEESTER, H. J. 2005. Genetic engineering of terpenoid metabolism attracts, bodyguards to Arabidopsis. *Science* 309:2070–2072.

KÖLLNER, T. G., SCHNEE, C., GERSHENZON, J., and DEGENHARDT, J. 2004. The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distribution. *Phytochemistry* 65:1895–1902.

LOIVAMAKI, M., MUMM, R., DICKE, M., and SCHNITZLER, J. P. 2008. Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proc. Natl. Acad. Sci. USA* 105:17430–17435.

MUMM, R. and HILKER, M. 2005. The significance of background odour for an egg parasitoid to detect plants with host eggs. *Chem. Sens.* 30:337–343.

NGUMBI, E. N., NGI-SONG, A. J., NJAGI, E. N. M., TORTO, R., WADHAMS, L. J., BIRKETT, M. A., PICKETT, J. A., OVERHOLT, W. A., and TORTO, B. 2005. Responses of the stem borer larval endoparasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) to plant derived synomones: Laboratory and field cage experiments. *Biocontrol Sci. Techn.* 15:271–279.

NIINEMETS, U., LORETO, F., and REICHSTEIN, M. 2004. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends Plant Sci.* 9:180–186.

PARÉ, P. W. and TUMLINSON, J. H. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* 121:325–331.

PAREJA, M., MOHIB, A., BIRKETT, M. A., DUFOUR, S., and GLINWOOD, R. T. 2009. Multivariate statistics coupled to generalized linear models reveal complex use of chemical cues by a parasitoid. *Anim. Behav.* 77:901–909.

PASCHOLD, A., HALITSCHKE. R., and BALDWIN, I. T. 2006. Using 'mute' plants to translate volatile signals. *Plant J.* 45:275–291.

PENUELAS, J., FILELLA, I., STEFANESCU, C., and LLUSIA, J. 2005. Caterpillars of *Euphydryas aurinia* (Lepidoptera: Nymphalidae) feeding on *Succisa pratensis* leaves induce large foliar emissions of methanol. *New Phytol.* 167:851–857.

PICHERSKY, E., NOEL, J. P., and DUDAREVA, N. 2006. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* 311:808–811.

RASMANN, S. and TURLINGS, T. C. J. 2008. First insights into specificity of belowground tritrophic interactions. *Oikos* 117:362–369.

SCHNEE, C., KÖLLNER, T. G., HELD, M., TURLINGS, T. C. J., GERSHENZON, J., and DEGENHARDT, J. 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proc. Natl. Acad. Sci. USA* 103:1129–1134.

SCHRÖDER, R. and HILKER, M. 2008. The relevance of background odor in resource location by insects: A behavioral approach. *Bioscience* 58:308–316.

TAKÁCS, S., GRIES, G., and GRIES, R. 1997. Semiochemical-mediated location of host habitat by *Apanteles carpatus* (Say) (Hymenoptera: Braconidae), a parasitoid of clothes moth larvae. *J. Chem. Ecol.* 23:459–472.

TAMÒ, C., RICARD, I., HELD, M., DAVISON, A. C., and TURLINGS, T. C. J. 2006. A comparison of naive and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. *Anim. Biol.* 56:205–220.

TON, J., D'ALESSANDRO, M., JOURDIE, V., JAKAB, G., KARLEN, D., HELD, M., MAUCH-MANI, B., and TURLINGS, T. C. J. 2007. Priming by airborne signals boosts direct and indirect resistance in maize. *Plant J.* 49:16–26.

TURLINGS, T. C. J., DAVISON, A. C., and TAMÒ, C. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.* 29:45–55.

TURLINGS, T. C. J., LENGWILER, U. B., BERNASCONI, M. L., and WECHSLER, D. 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207:146–152.

TURLINGS, T. C. J., TUMLINSON, J. H., ELLER, F. J., and LEWIS, W. J. 1991a. Larval-damaged plants—Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomol. Exp. Appl.* 58:75–82.

TURLINGS, T. C. J., TUMLINSON, J. H., HEATH, R. R., PROVEAUX, A. T., and DOOLITTLE, R. E. 1991b. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17:2235–2251.

TURLINGS, T. C. J., TUMLINSON, J. H., and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–1253.

TURLINGS, T. C. J. and WÄCKERS, F. 2004. Recruitment of predators and parasitoids by herbivore-injured plants, pp. 21–75, in R. T. Cardé and J. G. Millar (eds.). Advances in Insect Chemical Ecology. Cambridge University Press. Cambridge.

VAN DAM, N. M. and POPPY, G. M. 2008. Why plant volatile analysis needs bioinformatics—detecting signal from noise in increasingly complex profiles. *Plant Biol.* 10:29–37.

VON DAHL, C. C., HAVECKER, M., SCHLOGL, R., and BALDWIN, I. T. 2006. Caterpillar-elicited methanol emission: a new signal in plant-herbivore interactions? *Plant J.* 46:948–960.

YAN, Z. G. and WANG, C. Z. 2006. Identification of *Mythmna separata*-induced maize volatile synomones that attract the parasitoid *Campoletis chlorideae*. *J. Appl. Entomol.* 130:213–219.