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Attraction of parasitic wasps by caterpillar-damaged plants

Ted C. J. Turlings and Maria Elena Fritzsche

Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, CH-2007, Neuchâtel, Switzerland

Abstract. Plant volatiles emitted in response to herbivory have been suggested to function as signals to attract natural enemies of herbivores. Most known examples of induced plant volatiles used by natural enemies involve parasitoids that locate caterpillars by means of odours emitted by plants after caterpillar attack. We study the tritrophic system that comprises the parasitoid *Cotesia marginiventris*, host caterpillars from the genus *Spodoptera*, and maize plants. Among the volatiles emitted by caterpillar-damaged maize plants, sesquiterpenes and indole are particularly attractive to the parasitoid. The usefulness of these plant volatiles for parasitoids is obvious. Less clear is their benefit to plants that emit them, as in most cases parasitization does not immediately stop caterpillars from damaging plants. However, plants appear to benefit directly from attracting *C. marginiventris*, as parasitized caterpillars consume considerably less plant tissue than unparasitized caterpillars. It is expected that in systems where parasitoids significantly reduce herbivory, they have contributed to selective pressures that have shaped the phenomenon of herbivore-induced volatile emissions by plants.

1999 Insect–plant interactions and induced plant defence. Wiley, Chichester (Novartis Foundation Symposium 223) p 21–38

Many plants respond to herbivory with the production of defence chemicals (Karban & Baldwin 1997). Several of these induced substances are volatiles and result in odours that are readily used by natural enemies (predators and parasitoids) to locate herbivores. It has been suggested that one of the functions of induced volatiles is to serve as signals to attract natural enemies, as plants' fitness may benefit from the interaction (e.g. Dicke et al 1990a, Dicke 1995, Sabelis & De Jong 1988, Turlings et al 1995). However, the benefit to an individual plant of attracting parasitoids is not always clear, because parasitized caterpillars will in most cases continue to feed and cause significant damage to a plant. Here we will discuss several aspects of parasitoid attraction by means of herbivore-induced plant odours and use results from our own studies to argue that parasitoids contribute to selective forces that have resulted in and maintained the phenomenon of herbivore-induced plant volatile emission.

Caterpillar-induced plant volatiles

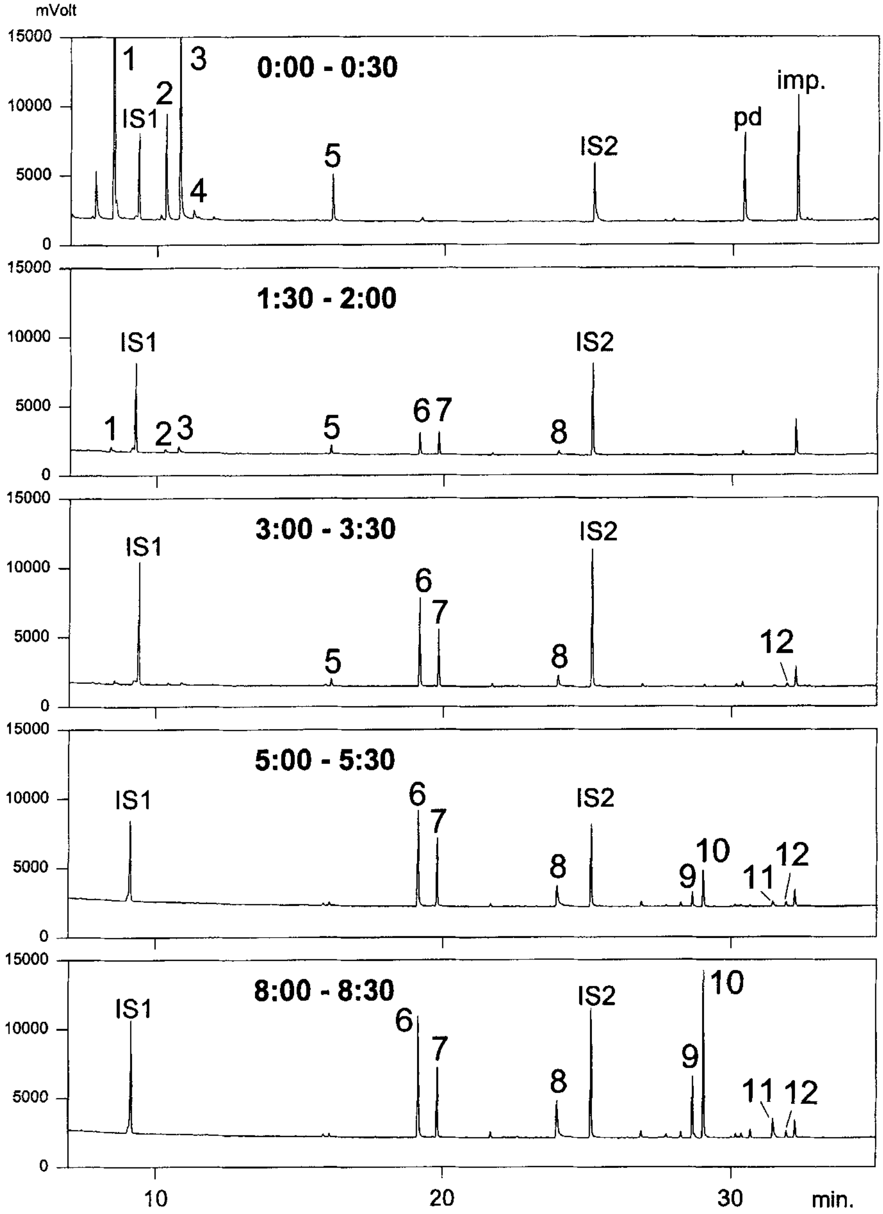
Changes in volatile emissions in response to caterpillar feeding are easily demonstrated with young maize plants. The resulting odour can even be detected with the human nose several hours after initial insect feeding. The volatiles that are involved can be collected on adsorbents and gas chromatographic analyses of extracts of these collected volatiles show dramatic differences between caterpillar-damaged and healthy plants (e.g. Turlings et al 1990, Takabayashi et al 1995). The response of the plant is triggered by elicitors in the oral secretion of caterpillars (Turlings et al 1993a). One such elicitor (volicitin) was identified from the regurgitant of *Spodoptera exigua* (Alborn et al 1997, Tumlinson 1999, this volume).

Figure 1 illustrates the temporal changes in volatile releases that are seen after a maize plant is mechanically damaged and the damaged sites are treated with caterpillar regurgitant (in this case from *S. littoralis*). At 9 am 8-day-old maize plants were treated and immediately after treatment volatiles were trapped on filters every 0.5 h for a period of 12 h (Turlings et al 1998). Analyses of the collected odours (Fig. 1) show that immediately after damage the plants emit typical green-leafy aldehydes, alcohols and acetates that 'bleed' directly from the damaged sites. Without any further damage the emission of these highly volatile compounds will cease within hours. After 2–3 h the first induced volatiles (monoterpenes and indole) are emitted. It takes a few hours more before the emission of sequiterpenes is observed (Fig. 1). The emission of the induced compounds lasts throughout the entire collection period, but slowly wanes after reaching a maximum late during the day. In the context of their role in defence against herbivores, the observed timing of emissions is expected to be fast enough to allow the plant to attract the natural enemies long before the herbivores have done significant damage to the plant.

Attraction of parasitoids by means of herbivore-induced plant volatiles

Table 1 lists the studies that have demonstrated the importance of herbivore-induced plant odours in host location by parasitoids. Relatively few systems have

FIG. 1. (*Opposite*) Chromatographic profiles of odours collected from maize seedlings (var. Ioana) at different times after damage and treatment with caterpillar regurgitant. The numbers with the peaks represent: 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexen-1-ol; 4, (*E*)-2-hexen-1-ol; 5, (*Z*)-3-hexen-1-yl acetate; 6, linalool; 7, (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 8, indole; 9, (*E*)-α-bergamotene; 10, (*E*)-β-farnesene; 11, (*E*)-nerolidol; 12, (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Two remaining volatiles did not originate from the plants; pentadecane (pd) was released from the regurgitant that was used to treat the plants and imp. represents an impurity in the air. IS1 and IS2 are the internal standards *n*-octane and *n*-nonyl-acetate. Taken from Turlings et al (1998) with permission, copyright Springer-Verlag.


mVolt
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9
10
11
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min.

TABLE 1 **Examples of parasitoids using caterpillar-induced plant odours to locate their hosts**

Parasitoid	*Herbivore(s)*	*Plant(s)*	*Specifics on the interactions*	*Selected references*
Cotesia marginiventris	*Spodoptera* spp. *Helicoverpa zea* *Trichoplusia ni*	Maize Cotton Cowpea	Plants respond to herbivory with the emission of volatiles that are attractive to the parasitoid. Factors in the regurgitant of the caterpillars elicit the emissions. One such elicitor has been identified and was named volicitin.	Turlings et al (1990, 1991a,b) Alborn et al (1997) Tumlinson (1999, this volume)
Microplitis croceipes	*Helicoverpa* and *Heliothis* spp.	Cotton Cowpea Maize	The wasp is strongly attracted to volatiles emitted by the plants after they have been damaged by caterpillars or after treatment with caterpillar regurgitant.	McCall et al (1993) Turlings et al (1993a) Röse et al (1996)
Cotesia glomerata, *Cotesia rubecula*	*Pieris* spp.	Cabbage and related sub-species	The parasitoids are attracted to caterpillar-damaged plants due to an increase in odour emitted by the plant. β-glucosidase in the caterpillar oral secretion elicits this reaction in the plant.	Steinberg et al (1993) Agelopoulos & Keller (1994) Mattiacci et al (1994, 1995)
Cotesia kariyai	*Pseudaletia separata*	Maize	Maize plants eaten by early instar larvae of the herbivore show a significant increase in odour emissions and become attractive to the parasitoid. The plant does not respond to late instar larvae.	Takabayashi et al (1995)
Cotesia flavipes	*Chilo partellus*	Maize	The stem borer causes maize plants to systemically emit volatiles that attract the parasitoid.	Potting et al (1995)
Cardiochiles nigriceps	*Heliothis virescens*	Cotton Tobacco	Caterpillar feeding induces a specific emission of volatiles, which the parasitoid can distinguish from emissions induced by a non-host caterpillar.	De Moraes et al (1998)

been studied, but all show the importance of plant-provided signals, suggesting that the phenomenon is very common. These interactions have been mainly studied for parasitoids that attack Lepidoptera, but natural enemies that attack herbivores from other orders also make effective use of plant odours. One well studied example involves predatory mites that use induced plant odours to locate herbivorous mites, as discussed by Dicke (1999, this volume). In the case of mites, some of the individual compounds that are emitted by infested plants have been shown to be attractive to the predators (Dicke et al 1990b). For parasitoids, however, little is known about which of the plant volatiles are actually attractive.

Which of the compounds released from the plant are the most important in attracting parasitoids?

A commonly asked question concerns the importance of the individual plant-released volatile compounds for the attraction of parasitoids. The keen ability of parasitoids to learn any odour that is associated with the presence of host odour (Lewis & Tumlinson 1988, Turlings et al 1993b, Vet et al 1995) suggest that the entire blend released by a plant is important. Still, it can be expected that some compounds are more attractive (or better perceived) than others. This is the case for several specialist parasitoids. *Heydenia unica* is attracted to terpenoids associated with its host, the southern pine beetle (Camors & Paine 1972). Elzen et al (1984) found that several terpenoids released from cotton plants are particularly attractive to the parasitoid *Campoletis sonorensis* and Udayagiri & Jones (1992) showed that fractions of maize volatiles containing sesquiterpenes, aldehydes, a ketone and esters were much more attractive to the specialist parasitoid *Macrocentrus grandii* than polar fractions containing alcohols. The following experiment involving the parasitoid *Cotesia marginiventris* and its responses to volatiles emitted by caterpillar-damaged maize plants illustrates that it can be difficult to determine the relative importance of particular compounds.

C. marginiventris is a solitary endoparasitoid that can develop in caterpillars of many different species. As a consequence, it can find suitable host caterpillars on many different plant species. One such plant is maize and a variety of experiments have shown that caterpillar-induced maize volatiles are very attractive to *C. marginiventris*. Maize volatiles are much more attractive than odours from the caterpillars or their faeces (Turlings et al 1991a). In one experiment the odours from maize seedlings damaged by caterpillars (*Spodoptera exigua*) were collected in large amounts and extracts of these collections were placed on strips of paper to test their attractiveness in a flight tunnel (Turlings et al 1991b). At an optimal dose, about 75% of female wasps tested were found to be attracted to the extract (Turlings et al 1991b). With this dose, an experiment was designed that allowed us

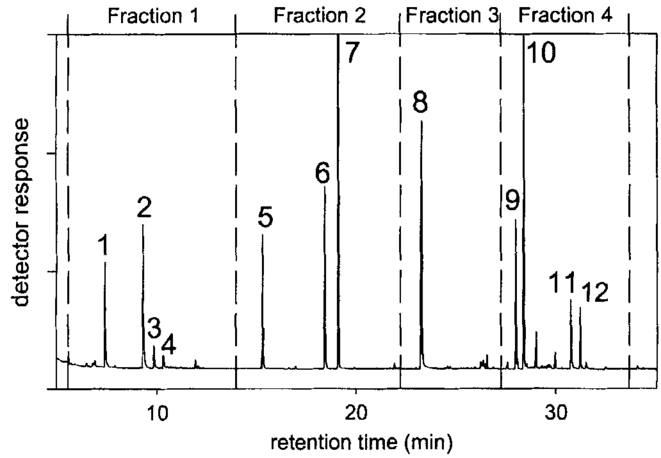

FIG. 2. Chromatogram of volatiles collected in mass quantities from maize seedlings eaten by *Spodoptera* larvae. The four fractions that were taken for bioassays with the parasitoid *C. marginiventris* are indicated. Peak identities are given in the legend for Fig. 1.

to exclude parts of the total odour blend to determine how this affects the attractiveness of the extracts.

Odours of the complete plant-host complex were collected as described by Turlings et al (1991b). Using a technique first described by Brownlee & Silverstein (1968), we collected four fractions (see Fig. 2) of the total odour blend with a preparative gas chromatograph onto glass capillary tubes (see details in Turlings et al 1991b). In a first test, a recombination of all four fractions was compared with different recombinations of only three fractions. One at a time, recombinations were applied on a strip of paper (1×5 cm) as described by Turlings et al (1991b) and tested for attractiveness in a flight tunnel. Six 2-day-old experienced wasps (two groups of three) were allowed to fly to a single recombination and we recorded how many of them flew to the odour source. The recombinations were tested 'blind' in random order. The experiment was replicated five times on different days. Positive responses often resulted in landings on the paper strip, but a number of females approached a target strip within 3 cm without landing on it. Both these behaviours were recorded separately. The results in Fig. 3A show that the most attractive recombinations included fractions 1, 3 and 4. Surprisingly, this was more attractive than a recombination of all fractions.

Similar results were obtained with a choice experiment. The principal of this experiment was the same, except that all treatments were placed in the flight tunnel at the same time. This time only the four possible recombinations of three

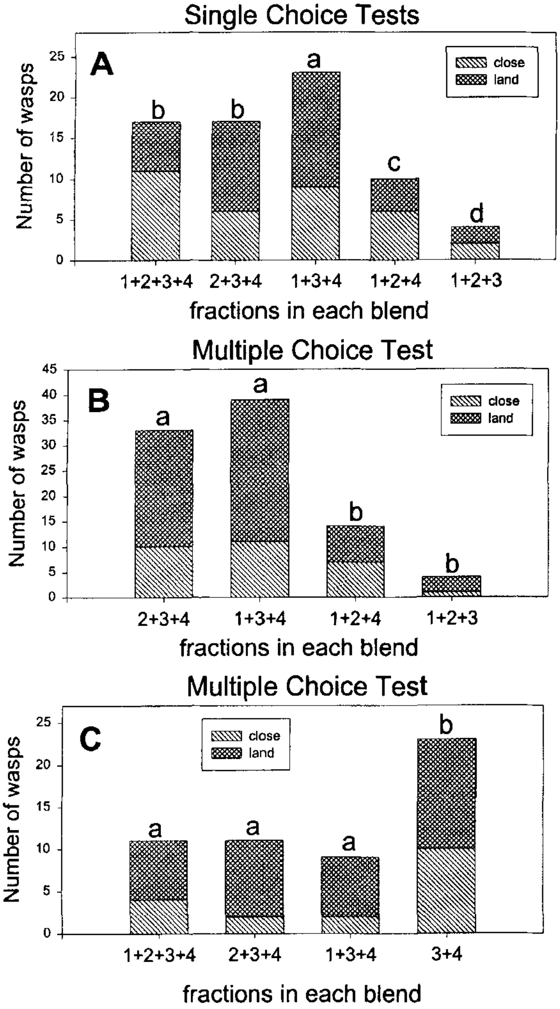

FIG. 3. Responses of wasps to recombinations of different odour fractions (see Fig. 2) placed on paper strips in a flight tunnel. (A) single choice tests, (B) and (C) multiple choice tests. Responses are divided between wasps that approached the target to within 3 cm (close) and wasps that landed on the target (land). The letters with each bar indicate significant differences in attractiveness among the test samples (Fisher's PLSD after ANOVA, $P < 0.05$).

fractions were used. They were applied on four different paper strips placed 10 cm apart perpendicular to the wind direction. The order in which they were placed was random. Groups of five experienced female wasps were released and their preferences (3 cm approaches and landings) were recorded. After each group of five the position of the paper strips was shifted one position to the right (the most right strip would move to the most left position), and a new group of five female wasps was released. This was repeated five times on a particular day and the entire experiment was repeated five times on different days. Again the recombination of fractions 1, 3 and 4 was the most attractive (Fig. 3B).

The main conclusion from this experiment is that fraction 2, which contains (*Z*)-3-hexen-1-yl acetate, linalool and (3*E*)-4,8-dimethyl-1,3,7-nonatriene, was the least attractive and may even have been repellent or masked the attractive fractions. This is surprising as these compounds do contribute significantly to the blend of induced volatiles. The results also indicated that fraction 1 did not contribute to the attractiveness of the entire blend.

One more experiment was conducted to further test the importance of fractions 1 and 2. For this, wasps were again offered four odours, one containing all four fractions, one without fraction 1, one without fraction 2, and one without both fractions 1 and 2. Most wasps flew to this last combination, which only included fractions 3 and 4 (Fig. 3C).

It is possible that the release rates of the highly volatile compounds in fractions 1 and 2 deviated too much from a natural release and thus cause repellence or masking of the attractive odours. It was previously found that when the entire blend was tested at very high concentrations its attractiveness also dropped significantly (Turlings et al 1991b). The negative effect of the two first fractions may therefore have been the result of limitations of the experimental procedure. Dose–response tests with these factions could perhaps show this. For now, it can be concluded that fractions that contain indole and several sesquiterpenes are highly attractive to the wasp.

Does a plant benefit from attracting parasitoids?

Parasitoid larvae do not kill their hosts immediately. The immature parasitoid develops within a living host, which in many cases continues to feed and grow. These so-called koinobiont parasitoids which allow further development of their host are common among larval parasitoids of Lepidoptera. Frequently, parasitization causes the caterpillars to eat even more than healthy caterpillars (e.g. Parker & Pinnell 1973, Byers et al 1993). In those cases, plants may not benefit directly from attracting parasitoids and it is unlikely that parasitoids contribute to selective pressures that favour plants to emit volatiles in response to herbivory. It seems, however, that there are numerous parasitoids that do reduce herbivory by their hosts and that they therefore may have direct positive effect on plant fitness (Beckage 1985). We are investigating this possibility for *C. marginiventris* and how this parasitoid may affect plant fitness.

One approach has been to compare the development of parasitized and unparasitized caterpillars (*Spodoptera littoralis*). Insects were kept under ambient

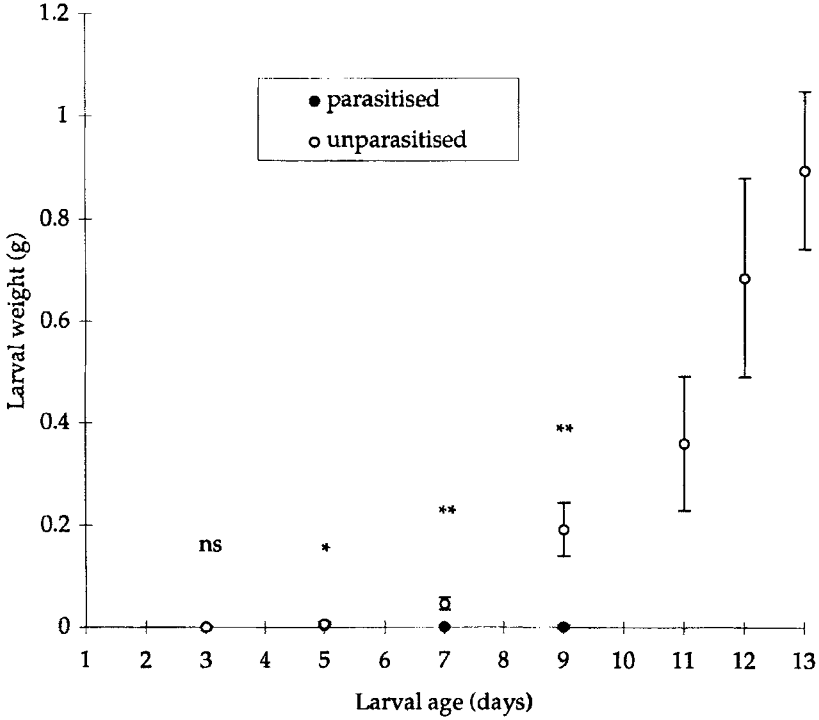

FIG. 4. Comparison between the weight gain of parasitized and unparasitized *Spodoptera littoralis* larvae. Six days after parasitization, larvae have gained very little weight. At this point the parasitoid larvae emerges from its host and the host dies, while unparasitized larvae continue to feed and grow considerably. The asterisks indicate significant differences in the weight of parasitized and unparasitized caterpillars (simple factorial ANOVA; ns, not significant; $*P<0.02$; $**P<0.0001$).

light (natural) and temperature in our laboratory. Three-day-old caterpillars that had been feeding on maize seedlings (var. Delprim) were offered to 2-day-old mated *C. marginiventris* females. Caterpillars that were observed to be stung by a parasitoid were removed and placed singly with an ample supply of maize leaves as food. Control (unparasitized) caterpillars that originated from the same egg batch were held the same way. Every two days the caterpillars were weighed and every day the leaves were replaced with fresh ones. The average weight gain of parasitized larvae, as shown in Fig. 4, was significantly reduced starting 2 days after parasitization. This agrees with previous reports on reduced consumption by caterpillars parasitized by *C. marginiventris* (Ashley 1983, Jalali et al 1988). The reduction in feeding results in less leaf damage (Table 2) and increases the chances

TABLE 2 Dry-weight of leaves from 5-week-old maize plants damaged by unparasitized or parasitized *Spodoptera litoralis* caterpillars

Treatment	*Dry weight (g) of leaves (average ± SD)*
Control plants ($n = 16$)	0.78 ± 0.23
Plants eaten by unparasitized caterpillars ($n = 16$)	0.43 ± 0.20
Plants eaten by parasitized caterpillars ($n = 22$)	0.77 ± 0.17

Caterpillars (3 d old) were placed singly on the plants and the leaves were dried after pupa formation by the healthy caterpillars. Factorial analysis (ANOVA) showed a significant difference between treatments, $P < 0.0001$, $F = 17.081$.

of plants surviving a caterpillar attack (M. E. Fritzsche & T. C. J. Turlings, unpublished data).

Conclusions

Parasitoids make effective use of herbivore-induced plant volatiles to locate hosts for their offspring. The effects of secondary plant metabolites on insects that interact with plants have undoubtedly led to selection pressures that resulted in modifications in the production and release of these substances. The third trophic level of natural enemies of herbivores is likely also to have contributed to these selective pressures. Despite the fact that they do not immediately kill their host, many parasitoids significantly reduce plant damage caused by herbivores and thus may positively affect plant fitness. Plants that benefit from parasitoids are expected to have adapted the release of odours so as to optimize their attractiveness to these insects. However, this may be entirely different in cases where parasitoids and predators have no immediate effect on herbivory. If the attraction of the third trophic level is an important function of induced plant volatiles, comparative studies may reveal that induced plant signals occur principally in systems where plant fitness is positively affected by natural enemies of herbivores.

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DISCUSSION

Boland: You used preparative GC to separate the volatiles into four groups. This technique may seriously affect the composition, at least in the high boiling components. Did you check that in the effluent the original ratio is maintained?

Turlings: It is difficult to re-collect some of the highly volatile compounds in the glass tube. However, we played with this and in the end we were able to re-collect more than 90% of the highest volatility compounds by using some Super-Q absorbent in the glass tube and submerging the tube in liquid nitrogen. Therefore I am confident that we were close to the original ratios. This doesn't necessarily mean that we got out what we injected in, because decomposition may take place. But judging by the response of the parasitoids to the re-collected volatiles, I'm sure that we got the attractive compounds.

Pickett: How did you eliminate the effects of variation in the volatility of the different compounds in the bioassay work? When you put the green leaf compounds on the filter paper, they will come off more quickly, whereas fraction 4 will come off slowly. The terpenoids are also held by the paper to some extent.

Turlings: We didn't. We did not do anything in this particular experiment to slow down the release rates of these compounds. If we did, we would possibly get a more natural release rate of the highly volatile compounds, but then a very unnatural release of the less volatile compounds. In this case I just applied the solvent with the volatiles directly onto the paper, waited just a minute to let the solvent evaporate and then I placed it in the wind tunnel. I used the same strip of paper throughout an experiment that may last 30–40 minutes. So there is likely a range of different release rates for the various compounds.

Gershenzon: The compounds may affect each other's release rate so that the volatility of a substance in a mixture is very often different from volatility when measured in pure form. Hence, it would be interesting to follow up to see what the headspace composition of these fractions really shows you, in comparison to the composition of what you have applied to the filter paper.

Turlings: If you look at those headspaces you get complicated results that depend on how long you collect for and precisely when you collect. It is far more useful to start working on a technique to get those release rates more comparable to the natural situation.

Gershenzon: Regarding your synthetic blend of volatiles, did you mix and test your blend on the same day, or mix it up on one day and test it a few days later? I'm curious about the stability of the individual compounds in these blends. Was the chromatogram that you showed us obtained from a sample taken at the same time that you conducted the bioassay?

Turlings: I don't remember how long a time separated that chromatogram and the bioassay. We used just one synthetic blend for the whole series of experiments, and that lasted about 3–4 weeks.

Pickett: Certainly the farnesene and the homoterpenes are unstable.

Turlings: They're not unstable if you look at them in terms of the natural blend. We do a lot of re-analysis of samples that we collected several months previously, and we get the same results. Admittedly, these are kept at −70 °C.

Gershenzon: This is interesting; they tend to be more stable in their natural context than when they are purified.

Pickett: Purified farnesenes oxidize very quickly.

Dietrich: Can you further fractionate fractions 3 and 4?

Turlings: Yes, that is something that would be interesting to look at in the future, but we haven't done it yet.

Beale: Fraction 2 as a whole had a negative effect on the bioactivity of 3 plus 4. Therefore, have you added back the individual components of 2?

Turlings: We haven't done that yet.

Vet: You are saying that fraction 2 changes the responsiveness to 3 and 4, and you are talking in terms of a deterrent effect. But might it instead be the case that fraction 2 is actually making the signal from the other fractions less clear? That is, if 3 and 4 are really the fractions that elicit the parasitoid behaviour and you add something else, the clarity of the signal will be reduced. Consequently, it misleadingly looks like fraction 2 has a deterrent effect.

Turlings: That's definitely a possibility. I think we have to get rid of that problem with the release rates and then we can start playing around with adding and leaving out certain compounds.

Tumlinson: This could be something to do with learning. These wasps are experienced at detecting the odour of caterpillars feeding on plants, and this signal depends on what the plants were releasing at the time. It's hard to say that some compounds are more important than others, because the plants release different blends at different times of the day.

Vet: So 1 and 2 may not have been there during the training and therefore the insects really go for 3 and 4. And when you train them on immediate plant response — basically on the C_6 compounds — you may get more of a response to fractions 1 and 2.

Turlings: I did an experiment that sort of addresses this issue, but I didn't do it for that purpose. We compared fresh damage with old damage, and tested those next to each other in the wind tunnel. We gave a group of wasps experience with fresh damage (mainly the green leafy volatiles released) and then another group of wasps had experience with the old damage (terpenoids). Wasps that have had experience with old damage show a much clearer preference for old damage, but the ones that had experience with fresh damage were still quite attracted to the old damage.

Poppy: I suppose another way of looking at this is asking whether you are dealing with recognition of the correct 'signal', or recognition of an incorrect 'signal'. With aphids, for instance, the parasitoid *Aphidius ervi* has an innate ability to discriminate between bean plants infested by its preferred host, *Acyrthosiphon* *pisum*, from bean plants infested by the non-host, *Aphis fabae*. What we don't know at this stage is whether that's because *A. fabae* induces the plant to produce an incorrect 'signal', which the parasitoids recognize and do not respond to, or because this aphid does not induce the plant to produce the correct 'signal'.

In terms of fractions 1 and 2, is it actually just a dilution factor or is there actually an incorrect signal that's been recognized so the parasitoids no longer respond to the correct signal? Are they so plastic that sometimes you can get more discrimination if that incorrect signal has been associated with a negative experience?

Baldwin: Since you have all demonstrated that associative learning is fundamentally important in this process, and in the light of this discussion of trying to understand the source of variation in volatile emissions from plants, I was wondering whether or not in your tests purified volicitin generates the same degree of variability that you see when you test the whole spit. You have talked about many different sources of variation: when you replace a herbivore with a defined chemical substance, does some of that variation go away or does the emission from the plant continue to be variable?

Tumlinson: Whether the seedlings are incubated in spit or in volicitin doesn't alter the variation in volatile release with time of day. Of course, volicitin isn't the only active compound in the spit: we know that there's at least one other compound that has activity. I'm not sure that testing pure volicitin is therefore going to give you all the answers. We see a lot of variability with the plants no matter what we do.

Haukioja: Insect spit contains lots of microorganisms: do you know whether these microorganisms produce the significant signals, or is it something from the insect itself?

Tumlinson: No.

Haukioja: Would it be possible to kill the microorganisms in the spit by irradiation, for example, so that you could test this?

Tumlinson: I don't think that would be the experiment. Once you have the spit the compounds are there, so killing the microorganisms at that point is not going to answer to the question.

Turlings: Killing microorganisms would definitely not reduce the activity of the spit: if you boil the spit you still have activity, and it is also active when it has been sterile-filtered. But as Jim Tumlinson said, this still doesn't rule out the possibility that the elicitor is already produced by microorganisms at this stage.

Schultz: At the risk of interrupting the terpenoid flow here, I'm curious about the indole. The question I've been wondering about for a long time is, if a plant is exposed to indole, does it have physiological activity? After all, indole is volatile and may be perceived by plants as a form of growth hormone or signal.

Firn: Indole tends to be pretty inactive in changing plant growth and development at realistic concentrations.

When plants are attacked by fungi, do they give off volatiles?

Pickett: Yes.

Firn: How do fungal-induced volatiles compare with the volatiles induced by an insect pest? How can a parasite discriminate between the signals?

Pickett: In the situation that we know best, which is *Alternaria brassicae* on oilseed rape (*Brassica napus*), the *A. brassicae* causes a different quantitative profile and the generation of some novel compounds. In fact, one is so novel we don't know what it is yet.

Firn: Is there a difference in parasitism when comparing plants carrying a fungal infection and healthy plants?

Pickett: This would most likely complicate the studies.

Dicke: The volatile profile released by lima bean plants infected with *Pseudomonas* bacteria is completely different from the profile induced by spider mite infection, as I will show in my paper (Dicke 1999, this volume).

Vet: Some parasitoids already have problems when two different herbivores are feeding on the same host plant.

Firn: The message emerging here is that the information content in volatile mixes is extremely high, especially when one considers mixed plant communities with multiple pests and diseases.

Dicke: That might be very relevant in the context of what Ted Turlings showed, because if an attacked plant is in the midst of plants that have not been attacked but which have still been damaged by wind, for instance, they will produce the green leaf volatiles but they don't produce the terpenoids. Thus there will be a plant that emits the complete mixture surrounded by plants that emit only part of the mixture.

Firn: Presumably the signals from any single plant species will be blended with signals from other species of plants producing different volatiles. The volatiles sensed by any parasite will therefore be made up of many compounds from a wide range of plants. This means that the signal will be extremely 'noisy', with high spatial and temporal variation.

Wadhams: On this issue of how insects can perceive a point source, Tom Baker has recently published an interesting paper on cell pairings in Lepidopteran male moths (Baker 1998). This focuses the orientation of the male to a point source. Because of a temporal coding that occurs, not only are they able to look at ratios, but they look at the ratios at an instantaneous point in time. This applies even more when you're talking about phytophagous insects, and host location and herbivores. Most herbivores are responding at a peripheral level to ubiquitous plant compounds, yet they're still able to differentiate their host from a non-host. The way we think they do that is by looking for this instantaneous stimulation of the two olfactory cells, which says that a particular signal comes from that plant and it's not fortuitous mixing of signals from two plants giving the same appropriate ratio.

Haukioja: As you mentioned in your paper, in some cases parasitized herbivore larvae actually consume more than unparasitized larvae. However, in the case you described it was clear that the parasitized larvae ate less of the plant. Do you have any idea of whether in this case reduced consumption by the larva after parasitization is perhaps specific for this herbivore or specific for this parasitoid? If there are differences, for instance, in how parasitoids manipulate larval feeding, for plants to really benefit, they should attract those parasitoids which cause the larva to eat less.

Turlings: This particular parasitoid, independent of which species it attacks, will reduce the feeding rate. Whether all caterpillars that are feeding on this particular plant are attacked by parasitoids that reduce feeding rates, I don't know. I don't think that it's up to the plant to send out a signal that only attracts this specific parasitoid and not others, because if the others are interested in finding the caterpillars they can pick up the same signal.

Vet: You are working with a solitary parasitoid. We have two species of parasitoid being attracted by the plants. One is the solitary parasitoid, and in general the solitary parasitoids have a limited amount of resources that they use from the host and they generally tend to reduce the growth after parasitization. The gregarious parasitoid, which is also attracted, can be quite different. It is known from polyembryonic parasitoids (which lay one egg in a host, but this then multiplies to as many as a few thousand individuals), that they can stimulate the feeding behaviour of the host by 50% or more, so these are terrible for the plant. With our gregarious parasitoid, the effect on the growth of the herbivore depends on the clutch size that is being laid inside the host: if there is only a small clutch, host growth (and thus feeding rate) is reduced to the point where it's still less than an unparasitized host. But if there are many eggs, this stimulates the host to grow. You can see how difficult it can be if you don't look at the combination of species that can use this plant information — the plant cannot choose who is going to make use of the signals it releases.

Gierl: In your profiles of the volatiles released by plants, I was surprised to see that in the absence of induction there was absolutely no signal. How do the herbivores find their host plants in the first place?

Turlings: As far as our detection limits are concerned, it depends on which genotype of plant you look at. The example that I showed is fairly extreme: we really don't see anything. But in other cases linalool and α-farnesene are produced by undamaged plants, sometimes in quite significant amounts.

Gierl: And are these signals overlapping signals that are then emitted to attract the parasitoids?

Turlings: Yes.

Tumlinson: Undamaged plants do release volatiles, but they release them at very different levels. Typically, in some of our experiments with a cotton plant that had

6–8 true leaves, when it was damaged by several larvae we were getting 8–10 μg/h, whereas from an undamaged plant we were getting maybe 400 ng/h.

So many of our experiments are artificial: we take these plants and take great care not to damage them, and we put them in our volatile collection systems. In fact if you get a little bit of damage, the compounds that we always see that indicate stress just jump at you. I suspect that in the natural situation, due to wind damage and so forth and so on, you are going to get a lot of compounds released from the plants.

Wadhams: I agree. Intact plants give off almost no volatiles, but the insects can still detect those levels. In coupled GC/electrophysiological runs where we are using the insect's antennae as a detector, we can, in some instances, have an absolutely flat GC baseline but the insect's olfactory cells still respond strongly. There can be several orders of magnitude difference between the insect's ability to detect a compound and our ability to see it with current techniques.

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General discussion I

Poppy: Our discussions of the co-evolutionary aspects of herbivore-induced volatiles in tritrophic interactions, and how plants can benefit if the larval consumption rate is reduced, still don't answer the key question of what is actually happening within the plant. Is the plant actually generating this 'signal' as a response to herbivore attack and therefore those plants that do it well are selected over evolutionary time? Or, as we believe is the case for aphids, is the herbivore saliva actually causing up- and down-regulation of various biosynthetic pathways within the plant to make that plant more suitable to the herbivore, and the parasitoids are exploiting volatiles which are produced as a consequence of this up- and down-regulation? We are still not sure which is the governing factor: are the plants actively responding and sending out SOS signals, or are the volatiles produced as a by-product of being attacked?

Baldwin: In thinking about the function of these signals, when you are devoid of information it is sometimes helpful to approach this question from the engineering standpoint, even though that's not the way evolution works (evolution clearly functions as a tinker rather than an engineer). Richard Karban came up with an alternative idea about how induced defences function — his 'moving target' model (Adler & Karban 1994). In this model he argued that what was important functionally for an induced response was not that it was a directional response towards a phenotypic state that was necessarily more defended, but the change in phenotypic state was a way to generate variation. And it is the variance *per se* that functions defensively. When you're dealing with an information-based defence, which as Louise Vet has pointed out so many times, is not controlled by the plant — it is just thrown out and the rest of the ecological community responds in a highly complex way — there may be a lot of selection for generating variation in that signal. I wonder whether when we are designing analytical procedures to measure these responses, we are actually throwing away part of the functional component.

Vet: I think variation may mean something different for a herbivore than for their natural enemies. Learning seems to play an essential role for natural enemies. It may be that plants can indeed disturb the attraction of the herbivore by creating variation. And because the natural enemies are such good learners, they may pick up the essential variation and the signal may therefore still be of benefit to the plant. The plants may be able to exploit the differential response of herbivores and their natural enemies.

Firn: The fact that many volatiles are inducible by mechanical damage, herbivory or infection should not necessarily be regarded as remarkable. If organisms gain fitness by possessing mechanisms to generate and retain chemical diversity (Jones & Firn 1991), then it is predictable that mechanisms that give cost reductions will be selected.

One such mechanism would be inducibility. The linking of the control elements of the biosynthetic pathway with an appropriate sensor to give inducibility could occur early in the evolutionary history of the pathway. The gains from subsequent selection improving this inducibility of the overall pathway might be expected to exceed the gains of making each later stage of the pathway inducible. It might be expected that more than one sensor would be linked to the inducible elements such that different cues might all enhance the flux through the pathway, with different individual compounds in the resulting mixture conferring a benefit under different circumstances. This would explain why we have heard of so many stimuli that can induce volatile production (mechanical damage, insects, bacteria, fungi, etc.) and why hundreds of different compounds might be induced by any one stimulus.

Schultz: The variance idea is a more useful explanation for variation in resistance traits by plants that act on the herbivore. I think there's value in being undependable with respect to the herbivore. It is essentially a resistance management strategy: you make the resistance variable so the herbivore has trouble tracking it. In this case we want the parasitoid to track this variation.

Baldwin: But you cannot guarantee that only the parasitoid picks up on it. As long as you can't guarantee that, and you've got an organism (the parasitoid) which is potentially a quicker learner than another organism (the herbivore), perhaps changing what a plant emits is a way to make sure that it's only the parasitoid that picks it up.

Schultz: That's true, but the evidence so far is that the suite of plant compounds that herbivores make use of isn't the same as the suite used by the parasitoids.

Vet: Do we know that for sure?

Städler: There are few examples. As far as I am aware, those examples that we do have indicate that this long-range orientation of herbivores to host plants does not exist. It is very different from the parasitoid situation. I would speculate that host plants that put out such compounds would be wiped out pretty soon.

Dicke: But there are very few examples where it has been shown that parasitoids are attracted over a range of 50–100 m. Most of these studies have been done in the lab — there are hardly any field data on this.

Turlings: As far as the few examples of herbivores responding to induced volatiles are concerned, the only cases where you find real attractiveness is when you look at beetles. Other examples show more repellency. We have shown that aphids are repelled by induced volatiles of maize plants.

Dicke: Peter Landolt (1993) showed that there is attraction of cabbage looper moths towards plants infested by conspecific larvae, but when they oviposit, they will oviposit on the plants that are close by, but are not actually infested by herbivores. So that is still an initial attraction that is used to locate suitable host plants.

Turlings: He found for cotton that infested plants were attractive to the moth *Trichoplusia ni*, but it preferred to oviposit on undamaged plants. For cabbage he found that healthy plants were more attractive than plants damaged by conspecifics. In both cases the moth avoids ovipositing on plants that are already infested. I would call that repellency.

Tumlinson: We shouldn't forget the bark beetle situation, where attack on the tree by the beetles induces the tree to produce volatiles that attract large numbers of beetles.

Schultz: Do these volatiles overlap with parasitoid attractants?

Tumlinson: Yes, parasitoids are also attracted.

Dicke: I was surprised, because in the 1980s I published on the response of spider mites towards volatiles from spider mite-infested plants (Dicke 1986). In this case the spider mites were repelled, and I thought that would prove to be a more general result. But now I can come up with more examples where herbivores are attracted than where herbivores are repelled by infested plants (see Dicke & Vet 1998). It might have to do with the herbivore density and consequently the amounts of volatiles that are emitted anyway, as was the case in my study. When the amount of volatiles increases, it is a great risk to the plant because it exposes itself and so herbivores then could exploit these volatiles that are produced.

Pickett: In our studies, we have both situations. Some of the infestation signals are used as attractants, admittedly under artificial circumstances, and some are used as repellents. However, some of the repellents can be highly effective in the field.

Wadhams: The sort of compounds that we are looking at, as far as I can see, are very similar, I just don't have a good documented case of a parasitoid and a herbivore where we know that they are looking for exactly the same compounds.

Pickett: Collectively, I think we ought to look more at this. We have excellent model systems; we have simply not yet done the work. With the parasitoid *Aphidius ervi* and the pea aphid *Acyrthosiphon pisum* we can look very carefully to see exactly which compounds are involved.

Firn: Are there parasites of parasites?

Pickett: Yes. There exists a complicated guild of hyperparasitoids.

Firn: Do these parasites use plant volatiles to detect their hosts?

Wadhams: There's one compound in the bean aphid/parasitoid complex, which we think is particularly associated with the parasitoid recognizing the aphid/host plant complex, and this may also be a pheromone for the hyperparasitoid.

Vet: Perhaps there is a reason why you can expect herbivores to respond differently compared to their natural enemies, because herbivores tend to have just one host plant species. One trophic level up, parasitoids generally have more than one host. This by itself expands the plant range immediately. Now if you assume that a generalist insect needs to learn more than a specialist, you would expect that herbivores will use more specific plant cues than the parasitoids one trophic level up.

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