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The Significance of Background Odour for an Egg Parasitoid to Detect Plants with Host Eggs

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Abstract

Scots pine has been shown to produce a volatile bouquet that attracts egg parasitoids in response to oviposition of the herbivorous sawfly *Diprion pini*. Previous analyses of headspace volatiles of oviposition-induced pine twigs revealed only quantitative changes; in particular, the sesquiterpene (*E*)-β-farnesene was emitted in significantly higher quantities by oviposition-induced pine. Here we investigated whether (*E*)-β-farnesene attracted the egg parasitoid *Chrysonotomyia ruforum*. We tested the behavioural response of *C. ruforum* females to different concentrations of (*E*)-β-farnesene. Egg parasitoids did not respond to this sesquiterpene at either concentration tested. However, they did respond significantly to (*E*)-β-farnesene when this compound was offered in combination with the volatile blend emitted from pine twigs without eggs. This response was dependent on the applied concentration of (*E*)-β-farnesene. Further bioassays with other components [(*E*)-β-caryophyllene, δ-cadinene] of the odour blend of pine were conducted in combination with the volatile blend from egg-free pine as background odour. None of the compounds tested against the background of odour from an egg-free pine twig were attractive to the egg parasitoid. These results suggest that the egg parasitoids responded specifically to (*E*)-β-farnesene, but only when this compound was experienced in the 'right' context, i.e. when contrasted with a background odour of non-oviposition-induced pine volatiles.

Key words: *Diprion pini*, egg deposition, (*E*)-β-farnesene, induced defense, *Pinus sylvestris*, sesquiterpenes

Introduction

Carnivorous arthropods are known to use volatile cues that are released by plants infested with herbivorous insects (e.g. Dicke and van Loon, 2000; Hilker and Meiners, 2002; Hilker *et al.*, 2002b; Turlings *et al.*, 2002). Plants emit a complex blend of compounds which, in response to herbivory, may be altered either qualitatively or quantitatively (e.g. Dicke, 1999). Qualitative alterations comprise the production of novel compounds that are not emitted by uninfested plants. On the other hand, plants may also respond to herbivore infestation by emission of a volatile pattern that is qualitatively similar to the blend emitted by intact or mechanically damaged plants. In this case, the emission rate is much higher or the quantitative composition of the blend is changed (Dicke and van Poecke, 2002). Though herbivore-induced plant volatiles can provide carnivores like parasitoids with specific information about the attacking herbivore, the composition of plant volatile blends can also be highly variable (see the overview given by Dicke, 1999). The high geno- and phenotypic variability of herbivore-induced plant volatiles might limit the reliability of these cues for parasitoids (Dicke and Vet, 1999; Dicke and Hilker, 2003). Many parasitoids have the ability to learn plant odours associatively during a host encounter, thus enabling them to adjust their responses to varying host-related cues (Turlings *et al.*, 1993; Vet *et al.*, 1995).

For the understanding of chemical communication mechanisms in tritrophic systems, one of the most intriguing questions is which differences between herbivore-induced and non-induced volatile blends are used by parasitoids to discriminate the 'right' from the 'wrong' blend. In this study, we investigated this question for an egg parasitoid responding to plant volatiles induced by the egg deposition of its herbivorous host. Egg deposition of the herbivorous sawfly *Diprion pini* L. (Hymenoptera, Diprionidae) induced Scots pine (*Pinus sylvestris* L.) to emit volatiles that attract the specialized egg parasitoid *Chrysonotomyia ruforum* Krausse (Hymenoptera, Eulophidae) (Hilker *et al.*, 2002a). However, egg parasitoids first need to learn to respond to oviposition-induced pine volatiles (Mumm *et al.*, 2005).

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Volatiles from artificially wounded pines did not attract female *C. ruforum*. The artificial wounding mimicked the damage of the pine needles inflicted by sawfly females with their sclerotized ovipositor valves prior to egg deposition. Moreover, the application of jasmonic acid (JA) induced pines to emit volatiles that attracted females of *C. ruforum* (Hilker *et al.*, 2002a). This phytohormone is known to be widely involved in herbivore-induced defence responses in plants (reviewed by de Bruxelles and Roberts, 2001; Gatehouse, 2002; Schaller and Weiler, 2002), including coniferous trees (e.g. Martin *et al.*, 2002; Hudgins *et al.*, 2003). Several studies demonstrate that volatiles from pine might be involved in the attraction of parasitoids of pine feeding herbivores (Sullivan *et al.*, 2000; Völkl, 2000; Sullivan and Berisford, 2004).

In order to detect the pine volatiles induced by egg deposition of *D. pini*, we analysed the volatiles of the headspace of oviposition-induced pine twigs and compared them with volatiles from artificially wounded (non-attractive) pine twigs. Additionally, odour from JA-treated pine twigs was compared with odour from untreated and undamaged pine twigs. Neither egg deposition nor JA-treatment induced a qualitative change in the volatile blend of pine compared to the respective controls (Mumm *et al.*, 2003). Oviposition-induced and JA-treated pine twigs emitted the same compounds, i.e. mainly mono- and sesquiterpenes, as the respective controls. Except for one component, no significant quantitative changes were detected when comparing oviposition-induced twigs and the respective controls. However, the sesquiterpene (*E*)-β-farnesene was emitted in significantly higher amounts by oviposition-induced pine twigs compared to the controls (Mumm *et al.*, 2003). Interestingly, among three other sesquiterpenes (α-muurolene, γ-cadinene and δ-cadinene), (*E*)-β-farnesene was also emitted in significantly higher amounts by pine twigs treated with JA compared with untreated controls. Thus, (*E*)-β-farnesene was the only component that was released in significantly larger amounts from pine twigs induced by egg deposition and those treated with JA (Mumm *et al.*, 2003).

In this study, we investigated whether (*E*)-β-farnesene is utilized by *C. ruforum* as a chemical cue to locate pine infested with eggs of *D. pini*. The behavioural response of *C. ruforum* to different concentrations of (*E*)-β-farnesene was tested in an olfactometer. Background odour is known to affect the response of insects to single volatile components (Smith, 1998; Kelling *et al.*, 2002). Therefore, we hypothesized that *C. ruforum* shows a different behavioural response to (*E*)-β-farnesene if this is offered in combination with the natural volatile blend of the host plant.

In order to test whether the egg parasitoid's behavioural response which we detected for (*E*)-β-farnesene was specific for this sesquiterpene, the parasitoid's response to two further terpenoid components was also studied. (*E*)-β-Caryophyllene was chosen since it was identified as the predominating sesquiterpene in the headspace of attractive oviposition-induced and JA-treated pine (Mumm *et al.*, 2003). However, neither egg deposition nor treatment with JA resulted in an increased emission of (*E*)-β-caryophyllene compared with the respective controls (Mumm *et al.*, 2003). Furthermore, δ-cadinene was selected as test component since it was emitted in significantly higher amounts by the attractive twigs treated with JA when compared with untreated controls. However, oviposition-induced twigs did not release higher amounts of this terpene (Mumm *et al.*, 2003). (*E*)-β-Caryophyllene and δ-cadinene were both tested on the background of volatiles from pine twigs without eggs.

Materials and methods

Plants and insects

Branches of *P. sylvestris* L. used for experiments and rearing were detached from crowns of 15- to 35-year-old trees in the forests near Berlin. All stems were cleaned and sterilized according to the method of Moore and Clark (1968). *Diprion pini* L. was reared continuously in the laboratory on cut pine twigs as described by Bombosch and Ramakers (1976) and Eichhorn (1976) at 25 ± 1°C, L/D 18:6 h, and 70% relative humidity. The egg parasitoid *C. ruforum* Krausse was obtained from parasitized eggs of *D. pini* and *Neodiprion sertifer* Geoffroy collected in the field in France (near Fontainebleau) and central and southern Finland. The origin of the egg parasitoid is known to have no effect on its response to odours such as host sex pheromones (Hilker *et al.*, 2000). Thus, we did not separate parasitoids from the different collection sites. Parasitized eggs were kept in Petri dishes (i.d. 9 cm) in a climate chamber at 10°C. To induce parasitoid emergence, needles with parasitized eggs were placed in a climate chamber at 25°C, with an 18:6 L:D photoperiod and 70% relative humidity. Emerging adults were collected daily and transferred in small Perspex tubes (75 mm long, 15 mm i.d.) covered with gauze at one end. A cotton-wool plug moistened with an aqueous honey solution closed the other end. The parasitoids were kept at 10°C, 18:6 L:D, until they were used for bioassays.

Naive egg parasitoids were shown not to respond to oviposition-induced pine volatiles (Mumm *et al.*, 2005). Therefore, parasitoid females used for the bioassays were experienced with the plant–host complex as described by Mumm *et al.* (2005). Two days prior to the bioassays, parasitoids were given contact with male parasitoids and a plant–host complex, consisting of a pine twig carrying eggs of *D. pini*, adult sawflies, and a cotton-wool pad with aqueous honey solution. After 24 h, parasitoids were removed from the plant–host complex and kept in clean Perspex tubes provided with the aqueous honey solution only for another 24 h prior to the experiments.

Olfactometer bioassay—general procedures and data collection

All bioassays were conducted in a four-arm olfactometer (Pettersson, 1970; Vet *et al.*, 1983) as described in detail by

Hilker *et al.* (2002a). The airflow was adjusted to 155 ml/min. When starting a bioassay, a parasitoid female was introduced into the arena of the olfactometer. We recorded how long the parasitoid spent walking within each of the four odour fields over a period of 600 s using the Observer program 3.0 (Noldus, Wageningen, The Netherlands). Data obtained from parasitoids that walked for <300 s were discarded.

Chemicals

(*E*)-β-Farnesene was synthesized according to a modified procedure of Kang *et al.* (1987). To a solution of farnesyl chloride (0.25 g, 1 mM, Aldrich, Steilheim, Germany) in dry THF (5 ml, Aldrich), 18-crown-8-ether (0.53 g, 2 mmol, Aldrich) was added followed by potassium-*tert*-butoxide (KOtBu, 1.1 g, 10 mM, Aldrich). The reaction mixture was stirred at 40°C for 24 h. To stop the reaction, the solution was mixed with 30 ml *tert*-butyl methylether, washed with water and saturated NaCl solution. The organic phase was dried over Na_2SO_4 sicc. and the solvent was removed *in vacuo*. The crude product was separated and purified by solid phase extraction with silica gel as stationary phase. (*E*)-β-Farnesene was eluted with n-hexane:ethyl acetate (9.5:0.5, v/v). With this method, (*E*)-β-farnesene of 99% purity with a recovery rate of 37% was obtained. The other terpenes were purchased as reference compounds from commercial companies: (*E*)-β-caryophyllene (80%; Aldrich), and δ-cadinene (>97%; Fluka, Buchs, Switzerland).

Plant treatments

We tested whether single terpene components attracted female egg parasitoids when offered in combination with odour from pine twigs that were not induced by egg deposition but were artificially wounded instead. Artificially wounded pine twigs were taken as background odour because the chemical analyses had compared the headspace of oviposition-induced twigs and artificially wounded ones (Mumm *et al.*, 2003). Oviposition-induced twigs were not compared just with untreated twigs, since wounding of pine needles which occurs also naturally during egg deposition might change the composition of volatiles. However, the behavioural bioassays had revealed that odour from wounded twigs did not attract the egg parasitoids (Hilker *et al.*, 2002a). Thus, a comparison of the headspace of wounded twigs and oviposition-induced ones made it possible to exclude all components released due to the pure wounding as potential key components causing the attractiveness.

For our experiments, small pine twigs (10–12 cm) were cut and provided with tap water. The treatment of the artificially wounded pine twigs were conducted as described by Mumm *et al.* (2003). Eight pine needles of a twig were slit tangentially with a clean insect needle. Wounded pine twigs were placed in water for 72 h at 25°C, 18:6 L:D and 60% relative humidity. Prior to the bioassay, a ~5 cm long part of the twigs was cut and wrapped tightly with Parafilm®. This part of the twig was placed into the odour source flask of the olfactometer.

Response of egg parasitoids to (*E*)-β-farnesene

(*E*)-β-Farnesene was diluted in n-hexane (Roth, Karlsruhe, Germany). In all experiments, 0.1 ml of the diluted (*E*)-β-farnesene was applied to a piece of filter paper (~16 cm^2). After 90 s, when most of the solvent had evaporated, the filter paper was placed into a glass flask (250 ml) of the olfactometer. Purified air flowing into the test field of the olfactometer passed through the flask which contained the odour sample. A filter paper with 0.1 ml hexane was placed into glass jars providing the odour for each of the three control fields (1–3). A female egg parasitoid was then introduced into the olfactometer. New filter papers with terpenes or hexane were used for each parasitoid. Three different concentrations were tested: 0.01, 0.1, and 1 μg/μl hexane.

Response of egg parasitoids to single terpenes in combination with pine volatiles

In this experiment, we assessed the responses of egg parasitoids to (*E*)-β-farnesene and two other sesquiterpenes [(*E*)-β-caryophyllene, δ-cadinene] that were offered in combination with pine volatiles. Pine twigs were artificially wounded as described above. Volatiles of artificially wounded pine twigs were mixed with single sesquiterpenes and offered in the test field of the olfactometer. For this setup, the olfactometer was modified as follows: the air flow of the test field was split and was passed through a glass flask (250 ml) containing the artificially wounded pine twig and through a second flask which contained the filter paper with a terpene. After the air had passed the two sample flasks, the air flows were joined together again. Thus, volatiles of the pine twig and the applied terpenes were mixed just before entering the arena of the olfactometer. Control fields were provided with 0.1 ml hexane. A volume of 0.1 ml of the tested terpenes or solvent was applied to filter paper disks (~16 cm^2). (*E*)-β-Farnesene was tested in concentrations of 0.01, 0.1 and 1 μg/μl hexane, (*E*)-β-caryophyllene and δ-cadinene were tested in concentrations of 0.1 and 1 μg/μl hexane. New filter paper disks with terpenes or hexane were used for each parasitoid. Each artificially wounded pine twig was changed after 8–10 parasitoids tested.

Data analysis

Data were statistically evaluated using the Friedman ANOVA and the Wilcoxon–Wilcox test for multiple comparisons (Köhler *et al.*, 1995) using the software program SPSS 11.0 (SPSS Inc., USA). Statistical significant response to test odour became especially evident when duration of parasitoid walking within the test odour field was strongly different from duration of walking within the opposite control field (i.e. field 2; compare Tables 1 and 2).

Table 1 Response of female egg parasitoids *C. ruforum* to different concentrations of synthetic (*E*)-β-farnesene alone (left) and offered in combination with volatiles from non-oviposition-induced *Pinus sylvestris* twigs (right)

(*E*)-β-farnesene							(*E*)-β-farnesene combined with pine volatiles					
Concentration	Walking duration [s]				*n*	Statistics	Walking duration [s]				*n*	Statistics
	T	1	2	3			T	1	2	3		
0.01 μg/μl	103 (75–214)	125 (61–210)	114 (27–214)	48 (24–165)	20	n.s.	100 (49–201)	158 (74–261)	101 (58–185)	90 (21–141)	27	n.s.
0.1 μg/μl	58 (26–160)	71 (33–227)	98 (62–263)	79 (58–156)	20	n.s.	141^{a} (94–282)	112^{ab} (36–210)	37^{b} (13–152)	24^{b} (0–76)	21	$P = 0.003$
1 μg/μl	98 (47–157)	73 (17–180)	95 (48–148)	147 (49–221)	22	n.s.	78^{a} (15–133)	113^{ab} (51–226)	155^{b} (114–197)	92^{ab} (34–134)	21	$P = 0.007$

Median values and interquartile range (parentheses) of the time parasitoid females spent in test (T) and control fields (1–3) of a four-arm olfactometer are given. Test field with 0.1 ml of (*E*)-β-farnesene or 0.1 ml of (*E*)-β-farnesene with pine volatiles; (*E*)-β-farnesene was applied on filter paper; 1, 2, 3, = three control fields with 0.1 ml of hexane (solvent) applied on filter paper. n.s., non-significant ($P > 0.05$) difference evaluated by a Friedman analysis of variance. Different letters indicate significant ($P < 0.05$) differences evaluated by the Wilcoxon–Wilcox test.

Table 2 Response of female egg parasitoids *C. ruforum* to different concentrations of synthetic sesquiterpenes (*E*)-β-caryophyllene and δ-cadinene offered in combination with volatiles from non-oviposition-induced *Pinus sylvestris* twigs

Concentration		Walking duration [s]				*n*	Statistics
		T	1	2	3		
0.1 μg/μl	(*E*)-β-caryophyllene	130 (73–216)	72 (6–147)	44 (13–122)	103 (12–268)	25	n.s. (p=0.24)
	δ-cadinene	196 (77–243)	101 (51–195)	101 (41–170)	140 (60–194)	31	n.s. (p=0.08)
1 μg/μl	(*E*)-β-caryophyllene	144 (59–229)	122 (46–189)	95 (13–145)	101 (8–169)	22	n.s. (p=0.40)
	δ-cadinene	153 (66–292)	148 (80–203)	95 (54–111)	98 (58–216)	23	n.s. (p=0.24)

Median values and interquartile range (parentheses) of the time parasitoid females spent in test (T) and control fields (1–3) of a four-arm olfactometer are given. Test field with 0.1 ml of the sesquiterpenes with pine volatiles; (*E*)-β-farnesene was applied on filter paper; 1, 2, 3, = three control fields with 0.1 ml of hexane (solvent) applied on filter paper. n.s., non-significant ($P > 0.05$) difference evaluated by a Friedman analysis of variance.

Results

Response of egg parasitoids to (*E*)-β-farnesene

Female egg parasitoids showed no significant response to any of the applied concentrations (0.01, 0.1 and 1 μg/μl) of (*E*)-β-farnesene (Table 1). Therefore, (*E*)-β-farnesene *per se* was neither attractive nor repellent to the egg parasitoids at the concentrations tested.

Response of egg parasitoids (*E*)-β-farnesene in combination with pine volatiles

In order to elucidate whether *C. ruforum* responds to (*E*)-β-farnesene together with pine volatiles as background odour, we offered (*E*)-β-farnesene in combination with volatiles of artificially wounded pines. Volatiles of artificially wounded pine twigs were not attractive *per se* for female *C. ruforum* (Hilker *et al.*, 2002a). When offered together with volatiles from artificially wounded twigs, (*E*)-β-farnesene applied at a concentration of 0.01 μg/μl elicited no significant response in *C. ruforum.* In contrast, a 10-fold higher concentration (0.1 μg/μl) of (*E*)-β-farnesene combined with pine volatiles significantly attracted egg parasitoids (Table 1). However, when (*E*)-β-farnesene was offered at a concentration of 1 μg/μl on the background of volatiles from a wounded pine twig, the attractiveness switched to a repellent effect. Parasitoids avoided the test-field in the olfactometer compared with the control fields (Table 1). Thus, there is a dose-dependent effect of (*E*)-β-farnesene combined with volatiles of artificially wounded pine.

Response of egg parasitoids to single sesquiterpenes in combination with pine volatiles

To test whether the parasitoids' response to (*E*)-β-farnesene in combination with pine volatiles is specific for this compound, we used two other sesquiterpenes present in the headspace of *P. sylvestris*, i.e. (*E*)-β-caryophyllene and δ-cadinene. The sesquiterpenes (*E*)-β-caryophyllene and δ-cadinene were tested in combination with volatiles from wounded pine twigs at the concentrations at which (*E*)-β-farnesene was active (0.1 and 1 μg/μl). The egg parasitoid *C. ruforum* did not respond to the 0.1 μg/μl concentration of (*E*)-β-caryophyllene in the olfactometer (Table 2). δ-Cadinene tended to attract *C. ruforum* at the 0.1 μl/μg concentration, but this was not statistically significant (Table 2). The 1 μg/μl concentration of (*E*)-β-caryophyllene or δ-cadinene had neither an attractive nor a repellent effect to the egg parasitoids (Table 2).

Discussion

Females of the egg parasitoid *C. ruforum* were not attracted by different concentrations of (*E*)-β-farnesene alone. However, when this component was offered against a background of a non-attractive natural blend of pine volatiles, this combination became attractive when tested at the intermediate concentration. The combination became repellent when tested with a high concentration of (*E*)-β-farnesene. When combining the non-attractive natural blend of pine volatiles with other terpenoid components than (*E*)-β-farnesene, no such effects were detected at either concentration tested.

Rutledge (1996) gives an overview of studies which show that single volatile constituents of the host plant were able to attract parasitoids. (*E*)-β-Farnesene is a common sesquiterpene that is released by many plants and also herbivorous insects, e.g. aphids (Nault and Bowers, 1974). Numerous studies have shown increased amounts or *de novo* production of this sesquiterpene in plants induced by feeding herbivores (e.g. Bolter *et al.*, 1997; De Moraes *et al.*, 1998; Turlings *et al.*, 1998; Gols *et al.*, 1999; Paré and Tumlinson, 1999; Röse and Tumlinson, 2004), by mechanical damage (e.g. McAuslane and Alborn, 1998) and by treatment with JA (e.g. Rodriguez-Saona *et al.*, 2001; Schmelz *et al.*, 2001). Numerous insect species are able to respond to (*E*)-β-farnesene, including herbivorous insects (e.g. Koshier *et al.*, 2000; Bengtsson *et al.*, 2001). Both predators and parasitoids show EAG responses (e.g. Al Abassi *et al.*, 2000; Du *et al.*, 1998; Weissbecker *et al.*, 2000; Zhu *et al.*, 1999) as well as behaviourally responses towards (*E*)-β-farnesene (Du *et al.*, 1998; Francis *et al.*, 2004; Micha and Wyss, 1996). However, in our study we could not show any behavioural response of *C. ruforum* to (*E*)-β-farnesene *per se.*

Synergistic effects of (*E*)-β-farnesene with other infochemicals mediating insect–plant interactions are well known. For example, (*E*)-β-farnesene emitted by apples (*Malus* spp.) acts as a synergist by significantly enhancing the attractiveness of the sex pheromone of *Cydia pomonella* (codlemone) for male codling moths (Yang *et al.*, 2004). On the other hand, the response of the aphid *Lipaphis erysimi* to its alarm pheromone (*E*)-β-farnesene was significantly increased when (*E*)-β-farnesene was combined with plant-derived isothiocyanates (Dawson *et al.*, 1987). However, in all these cases at least two different compounds that originate from different trophic levels (host plant and herbivore) were combined and then act in an additive or synergistic way.

This is a different to the results in our experiments, where qualitatively no novel volatile mixture was composed, but only the ratio of a single compound ((*E*)-β-farnesene) to the whole blend was changed. (*E*)-β-Farnesene is present in the headspace of untreated pine twigs, artificially damaged ones, JA-treated ones and oviposition-induced ones. Only the two latter types emit odour that is attractive to the egg parasitoid (Mumm *et al.*, 2003). Thus, the attractive effect of the combination of (*E*)-β-farnesene at its intermediate concentration and the natural volatile blend of a non-attractive pine twig is not due to a synergism *sensu stricto* because neither the pure (*E*)-β-farnesene nor the volatiles of artificially wounded pines were attractive *per se* to the parasitoids.

Recently, two further tritrophic studies showed the importance of plant background odour when testing the behavioural response of carnivorous arthropods to single components of the natural plant headspace or to a mixture of specific components of the natural blend: (i) a synthetic mixture of volatiles composed of constituents of the headspace of spruce logs (*Picea abies*) infested by bark beetles was only as attractive as infested logs for parasitoids of bark beetles when this mixture was offered at the background of odour from uninfested logs. Neither the synthetic mixture nor the odours of uninfested logs were attractive *per se* (Pettersson, 2001; Pettersson *et al.*, 2001); and (ii) lima bean plants (*Phaseolus lunatus*) infested by spider mites (*Tetranychus urticae*) are known to produce methyl salicylate, thus attracting predatory mites (de Boer and Dicke, 2004). Methyl salicylate at low and intermediate concentrations did not attract predatory mites, nor did volatiles from uninfested lima bean plants. However, when methyl salicylate was offered against a background of odour from uninfested plants, this combination attracted the predatory mites.

The attractiveness of (*E*)-β-farnesene mixed with non-oviposition-induced pine volatiles was dose-dependent. Only the concentration of 100 ng/μl (*E*)-β-farnesene attracted *C. ruforum* females, whereas 10 ng/μl did not. Electrophysiological studies of the antennal olfactory system of *Musca domestica* revealed that background odour could increase the response of the antenna to low concentrations of odour pulses, whereas responses to higher concentrations of volatile stimuli decreased when a background odour was present (Kelling *et al.*, 2002).

We do not know what physiological mechanisms are responsible for the behavioural effects that we have detected here. Our results show that background odour was essential for the egg parasitoid to respond behaviourally to (*E*)-β-farnesene. Our data suggest that *C. ruforum* is comparing the 'contrast' between this sesquiterpene and other pine volatiles. Similarly, the ability of hymenopteran insects to detect and discriminate different colour patterns is primarily based on the contrast of single colours rather than specific colour characteristics (Hempel *et al.*, 2002; Fischer *et al.*, 2003, 2004). However, bees detected and discriminated specific colour patterns better than others, although the contrast of the colours was the same (Hempel *et al.*, 2001, 2002). A specific ratio of (*E*)-β-farnesene within a pine volatile background might 'tell' the egg parasitoid where to find host eggs. If the ratio is 'wrong', i.e. either too low (as in the experiment with 10 ng/μl) or too high (1 μg/μl), parasitoids may not show a response or as in the latter case were even repelled. Further studies need to investigate whether the whole pine volatile bouquet is necessary as the background odour to elicit the behavioural response of the egg parasitoids or whether a combination of single (key) components with (*E*)-β-farnesene elicit adequate responses in *C. ruforum*, as suggested for other parasitoids (Vet *et al.*, 1998; Dicke and Vet, 1999).

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