

Choosy egg parasitoids: Specificity of oviposition-induced pine volatiles exploited by an egg parasitoid of pine sawflies

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Abstract

Generalist parasitoids are well-known to be able to cope with the high genotypic and phenotypic plasticity of plant volatiles by learning odours during their host encounters. In contrast, specialised parasitoids often respond innately to host-specific cues. Previous studies have shown that females of the specialised egg parasitoid *Chrysonotomyia ruforum* Krausse (Hymenoptera: Eulophidae) are attracted to volatiles from *Pinus sylvestris* L. induced by the egg deposition of its host *Diprion pini* L. (Hymenoptera: Diprionidae), when they have previously experienced pine twigs with host eggs. In this study we investigated by olfactometer bioassays how specifically *C. ruforum* responded to oviposition-induced plant volatiles. Furthermore, we studied whether parasitoids show an innate response to oviposition-induced pine volatiles. Naïve parasitoids were not attracted to oviposition-induced pine volatiles. The attractiveness of volatiles from pines carrying eggs was shown to be specific for the pine and herbivore species, respectively (species specificity). We also tested whether not only oviposition, but also larval feeding, induces attractive volatiles (developmental stage specificity). The feeding of *D. pini* larvae did not induce the emission of *P. sylvestris* volatiles attractive to the egg parasitoid. Our results show that a specialist egg parasitoid does not innately show a positive response to oviposition-induced plant volatiles, but needs to learn them. Furthermore, the results show that *C. ruforum* as a specialist does not learn a wide range of volatiles as some generalists do, but instead learns only a very specific oviposition-induced plant volatile pattern, i.e., a pattern induced by the most preferred host species laying eggs on the most preferred food plant.

Introduction

Parasitoids of herbivores are known to use plant volatiles when foraging for hosts (e.g., Vinson, 1991; Vet & Dicke, 1992; Rutledge, 1996; Steidle & van Loon, 2003). Herbivore damage caused by feeding or egg deposition may induce qualitative and quantitative changes in the plant's volatile blend (e.g., Dicke, 1994; Turlings & Fritzsche, 1999; Dicke & van Loon, 2000; Hilker & Meiners, 2002). Such induced plant volatiles may be specific for a herbivore species (e.g., De Moraes et al., 1998; Du et al., 1998; Turlings et al., 1998,

2002) or a particular developmental stage of the herbivore (e.g., Takabayashi et al., 1995; Gouinguéné et al., 2003). Moreover, even related plant species or conspecific varieties attacked by the same herbivore species emit specific volatile patterns (Takabayashi & Dicke, 1996; Geervliet et al., 1997; Gouinguéné et al., 2001). The ability of parasitoids to discriminate between specific plant volatile blends has been reported for numerous tritrophic systems (reviewed by Dicke, 1999; Fritzsche-Hoballah et al., 2002).

The high geno- and phenotypic variability of herbivore-induced plant volatiles might limit the reliability of these cues for parasitoids to find a suitable host (reviewed by Dicke & Vet, 1999; Dicke & Hilker, 2003). Parasitoids show either a fixed innate response to specific cues or deal with this variability by learning odours associatively when encountering hosts, thus enabling them to adjust or

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reinforce their responses to changes in host-related cues (Turlings et al., 1993; Vet et al., 1995). Specialised parasitoids, attacking a few herbivore species that feed upon only few plant species, are generally thought to innately respond to infochemicals and to use primarily specific cues for finding a host (Vet & Dicke, 1992; Steidle & van Loon, 2003).

In a previous study, Hilker et al. (2002) demonstrated that the egg parasitoid *Chrysonotomyia ruforum* (Krausse) (Hymenoptera: Eulophidae) was attracted to volatiles from *Pinus sylvestris* L. (Pinales: Pinaceae) after egg deposition of the herbivorous sawfly *Diprion pini* L. (Hymenoptera: Diprionidae). In contrast, volatiles from pine twigs without eggs that were artificially wounded to mimic the mechanical damage inflicted by the oviposition of the female sawfly were not attractive to *C. ruforum* (Hilker et al., 2002). Oviposition by *D. pini* induces not only a local response, but also a systemic reaction in pine needles without eggs but adjacent to those carrying sawfly eggs. Thus, egg parasitoids were attracted to pine volatiles that were induced by egg deposition (Hilker et al., 2002).

The host range of *C. ruforum* is restricted to eggs of members of the subfamily Diprioninae (Pschorn-Walcher & Eichhorn, 1973; Eichhorn & Pschorn-Walcher, 1976) that mainly feed on pines (*Pinus* spp.) in Europe (Pschorn-Walcher, 1982). According to the definition given by Steidle & van Loon (2003), the egg parasitoid *C. ruforum* is therefore considered as a specialist regarding the host and the host plant level.

In this study we investigated the specificity of chemical cues used by the egg parasitoid *C. ruforum* for host search. First, we investigated whether female egg parasitoids responded innately to oviposition-induced pine volatiles. Second, we tested whether the induction of plant volatiles by egg deposition was specific for the plant or herbivore species (species specificity). In addition, we studied whether larval feeding also induces volatiles in *P. sylvestris* that attract female *C. ruforum*, as do eggs of *D. pini* (developmental stage specificity).

To examine the plant specificity of this tritrophic interaction, we tested whether volatiles from the Austrian black pine (*Pinus nigra* Arnold var. *nigra*) carrying eggs of *D. pini*, attract *C. ruforum* females. It has been reported that *D. pini* accepts *P. nigra* as a host for oviposition (Eliescu, 1932; Zivojinovic, 1954; Auger et al., 1994; Barre et al., 2002). It has also been reported that eggs of *D. pini* laid on *P. nigra* are attacked by *C. ruforum* (Zivojinovic, 1954). *Pinus nigra* var. *nigra* is naturally distributed mainly in south-eastern Europe (Austria, Italy, Balkan Peninsula) but has been cultivated for parks and forests worldwide (Krüssmann, 1983; Schütt et al., 1992; Rafii et al., 1996).

Herbivore specificity was studied by testing the response of *C. ruforum* to volatiles from *P. sylvestris* twigs on which eggs of *Gilpinia pallida* Klug or *Neodiprion sertifer* Geoffroy were deposited. The diprionid species are frequently occurring herbivorous sawflies of Scots pine (*P. sylvestris*) (Pschorn-Walcher, 1982). Eggs of *N. sertifer* and *G. pallida* are both known to be suitable hosts for *C. ruforum* (Pschorn-Walcher & Eichhorn, 1973; Pschorn-Walcher, 1988).

Materials and methods

Plants and insects

Branches of *P. sylvestris* used for the experiments and insect rearing were detached from crowns of 15- to 35-year-old trees in the forests near Berlin. Branches from *P. nigra* var. *nigra* were cut from a 14-year-old pine stand near Berlin. For the experiments, small twigs were detached from these branches (see below for further details). For the rearing of sawflies, all stems were cleaned and sterilized according to Moore & Clark's method (1968).

Diprion pini was reared continuously in the laboratory on cut pine twigs, as described by Bombosch & Ramakers (1976) and Eichhorn (1976) at 25 ± 1 °C, L18:D6 photoperiod, and 70% r.h. The laboratory culture of *D. pini* was started with cocoons collected in the field in France (by C. Géri, INRA, Orléans). Adults of *G. pallida* were reared from cocoons (collected in the field in Finland) under the same conditions as described for *D. pini*. *Neodiprion sertifer* adults emerged from cocoons collected by A. Martini (University of Bologna, Italy) from pine trees in Italy. A continuous laboratory rearing of this species was not established.

The egg parasitoid *C. ruforum* was obtained from parasitized eggs of *D. pini* and *N. sertifer* collected in the field in France (near Fontainebleau) and in southern and central Finland. Parasitized eggs were kept in Petri dishes (9 cm inner diameter) in a climate chamber at 10 °C, L18:D6 photoperiod, and 70% r.h. To induce parasitoid emergence, needles with parasitized eggs were placed in a climate chamber at 25 °C, L18:D6 photoperiod, and 70% r.h. Emerging adults were collected daily and transferred in small perspex tubes (75 mm long, 15 mm in diameter) covered with gauze at one end. A cotton-wool plug moistened with an aqueous honey solution closed the other end. About 20 male and 20 female parasitoids were confined per tube. Mating was normally observed soon after emergence. The parasitoids were kept at 10 °C and L18:D6 until they were used for bioassays. Parasitoids used for bioassays were 2–16 days old.

To test whether the response to volatiles from differently treated pine twigs was dependent on the plant–host complex

experienced by the parasitoids, two types of treatments were investigated: (i) parasitoids were given the chance to experience the same plant–host complex that was offered in the olfactometer (within experiment), or the (ii) experienced and tested plant–host complexes were different (cross-experiment). Naïve wasps had no contact with unparasitized sawfly eggs prior to the experiments. When testing experienced female parasitoids, these had prior contact with unparasitized sawfly eggs on pine twigs for a period of 24 h. After this exposure time, female parasitoids were kept isolated from host eggs on pine twigs for 24 h in a Petri dish (see Hilker et al., 2002). This lag period was thought to enhance the parasitoid's motivation to forage for hosts. It was previously shown that the responsiveness of *C. ruforum* to oviposition-induced pine volatiles was not negatively influenced by this procedure (Hilker et al., 2002).

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. (2002). The airflow was adjusted to 155 ml min⁻¹. When starting a bioassay, a parasitoid female was introduced into the arena of the olfactometer. We recorded how much time the parasitoid was present within each of the four odour fields over a period of 600 s using the Observer program 3.0 (Noldus Information Technology, Wageningen, The Netherlands). Data obtained from parasitoids that walked for less than 300 s were discarded. For each treatment, 22–40 parasitoids and 4–6 plant samples were tested. Data were statistically evaluated using a Friedman ANOVA and the Wilcoxon–Wilcox test for multiple comparisons (Köhler et al., 1995) using the software program SPSS 11.0. (SPSS Inc., USA). We termed an odour 'attractive' when the parasitoid preferred walking in the olfactometer field provided with this odour, as significantly longer walking periods in the odour field are usually interpreted as a response of the parasitoid to an attractive odour (Hilker et al., 2002).

Plant treatments general

Small pine twigs (10–15 cm) of *P. sylvestris* or *P. nigra* laden with host eggs were obtained, similarly to the method described by Hilker et al. (2002). Two female and two male sawflies were confined with the twigs that were provided with tap water in a glass cylinder (height 22 cm, inner diameter 15 cm) covered by a gauze lid. Females were allowed to mate and to lay eggs. After a period of 72 h, the sawflies were removed from the twigs. Twigs carrying at least four egg masses were removed from the tap water and then tightly wrapped with parafilm at the cut end when used for the bioassays.

Species specificity

In order to investigate whether the emission of attractive plant volatiles induced by oviposition is specific for the tritrophic system *P. sylvestris*–*D. pini*–*C. ruforum*, we tested the attractiveness of the volatiles from pine twigs subjected to the following treatments, changing either the plant or herbivore species or both:

(a) *Plant specificity.* To study how *C. ruforum* reacts to volatiles from egg-laden twigs other than from *P. sylvestris* (Hilker et al., 2002), the response of experienced parasitoid females (see above) to volatiles from twigs of *P. nigra* var. *nigra* carrying eggs of *D. pini* was tested. Twigs were treated as described above.

(b) *Herbivore specificity.* We tested whether egg deposition by *G. pallida* or *N. sertifer* induces *P. sylvestris* to emit volatiles that attract the egg parasitoid *C. ruforum*. Pine twigs that were carrying at least four egg masses of these species for a period of 72 h were used for the bioassays as described above.

Developmental stage specificity

To investigate whether not only oviposition, but also feeding of sawfly larvae induces the emission of volatiles attractive to the egg parasitoids, two different bioassays were performed. First, 25 young *D. pini* larvae (L1 and L2) were allowed to feed on small *P. sylvestris* twigs (ca. 10 cm long) for 24 h. Pine twigs were used for olfactometer bioassays immediately after removal of the larvae. In contrast to the treatment period of 72 h used for the oviposition-exposed plants, we reduced the period of treatment in these feeding-exposed plants because the twigs would otherwise have been completely consumed.

In order to carry out an experiment after a treatment period of 72 h, we modified the set-up as follows. We used the fact that oviposition-induced volatiles are not only emitted locally at the site of egg deposition, but also systemically in adjacent, egg-free parts of a twig after 72 h (Hilker et al., 2002). If larval feeding induces attractive volatiles in pine twigs, we hypothesized that this response would also be systemic. Therefore, we modified the treatment procedure and used a method for systemic induction described by Hilker et al. (2002): 25 young larvae of *D. pini* (L1 and L2) were placed on the lower half of a pine twig (ca. 15 cm long), while the upper half of the twig was covered with polyethylene terephthalate (PET) foil to prevent feeding. The bag was ventilated with purified air through an in- and outlet. After a feeding period of 72 h, the upper half of the twig was cut and the foil was removed. The cut end of the upper twig was tightly wrapped with Parafilm®. The response of *C. ruforum* females to volatiles from the upper part of the twig was tested in the olfactometer.

Table 1 Parasitoid experience. Responses of naïve (A1) or experienced (A2) *Chrysonotomyia ruforum* females to volatiles from *Pinus* twigs induced by egg deposition of *Diprion pini* offered in a test field (Test) of a four-arm olfactometer. Control fields (1–3) were supplied with clean air. The time that the parasitoid females were present in the test and control fields are given over an observation period of 600 s. Median values and interquartile ranges (parentheses) are given

		Plant/host complex during experience	Plant/host complex for bioassay	Duration of stay (s)			n	Statistics	
				Test	Control fields				
Naïve	A1	–	<i>P. sylvestris</i> – <i>D. pini</i>	95 (24–283)	113 (19–280)	108 (33–326)	86 (6–154)	22	ns (P = 0.274)
Experienced	A2	<i>P. sylvestris</i> – <i>D. pini</i>	<i>P. sylvestris</i> – <i>D. pini</i>	387 ^a (331–437)	45 ^b (15–98)	33 ^b (10–62)	116 ^b (52–166)	27	*** (P < 0.001)

***Indicates a significant (P < 0.001), and ns a non-significant (P > 0.05) difference evaluated by a Friedman ANOVA. Different letters indicate significant (P < 0.001) differences evaluated by the Wilcoxon–Wilcox test.

Results

Significance of parasitoid experience

Naïve *C. ruforum* females were not attracted to *P. sylvestris* volatiles induced by oviposition of *D. pini* (Table 1, A1). On the other hand, egg parasitoids that had previous experience with this plant–host complex were significantly attracted to oviposition-induced pine volatiles (Table 1, A2). Thus, a previous access to a plant–host complex was essential for *C. ruforum* to learn to respond to oviposition-

induced pine volatiles. Therefore, all the following experiments were only conducted with experienced parasitoids.

Species specificity

Volatiles from *P. nigra* with eggs of *D. pini* did not attract female *C. ruforum*, even though the parasitoids had experienced the same plant–host complex prior to the bioassay (Table 2, B1). Neither were volatiles from *P. sylvestris* carrying eggs of *G. pallida* attractive to female *C. ruforum* (Table 2, B2). In contrast, parasitoids were significantly

Table 2 Species specificity of oviposition-induced plant volatiles. Responses of experienced (B1–3, C1–4) *Chrysonotomyia ruforum* females to volatiles from twigs of two *Pinus* species carrying eggs of different sawfly species offered in a test field (Test) of a four-arm olfactometer. Control fields (1–3) were supplied with clean air. The time the parasitoid females were present in the test and control fields are given over an observation period of 600 s. Median values and interquartile ranges (parentheses) are given

		Plant/host complex during experience	Plant/host complex for bioassay	Duration of stay (s)			n	Statistics	
				Test	Control fields				
Within experiments	B1	<i>P. nigra</i> – <i>D. pini</i>	<i>P. nigra</i> – <i>D. pini</i>	95 (49–134)	211 (87–295)	186 (74–251)	69 (20–177)	25	ns (P = 0.069)
	B2	<i>P. sylvestris</i> – <i>G. pallida</i>	<i>P. sylvestris</i> – <i>G. pallida</i>	161 (73–216)	107 (59–170)	148 (54–242)	139 (76–236)	30	ns (P = 0.378)
	B3	<i>P. sylvestris</i> – <i>N. sertifer</i>	<i>P. sylvestris</i> – <i>N. sertifer</i>	209 ^a (140–403)	69 ^{ab} (14–167)	104 ^b (0–176)	83 ^b (38–155)	23	** (P = 0.006)
Cross experiments	C1	<i>P. sylvestris</i> – <i>D. pini</i>	<i>P. nigra</i> – <i>D. pini</i>	92 (39–188)	164 (39–188)	136 (42–227)	102 (29–186)	27	ns (P = 0.39)
	C2	<i>P. nigra</i> – <i>D. pini</i>	<i>P. sylvestris</i> – <i>D. pini</i>	136 (68–206)	147 (82–182)	139 (82–227)	116 (93–165)	26	ns (P = 0.93)
	C3	<i>P. sylvestris</i> – <i>D. pini</i>	<i>P. sylvestris</i> – <i>G. pallida</i>	113 (82–196)	160 (92–214)	138 (87–220)	96 (56–117)	25	ns (P = 0.336)
	C4	<i>P. sylvestris</i> – <i>G. pallida</i>	<i>P. sylvestris</i> – <i>D. pini</i>	115 (55–232)	101 (40–205)	89 (44–189)	176 (107–240)	28	ns (P = 0.296)

**Indicates a significant (P < 0.01) and ns a non-significant (P > 0.05) difference evaluated by a Friedman ANOVA. Different letters indicate significant (P < 0.05) differences evaluated by the Wilcoxon–Wilcox test.

attracted to volatiles from *P. sylvestris* induced by egg deposition of *N. sertifer* (Table 2, B3).

In cross-experiments, the parasitoid had experienced a plant–host complex different from that tested in the olfactometer assay. Parasitoids that had experienced odours from the *P. sylvestris*–*D. pini* complex did not respond significantly to volatiles from *P. nigra* carrying *D. pini* eggs (Table 2, C1), nor did they show a significant response when they had experienced volatiles from the *P. nigra*–*D. pini* complex and were tested for their response to *P. sylvestris* twigs with eggs of *D. pini* (Table 2, C2). Parasitoids that had been exposed to volatiles from *P. sylvestris* laden with eggs of the major host *D. pini* did not respond significantly to volatiles from *P. sylvestris* with eggs of *G. pallida* (Table 2, C3 and compare with B2). Volatiles from *P. sylvestris* with *D. pini* eggs were no longer attractive to the parasitoids, if they did not experience the major host prior to the bioassay (Table 2, C4). Thus, *C. ruforum* responded only to *P. sylvestris* volatiles induced by the egg deposition of the major hosts (*D. pini* and *N. sertifer*) when these hosts had been experienced prior to the bioassay.

Developmental stage specificity

Larval feeding for a period of 24 h did not locally induce the emission of volatiles in *P. sylvestris* twigs that attract the egg parasitoid *C. ruforum* (Friedman-ANOVA: $\chi^2 = 3.57$, $P = 0.311$; $n = 23$). Nor were parasitoids attracted to volatiles from undamaged twig parts when 25 larvae had fed on adjacent parts for 72 h (Friedman-ANOVA: $\chi^2 = 0.60$, $P = 0.896$; $n = 22$).

Discussion

The egg parasitoid *C. ruforum* did not show an innate response to volatiles from *P. sylvestris* induced by the oviposition of *D. pini*. However, they were able to significantly respond to those volatiles that had previously been experienced, and could be associated with the presence of host eggs (Table 1, A1–2).

According to a literature survey by Steidle & van Loon (2003), the learning behaviour of specialised parasitoids such as *C. ruforum* has been demonstrated in significantly fewer species compared to parasitoids with a broader foraging range. Instead, specialised parasitoids searching for a host were primarily shown to respond innately to specific chemical cues from the host, the host's plant, or from both trophic levels. The egg parasitoid *C. ruforum* is known to respond innately to the sex pheromones of its sawfly hosts (Hilker et al., 2000). Although these sex pheromones are highly reliable for identifying habitats containing adult hosts, their detectability is low regarding their amount and in particular their appearance in time (Vinson, 1998;

Steidle & van Loon, 2002; for discussion). On the other hand, pine volatiles that are induced by egg deposition indicate the presence of host eggs, but may not be as reliable due to high qualitative and quantitative variation (Vet & Dicke, 1992).

We suggest that the learning of abundant oviposition-induced plant cues is beneficial for the specialised egg parasitoid *C. ruforum* and argue as follows:

(i) Pines possess a high qualitative and quantitative variability in the composition of secondary terpenoid compounds between genotypes (Sjödén et al., 2000; Petrakis et al., 2001; Krauze-Baranowska et al., 2002), within trees (e.g., Barnola et al., 1997; Forrest et al., 2000; Latta et al., 2000), and individual tissues (e.g., Sjödén et al., 1996, 2000; Litvak & Monson, 1998; Kleinhentz et al., 1999; Latta et al., 2000; Manninen et al., 2002). Furthermore, the emission rates of terpenes are strongly affected by environmental factors such as temperature and light intensity (e.g., Tingey et al., 1991). Thus, an egg parasitoid searching for host eggs within a pine tree needs to cope with volatile bouquets that might vary with the genotype, the position within the tree, the herbivores attacking the tree, the season, the time of day, and other environmental factors.

(ii) Mumm et al. (2003) were able to demonstrate that oviposition by *D. pini* on *P. sylvestris* does not result in qualitative, but small quantitative changes in the pine volatile pattern. These results show that the egg parasitoid *C. ruforum* is able to detect very small changes of the pine volatile pattern after having experienced that this volatile pattern is associated with eggs of the major host. A fixed innate response to such small quantitative changes of the pine's volatile pattern induced by egg deposition might be nonadaptive when taking into account that this pattern might vary in dependence of numerous other factors. Instead, learning could be a favourable strategy enabling *C. ruforum* to respond very flexibly to varying conditions and to adjust its response to finely tuned, small quantitative volatile changes induced by host egg deposition.

The response of *C. ruforum* to oviposition-induced pine volatiles was shown to be specific for the plant species. Volatiles emitted from *P. sylvestris* after egg deposition of *D. pini* were attractive to *C. ruforum* when this plant–host complex had previously been experienced (Table 1, A2 and Table 2, C2), as was also shown in a previous study (Hilker et al., 2002). Surprisingly, *C. ruforum* females that had previous experience with the *P. nigra*–*D. pini* complex did not respond significantly to volatiles from the same plant–host complex (Table 2, B1). These results suggest that *C. ruforum* does not learn to respond to odours from *P. nigra* carrying eggs of *D. pini*.

The positive response of egg parasitoids to oviposition-induced *P. sylvestris* volatiles and their 'non-response' to

egg-carrying *P. nigra* twigs might be due to quantitative and qualitative differences between the volatile patterns of the two pine species. Detailed analyses of the volatiles from the headspace of egg-laden *P. nigra* and *P. sylvestris* revealed that only 50% of identified compounds were present in both species (Mumm et al., 2003, 2004). The quantitative composition of the compounds detected in both species was clearly shown to be different (Mumm et al., 2004). Thus, the headspace of egg-laden *P. nigra* twigs might lack components that are necessary for *C. ruforum* to become attracted. Furthermore, the egg-carrying *P. nigra* twigs might release such a quantitative composition of volatiles that cannot be associated by *C. ruforum* with the presence of suitable hosts. Moreover, compounds emitted by egg-laden *P. nigra* may mask the attractiveness of other key compounds in the volatile mixture (Chandra & Smith, 1998; Laloi et al., 2000; Meiners et al., 2003, and references therein).

Pinus nigra is known as a host plant of poor quality for *D. pini*. Egg development and larval performance of the sawfly is significantly reduced when compared to *P. sylvestris* (Auger et al., 1994; Barre et al., 2002). Sawfly eggs laid on a suboptimal plant might not be preferred hosts for *C. ruforum* to forage for, because the parasitoid's development might also be negatively affected on such a plant, as was shown for other parasitoids (Hofstetter & Raffa, 1997; Turlings & Benrey, 1998; Hunter, 2003; Sznajder & Harvey, 2003; Takasu & Lewis, 2003). Meiners et al. (2000) also showed that the response of the eulophid egg parasitoid *Oomyzus gallerucae* to elm volatiles induced by the oviposition of its host, the elm leaf beetle, is specific for the plant species most favourable for the beetle.

The response of *C. ruforum* to oviposition-induced pine volatiles was specific for the herbivore species that laid eggs on *P. sylvestris*. Oviposition by *D. pini* and *N. sertifer* induced the emission of pine volatiles attractive to *C. ruforum*, whereas egg deposition by *G. pallida* did not. Egg depositions of closely related sawfly species obviously elicit different responses in *P. sylvestris*, which can or cannot be learnt by *C. ruforum*. The sawflies *D. pini* and *N. sertifer* represent major defoliators of *P. sylvestris* in Europe by causing severe damage to coniferous forests during an outbreak (Pschorn-Walcher, 1982). On the other hand, *G. pallida* is thought to be only a marginal pest (Pschorn-Walcher, 1982). Oviposition-induced responses might have evolved in *P. sylvestris* to specific major pest insects as a counter-adaptation to the high damage caused by the insects. *Chrysonotomyia ruforum* might have developed a specific ability to learn pine volatiles induced by oviposition of the favoured and most abundant host species, in order to search for hosts at those sites where maximum host availability, and thus maximum reproduction, is possible.

To be able to respond in a differentiated manner, plants must be able to 'recognise' the egg-laying species. Herbivore-borne elicitors are assumed to be responsible for the recognition process by plants (Stout & Bostock, 1999). The elicitor inducing the production of attractive volatiles in *P. sylvestris* after egg deposition by *D. pini* is located in the oviduct secretion of the sawfly females (Hilker et al., 2002). Eggs are coated by the oviduct secretion when they are laid into pine needles. Further studies are needed to elucidate whether *P. sylvestris* 'recognises' the ovipositing diprionid species by chemical differences between the oviduct secretions, or by other means such as differences in the wounding of the pine needles prior to egg deposition.

Egg parasitoids were not attracted to volatiles from pine twigs that had been damaged by feeding sawfly larvae. Therefore, the response of the egg parasitoid *C. ruforum* is specific for pine odours induced by a suitable developmental stage that can be parasitized. From the plant's perspective, the production of oviposition-induced volatiles seems to be a specific response to egg deposition and not a general reaction to herbivore damage. This specific plant response might be mediated by the elicitor in the oviduct secretion (see above). Furthermore, foraging *C. ruforum* that would rely on plant odours induced by larval feeding might be especially fooled because *D. pini* females avoided laying eggs on pine twigs treated with the oral secretions of conspecific larvae, and thus sawfly females might move from larvally infested sites to uninfested ones to avoid intra-specific competition (Hilker & Weitzel, 1991).

In conclusion, the egg parasitoid *C. ruforum* specialized on diprionid hosts was shown to be able to learn cues specific for the plant species that is most beneficial for herbivore performance, for the herbivore species most abundant, and for the developmental stage (i.e., the egg stage) suitable for parasitization. Our results show that a specialist egg parasitoid does not innately respond to oviposition-induced plant volatiles, but is able to learn using those volatile patterns for host location that seem most beneficial.

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