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Unusual mechanisms involved in learning of oviposition-induced host plant odours in an egg parasitoid?

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Parasitoids of herbivorous larvae are well known to be able to associatively learn a response to feeding-induced plant volatiles. However, little is known of the abilities of egg parasitoids to learn a response to herbivore oviposition-induced plant volatiles (OVIV). The eulophid wasp *Chrysonotomyia ruforum* is known to be attracted by OVIV from Scots pine released 3 days after host egg deposition. This study aimed to elucidate (1) the impact of the nutritional state of the parasitoid on its response to OVIV and (2) the effect of learning and response to the temporal change of OVIV. Supply of food as a reward during associative learning trials inevitably influences the nutritional state and thus leads to an experimental constraint. Nevertheless, the results indicate that the parasitoids have to associate OVIV with host presence and that they need to be supplied with food to learn and to respond to OVIV. Furthermore, the parasitoids' positive response to OVIV from pine twigs laden with eggs for 3 days was independent of the induction time of pine twigs experienced. However, a positive response was given only to OVIV from pine twigs with 3-day-old eggs, while OVIV from twigs with other induction times (1, 2, 4 days) were not attractive. Thus, our findings indicate an unusual learning process in this specialized egg parasitoid. We suggest that the parasitoid is using a learning strategy by which common information is filtered out of similar odour blends (here: 1- to 4-day-long induced pine twigs), but a behavioural response to this learned information becomes evident only in a specific odour context (here: 3-day-long induced twigs).

Keywords: behavioural plasticity; *Chrysonotomyia ruforum*; *Diprion pini*; experience; nutrition; odour learning; parasitoid; *Pinus sylvestris*

In the past three decades associative learning of plant odours has been studied in several insect species (Gould 1993; Papaj & Lewis 1993; Smid 2006). It became evident that many insects are able to associate specific plant odours as a conditioned stimulus with a rewarding unconditioned stimulus such as food and/or host resources (e.g. Daly & Smith 2000; Laloi et al. 2000; Meiners et al. 2003). However, the exact conditions necessary for associative learning of complex plant volatile blends are not well elucidated yet.

Parasitic wasps attacking herbivorous insects are known to use both plant and herbivore odour to locate their hosts (Vet et al. 1995; Quicke 1997; Vinson 1998; Steidle & van Loon 2003; Takabayashi et al. 2006). The volatiles released from plants and herbivores may vary due to numerous abiotic and biotic factors, thus exposing the parasitoids to a highly complex and variable odourous environment (Hilker & McNeil, in press). Parasitoid responses to phenotypic changes of plant volatiles have been studied intensively with respect to changes induced by herbivore attack or herbivore egg deposition (Turlings & Benrey 1998; Kessler & Baldwin 2001; Hilker & Meiners 2002, 2006; Turlings et al. 2002; Mumm & Hilker 2006). The changes of plant odour may be specific for the herbivore

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species and herbivore phenotype (De Moraes et al. 1998; Meiners et al. 2000; Turlings et al. 2002; Mumm et al. 2005), thus providing information on the most suitable host. To cope with high variability of plant volatiles, a parasitoid should show appropriate phenotypic plasticity to successfully locate hosts.

Parasitoid responses to plant odour are known to change due to experience (Vet et al. 1995) but also due to age (Kester & Barbosa 1991; Bertschy et al. 1997), egg load (Vet & Dicke 1992) and nutritional status (Lewis & Takasu 1990; Jacob & Evans 2001). The ability to learn by experience is widespread among parasitoids (Turlings et al. 1993; Steidle & van Loon 2003; Smid 2006) and has been found to significantly improve host and food finding (Lewis & Martin 1990; Papaj & Vet 1990; Steidle 1998). Many adult parasitoids are able to learn associatively to respond to cues they have experienced in the presence of hosts or food (Turlings et al. 1993; Vet et al. 1995; Smid 2006).

Learning abilities may be essential for parasitoids attacking many host species or hosts with a wide range of host plants (Vet & Dicke 1992), but they may even support foraging by specialized parasitoids that also have a genetic predisposition to specific cues (Steidle & van Loon 2003). If specific host or food cues are available only in low amounts or only during a short time period, a learned response to other more general cues also associated with the host or food might serve as 'back-up' trait for specialized parasitoids to intensify or prolong successful foraging. For example, the response of the egg parasitoid *Chrysonotomyia ruforum* to pine volatiles induced by host egg deposition may be considered such a back-up trait (Hilker et al. 2002). This eulophid wasp is specialized on diprionid sawflies feeding on pine (Pschorn-Walcher & Eichhorn 1973; Eichhorn & Pschorn-Walcher 1976; Pschorn-Walcher 1982). It is known to respond innately to sex pheromones of the most abundant host species, that is, the sawflies *Neodiprion sertifer* and *Diprion pini* (Hilker et al. 2000). In addition to the innate response to host cues, this egg parasitoid is attracted to oviposition-induced pine volatiles (OVIV) when these have been experienced before. Odour of pine laden with 3-day-old eggs is attractive (Hilker et al. 2002). Whereas the presence of sex pheromones might indicate future egg laying or freshly laid host eggs to *C. ruforum*, the OVIV indicate that eggs have been laid 3 days before. Thus, the ability to detect OVIV might serve as a back-up trait for *C. ruforum* to locate host eggs even when sex pheromones have evaporated.

Naïve and hungry *C. ruforum* were found not to respond to oviposition-induced pine volatiles (Mumm et al. 2005). So far, this egg parasitoid was shown to respond to odours emitted from pine twigs after 3 days of sawfly egg deposition only when it has experienced the plant–host complex with additional availability of honey as food source. The plant–host complex offered during experience consisted of pine twigs that emitted odours after 1 day of sawfly egg deposition (Hilker et al. 2002; Mumm et al. 2005).

The study presented here aimed to elucidate the parameters that affect learning and thus response of *C. ruforum* to OVIV released from pine twigs.

First, we studied the importance of the nutritional state of the egg parasitoid and investigated the question of whether the naïve parasitoids tested so far did not respond to OVIV simply because they were hungry and not fed prior to the experience phase and bioassay.

Second, we investigated the parameters (rewards) that might be important during the experience phase and thus might be necessary for associative learning by *C. ruforum*. We hypothesized that parasitoids fed honey prior to the experience phase need to associate during an experience phase OVIV (conditioned stimulus) with (1) food as reward (unconditioned stimulus), (2) host eggs but no food as reward or (3) both food and host eggs as rewards (Gould 1993).

Third, we aimed to elucidate whether the positive response of the parasitoids to OVIV from pine twigs laden with 3-day-old eggs was affected by experience of OVIV from pine twigs with induction times other than those tested so far (1 day; Hilker et al. 2002). Additionally, we examined the attractiveness of odours that are emitted from pine twigs after different time points (1–4 days) of sawfly egg deposition. It is well known that herbivore-induced volatiles change over time (Loughrin et al. 1994; Turlings et al. 1998). Thus, the response of the egg parasitoid to odour of pine twigs laden with eggs may change with changing induction times.

METHODS

Plants and Insects

Large branches of *Pinus sylvestris* L. (Pinales, Pinaceae) were detached from 15- to 30-year-old trees in a forest near Berlin, Germany and brought into the laboratory where the stems were cleaned and sterilized according to the method of Moore & Clark (1968). For all experiments, small twigs (about 20 cm length) were cut from these branches and treated as described below.

The sawfly *D. pini* L. (Hymenoptera, Diprionidae) was reared continuously in the laboratory on cut pine branches (about 60 cm length) as described by Bombosch & Ramakers (1976) and Eichhorn (1976) at 20 ± 1°C, 65% RH and 18:6 h light:dark (LD) cycles. Adult females lay up to 20 eggs per needle (about 100–150 eggs per female). The larvae of this species may heavily damage pine forests. Cocoons overwinter mainly in the soil (Pschorn-Walcher 1982; Brauns 1991).

The egg parasitoid *C. ruforum* (Krausse; Hymenoptera, Eulophidae) was obtained from parasitized host eggs collected in the field in southern Finland. This parasitoid species may considerably limit population densities of *D. pini* (Pschorn-Walcher 1982). In addition to *D. pini*, eggs of a few other diprionid species feeding on pine are attacked also by *C. ruforum* (Eichhorn & Pschorn-Walcher 1976). Pine needles with parasitized eggs were brought into the laboratory and kept in petri dishes at 5°C. To initiate parasitoid emergence, needles with parasitized eggs were transferred to 20 ± 1°C, 65% RH and 18:6 h LD. Emerging adults were collected daily, transferred into a 20-ml vial and kept at 10 ± 1°C, 65% RH and 18:6 h LD until they were used for conditioning. The time between emergence and conditioning is referred to here as 'storage phase'. Due