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Odour preference of a parasitic wasp depends on order of learning

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Abstract. Female *Leptopilina boulardi* wasps, larval parasites of *Drosophila melanogaster*, can learn to respond to more than one odour by associating these odours with oviposition experience. These wasps can memorise and respond to at least two different odours, and prefer the last one learnt.

Key words. Leptopilina boulardi; Hymenoptera; Eucoilidae; parasitoid; olfaction; learning; memory; olfactometer.

There is increasing evidence that insects can learn to respond to different environmental stimuli like odours, colours and shapes. Work on olfactory learning showed that classical conditioning is an important mechanism underlying behavioural plasticity in insects. The response to an odour can be changed after an association of this odour with a reward, such as food[1,2], oviposition[3,4], or a kairomone[5-7]. Koltermann[8,9] described a time-linked memory in bees. Bees relate the time of day to the learning of an odour and can be trained to respond to different odours at different times. Koltermann[9] suggested that this type of behaviour may be an example of 'state-dependent learning'[10], in which bees would associate an odour with a certain inherent physiological state. The work of Lewis and Takasu[1] showed that *Microplitis croceipes*, a larval parasitoid, can learn two novel odours associated with separate host and food resources, and make a choice between these odours on the basis of its relative host and food needs. As in bees, the memory for a particular odour seems to depend on the physiological state of the insects, as hungry wasps chose the food-associated odour, and well-fed wasps the oviposition-associated odour. Interestingly, foraging bees cannot learn two kinds of flowers at the same time of day[11]. The question arises whether insects can remember different odours independently of their physiological state. In this paper we analyse this problem by studying the effects of two successive conditionings to different odours on the orientation behaviour of *Leptopilina boulardi* Barb. et al. (Hymenoptera; Eucoilidae), a larval parasitoid of *Drosophila melanogaster*[12,13]. These odours were associated with the same reward, i.e. oviposition, so that influences of competing biological needs were avoided[1].

Materials and methods

Bioassays were performed in a four-armed airflow olfactometer designed by Pettersson[14], similar to the one described in detail by Vet et al.[15] with some modifications[3]. The olfactometer consisted of an exposure chamber connected to four arms through which air flowed (200 ml/min) into the chamber. The air was sucked through a central hole in the bottom of the chamber and created four distinct fields of equal area. Insects were introduced into the centre of the chamber and were observed for 1 min. During that time we measured the time spent in the different fields, of which one or more could be permeated with odour. One day before testing, the female wasps were allowed to oviposit in host larvae during two periods of 30 min with a 1-h interim period. For the purpose of conditioning, the wasps were kept in an airflow which could be permeated with odour, and in the presence of agar-agar infested with host larvae. Artificial strawberry (S) and banana odours (B) (supplied by Haarmann & Reimer, 92000 Nanterre, France, and used in an undiluted form) served as odour sources in the experiments. From previous work[16] it is known that both odours can be learnt by the parasitoids. Odourless air (O, pseudoconditioning) served as an odour control.

In this experiment we studied the effect of conditioning to a new odour on the previously learned odour. For this purpose we tested differently-treated groups of female wasps, i.e. BS, BO, OB, OS and OO, in the olfactometer where one field was permeated with B. BS females were successively conditioned to B and S before being tested in the olfactometer. BO females were first conditioned to B and then allowed to oviposit in an odourless airflow (O), and served as a reference. This group was expected to respond better to B than the BS group if the conditioning to S affected the memory for B. OB females, first pseudoconditioned (O) and thereafter conditioned to B, were included to compare with BO females, to show whether pseudoconditioning after conditioning to B would influence the response of these females to B. Possible effects of so-called cross-induction[17] through conditioning to a second odour (to S) on the response to B were studied by including a group of OS females which were pseudoconditioned before being conditioned to S. Finally, the responses of wasps naive to odours and which had experienced two pseudoconditionings (OO females), were also measured.

Results and discussion

Figure 1 represents the responses to B for the five groups, i.e. OO, OS, OB, BO and BS. The differences between the groups were caused by significantly higher percentages of the time being spent in the B field of the olfactometer by the BO, OB and BS groups compared to the other groups. The responses between the BO, OB and BS groups, and those between the OO and the OS groups, did not differ significantly from each other. These results suggest that the memory for B is not affected by a subsequent conditioning to S, since BS females responded as well to B as did BO and OB females. The response of BS females to B cannot be explained by cross-induction through the conditioning to S, because OS females did not respond significantly better to B than OO females.

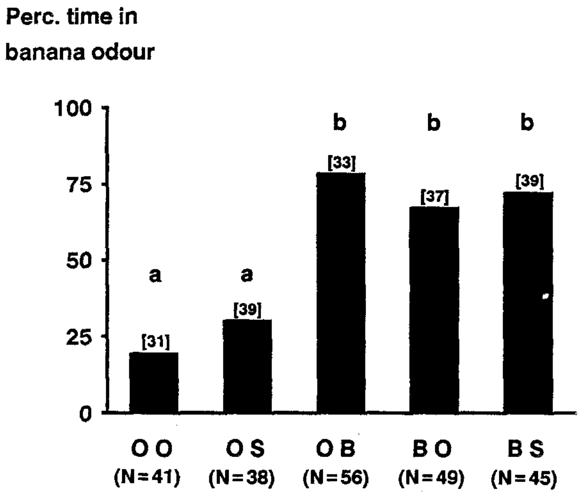

Figure 1. Percentage of time (with SD) spent in banana odour by differently conditioned groups of wasps, i.e. OO, OS, OB, BO and BS (for explanation see text). Different letters represent significant differences at $p < 0.01$. (Multiple comparison test, after Kruskal-Wallis, $p \ll 0.0001$).

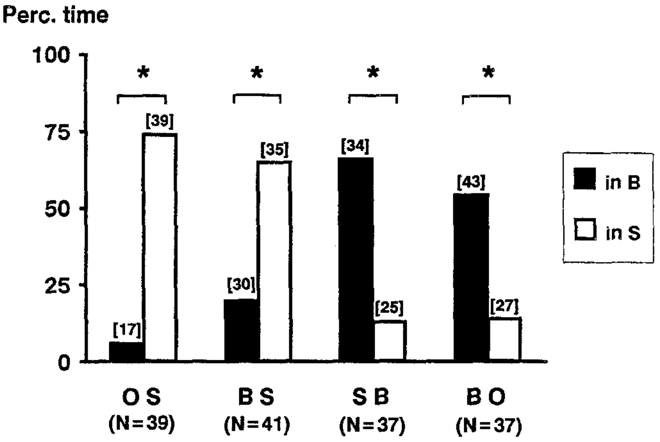

Figure 2. Percentage of time (with SD) spent in banana and in strawberry odour by 4 differently conditioned groups of wasps, i.e. OS, BS, SB and BO (for explanation see text); *, significantly different at $p < 0.05$ (Friedman test).

One can argue that the insects cannot be successfully conditioned to a new odour after having learnt a first one. A high percentage of bees, for example, do not switch from the first odour learnt to an alternative one[8]. This fact, together with possible effects of cross-induction, complicated the interpretation of the results obtained by Koltermann[8]. In the experiment described above, only the responses to B were measured. Therefore, we tested in another experiment whether the second odour (S) was really learnt by the BS wasps. In this experiment we also wanted to determine whether BS females have an odour preference if both odours are indeed learnt. We created a choice situation by permeating one field of the olfactometer with B and another one with S. In this situation we tested BS females, the control treatments BO and OS, and also a group where the learning order was reversed (SB females). The data presented in figure 2 show that BO and OS females preferred the odour they had already experienced. BS females responded in a way similar to OS females, which proves that there is no problem in conditioning the females to a second odour, and that BS females preferred S to B. It also confirmed our observation in the first experiment that BS females readily accepted substrates with larvae during the conditioning to S. SB females, however, preferred B to S, demonstrating that the odour preference is determined by the order in which the odours are learnt. The results of these experiments show that the female wasps can memorise more than one host-associated odour independently of their physiological state, and prefer the last one learnt.

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The induction of secondary seed dormancy by oxygen deficiency in a barnyard grass *Echinochloa crus-galli*

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Abstract. At 25 °C, secondary dormancy was induced in seeds of *E. crus-galli* exposed for 100 days to oxygen deficiency. By contrast, hypoxia did not induce dormancy at 15 °C or prevent dormancy termination at 7 °C. Secondary dormancy was terminated after 2 months stratification at 7 °C. Oxygen deficiency may increase the proportion of dormant seeds in the soil, and affect the dynamics of the barnyard-grass soil seed bank.

Key words. Barnyard grass; *Echinochloa crus-galli*; oxygen deficiency; seed dormancy; soil seed bank; stratification; temperature.

In some species, secondary seed dormancy may be induced after the primary one has been terminated by afterripening (exposure of dry seed to high temperature) and/or stratification (exposure of moist seed to cold). The induction of secondary dormancy usually requires a prolonged period at conditions adverse to germination, e. g. high or low temperatures, darkness, or water stress[2], and may be viewed as an adaptation for survival. Induction of secondary dormancy by oxygen deficiency has been observed rather rarely[3].

We investigated the induction of secondary seed dormancy in a barnyard grass, *Echinochloa crus-galli* (L.) P. Beauv. In this species, a fraction of seeds (typically 1–20% in different populations) terminates dormancy after 3 months of afterripening at 25 °C[4], and this proportion may be increased by high temperature[5]. In the rest of the seed population, dormancy may be terminated by stratification at 1–10 °C[6]. Experimental induction and termination of secondary dormancy has not been studied as yet.

Material and methods

The seed material was collected at 2 localities in western Czechoslovakia, 20 km north of Prague. The seeds (spikelets consisting of a caryopsis enclosed in lemma, palea and glumae) were swept with an entomology net from plants growing within maize or sugar beet crops. The material collected at Odolena Voda (OV) and Kozomín (K) in 1990 was afterripened at 25 °C and 30% air relative humidity, for 8 months. Material FS was collected in 1989 at Odolena Voda (germination rate after 3 months of afterripening 5.0%). A part of this material was buried in the field from October 1989 to April 1990. This treatment terminated dormancy in about 95% of the seeds. The post-dormant material (F) was stored at room conditions (20 °C, 40% relative humidity) until the beginning of the experiments. The proportion of non-dormant seeds at the start of the experiment was 91.8, 21.6, 5.0 and 1.2% in F, OV, FS and K material, respectively.

The oxygen deficiency treatment consisted of submerging a packet of the seeds in water and carefully expelling the remaining air. The samples of F, OV and K seeds were then placed at a constant 7, 15, and 25 °C, in darkness. The stratification treatment for termination of secondary dormancy consisted of burying the packets of seeds in moist sand, and incubating them at 7 °C in darkness. In this experiment we used the samples of F and OV seeds