Why are seed cones of Swiss stone pine (*Pinus cembra*) not attacked by the specialized pine cone weevil, *Pissodes validirostris*? A case of host selection vs. host suitability

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Abstract

The pine cone weevil, *Pissodes validirostris* Gyll. (Coleoptera: Curculionidae), attacks seed cones of most Eurasian pine species, except these of Swiss stone pine (*Pinus cembra* L.). Behavioural responses of adult weevils to cone volatile emissions of Swiss stone pine and to those of a common host, mountain pine (*Pinus uncinata* Ram.), were compared in an olfactometer. Weevils were significantly attracted by the volatile blend emitted by mountain pine, but Swiss stone pine volatiles elicited an inverse response, with most weevils moving in the opposite direction to the odour source. However, the majority of second instar weevil larvae that were extracted from mountain pine cones and transferred into Swiss stone pine cones were capable of developing to the adult stage. This suggests that Swiss stone pine cones do not contain strong feeding deterrents that could prevent larval development. The possible factors involved in the absence of colonization of Swiss stone pine cones by cone weevils are discussed.

Introduction

Phytophagous insects are known to preferentially colonize plants that are taxonomically related to their usual host. In many cases, plant chemistry may be responsible for the absence of insect attack on a given plant species (Bernays & Chapman, 1994). However, it is unclear whether the effects of particular plant chemical defenses prevent insect colonization indefinitely, or simply delay it (Jones & Lawton, 1991).

The pine cone weevil, *Pissodes validirostris* Gyll. (Coleoptera: Curculionidae) is a major cone pest of *Pinus* spp. in Eurasia (Annila & Hiltunen, 1977; Roques, 1976, 1983). Weevil larvae are specialized in pine cone exploitation and cannot develop in any other habitat. After overwintering in trunk bark crevices, adult weevils feed on the cone surface until sexual maturation. Mating takes place on cones and leader shoots, and females lay eggs into the scales of 2nd year cones, when the cone moisture content peaks. Both larval development (4 larval instars) and pupation occur within the same cone (Roques, 1976). Although it attacks most of the pine species native to Europe, *P. validirostris* has never been observed on Swiss stone pine (SPP), *Pinus cembra* L., an alpine conifer (Roques, 1983). Moreover, the cone entomofauna of SPP is limited to only three insect species, none of which is a cone specialist (Roques, 1983; Dormont & Roques, 1999). By comparison, seed cones of sympatric conifers growing at high altitude in the Alps, such as those of European larch (*Larix deciduas* Mill.) and mountain pine (*Pinus uncinata* Ram.), host 3–4 times more phytophagous insect species in a smaller or equally sized host plant (Roques, 1983). The absence of the cone weevil in *P. cembra* is surprising, particularly as mountain pine cones are heavily...
infested by weevil larvae in French and Swiss stands where the two pine species are mixed (Roques, 1983; Roques et al., 1983). Differences in cone cues provided by the two pine species may be responsible for the choice of adult weevils. In most specialist cone insects (e.g., Strobilomyia cone flies), orientation towards the host is achieved by flying adults, which use the visual and olfactory cues supplied by the cones (Turgeon et al., 1994). In contrast, adult cone weevils emerging from the overwintering sites mostly disperse by walking to the nearest cone-bearing pines (Annila, 1975). Short-range host detection within the tree is then influenced by both cone cues, negative geotaxis and positive phototaxis, the weevils attacking at first the cones developing on the leader shoots (Roques, 1988). A similar host selection behaviour has been observed in the white pine weevil, Pissodes strobi Peck (Vandersar & Borden, 1977).

The limited insect colonization of SPP cones has led to suggestions that volatiles or oleoresin emitted by these cones may act as a chemical barrier against insect attack (Dormont & Roques, 1999). Preliminary investigations have shown that mountain pine cones sprayed with a solution of cone volatiles from SPP are not attacked by cone weevils (Dormont et al., 1997). Although the cone volatiles of both pine species differ in the relative level of a few monoterpenes (Dormont et al., 1998), it is still unclear whether the stone pine emissions repelled adult weevils or only masked those of mountain pine. In addition, no information exists about the likelihood of weevil larvae developing on stone pine cones if egg-laying succeeds.

Therefore, the objectives of this study were:
(i) to compare the behavioral responses of walking adult P. validirostris to mountain pine and SPP cone volatiles in laboratory olfactometer bioassays, and
(ii) to determine whether cone weevil larvae extracted from mountain pine cones and transferred into SPP cones can successfully develop to the adult stage.

Materials and methods

**Insect and cone collections.** Cone weevil larvae and adults were collected in a natural mountain pine forest located at Montgenèvre (44°55′ N, 6°44′ E, 1950 m elevation) in the southern French Alps. The timing of collection was chosen according to observations of weevil life cycle in the Alps carried out by Roques et al. (1983). Weevil-attacked cones were collected in June 1994 and June 1995, approximately one week after egg-laying punctures were first observed on the scales of 2nd-year cones. A total of 30 different trees were sampled. Adult weevils were collected in 1996 by beating pine branches as soon as a few cones had feeding punctures on their surface (by 12 June). A total of 30 weevils, 19 males and 11 females, were obtained. The insects were immediately taken to a nearby field laboratory, placed in individual plastic boxes containing fresh mountain pine shoots, and stored inside at 18 ± 3 °C. Sound mountain pine cones were sampled at the same location whilst sound SPP cones were simultaneously collected in the nearby forest of Les Ayes (44°51′ N, 6°36′ E, 1900–2100 m elevation) where the two pine species are mixed.

**Transfer of weevil larvae from mountain pine cones to SPP cones.** The transfer tests were carried out in the laboratory during late June 1994. Using a pin, a small hole ca. 8 mm deep was dug through the scale surface of 15 fresh SPP cones. Fifteen 2nd-instar larvae were extracted from mountain pine cones and placed individually within each hole. Difficulties inherent to the use of 1st-instar larvae, which are very fragile (3% survival rate after transfer to an artificial diet; Roques, 1976), led to the preferential use of 2nd-instar larvae in the tests. The treated cones were reared under laboratory conditions (20 ± 2 °C). In order to test for the effect of the transfer technique on larval development and survival, 15 larvae were similarly reintroduced into 15 sound mountain pine cones. In parallel, 30 weevil-attacked mountain pine cones were stored undissected in boxes as a control for estimating the duration of insect development under laboratory conditions. We considered the adult emergence period complete 15 days after emergence of the first imago. After this time all cones were dissected in order to look for both the patterns of larval damage and the possible presence of dead larvae.

In 1995, additional tests were carried out in situ; i.e., without removing the cones from the trees. These experiments were conducted in the forest of Les Ayes, at 1900 m altitude. Five cone-bearing mountain pine trees, and five cone-bearing SPP trees were randomly selected, and larvae were transferred into sound cones located at the tree tops, using the same technique as in 1994. Twenty 2nd-instar larvae were transferred into SPP cones and an equal number were transferred into mountain pine cones. In late summer, all these cones were collected and dissected. The duration of larval development under natural conditions was assessed through regular collections of weevil-infested
cones on mountain pine trees growing close to those used in the study.

Responses of adult cone weevils to cone volatiles. Behavioural bioassays were carried out during mid-June 1996 using adults freshly collected from mountain pine. Tests were done using an olfactometer design derived from those used by Pierce et al. (1981) and Rochat (1991). The design consisted of a plastic rectangular arena (30 × 12 × 8 cm) with two holes cut on the arena floor. The holes were 2.5 cm in diameter, spaced 20 cm apart, and closed by a small circular grid. A circular plexiglass container (6 cm in diameter, 12 cm high) was placed under each hole and pierced at the bottom in order to allow air to enter. Airflow was generated by a pump connected to the olfactometer at the center of the floor, which provided a continuous movement of air from outside through each container’s grid holes, as well as within the arena. The airflow rate was measured using an air flow-meter placed between the pump and the olfactometer, and was maintained at 400 ml min⁻¹. Airflow movements within the arena were assessed using chemical smoke (a mixture of ammoniac + chlorhydric acid), and airflow rate was adjusted so that insects at the center of the arena could perceive both odor sources without any air turbulence within the arena. The tests were done in a darkened room equipped with two red lights (40 Lux) placed 50 cm above the arena. The volatile source consisted of a fresh cone placed in one of the containers. In order to record insect movements, the arena floor was completely covered with a white paper sheet, which was divided in four equal parts named A, B, C, and D. Section D included the hole corresponding to the container with the cone, section A included the opposite hole. Following every test, the paper sheet was replaced and the entire assembly was washed using a solvent mixture of alcohol-pentane-acetone (1:1:1). The temperature of the laboratory room was held at 19 ± 2 °C during the experiments.

One hour prior to the experiment, the individual plastic boxes containing the 30 newly collected adult weevils were transferred to the testing room. Each experiment consisted of releasing an individual in the center of the arena and recording its behaviour for 10 min. All weevils were submitted in a random order to the following tests, which were applied successively: (i) no cone in either of the two containers, in order to record weevil activity in the absence of an odour source; (ii) a mountain pine cone vs. no cone; (iii) a SPP cone vs. no cone. Each weevil’s position was continuously observed, and the total length of time (in seconds) spent by an individual in each part of the olfactometer was summed over the 10-min period.

Data analysis. The number of insects still alive at each stage of development (larvae, pupae, and newly emerged adults) after transfer was compared between SPP and mountain pine cones using a Fisher exact test (P = 0.05) (Statistica 5.0 Microsoft©). The mean duration of weevil development from transfer to adult emergence in SPP and moutain pine cones was compared using a Mann–Whitney U test (P < 0.05) (Statistica 5.0 Microsoft©). To test for weevil responses to cone volatiles, the mean cumulative time spent by adults in each part of the olfactometer was compared between the 3 olfactory situations (no odour, presence of mountain pine cones, presence of SPP cones) using the non-parametric Kruskall–Wallis test (P < 0.05) (Statistica 5.0 Microsoft©).

Results

Transfer of weevil larvae from mountain pine cones to SPP cones. In 1994, 11 of 15 weevil larvae transferred into a SPP cone succeeded in developing from the 2nd-instar to the adult stage (Figure 1A). This survival rate was not significantly different from that observed when the larvae were transferred into mountain pine cones; more than half of the larvae reached the adult stage in both pine species (Fisher exact test, P = 0.17). A much lower proportion of larvae reached the adult stage in the 1995 field experiments (Figure 1B). Most of these larvae were found dead within the hole into which they were originally placed. The number of larvae still alive at each stage of development did not differ between SPP cones and mountain pine cones in either laboratory or field experiments (P = 0.10 in 1994; P = 0.29 in 1995). The mean duration of weevil development from transfer to adult emergence did not differ significantly between pine species in 1994 (39 days in SPP vs. 34 days in mountain pine; U = 4.2, P = 0.57) or 1995 (42 days in SPP vs. 45 days in mountain pine; U = 5.0, P = 0.39). However, adult emergence occurred an average of 11 days (1994) or 8 days (1995) later than that observed in undissected control mountain pine cones.

Response of adult weevils to cone odors. When a mountain pine cone was introduced into the container connected to section D, the insects spent significantly
Table 1. Mean time spent by adults of *Pissodes validirostris* in each part of the olfactometer when submitted to different odor stimuli. Values are expressed by the average number of seconds (± s.e.) spent by weevils in each part of the arena over a 10-min period (n = 30 adult weevils tested for each odor source). The arena floor was divided in 4 equal parts named A, B, C, and D. Section D included the hole corresponding to the container with the cone, section A included the opposite hole.

<table>
<thead>
<tr>
<th>Odor source</th>
<th>Part of the olfactometer</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cone</td>
<td>170.4 (41.5)a</td>
<td>128.0 (28.7)a</td>
<td>139.4 (24.1)a</td>
<td>161.8 (34.7)a</td>
<td></td>
</tr>
<tr>
<td>Mountain pine cone</td>
<td>110.9 (44.7)b</td>
<td>93.8 (33.9)b</td>
<td>153.1 (39.4)b</td>
<td>242.1 (65.9)b</td>
<td></td>
</tr>
<tr>
<td>Swiss stone pine cone</td>
<td>289.3 (43.4)c</td>
<td>143.5 (39.6)c</td>
<td>141.6 (19.5)a</td>
<td>25.6 (12.7)c</td>
<td></td>
</tr>
</tbody>
</table>

1 Values in the same column followed by the same letter are not significantly different (P > 0.05; Kruskal–Wallis test).

Discussion

Cone weevils originating from mountain pine were successfully reared from 2nd larval instar to adulthood within cones of SPP, although the latter pine species has never been observed as a host in the wild. Indeed, the observed larval mortality was high, especially during the field tests where 82.5% of the larvae died following transfer to SPP cones whilst such a transfer only resulted in a total larval mortality of 33.3% in the 1994 laboratory experiments. Annila (1975) has reported that 25.4% of the weevil larvae developing in cones of Scots pine (*Pinus sylvestris* L.) in a Finnish seed orchard died because of natural mortality factors such as resin flow, starvation and failure to emerge. In the same seed orchard, the total larval mortality (excluding parasitism and predation) reached up to 58.8% when weevils naturally colonized cones of lodgepole pine (*P. contorta* Loud.), an exotic pine introduced to Europe. In the latter case, the major mortality factor appeared to be an important resin flow emitted by the cone tissues in reaction to larval penetration and first feeding. Annila (1975) also reported a total egg mortality of 12.0% in *P. sylvestris* and 38.4% in *P. contorta*, eggs having been mostly killed by pitch exuding from the walls of egg punctures. Roques (1976) made similar observations on Scots pine cones attacked by *P. validirostris* in France. Such a resinous defence reaction is characteristic of conifer structures such as cones and buds and it largely affects establishment and survival of larvae (McCulough & Wagner, 1993). Therefore, a highly resinous cone like that of SSP could have been suspected to limit seriously the early survival of weevil larvae. However, larval survival did not differ significantly in cones of SSP and cones of the original host, mountain pine, when the transfer conditions were identical. The higher mortality observed on both species in field tests could be therefore attributed to the transfer methodology. Digging a hole on a cone still developing on a tree might have resulted in a much more abundant and continuous resin flow than the same technique did on a drying cone separated from the tree. Eliason & McCulough (1997) observed a similarly high mortality (70 to 100%) of 1st and 2nd larval instars of three different types of insect pest (*Dioryctria zimmermani* Grote, *Neodiprion sertifer* Geoffroy, *Chionapsis pinifoliae* Fitch) after their transfer to Scots pine trees in the field.

Although no survey of compounds, such as tannins or phenols, in cones has yet been attempted, it is unlikely that SPP cones contain any strong feeding deterrent that prevent larval development of cone weevils. Roques (1988) noted that cone weevil larvae did not display any difference in their development on a semi-artificial medium when crushed tissues of
Figure 1. Mortality and survival at the different stages of development of the 2nd-instar larvae of *Pissodes validirostris* extracted from mountain pine cones and transferred into cones of either Swiss stone pine or mountain pine in 1994 (*n* = 30) and 1995 (*n* = 40). In the 1994 experiment (A), the cones were reared under laboratory conditions whilst they were left on trees in the 1995 experiment (B).
of four monoterpenes may be sufficient to modify the host selection behaviour of adult cone weevils which did not attack any of the treated cones (Dormont et al., 1997). Analysing cone oleoresin in Scots pine, Oshkaev (1981) reported a positive correlation between the proportion of α-pinene and cone weevil damage in Lithuania whilst Annila & Hiltunen (1977) found an inverse correlation for the same monoterpenes in Finland, as myrcene, 3-carene, β-phellandrene and p-cymene were in this case positively correlated with weevil attack. However, neither study considered cone volatiles. Olfactory responses of adults of the related Pissodes strobi Peck appeared similarly mediated by host volatile monoterpenes (Rieske & Raffa, 1993; Alfaro, 1996) but tree resistance to white pine weevils also depended on oleoresin and monoterpenes composition (Trudel et al., 1994).

Since Dethier et al. (1960) defined a repellent as ‘a chemical which causes an insect to make oriented movements away from its source’, the responses of cone weevils in olfactometer led us to assign a repellant effect to the cone odours of P. cembra. Such cones probably emit a much greater amount of volatile terpenoids than the cones naturally used as hosts by cone weevils (e.g., P. sylvestris, P. uncinata, P. nigra) (Dormont & Roques, unpubl.). Chemicals normally attractive to an insect may be repellent to the same insect at higher concentrations (Bernays & Chapman, 1994). Thus, it is possible that the high concentrations of some monoterpenes in the cone volatiles of SPP have slowed down or prevented cone colonization by insects.

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References


