

The ROLE of OLFACTION in SUBSTRATE LOCATION by *EUOPHRYUM CONFINE* (BROUN) (COLEOPTERA: CURCULIONIDAE)

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Abstract Olfaction behaviour of adult *Euophryum confine* was investigated using a wood substrate decayed by brown rot and white rot fungi. The first part of this study established whether adult weevils demonstrate a preference for wood modified by decay fungi over sound wood of the same species. Blocks of *Pinus nigra* (Arnold) sapwood were decayed with the brown rots *Coniophora puteana* (Schum.:Fr.) Karst, *Serpula lacrymans* (Wulf.:Fr.) Schroeter, and the white rot *Fibroporia vaillantii* (DC.:Fr.) Parmasto. *E. confine* adults demonstrated a significant attraction to wood decayed by *C. puteana* and *S. lacrymans*. The second part of the study investigated the role of volatiles released during wood decay in attracting *E. confine* adults. The role of ethanol and three volatiles released (α-pinene, 2-pentanone, and 2-heptanal) as potential attractants was investigated. This study found *E. confine* adults exhibit a significant attraction to α-pinene, 2-pentanone, and ethanol.

Key Words Weevil choice test α-pinene 2-pentanone ethanol

INTRODUCTION

Euophryum confine is a weevil native to New Zealand that has become established in the United Kingdom over the last 50 years. *E. confine* is considered a secondary pest of building timbers since they are found exclusively in timbers undergoing fungal decay. Both softwood and hardwood species are vulnerable to attack, although most infestations are recorded in Scots Pine (*Pinus sylvestris* L.) due to its extensive use as a building timber. *Coniophora puteana* (cellar rot) and *Serpula lacrymans* (dry rot) are both brown rot fungi that cause considerable decay to building timbers in the United Kingdom and abroad (Zabel and Morrell, 1992). Of lesser economic importance is the white rot *Fibroporia vaillantii* with which *E. confine* is occasionally found (Linscott, 1967). All three species of fungi, which decay both softwoods and hardwoods, are found in association with *E. confine* (Linscott, 1967; McClenaghan, 1985). This weevil is also common in British woodlands where it is found in both standing trees and fallen logs in which decay is present (Hickin, 1967).

Following its introduction, *E. confine* spread rapidly throughout the United Kingdom (Hickin, 1967). The mechanism by which these weevils disperse was undetermined for a number of years. Earlier studies failed to induce flight in adults by increasing the ambient temperature (Hum et al., 1980). Following research by our laboratory, relative humidity (<30%, at 20±1°C) was found to be important in inducing flight in this species (Green and Pitman, 2001). One hypothesis is that as wood dries out at low relative humidity, the surface hardness of the substrate increases, inhibiting the weevil's tunnelling. Given that these weevils are never found in decayed wood that has dried out, it suggests active decay is necessary for attack. Although the mechanism for dispersal has been determined, the mechanism by which this weevil detects wood of suitable condition to colonise has never been established. It is likely that *E. confine* responds to odour plumes emitted

when wood decays, rather than tactile cues or a random search strategy (Murlis et al., 1992; Hardie et al., 2001).

Korpi et al. (1999) determined the volatiles that are released by aspen (*Populus* sp.) when decayed by *C. puteana* and *S. lacrymans*. Given that *E. confine* have been found in association with both these species (Linscott, 1967; McClenaghan, 1985), any volatile emanating from wood decayed by either of these fungi may act as an attractant for this species. Three organic volatiles increased in emission when aspen was decayed by these fungi: α -pinene, 2-heptanal, and 2-pentanone. Of those volatiles, α -pinene was released in highest quantity when aspen was decayed by *C. puteana*.

In order to establish whether adult weevils are attracted to decaying wood for colonisation, two experiments were undertaken following work by Honda and Bowers (1996), Fadamiro and Wyatt (1995), and Oevering (2001). A two-choice test was set up to determine whether *E. confine* adults express preferences for wood they typically colonise (*Pinus* sp.) modified by different decay fungi. A second experiment investigated the attractiveness of three volatiles released by both *C. puteana* and *S. lacrymans* decaying wood (Korpi et al., 1999).

The response of *E. confine* to ethanol was also investigated following work by Montgomery and Wargo (1983) who demonstrated that a number of wood-boring beetles are attracted to it and other host-derived volatiles from oak seedlings. Ethanol is produced by fungal decay of wood under anaerobic conditions (Zabel and Morrell, 1992).

MATERIALS and METHODS

Mature, unsexed *E. confine* weevils of unknown age originating from a single colony in a standing beech (*Fagus* sp.) (Brickhill, Buckinghamshire, U.K. Grid reference: SP9032) were used in this experiment. Weevils were speciated from their close relatives, *E. rufum*, using morphological traits as described in Thompson (1989).

The fungi used in this experiment were grown from verified cultures (Building Research Establishment, Garston, Hertfordshire, United Kingdom) on malt extract agar (MERCK 5398). A

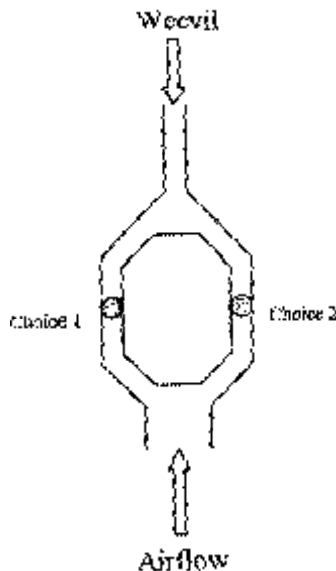


Figure 1. Two-choice experimental apparatus.

2mm veneer of *Pinus nigra* was sectioned into 10mm square blocks, autoclaved, then exposed to decay fungi for 7 days at 24°C±1°C and 90% RH (±5%).

A two-choice experimental arena was constructed as in Figure 1. This consisted of a Perspex entrance tube (10 mm dia.) leading to two glass vials (15 mm diam. 20 mm high). Once a weevil had entered a vial, they could not exit since a coating of Fluon® was placed around their rims. Individual weevils were placed in the neck of the tube in a continuous airflow (0.4 litres/minute) supplied by a pump (Algarde, 3000s). The air pump was connected through the glass vials using odourless nylon tubing (PEAK airline) via a regulatory valve (Algarde in-line airline valve) used to reduce airflow over the test materials.

Test materials were placed in one of the vials and left for one hour before experiments were conducted. Organic volatiles (0.5 ml) were dispensed onto filter paper (10 mm dia. Whatman) before being placed in the vials.

The following choice tests were conducted: Blank vs. Blank (Control); Blank vs. Undecayed *Pinus nigra* sapwood; Blank vs. Decayed *P. nigra* (*Coniophora puteana*); Blank vs. Decayed *P. nigra* (*Serpula lacrymans*); Blank vs. Decayed *P. nigra* (*Fibroporia vaillantii*); Blank vs. Malt extract agar (MEA); Blank vs. MEA + *C. puteana*; Blank vs. MEA + *S. lacrymans*; Blank vs. MEA + *F. vaillantii*; Blank vs. Filter paper (Whatman); Blank vs. (+)- α -pinene ($\leq 97.0\%$, Fluka 80604); Blank vs. 2-heptanal ($\leq 95.0\%$, Fluka OJ 75170); Blank vs. 2-pentanone ($\leq 99.0\%$, Fluka OJ 68950); Blank vs. ethanol (96% v/v, BDH 28719); Blank vs. α -pinene + ethanol; Ethanol vs. α -pinene.

All experiments were conducted in visually neutral surroundings at 20°C±1°C, 65% RH (±5%) and replicated 40 times. Weevils were monitored for 2 hours, or until a choice was made. The numbers of adults entering each vial was compared using χ^2 analysis, with the null hypothesis that weevils demonstrate no preference (Bailey, 1995).

RESULTS

Results are presented in Table 1. The apparatus showed no bias between vials and no significant attraction was demonstrated for sound wood over a blank vial. Significant preferences were shown for *C. puteana* and *S. lacrymans* volatiles when decaying wood, though only for *C.*

Table 1. Influence of olfactory cues on *E. confine* chemotaxis

Choice 1	Choice 2	Capture 1	Capture 2	Total
Blank	Blank (Control)	23	17	40
Blank	Undecayed <i>Pinus nigra</i> sapwood	26	14	40
Blank	Decayed <i>Pinus nigra</i> (<i>Coniophora puteana</i>)	11*	29*	40
Blank	Decayed <i>Pinus nigra</i> (<i>Serpula lacrymans</i>)	13*	27*	40
Blank	Decayed <i>Pinus nigra</i> (<i>Fibroporia vaillantii</i>)	17	23	40
Blank	Malt extract agar	16	24	40
Blank	<i>C. puteana</i>	8*	32*	40
Blank	<i>S. lacrymans</i>	15	25	40
Blank	<i>F. vaillantii</i>	21	19	40
Blank	Filter paper	16	24	40
Blank	α -pinene	12*	28*	40
Blank	2-heptanal	29*	11*	40
Blank	2-pentanone	12*	28*	40
Blank	ethanol	9*	31*	40
Blank	α -pinene + ethanol	13*	27*	40
Ethanol	α -pinene	25	15	40

*Significant results using χ^2 analysis at $P \leq 0.05$ with 1 degree of freedom.

puteana when grown on agar. There was a significant response to α -pinene and to ethanol on their own and when tested together. *E. confine* showed no preference for ethanol over α -pinene when tested against each other. There was also a significant preference for blank vials over 2-heptanal, suggesting this volatile to be a repellent.

DISCUSSION

The results from this study show that *E. confine* uses olfactory cues to locate potential food sources. Of the volatiles tested, *E. confine* demonstrated positive orientation towards α -pinene and 2-pentanone, suggesting that these may be key compounds in attracting these weevils to decaying timbers. α -pinene is released by freshly cut sound timber and has been shown to be an attractant to pine weevil, *Hylobius sp.* which are attracted to freshly cut stumps and recently wounded trees (Schroeder, 1988; Lindl w et al., 1993). α -pinene is also released by wood undergoing fungal decay (Korpi et al., 1999). It may be that volatile concentration determines the attractiveness of decayed wood to *E. confine* adults. Wood undergoing fungal decay is suitable for colonisation, whereas a freshly cut (un-decayed) stump would be unsuitable for this species, though no monitoring for attraction has yet been undertaken.

Tilles et al. (1986) and Shore and Lindgren (1996) showed that ethanol was a synergist for α -pinene in attracting the ambrosia beetle *Trypodendron lineatum* (Oliv.) (Coleoptera: Scolytidae) to potential food sources. Synergism of this kind was not seen with *E. confine*. Ethanol would not usually be produced when structural wood undergoes decay, as it is not produced under aerobic conditions.

The positive response exhibited to 2-pentanone has not been previously reported for any other wood-boring insect. Further research will be conducted to this and the weevils' negative response to 2-heptanal. Future work will also examine the antennal morphology of *E. confine* in relation to their response to tactile and chemical stimuli and the importance of wood decay level in determining substrate attraction.

In conclusion, this study has shown for the first time that adult *E. confine* use olfaction to detect suitable substrate. Volatiles released by the decay fungi commonly associated with this pest have been shown to influence the response of this species.

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