ATTRACTION OF ADULT *COSTELYTRA ZEALANDICA* TO 4-HYDROXYBENZALDEHYDE

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ABSTRACT

Field experiments were conducted to determine the attraction of adult *Costelytra zealandica* (White) to phenol, the sex pheromone for this species, and the phenolic aldehyde, 4-hydroxybenzaldehyde, when presented separately and in a mixture in traps. Aqueous phenol and 4-hydroxybenzaldehyde in acetone were equally attractive to beetles when presented separately in traps; a mixture of aqueous phenol and 4-hydroxybenzaldehyde in acetone also attracted similar numbers of beetles to either chemical alone. When the two chemicals were presented in separate tubes in the same trap, ca 2-fold greater numbers of beetles were attracted than with either component alone. Supplementary experiments showed solvent type and age of solution had no influence on the attractiveness of 4-hydroxybenzaldehyde when presented alone in traps. A possible explanation for the role of 4-hydroxybenzaldehyde, a component of some host plants of *C. zealandica*, acting in concert with the sex pheromone of this species is briefly discussed.

Keywords: *Costelytra zealandica*, sex pheromone, semiochemical, phenol, 4-hydroxybenzaldehyde, trap.

INTRODUCTION

Costelytra zealandica (White) (Coleoptera: Scarabaeidae) is a common native insect pest of ryegrass-white clover pastures in New Zealand. Estimates of the value of losses caused by the soil-dwelling larvae of this species range between \$41-89 million per annum (Garnham & Barlow 1993). Semiochemicals for *C. zealandica* have been investigated over many years following the discovery by Osborne & Hoyt (1969, 1970) that the adhesive Pliobond® (Goodyear Tire & Rubber Company, USA) and Durez 12687, a phenol formaldehyde resin believed to be a component of the adhesive, were powerful attractants to male beetles. Subsequent research identified that phenol was a sex pheromone for this species (Henzell & Lowe 1970) and is produced by bacteria in the collateral glands of females (Hoyt et al. 1971). Aqueous phenol solutions were also shown to be attractive to male beetles (Henzell 1970).

The discovery that moistened Durez 12687 was considerably more attractive to male beetles than aqueous phenol solutions or crystalline phenol when the release rates of phenol were similar could not be explained (Boyd 1975). Osborne & Boyd (1975) suggested either an unidentified potent attractant or an odour synergist was present in Durez 12687 and this accounted for superior catches in traps baited with this substance. Although further chemical analysis of Durez 12687 was carried out (Boyd 1975), the identity of another attractant or synergist chemical was never confirmed.

Studies on phenolic compounds related to those found in Durez 12687 have also been carried out and one, reputedly, displayed synergistic activity with phenol (Osborne 1983). This compound was 4-hydroxybenzaldehyde which, when left to stand in acetone for 2-3 weeks, increased trap catches of male beetles by 5-6 fold over aqueous phenol alone. On standing at room temperature, acetone and 4-hydroxybenzaldehyde will react to form 4-(4-hydroxyphenyl)-3-butene-2-one (Jacobsen et al. 1976), and it was this compound Osborne (1983) suggested could be the odour synergist. Unfortunately,

subsequent field tests with 'aged' 4-hydroxybenzaldehyde and acetone in combination with phenol were inconclusive.

The aim of this study was to revisit the earlier claim by Osborne (1983) that the addition of 4-hydroxybenzaldehyde in acetone enhanced the potency of phenol as a sex attractant for *C. zealandica*.

METHODS AND MATERIALS

Main experiments

Field experiments were established at three farms on the Canterbury Plains where *C. zealandica* is a persistent pest. Simple water pan traps of the type described by Osborne & Hoyt (1970) were set out at for three nights at Sites 1 and 2, and four nights at Site 3 during the peak of the beetle flight period in late spring. Traps were placed on the surface of paddocks at ca 20 m intervals in a grid pattern and treatments were replicated four times in a randomised block design. The chemicals (Fig. 1, Table 1) were placed in the traps about 30 min before the beetle flights began at dusk each evening. Phenol (Sigma-Aldrich Corporation, USA; 99% purity, MWt 94.1, vp 0.627 mm Hg at 10°C) (10 mg) and 4-hydroxybenzaldehyde (Sigma-Aldrich Corporation, USA; 98% purity, MWt 122.1, vp 0.0014 mm Hg at 10°C) (10 mg) were dissolved in water (1 ml) or acetone (1 ml), respectively. For treatments 1, 2, 3 and 5, component chemicals were placed in a single 10 ml glass tube in a trap, but for treatment 4 the chemicals were placed in separate tubes and both tubes were placed adjacent in the same trap. Control traps (no attractant chemical) were not set out during the experiments because previous work had shown very few beetles were captured in such traps.

FIGURE 1: The structural formula of (a) phenol and (b) 4 hydroxybenzaldehyde.

At the cessation of flights each evening, the content of each trap was collected and stored in a sealed plastic bag for counting at a later time. At each site, trap catches for each treatment on all nights were pooled for analysis of variance; when a significant ($P < 0.05$) *F*-ratio was obtained, means were compared by a least significant difference (LSD) test ($P = 0.05$).

Supplementary experiments

Results from the main experiments indicated 4-hydroxybenzaldehyde in acetone was an attractant for *C. zealandica* beetles (Table 1). However, it was not clear whether 4-hydroxybenzaldehyde or a reaction product was attractive. Using the same traps and four replicates per treatment on two separate evenings towards the end of the beetle flight season, two supplementary experiments were carried out. On the first night, the effect of ageing 4-hydroxybenzaldehyde (10 mg) in acetone (1 ml) for 3 weeks was compared with freshly-prepared solutions (one from which the acetone was rapidly evaporated after preparation) and 4-hydroxybenzaldehyde dissolved in ethanol. On the second night, freshly-prepared acetone and ethanol solutions of 4-hydroxybenzaldehyde (10 mg in 1 ml, respectively) were compared with ethanol and acetone alone, and aqueous phenol (10 mg in 1 ml). Trap catch data for each evening were subjected to analysis of variance and means were separated by a LSD test ($P = 0.05$).

RESULTS

Main experiments

Beetles were trapped at all three sites, although markedly more beetles were captured at Site 1 than at Sites 2 and 3 (Table 1). The trapping data confirmed aqueous phenol (treatment 1) was an attractant for adult *C. zealandica*. The presence of acetone in aqueous phenol solutions (treatment 2) did not significantly influence the attractiveness of phenol although lower average trap catches were recorded at all three sites when compared with aqueous phenol alone. The addition of 4-hydroxybenzaldehyde in acetone to aqueous phenol solutions (treatment 3) did not significantly increase the catch of beetles over that of aqueous phenol alone. When aqueous phenol and 4-hydroxybenzaldehyde were presented in separate tubes in the same trap (treatment 4), significantly more beetles were caught at all three sites than in traps baited with aqueous phenol alone, and aqueous phenol with 4-hydroxybenzaldehyde in acetone mixed in the same tube (treatment 3). When 4-hydroxybenzaldehyde in acetone was presented alone (treatment 5), these traps captured similar numbers of beetles to those in treatments 1-3, but significantly fewer beetles than in treatment 4.

TABLE 1: Mean trap per catch, per night of adult *Costelytra zealandica* at three sites in Canterbury in response to phenol and 4-hydroxybenzaldehyde.

Treatment	Site 1		Site 2		Site 3	
	Mean	SE	Mean	SE	Mean	SE
1. Phenol	338.0	70.5	69.0	4.0	30.5	2.9
2. Phenol&acetone	276.5	26.0	44.0	11.8	25.5	4.4
3. Phenol&4-hydroxybenzaldehyde ¹	443.8	64.3	81.5	12.2	41.3	7.5
4. Phenol&4-hydroxybenzaldehyde ²	835.5	44.8	126.5	11.3	180.5	45.0
5. 4-hydroxybenzaldehyde	413.0	75.5	50.8	12.3	106.5	5.8
LSD (P=0.05)	183		33		64	

¹Treatment 3: phenol and 4-hydroxybenzaldehyde mixed in one tube in traps.

²Treatment 4: phenol and 4-hydroxybenzaldehyde placed in separate adjacent tubes in traps.

Supplementary experiments

When the influence of solvent type and 'ageing' of 4-hydroxybenzaldehyde in acetone were tested in supplementary experiments (Table 2), there were no significant differences between average trap catches for all treatments on night 1, although twice as many beetles were captured in traps baited with freshly-prepared 4-hydroxybenzaldehyde from which the acetone had been evaporated immediately after preparation. On night 2, again, no significant differences in average trap catches occurred between traps baited with 4-hydroxybenzaldehyde dissolved in acetone or ethanol and aqueous phenol, and very few beetles were caught in traps in which acetone or ethanol alone was present.

DISCUSSION

Phenol is regarded to be the primary sex pheromone for *C. zealandica* (Henzell & Lowe 1970) and was the first sex pheromone identified for scarab beetles (Leal 1998). More recently, phenol has been identified from whole body extracts of *Melolontha hippocastani* F. (Ruther et al. 2001), and has been shown to attract adult male *M. hippocastani* and *M. melolontha* L. (Ruther et al. 2002). Although several studies have now demonstrated the attractiveness of phenol to adult male *C. zealandica*, few experiments have been carried out to explore its attractiveness in combination with other chemicals. This is warranted since there has been increased recognition of the role of host plant volatile chemicals interacting with pheromones in the attraction of insects (e.g. Dickens 1989; Yarden & Shani 1994; Ruther et al. 2002; Yang et al. 2004).

TABLE 2. Supplementary experiments on the influence of solvent type with 4-hydroxybenzaldehyde on the mean catch per trap, per night of adult *Costelytra zealandica*.

Treatment	Mean	SE
Night 1		
4-hydroxybenzaldehyde in acetone, freshly prepared	102.2	45.8
4-hydroxybenzaldehyde in acetone, aged 3 weeks	85.7	15.1
4-hydroxybenzaldehyde freshly prepared, acetone evaporated rapidly	207.5	27.9
4-hydroxybenzaldehyde in ethanol	102.5	44.4
LSD (P=0.05)	126	
Night 2		
Acetone	1.0	0.6
4-hydroxybenzaldehyde in acetone	67.6	11.4
Ethanol	2.0	2.5
4-hydroxybenzaldehyde in ethanol	68.4	12.0
Phenol in water	87.2	12.3
LSD (P=0.05)	28	

The assertion by Osborne (1983) that an odour synergist for phenol may be formed from the reaction between 4-hydroxybenzaldehyde and acetone, and would result in substantially increased trap catches, was not confirmed in this study. Mixtures of aqueous phenol and 4-hydroxybenzaldehyde in acetone prepared in a manner similar to that described by Osborne (1983) and left to 'age' for ca 3 weeks were no more attractive to adult *C. zealandica* than phenol alone (Table 1). A similar result was recorded in the supplementary experiment on night 1. Although no authentic sample of the reaction product between acetone and 4-hydroxybenzaldehyde was obtained and tested, it would not now seem relevant because 4-hydroxybenzaldehyde in ethanol (where no reaction product is formed) proved to be equally attractive as 4-hydroxybenzaldehyde in acetone, and aqueous phenol in the supplementary experiment on night 2. The most significant discovery from these experiments was that 4-hydroxybenzaldehyde proved to be an attractant equivalent to phenol. The observation that, on average, approximately 2-fold greater numbers of beetles were caught in traps when phenol and 4-hydroxybenzaldehyde were presented in separate tubes in the same trap than when they were mixed in one tube was also novel (Table 1). This response could more accurately be described as additive, rather than synergistic as suggested by Osborne (1983).

A possible explanation for these observations is that when 4-hydroxybenzaldehyde in acetone is presented separately in a trap, acetone rapidly evaporates and leaves a deposit of 4-hydroxybenzaldehyde (and, possibly some reaction product from 'aged' solutions) that releases vapour by sublimation (Budavari et al. 1989). When aqueous phenol is placed in traps, phenol would volatilise from solution so when aqueous phenol and an acetone solution of 4-hydroxybenzaldehyde were mixed in a tube, only phenol would be readily released because sublimation of 4-hydroxybenzaldehyde would not occur. Further, there is the possibility that the phenolic compounds are deprotonated in the solvents (water and acetone) and transformed to non-volatile anions thus reducing their release rates. Phenol has an estimated vapour pressure 63-fold greater than 4-hydroxybenzaldehyde at 10°C (www.chemical-ecology.net/java/jav-vp.htm, accessed 3 April 2009) and thus the aerial concentration of phenol immediately around the trap was likely to be significantly higher than that of 4-hydroxybenzaldehyde.

Several biological explanations may account for the improved attractiveness of phenol and 4-hydroxybenzaldehyde to adult *C. zealandica* when presented in separate tubes in

the same trap. One relates to the role of host plant chemicals working in combination with pheromones. Budavari et al. (1989) state 4-hydroxybenzaldehyde is widely distributed in plants in small amounts, and Whitehead et al. (1979) have shown it is found in the roots, and surrounding soil, of ryegrass and white clover plants, which are favoured hosts of *C. zealandica* and the dominant plant species in New Zealand pastures. Several other scarab species show increased responses when a combination of sex pheromone and host plant chemicals are presented (Yarden & Shani 1994; Ruther et al. 2002), and this could be a further example. The adaptive advantage of a response by an insect species to a combination of sex pheromone and host plant volatiles could be that mating and oviposition would occur on or near the host plant and thus favour the development and survival of offspring.

An alternative explanation relates to the respective roles of chemicals in multi-component sex pheromones. Although phenol is reputedly the main constituent of the sex pheromone of *C. zealandica* (Henzell & Lowe 1970), gas chromatographic analysis of ether extracts of adult females revealed numerous unidentified peaks in addition to phenol. It is possible one or more of these chemicals may act as a short-range aphrodisiac and enhance the capture of males in a trap. It is suggested that 4-hydroxybenzaldehyde performs this function with the African sugar-cane borer, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) (Kunesch et al. 1981). Interestingly, 4-hydroxybenzaldehyde is also implicated in the chemical ecology of several dytiscid beetle species as a component of defence secretions (e.g. Staddon 1979), as a phagostimulant for a scolytid beetle (Baker et al. 1968) and as a component of the pheromone produced by males of a leaf-footed bug (Aldrich et al. 1979). More detailed behavioural analysis of the response of *C. zealandica* to phenol and 4-hydroxybenzaldehyde is needed to resolve their respective roles in the chemical communication of this species.

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