

**Upwind flight of *Prostephanus truncatus* (Horn) is mediated  
by aggregation pheromone but not food volatiles**

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## Abstract

Flight bioassays were carried out to investigate the response of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to host-maize materials in comparison to the male-produced aggregation pheromone. Upwind attraction of flying *P. truncatus* is mediated by the male-produced aggregation pheromone. In contrast, beetles showed no response to maize grains or its volatiles, and no interactions between aggregation pheromone and maize volatiles were recorded. This absence of upwind flight to food volatiles, or any synergism between pheromone and food volatiles suggests that the male-produced aggregation pheromone is the only known long-range semiochemical used by *P. truncatus* for dispersal and host selection. These results are in contrast with those on several other stored-product species. We propose that being principally a wood borer which is not restricted to food stores, *P. truncatus* may not use maize volatiles as long-range host finding kairomones in nature.

## Introduction

Many phytophagous insects use host-derived kairomones, pheromones or both to locate suitable hosts (Metcalf, 1987), including stored-product insects (Barrer, 1983; Stubbs *et al.*, 1985; Pierce *et al.*, 1990; Subramanyam *et al.*, 1992; Dowdy *et al.*, 1993; Phillips *et al.*, 1993). For *Carpophilus* spp. food volatiles and pheromones are known to interact in stimulating a response (e.g. Bartelt *et al.*, 1992; Hengchen *et al.*, 1992; James *et al.*, 1994). For other species aggregation pheromones act synergistically with food volatiles (e.g. Walgenbach *et al.*, 1987; Dowd & Bartelt, 1991; Dowdy *et al.*, 1993). A knowledge of the interaction between stored-products insects and their plant hosts may have important practical applications such as in the development of food-baited trapping systems.

The larger grain borer, *Prostephanus truncatus* (Horn) is a member of the coleopteran family Bostrichidae, which consists principally of wood borers (Chittenden, 1911). Although it is a serious pest of farm-stored maize and cassava in the tropics (Hodges, 1986), large widespread populations of *P. truncatus* occur in the bush, suggesting that the major reservoir for the pest is probably wood (Rees *et al.*, 1990; Nang'ayo *et al.*, 1993).

The two-component male-produced aggregation pheromone of *P. truncatus* elicits attraction of walking conspecifics in laboratory bioassays (Cork *et al.*, 1991; Boughton & Fadamiro, 1996), as well as flying beetles in wind tunnels (Fadamiro, 1995), and in the field (Dendy *et al.*, 1989; Farrell & Key, 1992; Tigar *et al.*, 1993). *Prostephanus truncatus* adults are attracted over short distances to maize grain (Hodges, 1994, citing Detmers, 1990) and dried cassava (Wright *et al.*, 1993). However, field studies in Africa (Wright *et al.*, 1993), and Central America (Tigar *et al.*, 1994) suggest that *P. truncatus* is not attracted at long-range to maize grain or cob or to dried cassava.

Coupled gas chromatographic (GC)-electroantennographic detection (EAD) analyses using *P. truncatus* have led to the identification of ten antennally-active compounds in maize volatiles, and nine in cassava volatiles (Pike *et al.*, 1994). The bioactivity of these compounds was confirmed using a pitfall bioassay. As a next logical step to the above initial positive results, we were interested in the study of upwind flight behaviour of *P. truncatus* to different blends of the active components of the maize volatiles that had been identified by Pike *et al.* (1994).

We report the results of the wind tunnel studies on the responses of *P. truncatus* to maize-grain volatiles and aggregation pheromone alone and together.

## Materials and methods

### *Wind tunnel bioassays*

Adult *P. truncatus* (Tanzanian strain which had been cultured in the lab for up to 5 years) were reared on whole, clean maize at  $30\pm 1^\circ\text{C}$  and  $65\pm 5\%$  r.h. under a L12:D12 photoperiod. Beetles of known age were collected from cultures established on milled grain that passed through an Endecotts sieve, 3.35 mm mesh, to ease removal of beetles (Fadamiro, 1995). Intact beetles used in bioassays were sexed using the method of Shires & McCarthy (1976). Flying beetles were bioassayed 10-12 h into the photophase in a clear, rectangular, glass wind tunnel, 160 x 75 x 75 cm (Fadamiro, 1995), at  $29\pm 1^\circ\text{C}$ ,  $30\pm 5\%$  r.h., 3700 lux, and  $20\pm 1$  cm/s wind speed.

### *Pheromone*

The male-produced aggregation pheromone of *P. truncatus* has two components, 1-methylethyl(2*E*)-2-methyl-2-pentenoate (Trunc-call 1 or T1) and 1-methylethyl(*E,E*)-2,4-dimethyl-2,4-heptadienoate (Trunc-call 2 or T2) (Cork *et al.*, 1991, D. Hall, pers. comm.). A 1:1 blend of T1:T2 and dichloromethane solvent were

supplied by the Natural Resources Institute, Chatham, UK (NRI) in polythene vial dispensers (20 x 9 x 1.5 mm). Both were stored separately at -50°C before use. An optimal 0.2 mg dose of the pheromone was used in the bioassays (Fadamiro, 1995).

### *Maize volatiles*

Pesticide-free, clean, American yellow maize grain packaged in thick polythene bags were frozen and then stored in a cold room at 3-4°C. Prior to use in bioassays, whole maize grains were allowed to warm up to 20±5°C overnight. Volatiles from freshly harvested whole maize were collected (Pike *et al.*, 1994) by placing 3 kg of grain in a 3 l Buchner flask and drawing in air (2 l/min) through a filter of activated charcoal via a tube to the base of the flask and out through a collecting filter containing Porapak Q (50-80 mesh; 200 mg). The Porapak was pre-cleaned by washing thoroughly with dichloromethane and drying under nitrogen. Breakthrough of volatiles was prevented by connecting two filters in series. Collections were made for 7-day periods at 27°C and 70% r.h. Trapped volatiles were eluted with dichloromethane (4 x 500 µl) purified by passage through neutral alumina. Samples were analysed by capillary GC on (25m x 0.32 mm i.d. columns) coated with polar CP-Wax 52CB (Chrompack) or non-polar CP-Sil 5CB (Chrompack) with helium carrier gas at 0.4 kg/cm<sup>2</sup>, splitless injection and oven temperature held at 50°C for 2 min then programmed from 50°C to 230°C at 6°C/min. Quantitative analyses used flame ionisation detection (220°C). Six blends of five synthetic maize volatiles were tested, as was the natural blend of captured maize volatiles (Table 1).

### *Response to maize versus pheromone*

Five treatments, presented singly, were tested in the wind tunnel. These were:

- 1) no-food control, comprising of an empty 9 cm petri dish + solvent vial
- 2) whole-grain maize, 9 cm petri dish containing 50 mg of fresh maize + solvent vial.

- 3) infested maize, 9 cm petri dish containing 50 mg of *P. truncatus*-infested maize + solvent vial.
- 4) pheromone, empty 9 cm petri dish + 0.2 mg pheromone vial.
- 5) maize-pheromone, 9 cm petri dish containing 50 mg of fresh grain + 0.2 mg pheromone vial (to test for interaction between maize and pheromone).

The two constituents of each treatment were placed  $\leq 1$  cm apart on a 20 cm high platform placed 35 cm downwind of the air-outlet screen, and 70 cm upwind of a beetle release platform. For each treatment, 10 unsexed beetles were placed on the release platform. Data recorded during the following 10 min were: number of beetles taking-off; number landing upwind of the release platform within  $\leq 50$  cm to the upwind screen; number orienting to treatment by flying upwind at the level of and to within 10 cm of the treatment; and number landing on the upwind platform holding the treatment. Each treatment was tested six times with a fresh group of 10 beetles. Data were made using Kruskal-Wallis (non-parametric) test (Sokal & Rohlf, 1981).

#### *Response to maize volatiles versus pheromone*

A dose response experiment was undertaken with 20, 50, 100 & 200  $\mu$ l of blend 1 (Table 1), the complete synthetic blend. This was compared to 20  $\mu$ l of the solvent control (dichloromethane), and 0.2 mg of the synthetic aggregation pheromone. The blend doses were dispensed onto a small triangular piece of filter paper using a disposable microcap pipette. The filter paper was then suspended by a 20 cm high plastic peg clamp 70 cm upwind of the beetle release platform. Twenty unsexed beetles were released and the following data recorded for 10 min: numbers of beetles taking-off; numbers orienting (as above); and number landing on the treatment source (the peg or the filter paper). There were three replicates per treatment.

In a second experiment, nine treatments were tested singly in randomised order: the seven synthetic maize volatile blends (100  $\mu$ l dose); 100  $\mu$ l of dichloromethane solvent control; and 0.2 mg of pheromone. All nine treatments were

run in a day and replicated four times, except for blend 7 (Table 1) which was replicated only twice due to shortage of material. Responses of 20 released beetles, per stimulus were observed as above for 7.5 min.

*Two-choice experiment: pheromone+solvent versus pheromone+ maize volatiles*

This experiment was designed to test if flying beetles would discriminate between pheromone alone and a pheromone+host volatiles source when simultaneously presented in the wind tunnel.

The design of this experiment was similar to that described by Fadamiro *et al.* (1996). The two sources were suspended 20 cm high, 12.5 cm apart and 100 cm upwind of the centered beetle release platform. One of the sources consisted of 0.2 mg pheromone in a polythene vial plus 100  $\mu$ l of the dichloromethane solvent placed closely side by side. The other source was a vial with 0.2 mg of pheromone beside a vial containing 100  $\mu$ l of blend 7 (Table 1). Ten male and 10 female beetles were released together downwind of the two odour sources and the data recorded for 10 min were: number of beetles orienting to each source; and number and sex of beetles landing on each source. The two sources were alternated between tests to control for possible bias in the wind tunnel. This experiment was repeated six times and data were analysed using Chi-square tests (Parker, 1979).

## Results

*Response to maize versus pheromone*

The numbers of beetles taking off were not significantly different between the five treatments (Table 2). However, significantly more beetles landed upwind, oriented upwind, and landed on the treatment source in the two treatments containing

pheromone than in the other three treatments which were not different from each other (Table 2).

#### *Response to maize volatiles versus pheromone*

With the exception of the 100 µl dose of maize volatile blend 1, to which three beetles oriented (most probably by chance), no flying beetle oriented to or landed on the source of maize volatiles at any dose (Table 3). However, about 65% of flying beetles landed on the pheromone treatment source.

In the second experiment, flying *P. truncatus* were generally unresponsive to all seven volatile blends including blend 7, the natural maize volatiles (Table 4). However, over 80% of flying beetles responded to the pheromone source.

#### *Two-choice experiment: pheromone+solvent versus pheromone+ maize volatiles*

There were no significant differences in the numbers or sex ratios of beetles orienting to, and landing on sources of pheromone+solvent, or pheromone+maize volatiles (Table 5).

## Discussion

Apart from confirming pheromone-mediated upwind flight of *P. truncatus*, the results of the first two experiments (Tables 2 & 3) suggest that *P. truncatus* are not attracted in upwind flight from long range ( $\geq 70$  cm) to maize or maize volatiles. This contrasts with the results of 'pitfall' bioassays in *P. truncatus* responded over a short range ( $\leq 5$  cm) to maize and cassava volatiles (Pike *et al.*, 1994).

Stored-product beetles that respond positively to host-plant volatiles include: *Trogoderma* spp. (Dermestidae) (Nara *et al.*, 1981), *Oryzaephilus* spp. (Cucujidae) (Mikolajczak *et al.*, 1984; Stubbs *et al.*, 1985; White, 1989; Pierce *et al.*, 1990), *Tribolium* spp. (Tenebrionidae) (Phillips *et al.*, 1993), and *Sitophilus* spp. (Curculionidae) (Phillips *et al.*, 1993; Pike *et al.*, 1994). Dowdy *et al.* (1993) report



that *Rhyzopertha dominica* (Bostrichidae) was more responsive to infested than clean wheat, possibly because of pheromone in infested grain. Pierce *et al.* (1990) concluded that *Oryzaephilus* spp. may use some components of host volatiles as host-finding kairomones in nature. However, the majority of the above mentioned studies employed pitfall olfactometers (walking bioassays). Only rarely, e.g. for *Carpophilus lugubris* Murray (Nitidulidae) has response by flying beetles to food odours been recorded in the wind tunnel (Lin & Phelan, 1991), and in the field (Lin *et al.*, 1992). It seems reasonable that food volatiles could mediate food finding in the dusky sap beetle, *C. lugubris* and other nitidulids, since they are naturally closely associated with ripening fruits and vegetables (Lin *et al.*, 1992).

A flight bioassay may be a more realistic model of beetle field responses than bioassays employing walking beetles (Domek *et al.*, 1990), and can act as a good predictor of chemically mediated host finding in the field (Lin & Phelan, 1991; Lin *et al.*, 1992). This may account for the contrast between our results and those of Pike *et al.* (1994). However, our results support the results of field studies in Togo (Wright *et al.*, 1993) and Mexico (Tigar *et al.*, 1994) which detected no long-range attraction of adult *P. truncatus* to maize grain or cobs.

In neither single- nor two-choice flight bioassays did the addition of maize to a pheromone source alter the level of beetles' response (Tables 2 & 4). The absence of interaction between aggregation pheromone and maize volatiles is in contrast to many studies in which synergism occurred between the aggregation pheromones and host volatiles, e.g. for: *Carpophilus* spp. (Nitidulidae) (Dowd & Bartelt, 1991; Bartelt *et al.*, 1992; Lin *et al.*, 1992; James *et al.*, 1994); *Sitophilus* spp. (Curculionidae) (Walgenbach *et al.*, 1987); and *Oryzaephilus* spp. (Cucujidae) (Oehlschlager *et al.*, 1988).

Because *P. truncatus* is principally a wood borer (Chittenden, 1911) with great abundance in the natural environment (Nang'ayo *et al.*, 1993), one might not expect it to be specifically attracted to maize-plant materials. Hodges (1994) has

suggested that *P. truncatus* bores test burrows into any sufficiently soft substrate, eventually locating suitable foods such as maize and cassave. A similar means of host selection is apparently used by some Scolytidae attacking wood (Wood, 1982). Kennedy's (1986) hypothesis of a requisite dispersal phase prior to responding to olfactory stimuli seems unlikely to apply to *P. truncatus*, since it did to require a dispersal phase before orienting to pheromone. On-going field trapping studies in Kenya have shown that the addition of synthetic maize volatiles to pheromone makes no contribution to trap catch, thus providing further evidence that they play no role in host selection (Hodges, unpublished data).

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Volatile blend	Components / ratio
1	Nonanal 8: Hecanal 8: Hexanoic acid 50 (50 ng/μl): Nonanoic acid 25: Vanillin 1: 2 phenyl-ethanol 6
2	Blend 1 plus tetradecanal 2 ng/μl
3	Blend 1 minus hexanoic acid
4	Blend 1 minus nonanoic acid
5	Hexanoic (50 ng/μl) and Nonanoic acids in 2:1 ratio
6	Blend 1 plus 2-octenal (5 ng/μl), 2-nonenal (5 ng/μl), octan-3-ol (3 ng/ul), and octen-3-ol (2 ng/μl).
7	A whole entrainment sample from maize ( natural maize volatiles)

**Table 1.** The composition of synthetic and natural maize volatile mixtures used in this study.



Treatment	No. taking-off	No. landing upwind	No. orienting to treatment source	No. landing on treatment source
No-food	24	6 a	0 a	0 a
Clean maize	25	8 a	5 a	0 a
Infested maize	23	9 a	4 a	1 a
Pheromone	21	17 b	15 b	15 b
Maize+pheromone	22	18 b	17 b	17 b
<i>P</i> -value	= 0.62	= 0.02	< 0.0006	< 0.0001

**Table 2.** Responses of *P. truncatus* to whole-grain maize grain *versus* pheromone. A total of 60 beetles were released per treatment in 6 replicates. Values in the same column having the same letter are not significantly different (at  $P < 0.05$ ). Chi-square values for numbers taking off, landing upwind, orienting and landing on the treatment source are 2.63, 12.27, 19.43 and 25.61, respectively.

Treatment	No. taking-off	No. orienting to treatment source	No. landing on treatment source
Blend 1, 20 $\mu$ l	9	0	0
Blend 1, 50 $\mu$ l	14	0	0
Blend 1, 100 $\mu$ l	14	3	0
Blend 1, 200 $\mu$ l	12	0	0
20 $\mu$ l dichloromethane solvent control	20	0	0
0.2 mg pheromone	14	9	9

**Table 3.** Response of *P. truncatus* to different doses of synthetic maize volatile blend 1. A total of sixty beetles were released per treatment.

Treatment	No. taking-off	No. orienting to treatment source	No. landing on treatment source
Blend 1	15	0	0
Blend 2	17	0	0
Blend 3	15	0	0
Blend 4	11	0	0
Blend 5	11	1	0
Blend 6	12	1	0
Blend 7	7	0	0
Solvent control	13	0	0
0.2 mg pheromone	14	12	11

Table 4. Responses of *P. truncatus* to maize volatiles versus pheromone.

Treatments presented singly in 100  $\mu$ l doses. Eighty beetles were tested per treatment except for blend 7 for which only 40 beetles were tested.

Treatment	No. orienting to source	No. landing on source	Sex ratio of beetles that landed on source (male:female)
Pheromone+Solvent	22	15	7:8
Pheromone+Volatile	20	17	9:8
P - value	> 0.5	> 0.5	> 0.5

Table 5. Response of *P. truncatus* to two simultaneously presented sources of pheromone or pheromone plus maize volatiles (Blend 7). Chi-square values for numbers orienting, landing, and sex ratio are 0.024, 0.031, and 0.125, respectively.