Studies of responses of stored-product pests, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* Motsch., to food volatiles

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Abstract

A laboratory bioassay was used to demonstrate attraction of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to volatiles from maize and cassava trapped on Porapak resin. Attraction of maize weevils, *Sitophilus zeamais* Motsch., to trapped maize volatiles was also shown. The volatile collections were examined by gas chromatography (GC) linked to electroantennographic (EAG) recording and by GC linked to mass spectrometry.

With *P. truncatus*, up to nine EAG-active components were detected in cassava volatiles and ten in maize volatiles, with at least five of the components common to the two food sources. Nonanal was identified as the major active component of cassava volatiles, and hexanoic acid of maize volatiles. Actonal, nonanoic acid and vanillin were also identified as EAG-active components.

In contrast, with *S. zeamais* only one EAG-active component revealed the presence of 1-hydroxyheptan-2-one, a novel compound in maize volatiles, but this did not elicit an EAG response from *S. zeamais*. The remaining unidentified compounds will be identified and evaluated using bioassays, in anticipation that the results will help to improve trapping methods and contribute to greater understanding of stored-product pest ecology and management.

Introduction

There is considerable interest in the behavioural responses of stored-product insects to host-plant volatiles (Barrer 1983; Subramanyam et al. 1992). Table 1 details a number of the studies that have been published.

These studies are of interest because the stored-product pests represent a special class of phytophagous insects that appear to be highly adapted to surviving on numerous commodities stored for human or animal consumption. Increased understanding of the interaction between the insects and the commodity will serve to increase our knowledge of stored-product pest ecology. The results also have important practical applications in the development of monitoring systems for stored-product pests based on attraction to food lures. Furthermore, interactions between food volatiles and pheromones have been reported for several species (e.g. Walgenbach et al. 1987; Dowdy et al. 1993; Phillips et al. 1993) and understanding of these interactions is necessary for optimisation of pheromone-based monitoring systems.

Prostephanus truncatus (Horn) or the larger grain borer is a stored-product pest which originated in Central America but has become a major problem in Africa after its introduction in the late 1970s (Hodges 1986).

This paper describes laboratory investigations of the responses of this species to food volatiles. Apart from their practical implications, the results of this work will provide interesting comparisons with those on other species because it is becoming apparent that *P. truncatus* has only adapted to storage situations relatively recently and certainly is not restricted to this type of habitat (Rees et al. 1990). Results are also presented on attraction of *Sitophilus zeamais* Motsch. to food volatiles.

Materials and Methods

Sample collection

The maize used in the trial was freshly-harvested white maize from Dar es Salaam, Tanzania. The cassava was bought as fresh intact tubers in the U.K. and originated from the Caribbean. The tubers were peeled, chipped and dried before use. Volatiles from whole maize (3 kg) or cassava chips (400 g) were collected by placing the sample in a 3-L Buchner flask and drawing in air (2 L/minute) 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, and the absence of significant breakthrough of volatiles was prevented by connecting two filters in series. Collections were made for seven-day periods at 27°C and 70% r.h.. Trapped volatiles were eluted with dichloromethane (4×500 μL purified by passage through neutral alumina.

Analyses of volatiles

Samples were analysed by capillary gas chromatography (GC) on columns (25 m × 0.32 mm i.d.) coated with polar CP-Wax 52CB (Chrompack) or non-polar CP-Sil 5CB (Chrompack) with helium carrier gas at 0.4 kg/cm^2, 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). In linked gas chromatography-mass spectrometry (GC-MS), the same GC columns and conditions were used, and the GC was interfaced to a Finnigan-MAT ITD 700 operated in electron impact (EI) or chemical ionisation (CI) with *iso*-butane. Linked gas chromatography-electroantennography (GC-EAG) analyses also used the same GC columns and conditions, as described by Cork et al. (1990). GC retention data are presented as equivalent chain lengths (ECLs) relative to the retention times of straight-chain acetates.

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Bioassay

The *S. zeamais* and *P. truncatus* used for bioassay and EAG studies originated from Zimbabwe and Tanzania respectively. The insects were kept in culture on whole maize at 27°C and 70% r.h. All the insects used for experimental purposes were between four and six weeks old and were not sexed.

The bioassay used in this trial was based on the method developed at CSL, Slough, U.K. (J. Chambers, per. comm.). The materials used comprised 200 mm formica-based arenas each with a single, central pitfall trap (3" × 1" glass tube). The arena surfaces were covered in filter paper (Whatman No. 1) to provide a uniform surface and to facilitate insect movements, and each arena was covered with a lid to prevent insect escape. Insects were bioassayed individually. For *S. zeamais* the glass pitfall tube stood proud of the arena at a height of 5 mm. Initial trails had suggested that such a position increased the sensitivity of the test. Work is ongoing to investigate this matter fully. Forty arenas were operated simultaneously and the exposure period was standardised at one hour for *S. zeamais* and two hours for *P. truncatus*. Twenty of the traps contained a sample: the remainder used the solvent alone as a control. The sample (20 μL) was introduced into the base of each experimental pitfall trap on a triangle of filter paper (Whatman No. 1; 2 cm^2).

For the *S. zeamais* bioassay, dilutions of the volatile extract were prepared and tested. The original strengths of maize and cassava volatiles have only been tested against *P. truncatus* to date. Five replicates were performed for each treatment, and differences between the numbers of insects trapped in the treatment and unbaited control were compared using *t* tests.

Results

Attraction of *P. truncatus* to maize and cassava volatiles

In the bioassay, *P. truncatus* was significantly attracted to collections of volatiles from both maize and cassava (Table 2). In linked GC-EAG analyses of cassava volatiles, nine EAG-active components were detected using the polar GC column (Table 3) and eight on the non-polar column. In GC-EAG analyses of the maize volatiles on the polar GC column, ten active components were detected, and some of these can be correlated by their retention times and mass spectra with components of the cassava volatiles (Table 3). The major component of the cassava volatiles was EAG-active and was identified as nonanal by comparison of GC retention times and mass spectra with those of synthetic material. Nonanoic acid and decanal were also identified as EAG-active components. In the maize volatiles, the major component was EAG-active and was identified as hexanoic acid. Nonanoic acid and decanal were also detected as EAG-active components. Vanillin (4-hydroxy-3-methoxybenzaldehyde) was present at significant levels in the maize volatiles and caused an EAG response. It was present at trace levels in the cassava volatiles (< 0.2% of the major component) and was not detected by the insects.

Attraction of *S. zeamais* to maize volatiles

S. zeamais was significantly attracted to collections of maize volatiles, and the effect was concentration dependent (Table 4).

The volatile collection elicited a strong EAG response from *S. zeamais*. Duplicate GC-EAG analyses were carried out on seven different insects, and a single EAG-active component was detected with retention times (ECLs) of 9.65 on the polar and 6.46 on the non-polar GC columns. GC-MS analyses on the polar column in CI mode indicated a molecular weight of 130, and in EI mode showed fragment ions at m/z 99 (35%), 71 (40%), 55 (20%), 43 (100%) and 41 (60%), although a component with similar mass spectrum could not be detected at the retention time of the EAG-active component on the non-polar column. 1-Hydroxyheptan-2-one was proposed as a structure and synthesised. The mass spectra and retention time of this compound on the polar column were identical to those of the component in the maize volatiles, and this was detected in analyses on the non-polar column at a retention time of 6.19, close to but not identical to that of the EAG-active component. The synthetic 1-hydroxyheptan-2-one was inactive in the bioassay and elicited no EAG response from *S. zeamais*.

Work is continuing to determine the structure of the EAG-active component in maize volatiles. The GC retention times of this are also similar to those of the diastereomers of the sex pheromone of *S. zeamais*, 5-hydroxy-4-methylheptan-3-one (Phillips et al. 1985), 9.60 and 9.75 on the polar column and 6.35 and 6.43 on the non-polar column. The pheromone could not be detected in the maize volatiles by GC-EAG or GC-MS analyses.

Discussion

The results reported here show that *P. truncatus* is attracted to volatiles from cassava and maize collected on Porapak. These volatiles elicit an EAG response from the beetles, the EAG-active components have been characterised by linked GC-EAG analyses, and there are several active components in common. Some of these components have been identified and work is continuing to identify the remainder. It is anticipated that those components which are detected by the antennal receptors will play a role in the behavioural response of the insect, and the synthetic compounds will be evaluated in the behavioural bioassay.

If positive behavioural responses are produced over the short range used in this bioassay further experiments will be conducted to test responses over a greater range.

Hexanoic acid, identified as the major component of the maize volatiles used in this trial was implicated in attraction of *S. zeamais* to cereals by Honda and Oshawa (1990), and nonanal, the major component of the cassava volatiles, has been shown to attract silvanid beetles both alone (Mikolajczak et al. 1984; Oehlschlager et al. 1988) and in combination with the aggregation pheromones (Mushobozy et al. 1993). Vanillin and maltol (3-hydroxy-2-methylpyr-4-one) were shown to synergise the attractiveness of the pheromones of both *S. oryzae* (L.) and *Tribolium castaneum* (Herbst.) by Phillips et al. (1993): although vanillin was found in both cassava and maize volatiles, maltol was not detected in either.

In contrast to *P. truncatus*, which showed EAG responses to several components of the maize volatiles, *S. zeamais* responded to only one component in linked GC-EAG analyses. An initial attempt at identification of the chemical structure of this showed the presence of 1-hydroxyheptan-2-one in the volatiles, although this was not the active component. This is the first time this compound has been identified in cereal volatiles, and is presumably a fatty acid oxidation product. It is structurally related to 2-hydroxyoctan-3-one, a pheromone component of *Xylotrechus* spp. (Coleoptera: Cerambycidae) (Kuwahara et al. 1987). Work is continuing to establish the identity of the component which elicits EAG and behavioural activity from *S. zeamais*.

Table 1. Published studies on the behavioural responses of stored-product insects to host plant volatiles

Oryzaephilus sp.	(Coleoptera: Silvanidae)	Mikolajczak et al.	1984
		Stubbs et al.	1985
		Pierce et al.	1990, 1991
Trogoderma sp.	(Coleoptera: Dermestidae)	Nara et al.	1981
Sitophilus sp.	(Coleoptera: Curculionidae)	Trematerra and Girgenti	1989
		Honda and Oshawa	1990
		Phillips et al.	1993
Tribolium sp.	(Coleoptera: Tenebrionidae)	Phillips et al.	1993
Rhyzopertha dominica	(Coleoptera: Bostrichidae)	Dowdy et al.	1993

Table 2. Attraction of *P. truncatus* to trapped volatiles from maize and cassava (numbers trapped in 20 individual bioassays).

Replicate	Maize		Cassava	
	Treatment	Control	Treatment	Control
1	13	5	13	5
2	12	7	8	4
3	8	2	12	4
4	6	1	12	3
5	9	5	13	5
Mean	9.6	4	11.6	4.2
SD	2.88	2.45	2.07	0.84
t	3.31		7.4	
P	0.01		>0.0001	

Table 3. Active components detected in GC-EAG analyses with *P. truncatus* of cassava and maize volatiles on a polar GC column

Cassava volatiles			Maize volatiles			
ECL[a]	n[b]	Relative amount	ECL[a]	n[b]	Relative amount	Identification
7.11	9	100	7.18	4	16	nonanal
7.49	6	0.6				
7.64	8	0.8	7.74	3	4	
8.21	8	13				decanal
8.39	9	1.8	8.33	5	2	
8.55	5	12				
8.75	7	40	8.81	5	15	
			9.10	3	0.2	
			11.76	7	100	hexanoic acid
			12.37	3	4	
14.65	4	0.2				
14.88	6	37	14.86	7	50	nonanoic acid
			18.26	3	2	
			18.51	6	6	vanillin

[a] Retention data as equivalent chain lengths.
[b] Number of times observed in three replicate analyses on three insects.

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Table 4. Attraction of *S. zeamais* to dilutions of trapped volatiles from maize (numbers trapped in 20 individual bioassays)

Replicate	× 1.0	Control	× 0.1	Control	× 0.01	Control	× 0.001	Control
1	0	1	13	5	10	5	3	3
2	12	2	15	8	7	4	4	2
3	14	3	12	3	5	4	3	3
4	15	6	12	4	7	4	5	4
5	14	2	11	4	6	5	7	6
Mean	12.8	2.8	12.6	4.8	7	4.4	4.4	3.6
SD	2.39	1.92	1.52	1.72	1.87	0.54	1.67	1.51
t	5.17		7.12		2.98		0.792	
P	0.0009		0.0001		0.0175		0.4510	

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