

# ROLE OF ODOR VOLATILES IN THE STORED-GRAIN ECOSYSTEM

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## Abstract

Insects, mites and microflora produce distinct volatiles that combine with the oxygen, nitrogen and carbon dioxide of intergranular air in the stored-grain ecosystem. Many of these volatiles, individually and collectively, affect the behaviour, development, and population growth of pest organisms in the composite gaseous environment. In the laboratory *Sitophilus granarius*, *S. oryzae*, and *Tribolium castaneum* were generally repelled by most of the four fungal and one acarine volatiles; *T. castaneum* larvae, however, were attracted to 1-octanol and 3-methyl-1-butanol. Oviposition of *T. castaneum* was reduced by 50% during the first 2 d when beetles were exposed to 3-methyl-1-butanol. Developmental time and rate of growth of *T. castaneum* population was unaffected by exposure to trigeane, but greatly reduced by exposure to 3-methyl-1-butanol.

## Introduction

In stored-grain ecosystems populations of microflora and arthropods interact among the members of their own group or between individual members of each of the two groups. Direct and indirect actions of these organisms eventually result in qualitative and quantitative deterioration of human food and livestock feed. Although a favorable combination of temperature and relative humidity sets off the destructive activity of the pest organisms, the organisms themselves modify the environment through their feeding, increasing numbers, and production of excrement and metabolites. Because most of the storage pests have aerobic respiration, accumulation of carbon dioxide and depletion of oxygen caused by their metabolic activity are the most obvious result of physiological processes involved in an active colony of insects, mites, and microflora in stored grain (Sinha et al. 1986, Mills 1986). Other metabolic products produced by microflora, such as antibiotics and mycotoxins, have been studied with considerable interest during the last few decades. But there is yet another group of inconspicuous metabolites, the volatile chemicals, which are regularly produced by most pest organisms in stored-grain ecosystems. In pre-harvest, agro-ecosystems various plant volatiles play a decisive role in the co-evolution of plants and insects, and volatile secondary plant compounds act as semiochemicals having specific functions in insect populations and host-plant selection (Metcalf 1987). Insect volatiles such as pheromones, and allomones have also been used extensively for detecting and even

controlling pest insects in field crops (Roelfs and Carde 1974). In stored-grain ecosystems, pheromones and other volatiles have been studied and successfully used for monitoring and controlling stored-product insects (Burkholder and Ma 1985). Also, several volatiles produced by mites have been isolated by Kuwahara and his group (Kuwahara et al. 1980). Despite studies on these chemical compounds and their specific use as part of an integrated pest management program, we know little about the individual and collective effects of the volatiles produced by microflora and arthropod species in actual stored-grain ecosystems. Limited holistic studies have been conducted on odor and mycotoxin formation in cereals during granary storage (Abramson et al. 1980) and on odor-volatiles associated with mite-infested bin-stored wheat (Tuma et al. 1990). These studies describe only the association of volatiles with particular species of microflora or mites rather than the positive or negative impact of these volatiles, diffused singly or collectively, on insect, mite or fungal species commonly found in the stored-grain ecosystem.

This report summarizes: (1) the locomotory response produced by four fungal and one acarine volatile on the adults and larvae of two major stored-product insects: the granary weevil, *Sitophilus granarius* (L.) and the red flour beetle, *Tribolium castaneum* (Herbst); (2) the effect of the fungal volatile, 3-methyl-1-butanol, on the oviposition of *T. castaneum*; and (3) the effect of the acarine volatiles tridecane and 3-methyl-1-butanol on development and reproduction of *T. castaneum*.

## Materials and Methods

### Locomotory response of *S. granarius* and *T. castaneum*

Fifty adult *S. granarius*, and adult or 4th instar larvae of *T. castaneum* were placed in sealed chambers to observe their responses to the presence of four separate fungal and one acarine volatile placed at one end of the apparatus. The chamber consisted of two 0.24 l glass jars connected together by attaching them to their screw-on lids which were soldered together. A 50-mm hole was previously cut out of each lid and a coarse wire mesh placed between them. The mesh was coarse enough to allow insects to travel freely between jars yet preventing grain from spilling out when jars were connected or separated.

A filter paper (No. 1 Whatman, 4.25 cm dia.) was placed at the bottom of each jar which was filled with wheat (cv. Katepwa), previously conditioned to 14.0% MC. The composite lid was screwed to this jar and the rim wrapped with PVC electrician's tape to form an airtight seal. Either 10.0-, 1.0-, or 0.1- $\mu$ l quantities of a volatile liquid was placed on the filter paper in the empty jar using a 10-ml gas-tight syringe; this jar was

then immediately filled with wheat. Twenty-five insects were added to the top of each jar which was tapped to move the insects into the grain. The jar with the attached lid was inverted and fastened to the second jar, the entire assembly was placed horizontally, and the second rim sealed with tape. The jars were held at  $30 \pm 1.0^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity (RH) for 24 h, before being separated, and the insects in each jar counted.

The fungal volatiles used were 1-octanol, 1-octen-3-ol, 3-methyl-1-butanol, and 3-octanone and the acarine volatile was tridecane. Ten replicates were used for each insect, volatile and concentration. Statistical assessment was done by analysis of variance and Duncan's multiple range test after an arcsine transformation. Significant differences in the response of insects exposed to volatiles and those in the control sets were determined by using Dunnett's test, 2-sided,  $\alpha = 0.05$  (Steel and Torre 1980).

### Oviposition tests with *T. castaneum*

Adult *T. castaneum* Herbst were taken from a healthy, mixed-age laboratory culture maintained on enriched white flour and brewer's yeast (20:1) at  $27.5 \pm 1.0^\circ\text{C}$  and  $65 \pm 5\%$  RH. Groups of 200 adults were collected into glass vials by aspiration. Ten grams of enriched white flour, previously sieved through a No. 80 mesh sieve (aperture 0.177 mm), were placed into plastic petri dishes (85 mm dia  $\times$  13 mm high). Two hundred adult insects were placed into each petri dish and the tops held in place by two pieces of masking tape. Sixteen petri dishes containing insects were placed into each of two glass desiccators which contained saturated sodium chloride solution to maintain a relative humidity of 75% RH. To acclimatize them with experimental conditions the beetles were allowed to oviposit in darkness at  $30 \pm 0.5^\circ\text{C}$  for 3 days then adults, eggs, and flour were separated using a No. 20 mesh and the eggs were counted. Adults were placed into the loosely covered petri dishes and flour again and then into desiccators. A small, open, glass petri dish containing 1 ml of 3-methyl-1-butanol was placed in one of the two desiccators which were sealed with non-ventilated glass tops and incubated at  $30 \pm 0.5^\circ\text{C}$ . Eggs were sieved and counted every 24 h for each assessment with 16 replicates. An additional 1 ml of 3-methyl-1-butanol was added after the second count because the chemical had evaporated. An identical control test was conducted in the other desiccator not containing the volatile compound. The results were analyzed by Student's t-test.

### Development and reproduction of *T. castaneum*

Ten, 1-day-old eggs of *T. castaneum* were placed in 70 mm high  $\times$  26 mm inner diameter glass vials containing 16 g of crushed wheat (*Triticum aestivum* cv. Katepwa, Canada Western Hard Red Spring No. 2 grade) ground by a plate mill (model 4-E, The

Straub Co., Croydon, Pennsylvania). Eggs were collected by first placing adult insects on white wheat flour, previously passed through a No. 80 mesh sieve (aperture 0.177 mm) and incubating these cultures for 24 h at 30°C and 70% RH. The adults were then removed and the eggs sieved using a No. 60 mesh sieve (aperture 0.250 mm).

Each vial was capped with a snap-on plastic lid, fitted with 40-mesh wire screen (aperture, 0.425 mm) over a circular opening (diameter 12 mm) and lined with a No. 5 Whatman filter paper for ventilation and to prevent escape of insects.

Two identical 9.50-l capacity glass desiccators were each filled with 500 ml of saturated KOH solution to maintain a 70% RH environment, and 60 vials containing eggs on crushed wheat were placed in each. A small glass petri dish containing 1 ml of tridecane or 3-methyl-1-butanol was added to one desiccator then both were placed into separated growth chambers maintained at  $30 \pm 0.5^\circ\text{C}$  and  $70 \pm 5\%$  RH. Each desiccator was aerated weekly and the specific volatile compound was replenished weekly as it evaporated.

Samplings began at 28 d after initiation and every 14 d thereafter with 10 vials from each desiccator being removed at a time, and total numbers of adults in each vial counted; the presence of larvae and pupae was noted. For statistical analysis insect counts from ten vials containing a volatile were compared with those from ten control vials. The data were tested for normality and those that were normal were subjected to Students' t-test, and those that were not normal were subjected to Kruskal-Wallis nonparametric test (Chi-square approximation) using SAS procedure NPAR 1 WAY (SAS Institute 1985).

## Results and Discussion

### Locomotory response of *S. granarius* and *T. castaneum*

Generally, adult and larval stages of the two insect species tested were either repelled or remained neutral when they were exposed to the five volatiles, except in the case of *T. castaneum* larvae which were attracted to 3-methyl-1-butanol and 1-octanol. The degree of repellency was generally proportional to the concentration of the compound used. Controls with no chemical indicated no significant positive or negative response (Fig. 1A-D). *S. granarius* was repelled most strongly by 3-octanone at a  $10 \mu\text{l}$  concentration; there was no response at the  $0.1 \mu\text{l}$  level (Fig. 1B). This species did not show any response to 3-methyl-1-butanol within the range of concentrations tested. *S. granarius* was repelled significantly ( $P < 0.01$ ) by the acarine volatile, tridecane, only at a  $10 \mu\text{l}$  concentration (Fig. 1A).

*Tribolium castaneum* adults also were repelled most strongly by 3-octanone, but only at a 10  $\mu\text{l}$  concentration (Fig. 1C). *T. castaneum* showed no significant response to 1-octanol regardless of the concentration used. This species, unlike *S. granarius*, was repelled by 3-methyl-1-butanol at a 10  $\mu\text{l}$  level ( $P < 0.05$ ). *T. castaneum* adults showed significantly strong repellency ( $P < 0.01$ ) to tridecane at all concentrations.

*T. castaneum* larvae were repelled most strongly by 1-octen-3-ol at all three concentrations (Fig. 1D). Larvae did not react to 3-octanone unlike the adults which were repelled strongly at a 10  $\mu\text{l}$  concentration. It is noteworthy that 3-methyl-1-butanol attracted larvae while strongly repelling adults at a 10  $\mu\text{l}$  concentration. Larvae were also attracted to 1-octanol at a 0.1  $\mu\text{l}$  concentration, whereas the adults showed no response to this compound at any concentration. *T. castaneum* larvae were repelled by tridecane only at the two lower concentrations of 1.0 and 0.1  $\mu\text{l}$ .

### Oviposition response of *T. castaneum* to fungal volatile

Oviposition of *T. castaneum* adults was reduced by 50% during the first 2 d when the beetles were exposed to the most common fungal volatile, 3-methyl-1-butanol (Fig. 2A). It seems that brief but continuous exposure to this volatile progressively reduced egg production. Such interspecific antagonistic relations between fungi and insects is likely to have selective advantage in favor of the fungus which produces the allomone, 3-methyl-1-butanol (Abramson et al. 1980). Thus the storage fungi, such as *Penicillium* and *Aspergillus*, which abundantly occur in beetle-infested stored grain in many parts of the world seem to have a definite role in suppressing the population of one of the most common stored grain beetles even when fungi are not in physical contact with the beetles in the stored-grain ecosystems.

### Development and reproduction of *T. castaneum* in an environment containing acarine and fungal volatiles

There was no significant difference in the progeny production of *T. castaneum* when individuals were exposed from the egg stage and incubated for 14 weeks in tridecane-free and tridecane-containing environments (Fig. 2B). Except for week 14, adult numbers remained close in both control and test vials; a slight increase in the counts in both sets on week 14 could be caused by factors other than volatile effects. Thus, one of the most common volatiles produced by stored-product mites seems to have no effect on development and reproduction of a common stored-grain beetle, *T. castaneum*.

In contrast, when the environment contained a fungal volatile, 3-methyl-1-butanol, progeny production of *T. castaneum* were consistently and significantly reduced at each

period of assessment as compared to those reared in an environment lacking this volatile (Fig. 2C). Therefore, the common fungal volatile, 3-methyl-1-butanol seems to have an antagonistic effect on the population growth of *T. castaneum*. It is noteworthy that similar negative effects of this major fungal allomone were also found in the oviposition experiments with the same species of stored-product insect.

### Ecological and evolutionary considerations

The preliminary results of this investigation open a new dimension to our understanding of the broad multiple roles of various volatiles produced by many common microflora, mites and insects in the stored-grain ecosystem. Because of our need to develop a better method of detection of these pests in infested produce, past research work on volatiles concentrated mainly on the locomotory behavioral aspects (aggregation and attraction potential of a volatile to facilitate insect detection trap development) of insects in relation to specific volatiles (Burkholder and Ma 1985, Pierce et al. 1989).

Whether a volatile produced by one pest species promotes or reduces population growth of other species sharing the same habitat, is, however, an important question from ecological and evolutionary standpoints; and these relationships should be studied to ensure sound strategies for long-term stored-grain pest management. If the numbers of a pest species can be reduced by another species that does not even have direct physical contact with the victim species, selective advantage of that volatile-producing species will be considerable. We must, however, study these special attributes of volatile-producing species within the whole ecosystem context, so that we will be able to effectively manipulate volatile odors to our advantage.

### Acknowledgements

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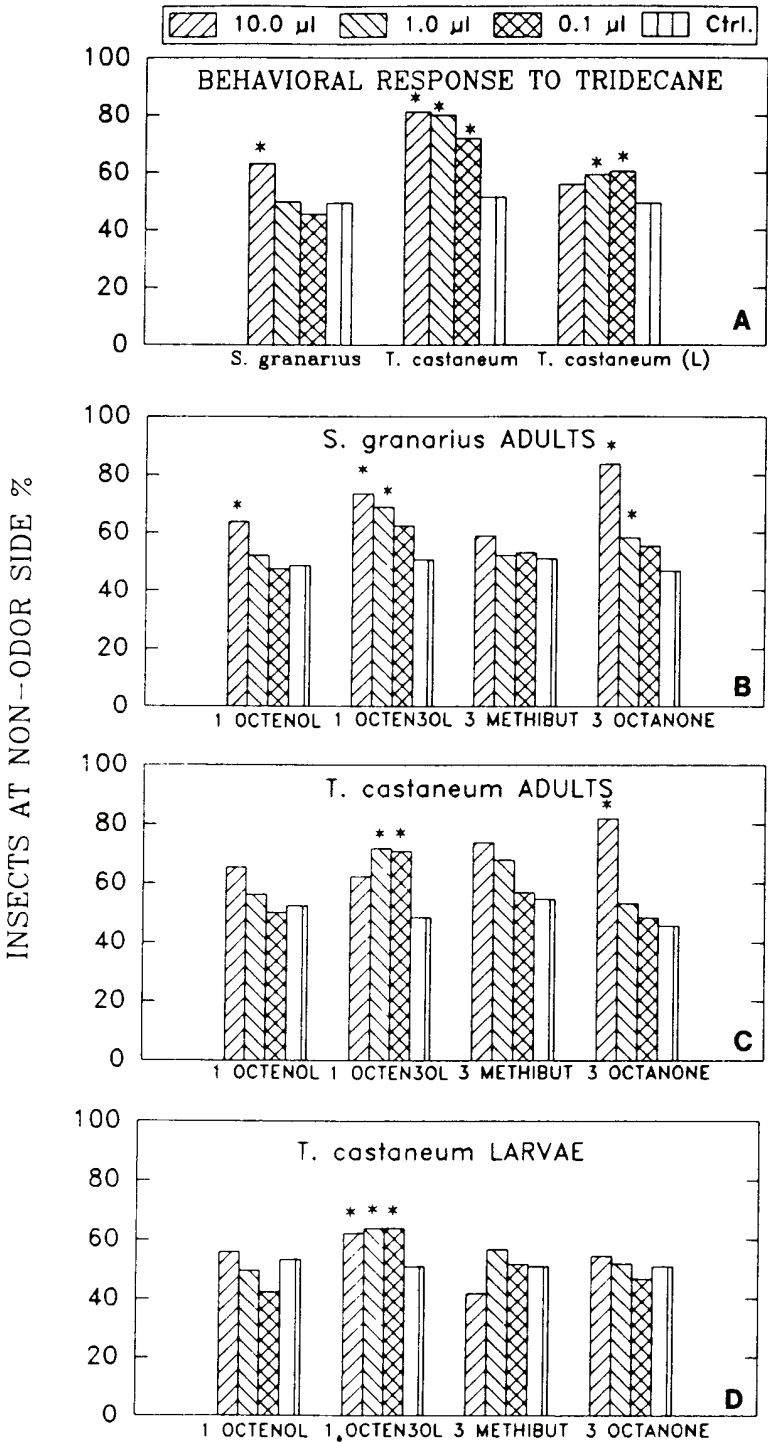
### Figure Captions

Fig. 1. Locomotory response of *Sitophilus granarius* and *Tribolium castaneum* to different concentrations of acarine (tridecane) and fungal volatiles of 30°C and 70% RH, bars marked with asterisks indicate significant difference from control ( $P < 0.05$ ). A.

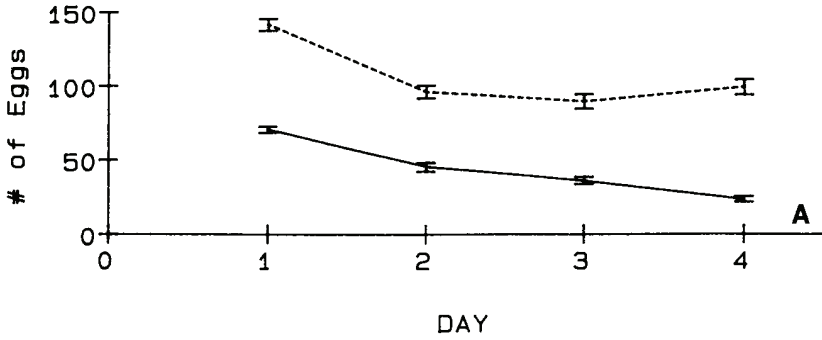
Response to tridecane by *S. granarius* (adults) and *T. castaneum* (adults and larvae). B. *S. granarius* adult response to 1-octanol, 1-octen-3-ol, 3-methyl-1-butenol, and 3-octanone. C and D. *T. castaneum* adult and larval response to the four fungal isolates listed in B.

Fig. 2A. Number of eggs yielded from 200 mixed-sex adults *Tribolium castaneum* were exposed to 3-methyl-1-butenol during 24 h at 30°C and 75% RH. The broken line represents control and the solid line tests with the volatile. B. Number of adults developed from 10 eggs of *T. castaneum* when these were exposed to tridecane from 14 weeks at 30°C and 70% RH. C. Number of adults developed from 10 eggs of *T. castaneum* where these were exposed to 3-methyl-1-butenol for 8 weeks at 30°C and 70% RH. The broken line represents control and the solid line tests the volatile.

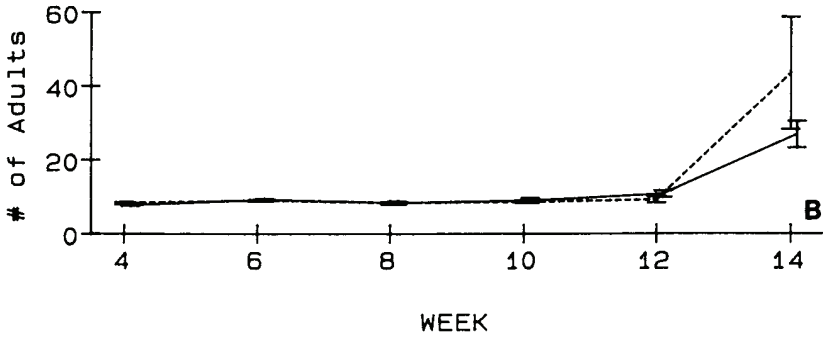




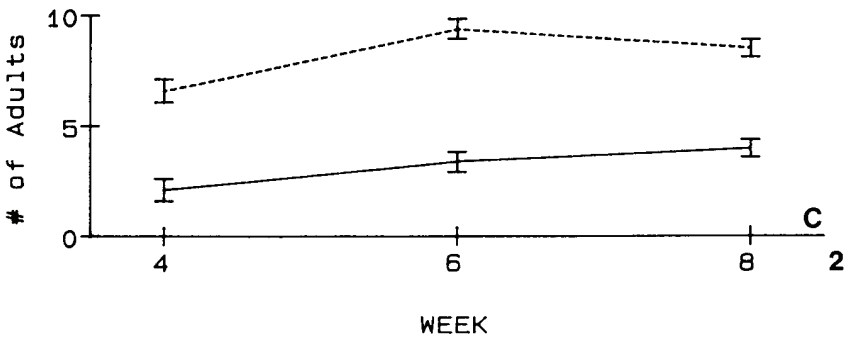
VOLATILE: 3-METHYL-1-BUTANOL  
Mean +/- standard error



VOLATILE: TRIDECANE  
Mean +/- Standard error



VOLATILE: 3-METHYL-1-BUTANOL  
Mean +/- Standard error



LE ROLE DES ODEURS VOLATILES DANS L'ECOSYSTEME  
DES STOCKS DE GRAINS

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RESUME

Les insectes, les acariens et la microflore produisent des substances volatiles distinctes qui se combinent à l'oxygène, l'azote et le dioxyde de carbone de l'air intergranulaire des écosystèmes des grains stockés. Plusieurs de ces substances affectent le comportement et l'abondance des parasites vivant dans un environnement gazeux, individuellement ou collectivement. Nous présenterons un survol de cet aspect complexe de la fonction d'un écosystème en nous basant sur les publications et les données collectées en grenier et en laboratoire. En laboratoire, *Ahasverus advena*, *Cryptolestes ferrugineus*, *Sitophilus granarius*, *S. oryzae* et *Tribolium castaneum* ont pu généralement être classés par quatre substances fongiques et une substance acarienne volatile ; la larve de *T. castaneum*, cependant, a été attirée par le 1-octanol et le 3-méthyl-1-butanol. La croissance et la reproduction de *T. castaneus* ont semblé ne pas être affectées par le tridécane. La fréquence et la saison d'apparition des substances volatiles d'origines fongique et acarienne dans les greniers fermiers y sont discutées.