

## **The Growth of *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) on Feed Media Dosed with Flavour Volatiles Found in Dry Cocoa Beans**

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**Abstract:** Acetophenone, ethyl butyrate and 2-phenyl ethanol were used as additives to media on which *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) were cultured for 65 days. Acetophenone and ethyl butyrate had a positive impact on the weight and multiplication of the insects. The weight and rate of multiplication was higher in feed media containing acetophenone alone. The amount of the flavour volatiles added was high enough to deter the *Lasioderma* from feeding well, which affected their growth. This attraction to specific flavour volatiles in some stored products could be used in the formulation of effective control measures for insect pests.

**Key words:** Acetophenone, ethyl butyrate, 2-phenyl ethanol, feed media, *Tribolium*, *Lasioderma*

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### **INTRODUCTION**

Successful host selection by insects depends on the execution of appropriate behavioural responses to an array of cues, including volatile chemicals (Vet *et al.*, 1990). There is now well-documented evidence that the mixture of such chemicals emitted by plants can change quantitatively in response to feeding damage (Turlings *et al.*, 1990, 1991; Vet and Dicke, 1992; Tumlinson *et al.*, 1993; Steinberg *et al.*, 1993; Takabayashi *et al.*, 1994). Previous investigations of both pheromones and food attractants have involved successive fractionation of the collected volatiles and subsequent behavioural testing, however this approach suffers several drawbacks. To determine the response to every component in a complex mixture involves considerable effort and, in addition, the presence of a repellent material in a fraction may mask the effect of an attractant (Peter *et al.*, 1989).

There is considerable interest in the behavioural responses of stored-product insects to host-plant volatiles (Barrer, 1983; Subramanyam *et al.*, 1992). Stored-product pests represent a special class of phytophagous insects that appear to be highly adapted to survive on numerous commodities stored for human or animal consumption. Interactions between food volatiles and pheromones have been reported for several species (Walgenbach *et al.*, 1987; Dowdy *et al.*, 1993; Phillips *et al.*, 1993) and understanding interactions is

prerequisite for optimisation of pheromone-based monitoring systems.

Dry cocoa beans like any stored products are vulnerable to infestation by numerous species of insects and mites, many of which can cause serious nutritional damage and economic loss (Jonfia-Essien, 2006). In an earlier study to find out factors responsible for insect attack on the cocoa beans, flavour volatiles were identified to play a major role. This study therefore aims at using the significant flavour volatiles in a food source to control the growth of two common insect pests of stored products. The hypothesis of the study is that different volatiles have different effects on the feeding behaviour of insects.

### **MATERIALS AND METHODS**

**Materials:** Young fresh adult *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) were obtained from the Central Science Laboratory, York, UK and cultured on wholemeal wheat flour and brewer's yeast.

**Methods:** Twenty adults of *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) were introduced separately into culture bottles containing 150 g each of wholemeal wheat flour and brewer's yeast (20:1 by weight) plus 0.5 mL of three flavour volatiles, singly and in various combinations. The flavour volatiles used were

acetophenone, ethyl butyrate and 2-phenyl ethanol at concentrations of 50 and 25  $\mu\text{L mL}^{-1}$  for *Tribolium* and *Lasioderma* respectively. The treatments were eight in total including one control and they were all replicated three times. The control contained only the feed media 150 g wholemeal wheat flour and brewer's yeast. The cultures were maintained at  $30\pm 1^\circ\text{C}$  and relative humidity of  $70\pm 2\%$  in the dark for 65 days in 1 litre Kilner jars. Adult insect counts were made on days 37, 51 and 65. The mean weight of single insects was determined on day 40 by weighing 3 replicates of 10 insects using a side loading analytical balance. The study was conducted at the Division of Agriculture and Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus in 2005.

### RESULTS

**Weight of insects:** Feeding of insects occurred in all of the cultures with and without the addition of the flavour volatiles. Compared with their weight before the experiment, adults of *Tribolium* were twice as heavy at the end of the experiment. The *Tribolium* that fed on media containing acetophenone or ethyl butyrate were heavier than the control (Table 1). Those that fed on media containing all three flavour volatiles weighed the least, followed by the acetophenone and 2-phenyl ethanol combination. In contrast, there was only a slight increase in weight of the *Lasioderma* that fed on media containing acetophenone, ethyl butyrate or both.

**Multiplication of insects:** Multiplication of the insects also occurred in all the different treatments (Fig. 1 and 2) and increased numbers of *Tribolium* were present after 37 days of incubation. Those that fed on the control medium had the highest population followed by those that fed on media containing acetophenone and ethyl butyrate, acetophenone alone and ethyl butyrate alone. The lowest population occurred on the medium containing the combination of ethyl butyrate and 2-phenyl ethanol. By

Table 1: Weight of adult insects after 40 days of feeding on feed media containing various combinations of flavour volatile

Feed media	Weight of insect (mg)	
	<i>Tribolium</i>	<i>Lasioderma</i>
AP	1.69 $\pm$ 0.001	0.54 $\pm$ 0.001
EB	1.60 $\pm$ 0.001	0.51 $\pm$ 0.001
PA	1.32 $\pm$ 0.010	0.45 $\pm$ 0.001
AP+EB	1.36 $\pm$ 0.002	0.49 $\pm$ 0.001
AP+PA	1.14 $\pm$ 0.005	0.45 $\pm$ 0.000
EB+PA	1.33 $\pm$ 0.002	0.45 $\pm$ 0.000
AP+EB+PA	1.10 $\pm$ 0.007	0.44 $\pm$ 0.000
Control	1.46 $\pm$ 0.001	0.46 $\pm$ 0.002
Insects before feeding	0.63 $\pm$ 0.009	0.45 $\pm$ 0.002

AP-acetophenone; EB-ethyl butyrate; PA-2-phenyl ethanol

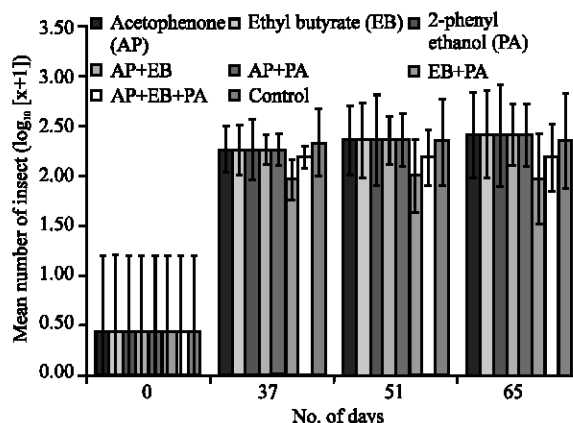


Fig. 1: Populations of adult *Tribolium* after feeding on media containing various combinations of flavour volatiles

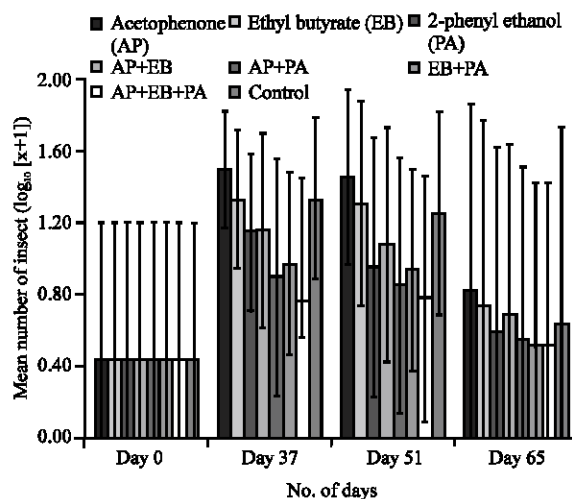


Fig. 2: Populations of adult *Lasioderma* after feeding on feed media containing various combinations of flavour volatiles

day 51, there had been a further increase in the population of *Tribolium* in the control and acetophenone treatments.

In contrast, the population of *Tribolium* in the other treatments increased less. By day 65, *Tribolium* on feed medium containing acetophenone alone had the highest population, followed by the control and medium containing both acetophenone and ethyl butyrate.

Multiplication of *Lasioderma* was poor compared to that of *Tribolium*. There was a gradual increase in the population of *Lasioderma* up to day 51 but then the population declined by day 65 (Fig. 2). The feed treatment containing acetophenone had the highest population at day 37, followed by the control and ethyl butyrate treatment. The lowest population increase occurred on media containing the combination of acetophenone, ethyl

butyrate and 2-phenyl ethanol. At days 51 and 65, the same population trends were evident.

## DISCUSSION

The feeding analysis was very valuable because it showed the selective attraction of *Tribolium* and *Lasioderma* to some flavour volatiles found in cocoa beans. It was the only way by which translation of the attraction of the insects to the flavour volatiles into feeding could be ascertained (Jonfia-Essien, 2006).

Behavioural mechanisms provide a system of avoidance of non-host chemicals by which insects select their food, though the molecular basis for the action of chemical deterrents on both gustatory and olfactory sensory systems in insects is only poorly understood (Koul, 2004). In plant anti-herbivore chemistry, a strong link does not exist between feeding deterrence and internal toxicity in insects, which suggests that behavioural rejection is not an adaptation to ingested effects but more an outcome of deterrent receptors with chemical sensitivity (Mullin *et al.*, 1991, 1994).

The significant increase in the weight of *Tribolium* adults ( $p < 0.001$ ) 40 days after introduction is an indication that feeding occurred in all the feed media. This also implies that the insects were well nourished, with a positive impact on their multiplication. The weight of *Lasioderma*, in contrast, did not increase as much, which implies low feeding and poorly nourished with little effect on their multiplication. Thus, the feeding behaviour of *Tribolium* was completely different from that of *Lasioderma*. It is possible that the amount of the flavour volatiles was high enough to deter the *Lasioderma* from feeding, resulting in the reduction in the population between days 51 and 65 and thus acting as an antifeedant.

Any substance that reduces consumption by an insect is known as an antifeedant (Koul, 2004). It could also be considered as a peripherally mediated behaviour-modifying substance (i.e. acting directly on the chemosensilla in general and deterrent receptors in particular) resulting in feeding deterrence (Isman, 1994). This definition, however, excludes chemicals that suppress feeding by acting on the central nervous system (following ingestion or absorption), or substances that have sublethal toxicity to the insect (Isman, 2002).

Feeding deterrents with a wide diversity of structures are not known to interfere directly with insect taste cell responses to phagostimulants such as sugars (Lam and Frazier, 1991; Schoonhoven *et al.*, 1992). Presently the mode of action of feeding modifying chemicals in insect gustatory systems is largely unknown (Frazier, 1992; Schoonhoven *et al.*, 1992), though some molecular targets have been identified (Koul, 1997).

No two insect species are equipped with an identical sensory system. Each species has a unique sensory window, which can discriminate between host and non-host plants (Schoonhoven, 1982) and this was observed in the feeding behaviour between *Tribolium* and *Lasioderma*. Even in very closely related species the chemical senses show striking differences (Van Drongelen, 1979). The sensing of contact chemicals may in evolutionary terms be easily adapted to changing circumstances, as evidenced in two strains of *Mamestra brassicae* in response to sinigrin and naphthyl- $\beta$ -glucoside (Wieczorek, 1976). Insect feeding deterrents may also be perceived either by stimulation of specialized deterrent receptors or by distortion of the normal function of neurons that perceive phagostimulating compounds (Koul, 2004).

The significant difference in the weight and population of insects ( $p < 0.001$ ) fed on media containing the various flavour volatiles is an indication of different responses of the insects to different volatiles. It also supports the hypothesis that different volatiles have different effects on the feeding behaviour of insects. The highest weight and population increase of insects fed with media containing acetophenone indicated that the volatile could be used to boost the multiplication of the insect.

The body design of insects makes the apparently simple process of growing extremely complex. Although many insects, especially at immature stages, have elastic skeletons, continuous growth eventually requires that the skeleton be replaced with a larger one. The ability to replace an exoskeleton is currently believed to have evolved only once during animal evolution (Ewer, 2005). Findings on the effect of flavour volatiles on the weight of the insect are a challenge to the procedure of ecdysis which could influence further development on research.

In future breeding programmes for cocoa, it may be appropriate to try to reduce the amount of acetophenone in beans in order to reduce the activity of some insects. In addition, the flavour volatiles that were found to be deterrent to the insects could be increased.

## REFERENCES

- Barrer, P.M., 1983. A field demonstration of odour-based host-food finding behaviour in several species of stored grain insects. *J. Stored Prod. Res.*, 19: 105-110.
- Dowdy, A.K., R.W. Howard, L.M. Seitz and W.H. McGaughey, 1993. Response of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromone and wheat volatiles. *Environ. Entomol.*, 22: 965-970.

- Ewer, J., 2005. How the Ecdysozoan Changed its Coat. Public Library of Sci. Biol., pp: 3: e349.
- Frazier, J.L., 1992. How Animals Perceive Secondary Plant Compounds. In: Herbivores: Their Interaction with Secondary Plant Metabolites, Evolutionary and Ecological Processes, Rosenthal, G.A. and M.R. Berenbaum (Eds.). 2nd Edn., Vol. 2, Academic Press, San Diego, pp: 89-134.
- Isman, M.B., 1994. Botanical insecticides and antifeedants: New sources and perspectives. Pest. Res. J., 6: 11-19.
- Isman, M.B., 2002. Insect antifeedants. Pesticide Outlook, 13: 152-157.
- Jonfia-Essien, W.A., 2006. Effect of feeding damage by *Lasioderma* and *Tribolium* species on stored beans of cocoa. A Ph.D Thesis submitted to University of Nottingham.
- Koul, O., 1997. Molecular Targets for Feeding Deterrents in Phytophagous Insects. In: Ecology and Evolution of Plant Feeding Insects in Natural and Man-Made Environments, Raman, A. (Ed.). Int. Scientific Public., New Delhi, pp: 123-134.
- Koul, O., 2004. Insect Antifeedants. CRC Press, Raton, Florida, pp: 1005.
- Lam, P.Y.S. and J.L. Frazier, 1991. Rational Approach to Glucose Taste Chemoreceptor Inhibition as Novel Insect Antifeedants. In: Synthesis and Chemistry of Agrochemicals II, Baker, D.R., J.G. Fenyves and W.K. Moberg (Eds.). ACS Symposium Series 443. Am. Chem. Soc. Washington, D.C., pp: 400-412.
- Mullin, C.A., C.H. Mason, J. Chou and J.R. Linderman, 1991. Phytochemical Antagonism of  $\gamma$ -aminobutyric Acid Based Resistance in *Diabrotica*. In: Molecular Mechanisms of Insecticide Resistance: Diversity among Insects. Mullin C.A. and J.G. Scott (Eds.). ACS Symposium Series 505. Am. Chem. Soc. Washington, D.C., pp: 288-308.
- Mullin, C.A., S. Chyb, H. Eichenseer, B. Hollister and J.L. Frazier, 1994. Neuroreceptor mechanism in insect gustation, a pharmacological approach. J. Insect. Phys., 40: 913-931.
- Peter, R.W., J. Chambers, C.M. Walter, J.P.G. Wilkins and J.G. Millar, 1989. Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) collection, identification and bioassay of attractive volatiles from beetles and oats. J. Chem. Ecol., 15: 999-1013.
- Phillips, T.W., X.L. Jiang, W.E. Burkholder, J.K. Phillips and H.Q. Tran, 1993. Behavioural responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J. Chem. Ecol., 19: 723-734.
- Schoonhoven, L.M., 1982. Biological aspects of antifeedants. Entomologia Experimentalis et Applicata, 31: 57-69.
- Schoonhoven, L.M., W.M. Blaney and M.S.J. Simmonds, 1992. Secondary Coding of Feeding Deterrents in Phytophagous Insects. In: Insect Plant Interaction, Berneys. E.A. (Ed.). Vol. 4 CRC Press, Raton, Florida, pp: 59-79.
- Steinberg, S., M. Dicke and L.E.M. Vet, 1993. Relative importance of infochemicals from first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. J. Chem. Ecol., 19: 47-59.
- Subramanyam, B.H., V.F. Wright and E.E. Fleming, 1992. Laboratory evaluation of food baits for their relative ability to retain three species of stored-product beetles (Coleoptera). J. Agric. Entomol., 9: 117-127.
- Takabayashi, J., M. Dicke, S. Takahashi, M.A. Posthumus and T.A. Beek, 1994. Leaf age affects composition of herbivores synomones and attraction of predatory mites. J. Chem., 22: 373-386.
- Tumlinson, J.H., W.J. Lewis and L.E.M. Vet, 1993. How wasps find their hosts. Sci. Am., 268: 100-106.
- Turlings, C.T.J., J.H. Tumlinson, F.J. Eller and W.J. Lewis, 1991. Larval damaged plants: Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. Entomol. Exp. Appl., 58: 75-82.
- Turlings, C.T.J., J.H. Tumlinson and W.J. Lewis, 1990. Exploitation of herbivore-induced plant odours by host-seeking parasitic wasps. Science, 250: 1251-1252.
- Van Drongelen, W., 1979. Contact chemoreception of host plant specific chemicals in larvae of various *Yponomeuta* species (Lepidoptera). J. Compar. Physiol., 134: 265-279.
- Vet, L.E.M., W.J. Lewis, D.R. Papaj and J. Lenteren, 1990. A variable-response model for parasitoid foraging behaviour. J. Insect. Behav., 3: 471-490.
- Vet, L.E.M. and M. Dicke, 1992. Ecology of infochemical natural enemies in a tritrophic context. Ann. Rev. Entomol., 37: 141-172.
- Walgenbach, C.A., W.E. Burkholder, M.J. Curtis and Z.A. Khan, 1987. Laboratory trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol., 18: 763-767.
- Wieczorek, H., 1976. The glycoside receptor of the larvae of *Mamestra brassicae* L. (Lepidoptera: Noctuidae). J. Comparative Physiol., 106: 153-176.