THE RESPONSE OF INSECTS TO FUMIGANT CONCENTRATION GRADIENTS

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An apparatus has been developed at Slough that permits the observation of insects encountering a gas front or concentration gradient moving down a glass tube fixed to a fumigation chamber. Movement of gas was controlled by maintaining a slight positive pressure in the chamber and regulating flow by fitting a narrow bore capillary to the far end of the observation tube. Throughout observations, gas samples were taken in gas-tight syringes from side arms of the tube fitted with septa. For both methyl bromide and phosphine, gas tended to move along the apparatus as a front with only a limited amount of diffusion occurring on either side. With phosphine, higher concentrations of gas (over 0.5mg/l) were repellent to a susceptible strain of Sitophilus granarius but not to a phosphine-resistant strain. Resistant and susceptible strains of Oryzaephilus surinamensis displayed increased activity in the presence of phosphine but were attracted rather than repelled by the gas. No effect was observed for a resistant strain of Cryptolestes ferrugineus but a strain of Tribolium castaneum showed attractancy to a phosphine concentration of 0.5 mg/l. For methyl bromide again the susceptible strain of S. granarius was repelled by the gas but no such effect was observed in the resistant strain. O. surinamensis strains again showed evidence of attractancy.

Introduction

The possibility that insects may be able to move away from toxicants has long been recognised in the field of pest control. The avoidance of DDT-treated surfaces by mosquitoes was reported nearly 40 years ago (Kennedy, 1947). The provision of a harbourage in insecticide-treated arenas has been shown to increase the survival time of stored product beetles considerably (Pinniger, 1974, Wildey, 1977). In arenas enclosing filter papers partly treated with insecticide, avoidance of insecticidal surfaces has been demonstrated in Tribolium castaneum (Herbst) (Wildey, 1983) but not in Sitophilus granarius (L.) (Prickett and Ratcliffe, 1977).

The ability to avoid a sprayed surface is more easily demonstrable than avoidance of a gaseous toxicant. In an open system gases diffuse rapidly and concentration gradients are set up between the point of gas introduction or generation and points of leakage. Air movements caused

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by temperature fluctuations and external wind also influence the distribution of gas. If the stimulus is merely excitory an increase in the general level of activity occurs. In a system offering a choice between the presence or absence of stimulant, such as a partly treated surface, such an increase in activity will result in a bias in terms of numbers of individuals moving onto the untreated surface where receptors in the tarsi or mouthparts will not be stimulated. A repellent compound will cause a proportionally greater number of individuals to become segregated on the untreated part of the arena than that expected from any increase in activity. Because of the inevitable formation of concentration gradients, with gases the division of repellent and excitory responses is more difficult. However, monitoring of the attraction of male moths to the pheromone released from calling females has been pursued with some success (Marsh et al., 1978). Sex pheromones are active at very low concentrations and the orientation of males zig-zagging in and out of wind borne 'plumes' of pheromone in a way presents a situation comparable with arenas partly treated with insecticide.

Apart from sex pheromones, airborne stimuli emanating from the mandibular glands of larvae (Corbet, 1973), or from pairs of adults (Barrer, 1977), have been found important for oviposition in moths. Little work has been published, however, on the repellency of gases. Willis and Roth (1954) examined various levels of carbon dioxide for their repellent or attractant effects on flour beetles in an olfactometer. They observed that both sexes of T. castaneum were increasingly attracted to concentrations of CO_2 up to 15%, that levels up to 50% retained some attractancy, but that higher concentrations had a repellent effect. In contrast both sexes of Tribolium confusum du Val were attracted by concentrations up to 90%. The olfactory sensors in T. castaneum were tentatively identified as the sensilla basiconica of the antennal club segments.

In grain bulks the movement of insects and diffusion of gas is to some extent restricted. Most infestations start with an invasion of the bulk from insects in the retaining structure. The distribution of insects is never random and local areas of aggregation occur in response to temperature and moisture gradients and directional responses during dispersion arising from disturbance (Surtees, 1964; 1965). Retaining structures for bulk storage are rarely sufficiently gas-tight to permit retention of fumigant gases such as phosphine or carbon dioxide for longer than the minimum period required for control and hence many treatments are marginal and failures occur. If pests can orientate towards sites of leakage then prospects for successful control reduce accordingly. The present study was conducted to investigate the locomotory response of some common pests to various concentrations of the fumigants phosphine and methyl bromide. The apparatus employed enabled test insects to be observed before and during exposure to gas.

Methods

Apparatus

All experiments were performed in light in a constant temperature room maintained at 25°C, 60% r.h. and housing a $1.7 m^3$ fumigation chamber.

The apparatus for observing insects in the presence of increasing gas concentrations originally comprised a 1.5 m jointed tube of internal diameter 5 mm fitted with short, septa-sealed side arms for gas sampling, a valve for introducing insects and a tap for controlling release of fumigant from the chamber. Later, an essentially similar tube was employed featuring less joints and the wider internal bore of 8 mm. The tube was mounted horizontally using a spirit level and connected to the fumigation chamber through a 2 cm diameter port fitted with a drilled neoprene bung (Fig 1). At the far end of the tube a capillary tube was fitted to reduce the rate of gas flow.

For each experiment the fumigation chamber was dosed with the required level of fumigant. Phosphine was released from aluminium phosphide pellets and sufficient time was allowed for gas production to be completed before commencing experiments. Gas samples were taken by pressure locking gas syringes through the septa fitted to the side arm ports of the observation tube. Care was taken to insert each needle down into the main tube. To facilitate the observation of insects, the apparatus was marked off into four sections on each side of the centre port and point of introduction of insects, each section measuring about 18 cm. All side arms were coated with 'Fluon' (polytetrafluoroethylene) suspension to prevent the access of insects.

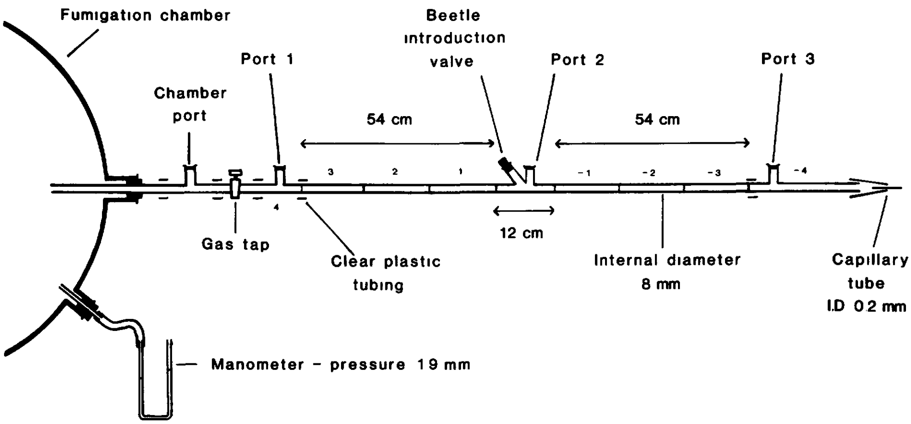

Fig 1. Apparatus for observing repellent or attractant effects of fumigants.

Control of gas movement

During initial tests on the apparatus it was found that the passage of gas through the apparatus was greatly influenced by any changes in atmospheric pressure. A water-filled manometer was therefore connected to the chamber and revealed that changes of up to 10 or 12 mm water gauge were registered within minutes of starting a flow of gas. A slow air stream from a compressed air source was applied to the chamber intermittently to maintain a slight positive pressure inside. Using a capillary tube of about 0.2 mm bore the head of pressure was adjusted so that the gas concentration at the central sampling port exceeded 50% of that in the chamber about five minutes after opening the tap. For the 8 mm bore apparatus the appropriate head of pressure in the chamber was equivalent to 18-20 mm of water for both methyl bromide and phosphine. This gave a movement along the tube of the steepest part of the gas concentration gradient of about 2.6 mm per second over the 78 cm separating the tap from the central sampling port. Observations on the rate of movement of *Oryzaephilus surinamensis* (L.) and *S. granarius* adults in the apparatus had established that both species when on the move habitually progressed at well over 5 mm/sec.

Test procedure

Most experiments were performed on strains of *S. granarius* and *O. surinamensis*, but one test included *T. castaneum* and *Cryptolestes ferrugineus* (Stephens) (Table 1). These latter species had difficulty in dispersing through the apparatus and required the assistance of a cotton thread laid along the length of the observation tube. Adult beetles of all strains were obtained from standard stock cultures reared at 25°C, 60% r.h. and were aged between 2 and 10 weeks at time of test. Prior to each experiment batches of twenty beetles were counted out into glass tubes. The effects of four concentrations of phosphine between 0.1 and 2.0 mg/l and two of methyl bromide (2 and 8 mg/l) were examined.

For each experimental run a batch of twenty beetles was inserted at the central point of the apparatus, the tap connecting the observation tube to the chamber was opened and a stop clock was started. At minute intervals the number of insects occurring in each of the eight marked zones was recorded. Four gas samples were withdrawn from the centre port 2 between the second and seventh minute after opening the tap, and a final sample was taken after 9 or 10 min. Samples were analysed using a gas chromatograph fitted with a flame photometric detector for phosphine or a flame ionisation detector for methyl bromide. Observations on the distribution of insects were ceased after 10 min and the tap was closed. Insects were removed from the apparatus by opening the sampling point after the tap, removing the capillary tube end piece and applying a suction pump and trap to the opened end of the observation tube. The pumping was continued a minute or two after removal of insects to flush all trace of gas from the apparatus.

For each species and strain at least seven runs in the presence of gas and seven controls with the tap closed were performed. Usually control runs and gas runs were alternated, and for each strain and gas concentration the full set of results was collected on a single day. Results for each batch of seven or more runs were collated and beetles were scored in relation to the distance moved away from the centre, four marks being allotted to each individual in zones +4 (towards chamber) or -4 (away from chamber) three in zones 3, etc. Averaged totals either side of the centre 5 min after the start of the experiment were compared and the time taken for 90% of the maximum score to be obtained on each side was calculated.

Table 1. Strains of insects included in experiments

Species		Origin	Resistance status
Sitophilus granarius	1.	Slough Laboratory, (pre 1945)	Susceptible
	2.	Canada, 1983	Selected for PH_3 resistance by E J Bond
	3.	Canada, 1983	Selected for CH_3Br tolerance by E J Bond
Oryzaephilus surinamensis	1.	Slough Laboratory, (pre 1958)	Susceptible
	2.	Bangladesh, 1983	PH_3 resistant
	3.	India, 1972	Malathion resistant
Tribolium castaneum	1.	Slough Laboratory, (pre 1963)	Susceptible
Cryptolestes ferrugineus	-	Bangladesh, 1983	PH_3 resistant

Results

The rate of gas movement

Gas appeared to progress down the tube as a front rather than by simple diffusion. Hence, with phosphine at 0.5 mg/l (330 ppm), whereas no gas was detected at the central port 2 min after opening the tap, nearly 90% of the full concentration was present after 5 minutes (Fig. 2). With methyl bromide at 8 mg/l (2,000 ppm) a similar rapid increase in gas concentration occurred between three and five minutes (Fig. 3). The linear diffusion rate of a gas in a system of known cross section area can be calculated from the standard rate of diffusion of hydrogen in air of 0.634 $/cm^2/sec$, and the relative density of the gas (Barker, 1974). Hence for phosphine the diffusion rate is $\frac{1}{\sqrt{17.00}} \times 0.634 = 0.154/cm^2/sec$, and for methyl bromide $\frac{1}{\sqrt{47.47}} \times 0.634 = 0.092/cm^2/sec$. Thus methyl bromide may be expected to diffuse down the tube at about 60% the rate of phosphine. The difference between the rates of gas movement shown in Figs 2 and 3 is not of this magnitude although the passage of methyl bromide was a little slower than that of phosphine. The total volume (length x cross sectional area) of the apparatus from tap to capillary end piece is approximately 156 x 0.503 cm^3 = 78.4 cm^3 and if back diffusion is ignored it would take $\frac{78.4}{2 \times 0.092 \times 0.503}$ seconds (14 min 7 sec) for about 50% of the maximum level of gas to be obtained at the centre position. Thus in practice gas moved down the tube at about three times the rate of passive diffusion, and it is apparent that this increase may be attributed to the very slight head of pressure in the chamber.

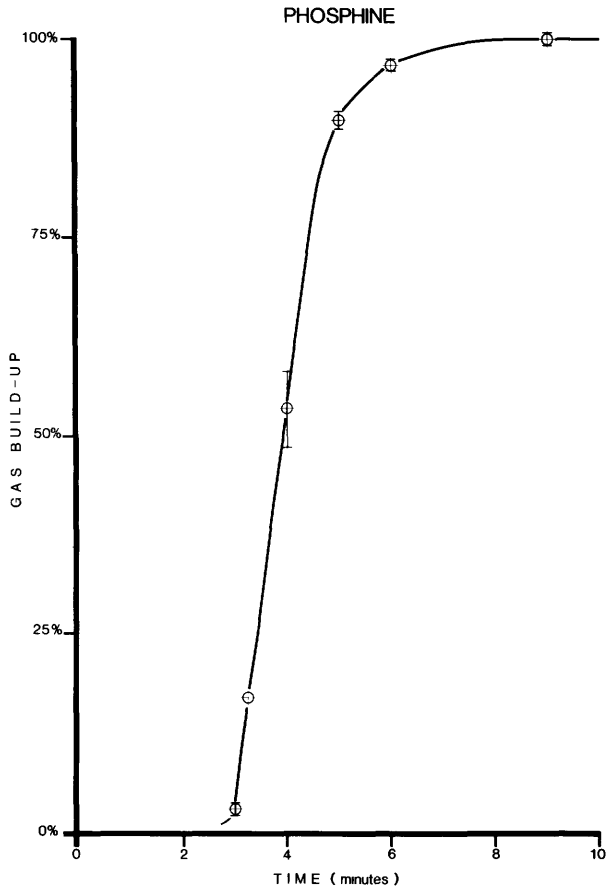

Fig 2. Movement of phosphine in the observation tube as estimated from gas samples taken at the centre port at intervals after opening the tap (mean and S.E. bars of 11 runs at 0.5 - 0.6 mg/l).

Response of insects to the concentration gradients

Methyl bromide - A concentration of 2 mg/l exerted a repellent effect against a laboratory susceptible strain of *S. granarius* but not against a strain selected for methyl bromide resistance (Table II). The movement of laboratory stock adults towards the chamber was checked from 3 minutes after opening the tap. *O. surinamensis* strains in contrast appeared to be attracted to this concentration, a more pronounced response being elicited from the susceptible strain. A reduction in this response was observed at 8 mg/l methyl bromide and again little effect could be discerned in the methyl bromide resistant strain of *S. granarius*. There was little evidence of a change in the level of activity in the presence of methyl bromide at these concentrations.

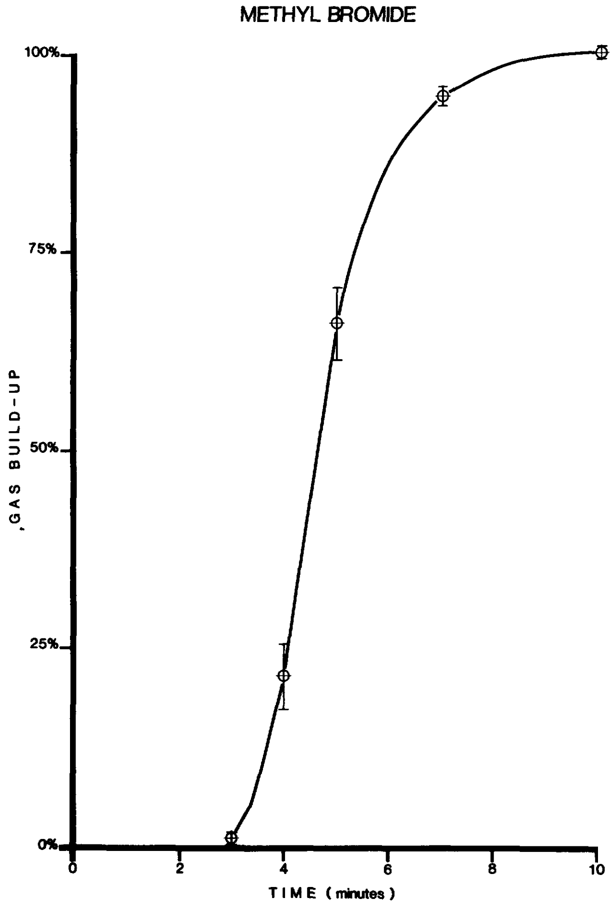

Fig 3. Movement of methyl bromide in the observation tube as estimated from gas samples taken at the centre port at intervals after opening the tap (mean and S.E. bars of 8 runs at 8 mg/l).

Phosphine - At 0.1 mg/l a phosphine resistant strain of O. surinamensis showed slight attractancy accompanied by an increase in the level of activity (Table III). No such effects were observed in strains of S. granarius, indeed in the laboratory strain activity appeared to be depressed. At 0.5 mg/l no effect was seen in a phosphine resistant strain of C. ferrugineus but the two strains of T. castaneum seemed to be attracted towards the gas. Both strains slowed their movement away from the chamber after about 4 min, in contrast to control in which movement on either side of the centre proceeded throughout the observation period (note times to reach 90% of maximum scores in Table III).

With higher concentrations of phosphine (Table III, 0.9 and 0.2 mg/1), both strains of O. surinamensis showed some indication of attraction but in the resistant strain the response was accompanied by hyperactivity, a factor which probably lowered the level of attraction observed. In contrast the laboratory stock of S. granarius was repelled by the gas at 0.9 mg/l and to a lesser extent at 2.0 mg/l phosphine, movement towards the chamber being checked after 3 or 4 minutes.

Discussion

In spite of the tentative nature of this preliminary investigation there are indications that concentrations of fumigant gases that occur in practice may either repel or attract beetle pests, and it is apparent that further consideration needs to be given to this area. The technique offers a method of controlling the rate of flow of gas in a tube by varying the size of the bore of a capillary end piece or the level of positive pressure in the chamber. Rates of atmosphere movement in the tube are comparable with the diffusion rates of lighter gases and a wind effect is thus avoided. The head of pressure necessary in the chamber is so small in relation to atmospheric pressure changes that this aspect may also be discounted as a source of variation in the response of insects.

Many factors require further investigation in order to refine the technique and increase its sensitivity. Firstly alternative methods of scoring insect movement could be worth exploring. Many conditions standardised in the present test procedure may need to be adjusted for different species. It is already apparent that for T. castaneum and C. ferrugineus a better type of surface in the glass tube is required for improving locomotion and the rate of gas flow may sometimes need to be slowed for comparison of species with different levels of activity. The current results indicate that resistant and susceptible strains do show differences in response and so a comparison of strains is desirable.

In these initial experiments beetles were presented for test in the relatively wide range of 2 to 10 weeks and were not sexed. In a brief comparison of adults of S. granarius aged two or three weeks with others aged 8-10 weeks, no obvious difference in response was evident but further testing with this and other species is necessary to eliminate this possible source of variation.

In experiments on insect dispersion there is always the possibility of results being influenced by the presence of pheromones, a movement of insects towards the pheromone source creating a bias. In some cases this problem can be resolved by experimenting on one sex at a time, but other attractants such as aggregation pheromones may still affect results.

Lastly, although every effort was made to standardise light conditions in the experimental room and checks were made with a photographic light meter to ensure that an even distribution of light occurred along the apparatus, the level of illumination may have affected results in some strains. It is known that beetle activity is influenced by cycles of light and dark (Bell and Kerslake, 1986) and thus the rate of movement may differ at different times of day. The fact that light may stimulate movement of insects in order to find a refuge is not necessarily a disadvantage in tests on their perception of gas concentrations. However its presence at high intensity may enable insects to observe the movements (however controlled) of experimental operators, or to discern various parts of the apparatus, and so be influenced in their direction of movement. Obviously, work is required at a range of light intensities.

In the fumigation of bulk grain the distribution of gas may be slow and hence any ability of insects to detect and move away from gas concentrations may be of survival value. The extent to which survival may be increased as a result of this ability has yet to be ascertained.

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Table II Effect of methyl bromide moving along a glass tube joined to a gas chamber on the movement of test insects (A = towards chamber, R = away from chamber)

Species and strain number (see Table I)		Runs with gas: No. of runs	Runs with gas: Mean scores at 5 minutes (A, R and S.E.* of A-R)	Runs with gas: Mins to reach 90% max score	Control runs: No. of runs	Control runs: Mean scores at 5 minutes (A, R and S.E.* of A-R)	Control runs: Mins to reach 90% max score	Total of scores in gas runs / Total in controls (Activity index)	Ratio of scores in gas runs / Ratio in controls (Repellancy <1, Attractancy >1)
2 mg/l									
S. granarius	1	7	11.6, 29.6 2.85	3/7	7	17.9, 16.7 3.63	9/9	1.19	0.37 $p<0.05$
	3	14	19.0, 20.3	6/8	14	15.3, 19.2	7/7	1.14	1.17
O. surinamensis	1	13	9.8, 3.8 1.54	9/8	22	6.6, 7.4 1.51	9/9	0.97	2.89 $p<0.05$
	3	7	10.8, 10.3	9/8	7	6.5, 8.3	9/7	1.43	1.34
8 mg/l									
S. granarius	3	7	19.6, 21.4	4/7	7	18.4, 17.7	3/8	1.13	0.88
O. surinamensis	1	7	15.3, 9.9	8/8	7	13.1, 11.0	9/8	1.04	1.30

* S.E. not shown if the difference between A and R in the gas run is less than (A + R) 4 unless the control bias is large enough to produce a 2 fold difference between score ratios in control and gas runs

Table III Effect of phosphine moving along a glass tube joined to a gas chamber on the movement of test insects (A = towards chamber, R = away from chamber)

Species and strain number (see Table I)		Runs with gas: No. of runs	Runs with gas: Mean scores at 5 minutes (A, R and S.E.* of A-R)	Runs with gas: Mins to reach 90% max score (A/R)	Control runs: No. of runs	Control runs: Mean scores at 5 minutes (A, R and S.E.* of A-R)	Control runs: Mins to reach 90% max score (A/R)	Total of scores in gas runs / Total in controls (Activity index	Ratio of scores in gas runs / Ratio in controls (Repellency <1 Attractancy >1)
0.1 mg/l									
S. granarius	1	7	11.1, 8.3	6/9	7	15.7, 12.1	8/9	0.70	1.04
	2	7	27.1, 27.9	6/5	7	28.6, 25.6	4/5	1.02	0.87
O. surinamensis	1	17	6.1, 7.1	8/8	22	4.8, 5.3	9/10	1.31	0.95
	2	17	12.0, 9.8	9/9	19	6.4, 8.1	9/10	1.50	1.55
0.5 mg/l									
C. ferrugineus		7	6.3, 11.7	7/4	7	5.9, 11.0	8/7	1.07	1.01
T. castaneum	1	7	16.4, 10.1 2.69	10/3	7	9.1, 16.0 3.98	8/8	1.06	2.85 p<0.05
	2	7	24.3, 9.6 2.62	8/4	7	17.0, 13.0 1.35	8/9	1.13	1.94 p>0.1
0.9 mg/l									
S. granarius	1	7	12.0, 25.6 2.85	3/6	7	22.7, 17.1 5.10	5/6	0.95	0.35 p<0.05
	2	7	20.3, 27.0	3/3	7	27.1, 31.4	4/4	0.80	0.86
O. surinamensis	1	7	12.9, 9.6	7/6	7	10.7, 15.0	8/7	0.87	1.88
	2	7	13.4, 16.6	9/4	7	5.9, 9.9	9/7	1.91	1.36
2.0 mg/l									
S. granarius	1	11	9.0, 18.1 3.22	4/9	9	12.6, 14.2 3.48	7/9	1.01	0.56 p>0.1

* see Table II