INFLUENCE OF REDUCED PRESSURES ON CALANDRA SPECIES, WITH SPECIAL REFERENCE TO FUMIGATION.

A Thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Science, University of London.

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ABSTRACT

This Thesis describes work done to investigate the effect of reduced pressures on the insects. (Calandra granaria and Calandra oryzae). The water loss from the insects and their mortality depend not only on the pressure maintained in the chamber in which the experiments were done but also on the humidity of the atmosphere surrounding the insects. The greatest response produced is at 2 cm. pressure, but at much lower pressures (3 - 4 cm.) the insects show a much reduced response.

The hydrogen cyanide sorbed and the mortality have been shown to depend on the air pressure in the chamber. More hydrogen cyanide is recovered from fumigated insects by crushing them before distillation and also by distilling them in the solution of PH 3.2 instead of PH 1.2.

There is practically no difference in the amount of hydrogen cyanide sorbed and in the amount recovered immediately after fumigation if the modified method (including crushing and lower acidity) is adopted when fumigation periods are small, like 1 - 4 hours, but when fumigation period is longer (12 hours) there appears to be some difference. C.granaria are found continuously to sorb hydrogen cyanide when the fumigation period is

increased from ½ to 16 hours. The rate of sorption is greater during the first hour, and subsequently decreases as the period of exposure increases.

Some hydrogen cyanide is retained in the insects' bodies, which could be recovered up to as long as 70 days after fumigation.

At the same dosage, as measured by the concentration-time product, when the insects are fumigated, it is found that high concentrations combined with short exposure periods produce greater sorption of fumigant than low concentrations for longer periods. The response produced under reduced pressure fumigation is greater than at atmospheric pressure.

GENERAL INTRODUCTION

Fumigation of stored products to control insect pests, is a common practice throughout the world. But fumigation under reduced pressures is practiced mainly in America and France where it has spread slowly over the past 35 years. Most of the previous work on fumigation under reduced pressure is confined to the application of a dose under reduced pressure, with the object of finding conditions under which complete mortality is obtained. Very short periods of fumigation have been shown to be sufficient to produce complete control under certain conditions, whereas comparatively long periods are required, if the pressure is not reduced. The insects in fumigation under reduced pressure are really subjected to two treatments namely, (1) a reduction of pressure and (2) a fumigation. It is of course likely that the treatments interact. Some workers have studied the effect of reduced pressures alone on the insects and most of them have taken as the response mortality, which may be associated with a variety of conditions of which loss of water from the insects may be one of the more important.

Part I of this thesis deals with the effect of reduced pressures at various humidities on the insects, taking water loss and mortality as the responses. Part II deals with fumigation under reduced pressures,

taking sorption of hydrogen cyanide as determined from the amount recovered from fumigated insects, and mortality as the responses. Since fumigation under reduced pressure has been claimed to be superior to fumigation at atmospheric fumigation, it was hoped that this kind of investigation would confirm or disprove this claim and would help to illuminate the mode of action of fumigation under reduced pressure. Part III deals with the sorption of hydrogen cyanide by the insects and its recovery from them. As the recovery of hydrogen cyanide from the insects immediately after fumigation provides an indirect measure of the amount sorbed by the insects during fumigation, some experiments are carried out in small, specially designed, fumigation apparatus to find the amount of hydrogen cyanide sorbed and the amount recovered and the difference, if any. In this part, some experiments are carried out with Calandra granaria with the object of determining the rate of sorption of hydrogen cyanide. Part IV deals with the recovery of hydrogen cyanide from batches of insects, fumigated at the same concentration-time products, but with different combinations of concentration and time. These experiments were carried out at three different pressures so as to study the influence of pressure on the insects under these conditions.

In this way, it has been possible to throw light on some of the fundamental problems connected with fumigation under reduced pressures.

PART I. EFFECT OF REDUCED PRESSURES ON THE INSECTS

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INTRODUCTION

Some experiments on the effect of reduced pressure on insects were carried out as early as 1670 by Robert Boyle but it was only recently, in 1929, that Lutz first demonstrated that insects, when subjected to reduced pressure, lose water. Many workers have subjected insects to reduced pressures, and obtained mortality responses but, except Livingston and Reed (1940), nobody has even attempted to find out the water loss from the treated insects. It is generally agreed that under reduced pressure, there is an oxygen deficiency, which acts as some kind of stimulus, the respiratory rate of the insects is increased, and there is increased evaporation of water from their bodies through the spiracles. This excessive loss of water is one of the factors responsible for death.

It is, therefore, very important to determine the water loss from insects subjected to various reduced pressures.

Some experiments were carried out in all glass vessels of 1075-1150 ml. capacity, by subjecting the insects to an absolute pressure of 2 cm. of mercury. It was found that the water loss from the insects was sufficient to change the humidity in the chamber, as shown by the change in

colour of cobalt thiocyanate paper put into the chamber. It was suspected therefore, that the insects would probably "regulate" their water loss as the air in the chamber became more and more humid. Hence it was decided to control the humidity in the chamber, to subject the insects to reduced pressures at various humidities, and to find out the magnitude of the water loss from them and also determine the mortality response of the treated insects.

REVIEW OF THE LITERATURE.

A search in the literature shows that as early as 1670, some experiments on the effect of reduced pressure on the insects were carried out by Robert Boyle. He was the first worker to note that if the exposures were long enough and the pressure reduced sufficiently, some species of insects were killed. Nearly 150 years later, Huber in 1814 carried out some experiments to investigate the ability of bees to survive low pressures and found that bees could withstand very low pressures. Jansette de Belleseme (1880) studied the ralative ability of insects to survive in a vacuum. He found that insects belonging to the group Hymenoptera could survive in a vacuum 5-6 hours at a temperature of 20°C, but cockroaches (Blattidae) for only 2 hours at 12°C. Cole (1905) was the first worker to show the importance of moisture to insects when subjected to reduced pressure. He found from two experiments that when adults of Calandra oryzae were subjected to a vacuum, in which the "mercury of the gauge was reduced to 5 inches", 50 per cent of the insects lived and fed up to 23 days when a dish of water was placed in an evacuated vessel, whereas

in another experiment without water, all the insects died within 7 days. He concluded from his two experiments that the deaths in the insects were due to severe desiccation. Roller (1906) while, observing the effect of gradually lowering the pressure upon the respiratory movements in grasshoppers, noted that the activity of the insects increased at first when the pressure was reduced and that considerable expansion of the abdomen occurred, the normal dimensions being regained when the vacuum was maintained for some time. At the end of the treatment the heart of the insect was found to be beating, though respiration had stopped. In November grasshoppers, however, respiratory movements ceased as the vacuum was produced. The insects survived the vacuum for 7 hours, but 8 hours proved fatal to them. Greenwood (1906) concluded that the ability of the insects to withstand extreme pressure changes was conferred by their tracheal mode of respiration. Babak and Foustka (1907) concluded that lack of oxygen was in some way a stimulus which promoted respiratory movements in the larvae of dragonfly. Mackie (1916) observed that the effect of a vacuum of "27½ inches" on all the stages of tobacco beetle L.<u>serricorne</u> was to expand the cellular tissues, and greatly to distend the bodies. When the insects were returned to atmospheric

pressure after 3 hours, many of them became active again. Mackie (1917) was the first worker to try a thermal-vacuum process for the control of L.serricorne infesting cigars. He suggested that the process in question caused the water content of all the insects comprehended therein, to change to gaseous form, thus all the insects were killed. Nagel (1921) reported that the larvae of Anobinn Striatum oliv. were unharmed by an exposure to a partial vacuum for 24 hours. Brassler (1925) reported that the storage of furs in an evacuated chamber was said to protect them from insects such as clothes moths, but Moll (1926) reported that the firms found that the vacuum alone was insufficient in practice, and had therefore combined it with heating at 40-50°C (104-122°F.) Cotton (1925) carried out an extensive piece of work on the relative ability of various stages of 20 insects to withstand a "vacuum of 28-29 inches". He found that in general, adult and pupal stages were more susceptible to the effect of vacuum than were the laval stages. Treated specimens of the larvae of the insects were stiff and brittle and appeared to have been thoroughly dried out by the process. He further observed that when the insects were subjected to "29 inch vacuum", all movement ceased in all stages of all species tried within a few minutes after the pump was started. If the air was restored in a short time, the insects

regained their normal activity. Lutz (1929) demonstrated that insects subjected to reduced pressures, lost moisture by evaporation more readily than at normal atmospheric pressure. Cotton (1932) found that the reduction of oxygen content of the atmosphere in a chamber, had a marked effect on the respiration of the insects and rendered them more susceptible to the effect of the fumigant. He concluded that oxygen deficiency associated with a reduced air pressure rather than the reduced pressure of itself was the factor affecting the insects. Wigglesworth (1935) found that with the insects kept in the mixtures of oxygen and nitrogen at atmospheric pressure, when the proportion of oxygen was reduced to one per cent, the spiracles of the insects were kept permanently open. Cotton (1932) concluded from his experiments that reduced oxygen content produced by reducing the atmospheric pressure increased the respiratory metabolism, but Woodworth (1936) got opposite results. He found that generally the reduction in pressure reduces respiratory metabolism. He explained the contradictory results by postulating two effects, opposite in nature, resulting from reduction in pressure. The first, a sudden increase in metabolism, and the second, a subsequent decrease in metabolism. Both effects were the

direct result of the change of pressure. Since reduced metabolism is the final and lasting effect of reduced pressure, he suggested that this metabolism should be called the "reduced pressure metabolism". The increased metabolism on the other hand, since it is temporary, and varies with the speed or degree of the change of pressure should be called the "reduced pressure compensatory metabolism". Moore and Carptenter (1938) observed that when the air pressure in the chamber was reduced below that of the atmosphere, certain species of insects first became very active and then slowly their activity decreased until they were rendered inactive, at a pressure of 10 mm. of mercury or less. Fisk and Shepard (1938) obtained 16-20 per cent mortality of T.confusum adults when subjected to an absolute pressure of 30 mm. of mercury for 90 minutes, when there was no water in the flask, but the mortality decreased to less than 1 per cent when a small amount of water was first added to the flask, indicating that the water loss from the insects' bodies in a dry atmosphere was considerable and could be fatal. Livingstone and Reed (1940) were the first workers to measure the water loss from the insects, subjected to vacuum. They found that the water loss and the mortality of the larvae and adults of L.serricorne, kept for 8 hours

at an air pressure of 2 inches of mercury were considerably greater in dry than in water-saturated air. They also found that an increase in the total amount of oxygen in the chamber prolonged the life of the insects, and, in the dry air, reduced the evaporation of water from their bodies. In moist air at a pressure of 2 inches of mercury no particular injurious effect was observed so that neither water loss nor lack of oxygen was important under these conditions. Reed and Vinsant (1942) reported treatment with the steam after reducing the air pressure raised the temperature in tobacco, in commercial packages, to about 160°F, and all the insects present were killed by this "thermal-vacuum" process. Bare, Tenhet and Reed (1946) while working with L.serricorne and E.elutella suggested that in the thermal-vacuum treatment, the insect mortality appeared to be caused by heat and vacuum combined, since a "very high vacuum of over 29 inches", alone, produced only negligible mortality. Wellington (1946) has reviewed the work done by other workers on the effects of low pressures on the insects and concludes as follows - "Extremely low pressure has only an indirectly adverse effect upon the insects, with the consequent reduction of oxygen, and the increased rate of evaporation playing roles of almost equal importance as the directly lethal factors. With insects

that ordinarily close their spiracles in dry air at normal pressure, the increased respiratory rate induced by oxygen-deficient air at low pressure results in a more rapid rate of evaporation through the opened spiracles. Insects that lack a closing mechanism for the spiracles will be subjected to increased evaporation at low pressure from the beginning. However, it does not necessarily follow that evaporation plays the most important part in hastening death under the unnatural conditions of extremely low pressure, since the time at which the fatal limit of desiccation is reached will be partly dependent upon the amount of free water contained within the insects. Consequently, it must be assumed that any mortality at a greatly reduced pressure is due to the effects of oxygen starvation combined with the effects of desiccation, and is not directly referable to the action of low pressure alone". Bare (1948) found that the reduced pressures, "above 28 inches on the mercury gauge", at the temperature of about 75°F produced a 100 per cent mortality of all the stages of E.elutella confined to a depth of 9 inches in the bales of Turkish tobacco, after an exposure period of 72 hours. The same treatment produced almost as great a mortality of all the stages, except the eggs of L.serricorne which required an exposure of as long as 10 days to "high vacuum" to produce 100 per cent mortality.

MATERIALS AND METHOD.

In these experiments, adults of Calandra granaria and Calandra oryzae were used. C. granaria was bred on English wheat at 25°C and 60-70 per cent R.H. in a constant temperature-humidity room, but C.oryzae was bred in an incubator at 22°C and 60-70 per cent R.H.

About 200 adults were placed in a 1 lb. jar which was 3/4th filled with wheat. The insects were allowed to remain for about 4 weeks, after which they were all removed. The young adults started emerging after about 5-6 weeks. They were separated and kept for about 3 weeks in bottled labelled "storage". Insects from the old cultures were collected after an interval of 4-5 days and about 1000 adults were kept per 1 lb. jars 3/4th filled with wheat, and the insects which were 3-4 weeks old were used for experimental purposes. The sex of the insects was not taken into consideration and they were selected at random from the storage jars.

Humidity Control in the Chambers.

Humidity in the all-glass chambers used was controlled by means of a drying agent or of salts wetted with a saturated aqueous solution, tubes containing one of these substances being kept in a chamber throughout the exposure

period. A large number of salts was tried and the following were finally selected, calcium chloride wetted with its saturated solution which maintained a relative humidity of 45 per cent, barium chloride wetted with its saturated solution which maintained a relative humidity of about 85 per cent. Phosphorous pentoxide, was used to maintain a low humidity, between 0 and 5 per cent. In one series of experiments humidity was not controlled but was allowed to change with the "water vapour" given off from the insects.

The humidity in each chamber was approximately measured by the final colour of a "cobalt thiocyanate paper" introduced before starting the experiment. Solomon's (1945) method was adopted in making the cobalt thiocyanate papers and some standards were prepared for comparing the colours obtained.

Colour Change in the Cobalt thiocyanate papers.

When phosphorous pentaoxide powder was used in the chamber, the colour of the cobalt thiocyanate paper was dark green or dark brown, but in presence of the saturated solution of calcium chloride, the colour of the paper was blue, and in presence of the saturated solution of barium chloride the colour of the paper was pink. Thus the colour of the paper indicated the humidity in the chamber

during treatment. In the chamber without any "buffer solution", the colour of the paper changed with the humidity in the chamber, which depended on the water loss from the insects. The paper was dark green or brown in the beginning when the chamber was evacuated, but as the insects were subjected to reduced pressure, they lost water, and this water raised the humidity in the chamber, as indicated by the change in the colour of the cobalt thiocyanate paper from dark green or brown to blue, to pinkish blue and finally to pink.

In another chamber without insects, the colour of the paper was dark green or brown when it was evacuated and remained unchanged showing that the humidity in the chamber was very low.

"Insect Cages".

The cages were small glass tubes about 5-6 cm. in length and about 1 cm. inner diameter, open at both ends. These tubes were closed at both ends with metal "caps", which were made by soldering fine wire gauze on one side of a metal ring. The use of muslin was avoided as it would have absorbed moisture and might have affected the results. Fifty insects were put in each of these cages and treated under reduced pressures at various humidities.

Measuring the "water loss" from the insects.

Each insect "cage" was weighed separately and into these weighed cages, fifty insects were placed and the cage plus insects weighed before and after treatment. The difference in the two weights gave the "water loss" from the insects, which was calculated as the percentage loss of the water content of the insects.

Method of evacuation.

The chamber was connected to the manometer and vacuum pump, which was then started and the chamber brought to a desired pressure. The pressure was read on the closed end manometer. The difference in the mercury level in two arms of the manometers gave the pressure in the chamber. Sometimes an open end manometer had to be used, when the pressure of 37 cm. or above was needed. In the open end manometer, the difference in the mercury levels when subtracted from atmospheric pressure, gave the pressure in cm. of mercury in the chamber. All the pressures used are referred to as "absolute" pressures in cm. of mercury.

Experimental procedure.

Fifty insects of each species were put separately into two weighed insect cages and were weighed to the

nearest 0.1 mg. An open glass tube about 6 cms. long and about 1 cm. internal diameter and containing cotton wool soaked in a saturated solution of calcium chloride or barium chloride was introduced into the "pyrex" glass chamber of about 1 litre capacity.

The insect cages and the tube containing the "buffer solution" or a small tissue paper "bag", containing phosphorous pentoxide were placed in the body of the chamber and one small piece of cobalt thiocyanate paper was placed in the neck. The chamber was evacuated to a particular pressure and kept in constant temperature room at 25°C for the required period. The chamber before opening was tested for leakage, by connecting it to the pumping system which was brought to pressure established in the chamber before the start of the experiment. On opening the stopcock of the chamber, if the mercury levels in the manometer remained unchanged, the chamber was taken as "air tight". Usually the pressure in the chamber was found to have risen by about 1 cm. of mercury, the rise being ascribed to the pressure exerted by the water vapour corresponding to the humidity at the end of the experiment. The air in the chamber was then brought to atmospheric pressure, and the insects taken out and weighed immediately. The "treated" insects were put in labelled glass tubes with some grains

of wheat and kept for counts of mortality in the constant temperature and humidity room.

"Control" Insects.

Sets of 50 adults of C.granaria and C.oryzae were always kept as controls, under the same conditions as those pertaining to the treated insects except that the air pressure was always atmospheric.

Control insects also were weighed before and after the experiment. There was no appreciable water loss or mortality in the controls.

Mortality results.

The insects were examined twice for the mortality results. The first recording was done on the 6th day after treatment and the second on the 10th day. Those insects which did not show any sign of movement of any part of the body when touched with a camel hair brush or a finger, were taken as dead. Usually it was easy to distinguish dead from alive, as the dead insects were dried up owing to desiccation, and their legs were folded and the bodies twisted.

Some observations on the activity of the insects, during and after reduced pressure treatment.

As the pressure fell in the chamber, the insects became

more and more active. At an absolute pressure of 2 cm. the insects became inactive within a few minutes, but at 4 or 8 cm. pressure, they were quite active and were seen to be moving about in their cages.

After treatment, when the pressure in the chamber had been restored to atmospheric, the activity of the insects was found to depend on the amount of water lost from their bodies. If the water loss was very small, they became active within a few minutes, but if the water loss was considerable, they took some hours to show appreciable movement and finally to become really active.

EXPERIMENTAL DESIGN

Most of the previous work on the effect of reduced pressures on the insects was confined to the investigation of one factor and the response it produced. In the present series of experiments, the investigation was extended to cover more than one factor. The object of the investigation was quantitatively to measure the water loss from C.granaria and C.oryzae when subjected to reduced pressures at various humidities for certain periods, and also to determine the mortality response. Three reduced pressures, three exposure periods and four humidities were selected. The three pressures chosen were 2, 4 and 8 cm. of mercury, the three periods tried were 4, 8 and 16 hours, while the three "controlled" humidities were about 5 per cent (or below) 45 per cent and 85 to 88 per cent. Relative Humidity. In one of the series, the humidity was not controlled, but was allowed to change with the escape of "water vapour" from the insects into the air of the chamber.

The results obtained were subjected to an analysis of variance to assess the importance of each factor separately and in combination with others.

The experiment was a fully factorial one involving four factors. Humidity was investigated at four levels, pressure at three, each at three exposure periods. There were two species.

PRELIMINARY EXPERIMENTS

(1) Determination of Water Content of the Insects.

Some preliminary experiments were carried out to determine the "water content" of C.granaria and C.oryzae. It was found that the water content of C.granaria was slightly higher than that of C.oryzae. Table 1 shows the water content, calculated as percentage loss of the body weight of insects before treatment.

TABLE 1.

Water Content of C.granaria and C.oryzae.

Treatment	% Water Loss from C.granaria	% Water Loss from C.oryzae
(1) Heating at 150-156°C for 6 hours.	54.8	51.8
x (2) Subjecting the insects to an abs. press. of 2 cm. of Hg. in presence phosphorous pentoxide (Humidity 5% or below) for 75 hours.	54.1	52.2
x (3) 2 cm. press. P_2O_5 96 hours.	54.5	48.2
same insects (a) 2 cm. 6 hours	15.2) Total	16.5) Total
same insects (b) 2 cm. P_2O_5 96 hours.	38.9) 54.1	31.9) 48.4
same insects x (4) (a) 3-4 mm. press. 48 hours	5.1) Total	8.3) Total
same insects (b) 2 cm. press. P_2O_5 96 hrs.	46.6) 51.7	39.8) 48.1
x (5) Atmospheric pressure - P_2O_5 44 days.	52.5	52.2
	Average 53.6	Average 50.1

x Temp. 52 - 64°F.

It is seen from the Table 1 that C.granaria has a water content about equal to 53.6 per cent of the body weight, whereas C.oryzae has a water content about equal to 50.1 per cent of the body weight.

(2) Effect of 3-4 mm. absolute pressure on C.granaria and C.oryzae.

In preliminary experiments on the effect of reduced pressures on C.granaria and C.oryzae some experiments were carried out to find the effect of pressures lower than those attainable in vacuum fumigation. The insects were subjected to an absolute pressure of 3-4 mm. of mercury for various periods, and it was found that there was considerable decrease in water loss from the insects, when compared with the water loss at 2 cm. absolute pressure or above. Table 2 shows the water loss and the mortality responses obtained with both species, when subjected to pressure of 3-4 mm. of mercury for various periods of exposure.

TABLE 2.

Water loss from C.granaria and C.oryzae, when subjected to the pressure of 3-4 mm. of mercury.

Pressure	Periods of Exposure	C.granaria % Water loss	C.granaria % Mortality	C.oryzae % Water loss	C.oryzae % Mortality
	4	5.2	0	5.6	0
	6	6.3	4	6.8	2
3-4 mm.	17	8.9	2	10.5	8
	24	15.9	16	13.6	18
	48	18.1	26	19.4	40

These results show that there is probably some kind of physical effect of such a low pressure on C.granaria and C.oryzae, as these same insects lost water at a much greater rate at pressure of 2 cm. of mercury and above. These results will be fully discussed later on.

RESULTS OF THE EXPERIMENT.

The results are given in Table 3. Per cent mortalities were first converted to angular transforms and the analysis of variance was carried out for both the responses studied (Tables 4 and 5). The following general conclusions can be drawn from the results.

(1) Both the species lose more water and give a higher mortality response as the pressure decreases from 8 to 2 cm. of mercury.

(2) When the humidity in the chamber decreases from 85 to 5 per cent, the water loss and the mortality of both species increase.

(3) As the period of exposure increases from 4 to 16 hours, the water loss as well as the mortality increases.

(4) Adults of C.oryzae are found to be more susceptible than adults of C.granaria. They lose more water and give higher mortality results.

(5) The mortality response of both species increases as the water loss is increased.

The effect of each factor separately or in combination with other factors was assessed by summing across other

factors. All the main factors proved to be significant at ,/ per cent probability level. The results are dealt with in details below. The mean of the response obtained, is plotted against the treatment in all the figures.

Pressure Effect.

It was found that the pressure had a pronounced effect on the insects. As the pressure decreased, the water loss and the mortality increased in both the species (figs.1 and 2). There appears to be a linear relationship between the pressure and the water loss response. The mean water loss increased from 21.9 to 56.6 per cent of the water content of the insects and the mean mortality increased from 26.9 to 64.2 angular units (20% to 81%) as the pressure decreased from 8 to 2 cm. of mercury.

TABLE 3.

Water loss and mortality responses of c.granaria and c.oryzae, when subjected to reduced pressures, under various humidities, for different periods.

C. granaria

Humidity	Exp. period in hours	2 cm. % water loss	2 cm. % mortality	2 cm. A.T. of % mortality.	4 cm. % water loss	4 cm. % mortality	4 cm. A.T. of % mortality	8 cm. % water loss	8 cm. % mortality	8 cm. A.T. of % mortality
5% R.H.	4	60.4	90	71.6	41.4	90	71.6	14.2	0	0
" "	8	87.0	100	90.0	65.7	96	78.5	24.5	14	22.0
" "	16	92.8	100	90.0	85.7	100	90.0	40.7	74	59.3
45% "	4	37.1	86	68.0	22.1	14	22.0	6.4	0	0
" "	8	53.6	90	71.6	30.9	48	43.9	13.0	0	0
" "	16	79.0	94	75.6	42.5	88	69.7	20.1	6	14.6
85% "	4	7.8	2	8.1	4.1	0	0	2.9	2	8.1
" "	8	11.3	0	0	9.6	0	0	4.3	0	0
" "	16	20.1	48	43.9	16.8	0	0	6.1	0	0
Humidity	4	16.3	0	0	12.0	4	11.5	7.6	0	0
not con-	8	20.2	6	14.2	13.6	0	0	10.9	0	0
trolled.	16	29.4	48	43.9	20.5	6	14.2	15.8	0	0

C. oryzae.

5% R.H.	4	91.7	100	90.0	81.6	100	90.0	37.4	90	71.6
" "	8	99.4	100	90.0	97.2	100	90.0	47.7	98	81.0
" "	16	98.2	100	90.0	98.3	100	90.0	70.3	100	90.0
45% "	4	78.7	100	90.0	58.8	100	90.0	16.9	10	18.4
" "	8	88.2	98	81.0	78.8	100	90.0	25.9	50	45.0
" "	16	90.8	100	90.0	91.1	100	90.0	39.0	84	66.4
85% "	4	25.6	7	15.3	18.9	18	25.1	8.7	4	11.5
" "	8	40.0	98	81.0	36.0	98	81.0	12.5	0	0
" "	16	63.2	96	78.5	44.8	100	90.0	18.2	12	20.3
Humidity	4	43.1	96	78.5	37.6	98	81.0	21.6	24	29.3
not con-	8	56.7	100	90.0	44.5	100	90.0	26.0	54	47.3
trolled	16	67.4	100	90.0	52.1	100	90.0	33.7	78	62.0

TABLE 4.

Analysis of Variance of the Mortality Response.

Items		Degrees of Freedom	Sum of Squares	Variance	Variance Ratio.
Pressure	P	2	19265.27	9632.63	30.7 xxx
Humidity	H	3	24418.88	8139.63	25.9 xxx
Exp. period	E	2	5142.73	2571.36	8.2 xxx
Species	S	1	28088.45	28088.45	89.5 xxx
P x H		6	1435.23	239.20	<1 n.s.
P x E		4	134.62	33.66	<1 n.s.
P x S		2	981.51	490.75	1.56 n.s.
H x E		6	242.96	40.49	<1 n.s.
H x S		3	4050.81	1350.27	4.3 xx
E x S		2	330.20	165.10	<1 n.s.
P x H x E		12	2730.39)	227.53	<1 n.s.
P x E x S		4	277.32)	69.33	<1 n.s.
H x E x S		6	1899.14)	316.52	1.0 n.s.
P x H x S		6	5144.12)	857.35	2.7 x
P x H x S x E		12	2506.73)	208.89	<1 n.s.
Total		71	96648.36		
Error		40	12557.70	313.94	

xxx means significant at .1% probability level

xx " " " 1% " "

x " " " 5% " "

n.s. " not significant.

TABLE 5.

Analysis of Variance of the Water Loss Response.

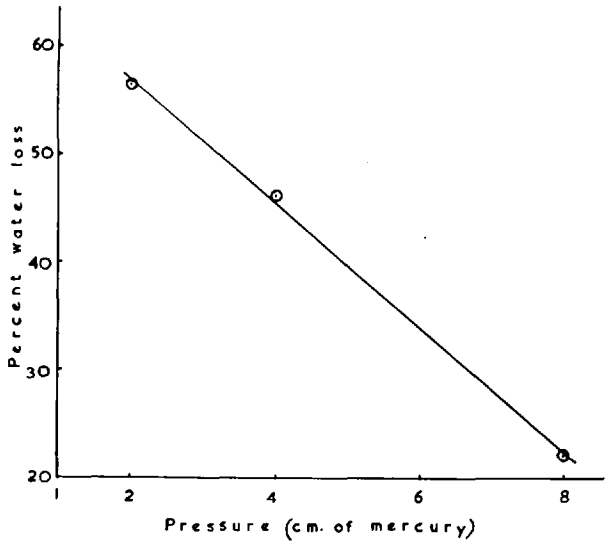
Items	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio	
Pressure P	2	15218.52	7609.26	154.5	xxx
Humidity H	3	25424.10	8474.70	172.1	xxx
Exp. period E	2	4874.51	2437.25	49.5	xxx
Species S	1	11105.48	11105.48	225.5	xxx
P x H	6	3264.68	544.11	11.04	xxx
P x E	4	136.49	34.12	<1	n.s.
P x S	2	756.73	378.36	7.7	xx
H x E	6	361.71	60.28	1.2	n.s.
H x S	3	191.94	63.98	1.3	n.s.
E x S	2	8.33	4.16	<1	n.s.
P x H x E	12	342.72)	28.56	<1	n.s.
P x H x S	6	662.37)	110.39	2.2	n.s.
P x S x E	4	85.67)	21.42	<1	n.s.
H x S x E	6	408.67)	68.11	1.4	n.s.
P x H x S x E	12	470.54)	39.21	<1	n.s.
Total	71	63312.46			
Error	40	1969.97	49.25		

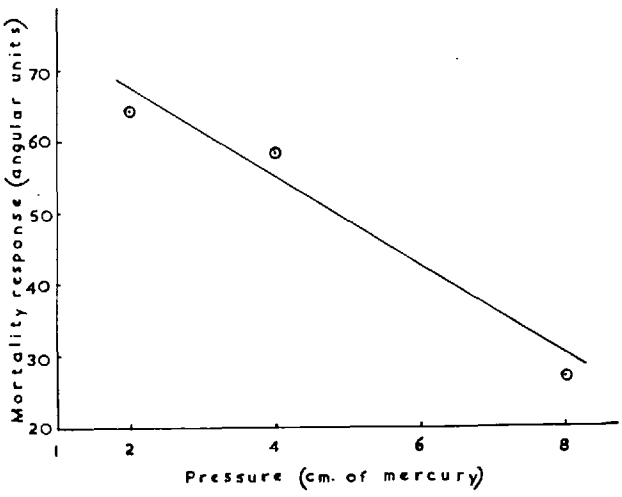
xxx means significant at .1% probability level.

xx " " " 1% " "

x " " " 5% " "

n.s. " not significant.





Figs. 1 & 2 - The water loss and mortality response at various pressures.

Humidity Effect.

The results are given in Table 6. Both the responses studied, depend on the humidity maintained in the chamber.

TABLE 6.

Mean Water Loss and Mortality Responses at Various Humidities.

Humidity	% Water Loss	Mortality in Angular Units.
5% R.H.	68.6	75.3
45% "	48.5	57.0
85% "	19.5	25.7
Humidity not controlled.	29.4	41.2

As the humidity decreases from 85 to 5% R.H. the loss of water as well as the mortality increases. The results show that when the humidity is not controlled, both the responses are intermediate between the responses produced at 85 and at 45% relative humidities.

Period Effect.

The loss of water and the mortality are both increased

as the exposure period increases from 4 to 16 hours. The results are given in Table 7.

TABLE 7.

Mean Water Loss and Mortality Responses When the Insects were Treated for Various Periods.

Exposure Period in Hours.	% Water Loss	Mortality in Angular Units.
4	31.4	39.7
8	41.6	49.4
16	51.5	60.3

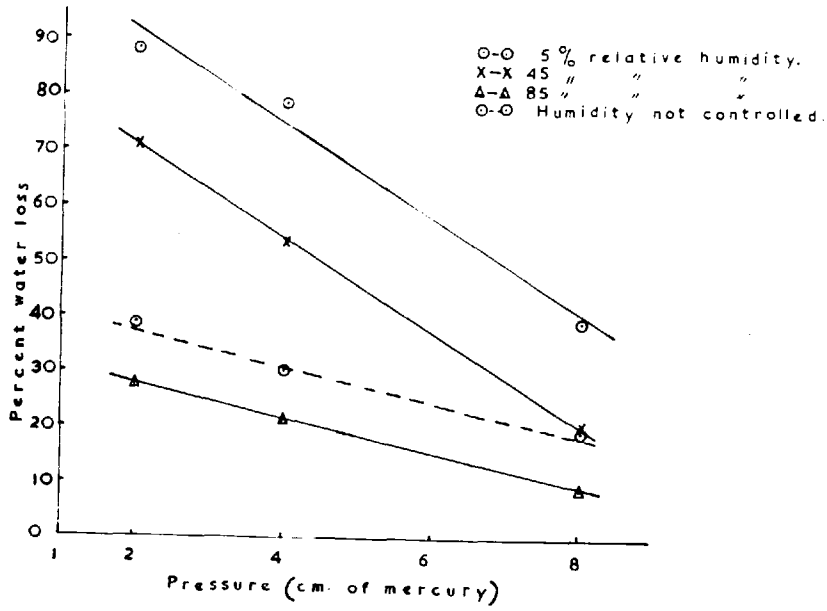
The insects lose water at a greater rate during the first 4 hours of exposure than later as they are treated for increasing periods.

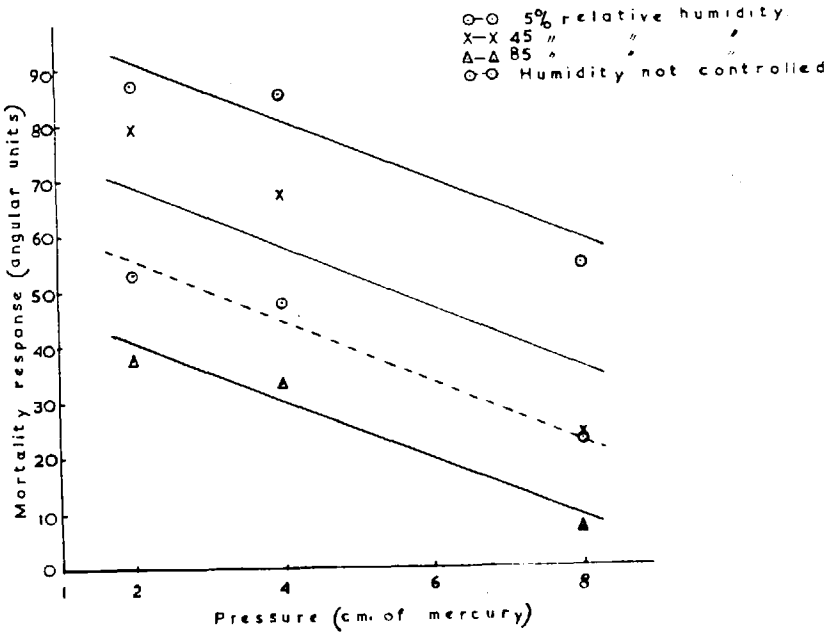
Species.

C.oryzae are found to be more susceptible than C.granaria. The mean loss of water from C.oryzae is 53.9% of the water content of the insects, whereas C.granaria lose only 29.1%. The mean mortality of C.oryzae is 69.6 angular units (88%), while the mortality of C.granaria is only 30.1 angular units (25%).

Effect of Pressure at Various Humidities.

The results are given in Table 8 and figs.3 and 4. This interaction was found to be significant at .1% probability level for the water loss response, but was not significant for the mortality response.





Figs. 3 & 4 - Pressure - Humidity Interaction.

TABLE 8.

Mean Water Loss and mortality response from both the species taken together when treated at various pressures under different humidities.

Humidity	% Water loss at various pressures			Mortality in angular units at various pressures		
	2 cm.	4 cm.	8 cm.	2 cm.	4 cm.	8 cm.
5 % R.H.	88.2	78.3	39.1	86.9	85.0	54.0
45 " "	71.2	54.0	20.2	79.4	67.6	24.0
85 " "	28.0	21.7	8.8	37.8	32.7	6.7
Humidity not controlled.	38.8	30.0	19.3	52.8	47.8	23.1

The results show that at any one pressure, the loss of water and the mortality depend on the humidity maintained in the chamber. At 4 cm. pressure, as the humidity decreases from 85 to 5% R.H. the mean loss of water increases from 21.7 to 78.3%, but at 8 cm. pressure, with the same changes of humidity the loss of water increases from 8.8 to 39.1%, showing that the humidity changes at a pressure of 4 cm. affect the insects more than the same changes at a pressure of 8 cm. At 2 cm. pressure, as the humidity decreased from 85 to 5% R.H., the water loss increased from 28.0 to 88.2%, which is not very different

from the effect at a pressure of 4 cm. At a humidity of 45% R.H. the water loss increases from 20.2 to 71.2%, when the pressure decreases from 8 to 2 cm. of mercury, but at 85% R.H. with the same pressure changes, the water loss increases only from 8.8 to 28.0%. The pressure changes at 45% relative humidity affect the insects to a greater extent than the same changes at 85% R.H. With the same pressure changes at 5 % R.H. the water loss increases from 39.1 to 88.2%. The effect due to the pressure changes at 5% R.H. was therefore practically the same as at 45% R.H. At any one pressure the mortality of both the species increases as the humidity decreases from 85 to 5%, and at any one humidity, the mortality increases, as the pressure decreases from 8 to 2 cm. of mercury.

Effect of Pressure on C.granaria and C.oryzae

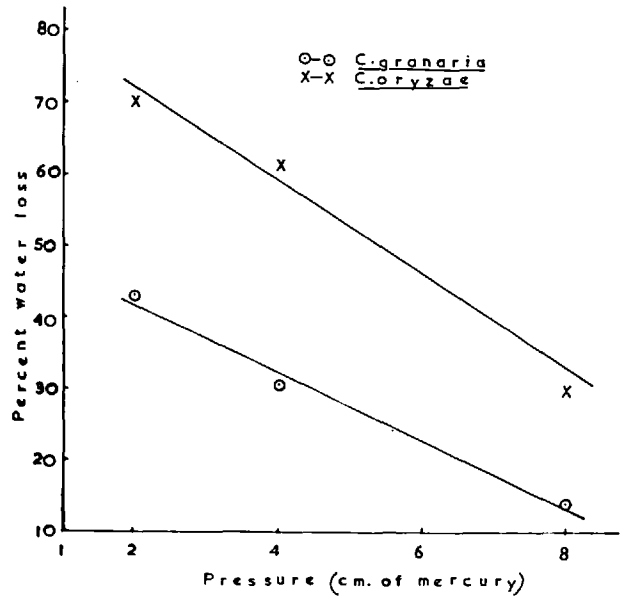
C.oryzae was found to be more susceptible than C.granaria at the pressures tried. The results are given in Table 9 and figs.5 and 6.

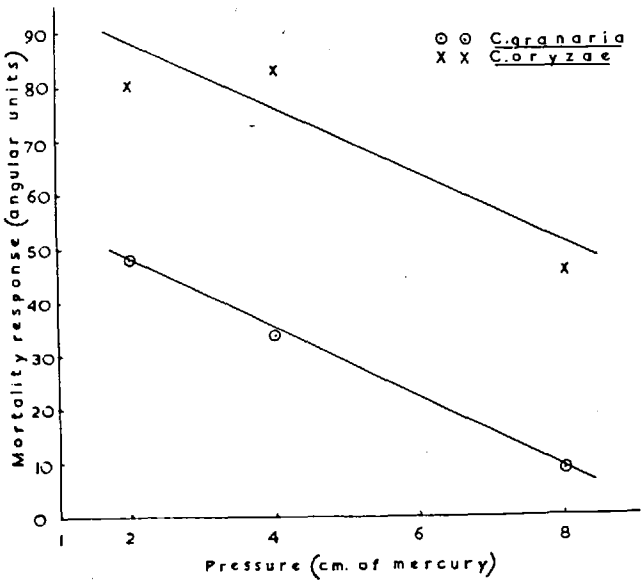
TABLE 9.

Mean Water Loss and Mortality Response of C.granaria and C.oryzae at various pressures.

Species	% Water Loss at Various Pressures.			Mortality in Angular Units at Various Pressures		
	2 cm.	4 cm.	8 cm.	2 cm.	4 cm.	8 cm.
C.granaria	42.9	30.4	13.9	48.1	33.4	8.6
C.oryzae	70.2	61.6	29.8	80.4	85.1	45.2

This interaction was found to be significant at 1% probability level for the water loss response, but was not significant for the mortality response. The results show that the difference in the water loss between the two species is greater at 4 cm. pressure than at 8 cm. This appears to be associated with the greater effect of treatment at a pressure of 4 cm. pressure on C.oryzae. With the decrease in pressure from 4 to 2 cm. both the species are affected to the same extent and the difference in the loss of water is practically the same at these two pressures. The mortality of both the species increased as the pressure decreased, and C.oryzae gave higher mortality response at all the pressures tried.





Figs. 5 & 6 - Pressure - Species Interaction.

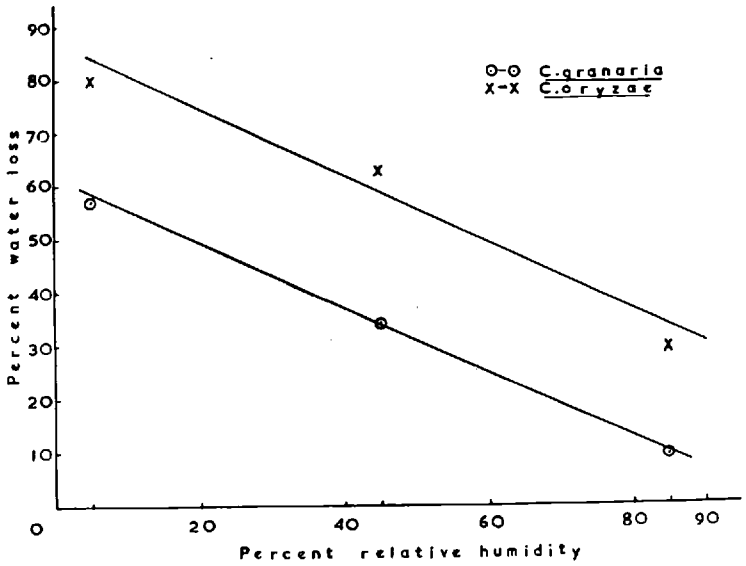
Effect of Humidity on C.granaria and C.oryzae.

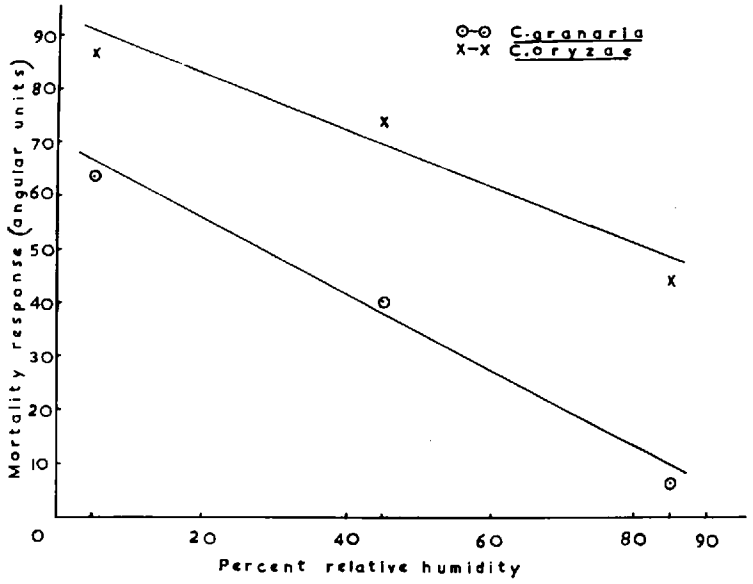
It was found that both the species lose more water and give higher mortality response as the humidity decreases from 85 to 5% R.H. The results are given in Table 10 and figs.7 and 8.

TABLE 10.

Mean Water Loss and Mortality Response of C.granaria and C.oryzae at various humidities.

Species	% Water Loss at Various Humidities				Mortality in angular units at various humidities.			
	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled
C.granaria	56.9	33.9	9.2	16.3	63.7	40.6	6.7	9.3
C.oryzae	80.2	63.1	29.8	42.5	86.9	73.4	44.7	73.1





Figs. 7 & 8 - Humidity - Species Interaction.

This interaction is significant at 1% probability level for the mortality response, but is not significant for the water loss response.

The water loss and the mortality of both the species increases as the humidity decreases, and C.oryzae was found to be more susceptible than C.granaria.

Effect of exposure period on C.granaria and C.oryzae.

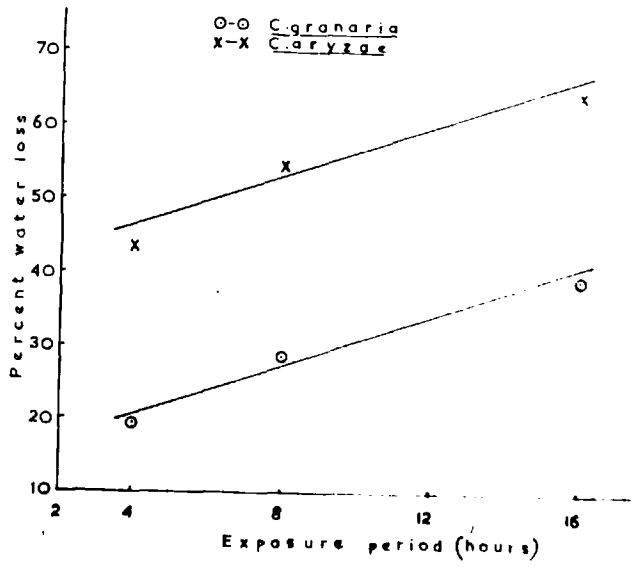
The results are given in Table 11 and figs.9 and 10.

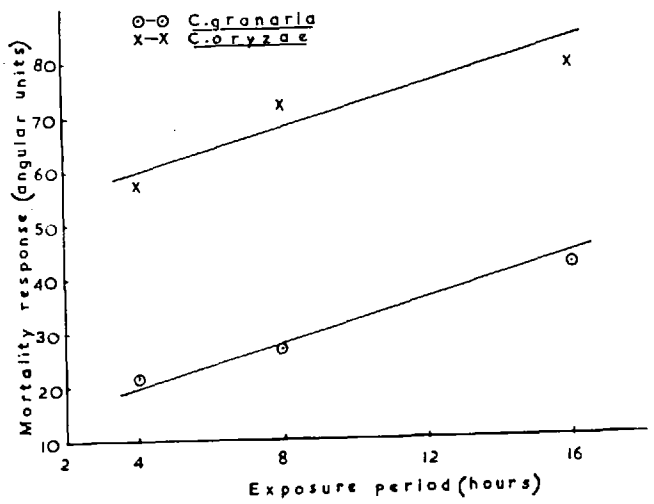
TABLE 11.

Mean Water Loss and Mortality response of C.granaria and C.oryzae at various exposure periods.

Species	% Water Loss at Various Exposure Periods			Mortality in Angular Units at Various Exposure Period		
	4 hours	8 hours	16 hours	4 hours	8 hours	16 hours
C.granaria	19.4	28.7	39.1	21.7	26.7	41.8
C.oryzae	43.4	54.4	63.9	57.6	72.2	78.9

This interaction was not significant for either of the responses. At all the periods tried, C.oryzae was found to be more susceptible than C.granaria. Both the species lost more water and gave higher mortality responses as the





Figs. 9 & 10 - Exposure period - Species Interaction.

exposure period increased. C.oryzae lost water at the average rate of about 11% of their water content per hour, during the first four hours of exposure, whereas C.granaria lost water at the rate of only 5% per hour, in the same period, and the rate of loss in both the species decreased as the exposure period increased from 4 to 16 hours.

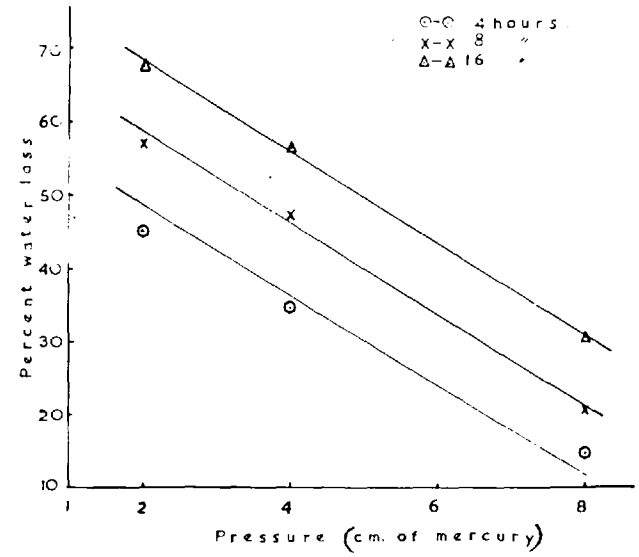
Effect of pressures at various periods.

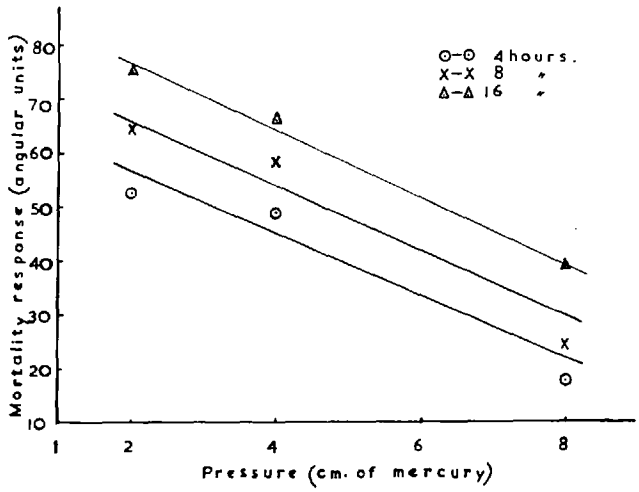
The results are given in Table 12 and figs.11 and 12.

TABLE 12.

Mean Water Loss and Mortality of both the species taken together, when treated under various pressures for different periods.

Exposure Period in Hours.	% Water Loss at Various Pressures.			Mortality in angular units at various pressures		
	2 cm.	4 cm.	8 cm.	2 cm.	4 cm.	8 cm.
4	45.1	34.6	14.5	52.7	48.9	17.4
8	57.0	47.0	20.6	64.7	59.2	24.4
16	67.6	56.5	30.5	75.3	66.7	39.0





Figs. 11 & 12 - Pressure - Exposure Period Interaction.

This interaction was not significant for either of the responses. The results show that at any period, water loss as well as the mortality of both species increases as the pressure decreases, and at a particular pressure, both the responses increase with the period.

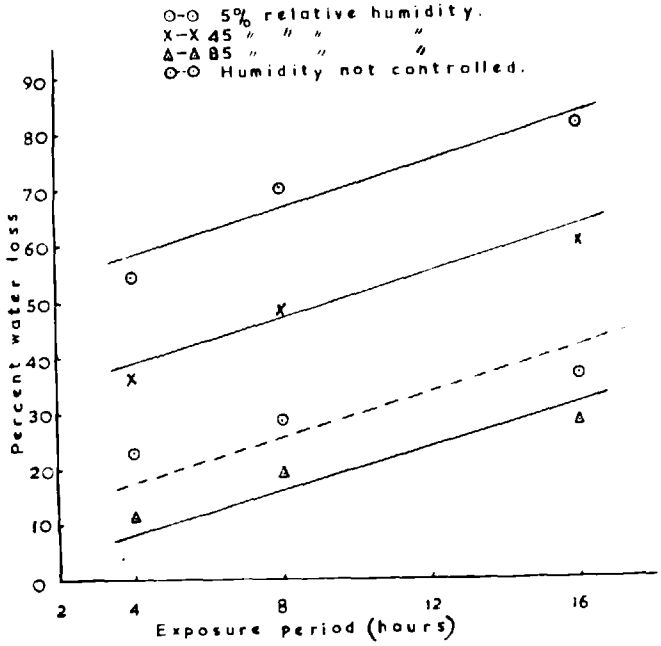
Effect of humidity at various periods.

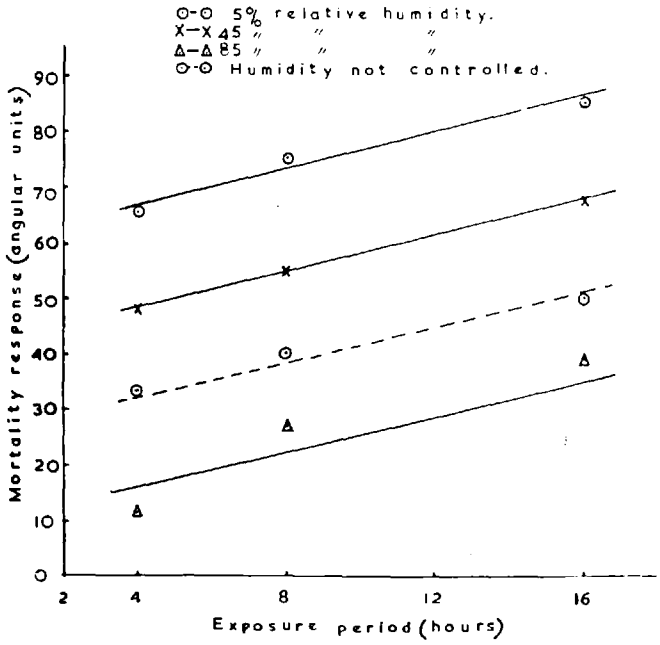
The results are given in Table 13 and figs. 13 and 14.

TABLE 13.

Mean Water Loss and Mortality of both the species taken together, when treated at various humidities for different periods.

Exposure period in hours	% Water Loss at Various Humidities				Mortality in angular units at various humidities			
	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled
4	54.4	36.6	11.3	23.0	65.8	48.1	11.3	33.4
8	70.2	48.4	18.9	28.7	75.2	55.2	27.0	40.2
16	81.0	60.4	28.2	36.5	84.9	67.7	38.8	50.0





Figs.13 & 14 - Humidity - Exposure period Interaction.

This interaction was not significant for either of the responses. At any particular exposure period, both the species lose more water and give higher mortality as the humidity decreases from 85 to 5% R.H. and at a particular humidity, the water loss and the mortality increase with the exposure period.

Triple interaction.

The results are given in Tables 14 - 17. None of the interactions were significant for the water loss response, and only one interaction between pressure, humidity and species was significant at 5% level for the mortality response. This shows that each factor produced its effect, but did not modify the effect of other factors.

TABLE 14.

Mean Water Loss and Mortality of C.granaria and C.oryzae, when treated under various pressures for different periods.

Exposure period in hours	Species	% Water Loss at Various Pressures.			Mortality in angular units at various pressures.		
		2 cm.	4 cm.	8 cm.	2 cm.	4 cm.	8 cm.
4	C.granaria	30.4	19.9	7.8	36.9	26.3	2.0
	C.oryzae	59.8	49.2	21.1	68.4	71.5	32.7
8	C.granaria	43.0	29.9	13.2	43.9	30.6	5.5
	C.oryzae.	71.1	64.1	28.0	85.5	87.7	43.3
16	C.granaria	55.3	41.4	20.7	63.4	43.5	18.4
	C.oryzae	79.9	71.6	40.3	87.1	90.0	59.7

TABLE 15.

Mean Water Loss and Mortality of C.granaria and C.oryzae, when treated at various humidities, under different pressures.

Pressure in cm.of mercury.	Species.	% Water Loss at Various Humidities.				Mortality in angular units at Various Humidities.			
		5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled
2	C.granaria	80.1	56.6	13.1	22.0	83.9	71.8	17.3	19.4
	C.oryzae	96.4	85.9	42.9	55.7	90.0	87.0	58.3	86.2
4	C.granaria	64.3	31.8	10.2	15.4	80.0	45.2	0	8.6
	C.oryzae	92.4	76.2	33.2	44.7	90.0	90.0	65.4	87.0
8	C.granaria	26.5	13.2	4.4	11.4	27.1	4.7	2.7	0
	C.oryzae	51.8	27.3	13.1	29.1	80.9	43.3	10.6	46.2

TABLE 16.

Mean Water Loss and Mortality of both the species taken together, when treated at various Humidities, under different pressures for three exposure periods.

Pressure in cm.of Mercury	Exposure period in hours	% Water Loss at Various Humidities.				Mortality in angular units at various Humidities.			
		5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled
2	(4	76.0	57.9	16.7	29.7	80.8	79.0	11.7	39.2
	(8	93.2	70.9	25.6	38.4	90.0	76.3	40.5	52.1
	(16	95.5	84.9	41.6	48.4	90.0	82.9	61.2	66.9
4	(4	61.5	40.4	11.5	24.8	80.8	56.0	12.5	46.2
	(8	81.4	54.9	22.8	29.0	84.2	66.9	40.5	45.0
	(16	92.0	66.8	30.8	36.3	90.0	79.9	45.0	52.1
8	(4	25.8	11.6	5.8	14.6	35.8	9.2	9.8	14.6
	(8	36.1	19.4	8.4	18.4	51.5	22.5	0	23.6
	(16	55.5	29.5	12.1	24.8	74.6	40.3	10.1	31.0

TABLE 17.

Mean Water Loss and Mortality of C.granaria and C.oryzae, when treated at Various Humidities for different periods.

Exposure period in hours	Species	% Water Loss at Various Humidities.				Mortality in angular units at Various Humidities.			
		5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled	5% R.H.	45% R.H.	85% R.H.	Humidity not Controlled
4	C.granaria	38.7	21.9	4.9	12.0	47.7	30.0	5.4	3.8
	C.oryzae	70.2	51.5	17.7	34.1	83.9	66.1	17.3	62.9
8	C.granaria	59.1	32.5	8.4	14.9	63.5	38.5	0	4.7
	C.oryzae	81.4	64.3	29.8	42.4	87.0	72.0	54.0	75.8
16	C.granaria	73.1	47.2	14.3	21.9	79.8	53.2	14.6	19.4
	C.oryzae	88.9	73.6	42.1	51.1	90.0	82.1	62.9	80.7

DISCUSSION

A search in the literature will show that most of the work on the effect of reduced pressures on insects has been based on the mortality responses obtained. It was Cole (1909) who first pointed out that insects, when subjected to reduced pressure, died by reason of desiccation. He realised the importance of a moist or dry atmosphere in the chamber under low pressure, but made no attempt to find out the water-loss from the insects under those conditions. Cotton (1925) observed that some larvae appeared to be dried when taken out of the chamber after treatment under reduced pressure. He found that adults of all the species of insects that he tried, dried up with an exposure of 7 hours, while certain larvae could survive exposure of even 24 hours to the same reduced pressure. These results could have been explained by actually measuring the water-loss from the treated insects, but Cotton did not attempt this measurement. Lutz (1929) was apparently the first worker to demonstrate that insects lose water when subjected to low pressure, but he also did not measure the water-loss from them. Fisk and Shepard (1938) were able to reduce the mortality in the control set of insects in their experiments from

16-20% to below 1% by exposing a little water in the chamber at reduced pressure, thus showing the importance of the evaporation of the water from the insects' bodies. The importance of measuring the water-loss from the insects was realised by Livingstone and Reed (1940) who found that the insect mortality depended on the amount of the water lost. They showed at a pressure of 2 inches of mercury and for an exposure period of 8 hours, the water lost and the mortality response were greater in the dry than in the wet atmosphere, other conditions being the same.

The present investigation is the first in which an attempt has been made to maintain definite humidities in the chambers under reduced pressures, and systematically to study the effect of reduced pressures on the insects with reference to loss of water from them and the mortality response produced. The results are consistent with those of Livingstone and Reed (1940).

We see from Table 8 and Figs. 3 and 4 that at any particular humidity, the loss of water as well as the mortality increased as the pressure decreased, and at any pressure, however low, both the responses depended on the humidity maintained in the chamber. It is found that at 8 cm. pressure, the water-loss increases from 8.8 to 39.1% of the water content of the insects, when the

humidity decreases from 85 to 5%, whereas at 4 cm. pressure the water-loss increases from 21.9 to 78.3%, with the same humidity changes. The difference in water-loss at these two pressures shows the extent to which the insects are affected by these pressures. Exposure to a pressure of 4 cm. produced a greater effect on the insects than exposure to a pressure of 8 cm. At both 45 and 5% relative humidities. The difference in the water-loss with the decrease in humidity, at 4cm. pressure is practically the same as at 2 cm. pressure, which shows that the humidity decrease from 85 to 8% has affected the insects to the same extent at these two pressures. At 85% R.H. the water-loss increases from 8.8% to 28.0% when the pressure decreases from 8 to 2 cm., whereas at 45% R.H. the same pressure changes increase the water-loss from 20.2 to 71.2. This shows that 45% relative humidity affects the insects to a greater extent than 85% R.H. at 4 and 2 cm. of mercury. At 5% R.H. the water-loss increases from 39.1% to 88.2% as the pressure decreases from 8 to 2 cm. of mercury. This shows that the pressure changes at 45% and 5% R.H. affect the insects to nearly the same extent. In view of these results, it is concluded that 5 - 45% relative humidities

at 2 - 4 cm. pressures produce greater response than the same humidities at 8 cm. pressure, or 85% R.H. at 2 - 4 cm. pressure. The mortality of both species increased as the humidity or the pressure decreased, keeping the other factor constant. When the humidity in the chamber was not controlled, both the responses produced with the pressure changes were found to be intermediate between the responses produced at 85 and 45% R.H. The results thus show that the humidity in the chamber plays an important part in regulating the water-loss from the insects. In small chambers of about 1 litre capacity, when the insects are treated under the reduced pressures, they lose sufficient water to raise the humidity, and the insects then again regulate their water-loss, thus emphasizing the importance of controlling the humidity in the small chambers, or alternatively, doing this kind of experiment in big chambers, where the humidity will not be appreciably altered by the water given off by the insects.

Adults of C. oryzae were found to be more susceptible than those of C. granaria under the conditions tried (Table 9 and Figs. 5 and 6). Salmond (1951) and Nahal (1952) obtained higher mortality response with C. oryzae than with C. granaria when treated under reduced pressures

of 2 - 8 cm. of mercury. High mortality results of C. oryzae appear to be associated with a more severe desiccation than that suffered by C. granaria. Both species lost more water as the exposure period increased from 4 to 16 hours (Table 11 and Figs. 9 and 10), but it is interesting to note that during the first 4 hours of exposure C. oryzae lost water at a greater rate than C. granaria, but the rate decreased asymptotically to that of C. granaria as the period of treatment increased from 4 to 16 hours. The rate at which the water is lost plays an important part in altering the humidity in the small chamber. As C. oryzae lose water at a greater rate than C. granaria, their share in saturating the atmosphere in the chamber will be greater, and C. granaria will thus be at advantage if adults of the two species are treated together, when the humidity is not controlled. Mortality caused by desiccation of course depends on the water-loss from the insects. Any factor that tends to reduce the water-loss reduces the mortality also. At 4 mm. pressure the water-loss and mortality are considerably less than at 2 cm. pressure, which was found to be most effective. When the insects were subjected to 4 mm. pressure for as long as 48 hours,

C. granaria and C. oryzae lost only 18i.% and 19.4% of their water content respectively, and the mortality was 26% and 40% respectively (Table 2). Moore and Carpenter (1938) found that C. oryzae at 1 - 2 mm. pressure sorbed less hydrogen cyanide and suffered a lower mortality than at 6 cm. pressure. They attributed these results to some kind of physical effect of the low pressure on the insects, such as collapse of tracheae. The water-loss and mortality results obtained lend support to their hypothesis. It is probably a collapse of the tracheae that reduces the loss of water and, in a fumigation, the sorption of fumigant, and in fact, sorption of hydrogen cyanide is considerably less at 2 mm. than at 6 cm. pressure. C. granaria and C. oryzae are affected by the reduction of pressure up to certain limit, beyond which the insects are less affected. It is therefore advisable in practice to try only those reduced pressures which have been found to produce the greatest response.

SUMMARY

The literature on the effect of reduced pressures on the insects is reviewed.

The method of studying the water-loss from the insects and controlling various humidities tried, is described.

Analysis of variance of the results is carried out and it is found that the water-loss and mortality of C. granaria and C. oryzae increase with the decrease in (1) pressure from 8 to 2 cm. of Hg., and (2) humidity from 85 to 5%.

C. oryzae were found to be more susceptible than C. granaria. They lost more water and gave a higher mortality response.

With the increase in exposure period, both the species lost more water and gave higher mortality responses and the rate of water-loss which was greater during first 4 hours of exposure slowly decreased as the period of exposure increased from 4 to 16 hours.

With the increase in the water-loss from the insects, the mortality in both species also increased.

It was found that at 4 mm. pressure, the water-loss as well as the mortality of both species was considerably less than that at 2 - 8 cm. pressure. This effect may be caused by some kind of physical reaction, such as the collapse of the tracheae at that pressure.

PART II. FUMIGATION UNDER REDUCED PRESSURES.

CONTENTS

INTRODUCTION

Insects damage crops in the field, and commodities in storage worth millions of pounds every year throughout the world. There is a constant "war" going on between the man and his enemy, the insect. Many methods are being tried and 'fumigation', i.e. subjecting the insects to the effect of the poisonous gases, is certainly one of the oldest and best method of killing the insects in 'closed' spaces. The poisonous gases enter the insects' bodies, through the spiracles and poison the insects. The amount of gas entering the insects' body, will depend among other conditions on the activity of the insect. Better results can be obtained, if by some means, the insects are forced to keep the spiracles open, or increase their respiratory rate, while they are subjected to the effect of the poisonous gases.

Oxygen deficient air has been shown to provide some kind of stimuli, which keeps the spiracles open for a long time, or increases the respiratory rate of the insects (Wigglesworth, (1935), Babak and Foust Ka (1907) and Buddenbrock and Rohr (1923)). Cotton (1932) and Cotton, Wagner and Young (1937), concluded that oxygen deficiency in the fumigation chamber under reduced pressure,

was the factor that had a marked effect on the respiration of the insects and rendered them more susceptible to the effect of the fumigant. Subjecting the insects to reduced pressures, is indirectly to subject them to oxygen deficient air, which increases their respiratory rate by an amount depending on the pressure maintained in the chamber. In that state, if the insects are subjected to the effect of the fumigant, they should sorb more gas. Fumigation under reduced pressure is based on the above principle, and is an improvement on the old technique of atmospheric fumigation.

Though this method of fumigation has been in use in America and other parts of the world, since 1915, practically no information is available on the influence of various reduced pressures on the sorption of the poisonous gases by the insects. Moore and Carpenter (1938), appear to be the only workers to find out the amount of hydrogen cyanide sorbed by the insects at reduced pressures.

Fisk and Shepard (1938) found that the median lethal dose of methyl bromide for Tribolium confusum decreased as the pressure in the chamber decreased.

In view of the scanty information available in the literature, it was felt that some light would be thrown on the process of fumigation under reduced pressure if the amounts of the gas sorbed by the insects, were

determined as well as the corresponding mortality.

The insects chosen for the investigation were the adults of Calandra granaria and C.oryzae and the fumigant was hydrogen cyanide which has the dual advantages of ease of manipulation and determination.

Review of the literature.

In this problem it has been found convenient to divide the literature in two separate parts, one dealing with the fumigation with hydrogen cyanide under reduced pressures and at atmospheric pressure, and the other part dealing with the recovery of hydrogen cyanide from the fumigated insects and other materials, and its determination.

Fumigation with Hydrogen Cyanide.

Fumigation of stored products and other materials with hydrogen cyanide is still a common practice, and often provides a practical method of killing the insect pests. Hydrogen cyanide has been in use for nearly 75 years, and though a considerable number of other fumigants have been tried and adopted for certain purposes, e.g. methyl bromide, carbon disulphide, ethylene oxide, carbon tetrachloride etc., hydrogen cyanide continues to be widely used, chiefly because of its high toxicity to the insects.

Fumigation under reduced pressures, or 'vacuum fumigation' as it is called, is practiced in U.S.A., France and to a limited extent in other parts of the world. The method originated 35 years ago and hydrogen cyanide, which was the fumigant first chosen, is still often used today.

According to Strand et al (1937), hydrogen cyanide was used as early as 1877 to kill museum pests, but its

first use at reduced pressure, was by Sasscer and Hawkins in 1915. Most workers have concentrated on the use of hydrogen cyanide under reduced pressures, in large scale fumigation and have concerned themselves with the complete control of the pests doing the damage. (Sasscer & Hawkins (1915), Sasscer & Sanford (1918), Sasscer & Dietz (1918), Nagel (1921), Smith (1923), Hagan (1931), Crumb & Chamberlin (1933), Rocci (1934), Young, Wagner and Cotton (1935), Lindgren (1936), McLaine & Munro (1937,1939) and Brubakar & Reed (1943).

There is practically no information available on the fumigation of insects with hydrogen cyanide under reduced pressures involving more than one factor. Moore and Carpenter (1938) found that the percentage mortality decreased as the air pressure increased. They also found that at atmospheric pressure, a concentration of about 6 mg. per litre was required to produce 95% mortality of T. confusum in a 10 minute exposure period, while at 1-2 mm air pressure, a dosage of only 0.44 mg. per litre was sufficient to produce the same kill.

The same workers in another paper on the sorption of hydrogen cyanide by the insects showed that there were pronounced differences among the different species of insects, both in the manner in which the fumigant was

sorbed, and in the amounts taken up. They found that the insects which were known to be difficult to kill, sorbed less hydrogen cyanide than did susceptible insects.

Fisk and Shepard (1938) found that the median lethal doses of methyl bromide for T. confusum increased as the air pressure in the chamber increased, from 2 cm. of mercury to atmospheric pressure. Salmond (1951) found that C.granaria and C.oryzae showed higher mortality response under reduced pressures than at atmospheric pressure, when fumigated with hydrogen cyanide. She was also able to show from the mortality responses of C.granaria and C.oryzae, that the technique of sustained vacuum fumigation was more effective than the technique in which the vacuum is dissipated in the process of admitting the fumigant.

Shepard (1939) states that "the granary weevil (C.granaria) is in general more resistant to fumigants than the rice weevil (C.oryzae). Busvine (1942) found that the median lethal dose of hydrogen cyanide in atmospheric fumigation for C.granaria was 22 mg. per litre, whereas for C.oryzae, it was 24 mg. per litre at 25 °C, when exposure period was 1 hour. Busvine's results appear to contradict the general statement of Shepard, but at reduced pressure, 6 cm. of mercury, C.oryzae has been found to be more susceptible to hydrogen cyanide

than C.granaria. (Moore & Carpenter (1938), Salmond (1951)). This shows how the same insects behave in a different way, under different conditions of the fumigation, each of which should be carefully controlled in an investigation.

Recovery of hydrogen cyanide from fumigated insects and its determination.

Fumigation, as a practical method of controlling insects, is an ancient practice but most investigations have been confined to applying known doses of fumigant and noting the mortality response. Until 1938 (Moore and Carpenter) no attempt was ever made to study the sorption of the fumigant by the insects. It was in 1944, that Sinclair and Ramsay first standardised a method for the determination of the minute amounts of hydrogen cyanide from the fumigated insects with picric acid, and Lindgren and Sinclair made the first attempt to recover and quantitatively determine the fumigant from fumigated scale insects.

The picric acid method for determination of hydrogen cyanide was used by Foy and Hyde (1937) and Nowosad and MacVicar (1940) for determination in the cyanophoric plants. These authors incubated the plant material for 24-48 hours at 30°C in a sealed tube, from the top of which was suspended a moist filter paper treated with

alkaline picrate solution. The hydrogen cyanide evolved changed the colour of the paper from yellow to reddish yellow or red, depending on the amount of hydrogen cyanide. The colour was extracted in water and the coloured solutions compared with standard solutions. Waller (1910), Adriano and Ynalvex (1932), Sullivan (1939) and Hogg and Ahlgren (1942) also used alkaline picrate in hydrogen cyanide determinations. Sullivan (1939) improved the method by measuring the colour intensity on a photometer. He reported that there was a greater sensitivity at lower concentrations of hydrogen cyanide, and suggested that aliquots containing more than 0.2 mg. of hydrogen cyanide should not be used.

Lindgren and Sinclair (1944,1945) and Pradhan and Bhatia (1951) are the only workers to use the alkaline picrate method for determining the hydrogen cyanide recovered from fumigated insects. Lindgren and Sinclair (1944) recovered more hydrogen cyanide from the non-resistant scale insects than from the resistant insects. They also recovered more hydrogen cyanide from the pupae of the walnut husk fly Rhagoletis completa, when fumigated at low relative humidities (15-20% R.H.) than at high humidities (70-90% R.H.). The same workers (1945) found that the recovery of hydrogen cyanide from fumigated house fly pupae (M.domestica) varied with the age of the pupae. They

showed that there was a definite increase in the amount of hydrogen cyanide recovered from the pupae with age. The greatest recovery was from pupae which were from 5-8 days old. Less hydrogen cyanide was recovered from pupae 3-5 days old, the recovery from which was about the same as that from pupae 1-3 days old.

Pradhan & Bhatia (1951) found that the more resistant insects needed a higher external concentration of hydrogen cyanide in the chamber. They are the first workers to attempt to make a balance sheet on the sorption and recovery of hydrogen cyanide from fumigated insects, and concluded that the sorption was greater than the recovery and the difference was the amount of hydrogen cyanide which was chemically changed into a compound, from which it was not possible to recover hydrogen cyanide by distillation.

Except for these few references, there appears to be no other paper on the sorption or recovery of hydrogen cyanide from fumigated insects.

MATERIALS AND METHOD.

Chemical Materials.

1. Hydrogen cyanide. 2. Sodium carbonate solution.
3. Picric acid solution. 4. Sulphuric acid.
5. Potassium cyanide solution.

Biological Materials.

Adults of *C. granaria* and *C. oryzae*.

1. Hydrogen cyanide.

Liquid hydrogen cyanide was used in all the experiments. Small ampoules of thin glass were filled with the liquid and sealed at the top. The ampoules were weighed before and after filling, the weight of liquid hydrogen cyanide being obtained by the difference.

In use the ampoules were introduced into the fumigation chambers and broken by shaking at the beginning of the fumigations. The dose of hydrogen cyanide was calculated from the weight of the fumigant and the volume of the chamber and was subject to an error of $\pm$ 1.5 mg. per litre, on account of the difficulty of filling ampoules with exactly the required weight of the fumigant.

2. Sodium carbonate solution.

A 5% solution of sodium carbonate was used to absorb the gaseous hydrogen cyanide. It was prepared by dissolving 25 gm. of sodium carbonate (analar) in a small

quantity of water, transferring the solution to a 500 ml. volumnetric flask, and making up to the mark with distilled water. I ml. of this solution was introduced into a micro-sintered glass bubbler by using a 5 ml. syringe and about 2 ml. of water were then added so as to cover the sintered glass disc with solution.

3. Picric acid solution.

A 1% picric acid solution was prepared and used as a colorimetric reagent for hydrogen cyanide. The proportion of picric acid and sodium carbonate solution was kept the same as that suggested by Sinclair and Ramsay (1944), 1 ml. of picric acid and 1 ml. of sodium carbonate solutions were measured out from separate stock solutions and mixed, when needed.

4. Sulphuric acid.

10 N. sulphuric acid solution was prepared by diluting 27.8 ml. of concentrated sulphuric acid to a volume of 100 ml. 1 - 2 small drops of the 10 N. solution were added to the 3 ml. of water which covered the crushed insects in the distillation tube and from water thus acidified the hydrogen cyanide extracted from the insects was removed by distillation in a current of air.

5. Potassium cyanide solution.

Potassium cyanide solution was used for preparing

the colorimeter calibration curve. 1 ml. of the standard solution contained 0.324 mg. of potassium cyanide, corresponding to 0.135 mg. of hydrogen cyanide. Known amounts of the solution were measured out in labelled test tubes, using a 5 ml. microburette, graduated to 0 • 01 ml. The colours were developed and the calibration curve prepared by plotting mg. of hydrogen cyanide against readings of the colorimeter.

Biological material.

The experimental insects were adults of Calandra granaria and Calandra oryzae. The insects were bred as described before and 3 - 4 weeks old adults were selected at random from the storage bottles for fumigation. Sex of the insects was not taken into consideration. 100 insects of each species were used for an experiment on the recovery of hydrogen cyanide after fumigation and another set of 100 insects was kept for determination of the mortality response. The insects were separated in specimen tubes and kept in the constant temperature and humidity room for 1 - 2 hours or more before fumigation.

Determination of hydrogen cyanide recovered from the fumigated insects:-

Technique.

The technique adopted in the present investigation

was practically the same as that standardised by Sinclair and Ramsay (1944), the broad principle of which was the formation of a colored compound, an isopurpurate, by solutions of alkaline picrate and cyanide on heating.

Klandiwetz (1859) was the first to note the formation of isopurpuric acid. The reaction may be formulated as follows:-

$$\underline{C_6H_3N3O7}+3KCN+3H_2O \longrightarrow \underline{C_8H_4KN_5O_6}+CO_2NHz+2KOH$$

Picric acid — Potassium isopurpurate.

The sodium carbonate solution containing the unknown amount of cyanide recovered from the fumigated insects by cool aeration and distillation is taken in a test tube, 1 ml. of 1% picric acid solution is added, and the volume then brought to the 10 ml. mark, if necessary. The tube is shaken to mix the reagents properly and then put in the boiling water bath for exactly 3 minutes. The tubes are shaken 3 - 4 times, while the colour is being developed. The solutions are taken out after the 3 minute heating period and kept in the constant temperature room for about 2 hours to cool. This period between heating and the instrument reading was found by Sinclair and Ramsay (1944) to give more consistent results than a shorter period. Each solution is then diluted to 25 ml., being the final volume of the solution ready for the colorimeter reading. For dilution, the solution is transferred to a 25 ml.

volumetric flask, the tube washed 3 times, the wash water also added to the solution in the flask and the solution brought to the mark. The water used for the dilution is also at the same temperature as that of the solution, being stored in the constant temperature room. In this way, temperature differences have been avoided and the temperature of the dilute solution is always about 25°C, irrespective of the fluctuating temperature of the laboratory. The colorimeter readings are taken immediately after the solutions are diluted to the final volume. The colour intensity of the various solutions is measured on the "Spekker" colorimeter, using green filters and 1 cm. cells to hold the solution.

All the unknown solutions were compared with the blank, which was prepared in exactly the same way as the standard solutions, except that solution of potassium cyanide was not added. The calibration curve was prepared with known amounts of the potassium cyanide solutions, the colorimeter readings were plotted against the mg. of hydrogen cyanide (corresponding to the potassium cyanide) and the curve thus obtained (Fig. 2) was used to read off the amount of hydrogen cyanide in mg. from the colorimeter readings of the unknown solutions. All the batches of fumigated insects used for the recovery of hydrogen cyanide were weighed before

treatment and the proportion of hydrogen cyanide recovered from them was expressed as mg./g. body wt.

Factors affecting the intensity of the colour developed.

The effect of the following factors on the intensity of the colour, which is produced by heating the alkaline picrate and the cyanide solution was determined.

1. Different volumes of the reagents.
2. Different initial volumes.
3. Period between heating and taking the colorimeter reading.

1. Effect of different volumes of the reagents:-

1 millilitre of 5% Na_2CO_3 solution to 1 ml. of 1% picric acid solution is the proportion suggested by Sinclair and Ramsay (1944) to determine minute amounts of hydrogen cyanide·keeping the volume of any one of the reagents constant, i.e. 1 ml., the volume of the other reagent was changed to 0.5, 1 and 1.5 ml., and the effect on the intensity of colour was found. Known amounts of standard potassium cyanide solution were added to the alkaline picrate solution and the volume brought to 10 ml. The heating period was 3 minutes and the hot solutions were allowed to cool in a constant temperature room for 2 hours. The remaining procedure was the same as that described previously. The results are given in Tables 1 and 2.

TABLE 1.

The effect of different amounts of picric acid solution on the colour intensity.

HCN in mg.(KCN solution used)	Colorimeter readings		
	.5 ml.picric acid	1 ml. picric acid	1.5 ml. picric acid
.006	.008	.012	.018
.048	.116	.155	.19
.096	.23	-	.38
.144	.343	.478	.545
.192	.423	.587	.718
.240	.49	.76	.902
.288	.558	.855	1.015

TABLE 2.

The effect of different amounts of sodium carbonate solution on the colour intensity.

HCN in mg.(KCN sol. used)	Colorimeter readings		
	.5 ml. Na_2CO_3	1 ml. Na_2CO_3	1.5 ml. Na_2CO_3
.006	.01	.01	.01
.048	.15	.146	.145
.096	.314	.311	.293
.144	.48	.45	.437
.192	.648	.585	.575
.240	.796	.742	.705
.288	.908	.849	.833

From Table 1 we see that the colour intensity varies with the amount of picric acid solution used, when all other factors are kept constant. The colorimeter readings are higher when 1.5 ml. of the picric acid solution are added than with 1 ml. and lower when .5 ml. is added. When 1.5 ml. of sodium carbonate solution are added the colorimeter readings are practically the same as when 1 ml. is added but are slightly higher when 0.5 ml. is added. (Table 2)

This shows the importance of rigidly keeping constant the volume of any reagent that is used in this colorimetric method of determining hydrogen cyanide. In all the experiments the volumes of picric acid and sodium carbonate solutions were kept constant at 1 ml. of each reagent.

2. Effect of different initial volumes.

The initial volume of the unknown solution as suggested by Sinclair and Ramsay (1944) is 10 millilitres. Some experiments were carried out to find the effect of different initial volumes on the intensity of colour with known amounts of potassium cyanide solution, when the final volume was 25 ml. and all other factors were kept constant. 5 ml., 10 ml., and 15 ml. were the three initial volumes chosen. The colours were prepared as previously described and the colorimeter readings taken.

The results are given in Table 3.

TABLE 3

Effect of different initial volumes on the colour intensity.

NCN in mg.	Colorimeter readings		
	5 ml.	10 ml.	15 ml.
.012	.048	.03	.024
.024	.096	.07	.054
.036	.147	.105	.085
.048	.2	.142	.117
.06	.241	.185	.16
.072	.3	.233	.202
.084	.35	.274	.235
.096	.383	.30	.26
.108	.436	.35	.291
.12	.45	.374	.31

The results show that the change in the initial volume also affects the development of the colour. The colorimeter readings are lower when the initial volume is 15 ml. than when it is 10 ml., and higher when it is 5 ml. (Table 3). These results emphasize the importance

of keeping the initial volume constant. The volume chosen was 10 ml. in the present series of experiments.

3. Effect of the period between heating and colour measurement on colour consistency.

Unknown solutions are heated for 3 minutes in the boiling water bath to develop the colour. The hot solutions are allowed to cool and then brought to the final volume of 25 ml. before the colorimeter readings are taken. It was considered important to investigate the effect of varying the period between heating and taking the colorimeter reading. The period suggested by Sinclair and Ramsay (1944) was 2 hours. The solutions containing known amounts of potassium cyanide were heated for 3 minutes and kept in a constant temperature room for about 14 hours, after which they were diluted to the final volume of 25 ml. and the colorimeter readings taken. The results are given in Table 4.

TABLE 4

Effect of 14 hours period between heating and taking colorimeter reading on the colour intensity.

HCN in mg.	Colorimeter readings.		
	14 hours period	2 hours period	
		Figs.from Table 1	Figs from Table 2
.006	.015	.012	.01 -
.048	.157	.155	.146
.096	.298	-	.311
.144	.468	.478	.45
.192	.627	.587	.585
.240	.756	.76	.742
.288	.857	.855	.849

We see from Table 4 that a period as long as 14 hours has practically no effect and the colour formed appeared to remain stable for this long period. The same dilute solutions just referred to were kept in a constant temperature room for a further period of 48 hours, after which the readings were taken again. The results are given in Table 5.

TABLE 5

Effect of 48 hour period between diluting and reading on colour intensity.

HCN in mg.	Colorimeter readings.	
	Readings taken x immediately	Readings taken after 48 hours
.006	.015	.017
.048	.157	.157
.096	.298	.295
.144	.468	.464
.192	.627	.618
.240	.756	.74
.288	.857	.843

x Figs. taken from Table 4.

A glance at the table shows that the readings are very slightly lower when taken after about 48 hours. The colour developed remains substantially unaffected for as long as 48 hours. In the experiments reported in this thesis, all the readings were taken within 2 - 4 hours after the solutions had been heated.

Temperature effect:-

Sinclair and Ramsay (1944) found that a rise of temperature caused an increase in the reading, but that

this rise was small in comparison with the temperature increase. Temperature differences were avoided by keeping the hot solutions in the constant temperature room to cool and by diluting with water kept in the constant temperature room. The colorimeter readings were taken immediately after the solutions had been diluted.

Filters:-

The solutions usually to be compared were of two colours, yellow and reddish yellow or red. Various filters were tried, and it was found that green filters as suggested by Sinclair and Ramsay (1944) were the most suitable. They covered a wide range on the colorimeter, corresponding to the minimum of .006 mg. and the maximum of .3 mg. of hydrogen cyanide.

Various solutions containing known amounts of potassium cyanide were prepared and the colorimeter readings taken with different filters. The results are given in Table 6 and Fig. 1.

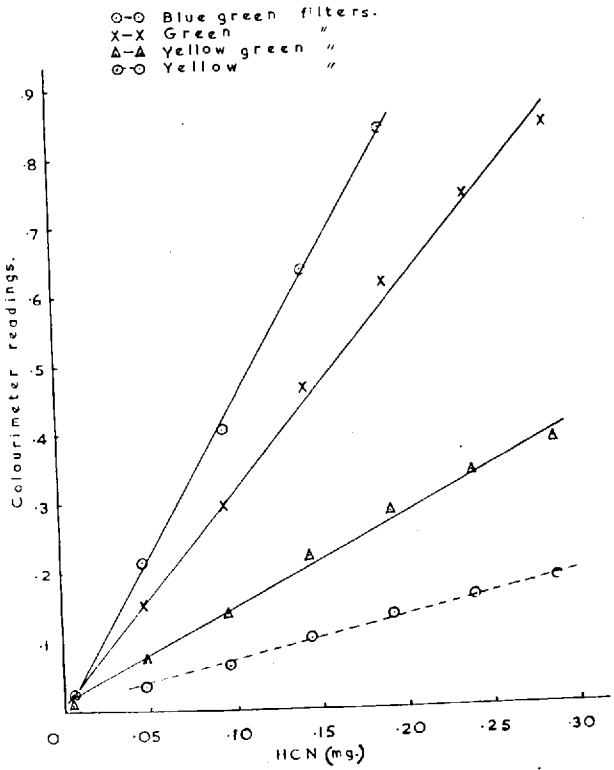


Fig.1 - Comparison of the sensitivity of the colourimeter using various filters.

TABLE 6

Colorimeter readings with different filters.

HCN in mg.	Colorimeter readings with diff. filters.			
	Blue-green	Green	Yellow-green	Yellow
.006	.026	.017 x	.011	-
.048	.215	.157	.076	.035
.096	.404	.295	.14	.063
.144	.635	.464	.219	.10
.192	.84	.618	.284	.133
.240	-	.74	.34	.157
.288	-	.843	.384	.18

x These figs taken from Table 5.

We see from Fig. 1 that the blue-green filters were the most sensitive but cyanide concentration up to only 0.2 mg. of hydrogen cyanide could be measured on the colorimeter with these filters. With green filters it was possible to measure up to 0.3 mg. of hydrogen cyanide, and they were as sensitive as the blue-green filters. For all colorimeter readings of unknown solutions and of the standard solutions, green filters were used. Yellow-green, and yellow filters were found to be useless.

CALIBRATION CURVE:

6.511 gm. of potassium cyanice (from British Drug Houses Ltd.) were dissolved in a small quantity of water, which was then transferred to a 1 litre volumetric flask and the solution diluted to the mark..50 ml. of this stock solution were taken in a 1 litre volumetric flask and diluted to the mark. This dilute solution was used for the preparation of the calibration curve. The potassium cyanide solution so prepared was first standardised with silver nitrate solution. The procedure adopted was that described by Vogel (Quantitative "Inorganic Analysis" P. 328). Silver nitrate solution was standardised with sodium chloride solution using potassium chromate as an indicator (Vogel, P. 311 - 312).

Potassium cyanide was found to be 99.56% pure. 1 ml. of this dilute solution will contain 0.324 mg. of potassium cyanide, corresponding to 0.135 mg. of HCN. Graded volume of this solution ranging from 0.05 to 2 ml. were taken in separate test tubes from a 5 ml. micro-burette graduated to .01 ml. 1 ml. of 5% sodium carbonate solution and 1 ml. of 1% picric acid solution were added to various measured quantities of potassium cyanide solution, and the volume brought to 10 ml. All the solutions after shaking were heated in the boiling water bath for 3 minutes. During this heating period

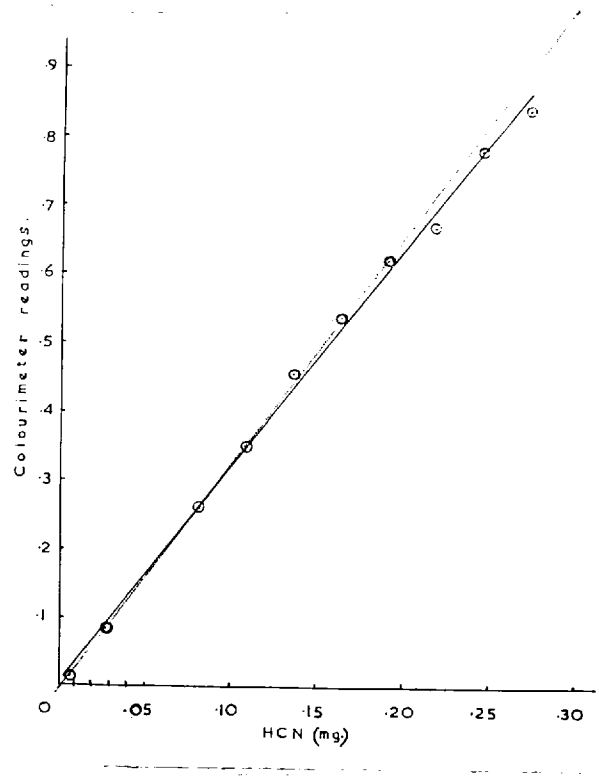


Fig.2 - Calibration Curve.

the solutions were shaken. The solutions were kept in a constant temperature room for about 2 hours to cool, and were then diluted to the final volume of 25 ml. and the colorimeter readings taken immediately, comparing the colour intensity with that of the blank. Duplicates were prepared and the results are given in Table 7 and Fig. 2.

TABLE 7

Colorimeter readings with known amounts of standard KCN solution.

KCN sol. in mg.	HCN in mg.	Colorimeter readings.		
		1st sample	2nd sample	Mean.
.05	.007	.015	.015	.015
.2	.027	.085	.086	.085
.6	.081	.263	.261	.262
.8	.108	.349	-	.349
1	.135	.454	-	.454
1.2	.162	.54	.536	.538
1.4	.189	.612	.632	.622
1.6	.216	.67	-	.67
1.8	.243	.772	.793	.782
2.0	.270	.856	.830	.843

Mean readings of the solutions containing known amounts of potassium cyanide were plotted against mg. of HCN (corresponding to the potassium cyanide). The curve thus obtained was used for determining the amounts of hydrogen cyanide in the unknown solutions. All the factors involved in the colour development were carefully controlled and adhered to, while working with the unknown solutions.

Distillation of unfumigated insects.

The alkaline picrate method of determining the minute amounts of hydrogen cyanide is quite sensitive and can be used to determine even as small a quantity of hydrogen cyanide as .006 mg. of HCN. The defect of the method is, that it is not specific. Any reducing compound e.g. aldehydes, ketones, hydrogen sulphide, etc., if distilled over, also form a coloured compound with alkaline picrate on heating. Pradhan Bhatia (1951), working with Tribolium confusum found that certain volatile reducing compounds were carried over with the distillate and affected the colour intensity.

In view of the fact that certain insects give off volatile reducing compounds it was found essential to determine if any such compound came over with the distillate from C. granaria and C. oryzae. 200 live

insects (100 of each species) were crushed in two distillation tubes and the air constantly passed through. As soon as the insects were crushed, which took about 15 minutes, the distillation tubes were put into the boiling water bath and air passed at the rate of about 10 ml. per minute for about 30 minutes. The bubblers containing sodium carbonate solution were kept in an ice bath. After 30 minutes of distillation, the contents of the bubblers were transferred to test tubes with 2 - 3 washings. 1 ml. of picric acid was added and the volume of the solution brought to 10 ml. The solutions were heated for 3 minutes in the boiling water bath, and the remaining procedure was the same as that described previously. It was found that the colorimeter reading was zero when these solutions were compared with the blank, prepared with 2 ml. of alkaline picrate. Distillation of unfumigated insects was repeated 3 times. These results show that the distillate of C. granaria and C. oryzae does not carry any reducing compound which affects the colour intensity. In the same way 2 distillations were carried out with dead and dried insects, which were starved to death and kept for over 2 months. The distillates in this case also showed no sign of the presence of any reducing compound.

Blank Solution.

The blank solution was prepared fresh for each experiment. 1 ml. of 5% sodium carbonate solution and 1 ml. of 1% picric acid solution were taken in a tube and diluted to the 10 ml. mark. The remaining procedure is exactly the same as with the unknown solutions. The colour of the blank is yellow, whereas that of the solutions containing hydrogen cyanide varied from reddish-yellow to cherry red, depending on the amount of the hydrogen cyanide distilled over.

Method of fumigating the insects:-

Pre-fumigation treatment.

200 adults of each species 3 - 4 weeks old were selected at random from the storage jars, without paying any attention to the sex of the insects. The insects were kept in a constant temperature room for at least 2 hours before they were fumigated or subjected to the pressure treatment. No food was supplied to the insects during this period. 100 adults of each species were put separately in glass tubes closed at both ends with fine muslin and a piece of thread. In two weighed insect cages, about 100 adults were weighed out accurately to the nearest 0.1 mg. to give the weight of the insects before treatment.

Method of fumigation.

A glass ampoule containing a known amount of liquid hydrogen cyanide was put in the body of the pyrex glass fumigation chamber, 1,075 to 1150 ml. capacity, and the four insect cages containing the insects were put in the neck of the chamber. The chamber was brought to the required pressure, and the ampoule was fractured by shaking the chamber, which was then kept in the constant temperature room. The insects were fumigated for the particular period desired and during this period the chamber was shaken 4 - 5 times. The insect cages were now in the body of the chamber. After testing for leakage with a manometer, the chamber was brought to atmospheric pressure and the insects taken out immediately. For fumigation at atmospheric pressure, the chamber was slightly evacuated and an ampoule containing hydrogen cyanide, broken. Immediately afterwards the chamber was brought to atmospheric pressure and the insects fumigated for a particular period.

Post fumigation treatment.

One set of the weighed insects was transferred at once to the distillation tubes and the hydrogen cyanide recovered. The other set of insects were kept for observation of mortality. Food was supplied to these insects and they were kept in the constant temperature room.

Mortality Results.

Counts were made 4 days after the fumigation. The control set went through the same pressure treatment, but was not fumigated. It was also kept in the constant temperature room. All the insects became inactive within a minute or two after an ampoule containing liquid hydrogen cyanide was broken in the chamber, and remained in that stage for sometime even after they were taken out of the fumigation chamber. The insects that showed movement of any part of the body were counted as alive. All the insects were touched with a camel hair brush or finger tip so as to stimulate them to make some kind of movement and it was not difficult after some practice to separate the live insects from the dead ones. The dead insects usually had their legs folded and bodies twisted and appeared to be somewhat dried. The control insects in general were all alive after the 4 day period except those treated at the lowest pressure, 2 cm. of mercury.

The mortality results are given in Table 12 and were analysed statistically for significance.

Temperature and humidity.

All the fumigations were carried out in a constant temperature-humidity room at 25°C and 65 - 70 % R.H.

The fumigated insects were also kept in the same room for 4 days, after which the mortality counts were made. The temperature effect on the insects was thus avoided. The fumigation chambers were always kept in the constant temperature and humidity room to attain the temperature of that room. The humidity was not strictly controlled. At atmospheric pressure, the fumigations were carried out at 65 - 70% R.H., but at reduced pressures, the humidity in the chamber depended on the vacuum produced. At 2 cm. pressure, the humidity is brought initially to below 10%, by evacuation of the chamber. The humidity rises considerably as water vapour evaporates from the insects. Control sets of insects were also kept under the same temperature and humidity conditions.

Description of apparatus and the method of recovering the hydrogen cyanide from the fumigated insects.

Lindgren and Sinclair (1944) appear to have been the first workers to attempt to recover hydrogen cyanide from fumigated insects. Two further attempts were made later, one by Lindgren and Sinclair (1945) and another by Prandhan and Bhatia (1951). But all these workers recovered the hydrogen cyanide by treating the insects whole. In the present investigations, it was decided to crush the insects and recover the hydrogen cyanide from

the crushed insects so as to find if this method was advantageous and if more hydrogen cyanide might be recovered by this modified procedure. The apparatus, ~~XXXXXX~~ as described by Sinclair and Ramsay (1944) is slightly modified so as to allow of the insects being crushed and is shown in Fig. 3 . Fumigated insects were put in the distilling tube which was flat-bottomed and contained about 2 ml. of water. The distilling tube was 6" in length and 1" internal diameter. This tube was closed with a rubber bung covered with aluminium foil. Through the bung passed a glass tube, (A) sufficiently lubricated with castor oil and flattened at the bottom and with a small hole (B) in the centre. This tube which served both for crushing and aeration reached the bottom of the distillation tube and was moved down on to the insects and then given a rotary motion so as to crush them. The appearance of the crushed insects is compared with that of whole in Fig. 4. A bent capillary tube passed through the other hole of the bung and was connected to the glass bubbler by means of a small piece of rubber tubing. Two, and sometimes three bubblers, each containing 1 ml. of 5% sodium carbonate solution, diluted to about 3 ml., were attached to the distillation tube so as to absorb the hydrogen cyanide liberated while the insects were being crushed and during distillation. Air purified by passing through soda lime was drawn through

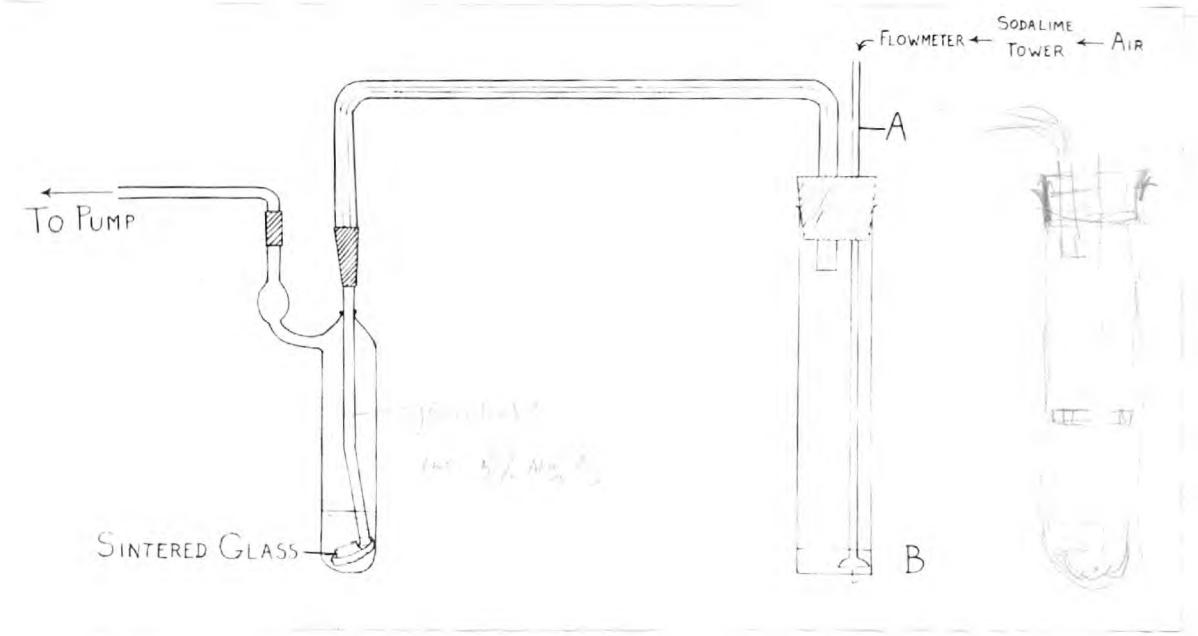


Fig. 3 - Distillation Apparatus.

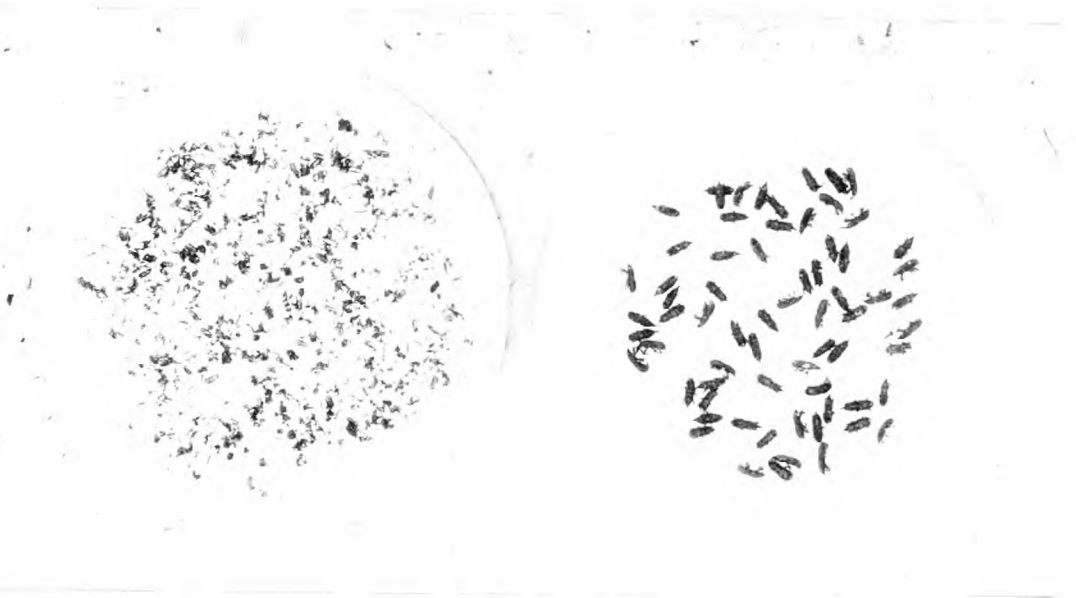


Fig.4 - The appearance of the crushed insects compared with the whole ones.

the system throughout the entire sequence of operations.

The rate of air flow was always controlled and was measured by the flow meter. The recovery of hydrogen cyanide from the fumigated insects was undertaken in two stages. In the first, air was drawn for 30 minutes, at room temperature, through the water in which the insects were crushed and which contained hydrogen cyanide, derived from the insects, in solution, and then through the bubblers where the fumigant was absorbed. This stage was followed immediately by the second, in which water and acid were added and the fumigant which passed from the insects into solution in the water was transferred to the solution in the bubblers by distillation in a current of air. Each species of insect from any one fumigation was treated separately. About 15 minutes was occupied in crushing the insects and during this operation air was drawn through the system at about 10 ml. per minute. The rate of flow was raised gradually to 150 ml. per minute and the cool aeration continued for 30 minutes.

The subsequent distillation followed immediately. The distillation tubes were put in the boiling water bath and the bubblers in ice bath and the air allowed to pass at the rate of about 10 ml. per minute for 30 minutes. 1 - 2 small drops of 10 N. sulphuric acid were added

through the aeration tube, followed by the addition of 1 ml. of water. After the 30 minute period of distillation, bubblers were disconnected from the distilling tube and emptied into the test tubes. The bubblers were washed 2 - 3 times and the wash water added to the solution. The HCN was determined as described before and the amount recovered from the fumigated insects was then calculated on gram body weight and expressed as mg. per g. body wt.

The method of HCN recovery from the fumigated insects differed from the one used by other workers in that the insects were crushed and the recovery of the HCN carried out by the two processes (a) ~~dry~~ Cool aeration and (b) ~~wet aeration~~ Distillation. The air was drawn through the distilling tube at a predetermined rate, which was measured by a flow meter, while the other workers did not measure the air flow rate, did not know how much air passed through the distilling tube to sweep away the fumigant during their 20 minute distillation period. Two, and sometimes three bubblers were used to trap the hydrogen cyanide. This precaution had to be taken as it was found that sometimes small amounts of hydrogen cyanide were trapped in the 2nd bubbler so, to be on the safe side, two bubblers were attached to the distillation tube. The use of the second bubbler was probably rendered necessary by the increased amounts of the fumigant which were recovered

by crushing the insects and by faster rates of air flow to remove the fumigant from the solution. Other workers have used only one bubbler per distillation.

The novel features of the method of recovery were found to increase materially the proportion of sorbed fumigant recovered and were adopted in all the determinations in this work.

Sensitivity of the method used in recovery and determination of hydrogen cyanide.

The apparatus previously described was first used for the distillation of known amounts of hydrogen cyanide from the standard solution of potassium cyanide. Known amounts of solution were taken in the distillation tube from a 5 ml. microburette and the solution diluted to about 3 ml. The tube was put in the boiling water bath, 1 - 2 small drops of 10 N. sulphuric acid were added through aeration tube, and the distillate collected in the solution of sodium carbonate in the bubblers. The bubblers were kept in the ice bath and, after about 30 minutes, were detached from the distillation tube and hydrogen cyanide determined as described before.

Another set of the coloured solutions was prepared by taking known amounts of the solution of potassium cyanide. The results of the hydrogen cyanide compared with the known amounts of cyanide in the solution of

potassium cyanide are given in Table 8.

TABLE 8

Hydrogen cyanide recovered in the distillate, compared with the standard KCN solutions.

HCN in mg. (corr. to KCN)	Colorimeter readings with	
	Standard KCN sol.	Distillate
.007	.015	.016
.014	.033	.034
.067	.217	.209
.135	.448	.435
.202	.644	.63
.270	.84	.82 1st bubbler
		.01 2nd "

It is clear from Table 8 that there is practically no difference in the amounts of HCN recovered by distillation and the amounts taken, showing that the method is quite satisfactory for the recovery of small amounts of HCN from the fumigated insects and is sensitive enough to distil over as small a quantity as .007 mg. of hydrogen cyanide. There is no appreciable loss of HCN on the

short rubber connections.

Crushing the insects before the distillation of the hydrogen cyanide from the fumigated insects.

Very little information is available on the recovery of hydrogen cyanide from fumigated insects and all the work reported (Lindgren and Sinclair, 1944, 1945 and Prandhan and Bhatia 1951) has been carried out with the fumigated insects distilled whole. While the work was in progress, it was thought desirable to find out whether more hydrogen cyanide might be recovered if the fumigated insects were crushed before the distillation.

A satisfactory method of crushing the insects in the distilling tube had to be developed. Air had constantly to be passed through the system so as to sweep away any fumigant liberated into the bubblers. These objects were achieved (by the method described before), and it was found that actually more hydrogen cyanide was recovered by crushing the insects before the distillation. The results are given in Table 9.

TABLE 9.

Recovery of hydrogen cyanide from the crushed and whole insects, fumigated at a pressure of 16 cm. of Mercury

Conc. used. mg. per litre	Exposure period in minutes	HCN recovered - mg./gm. body weight, from C.granaria			C.oryzae		
		Crushed	Whole	Increase in the recovery	Crushed	Whole	Increase in the recovery
15.7	15	.307	.213	.094	.329	.232	.097
25.75	"	.452	.283	.169	.513	.299	.214
9.87	30	.177	.117	.060	.244	.186	.058
22.38	"	.534	.309	.225	.383	.277	.106
8.19	60	.242	.135	.107	.233	.145	.088
15.98	"	.344	.251	.093	.373	.288	.085

All the fumigations were carried out at a pressure of 16 cm. of mercury and the hydrogen cyanide was recovered from 2 batches of C. granaria immediately after the fumigation and from C. oryzae after about 1½ hours. We see from Table 9 that more hydrogen cyanide is recovered from the crushed insects in all the experiments, but there appears to be no regular increment in the amount recovered by crushing the insects.

In view of the above results it was decided to carry out all the experiments on the recovery of hydrogen cyanide from the fumigated insects by crushing the insects and passing the air for 30 minutes at room temperature, (cool aeration), and by distillation for a further period of 30 minutes.

There are two distinct processes involved in the recovery of the fumigant from the fumigated insects: (a) crushing and cool aeration for 30 minutes and (b) distillation for 30 minutes. It was now decided to find out in which of these two processes more hydrogen cyanide was recovered. 400 insects (200 of each species) were fumigated for 30 minutes at atmospheric pressure. Hydrogen cyanide was recovered from C. granaria immediately after fumigation and from C. oryzae after about 1½ hours. 100 C. granaria were put in each of the two distilling tubes and 2 ml. of water were added. The fumigant was

collected in the bubblers containing solution of sodium carbonate. One of the two batches of the fumigated insects was crushed and the air passed through both the batches of the insects for 30 minutes to carry away the fumigant which was trapped in $Na_2 CO_3$ solution. After 30 minutes the bubblers were removed and another set of the bubblers containing fresh $Na_2 CO_3$ solution was attached to the two distilling tubes, and the distillation carried out for a further 30 minutes.

The first set of the bubblers contained hydrogen cyanide recovered from the crushed or normal insects during the cool aeration and the second set of the bubblers contained the hydrogen cyanide recovered during the distillation. The results are given in Table 10 and Fig. 5.

TABLE 10.

Recovery of hydrogen cyanide from C.granaria and C.oryzae by the two separate processes. The insects were fumigated for 30 minutes at atmospheric pressure.

Conc. used Mg./L.	HCN recovered - Mg/gm. body weight from											
	C.granaria						C.oryzae					
	Whole			Crushed			Whole			Crushed		
	Aeration	Distillation	Total	Aeration	Distillation	Total	Aeration	Distillation	Total	Aeration	Distillation	Total
48.7	.039	.140	.179	.138	.081	.219	Nil	.134	.134	.098	.109	.207
84.7	.067	.34	.407	.319	.152	.471	.022	.226	.248	.178	.126	.304

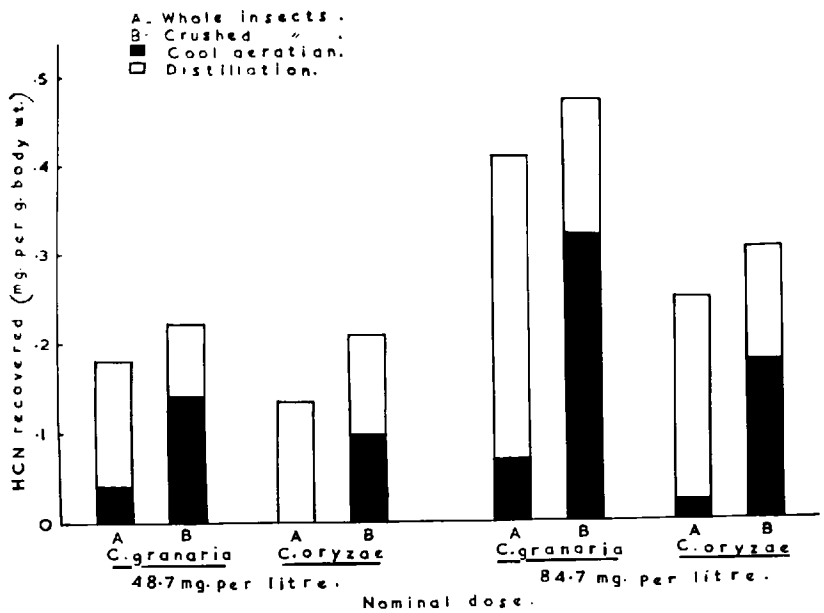


Fig.5 - Recovery of hydrogen cyanide during cool aeration and distillation from whole and crushed insects.

It is interesting to note that more than 50% of the total hydrogen cyanide recovery is obtained during the crushing and cool aeration, while very small amounts of hydrogen cyanide are obtained during the cool aeration when the insects are whole. It may be that the sorbed fumigant is readily available when the insects are crushed and is constantly carried away by the current of air, whereas when the insects are treated whole, the free fumigant has to diffuse out from the insects' bodies, which is a very slow process. The amount of fumigant recovered by distillation may consist of some free hydrogen cyanide plus the fumigant that has combined in the insects' bodies and has probably formed cyanohydrin-like compounds which liberate hydrogen cyanide on distillation. Thus the hydrogen cyanide recovered separately by these two processes may give a rough idea of the proportion of free and of hydrogan cyanide combined but recoverable.

At this stage, it is worth mentioning that the previous workers (Lindgren and Sinclair 1944, Lindgren 1949, and Prandhan and Bhatia 1951) recovered the hydrogen cyanide from the whole insects by distillation for 20 minutes, and it may be that their results might have been affected if they had also crushed the insects. These results will be discussed in detail later on.

Crushing the insects shows what an important part the technique plays in the recovery of the hydrogen cyanide from the fumigated insects. It appears that by crushing the insects, most internal tissues are exposed and easily give up the hydrogen cyanide at the high temperature of the boiling water.

PRELIMINARY EXPERIMENTS

A few experiments were carried out to find the dosage range suitable at the various pressures that were to be tried. For these preliminary experiments the two extreme pressures were chosen, i.e. a pressure of 2 cm. of mercury and atmospheric pressure. Three concentrations of hydrogen cyanide were used and the fumigation period was 60 minutes in all the experiments. Only *C. Granaria* were tried, 200 insects being fumigated each time, 100 in each cage, to serve as duplicates. Hydrogen dyanide was recovered from the fumigated insects, as previously described, the results being given in Table 11 and Fig. 6.

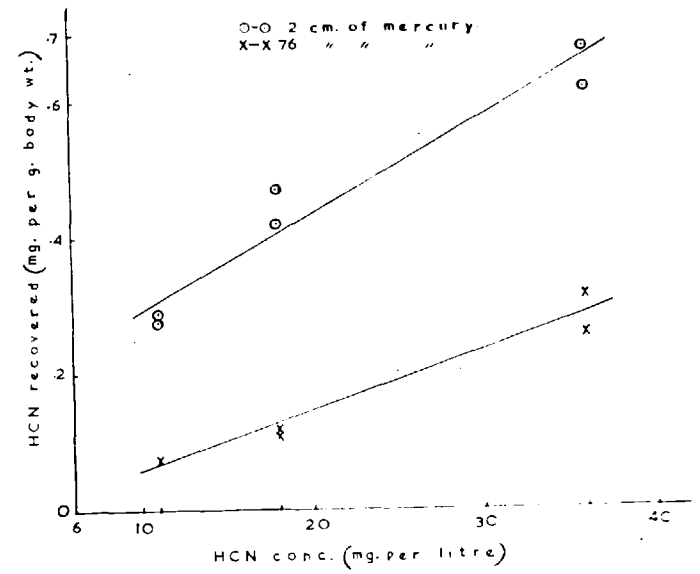


Fig.6 - Recovery of hydrogen cyanide from the insects fumigated at 2 and 76 cm. pressures. Duplicates plotted.

TABLE 11.

Recovery of Hydrogen cyanide from C. granaria, fumigated under 2 cm. and 76 cm. pressure, for 60 minutes at 25°C.

Conc. of HCN mg. per litre	Press. in cm. of mercury	HCN recovered. Mg/g.body wt. Replicate I	Replicate II
11 x	2	.288	.274
	76	.075	.074
18 x	2	.470	.419
	76	.117	.114
36 x	2	.618	.679
	76	.315	.353

x ± .6 mg. per litre.

It will seen that C. granaria sorbed more hydrogen cyanide when fumigated at pressure of 2 cm. of mercury than at atmospheric pressure, other conditions being the same. A glance at Fig. 6 shows that the provisional regression line at 2 cm. pressure is steeper than the one at atmospheric pressure, because of the greater effect of the increase in concentration from 11 to 36 mg. per litre at 2 cm. pressure.

From these results it was concluded that the pressure at which the insects were fumigated played an important part in the sorption of HCN and that the high amount of

hydrogen cyanide sorbed was largely responsible for the high mortalities obtained by fumigating under reduced pressure.

More extensive work was therefore carried out on the sorption of hydrogen cyanide and the mortalities in parallel experiments were also determined so as to find the influence of various reduced pressures on both species of Calandra, when fumigated with hydrogen cyanide.

EXPERIMENTAL DESIGN.

A search in the literature showed that most of the work on fumigation under reduced pressures had been carried out by varying one factor at a time, e.g. by applying a series of doses at a particular reduced pressure and observing the mortality. All the results obtained by other workers showed that less fumigant was needed to kill the insects at a reduced pressure than at atmospheric pressure. In view of these results it was decided to determine the amount of hydrogen cyanide sorbed by the insects when fumigated at various reduced pressures and also at atmospheric pressure, and at the same time to determine the mortality in duplicate batches of treated insects.

To investigate in details the influence of the various pressures, an experiment was designed with 4 factors. The pressure effect was investigated at 4 levels, using 3 concentrations each for 3 exposure periods and with two species of insects. The four pressures selected were 2 cm., 27 cm., 52 cm. of mercury and atmospheric pressure (75 - 76 cms.). The 3 concentrations were 12, 26 and 44 mg. per litre and the three exposure periods 30, 60 and 90 minutes. The responses of C. granaria and C. oryzae were determined.

RESULTS OF THE EXPERIMENT.

The results of the experiment are given in Table 12. Percent mortalities were converted into angular transforms and the results were subjected to an analysis of variance, which is given in Tables 13 and 14. In all the figures, mean of the response is plotted against the treatment. It was found that all the main factors were significant at the .1% probability level. The following general conclusions could be drawn from the results.

1. The hydrogen cyanide recovered and the mortality of the insects depend on the air pressure maintained in the chamber. With the decrease in pressure from atmospheric to 2 cm. of mercury recovery of hydrogen cyanide and the mortality of the insects increased.
2. More hydrogen cyanide was recovered from the fumigated insects and there was a higher mortality response, as the concentration of hydrogen cyanide in the chamber was increased from 12 to 44 mg. per litre.
3. Recovery of hydrogen cyanide as well as mortality of the insects increased with the increase in the exposure period from 30 to 90 minutes.
4. It was found that more hydrogen cyanide was recovered from the fumigated C. oryzae than from C. granaria, but the mortality response of C. granaria was higher than of C. oryzae.

5. The mortality in both species increased with the recovery of hydrogen cyanide.

All the factors are dealt with in detail, below. Each factor or an interaction was assessed by summing across all other treatments.

Pressure effect.

It was found that the hydrogen cyanide recovered and the mortality response of both the species decreased as the air pressure in the chamber increased from an absolute pressure of 2 cm. of mercury to atmospheric pressure. The results are given in Figs. 7 and 8.

TABLE 12.

Mortality and recovery of hydrogen cyanide from the insects, fumigated under various pressures, with different concentrations of hydrogen cyanide for different exposure periods.

C.granaria.

Conc. of HCN in mg. per litre	Exp. period in mins.	Pressure in cm. of Mercury											
		2			27			52			76		
		HCN recov. mg/gm body wt.	% mor-tal-ity	Angu. trans. of % mort-ality	HCN recov. mg/gm body wt.	% mor-tal-ity	Angu. trans. of % mort-ality	HCN recov. mg/gm. body wt.	% mor-tal-ity	Angu. trans. of % mort-ality	HCN recov. mg/gm. body wt.	% mor-tal-ity	Angu. trans. of % mort-ality
12	30	.394	97	80.0	.142	35	36.3	.079	8	16.4	.067	2	8.1
	60	.305	100	90.0	.166	56	48.4	.098	5	12.9	.066	4	11.5
	90	.263	100	90.0	.210	64	53.1	.109	16	23.6	.093	3	10.0
26	30	.556	100	90.0	.348	97	80.0	.221	77	61.3	.163	55	47.9
	60	.689	100	90.0	.431	100	90.0	.249	97	80.0	.159	72	58.1
	90	.764	100	90.0	.443	100	90.0	.238	99	84.3	.206	95	77.1
44	30	.723	100	90.0	.372	99	84.3	.348	96	78.5	.253	78	62.0
	60	.888	100	90.0	.563	100	90.0	.452	100	90.0	.287	73	58.7
	90	.802	100	90.0	.625	100	90.0	.536	100	90.0	.336	100	90.0
C.oryzae													
12	30	.484	100	90.0	.241	49	44.4	.102	1	5.7	.067	1	5.7
	60	.448	99	84.3	.262	61	51.4	.113	2	8.1	.068	2	8.1
	90	.485	100	90.0	.230	48	43.9	.141	2	8.1	.093	3	10.0
26	30	.602	100	90.0	.403	74	59.3	.273	40	39.2	.142	24	29.3
	60	.836	100	90.0	.488	94	75.8	.242	17	24.4	.256	75	60.0
	90	.781	100	90.0	.496	100	90.0	.261	86	68.0	.256	89	70.6
44	30	.850	100	90.0	.449	89	70.6	.243	53	46.7	.258	54	47.3
	60	.898	100	90.0	.634	100	90.0	.349	97	80.0	.316	51	45.6
	90	.949	100	90.0	.704	100	90.0	.394	98	81.0	.414	100	90.0

TABLE 13

Analysis of Variance of the mortality response of C. granaria and C. oryzae.

Items	Degrees of Freedom D.F.	Sum of Squares	Variance	Variance Ratio F.
Pressures P	3	23079.05	7693.02	121.0 xxx
Concentrations C	2	22750.11	11375.05	179.0 xxx
Exp.period E	2	2506.46	1253.23	19.72 xxx
Species S	1	1050.34	1050.34	16.53 xxx
C x P	6	7684.71	1280.78	20.15 xxx
C x E	4	533.75	133.44	2.1 n.s
C x S	2	307.58	153.79	2.42 ns
P x S	3	968.06	322.69	5.08 xx
P x E	6	1047.30	174.55	2.75 x
E x S	2	81.80	40.90	< 1 ns
P x C x E	12	1289.42	107.45)	n. s.
P x C x S	6	199.05	33.18)	n.s.
P x E x S	6	126.16	21.03)	n.s.
C x E x S	4	296.85	74.21)	N.S.
P x C x E x S	12	631.1	52.59)	
Error	40	2542.58	63.56	
Total	71	62551.74		

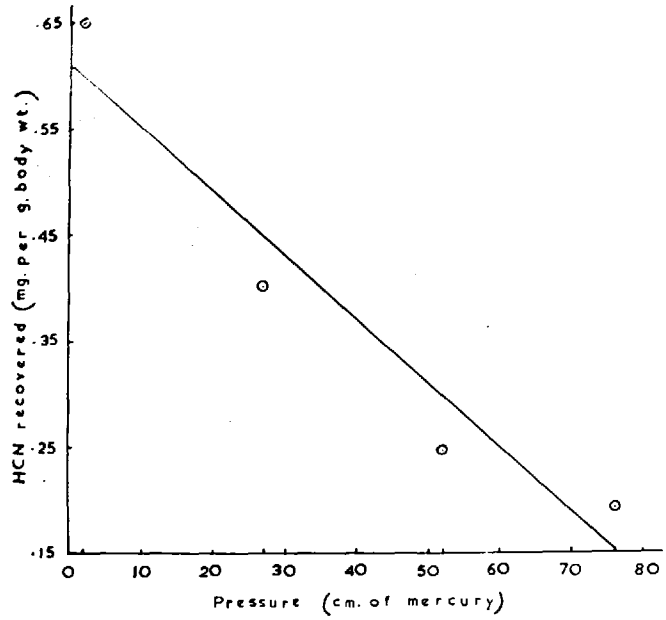
xxx significant at .1% probability level
xx " " 1% " "
x " " 5% " "
n.s. not significant.

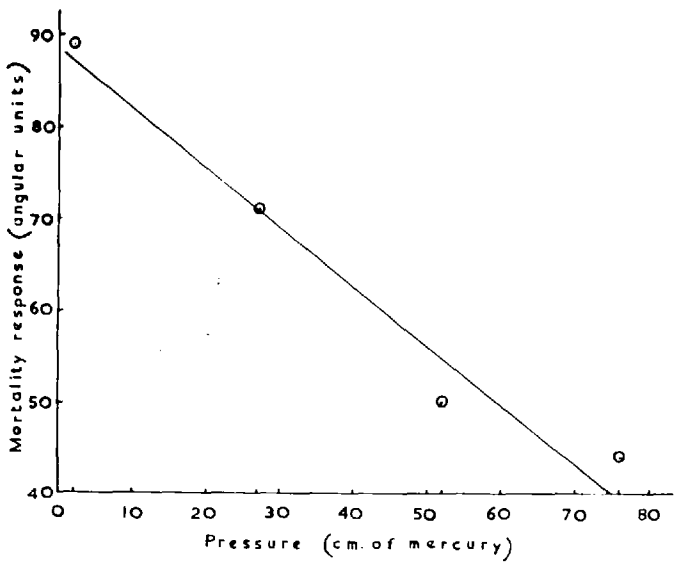
TABLE 14

Analysis of Variance of recovery response of C. granaris and C. oryzae.

Items	Degrees of Freedom D.F.	Sum of Squares	Variance	Variance Ratio F.
Concentrations C	2	1.3238	0.6619	648.29 xxx
Pressures P	3	2.26209	0.75403	738.4 xxx
Exp.periods E	2	0.09317	0.046585	45.62 xxx
Species S	1	0.03476	0.03476	34.04 xxx
C x P	6	0.100607	0.016768	16.43 xxx
C x E	4	0.052794	0.013198	12.93 xxx
C x S	2	0.004704	0.002352	2.30 ns.
P x E	6	0.012137	0.002023	1.98 ns
P x S	3	0.041191	0.01373	13.45 xxx
E x S	2	0.000334	0.000167	.016 ns
C x P x E	12	0.041215	0.003435	3.36 xx
C x P x S	6	0.022028	0.003671	3.595 x
P x E x S	6	0.004343	0.000724	ns.
C x E x S	4	0.003489	0.000872	ns.
C x P x E x S	12	0.014638	0.001220	
Error	22	0.0022470	0.001021	
Total	71	4.0113		

XXX significant at 0.1% probability level.
xx " " .1% " "
x " " 5% " "
n.s. not significant.





Figs.7 & 8 - Recovery of hydrogen cyanide and mortality response at various pressures.

They show that fumigation at 2 cm. pressure was the most effective and that, under the other conditions of the experiment, there was 100% mortality in both species. The mortality in both species was lowest when the insects were fumigated at atmospheric pressure (Fig. 8) One interesting fact that has emerged is that the insects have responded to different extents when the air pressure was decreased by 25 cm. of mercury. When the pressure decreased from 76 to 52 cm. the increase in the response was very small in comparison with that associated with the decrease in pressure from 52 to 27 and from 27 to 2 cm. of mercury. It is therefore not advisable to fumigate C. granaria and C. oryzae with hydrogen cyanide at a sustained pressure of 52 cm. of mercury as it produces practically the same results as does fumigation at atmospheric pressure.

Concentration effect.

The effect of the increase in the concentration of hydrogen cyanide in the chamber was to increase both mortality and sorption, as measured by recovery of hydrogen cyanide from the fumigated insects. The results are given in Table 15.

TABLE 15

Effect of concentration on the insects.

Concentration mg. per litre	Mean mortality response in angular units	Mean HCN recovered Mg/g body weight.
12	38.8	.197
26	71.9	.396
44	79.8	.527

Table 15 shows that the increase in mortality and in recovery of fumigant is greater at the lower range of concentrations and falls off at the higher ranges.

Effect of exposure period.

Equal increments of the period of exposure were found to produce a greater effect at short exposure periods, e.g. periods of 30 minutes, than at longer periods e.g. 90 minutes, although the increase in responses was continuous. The results are given in Table 16.

TABLE 16

Effect of exposure period on the insects.

Exposure period in minutes	Mean mortality response in angular units	Mean HCN recovered. Mg/g body weight.
30	56.4	.324
60	63.2	.386
90	70.8	.400

If the hydrogen cyanide recovered is taken as an index of the sorption by the insects, it is found that 1 g. insects sorbed hydrogen cyanide at the average rate of .011 mg./minute during first 30 minutes. The average rate decreased to about .002 mg./minute during 30 minutes, when the period was increased from 30 to 60 minutes. The average rate of sorption further decreased to less than .001 mg./minute, when the exposure period increased from 60 to 90 minutes. Mortality, expressed in angular units, steadily increased with the exposure period.

Species.

C. oryzae was found to absorb more fumigant than C. granaria but to be less susceptible. These results appear to show that the "internal resistance" of C. oryzae to the sorbed hydrogen cyanide is greater than of C. granaria.

Effect of pressures at various concentrations.

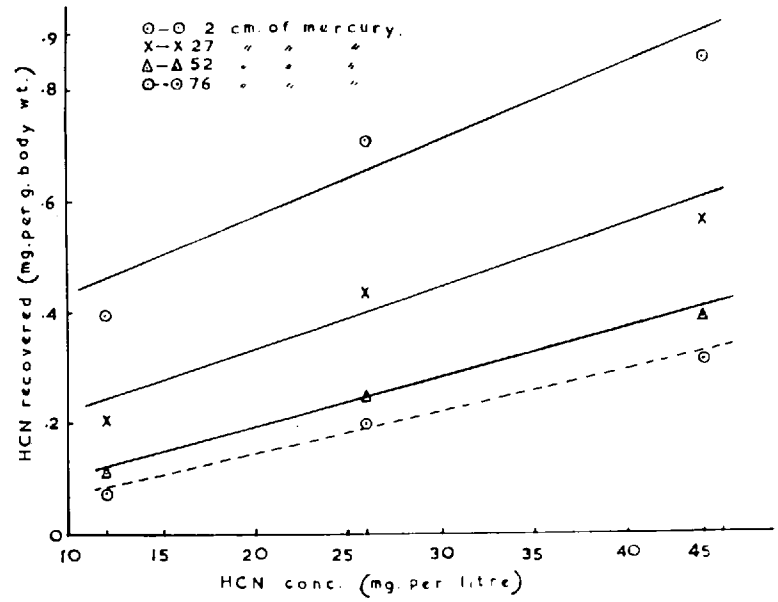
It was found that for both the responses this interaction was significant at .1% probability level. The results are given in Table 17 and Figs. 9 and 10.

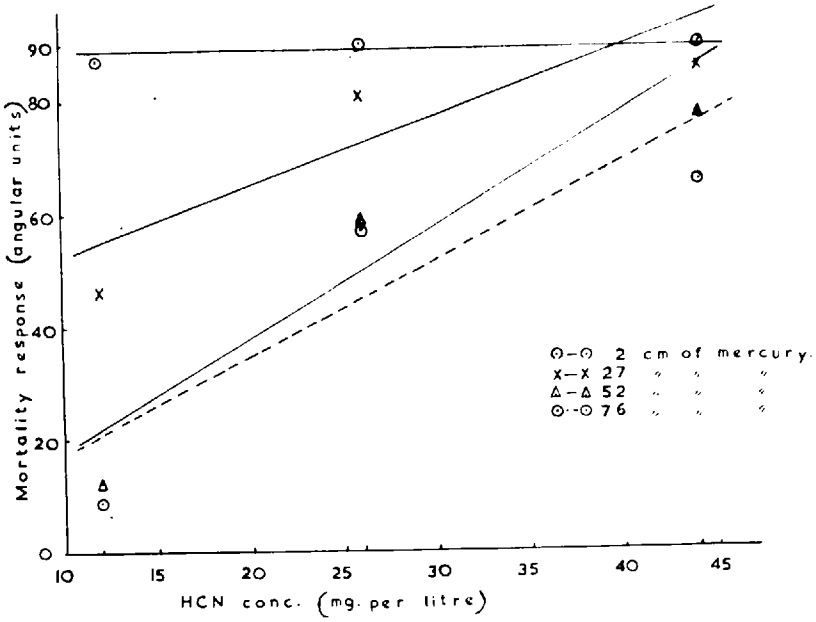
TABLE 17

Mean mortality response, in angular units, and hydrogen cyanide recovered from C. granaria and C. oryzae taken together at various concentrations and under different pressures.

Conc. in mg. per litre	Mortality response at various press.				HCN recovered at various pressures. Mg/g body weight.			
	2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
12	87.4	46.2	12.5	8.9	.397	.209	.107	.076
26	90.0	80.8	59.5	57.2	.703	.435	.247	.197
44	90.0	85.8	77.7	65.6	.851	.558	.387	.311

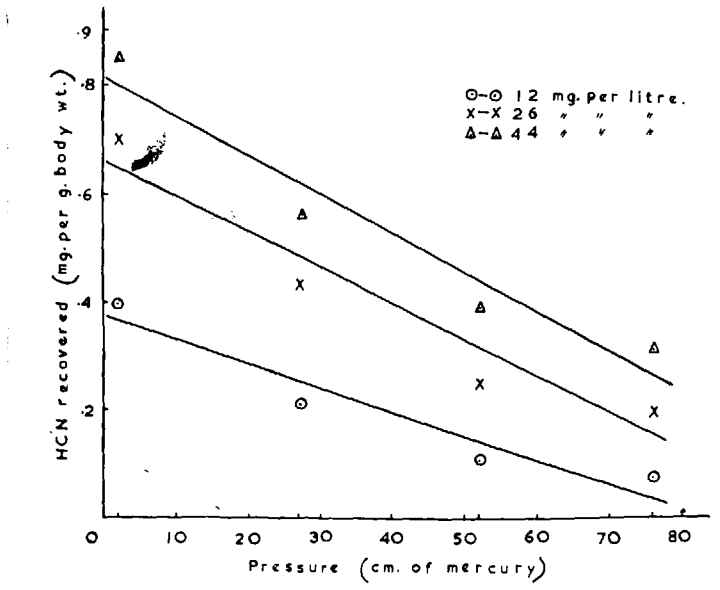
It was found that at any one pressure, both the mortality and the sorption of hydrogen cyanide of both species taken together increased as the concentration of hydrogen cyanide in the chamber increased, and that at one concentration, both responses decreased as the air pressure increased. We see from Table 17 and Figs. 9 and 10 that at a pressure of 2 cm. of mercury, a high mortality





Figs.9 & 10 - Pressure - Concentration Interactions.

occurred at all concentrations and that the amount of hydrogen cyanide recovered increased from 0.4 to 0.85 mg. per g. body weight, as the concentration increased from 12 to 44 mg. per litre. At other pressures the mortality response produced was greater when the concentration increased from 12 to 26 mg. per litre, i.e. by 14 mg., than with the increase in concentration from 26 to 44 mg. per litre, i.e. by 18 mg. At atmospheric pressure and 52 cm. pressure, the differences in HCN recovery were very small compared with the differences at other pressures, at all the concentrations tried. Fig. 9 shows that with the increase in concentration the "provisional regression lines" gradually became steeper as the pressure decreased from 76 to 2 cm. of mercury, when ~~mortality~~ recovery was the response, but the lines gradually flattened when ~~recovery~~ mortality was the response. At the pressures tried, the mortality regression line at a dose of 12 mg. per litre was steeper than the lines at other concentrations, but the recovery regression line became less steep with the same changes in concentration (Figs. 9A and 10A). It is concluded from these results that the fumigation of C. granaria and C. oryzae with hydrogen cyanide at 52 cm. pressure is not as efficient as at the other 2 reduced pressures, especially at 2 cm. pressure, where even at the lowest concentration tried,



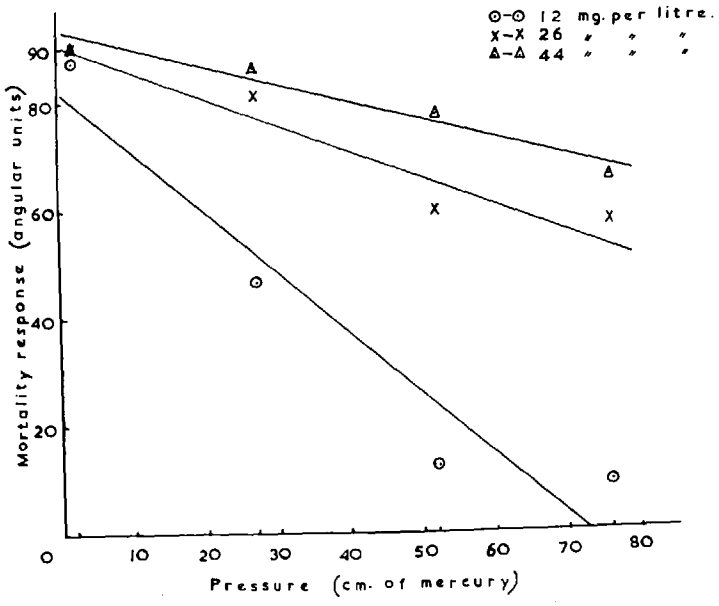


Fig. 9A & 10A. Pressure-Concentration Interaction.

12 mg. per litre, the mortality is very high.

Effect of pressures on C. granaria and C.oryzae.

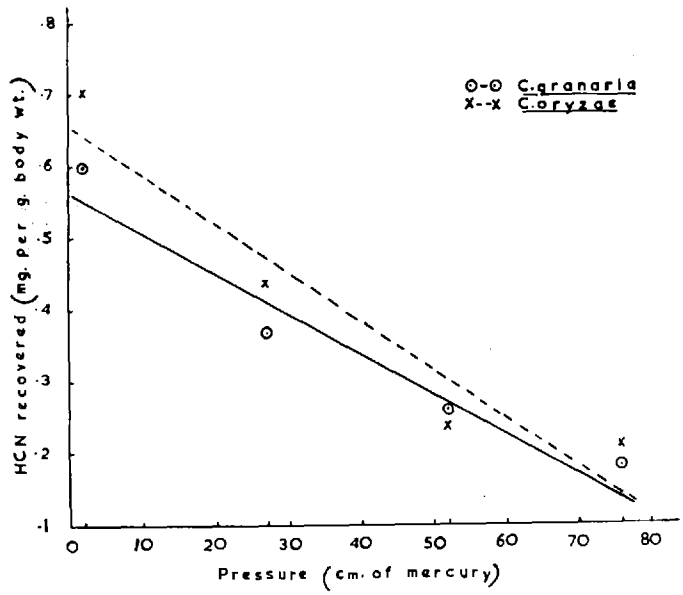
This interaction was found to be significant at the .1% probability level for the recovery response, and at 1% level for the mortality response. The results are given in Table 18, and Figs. 11 and 12.

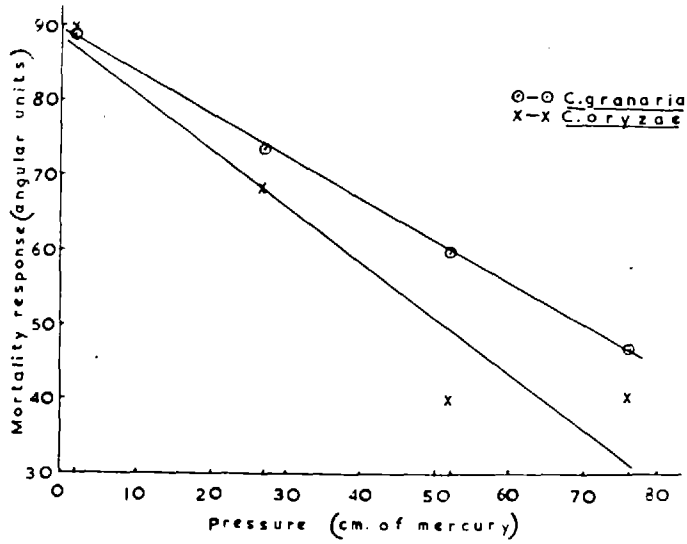
TABLE 18

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae at various pressures.

Species	Mortality response at various press.				HCN recovered at various pressures. Mg/g body wt.			
	2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
C.granaria.	88.9	73.6	59.7	47.0	.598	.367	.259	.181
C.oryzae.	89.4	68.4	40.1	40.7	.703	.434	.235	.208

The results show that both responses increase, with a decrease in pressure, and that C. oryzae is less susceptible but yields more fumigant in the recovery process than C. granaria. Both the species showed nearly 100% mortality at a pressure of 2 cm. but as the pressure increased the mortality of C. oryzae decreased faster than did that of C. granaria but more HCN was recovered from





Figs.11 & 12 - Pressure - Species Interactions.

C.oryzae than from C. granaria at the pressures tried. Both the species sorbed more hydrogen cyanide at 52 cm. pressure than at atmospheric pressure, but the difference was small compared with the differences found when the pressure was changed from 52 to 27 cm. and from 27 to 2 cm. of mercury. (Figs. 11 and 12).

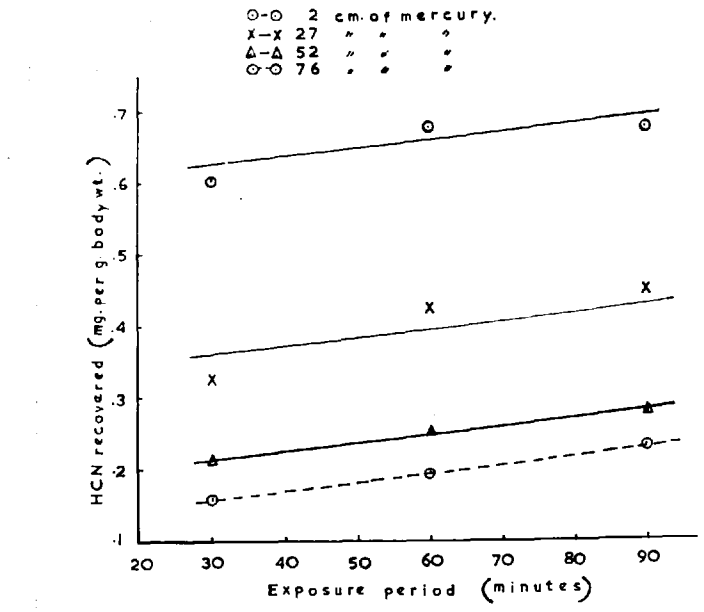
Effect of pressures at various periods.

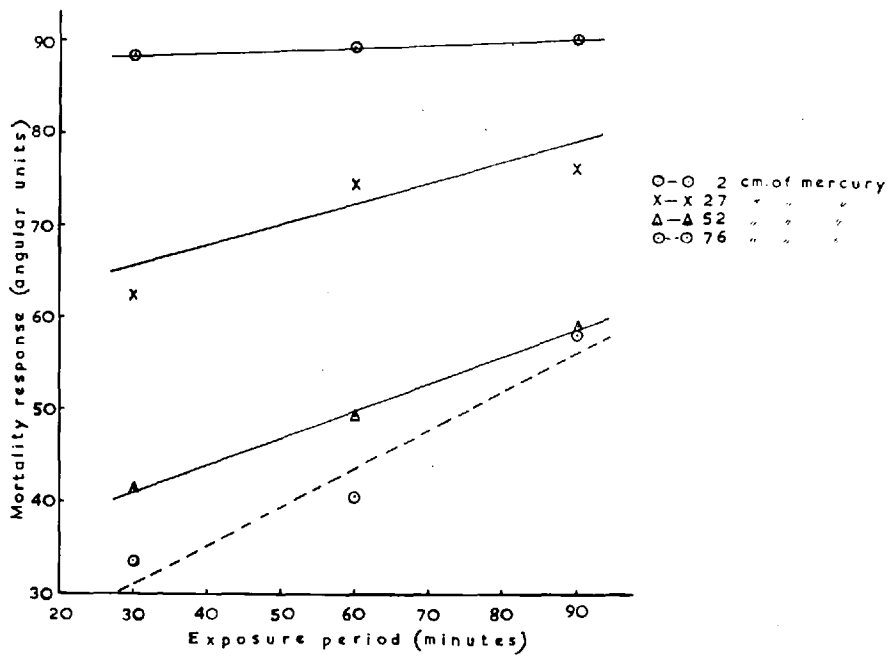
This interaction was significant at 5% probability level with the mortality response, but was not significant with the recovery response. The results are given in Table 19 and Figs. 13 and 14.

TABLE 19.

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae, taken together, for various pressures and exposure periods.

Exp.period in minutes	Mortality response at various press.				HCN recovered at various pressures. Mg/g body wt.			
	2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
30	88.3	62.5	41.3	33.4	.602	.326	.211	.158
60	89.0	74.3	49.2	40.3	.677	.424	.251	.192
90	90.0	76.1	59.1	57.9	.674	.451	.280	.233



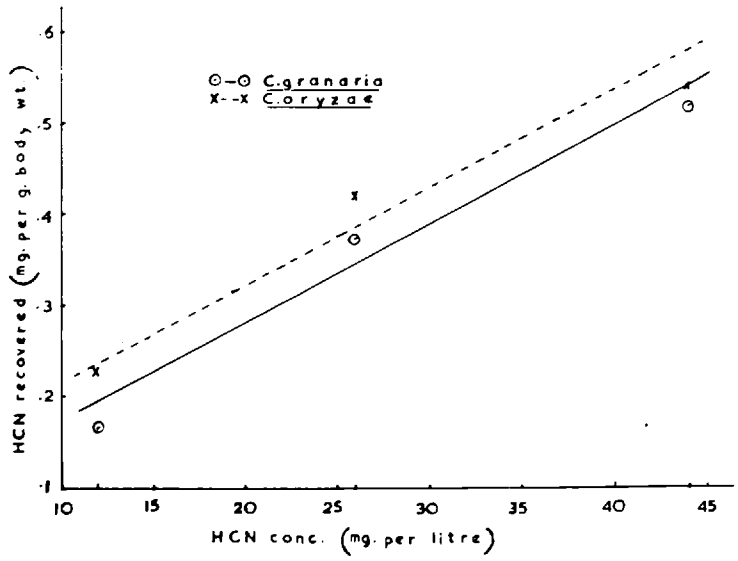


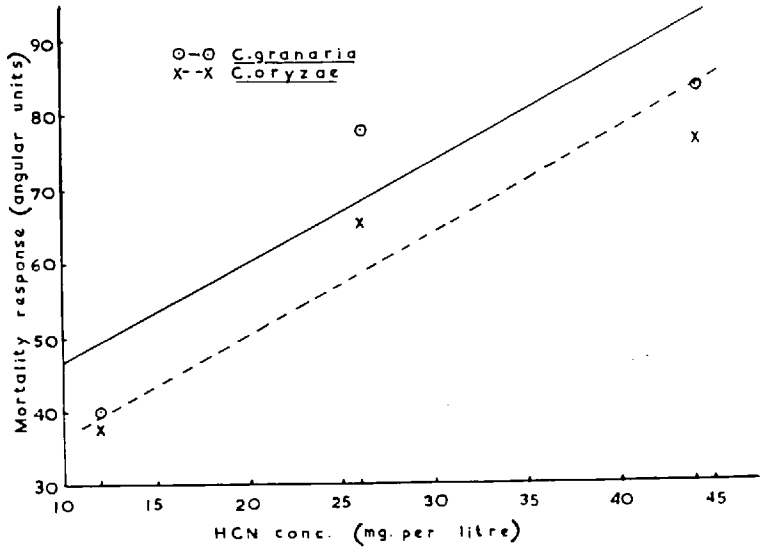
Figs. 13 & 14 - Pressure - Exp. period Interactions.

The mortality of both species and the hydrogen cyanide recovered, taken together, show that the response in both cases increased (1) with the increase in the exposure period from 30 to 90 minutes and (2) with the decrease in the air pressure from 76 to 2 cm. of mercury. A glance at Fig. 13 shows that with the decrease in pressure from 76 to 52 cm., the amount of HCN recovered increased, but to a lesser extent than with the decrease in air pressure from 52 to 27 and from 27 to 2 cm. pressure. Fig. 14 shows that at the periods tried, the provisional regression lines showing the mortality response become more flattened as the air pressure in the chamber decreases. There is nearly 100% mortality at an absolute pressure of 2 cm. of mercury, at all the three periods, whereas at atmospheric pressure, the mortality increases from 33.4 to 57.9 A.U. (30 to 72%) when the period increases from 30 to 90 minutes.

Effect of concentrations on C. granaria and C. oryzae.

This interaction was not significant for either of the responses. The results are given in Table 20 and Figs. 15 and 16.





Figs.15 & 16 - Concentration - Species Interactions.

TABLE 20

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae at various concentrations.

Species	Mortality response at various pressures			HCN recovered at various pressures, Mg/g body wt.		
	12mg/l	26mg/l	44mg/l	12mg/l	26mg/l	44mg/l
C.granaria	40.0	78.2	83.6	.166	.372	.515
C.oryzae	37.5	65.5	75.9	.228	.420	.538

The results show that both the species sorbed more hydrogen cyanide and gave higher mortality response as the concentration of hydrogen cyanide in the chamber is increased from 12 to 44 mg. per litre. It was found that less fumigant was recovered from C. granaria than from C. oryzae, but that the mortality of C. granaria was greater than that of C. oryzae. C. granaria offered less resistance to the sorbed hydrogen cyanide in their bodies than did C. oryzae. All the results point to the fact that the insects offer not only the resistance to the entry of the hydrogen cyanide into their bodies but also to the hydrogen cyanide sorbed. This hypothesis of surface and internal resistance of the insects to hydrogen cyanide was first put forward by Pradhan and Bhatia (1951) for other insects. The results will be discussed in detail

later on.

Effect of the exposure periods on C. granaria and C. oryzae.

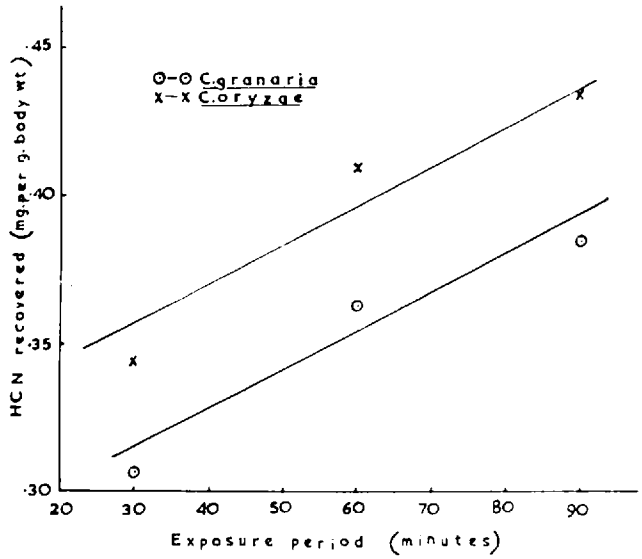
This interaction was not significant for either of the responses. The results are given in Table 21 and Figs. 17 and 18.

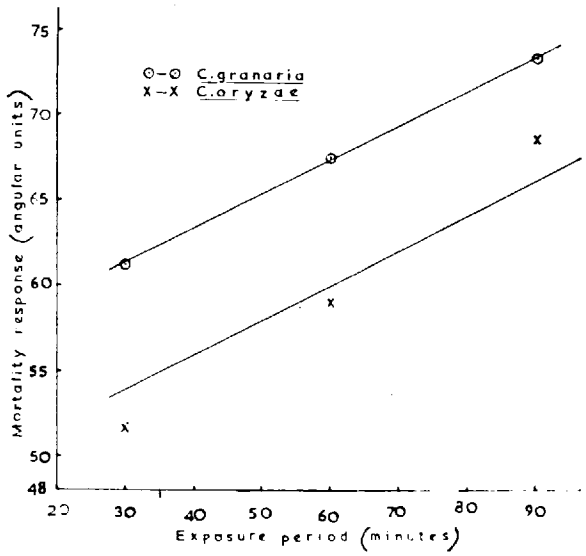
TABLE 21.

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae at various periods.

Species	Mortality response at various periods.			HCN recovered at various periods, Mg/G.body wt.		
	30 min.	60 min.	90 min.	30min.	60min	90min
C.granaria	61.2	67.5	73.2	.306	.363	.385
C.oryzae	51.5	59.0	68.5	.343	.409	.434

It was found that the mortality as well as the hydrogen cyanide recovered increased with the period of fumigation in both species. As mentioned previously also, less hydrogen cyanide was recovered from C. granaria than from C. oryzae, but the mortality of C. granaria was higher than that of C. oryzae. The rate of sorption of hydrogen cyanide in both species fell as the period of fumigation lengthened from 30 to 90 minutes. The rate





Figs.17 & 18 – Exp. period – Species Interactions.

of increase of mortality in both species also fell as exposure period was increased.

Effect of concentrations at various periods of fumigation.

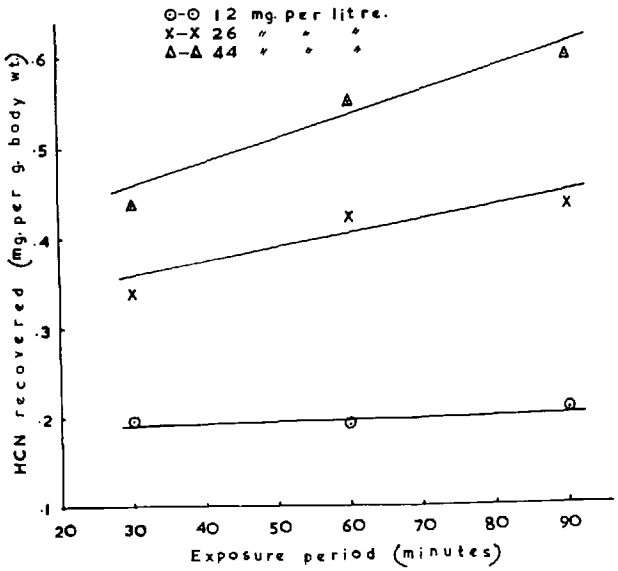
This interaction was found to be significant at .1% probability level for the recovery response, but was not significant for the mortality response. The results are given in Table 22 and Figs. 19 and 20.

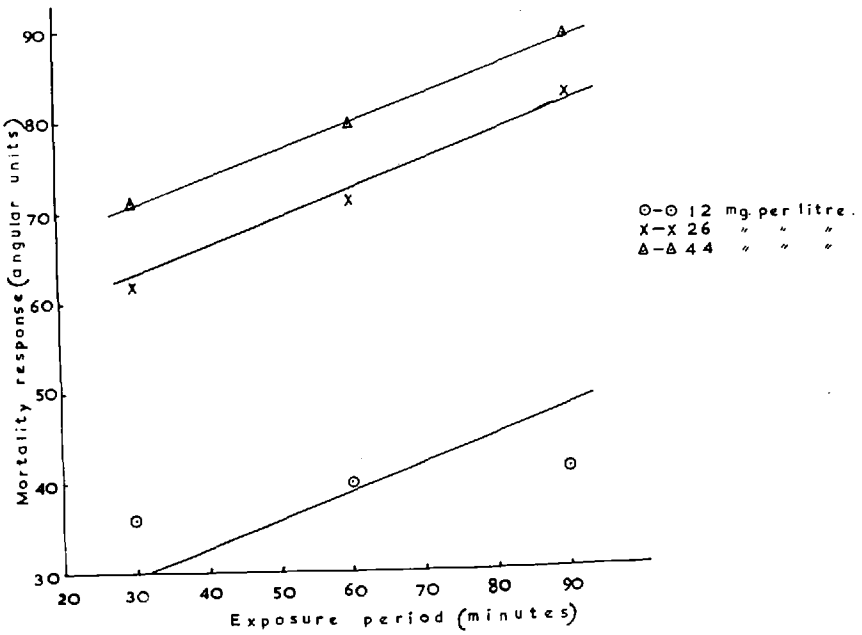
TABLE 22

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae taken together at various concentrations and periods.

Exposure period in minutes	Mortality response at various concentrations.			HCN recovered at various concentrations. Mg/g. body weight.		
	12mg/l	26mg/l	44mg/l	12mg/l	26mg/l	44mg/l
30	35.8	62.1	71.2	.197	.339	.437
60	39.3	71.0	79.3	.191	.419	.548
90	41.1	82.5	88.9	.203	.431	.595

It is interesting to note that at a dose of 12 mg. per litre the hydrogen cyanide recovered from both species when taken together, showed practically no difference, as the period increased from 30 to 90 minutes, and the mortality also showed little increase, but at the two other





Figs.19 & 20 - Concentration - Exp. period Interactions.

concentrations, the increase in the period exercised some influence and the provisional regression lines became steeper as the concentration increased from 12 to 44 mg. per litre. This change of slope appeared to be caused by the influence of the 90 minute exposure period at doses of 26 and 44 mg. per litre, whereas the 30 minute period produced less effect at the above concentrations (Fig. 20). When mortality response was taken into consideration, it was found that an increase in concentration of fumigant from 12 to 26 mg. per litre, i.e. by 14 mg., was associated with a larger increase in mortality than a concentration change from 26 to 44 mg. per litre, i.e. by 18 mg. (Fig. 19).

Triple interactions.

All the triple interactions were not significant for the mortality response, but for the recovery response the concentration pressure period interaction was significant at .1% probability level, and concentration-pressure-species at 5% level. The results are given in Tables 23 - 26.

The concentration-pressure-period interaction showed that the concentration and pressure changes modified the effect of the period on the insects. Concentration-pressure-species was also significant and this showed that concentration and pressure changes affected the two

species of Calandra to a different extent.

TABLE 23

Mean mortality response in angular units and HCN recovered from C. granaria and C. oryzae taken together at various concentrations and pressures for different periods.

Conc. in mg/l	Exp. period in min.	Mortality response at various press.				HCN recovered at various pressures. Mg/g.body wt.			
		2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
12	30	85.0	40.4	11.0	6.9	.439	.191	.090	.067
	60	87.1	49.9	10.5	9.8	.376	.214	.105	.067
	90	90.0	48.5	15.8	10.0	.374	.220	.125	.093
26	30	90.0	69.6	50.2	38.6	.579	.375	.247	.152
	60	90.0	82.9	52.2	59.0	.762	.459	.245	.207
	90	90.0	90.0	76.1	73.8	.772	.469	.249	.231
44	30	90.0	77.4	62.6	54.6	.786	.410	.295	.255
	60	90.0	90.0	85.0	52.1	.893	.598	.400	.301
	90	90.0	90.0	85.5	90.0	.874	.664	.465	.375

TABLE 24

Mean mortality response in angular units and hydrogen cyanide recovered from C. granaria and C. oryzae at various pressures and concentrations.

Conc. in mg/l.	Species	Mortality response at various press.				HCN recovered at various pressures. Mg/g body weight.			
		2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
12	C.granaria	86.7	45.9	17.6	9.9	.321	.173	.095	.075
	C.oryzae	88.1	46.6	7.3	7.9	.472	.244	.119	.076
26	C.granaria	90.0	86.7	75.2	61.0	.670	.407	.236	.176
	C.oryzae	90.0	75.0	43.9	53.3	.740	.462	.259	.218
44	C.granaria	90.0	88.1	86.2	70.2	.804	.520	.445	.292
	C.oryzae	90.0	83.5	69.2	61.0	.898	.596	.329	.329

TABLE 25

Mean mortality response in angular units and HCN recovered from C. granaria and C. oryzae when fumigated under various pressures for different periods.

Exp. period in min.	Species	Mortality response at various press.				HCN recovered at various pressures. Mg/g. body weight			
		2cm	27cm	52cm	76cm	2cm	27cm	52cm	76cm
30	C.granaria	86.7	66.9	52.1	39.3	.558	.287	.216	.161
	C.oryzae	90.0	58.1	30.5	27.4	.645	.364	.206	.156
60	C.granaria	90.0	76.1	61.0	42.8	.627	.387	.266	.171
	C.oryzae	88.1	72.4	37.5	37.9	.727	.461	.235	.213
90	C.granaria	90.0	77.7	66.0	59.0	.610	.426	.294	.212
	C.oryzae	90.0	74.6	52.4	56.9	.738	.477	.265	.254

TABLE 26.

Mean mortality response in angular units and HCN recovered from C.granaria and C.oryzae fumigated with various concentrations for different periods.

Exp. period in min.	Species	Mortality response at various concentr.			HCN recovered at various concentr. Mg/g. body weight.		
		12mg/l	26mg/l	44mg/l	12mg/l	26mg/l	44mg/l
30	C.granaria	35.2	69.8	78.7	.170	.322	.424
	C.oryzae	36.4	54.4	63.6	.223	.355	.450
60	C.granaria	40.7	79.5	82.2	.159	.382	.547
	C.oryzae	38.0	62.5	76.4	.223	.455	.549
90	C.granaria	44.2	85.3	90.0	.169	.413	.575
	C.oryzae	32.0	70.6	87.7	.237	.448	.615

DISCUSSION.

Fumigation under reduced pressures has been practised since 1918, but very little information of fundamental importance is available, as all the work reported has been carried out on the large scale and only one factor at a time has been studied, keeping other factors constant. Moore and Carpenter (1938) and Salmond (1951) who studied the effect of various air pressures on the insects when fumigated, found that higher mortalities were obtained at reduced pressures than at atmospheric pressure. The mortality obtained in the present series of experiments confirmed the findings of the above workers. Cotton, Wagner and Young (1937) found that oxygen deficiency in the chamber at the reduced pressures made the insects more susceptible to the effect of the fumigant. They fumigated batches of insects with the same dose of the ethylene oxide - carbon dioxide mixture under the same reduced pressure but with different oxygen contents (by introducing extra oxygen in one case) and found 100% mortality in one case when extra O_2 was not introduced and no mortality in the other case. They attributed the differences in the mortality in the two cases to differences in the oxygen content of the chamber. Wigglesworth (1935) found that

the respiratory rate of insects increased when the oxygen content decreased below 1%. (Their work was not with reduced pressures).

The results obtained by other workers are supported by the measurements of the water-loss and the hydrogen cyanide sorbed by C. granaria and C. oryzae in the present investigations. It was found that the insects lost considerably more water at the reduced pressures and sorbed more hydrogen cyanide than at atmospheric pressure. Severe desiccation caused by the loss of water and the higher amounts of hydrogen cyanide sorbed at the reduced pressures produced higher mortality responses than those produced by fumigation at atmospheric pressure.

From these results, one concludes that fumigation under reduced pressures is more effective than that at atmospheric pressure.

Moore and Carpenter (1938), Salmond (1951) showed that the mortality of insects, treated under various reduced pressures, increased as the air pressure in the chamber decreased from atmospheric pressure to 2 cm. of mercury. Young, Wagner and Cotton (1935) found that fumigation under a vacuum of 29" was more effective than under a vacuum of 26". Fish and Shepard (1938) found that the median lethal doses of methyl bromide for T.confusum decreased as the air pressure decreased from 76 to 2 cm. of

mercury. The mortality of the insects depends on the degree of vacuum maintained in the chamber. From the water-loss, hydrogen cyanide recovery and mortality results obtained in the experiments reported in this thesis, it is confirmed that the insects become more physiologically active as the pressure is decreased from 76 to 2 cm. of mercury and thus show a higher response.

It is clear from Figs. 3 and 4 (Part I) that the water-loss and the mortality at any one humidity depend on the air pressure in the chamber. As the air pressure is decreased from 8 to 2 cm. of mercury the insects become more active and lose more water, on account of oxygen deficiency in the chamber, and this desiccation again produces a higher mortality.

In the same way it is suggested that the higher amounts of hydrogen cyanide recovered from the insects fumigated at 2 cm. pressure compared with the amounts recovered from the insects fumigated at higher pressures is caused by the oxygen deficiency producing increased physiological activity. The mortality results, which largely depend on the hydrogen cyanide sorbed by the insects also increased with the decrease in the pressure (Figs. 7 and 8). It is therefore concluded from the results obtained that the physiological activity of the insects depends on the pressure maintained in the chamber and the mortality response

with which all the entomologists are concerned depends on the amount of hydrogen sorbed by the insects at the various reduced pressures. There are exceptions to most general conclusions, and so also to this one. Moore and Carpenter (1938) reported that C. oryzae fumigated at a pressure of 2 mm. of mercury sorbed less hydrogen cyanide and showed a smaller mortality response than at 2 - 6 cm. of mercury. They attributed this difference to the physical effect of such a high vacuum on C. oryzae bringing about a collapse of the tracheae of the insects. Their results have been confirmed by some experiments carried out on the water-loss from C. granaria and C. oryzae at 3 - 4 mm. pressure. It is clear from Table 2 (Part I) that the water lost was considerably less at 3 - 4 mm. than at 2 cm. pressure. For the results of this kind, there appears to be no other reason, but the collapse of the tracheae at such low pressures. It is physically impossible actually to demonstrate and prove such an hypothesis, but the results obtained support the above idea of physical effect on some insects at such low pressures.

The effect of increase in the concentration of hydrogen cyanide on the insects is to produce a higher mortality, at any one pressure (Figs. 9 and 9A). All workers report that higher mortalities are obtained with

increase in the concentration but most of them have tried only one pressure and have never compared the results obtained at various concentrations and pressures as has been done here. The interactions of the various factors will be discussed further, where they bear upon the estimation of recovery and mortality.

We see from Table 17, Figs. 9 and 9A, 10 and 10A that at 2 cm. pressure, there was a high mortality at all the concentrations tried, and the hydrogen cyanide recovered increased from about .4 to .85 mg. per g. body weight, whereas at atmospheric pressure the mortality increased from 8.9 to 65.6 A.U. (3 to 83%), and the recovery of hydrogen cyanide increased from .076 to .311 mg. (Table 17).

This gives some idea of the physiological activity of the insects at these 2 pressures. The amount of hydrogen cyanide sorbed at a dose of 12 mg. per litre at 2 cm. pressure appears to be sufficient to produce a very high mortality and further increase in the concentration brings about more sorption than is necessary to kill even the most resistant individual. Thus the results show that a dose of 12 mg. per litre of hydrogen cyanide is sufficient to produce 100% mortality of C. granaria and C. oryzae at 2 cm. pressure under the conditions tried, whereas as much as 44 mg. per litre are not sufficient to bring about 100% mortality in a fumigation at

atmospheric pressure. The recovery results have been of great help in throwing light on the mortality results. There is very little increase in the mortality when the pressure is decreased from 76 to 52 cm. of mercury, i.e. by 25 cm.,and the low value of this increase is parallelled by an equally small increase in the recovery of hydrogen cyanide, and in the line with these observations we may say that the insects are physiologically slighily more active at a pressure of 52 cm. than at atmospheric pressure.

With a further decrease in pressure from 52 to 2 cm. of mercury, the insects sorb more fumigant and thus show a higher mortality response. At a dose of 12 mg. per litre the amount of hydrogen cyanide recovered from the fumigated insects increases from .076 to .397 mg. per g. body weight, as the pressure decreases from 76 to 2 cm. of mercury, but this difference further increases when the dose is increased to 26 and 44 mg. per litre. The change of mortality response with the pressure is shown in Figs. 9 and 9A.

The effect of the period of fumigation at the concentrations tried is shown in Table 22 and Figs. 19 and 20. At a dose of 12 mg. per litre increase in period from 30 to 90 minutes has practically no effect on the insects, but at the other 2 doses both the amount of hydrogen

cyanide recovered and the mortality increase with the period. At all the three concentrations the greatest effect is produced during first 30 minutes of fumigation and further increase in the period produces very little effect. All these mortality results are parallelled by the results of recovery of hydrogen cyanide.

At all the periods tried, the increase in dose from 12 to 26 mg. per litre, i.e. 14 mg. per litre, produces a greater increase in mortality than does the increase in dose from 26 to 44 mg. per litre, i.e. 18 mg. per litre. This behaviour is also parallelled by that of the results of the recovery of fumigant.

Figs. 13 and 14 show that in fumigations at 2 cm. pressure, there was nearly 100% mortality even with an exposure period of 30 minutes, whereas at other pressures the mortality increased with the period, the effect being more pronounced at atmospheric pressure. This showed that fumigation under 2 cm. pressure for 30 minutes was sufficient to bring about a high mortality, but with the increase in pressure to 76 cm. of mercury, the mortality decreased considerably and fumigation for the short period of 30 minutes produced very little effect. It is suggested that an increase in physiological activity of the insects as the pressure is reduced is mainly responsible for these changes in the behaviour.

If we consider fumigation for the long period, 90 minutes, we find that even at atmospheric pressure there is a considerable mortality and so the differences in mortality associated with different pressures are much reduced. Insects were found to sorb hydrogen cyanide at a greater rate during the first 30 minutes of fumigation than during subsequent similar periods, at all the pressures tried.

Moore and Carpenter (1938) found that as much as .728 mg. of hydrogen cyanide per g. body weight were sorbed by C. oryzae in 2 minutes, when fumigated with a dose of 49 mg. per litre at 20°C under a pressure of 6 cm. of mercury, and that .955 mg. were sorbed in 30 minutes. From their results, it appears that most of the sorption takes place within first 2 minutes and then as the period increases, the amount sorbed increases but slowly.

In the present series of experiments, 30 minutes was the shortest period tried and the results taken in conjunction with those of Moore and Carpenter show that at the start of fumigation, sorption is rapid and that it falls off more or less exponentially with period of exposure.

It is found that mortality as well as the recovery of hydrogen cyanide increased with the exposure period in both species. Figs. 17 and 18 show that less fumigant

was recovered from C. granaria than from C. oryzae but that C. granaria was more susceptible than C. oryzae. These results may be explained with the help of the hypothesis put forward by Pradhan and Bhatia (1951). They suggested that the total "effective resistance" of a particular insect to hydrogen cyanide is the resultant of "surface resistance" and "internal resistance". By surface resistance they mean the resistance which insects offer to the entry of hydrogen cyanide into their bodies, and by internal resistance they mean the resistance offered by the insects to the sorbed hydrogen cyanide in their bodies. According to Pradhan and Bhatia, Table 21 and Figs. 17 and 18 would be taken to show that C. granaria sorbed less hydrogen cyanide than C. oryzae, showing that the surface resistance of C. granaria was greater than that of C. oryzae, but that C. granaria suffered a greater mortality than did C. oryzae, even with less hydrogen cyanide sorbed and therefore possessed a much lower internal resistance.

Previous work reported by other workers is mainly concerned with the mortality response obtained at a particular dose, which according to Pradhan and Bhatia is the total effective resistance offered by the insects. Until recently no attempt was made to correlate mortality with the amount of fumigant sorbed and it is this

correlation which has given rise to the idea of dividing "total" resistance into "surface" and "internal" resistance. As the correlation of mortality with sorption of hydrogen cyanide was not the main object of the present investigation, no extensive work was carried out on these lines, and this point has been discussed here only because the results appeared to follow the same trend as those reported by Pradhan and Bhatia.

Table 18 and Fig. 12 show that fumigation at a pressure of 2 cm. produced greater difference in the recovery of the fumigant from the two species than at other pressures. It may be assumed that all the insects become more physiologically active as the pressure is reduced, but to a different extent, with the result that the responses of different insects at various reduced pressures will depend on the extent to which they have been stimulated or depressed by the pressure changes.

SUMMARY

Literature on fumigation under reduced pressures with hydrogen cyanide and on the alkaline picrate method of estimating small amounts of hydrogen cyanide is reviewed.

It was found that more hydrogen cyanide was recovered by crushing the fumigated insects before distillation. More than 50 per cent of the total fumigant was recovered by crushing and cool aeration only, thus indicating approximately the free hydrogen cyanide available, while less than 50 per cent was recovered by distillation, which may consist largely of the hydrogen cyanide combined in the insects' bodies, but recoverable on distillation.

The results of recovery of hydrogen cyanide and those of mortality were subjected to an analysis of variance, and it was found that:-

(1) Pressure had a pronounced effect on the sorption of hydrogen cyanide by the insects, and on mortality. Fumigation under a pressure of 2 cm. was found to be the most effective, followed by fumigation at a pressure of 27 cm. Under the conditions tried, atmospheric pressure fumigation was found to be the most unsatisfactory, and that there was very little improvement produced by reducing the pressure

under which fumigation took place from 76 to 52 cm.

(2) With increase in concentration, the hydrogen cyanide sorbed as well as the mortality in both species increased. A dose of 12 mg. per litre was found to be sufficient to produce a very high mortality at 2 cm. pressure, whereas, a dose as high as 44 mg. per litre did not give a close approach to complete mortality at atmospheric pressure. There was a greater increase both in the recovery of hydrogen cyanide from the insects and in the mortality, when the concentration increased from 12 to 26 mg. per litre, i.e. by 14 mg., than when it increased from 26 to 44 mg. per litre, i.e. by 18 mg.

(3) The period for which the fumigation was carried out was found to produce its greatest effect during first 30 minutes of fumigation and the recovery of hydrogen cyanide from the insects as well as the mortality in both the species increased with the period. The rate of sorption of hydrogen cyanide was higher during the first 30 minutes, and as the period increased, the sorption rate decreased. A period of 30 minutes was found to be sufficient at a pressure of 2 cm. to produce a high mortality but at atmospheric pressure, even a period of 90 minutes did not produce a satisfactory kill of the insects.

(4) C.granaria sorbed less hydrogen cyanide than C.oryzae and both the species sorbed greater amounts of fumigant at reduced pressures than at atmospheric pressure. It appeared that a pressure of 2 cm. of mercury affected C.oryzae to a greater extent than C.granaria and the difference between the amount of fumigant recovered from batches of the two species is greater at a pressure of 2 cm. than at other pressures. There was 100 per cent mortality of both the species at 2 cm. pressure, but at other pressures, C.granaria showed a higher mortality than C.oryzae.

(5) Mortality in both the species was found to increase, as the insects sorbed more hydrogen cyanide.

RETENTION OF THE HYDROGEN CYANIDE

BY C.GRANARIA AND C.ORYZAE

RETENTION OF THE HYDROGEN CYANIDE BY C.GRANARIA AND C.ORYZAE.

When infested stored products, fresh or dried fruits or any other food is fumigated to kill the insect pests in it, attention is immediately drawn to the question, whether the material fumigated can be safely consumed by man or not. To answer this question, extensive work has been carried out to find how much and how long and in what form the fumigant sorbed is retained by the material.

Practically no information is available as to how the fumigants behave in the bodies of insects when they are sorbed.

Lindgren & Sinclair (1944) found that sorbed hydrogen cyanide completely dissipates from the citrus scale insects within 4 hours of the end of the fumigation. Preliminary experiments by the writer showed that C.granaria and C.oryzae did not behave like the citrus scale insects, when fumigated with hydrogen cyanide. From a few exploratory experiments it was found that C.granaria and C.oryzae were retaining some sorbed hydrogen cyanide in their bodies for several days and that this fumigant could be recovered in the usual way. Table 27 shows the amount of hydrogen cyanide recovered from C.oryzae up to 10 days after fumigation. (fig.21)

TABLE 27.

Recovery of hydrogen cyanide from the fumigated C.orysae at various times after the end of fumigation. Insects were fumigated at a pressure of 2 cm. of mercury for 90 minutes. Conc. used:- 8 mg. per litre (Insects were not "crushed"). Distillation period was 30 min.

Period between fumigation and recovery of fumigant.	Hydrogen Cyanide recovered from C.orysae mg. per g body weight.
0 day	.275
2 days	.216 x
6 "	.174 x
10 "	.157 x

x Hydrogen cyanide recovered, calculated on "dry" weight of the insects.

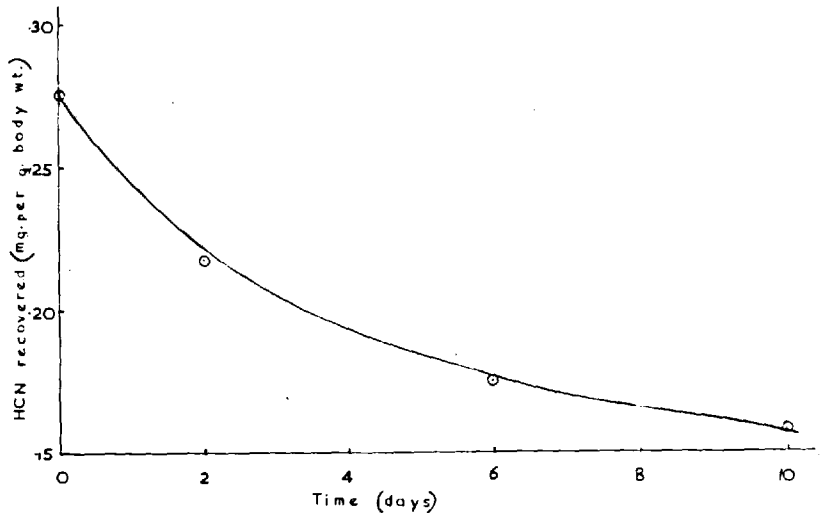


Fig. 21. Recovery of hydrogen cyanide from fumigated C.oryzae kept in still air for varying periods.

These reults are different from those obtained by Lindgren & Sinclair (1944) for the scale insects.

More extensive work was then undertaken to find how much hydrogen cyanide is retained by C.granaria and C.oryzae and for how long it is retained. All the insects were fumigated for 30 minutes at atmospheric pressure. 2,000 insects (1,000 of each species) were fumigated each time and 3 doses of hydrogen cyanide were tried. The insects were divided into batches each of which contained 100 individuals of one of the two species. One batch of each species was weighed before fumigation and the other batches of insects were weighed after fumigation. The hydrogen cyanide was distilled immediately after fumigation from the two batches of insects previously weighed. The procedure adopted was the same as in other experiments. The insects were "crushed" and 1-2 small drops of 10 N H_2SO_4 added to 3 ml. of water in the distilling tube as usual. The other batches of fumigated insects were separated out in groups of 100 each and were put into specimen tubes after weighing. No food was supplied to these fumigated insects. All the insects were kept in the constant temperature and humidity room, and the hydrogen cyanide was recovered from the fumigated insects at varying intervals of time. The results are given in the tables 28 and 29 and the figs.22 and 23.

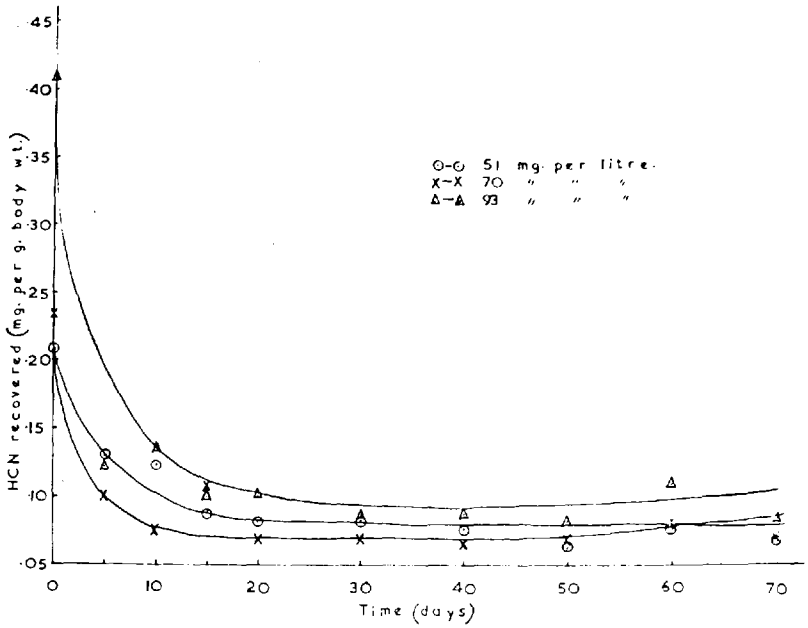


Fig. 22. Recovery of hyfrogen cyanide from fumigated <u>C.oryzae</u> kept in still air for varying periods.

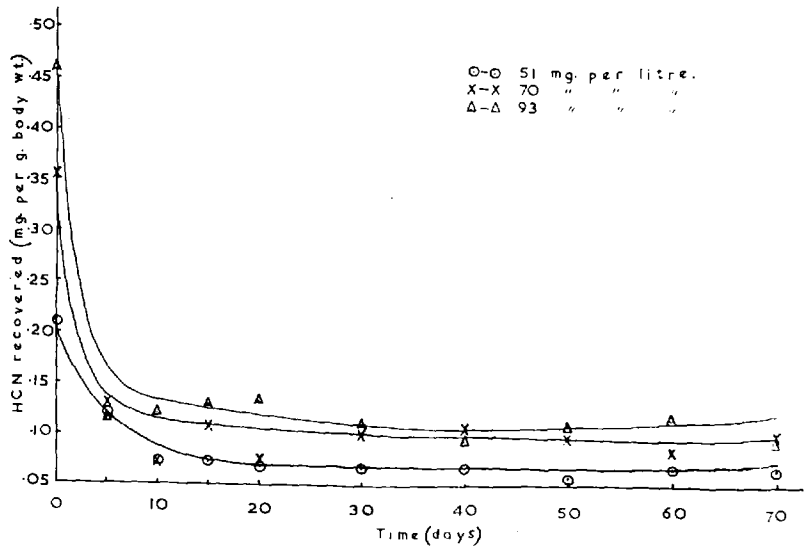


Fig. 23. Recovery of hydrogen cyanide from fumigated <u>C.granaria</u> kept in still air for varying periods.

It is interesting to note that C.granaria and C.oryzae retained as much as .07-.11 mg. of hydrogen cyanide per g. body weight and .07-.085 mg. per g. body weight respectively, for as long as 70 days after fumigation. The form in which the fumigant was retained was not determined. It may have been partly in the form of a cyanohydrin-like compound.

As expected, the rate of fall in the recovery of hydrogen cyanide was greatest immediately after fumigation and fell asymptotically to a lower level, the rate of fall being very small after 10 days. After fumigation with high doses, the recovery figures are more irregular. As the dose of hydrogen cyanide was increased, the amount sorbed and retained also increased. It was now certain that the hydrogen cyanide behaves in a different way in different insects e.g. sorbed hydrogen cyanide in citrus scale insects dissipates within 4 hours after fumigation (Lindgren & Sinclair (1944)) whereas in C.granaria and C.oryzae it is retained for 70 days after fumigation. The hydrogen cyanide recovered from the fumigated insects after 24 hours of fumigation was certainly less than the amount recovered immediately after fumigation. The question now arises as to what are the factors responsible for the fall in the recovery of hydrogen cyanide from the fumigated insects. Is it that hydrogen cyanide is slowly diffusing

TABLE 28.

Recovery of hydrogen cyanide from fumigated C. granaria on various days after fumigation. Atmos. press. 75-76 cm. Period of fumigation -30 min. Temp. 25° C.

Conc. in mg. per litre.	Wt. of the insects on 0 day.	Wt. of the insects on various days after fumigation.		HCN recovered Mg./Gm. body Wt. on various days after fumigation.	
	273.9 mg.	-	0 day	.212	0 day
	268.5 "	217.4	5 days	.127	5 days
	269.6 "	167.6	10 "	.082	10 "
	266.5 "	144.2	15 "	.079	15 "
51.5	278.7 "	147.8	20 "	.072	20 "
	270.4 "	146.3	30 "	.072	30 "
	265.4 "	142.5	40 "	.072	40 "
	266.7 "	144.2	50 "	.060	50 "
	266.9 "	144.3	60 "	.071	60 "
	265.9 "	150.1	70 "	.072	70 "
	254.5 "	-	0 day	.358	0 day
	267.8 "	225.9	5 days	.135	5 days
	254.2 "	145.7	10 "	.083	10 "
	265.1 "	145.2	15 "	.113	15 "
70.4	261.2 "	144.0	20 "	.080	20 "
	263.0 "	144.4	30 "	.102	30 "
	272.4 "	151.8	40 "	.110	40 "
	265.9 "	147.4	50 "	.098	50 "
	268.3 "	146.9	60 "	.090	60 "
	261.2 "	151.7	70 "	.103	70 "
	273.2 "	-	0 day	.461	0 day
	271.3 "	215.8	5 days	.125	5 days
	278.1 "	173.4	10 "	.129	10 "
	262.3 "	145.3	15 "	.137	15 "
93.0	265.2 "	155.7	20 "	.140	20 "
	272.8 "	151.0	30 "	.114	30 "
	269.7 "	148.6	40 "	.096	40 "
	258.4 "	141.9	50 "	.112	50 "
	270.1 "	152.2	60 "	.122	60 "
	272.9 "	158.6	70 "	.099	70 "

TABLE 29.

Recovery of hydrogen cyanide from fumigated C.oryzae on various days after fumigation. Atmos. press. 75-76 cm. Fumigation period - 30 min. Temp. 25° C.

Conc. in mg. per litre.	Wt. of the insects on 0 day	Wt. of the insects on various days after fumigation.		HCN recovered Mg./Gm. body Wt. on various days after fumigation.	
	265.1 mg.	-	0 day	.208	0 day
	260.6 "	179.3	5 days	.131	5 days
	301.7 "	158.7	10 "	.123	10 "
	264.2 "	132.4	15 "	.087	15 "
51.5	245.3 "	123.1	20 "	.081	20 "
	230.0 "	117.3	30 "	.083	30 "
	264.2 "	136.3	40 "	.076	40 "
	257.5 "	126.4	50 "	.052	50 "
	256.9 "	126.5	60 "	.078	60 "
	256.4 "	133.6	70 "	.070	70 "
	256.0 "	-	0 day	.234	0 day
	273.8 "	216.2	5 days	.102	5 days
	263.1 "	141.3	10 "	.078	10 "
	269.0 "	139.4	15 "	.108	15 "
70.4	256.6 "	131.1	20 "	.070	20 "
	262.0 "	133.9	30 "	.069	30 "
	268.2 "	136.0	40 "	.064	40 "
	259.0 "	134.1	50 "	.070	50 "
	262.2 "	134.1	60 "	.080	60 "
	258.2 "	136.6	70 "	.070	70 "
	263.6 "	-	0 day	.410	0 day
	259.5 "	174.1	5 days	.123	5 days
	265.0 "	143.1	10 "	.136	10 "
	258.0 "	136.2	15 "	.101	15 "
93.0	251.4 "	131.6	20 "	.103	20 "
	253.9 "	133.7	30 "	.087	30 "
	252.7 "	133.5	40 "	.087	40 "
	256.2 "	135.2	50 "	.082	50 "
	255.3 "	138.0	60 "	.11	60 "
	251.2 "	137.8	70 "	.084	70 "

away through the spiracles or other parts of the bodies of the insects after fumigation, or is it that, it is converted in some compound, which does not "liberate" hydrogen cyanide, even on distillation?

Some experiments were carried out to find the amount of hydrogen cyanide diffusing from the insects and the amount that could be recovered by distillation. After fumigation 600 insects (300 of each species) were fumigated, with hydrogen cyanide for 90 minutes. Only 2 pressures, 2 cm. of mercury and atmospheric pressure, were tried. Hydrogen cyanide was distilled from the previously weighed insects immediately after fumigation and the other fumigated insects, after weighing, were put into the outer cavity of a "Conway Unit", the inner cavity of which contained 1 ml. of 5% of a solution of sodium carbonate to which 2 ml. of water were added so as to fill the cavity up to the brim. 100 insects were put in each unit and this provided 2 replicates for each species. The unit was covered with a glass plate and kept in the constant temperature - humidity room. The solution of sodium carbonate absorbed all the hydrogen cyanide diffusing from the fumigated insects and the amount recovered by this method gave the amount of the hydrogen cyanide diffusing from the insects' bodies through the spiracles or through

some other parts. Each time, the solution was taken out in a test tube from the inner cavity of Conway Unit, and the cavity washed carefully 3 times and the wash water added to the solution. One ml. of 1 per cent picric acid was then added to the solution, which was then made up to 10 ml. The solution was heated for 3 minutes and the absorbed hydrogen cyanide determined as previously described. A fresh solution of sodium carbonate was again put in the inner cavity, which was filled to the brim as before and the experiment was continued. By this method it was possible to find out the amount of hydrogen cyanide diffusing from the insects' bodies during every 24 hours, for the first 3 days after fumigation.

TABLE 30.

Recovery of hydrogen cyanide from fumigated insects.
Exp. period 90 min. Temp. 25°C.

Expt. No.	Species.	Conc. in mg/l.	Pressure in cm. of Hg.	HCN recovered - mg./gm. body wt. by Distillation on 0 day (a)	Diffusion on 1st Day	2nd Day	3rd Day	6th Day	16th Day	Total	Distillation on 17th Day	Total Diffusion and Distillation. (b)	Conversion. a - b
(1)	C.gr.1	90.3	75.8	.799	.193	.046	.025	Nil	Nil	.264	.189	.453	.346
	C.gr.2				.203	.046	.025	.021	.Nil	.295	.164	.459	.340
	C.or.1				.163	.025	Nil	Nil	Nil	.188	.159	.347	.306
	C.or.2			.653	.203	.031	Nil	Nil	Nil	.234	.188	.422	.231
(2)	C.gr.1	118.6	76.7	.841	.217	.060	.022	.022	Nil	.321	.127	.448	.393
	C.gr.2.				-	.053	.023	Nil	Nil	-	.129	-	-
	C.or.1				.239	.045	Nil	Nil	Nil	.284	.176	.460	.315
	C.or.2			.775	.263	.048	Nil	Nil	Nil	.311	.171	.482	.293
									12th day		13th day		
(3)	C.gr.1	38.4	2	.609	.102	.026	.026	.026	Nil	.180	.182	.362	.247
	C.gr.2				.132	.026	.026	Nil	Nil	.184	.250	.434	.175
	C.or.1				.216	.045	.045	.049	Nil	.355	.212	.567	.319
	C.or.2			.886	.253	.045	.041	.082	.037	.458	.196	.654	.232
(4)	C.gr.1	51.6	2	.756	.142	.040	.029	.022	Nil	.233	.233	.466	.290
	C.gr.2				.149	.038	.026	.022	Nil	.235	.179	.414	.342
	C.or.1				.303	.066	.049	.074	.029	.521	.262	.783	.283
	C.or.2			1.066	.347	.066	.046	.077	.027	.563	.277	.840	.226

TABLE 30A.

Condensed data from Table 30. Mean of the two replicates is taken.

Expt. No.	Species.	HCN recovered by Mg./Gm. body Wt.			% HCN recovered by diffusion	% HCN recovered by distillation.	% HCN covert-ed.
		Distillation on 0 day	Diffusion	Distillation on 16th or 13th day			
1	*C.granaria*	.799	.279	.176	34.9	22.0	43.1
2		.841	.321	.128	38.2	15.2	46.6
3		.609	.182	.216	29.9	35.5	34.6
4		.756	.234	.206	31.0	27.3	41.7
1	*C.oryzae*	.653	.211	.173	32.3	26.5	41.2
2		.775	.297	.173	38.3	22.3	39.4
3		.886	.406	.204	45.8	23.0	31.2
4		1.066	.542	.269	50.9	25.2	23.9

The results are given in the Tables 30 and 30A.

As expected, more hydrogen cyanide was absorbed during the first period of 24 hours after fumigation than during subsequent periods. It is interesting to note that the traces of fumigant were recovered up to as long as the 12th day after fumigation, in one experiment, but in other experiments there was no recovery after the 6th day. 19-32 per cent of the amount sorbed diffused from the insects' bodies during first 24 hours after fumigation, and slowly this rate of diffusion decreased. When no more fumigant diffused in the Conway Units, some more hydrogen cyanide was recovered from the insects, which were by now considerably dessicated, by distillation in an air stream in the usual way. The amount thus recovered was presumed to be the proportion of hydrogen cyanide that combined in the insects' bodies and probably formed cyanohydrin-like compounds, from which it was possible to recover hydrogen cyanide by distillation, whereas the amount obtained by way of diffusion appears to be the free hydrogen cyanide which slowly diffused from the insects' bodies.

The total amount of hydrogen cyanide recovered by these two methods was still less than the amount sorbed, (as determined by cool aeration and distillation of another set of the insects immediately after fumigation) and this

difference was attributed to the formation of a compound in the insects' bodies, which did not liberate hydrogen cyanide even on distillation or in other words, the hydrogen cyanide which was irreversibly combined in the insects' bodies.

In these experiments, it is assumed that all the insects sorb the same or nearly the same amount of hydrogen cyanide. Thus it can be said that 30-50 per cent of the sorbed hydrogen cyanide diffuses away from the insects' bodies, and that 15-35 per cent is retained as a compound probably cyanohydrin, which liberates hydrogen cyanide on distillation and finally that 24-46 per cent is converted into some compound from which it is not possible to recover hydrogen cyanide by the methods available (See Table 30A).

This kind of research, though not directly of economic importance, is interesting in itself, and for its place in the fundamental investigation of the mechanism of fumigation. Many questions yet remain unanswered -

(1) What are the different compounds formed by the hydrogen cyanide in the insects' bodies?

(2) Why does the hydrogen cyanide sorbed by the insects react in a different way in different insects? e.g. In fumigated citrus scale insects, the hydrogen cyanide sorbed

dissipates within 4 hours from the end of the fumigation and thereafter no more can be recovered (Lindgren & Sinclair (1944)) whereas sorbed hydrogen cyanide is retained for as long as 70 days after fumigation of C.granaria and C.oryzae

(3) At what rate is the hydrogen cyanide diffusing away from the insects' bodies, and being converted into various compounds?

It is hoped that someone will take up the research on these lines and try to throw light on the above questions.

PART III. SORPTION AND RECOVERY OF HYDROGEN CYANIDE FROM THE FUMIGATED INSECTS.

CONTENTS

INTRODUCTION.

The recovery of hydrogen cyanide from the insects immediately after fumigation indicates indirectly the amount of hydrogen cyanide sorbed by the insects during the fumigation, but leaves one question unanswered, viz. is there any chemical conversion of hydrogen cyanide in the insects' bodies during the fumigation period? As has been shown in the previous part of this thesis by crushing the insects and then distilling the hydrogen cyanide from the crushed insects in a suspension of pH about 3.2, more hydrogen cyanide could be recovered than from a suspension of pH about 1.2. Unless the method of recovering the hydrogen cyanide from the fumigated insects is perfect, it is not fair to assume, that the fumigant recovered from the fumigated insects is the same as the amount sorbed by the insects. Pradhan and Bhatia (1951) found that the sorption of hydrogen cyanide by the insects was greater than the recovery, from the insects.

In view of the results obtained by the above mentioned workers, it was considered essential to endeavour to throw some light on this problem of sorption and recovery of hydrogen cyanide by using the modified and improved method

adopted in the present work. Some experiments were therefore carried out with C. granaria and C. oryzae in a small especially designed fumigation apparatus to find the difference, if any, between the sorption and recovery of hydrogen cyanide.

REVIEW OF THE LITERATURE.

Since practically no information is available on the sorption and recovery of hydrogen cyanide from the fumigated insects, a clear picture of the entry and behaviour of the gas in the insects' bodies cannot be formed. Moore and Carpenter (1938) determined the amount of hydrogen cyanide sorbed, but never tried to recover the hydrogen cyanide from the fumigated insects. Lindgren and Sinclair (1944,1945) recovered the hydrogen cyanide from the fumigated insects, but made no attempt to find the amount sorbed by the insects. Pradhan and Bhatia (1951) appear to be the only workers to attempt the determination of the sorption as well as recovery of the hydrogen cyanide from the fumigated insects. They found that the hydrogen cyanide sorbed was considerably more than the amount recovered, and they attributed the difference to the conversion of some hydrogen cyanide in the insects' bodies into some compound, from which it was not possible to recover hydrogen cyanide.

These are the few references available and the picture of sorption and recovery of hydrogen cyanide from the insects, as presented to us today, is still far from complete.

MATERIALS AND METHOD.

All the biological and chemical materials were the same as previously described. The hydrogen cyanide in all cases was determined by the alkaline picrate method.

Description of the fumigation apparatus.

The apparatus was especially designed for investigating the sorption of gaseous hydrogen cyanide by the insects. (fig.1.) It consists of a gas chamber A, insect chamber B, and a stopper C. The insect chamber is provided with a big hole D, about 1 cm. in diameter and a small hole E. This chamber fits in the barrel of the gas chamber and is closed with the stopper. The two chambers are connected to each other through the big hole, or the insect chamber is connected to the capillary tube F, through the small hole. In either case, the other side is disconnected from the insect chamber. The capacity of the gas chamber and insect chamber is 11.56 and 10.16 c.c. respectively. The method of fumigating the insects and determining the sorption is described below.

Method of fumigating the Insects.

The gas chamber is completely evacuated by attaching the apparatus to the vacuum pump, which is kept running for about

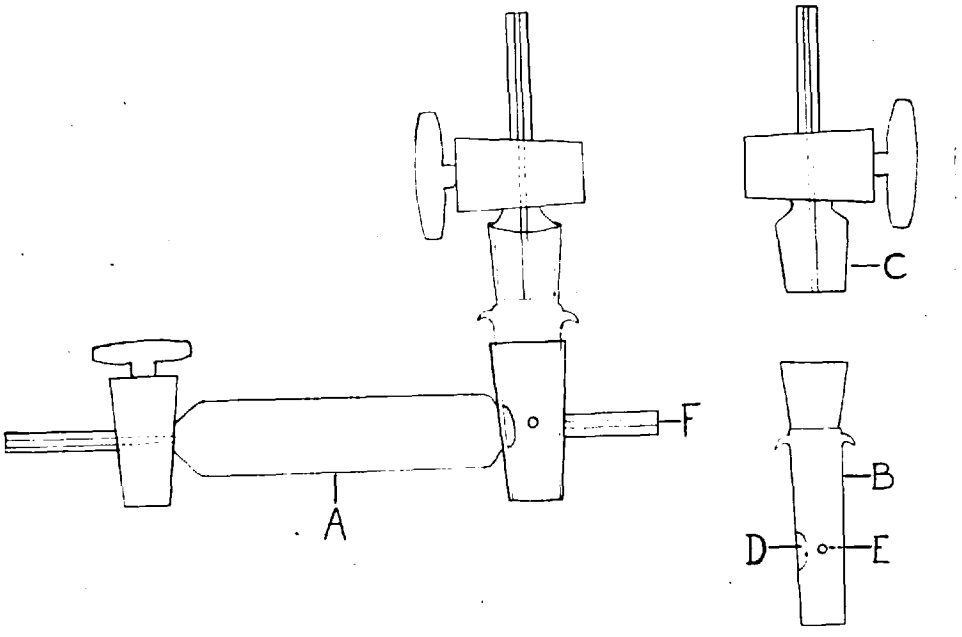


Fig.1 - Fumigation apparatus for determining sorption of hydrogen cyanide by the insects.

15 minutes. The gas sample is then taken into the gas chamber from the big reservoir, in which the desired concentration has been established. The first sample is taken 4-5 hours after an ampoule containing a weighed amount of hydrogen cyanide is broken in the big reservoir. The stop-cocks are kept open for about 3 minutes for the diffusion of the gas to occur. The insects are weighed before they are put in the insect chamber, which is then kept open to gas chamber for the particular period of fumigation. Within 1 minute after the insect chamber is opened to the gas chamber, all the insects become inactive and in that condition they are spread out in the insect chamber. The insects were fumigated at 25°C. It was found from the preliminary experiments that 5 to 10 minutes were needed for substantially complete diffusion of the gaseous hydrogen cyanide from one chamber to the other. (Table 1), and this period is of course very small compared with the fumigation periods employed.

Method of studying the sorption of hydrogen cyanide by the insects.

The insects were fumigated as described above. After the fumigation period is over, the gaseous hydrogen cyanide is first collected from the insect chamber, to which two

bubblers, containing sodium carbonate solution are attached by short pieces of rubber tube. The air is passed through the chamber to sweep away the hydrogen cyanide which is trapped in the bubblers. The rate of passage of air is slowly increased from about 10 c.c./minute to over 250 c.c./minute, and within 15-20 minutes, all the hydrogen cyanide is collected in the bubblers. The insects are then removed, care being taken first to wipe the grease from the neck of the chamber, so that the insects may not be contaminated there. The insect chamber is then again connected to the gas chamber, and air passed through the whole apparatus so as to sweep away the hydrogen cyanide from the gas chamber. The amount of hydrogen cyanide collected from the insect chamber, represents the gas remaining in the chamber plus the gas superficially held on the surface of the insects. The amount of hydrogen cyanide in the air space of the insect chamber is calculated from the concentration deduced from the amount obtained from the gas chamber. In general, the total amount of hydrogen cyanide obtained from both the chambers together was found to be less than the blank and the difference was the amount of hydrogen cyanide sorbed by the insects in their bodies during a particular fumigation period. Both the species

of Calandra were fumigated at atmospheric pressure for 1,4 and 12 hours, with doses of 4, 9.1 and 15.1 mg. per litre, and the amount sorbed was determined as above described.

Recovery of hydrogen cyanide from the fumigated insects.

The hydrogen cyanide was recovered from the fumigated insects exactly in the same way as previously described, except that the pH of the suspension, this time was about 3.2

It was thus possible to find the amount of hydrogen cyanide sorbed by the insects and also to recover the hydrogen cyanide from the same insects. This provided the complete picture of the sorption and recovery of the fumigant from C.granaria and C.oryzae when fumigated with hydrogen cyanide.

Reservoir. This was a pyrex glass flask of about 25 litres capacity, covered with a heavy metal plate. The plate was tin-plated on the inside so as to avoid loss of hydrogen cyanide on the metal. An ampoule containing a known amount of liquid hydrogen cyanide rested on a platform suspended from the plate. A wide glass tube of about 1 cm. internal

diameter with a stop-cock was fitted in the plate, for the evacuation of the flask and another capillary tube with a stop-cock was used for taking various samples into the gas chamber of the fumigation apparatus. The flask was evacuated to about 4 cm. of Hg. and an ampoule containing liquid hydrogen cyanide was broken with a solid glass rod which passed through the metal plate immediately above the ampoule platform. The flask was then brought to atmospheric pressure and shaken many times before any sample was taken. A tinned plate was suspended from the top of the flask and helped in mixing the gaseous hydrogen cyanide with the air in the reservoir when the flask was shaken. A desired concentration was thus established in the flask and various samples taken to fumigate the insects.

pH of the Solution during Distillation.

A few experiments were carried out to recover the hydrogen cyanide from the fumigated insects, using solutions of different pH. In the previous experiments, 1-2 small drops of 10 N sulphuric acid were added to 3 ml. of water and the pH of the solution, when determined on pH meter was found to be about 1.2 Some 10 N sulphuric acid was then diluted 200 times and 1-2 small drops of this dilute acid added to 3 ml. of water made the pH of the solution about 3.2 These two solutions of pH 1.2 and 3.2 respectively were first tried and the hydrogen cyanide recovered from two batches of the insects fumigated together in the same chamber and otherwise under other identical conditions. The results are given in Table31. It was found that more hydrogen cyanide was recovered when the solution of pH 3.2 was used. In these experiments also, as with the crushing of the insects, the increment was not regular, but in all the distillations carried out with both the species, more hydrogen cyanide was recovered as the pH of the solutions increased from 1.2 to 3.2. The results were promising and some 10 N sulphuric acid was this time diluted 40,000 times, and 1-2 small drops of this dilute acid, when added to 3 ml. of water, made the pH of the liquid about 4.7 This solution was then

tried. It was found that there was practically no difference in the recoveries of hydrogen cyanide from the fumigated insects when the solutions of pH 3.2 and 4.7 were used for the distillations. In all the experiments reported in this part of the thesis, the recovery of hydrogen cyanide from the fumigated insects was carried out with the solution of pH about 3.2

TABLE 3.I.

Recovery of HCN from the insects immediately after fumigation using solutions of different pH.

Pressure = 2-4 cm. of Hg.
Exposure period = 90 minutes. Temp. 25°C.

HCN Conc. mg. per litre.	Species tried.	pH of the solution.	HCN recovered mg/gm. body weight.	Increment.	Remark.
10.24	C.granaria	1.2 3.2	.378 .426	.048	
24.98	C.granaria	1.2 3.2	.691 .866	.175	
14.76	1) C.granaria	1.2 3.2	.393 .480	.087	
	2) C.granaria	1.2 3.2	.213 .270	.057	HCN recovered after about 20 hours.
4.89	1) C.granaria	1.2 3.2	.164 .182	.018	
	2) C.granaria	1.2 3.2	.128 .157	.029	HCN recovered after about 2 hours.
8.55	1) C.granaria	3.2 4.7	.324 .327	-	
	2) C.granaria	3.2 4.7	.291 .297	-	HCN recovered after about 2 hours.
22.0	1) C.oryzae	1.2 3.2	.639 .840	.210	
	2) C.oryzae	1.2 3.2	.572 .709	.137	HCN recovered after about 2 hours.
40.65	C.oryzae	1.2 3.2	.954 1.153	.199	

PRELIMINARY EXPERIMENTS

(1) Diffusion of gaseous hydrogen cyanide from the gas to the insect chamber and

(2) Sample Variation.

Diffusion of gas from one chamber to the other.

A few experiments were carried out to determine the time needed for the gas to diffuse from one chamber to the other so that there was no measurable difference of concentration. Various concentrations were established in the 5 litre flask and gas samples taken in the completely evacuated gas chamber of the fumigation apparatus. The insect chamber was then connected to the gas chamber for various periods and the gas was collected separately from both the chambers, as described before. The hydrogen cyanide collected in 2 sets of bubblers gave separately the amount present in each chamber. The hydrogen cyanide obtained from the insect chamber was the gas that diffused from the gas chamber in the particular period.

The results are given in Table 1.

TABLE 1.

Diffusion of gaseous hydrogen cyanide from one chamber to the other at various periods.

Sample No.	Diffusion Period in Minutes.	HCN obtained from gas chamber (in mg.)	HCN obtained from insect chamber (in mg.)	Theoretical amount of HCN in insect chamber (in mg.)
(1)	15	.052	.044	.046
(2)	15	.050	.043	.044
(1)	10	.082	.073	.072
(2)	10	.081	.073	.071
(1)	15	.101	.090	.089
(2)	x 16 hours	.102	.084	.090
(1)	5	.130	.106	.114
(2)	5	.128	.101	.113
(3)	10	.128	.107	.113

x To study the loss, due to grease and leakage.

One sees from the Table 1 that 5-10 minutes are sufficient for the complete diffusion of the gas. In the experiments reported in this part of the thesis, the minimum period used for the sorption of the hydrogen cyanide by the insects is 30 minutes, which is nearly 6 times the period needed for the complete diffusion of the gas from one chamber to the other.

Sample Variation.

Before the critical fumigations were carried out in the apparatus, it was decided to do some preliminary experiments on the sample variations. A desired concentration was established in the 25 litre flask and various gas samples taken in the gas chamber. The insect chamber was connected to the gas chamber for various periods, and the gas was collected from the whole apparatus and not from each chamber separately. The flask was shaken many times before any sample was taken after various periods. The results are given in Table 2.

The Table 2 shows that there was no great difference between any two samples and that there was no appreciable loss of hydrogen cyanide during the fumigation caused by

TABLE 2.

Sample Variation.

Sample No.	Period between ampoule breaking and sample taking in hours.	Insect chamber connected to gas chamber for various period in hours.	Hydrogen cyanide obtained from the apparatus.
1	2	3½	.057 mg.
2	7	¼	.055 "
3	9½	16	.054 "
4	27	2	.055 "
1	3	2	.125 "
2	6½	16	.121 "
3	25	¼	.120 "
4	30	8 days	.116 "
5	9 days	½	.106 "
6	" "	¼	.102 "

leakage or sorption on grease for as long as 8 days. When a particular concentration is established in the big reservoir, there is a noticeable loss of hydrogen cyanide in 9 days, which is probably caused mainly by leakage. It was thus found that this apparatus was quite satisfactory for studying the sorption of hydrogen cyanide by the insects with various concentrations and for the different periods.

Sorption of hydrogen cyanide by C.granaria.

In the small apparatus, when C.granaria were fumigated for 12 hours, it was found that the amount of hydrogen cyanide remaining in the apparatus after fumigation was nearly half the amount, initially taken. As it was decided to do some fumigations for 12 hours, to find the effect of longer periods on the sorption and chemical conversion of hydrogen cyanide in the C.granaria, it was essential to determine how quickly the concentration of hydrogen cyanide was falling in the apparatus, in other words, to find out the rate at which the hydrogen cyanide was sorbed by the C.granaria.

The insects were fumigated as previously described for various periods from ½ to 16 hours and 3 concentrations

of hydrogen cyanide 4, 9.1 and 15.1 mg./l. were tried. As soon as the exposure period had expired, the apparatus was connected to the bubblers containing the solution of sodium carbonate, by means of a short piece of rubber tubing. The air was passed through the apparatus as a whole, and after 20-25 minutes, the bubblers were disconnected and the hydrogen cyanide in them determined. Two blanks were carried out to find the amount of hydrogen cyanide in the fumigation apparatus, which provided the initial amount taken. The hydrogen cyanide collected in the bubblers after the fumigation of the insects for various periods, gave the amount remaining in the apparatus, which was still available to the insects. The difference between the amount of hydrogen cyanide in the blank, and the amount remaining in the apparatus after fumigation, gave the amount sorbed by the insects in the particular period. All the results were calculated as mg. of hydrogen cyanide sorbed per gram body weight of the insects before fumigation.

The results are given in Table 3 and figs.2 and 3.

TABLE 3.

Sorption of hydrogen cyanide by C.granaria.

Blank hydrogen cyanide in mg. (a)	Exposure Period in Hours.	Hydrogen cyanide in the apparatus after fumigation. (b)	Hydrogen cyanide sorbed by the C.granaria. (a-b)	Wt. of the Insects.	Hydrogen cyanide sorbed per mg./g. body wt.
	½	.078 mg.	.009 mg.	288.9 mg.	.031
.087	1	.076 "	.011 "	272.2 "	.040
equivalent	2	.078 "	.009 "	282.7 "	.032
to	4	.067 "	.020 "	279.1 "	.072
4 mg. per	8	.055 "	.032 "	279.2 "	.115
litre	12	.044 "	.043 "	280.9 "	.153
	16	.042 "	.045 "	275.7 "	.163
	½	.185 "	.012 "	275.1 "	.044
.197	1	.176 "	.021 "	273.3 "	.077
equivalent	2	.165 "	.032 "	281.1 "	.114
to	4	.136 "	.061 "	267.7 "	.228
9.1 mg. per	8	.141 "	.056 "	272.9 "	.205
litre	12	.126 "	.071 "	273.0 "	.260
	16	.107 "	.090 "	269.1 "	.334
	1	.304 "	.024 "	274.7 "	.087
.328	2	.297 "	.031 "	229.8 " *	.135
equivalent	4	.280 "	.048 "	238.8 " *	.201
to	8	.240 "	.088 "	270.1 "	.326
15.1 mg. per	12	.207 "	.121 "	272.1 "	.445
litre	16	.181 "	.147 "	271.5 "	.541

*less than 100 insects were taken.

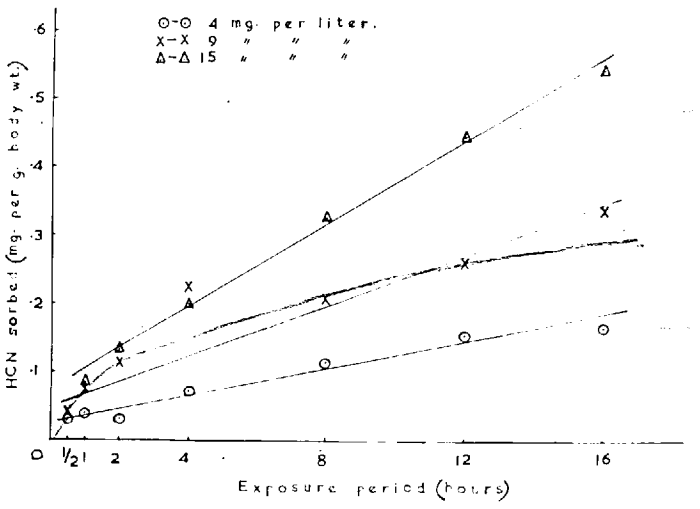


Fig.2 - Sorption of hydrogen cyanide by *C. granaria* while exposed to the fumigant for various periods.

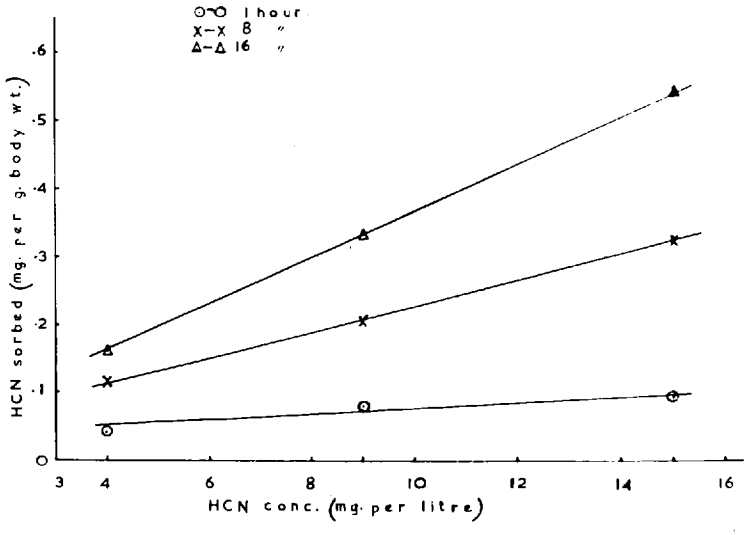


Fig.3 - Sorption of hydrogen cyanide by *C. granaria* at various concentrations.

We see from Table 3 and figs.2 and 3 that as the initial amount of hydrogen cyanide taken in the fumigation apparatus increases, the sorption of the fumigant by the insects also increases.

When adults of C.granaria are fumigated for 1 hour there is very little difference in the amount of hydrogen cyanide sorbed with the concentration changes, but the differences become more pronounced at longer exposure periods such as 12-16 hours. The average rate of sorption increases from .01 to .034 mg. per hour, when the weight of hydrogen cyanide taken in the apparatus increases from .087 to .328 mg. These results show that at atmospheric pressure, insects are continuously sorbing hydrogen cyanide right up to the end of a 16 hour period of fumigation, and that there is no sign of saturation of the insects' tissues with the hydrogen cyanide sorbed. The rate of sorption, as expected, is greatest at the beginning of the fumigation, and slowly decreases as the exposure period increases.

Sorption and recovery of hydrogen cyanide from the insects.

The insects were fumigated for various periods and the amounts of hydrogen cyanide sorbed and recovered, were determined as previously described. The results are given in Tables 4 and 5. The results show that there is practically

no difference in the amounts sorbed and recovered, when the fumigation periods are short, viz. 1-4 hours. When the fumigation period is longer, 12 hours, there appears to be some difference in the amount sorbed and recovered. The slight differences obtained with 1 and 4 hour fumigations cannot certainly be distinguished from errors of manipulation etc. such as those involved in taking various samples, or in the recovery of hydrogen cyanide from the fumigated insects. It is very difficult to say if such small differences are to be taken as the amount of hydrogen cyanide chemically converted.

C.oryzae was found to be more sorptive than C.granaria. It appears that in 12 hour fumigation, the differences in sorption and recovery of hydrogen cyanide were greater for C.oryzae than for C.granaria. The difference between the amount sorbed and recovered is attributed to the amount of hydrogen cyanide changed in the insects' bodies to some compound, from which it is not possible to recover hydrogen cyanide by the method adopted.

Pradhan and Bhatia (1951) fumigated the insects for 30 minutes only, and found that the hydrogen cyanide sorbed was greater than the amount recovered, but the results presented in this thesis (Tables 4 and 5) showed no considerable difference, hence some experiments were carried

out with the technique adopted by the above mentioned workers. They recovered the hydrogen cyanide from the whole insects and used one drop of 10 N H_2SO_4 in 1.5 ml. of water (pH = about 1.2). The distillation period in their case was 20 minutes. In the writer's experiments just reported the insects were crushed and the pH of the solution was about 3.2 The distillation period in both the above methods tried was kept the same, i.e. 30 minutes. The hydrogen cyanide was recovered and determined as described before. The results are given in Table 6.

The results show that substantially less hydrogen cyanide is recovered by the method adopted by Pradhan and Bhatia, and that the method as modified for this work did help in recovering higher amounts of hydrogen cyanide from the fumigated insects. It was thus possible to compare the recovery results by both the methods and to show that the so-called "conversion" during small fumigation periods may be ascribed to the imperfect technique employed for the recovery of hydrogen cyanide.

Table 5.

Sorption and recovery of hydrogen cyanide from C.oryzae, fumigated at atmospheric pressure (75-76 cm. of Mercury). Temp. 25°C.

Exposure period in hours	Conc. of HCN in mg. before fumigation Blank.	Conc. of HCN in mg. after fumigation. Gas Chamber (a)	Conc. of HCN in mg. after fumigation. Insect Chamber and Insects.	Conc. of HCN in mg. in insect chamber alone (b)	Total Amount recovered in mg. from both the chambers after fumigation (a + b) = c.	Amount sorbed by the insects in mg. Blank - c.	Amount recovered in mg. from insects. Aeration of insect chamber containing insects. (d)	Amount recovered in mg. from insects. Aeration and distillation. (e)	Total Amount recovered in mg. (d + e)	Weight of the insects in mg.	Amount sorbed by the insects mg./gm. body wt. (f)	Amount recovered from the insects mg./gm. body wt. (g)	Amount converted mg./gm. body wt. (f - g)
1	.175, equivalent to 8.1 mg. per litre	.084	.076	.074	.160	.015	Nil	.018	.018	283.3	.053	.063	-
4		.069	.062	.061	.131	.044	Nil	.037	.037	289.1	.152	.128	.024
12		.037	.034	.033	.071	.104	Nil	.070	.070	287.3	.362	.244	.118
1	.264, equivalent to 12.2 mg. per litre	.123	.113	.108	.231	.033	.005	.026	.031	284.7	.116	.109	.007
4		.105	.092	.092	.197	.067	Nil	.048	.048	255.4	.262	.188	.074
12		.067	.061	.059	.128	.136	Nil	.094	.094	287.8	.473	.327	.146
1	.334, equivalent to 15.4 mg. per litre	.159	.141	.140	.300	.034	Nil	.029	.029	298.1	.114	.097	.017
4		.135	.124	.119	.254	.080	.005	.071	.076	283.4	.282	.268	.014
12		.089	.080	.078	.169	.165	Nil	.136	.136	294.4	.561	.462	.099

TABLE 6.

Two Methods of recovering the Hydrogen Cyanide compared.

Treatment	Blank HCN in mg. (a)	HCN in mg. in the apparatus after fumigation (b)	HCN in mg. sorbed by the insects (a-b)	Exp. period in hours	Wt. of the insects in mg.	Sorption mg./gm. body wt.	HCN recovered in mg.	HCN recovered mg./gm. body wt.	Conversion mg./gm. body wt.
C.gr. (No crushing, PH = 1.2)	.264	.210	.054	4	262.0	.206	.028	.107	.099
C.gr. (Crushing, PH = 3.2x)		.222	.042		256.7	.164	.040	.156	.008
C.gr. (No crushing, PH = 1.2)	.264	.164	.100	12	258.1	.388	.058	.225	.163
C.gr. (Crushing, PH = 3.2x)		.162	.102		258.7	.394	.085	.329	.065
C.or. (No crushing, PH = 1.2)	.264	.199	.065	4	280.3	.232	.040	.143	.089
C.or. (Crushing, PH = 3.2x)		.197	.067		255.4	.262	.048	.188	.074
C.or. (No crushing, PH = 1.2)	.264	.120	.144	12	283.9	.507	.070	.247	.260
C.or. (Crushing, PH = 3.2x)		.128	.136		287.8	.473	.094	.327	.146
(No crushing, PH = 1.2)	.331	.291	.040	2	265.4	.151	.032	.121	.030
(Crushing, PH = 3.2)		.290	.041		259.5	.158	.042	.162	Nil
C.gr. (No crushing, PH = 1.2)	.389	.333	.056	2	300.7	.186	.034	.113	.073
(Crushing, PH = 3.2)		.344	.045		265.8	.169	.041	.154	.015
(No Crushing, PH = 1.2)	.389	.324	.065	4	279.9	.232	.052	.186	.046
(Crushing, PH = 3.2)		.330	.059		248.2	.238	.055	.222	.016
C.gr. (No crushing, PH = 1.2)	.389	.244	.145	12	266.7	.544	.100	.375	.169
(Crushing, PH = 3.2)		.238	.151		263.7	.573	.120	.455	.118

*These figures taken from Tables 4 and 5.

DISCUSSION.

Some experiments were carried out with C.granaria to find the hydrogen cyanide sorbed, and the results show that the insects are continuously sorbing hydrogen cyanide up to a period of 16 hours. (Table 3, figs. 2 and 3). As the period of fumigation increases from ½ hour to 16 hours, the sorption also increases, but to a different extent at different concentrations. The effect of exposure period is more pronounced at a dose of 15 mg./l. than at the other two concentrations, as can be seen from the slope of the straight lines. As the concentration increases from 4 to 15 mg./l. the lines become steeper and show that the increase in the amount of hydrogen cyanide sorbed during fumigations of from 1 to 4 hours is less than the increase during fumigations of from 4 to 16 hours. The concentration changes produce very little response during fumigation of 1-2 hours duration compared with the response produced in a fumigation lasting 12-16 hours. The difference in response is especially marked when comparison is made between the shortest and longest periods. The results agree, in general with those obtained previously (Table 12 pt.II).

This table shows that, with the increase in the dose from 12 to 44 mg. per litre the hydrogen cyanide recovered from C.granaria increased from .066 to .287 mg./gm. body

weight, when the fumigation period was 1 hour. It is concluded that concentration changes produce greater effects when the exposure period is from 8-16 hours, than at shorter periods, such as 1-2 hours, and that fumigation with hydrogen cyanide at atmospheric pressure for short exposure periods should therefore be avoided.

Peters (1936) states "small amounts of hydrogen cyanide cause, in the case of the granary weevil, a shock or stupefying effect that results in cessation of respiration. The hydrocyanic acid can then only enter the body by diffusion, for which high concentrations and long exposures are necessary", (therefore relatively greater resistance of C.granaria for hydrogen cyanide). Presumably the diffusion referred to is diffusion through the cuticle. Mackie and Carter (1937) concluded - "One factor not generally considered among those engaged in fumigation of grain is what may be called protective stupefaction, which occurs when concentration of a gaseous insecticide is not sufficiently strong to kill an insect immediately but knocks it out and causes a suspension of its normal breathing function, thus protecting it against the action of a fumigant. Lindgren (1938) reported that C.granaria was readily stupefied by a low concentration of hydrocyanic acid.

Neither of these workers have determined sorption, and it is difficult to give reasons for the mortality response that Lindgren obtained with the double treatment. He obtained a lower mortality of C.granaria, when first fumigated with small amount of hydrogen cyanide and then fumigated again with high concentration. These results he compared with another batch of the insects, which was fumigated with a similar dose, but did not undergo the pretreatment. The sorption results presented in this thesis show no indication of the so-called "protective stupefaction". The following facts summarise the evidence on which these statements are based.

At no stage from ½ to 16 hours has there been any sign of a sudden increase in the hydrogen cyanide sorbed. The results obtained with doses from 12 to 44 mg./l. (Table/2 pt.2) and with doses of from 4 to 15 mg./l. (Table 3), when compared for a fumigation period of 1 hour show in general a good agreement. In the apparatus used for the determination of sorption, the hydrogen cyanide taken was only 0.087 mg. (equivalent to a dose of 4 mg. per litre) and this hydrogen cyanide had to diffuse from the gas to the insect chamber. The insects therefore were subjected to very small amounts of hydrogen cyanide in the beginning of the fumigation, which can result in cessation of respiration and thus greatly reduce the rate of sorption

of gas. Such a phenomenon did not take place and the insects continued to sorb the hydrogen cyanide. In the previous experiments an ampoule containing a certain amount of liquid hydrogen cyanide was fractured and the insects were subjected to a high concentration from the beginning. Comparing both the methods of fumigation, it is found that the results do not support any such phenomenon as protective stupefaction. But at the same time, it is also certain that the changes in concentration of hydrogen cyanide produce little effect on the sorption by C.granaria, when fumigated for 1 hour, whereas when fumigated for 16 hours, the same insects with the same changes in concentration show considerably increased response. The results thus show that adults of C.granaria are able to offer resistance to the entry of hydrogen cyanide into their bodies, but it is difficult to believe that their respiratory system is not functioning. If we accept the idea that cessation of respiration is the mechanism in question, then this mechanism must operate for the whole fumigation period, which is as long as 16 hours, as at no stage is there any tendency to a sudden increase in sorption. The results obtained in experiments conducted with equal concentration-time products also show that the amounts of hydrogen cyanide sorbed during 12 hours of fumigation with doses of 3-9 mg. per litre

(Table 2 pt.IV) are also comparable to the sorption results obtained, when the insects are fumigated in the small apparatus. From all these results it is concluded that adults of C.granaria do not show anything like protective stupefaction, and that the amount of hydrogen cyanide sorbed steadily increases with the exposure period.

An attempt has been made to determine the amount of hydrogen cyanide sorbed and the amount that could be recovered from the fumigated insects. The results (Tables 4 and 5) show that at shorter fumigation periods, such as 1-4 hours, there is practically no difference between the sorption and recovery of hydrogen cyanide, but at longer periods such as 12 hours the amount recovered is less than the amount sorbed, showing that some of the sorbed hydrogen cyanide is probably changed chemically in the insects' bodies. Pradhan and Bhatia (1951) fumigated larvae of Corcyra cephalonica for 30 minutes and found that the amount sorbed was greater than the amount recovered and attributed the difference to the amount of hydrogen cyanide chemically changed into some irreversible compound. It has been shown that an imperfect technique for the recovery of hydrogen cyanide may well be the cause of this conclusion. Table 6 clearly shows that the same conclusions would have been drawn if the method of recovering the hydrogen cyanide from the

fumigated insects had not been modified. It is difficult to say, how far the results of Pradhan and Bhatia will be affected, if they were to crush the insects and use the solution of pH about 5.8, for distilling the hydrogen cyanide, but the results do emphasize the importance of "perfect technique" in this kind of work.

It will be interesting to find the chemical compounds formed, if any, and to determine their effect on the mortality of the insects, but from the present work, it is not possible to throw light on such problems.

SUMMARY.

The insects were fumigated in an especially designed fumigation apparatus and it was found that C.granaria continuously sorbed hydrogen cyanide up to 16 hours and showed no sign of the tissues becoming saturated. The results obtained show that there is nothing like protective stupefaction in C.granaria, as reported by other workers.

The amount of hydrogen cyanide recovered from C.granaria and C.oryzae was practically the same as the amount sorbed, when the insects were fumigated for 1-4 hours, but there was some difference if the insects were fumigated for 12 hours, when the amount of hydrogen cyanide sorbed was greater than the amount recovered. The difference is attributed to the conversion of some hydrogen cyanide irreversibly in the insects' bodies into some stable compound.

C.oryzae were found to sorb more hydrogen cyanide than C.granaria under the conditions tried.

It has been shown, how^an imperfect technique for the recovery of hydrogen cyanide from the fumigated insects can lead to wrong conclusions.

Part IV FUMIGATION UNDER REDUCED PRESSURES

WITH SAME CONCENTRATION-TIME PRODUCTS

CONTENTS

INTRODUCTION.

In large scale fumigation at atmospheric pressure, an exposure period of 24 hours or more is common practice, but shorter periods like 2-4 hours have usually been avoided, whereas in fumigation under reduced pressures, 4 hours exposure period is the highest ever tried. The tendency in recent years has been to save time and combine higher concentrations with shorter periods to obtain satisfactory control of the insect pests. The relation between exposure time and concentration of the fumigant to produce a constant mortality is expressed by a simple equation C x T = W (Haber's formula) where C = concentration, T = time and W = constant mortality. Some deviations from this formula have been reported at very low concentrations, also at very short exposure periods. (Salmond 1951 and Nahal 1952).

A search in the literature showed that no information is available on the sorption of the fumigant by the insects, when fumigated for various periods at any one concentration-time product. It was thought that this kind of study might help in finding the probable reason for the deviations reported, some experiments were therefore carried out in order to investigate the effect of the same

concentration-time product, built up in different ways, on Calandra species at various pressures, by determining both the sorption by the insects as measured by the recovery of hydrogen cyanide, and mortality.

REVIEW OF THE LITERATURE.

Haber first put forward a formula to show the relationship between concentration and exposure time to produce a constant mortality. C x T = W, where C = concentration, T = exposure time and W = a constant mortality. Two types of deviations, one at shorter periods and the other at lower concentrations have been reported, both resulting in increased value of W. (Knight (1925), Brinely & Baker (1927), Carpenter (1927), and Peters (1936)).

Peters (1936) regards the deviation with high concentration and short exposure period, as being brought about by saturation of the tissues of the organism, and proposes the following correction- $(C - Cs) \times t = W$, where Cs is the excess of concentration over the saturation of the tissues. (Busvine (1938) obtained some inconsistent results with C.granaria, which according to him, were probably associated with the experiments either at the ½ hour or the 20 hour exposure period. Salmond (1951), working with reduced pressures, obtained higher a mortality of C.granaria and of C.oryzae with higher concentrations of hydrogen cyanide combined with shorter exposure periods than at lower concentrations and longer exposure periods. She tried 12 minutes as the shortest period and 3 hours and 6 minutes as the longest period. Nahal (1952) found that with methyl bromide, the mortality of Calandra

granaria resulting from a given concentration-time product is less if this occurs at the centre of the sack than in the free space. He found that the curve for the free space always begins at a high level and falls, while that for the centre of the sack begins at a low level and then rises. He obtained widely different mortalities of Calandra species with hydrogen cyanide with which the difference in shape of the curves is more marked than with methyl bromide, independently of the concentration-time product, in the free space and in the centre of the sack. In all the methods of fumigation (American, French and Atmospheric), the insects in the centre of the sack are exposed to lower concentrations of hydrogen cyanide, especially at the beginning of the experiment, than the concentration in the free space. His work was with reduced pressure fumigation.

Except for these few references to the mortality of the insects at certain concentration-time products and some deviations reported from Haber's formula, there appears to be no other paper on the subject. Many hypotheses have been put forward, like protective stupefaction, neutralisation or excretion of the fumigant, and saturation of the tissues of the organism, to explain the deviations from Haber's formula, but surprisingly enough no one has attempted to study the sorption of the fumigant by the

insects, although the variation of sorption with dose would seem likely to have a direct bearing on Haber's rule and the deviations from it.

MATERIALS AND METHOD.

All the biological and chemical materials are the same as previously described. The hydrogen cyanide was recovered from the fumigated insects in the solution of pH about 3.2 at which most of the reversibly combined fumigant as well as the dissolved adsorbed fumigant was recovered, and was estimated by the alkaline picrate method. 300 insects (150 g. of each species) were fumigated each time. 100 insects were used for the distillation of hydrogen cyanide and 50 fumigated insects were kept for mortality determinations. At 2 and 37 cm. pressure, sometimes about 50 insects were used for recovery of hydrogen cyanide, as the amount of sorption was high and the determination became inaccurate (in that case only 200 insects were fumigated). All the experiments were carried out at 25°C in a constant temperature room.

Hydrogen cyanide concentrations.

Liquid hydrogen cyanide was used in all the experiments and ampoules containing known amounts of fumigant were fractured in glass chambers of 1075-1150 millilitre capacity to fumigate the insects. The concentrations of hydrogen cyanide needed for a fumigation of 12 hours were

very small, and the doses used were correct within ± .3 mg./l. for a 4 hour fumigation these were within ± .5 mg./l. and for 1 hour fumigation within ± 1 mg./l.

Mortality results in control set.

The insects kept as controls, were subjected to the same pressure treatment, but were not fumigated. At 2 cm. pressure, C.oryzae showed a high mortality, but the fumigation treatments produced complete mortality.

DESIGN OF THE EXPERIMENT.

From the preliminary experiments, it was found that the insects sorbed more hydrogen cyanide during 4 hours fumigation with a high concentration, than during 16 hours with a low concentration, at the same concentration-time (C-T) product. It was therefore decided to carry out some more extensive work on the sorption of hydrogen cyanide by Calandra species at various concentrations and exposure periods, all these combinations giving the same C-T products. Three concentration-time products were chosen, namely 36, 72 and 108 mg. hr. per litre, and 3 periods were tried, viz. 1, 4 and 12 hours. When the fumigation period was 1 hour the dose of hydrogen cyanide was 36 mg. per litre giving a C-T product of 36 mg. hr. per litre and with a 12 hour exposure period the dose was 3 mg. per litre in order to obtain the same C-T product. In this way all the combinations of concentrations and exposure periods were tried. Sorption as judged from the recovery of the hydrogen cyanide from the fumigated insects was the main response studied. Mortality results were also noted. Both the species of Calandra were fumigated, and three pressures namely, 2, 37 and 75-76 cm. of mercury were chosen.

PRELIMINARY EXPERIMENTS

Two experiments were carried out in the small fumigation apparatus in order to investigate the sorption of hydrogen cyanide by C.granaria during fumigation periods of 4 and 16 at the same concentration-time product. The appropriate concentration of hydrogen cyanide was established in the 5 litre flask and various samples taken in the gas chamber of the fumigation apparatus. The insects were fumigated and the hydrogen cyanide sorbed, determined as previously described.

The results are given in Table 1.

TABLE 1.

Sorption of hydrogen cyanide by C.granaria during fumigations of 4 hours and 16 hours at atmospheric pressure, at the same C-T product.

Exp.period in hours.	HCN conc. in the apparatus Blank (a)	HCN conc. remaining in the apparatus after fumigation. (b)	HCN sorbed by the insects. (a-b)	Wt. of the insects.	HCN sorbed mg./gm. body weight.
4	.395 mg.	.315 mg.	.080 mg.	240.9 mg.	.332
16	.100 "	.051 "	.049 "	251.8 mg.	.195

One sees from Table that more hydrogen cyanide is sorbed when a high concentration is combined with a short exposure period, than when a low concentration is combined with a long exposure period, to give a fixed concentration-time product. The results were fairly straight-forward, but this method of studying the effect had to be given up, as the fall in the concentration in the small fumigation apparatus during 16 hours was about 50 per cent, and the C-T product in that case was not the same as during 4 hours. It was therefore decided to continue this investigation in the larger glass chambers of 1075-1150 millilitre capacity, where the concentration fall, brought about by sorption on insects was negligible. Any desired C-T product could therefore be easily maintained, and all the subsequent work reported was carried out in these chambers.

RESULTS OF THE EXPERIMENT

The results are given in Table 2. As nearly all the doses proved to be lethal the mortality results were not analysed, and the analysis of Variance was carried out for the recovery response only. (Table 3). The following general conclusions can be drawn from the results:-

(1) With the increase in pressure from 2 to 76 cm. of mercury, the amount of fumigant recovered decreased.

(2) As the concentration-time product (C-T product) increased from 36 to 108 mg. hr. per litre, the hydrogen cyanide recovered also increased.

(3) The amount of hydrogen cyanide recovered, decreased, as the exposure period increased from 1 to 12 hours.

(4) More hydrogen cyanide was recovered from C.granaria than from C.oryzae, under the conditions tried.

The orthogonal nature of the experimental design enabled the effect of each factor separately or in combination with other factors to be assessed by summation across other factors. All the factors involved are dealt with in detail below.

Effect of the pressure.

It was found that both the species sorbed more fumigant as the pressure decreased from atmospheric to 2 cm. of mercury. The results are given in fig.1. The pressure effect was found to be significant at 0.1% probability level. The mean amount of hydrogen cyanide recovered from the insects when fumigated at atmospheric pressure was .429 mg. per g. body weight, and this increased to .883 mg. at 2 cm. pressure, thus proving the superiority of fumigation at reduced pressure in respect of the sorption of fumigant by insects.

Effect of exposure period.

Under the conditions tried, it was found that the hydrogen cyanide recovered, decreased as the period of fumigation increased from 1 to 12 hours. This effect was found to be significant at .1% probability level. The results are given in fig.2.

TABLE 2.

Mortality and HCN Recovery from C.granaria and C.oryzae at various treatments.

C.granaria.

Concentration time product in mg. per litre	Exposure period in hours	Pressure in cm. of Mercury. 2		37		76	
		HCN recovered mg./gm. body wt.	% mortality	HCN recovered mg./gm. body wt.	% mortality	HCN recovered mg./gm. body wt.	% mortality
36	1	.922	100	.470	100	.376	98
"	4	.674	100	.245	100	.174	52
"	12	.364	100	.174	96	.160	86
72	1	1.277	100	.956	100	.715	100
"	4	.864	100	.480	100	.352	98
"	12	.464	100	.340	100	.294	100
108	1	1.684	100	1.180	100	1.065	100
"	4	1.157	100	.709	100	.424	100
"	12	.760	100	.429	100	.386	100
		C.oryzae					
36	1	.844	100	.333	82	.306	92
"	4	.564	100	.256	54	.203	34
"	12	.409	100	.206	78	.165	94
72	1	.943	100	.874	100	.536	100
"	4	.855	100	.529	100	.444	94
"	12	.672	100	.307	100	.411	100
108	1	1.484	100	.839	100	.661	100
"	4	.984	100	.618	100	.504	100
"	12	.971	100	.464	100	.551	100

TABLE 3.

Analysis of Variance for Recovery Response.

Items	Degrees of Freedom	Sum of Squares.	Variance.	Variance Ratio.	
cons. time products. C	2	1.796591	.898295	148.8	xxx
Exp. Period E	2	1.829278	.914639	151.5	xxx
Pressures P	2	2.065290	1.032645	171.1	xxx
Species S	1	0.025005	.025005	4.1	x
C x E	4	0.129123	.032281	5.3	xx
C x P	4	0.091277	.022819	3.8	x
C x S	2	0.009400	.004700	<1	n.s.
E x P	4	0.148956	.037239	6.2	xx
E x S	2	0.195091	.097545	16.2	xxx
P x S	2	0.004499	.002249	<1	n.s.
C x E x P	8	0.051852)	.006481	1.1	n.s.
C x E x S	4	0.044731)	.011183	1.9	n.s.
E x P x S	4	0.032072)	.008018	1.3	n.s.
C x P x S	4	0.004962)	.001240	<1	n.s.
C x E x P x S	8	0.035378)	.004422		
Error	28	0.168995	0.006035		
Total	53	6.463505			

xxx significant at .1% probability level.

xx " " 1% " "

x " " 5% " "

n.s. not significant.

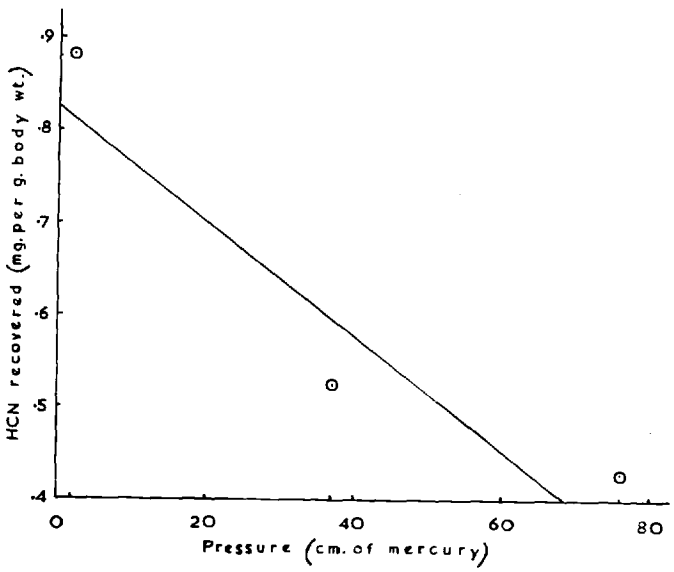


Fig.1 - Recovery of hydrogen cyanide at various pressures.

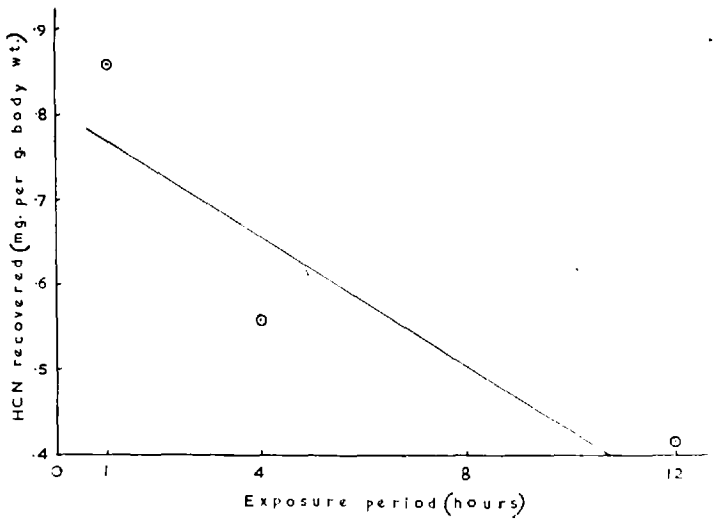


Fig.2 - Recovery of hydrogen cyanide at various Exp. periods.

The Mean amount of hydrogen cyanide recovered from the insects when fumigated for 1 hour was .859 mg/g. body weight, and the mean amount recovered when the insects were fumigated for 12 hours under otherwise identical conditions decreased to .418 mg.

Change in recovery as dosage increases.

As expected, the amount of fumigant recovered increased with the dosage. The results are given in Table 4.

TABLE 4

Mean hydrogen cyanide recovered at various C - T products.

C-T product in mg. per litre	Mean hydrogen cyanide recovered mg./g. body weight.
36	.380
72	.628
108	.826

As the C - T product increased from 36 to 108 mg. per litre the mean amount of hydrogen cyanide recovered from the fumigated insects increased from .380 to .826 mg/g. body weight.

Species.

More hydrogen cyanide was recovered from C. granaria than from C. oryzae. The mean amount of hydrogen cyanide recovered from C. granaria was .633 mg per g. body wt., whereas .590 mg. were recovered from C. oryzae.

Effect of C - T products at various pressures.

The results are given in Table 5 and Fig. 3. This interaction was found to be significant at 5% probability level.

TABLE 5

Mean hydrogen cyanide recovered from both the species taken together, when fumigated with different C-T products at various pressures.

C-T product in mg.hr. per litre	2 cm	37 cm	76 cm
36	.629	.281	.231
72	.846	.581	.459
108	1.173	.706	.598

It was found that at any one c - T product, the amount of hydrogen cyanide recovered from the insects depended on the pressure maintained in the chamber. As the pressure decreased from atmospheric to 2 cm. of Hg., the amount

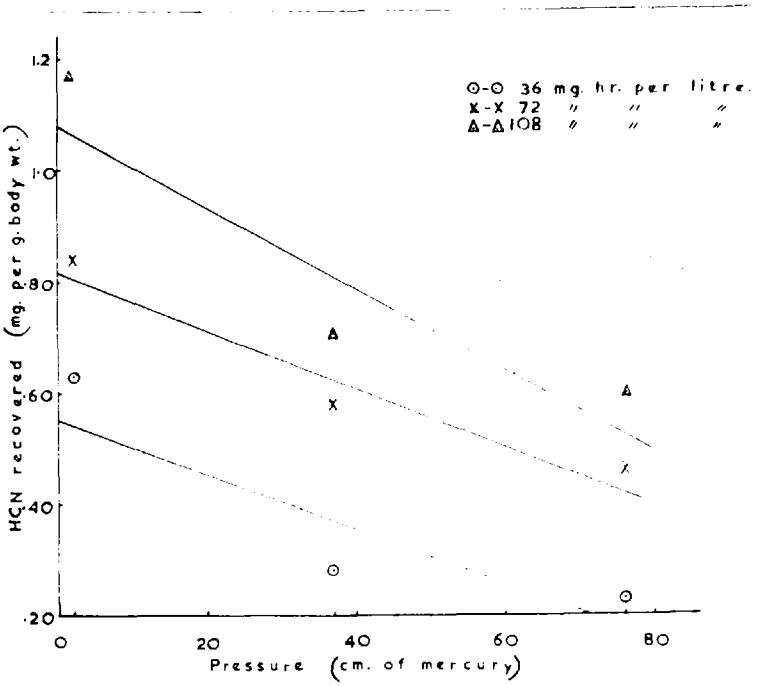


Fig.3 - Pressure - C-T Product Interaction.

recovered increased, showing that the insects sorbed more fumigant at the reduced pressures. As the C - T product increased from 36 to 108 mg.hr. per litre the amount of hydrogen cyanide recovered from the insects also increased, if the insects are fumigated at any one pressure.

Effect of exposure period at various C - T products.

The results are given in Table 6 and Fig. 4. This interaction was found to be significant at .1% probability level.

TABLE 6

Mean hydrogen cyanide recovered from both the species taken together when fumigated with different C - T products for various periods.

Exposure period in hours.	36 mg.hr. per litre	72 mg.hr. per litre.	108 mg.hr. per litre.
1	.542	.883	1.152
4	.353	.587	.733
12	.246	.415	.593

The results show that at any one C - T product, the amount of hydrogen cyanide sorbed depended on the exposure period. As the period increased from 1 to 12 hours, the amount of fumigant recovered from the insects decreased,

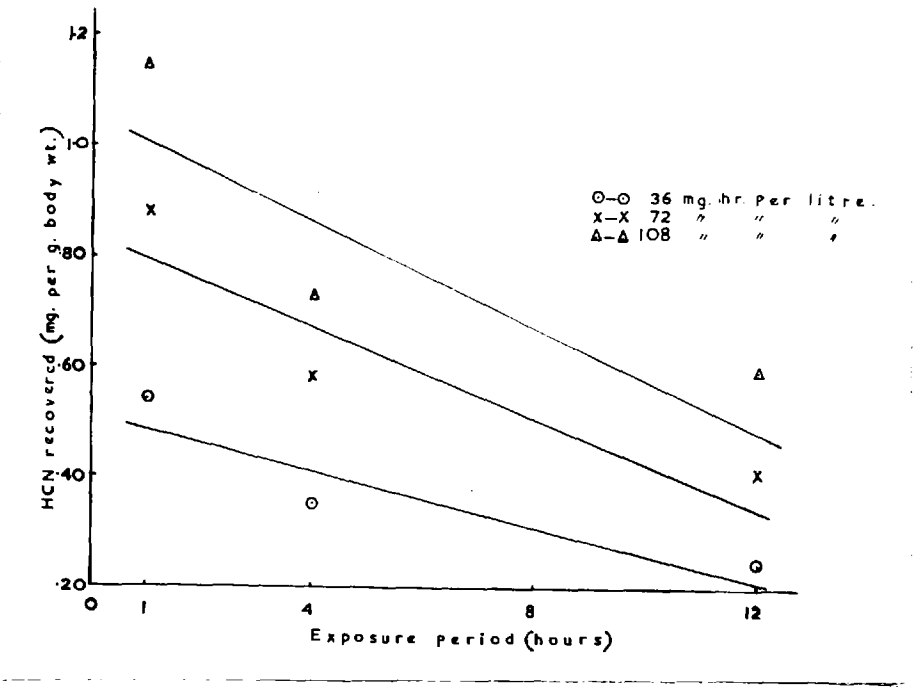


Fig.4 - Exp. period - C-T Product Interaction.

showing that high concentration combined with short exposure period affected the insects to a greater extent and produced a greater response than long periods and low concentrations. As the C - T product at any one period (which is the effect of the concentration) increased, the amount recovered increased but to a different extent at different periods. With the increase in C - T product from 36 to 108 mg.hr. per litre, the change of response produced, when insects are fumigated for 1 hour was greater than the response produced when the fumigation period was 12 hours.

Effect of C - T products on C. granaria and C. oryzae.

It was found that both the species sorbed more hydrogen cyanide as the C - T product increased from 36 to 108 mg.hr. per litre. (Table 7 and Fig. 5). More hydrogen cyanide was recovered from C. granaria than from C. oryzae, under the conditions tried.

TABLE 7

Mean hydrogen cyanide recovered from C. granaria and C. oryzae at various C - T products.

Species	36 mg.hr. per litre	72 mg.hr. per litre	108 mg.hr. per litre
C.granaria	.385	.638	.866
C.oryzae	.365	.619	.786

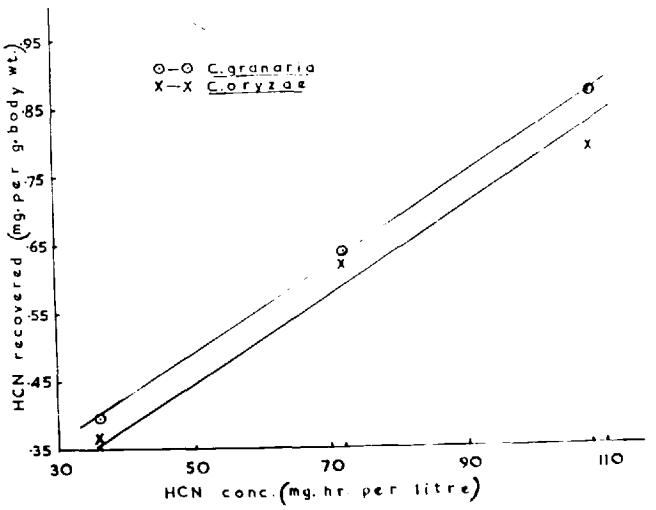


Fig.5 - C-T Product - Species Interaction.

Effect of pressures on C. granaria and C. oryzae.

Both the species sorbed more hydrogen cyanide as the pressure decreased from 76 to 2 cm. of mercury. (Table 8, Fig. 6).

TABLE 8

Mean hydrogen cyanide recovered from C. granaria and C. oryzae. when fumigated under 3 different pressures.

Species	2 cm.	37 cm.	76 cm.
C. granaria	.907	.554	.438
C. oryzae	.858	.492	.420

More hydrogen cyanide was recovered from C. granaria than from C. oryzae.

Effect of exposure periods at various pressures.

The results are given in Table 9 and Fig. 7. This interaction was found to be significant at 1% probability level.

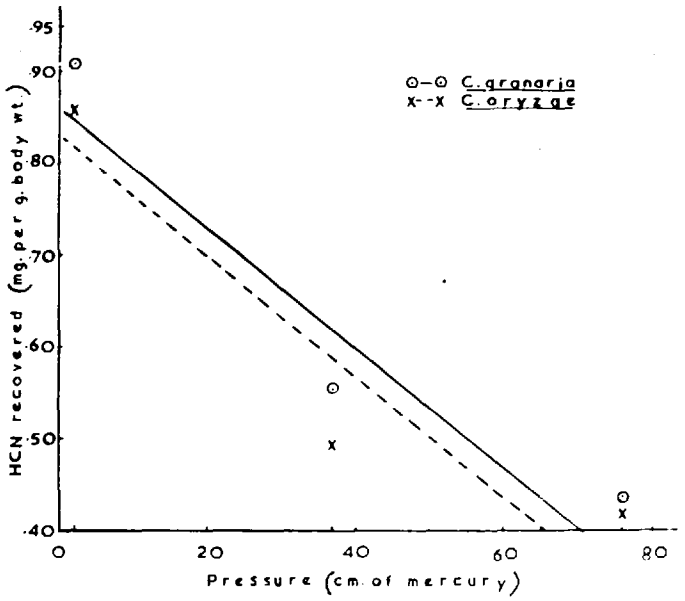


Fig.6 - Pressure - Species Interaction.

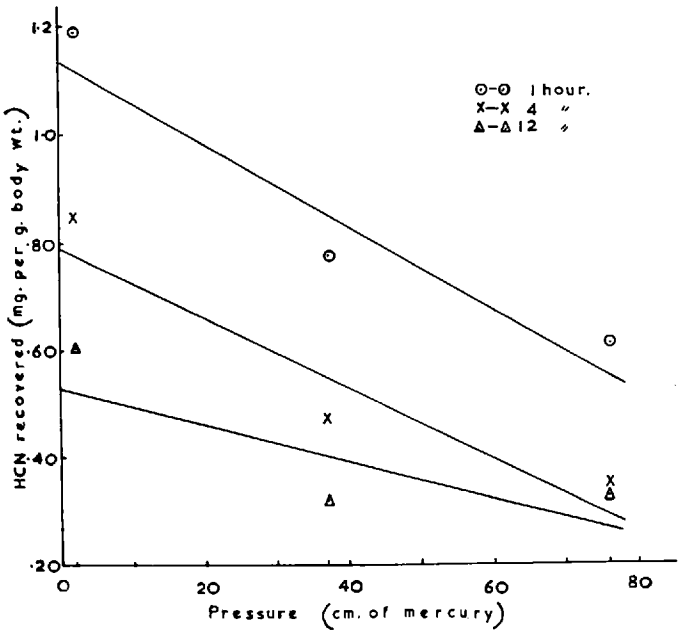


Fig.7 - Pressure - Exp. period Interaction.

TABLE 9

Mean hydrogen cyanide recovered from both species taken together, when fumigated for various periods, under different pressures.

Exposure period in hours.	2 cm.	37 cm.	76 cm.
1	1.192	.775	.610
4	.849	.473	.350
12	.607	.320	.328

It was found that at any one pressure, the fumigant recovered from both species decreased, as the exposure period increased from 1 to 12 hours. The hydrogen cyanide sorbed by both species at any one period increased as the pressure decreased from atmospheric to 2 cm. of Hg.

Effect of exposure period on C. granaria and C. oryzae.

The results are given in Table 10 and Fig. 8. This interaction was found to be significant at .1% probability level.

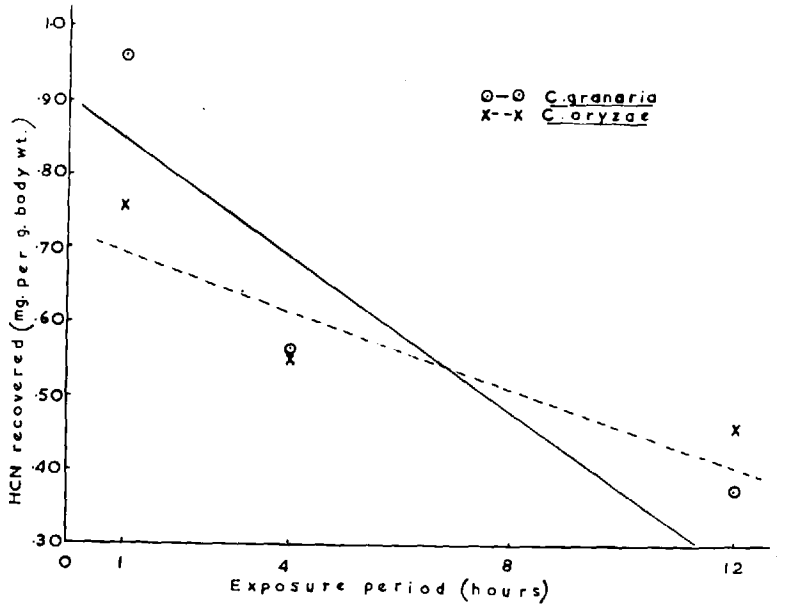


Fig.8 - Exp. period - Species Interaction.

TABLE 10.

Mean hydrogen cyanide recovered from C. granaria and C. oryzae, when fumigated for various periods.

Species	1 hour	4 hours	12 hours
C. granaria	.960	.564	.375
C. oryzae	.758	.551	.462

We see from Fig. 8 and Table 10 that, when the insects were fumigated for 1 hour more hydrogen cyanide was recovered from C. granaria than from C. oryzae, but the position was reversed when they were fumigated for 12 hours. With 4 hour fumigation period, there was practically no difference in the recovery from the two species.

Triple interactions.

None of these interactions proved to be significant. The results are given in Tables 11 - 14.

TABLE 11

Mean hydrogen cyanide recovered from both the species taken together when fumigated with various C-T products under different pressures for three exposure periods.

Abs. pressure in cm.of Hg.	Exposure period in hours.	36 mg.hr. per litre	72 mg.hr. per litre	108 mg.hr. per litre
2	1	.883	1.110	1.584
	4	.619	.859	1.070
	12	.386	.568	.865
37	1	.401	.915	1.010
	4	.250	.504	.663
	12	.190	.323	.446
76	1	.341	.625	.863
	4	.188	.398	.464
	12	.162	.352	.468

TABLE 12

Mean hydrogen cyanide recovered from C. granaria and C. oryzae when fumigated with various C - T products for different periods.

Exposure period in hours.	Species	36 mg.hr. per litre	72 mg.hr. per litre	108 mg.hr per litre
1	C. granaria	.589	.983	1.310
	C. oryzae	.494	.784	.995
4	C. granaria	.364	.565	.763
	C. oryzae	.341	.609	.702
12	C. granaria	.233	.366	.525
	C. oryzae	.260	.463	.662

TABLE 13

Mean hydrogen cyanide recovered from C. granaria and C. oryzae when fumigated under various pressures for different periods.

Exposure period in hours.	Species	2 cm.	37 cm.	76 cm.
1	C. granaria	1.294	.869	.716
	C. oryzae	1.090	.682	.501
4	C. granaria	.898	.478	.317
	C. oryzae	.801	.468	.384
12	C. granaria	.529	.314	.280
	C. oryzae	.684	.326	.376

TABLE 14

Mean hydrogen cyanide recovered from C. granaria and C. oryzae when fumigated with various C - T products under different pressures.

Abs. pressure in cm.of Hg.	Species	36 mg.hr. per litre	72 mg.hr. per litre	108 mg.hr. per litre.
2	C. granaria	.653	.868	1.200
	C. oryzae	.606	.823	1.146
37	C. granaria	.296	.592	.773
	C. oryzae	.265	.570	.640
76	C. granaria	.237	.454	.625
	C. oryzae	.225	.464	.572

DISCUSSION.

There is very little information available on the effect of a given C - T product used in different combinations of concentrations and exposure periods, on the mortality of insects. Haber was the first to put forward the formula to show the relationship between concentration and time to produce a constant mortality. C x T = W, where C = concentration, T = time and W = a constant mortality. Some deviations from this formula have been reported. Flury (1921), Peters (1936), Salmond (1951), Nahal (1952) etc. All the workers reported have taken mortality as the response. In the present series of experiments, mortality response as well as the recovery of hydrogen cyanide response were investigated, but nearly all the doses used proved to be lethal, hence the discussion will be mainly confined to the recovery response. Most of the workers, except Salmond and Nahal have fumigated the insects at atmospheric pressure, but in the present work two reduced pressures were also tried so as to investigate the influence of the reduced pressures on the insects under these conditions.

The results show that as the pressure decreased from atmospheric to 2 cm. of Hg., both the species sorb more hydrogen cyanide (Fig. 1). These results agree with those reported in the previous part. The effect of the pressure

has been discussed in detail in the last part, hence it suffices to say that when insects are subjected to reduced pressures they become more physiologically active and sorb more hydrogen cyanide. It appears from the results (Table 9 and Fig. 7) that at 2 cm. pressure, the amount of hydrogen cyanide recovered from the fumigated insects decreases as the period increases from 1 to 12 hours, but to a greater extent than at atmospheric pressure. There is practically no difference in the recovery of hydrogen cyanide when the insects are fumigated for 4 and 12 hours at atmospheric pressure. This shows that fumigation at a given C - T product for 4 hours with a certain concentration of hydrogen cyanide is as effective as fumigation for 12 hours with a low concentration as far as the sorption of the fumigant by the insects is concerned, but at 2 cm. pressure a high concentration combined with 4 hours appears to be far superior to fumigation for 12 hours with a correspondingly reduced concentration.

It is therefore concluded that the fumigation at reduced pressure of cm. of mercury should be carried out for short periods such as 1 - 4 hours, and long periods like 12 hours should be avoided.

It appears from the results (Table 10 and Fig. 8) that when the insects were fumigated for 1 hour with high

concentrations of hydrogen cyanide, C.granaria sorbed more hydrogen cyanide than C.oryzae, but there was practically no difference when they were fumigated for 4 hours. When the fumigation was carried out for 12 hours with low concentrations, C.oryzae sorbed more than C.granaria. These results show the relative sorption of hydrogen cyanide by the two species, under the different conditions of the experiments.

The results indicate that even at the same C - T product, the insects sorb different amounts of hydrogen cyanide when fumigated for different periods. Table 6 and Fig. 4 show that at any of the three C - T products tried, more hydrogen cyanide is recovered from the insects when fumigated for 1 hour with high concentrations of hydrogen cyanide than when fumigated at a low concentration for 12 hours. High concentrations combined with short periods produce a greater response than low concentrations for long periods. Salmond (1951), obtained higher mortality response of C.granaria and C.oryzae when fumigated at the same C - T product for 12 minutes with a high concentration of hydrogen cyanide than with a period of 3 hours and 6 minutes combined with a low concentration. Nahal (1952) fumigated C.granaria for 4 hours with methyl bromide and found that the mortality

resulting from a given C - T produce was less, if that occurred at the centre of the sack, than at free space. He showed that at these 2 sites the C - T curve had a different form. The curve for the free space always began at a high level and fell, while that for the centre of the sack began at a low level and rose. He also obtained a widely different mortality response both of C. granaria and C. oryzae with hydrogen cyanide.

The same trend was followed as with methyl bromide. The results of Salmond and Nahal can be explained by the recovery results obtained in the present work. Salmond reported high mortality when the insects are fumigated with high concentration for short periods and less mortality when the concentration is low, combined with long period. The recovery results show that the insects sorb more hydrogen cyanide when they are fumigated with high concentration for short period than with low concentration for long period. The results suggest that the high mortality obtained by Salmond with high concentration combined with short period may be caused by the high amount of hydrogen cyanice sorbed by the insects as indicated by the present experiments. Some mortality results obtained by the writer support this argument. Table 2 shows that when insects are fumigated with a dose of 36 mg. per litre for 1 hour,

More C. granaria and C. oryzae died than when fumigated with 9 mg. per litre for 4 hours, the C - T product being 36 mg.hr. per litre in both cases. More hydrogen cyanide was recovered in the first case than in the second. It is possible that the high mortality results obtained by Nahal, when the C - T curve starts at a high level, may be caused by the high amount of the fumigant sorbed by the insects. With the same C - T product, when the curve starts from low level, the fumigant sorbed may not be sufficient to produce the same level of mortality as in the first case. Nahal tried to explain his results by suggesting protective stupefaction at low concentrations as a possible reason. Protective stupefaction has been thoroughly discussed in the previous part and so far as the work presented in this thesis is concerned there is no evidence for such a phenomenon occurring. The reason for the low mortalities may be the smaller amount of hydrogen cyanide sorbed at the low concentrations.

Such hypotheses as saturation of the tissues of an organism at high concentration and neutralization or excretion of the fumigant at low concentration have been put forward to explain the deviations from Haber's formula. The recovery results show that the reason for the deviations reported may well be the different amounts of the fumigant

sorbed. When the insects are fumigated for 1 hour, the hydrogen cyanide recovered increases from .542 to 1.152, as the concentration increases from 36 to 108 mg. per litre (Table 6), showing that even at such high lethal concentrations the tissues of the insects do not show any sign of saturation.

In elucidating this problem no one has made any attempt in the past to study the sorption of the fumigant by the insects and from the present work it appears that such deviations as those reported by Salmond and Nahal as well as by other workers may be caused by the different amount of the fumigant sorbed at the same C - T products, obtained by different combinations of concentration and exposure time.

SUMMARY

The literature on the effect of concentration-time products is reviewed.

The writer's results show that both species sorb more hydrogen cyanide as the pressure decreases from atmospheric to 2 cm. of mercury.

Both species sorb more hydrogen cyanide as the C - T product increases from 36 to 108 mg.hr. per litre.

At the same C - T product, high concentrations combined with short exposure periods produce a greater response than low concentrations for long periods.

It appears from the results that more hydrogen cyanide is recovered from C. granaria than from C. oryzae when high concentrations are combined with a short period, 1 hour, but that the position is reversed at low concentration for a long period, 12 hours. Nearly the same amount of hydrogen cyanide is recovered from both the species when fumigated for 4 hours with intermediate concentrations.

It has been shown that the deviations reported from Haber's formula are probably caused by the different amount of hydrogen cyanide sorbed at different combinations of concentration and time.

GENERAL DISCUSSION

Most of the experiments on the effect of reduced pressures on insects by other workers have been carried out with other conditions remaining constant so as to determine the mortality with a particular pressure treatment. Livingston and Reed (1940) appear to be the only workers to show that, at a reduced pressure, 2 inches of mercury, insects lose less water and are less susceptible in water-saturated atmospheres than in dry atmospheres, other conditions being the same. In the present series of experiments (reported in Part I), an attempt has been made to control the humidity in the chamber, and to determine the water-loss and the susceptibility of the insects when treated under reduced pressures at various humidities. The results obtained show that the water-loss and the susceptibility of the insects, under any reduced pressure, however low, depend on the humidity maintained in the chamber. As the humidity decreases from 85 to 5%, both the responses increase. The water-loss and the percentage mortality of both the species used in the experiments increase as the pressure decreases from 8 to 2 cm. of mercury, other conditions, including the humidity, being the same. The greatest response occurs at a pressure of 2 cm. of mercury, but when insects from the same cultures are subjected to

a pressure of about 4 mm. of mercury, they lose less water and are less susceptible. Moore and Carpenter (1938) found that C. oryzae sorbed less hydrogen cyanide and became less susceptible at a pressure of about 2 mm., than at a pressure of 6 cm. According to these workers, there is some kind of physical effect on the insects, such as collapse of tracheae at very low pressures and this effect is held to account for the smaller loss of water and lower susceptibility.

The present work, therefore, confirms the existance of an optimum reduced pressure, below which susceptibility decreases. The optimum reduction of pressure should not be exceeded in practical control work and indeed it is not likely that it could be exceeded as leaks in the system, the water vapour pressure of commodities and the reduced pumping speed of the pump would preclude it.

When the insects are fumigated under reduced pressures, they will in fact show the effects of two treatments, (1) the effect of the reduced pressure and (2) the poisoning effect of the fumigant. The pressure effect has been discussed and it has been shown that the insects become physiologically more active as the pressure decreases. Some insects were fumigated with hydrogen cyanide under various reduced pressures and it was found that the amount of hydrogen cyanide recovered from the fumigated insects and the mortality obtained increased as the pressure

decreased from atmospheric to 2 cm. of mercury. The results agree with those obtained by other workers, most of whom have taken mortality only as the response. Salmond (1951) and Nahal (1952) obtained higher mortality response of C. granaria and C. oryzae with reduced pressure fumigation than with atmospheric pressure fumigation. It has been shown that the higher mortality results obtained with reduced pressure fumigation are associated with more hydrogen cyanide sorbed by the insects.

Recovery of hydrogen cyanide from the insects immediately after fumigation provides an indirect method of determining the sorption of hydrogen cyanide by the insects. But by crushing the insects and using the solution of pH 3 · 2 instead of 1 · 2, more hydrogen cyanide is recovered. These results showed that unless the method of recovering the hydrogen cyanide from the fumigated insects is perfect, it is not fair to assume that the hydrogen cyanide recovered is the same as the amount sorbed. Pradhan and Bhatia (1951) showed that the amount of hydrogen cyanide sorbed by the insects is more than the amount recovered and the difference they attributed to the amount of hydrogen cyanide converted in the insects' bodies. It has been shown by the method adopted by Pradhan and Bhatia and the same method as modified for the present work, that the differences in the sorption and the recovery of hydrogen cyanide, when the

fumigation periods are small, are due to the imperfect method employed for the recovery of hydrogen cyanide. Probably the results obtained by Pradhan and Bhatia might also be affected, if they were to crush the insects before distillation and use a solution of pH 3 - 2 for the liquid in which to suspend the insects. This shows the importance of perfecting the method employed for this kind of work.

When the insects are fumigated for 12 hours, the amount of hydrogen cyanide sorbed appears to be more than the amount recovered, and the difference is attributed to the amount chemically changed in the insects' bodies during the fumigation.

It was found that C. granaria continuously sorbed hydrogen cyanide, when fumigated at atmospheric pressure, right up to 16 hours of fumigation. Peters (1936) and Lindgren (1938) reported that there was protective stupefaction occurring in C. granaria. Neither of these workers have studied the sorption of hydrogen cyanide by the insects. The results obtained by the writer show that there is no cessation of respiration in C. granaria in low concentrations of hydrogen cyanide and that they sorb some hydrogen cyanide even from very small amounts taken in the gas chamber of the fumigation apparatus, which is then opened to the insect chamber. As the gas has to diffuse from one chamber to the other, the initial amount of fumigant

in the insect chamber, immediately after it is opened to the gas chamber, will be very small which can result in cessation of respiration. The sorption results obtained by this method of fumigation have been compared with the recovery results, when insects are fumigated in big chambers of 1075 - 1150 millilitre capacity by fracturing ampoules containing liquid hydrogen cyanide. In the latter method of fumigation, the insects are suddenly subjected to a high concentration and the amounts of hydrogen cyanide recovered from the insects show no great differences when compared with the amount sorbed by the insects, when fumigated in the small apparatus. Though the doses used in these two methods of fumigation are not the same, still the results definitely show that the insects are not closing their spiracles, when fumigated with small amounts of hydrogen cyanide. The other point in favour of the argument is that at no stage from ½ to 16 hours is there any sudden increase in the sorption of hydrogen cyanide by C. granaria. The amount sorbed continuously increases as the period of exposure increases from ½ to 16 hours. It has thus been shown that there is no such phenomenon as protective stupefaction occurring with C. granaria.

There is practically no information available on the amount of hydrogen cyanide sorbed by the insects, when

fumigated under reduced pressures with the same concentration-time products, but used in different concentration and time combinations. All the previous workers have taken the mortality as the response, and some of them have reported the deviations from Haber's formula, $C \times T = W$, where C is the concentration, T is the exposure time and W is the constant mortality. Salmond (1951) fumigated C. granaria and C. oryzae under reduced pressures with hydrogen cyanide and found that at the same concentration-time product, high concentrations combined with short exposure periods produced a higher mortality than for long periods with low concentrations.

Nahal (1952) with hydrogen cyanide obtained widely different mortality responses of C. granaria and C. oryzae, at the same concentration-time product, but formed in a different way. The recovery results obtained show that at any one concentration-time product, the amount of hydrogen cyanide recovered from the fumigated insects was more when the insects were fumigated for 1 hour with high concentrations of hydrogen cyanide than when fumigated for 12 hours with low concentrations.

These results show that the different mortality responses obtained by other workers may be associated with the different amounts of hydrogen cyanide sorbed by the insects.

The mortality results obtained in the present work also support the above argument, as a concentration of 36 mg. per litre for 1 hour's fumigation at atmospheric pressure produces a higher mortality than fumigation at a concentration of 9 mg. per litre for 4 hours, other conditions being the same. It has been shown that the recovery results show greater differences at reduced pressures than at atmospheric pressure.

All the results thus obtained lead to the conclusion that under reduced pressures, the insects become more physiologically active than at atmospheric pressure and that better results are achieved when the fumigations are carried out under reduced pressures, especially at as low a pressure as 2 cm. of mercury.

SUMMARY

Water-loss and mortality of Calandra granaria and C. oryzae under reduced pressure, however great the reduction, depended on the humidity maintained in the chamber. As the humidity decreased from 85 to 5% R.H. both the responses increased.

Both species lost more water and gave higher mortality response as the pressure decreased from 8 to 2 cm. of mercury, but at 4 mm. pressure there was some kind of physical effect on the insects, possibly as the collapse of tracheae, with the result that the water-loss and mortality considerably decreased at that pressure.

C. oryzae were found to be more susceptible than C. granaria, and to lose more water.

Both the responses increased with the exposure period, the rate of loss of water being high during first 4 hours of exposure, and slowly decreasing as the exposure period increased from 4 to 16 hours.

Mortality which is accompanied by desiccation increased as the insects lost more water.

When the insects were fumigated under reduced pressures, they were found to be more susceptible than when fumigated at atmospheric pressure. They sorbed more hydrogen cyanide and gave higher mortality response as the pressure decreased

from atmospheric to 2 cm. of mercury. Both the hydrogen cyanide sorbed and the mortality increased with the concentration of hydrogen cyanide, and with the period of exposure.

More hydrogen cyanide was recovered from C. oryzae than from C. granaria but C. oryzae had a lower mortality than C. granaria.

Mortality in both the species increased as more hydrogen cyanide was sorbed.

More hydrogen cyanide was recovered from the fumigated insects when they were crushed before distillation than when the whole insects were used. More than 50% of the total fumigant recovered was obtained during crushing and cool aeration and the rest during distillation, whereas when insects were used whole, very small amount of the total fumigant recovered was obtained during cool aeration.

More hydrogen cyanide can be recovered if distillation of the fumigated insects takes place from a suspension at a pH of 3.2 than from one at a pH of 1.2. A suspension of pH of 4.7 gave very little more hydrogen cyanide than did the suspension of pH of 3.2.

Both the species retained some of the sorbed hydrogen cyanide in their bodies for as long as 70 days after fumigation. About 30% of the hydrogen cyanide sorbed during fumigation was found to diffuse from the insects'

bodies in about 13 days after fumigation, and the amount recovered by distillation after about 13 days of fumigation was about 25%. The total amount of hydrogen cyanide obtained by diffusion and subsequent distillation was still less than the amount recovered from another batch of the insects immediately after fumigation. The difference can be attributed to the amount of hydrogen cyanide chemically changed in the insects' bodies into some compound from which it was not possible to recover hydrogen cyanide by the method used.

Adults of C. granaria were fumigated for various periods from ½ to 16 hours. The insects were continuously sorbing hydrogen cyanide and the amount sorbed depended on the concentration and the period of exposure. From the sorption results, it appeared that there was no such phenomenon as protective stupefaction occurring in C. granaria.

It was found that the hydrogen cyanide sorbed and the amount recovered immediately after fumigation were practically the same, when the fumigation periods were short, (like 1 - 4 hours). When the insects were fumigated for 12 hours, the hydrogen cyanide recovered was found to be less than the amount sorbed, the difference being attributed to the amount of hydrogen cyanide, which was chemically changed in the insects' bodies during fumigation.

It has been shown how an imperfect technique for the recovery of hydrogen cyanide from the fumigated insects can lead to wrong conclusions.

At the same concentration-time (C - T) product, more fumigant was recovered from both species when insects were fumigated for short periods with high concentrations of hydrogen cyanide than when they were fumigated for long periods with low concentrations.

More fumigant was recovered from both the species when fumigated under reduced pressures, than at atmospheric pressure.

As the C - T product increased from 36 to 108 mg.hr./litre the insects sorbed more hydrogen cyanide and showed no sign of their tissues becoming saturated with the sorbed fumigant.

More hydrogen cyanide was recovered from C. granaria than from C. oryzae when insects were fumigated for 1 hour with high concentrations of hydrogen cyanide, but the position was reversed when the insects were fumigated for 12 hours with low concentrations of hydrogen cyanide. Practically the same amount of fumigant was recovered from both species, when insects were fumigated for 4 hours with the intermediate concentrations of hydrogen cyanide.

APPENDIX.

The angular transformations of the percentage mortality responses are nearly proportional to the logarithmic measure of the dosage, on the commonly accepted view of the theory underlying biological assays (Finney 1952).

The dosage acting on the test insects is perhaps best estimated by the known recoveries of hydrogen cyanide from the fumigated insects. In the course of the analysis of variance of the results in terms of fumigant recovery, interactions are often found which may be interpreted in two quite distinct ways.

One such interaction is the extent to which the pressure reduction modifies the greater recovery of hydrogen cyanide for C.oryzae compared with C.granaria. (Table 18 and fig. 11 part II).

One interpretation would suggest that such modification was a result of the operation of the pressure reduction. The alternative way in which the significant pressure-species interaction may be interpreted, runs on these lines. The divergence shown in fig. 11, does not, however, appear when the fumigant recovery is measured on a logarithmic scale, i.e. the ratio of recovery on C.granaria to the

recovery on C.oryzae is substantially independent of the pressure at which the experiments are done.

As seems reasonable, we may regard the logarithmic measure of the recovery as the appropriate estimate of dosage to link with mortality response (in angular units), which is not the main purpose of this thesis. Taking this view, we would attribute the interaction to the change to the arithmetic scale adopted for the analysis of Variance.

Pressure effect;

The statement made on page 106 treats lines drawn straight for simplicity of presentation in the text figures (7 & 8 Part II), as if the points were in fact appropriately fitted by curves. This procedure has been justified by an analysis (Table I.A), which discloses that a curve of the sense described, better represents the experimental results than does a straight line.

Table I.A;- Demonstration of curvature of lines representing the influence of pressure on the mortality of insects and sorption of hydrogen cyanide.

(a) Influence of pressure on mortality.

Residual variance from table 13 pt. II. = 2.67

based on 40 d.f.

Residual variance from graph, when straight line is fitted to the points, = 16.000

based on 3 d.f.

Variance ratio 16.000/2.67 is 5.99, greater than 2.84, the upper 5% point of $F_{3,40}$.

(b) Influence of pressure on sorption.

Residual variance from Table 14 pt. II = 0.000,057

based on 22 d.f.

Residual variance from graph, when straight line is fitted to the points, = 0.003,070

based on 3 d.f.

Variance ratio 0.003,070/0.000,057 is 5.37 greater than 3.05 the upper 5% point of $F_{3,22}$.

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