THE RESPONSE OF THE CADELLE *Tenebroides mauritanicus* (L.) IN THE VACUUM FUMIGATION OF JUTE WITH METHYL BROMIDE

by

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NEW PNEUMATICAL EXPERIMENTS ABOUT RESPIRATION

XVIII

Of What Happened to Some Creeping Insects in our Vacuum

Notwithstanding the great Variety of Reptills, that Nature does almost everywhere produce; yet the inconvenient time and place, wherein the following Tryals were made, supplyed me with so few, that about these Animals I find among my Adversaria no more than the ensuing Notes.

Experiment I.

We took five or six Caterpillars of the same sort; but I could not tell to what ultimate species the Writers about insects referred them. These being put into a separable Receiver of a moderate size, had the Air drawn away from them, and carefully kept from returning. But notwithstanding this deprivation of Air, I found them, about an hour after, moving to and fro in the Receiver; and even above two hours after that, I could by shaking the Vessel, excite in them some motions, that I did not suspect to be Convulsive. But looking upon them again some time before I was to go to bed (which maybe was about 10 hours after they were first included) they seemed to be quite dead, and, though the Air were forthwith restored to them, they continued to appear so, till I went to Bed; yet, for Reasons elsewhere expressed, I thought fit to try, whether time might not at length recover them, and leaving them all night in the Receiver, I found the next day, that 3, if not 4 of them, were perfectly alive.

Robert Boyle
Philosophical Transactions of the
Royal Society, Volume V, pp. 2051-2056.
1670.

PREFACE

The work described in this thesis was done at the Science Service Laboratory, Canada Department of Agriculture, London, Ontario. Dr. Hubert Martin, Director of this Laboratory, kindly afforded full facilities for the prosecution of the study and also gave me valuable advice.

Dr. E.M. DuPorte, Entomology Department, Macdonald College, McGill University, was my supervisor of studies; the investigation itself and the preparation of the thesis were carried out under his direction. I am grateful to him for his interest and encouragement.

The equipment used in this study was made from my own original design.

In the experimental work described I was helped by three technical assistants in the Fumigation Section of the London Laboratory. Mr. C.T. Buckland was responsible for the maintenance and mechanical operation of the apparatus. Mr. T. Dumas, Assistant Chemist, made the chemical analyses, as described in the text, for the determination of the methyl bromide concentrations. Mr. E. Upitis was responsible for rearing the large populations of insects used, and for preparing them for use in the experiments. He also made the drawings for all the diagrams and charts used to illustrate this thesis.

I am also grateful to Mr. John Gates, Staff Photographer of the London Laboratory, for the photographic work.

London, Ontario.

April, 1958. H.A.U. Monro.

McGILL UNIVERSITY

FACULTY OF GRADUATE STUDIES AND RESEARCH

Ph.D. ENTOMOLOGY

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STATEMENT OF CONTRIBUTION TO KNOWLEDGE

The work described in this thesis has contributed to scientific knowledge in the following ways:

(1) For the first time it has been shown that the response of insects to poison gas at reduced pressures may follow a complex rather than a regular curve when mortality is related to pressure.

(2) A zone of high resistance between 30 and 50 mm. has been demonstrated with larvae and adults of *Tenebroides mauritanicus* (L.). Resistance in this zone is related to the effects of reduced pressure and is connected in some way with reduction of the visible activity of the insects at these pressures. The response in this zone is apparently independent of humidity.

(3) It has been shown that at pressures lower than the zone of high resistance the mortality of *T. mauritanicus* is due more to dessication than to the action of the fumigant.

(4) With *T. mauritanicus* there is an optimum pressure in the region of 75 to 150 mm., at which the insects are more susceptible to poisoning. This optimum occurs at a pressure where visible ac-

tivity is normal; below this pressure there is a progressive reduction in activity.

(5) The responses of two other insect species, represented by the larvae of *Tenebrio molitor* L. and the adults of *Tribolium confusum* Duv., are similar to those of *T. mauritanicus* but they occur at different pressure ranges.

(6) In the vacuum fumigation of jute the operating pressure and the humidity in different parts of the system are found to affect the mortality results more than the depth of the material which the fumigant has to penetrate.

(7) Another effective technique of vacuum fumigation has been found. In this technique, after an initial pressure of 25 mm. is produced in the system, atmospheric pressure is restored gradually over a period of two hours. This method was the most efficient of those studied for the treatment of bales of jute of low moisture content.

(8) This work has shown that in the study of vacuum fumigation the reactions of the insects may vary greatly with comparatively small changes in the pressure of the system. Because the biological aspect is critical, research in this field should not be carried out by chemical means alone.

April, 1958.

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INTRODUCTION

The present study was suggested by some observations made during field trials to test a new technique of vacuum fumigation proposed by Brown and Heuser (1953[a & b]). The results of these trials, which were described by Monro and King (1954), showed that in some treatments the distribution of insect mortalities within pressed bales of jute bags was the reverse of what was expected. The insects used in the trials were mature larvae of the cadelle <u>Tenebroides mauritanicus</u> (L.) and of the granary weevil <u>Sitophilus granarius</u> (L.). The mortalities at or near the centre of the bales were higher than those recorded near the surface or in the free air space of the fumigation chamber. In fumigation work, done either at atmospheric or reduced pressure, it has been considered that the main problem is to effect satisfactory penetration of the gas into the commodity, and that insect mortalities may be lower in the centre of a package than at the periphery.

This unexpected finding was considered of sufficient significance to justify an attempt to find a satisfactory explanation. It was necessary to discover whether or not these results were artifacts. If they were not, the investigation would undoubtedly reveal important information about the responses of insects during vacuum fumigation, information which might prove of interest in theory and of value in practice.

<u>Definition of Vacuum Fumigation</u>

"Vacuum fumigation" is a technique in which material to be disinfested or sterilised is placed in a chamber capable of withstanding an external pressure of one atmosphere. After the chamber is loaded and closed it is exhausted until a pre-selected pressure varying from 300 to 10 mm. Hg. is attained. After the required initial pressure is produced a fumigant (poison gas) is introduced and allowed to remain in the chamber for the length of time needed to effect the desired results on the offending organisms. Following the initial exhaustion of the chamber, various manipulations of the pressure of the system are effected either before, during, or after the introduction of the fumigant. These will be described below, and discussed in more detail. At the end of the prescribed exposure period atmospheric pressure is restored in the system, if this has not already been done as part of the process, and the gas-air mixture is then pumped out into the open air in a procedure known as "air-washing".

The primary advantage of vacuum fumigation is that, following the removal of a large proportion of the air from material undergoing treatment, the diffusion of the fumigant to all parts of the load is hastened and facilitated. It has also been shown that some species of insects are more susceptible to the toxic effect of the treatment as a result of their exposure to certain reduced pressures. However, as this thesis will show, it is not possible at present to generalise on the reactions of insects to the low pressures experienced during vacuum fumigation.

Since its introduction in practice about 1913 (Mackie, 1931) a special terminology has been used to describe the process and its variations. Workers in this field have rightly criticised much of the terminology as pure jargon, which is not scientifically descriptive (Johnson et al., 1938; Monro, 1941; Page et al., 1953). It was pointed out by all these authors that the term "vacuum fumigation" is actually a misnomer. More satisfactory terms would be "fumigation at reduced pressure" or "low pressure fumigation". Also, Page et al. (1953) proposed abbreviated descriptions to identify four of the variations in the manipulation of the vacuum fumigation process. These descriptions will be given below, in the review dealing with the vacuum fumigation of material.

Vacuum fumigation installations are now common throughout the world and are used in plant quarantine work and for the treatment of certain commodities, such as tobacco, cotton, packaged cereals, and jute bags, into which fumigants penetrate very slowly at atmospheric pressure.

Throughout this paper, when pressures are given in metric units, the abbreviated term "mm. pressure" refers to the pressure in millimetres of mercury (Hg.).

REVIEW OF PREVIOUS INVESTIGATIONS

In order to understand the effects of vacuum fumigation on insects it is necessary to study their responses to reduced pressures under three main sets of conditions:-

(1) In an empty chamber in the absence of the fumigant.

(2) In an empty chamber in the presence of the fumigant.

(3) In or on material during an actual fumigation.

It is also convenient to review previous work under these same general headings.

Effect on Insects of Reduced Pressures Alone

Robert Boyle (1670) made the first recorded observations on the effects of reduced pressure on insects. Boyle collected various insects, which he was unable to name accurately, such as caterpillars, flesh-flies,

flies, bees, wasps, butterflies, and a grasshopper. He subjected them to vacuums of unknown pressure for periods of ten hours or longer and noticed that several species were able to revive when restored to normal atmospheric pressure.

Francois Huber (1814), in a series of experiments on bees, showed that these insects can withstand very low pressures for short periods of time. The obervations of these older authors were purely qualitative and no attempt was made to explore the possibility of employing reduced pressures as a means of insect control. The first practical study was that of Cole (1906), who investigated the feeding, oviposition, and survival of Sitophilus oryza (L.) at reduced pressures. He found that at 1 inch Hg., in the presence of damp bread supplying both moisture and food, 5 out of 10 adults were active after 15 days. Another important observation was that at 5 inches of pressure in a dry bell jar all of ten weevils were dead after 7 days, and no feeding took place. But at the same pressure in the presence of a dish of water, from which all oxygen had been extracted, five of the weevils lived for 23 days and feeding and copulation took place. Cole commented as follows:- "These experiments emphasize the importance of moisture to grain weevils. In the first one it would have been concluded that the weevils died owing to the deprivation of oxygen, but the second experiment proves at once that the deaths were due to the severe dessication which would naturally be set up in the first case." It should hardly be necessary to point out that there would be less oxygen present in the second experiment than the first. In the second test part of the pressure would be exerted by the moisture, which, at the mean temperature of approximately 20° C. at which Cole did his experiments, would be 17.5 mm. Hg., or

0.69 inches Hg.

Back and Cotton (1926) made a thorough exploration of the possibility of using high vacuum as a practical means to control twenty important stored product insects and their stages. In experiments with the vacuum sustained between 1 and 2 inches pressure Hg., and at temperatures ranging from 60° to 70° F., all larvae, pupae, and adults of the species used were killed in 24 hours, except that 50 per cent of the larvae of Trogoderma ornatum (Say) survived. The eggs of only one species were used, those of Tineola bisselliella (Hum.), and of these 90 per cent survived. At higher pressures, from 2 to 6 inches and the same range of temperatures, in a concrete vault, some insects survived exposures of 4, 5 and 6 days, and after 7 days some larvae of Attagenus piceus (Oliv.), Tenebrio obscurus F., and T. ornatum survived. These authors made no mention of the humidity conditions in their experiments.

Lutz (1929) made a number of observations on the ability of various species of insects to withstand low pressures. He noted that 2 out of 10 adult fruit flies (Drosophila sp.) were able to survive 24 cycles of rapidly alternating changes of pressure from atmospheric to 22 mm. (the vapour pressure of water at normal summer temperatures).

Fisk and Shepard (1938), working with Tribolium confusum Duv., also reported that death at reduced pressures was accelerated in the absence of external moisture. Livingstone and Reed (1940) using Ephestia elutella (Hbn.) and Lasioderma serricorne (F.) were the first to weight insects before and after exposure to vacuum, and to demonstrate quantitatively that evaporation and mortality are directly related.

El Nahal (1953) in a detailed study of the response of S. granarius and *S. oryza* to reduced pressures, used factorially designed experiments to demonstrate the interaction of three pressure levels (20, 40, and 80 mm.) with three levels of humidity, exposure time, and temperature, respectively. He showed that the mortality of *S. oryza* increased sharply with decrease in pressure, but that this effect was less obvious with *S. granarius* at the pressures studied. Mortality was reduced by increasing the humidity at all pressures and was augmented by prolonging the exposure or increasing the temperature.

Bhambhani (1956) followed up the work of El Nahal by making similar experiments with the same two species, but he also measured the water loss by weighing the insects before and after exposure to the vacuum. Bhambhani confirmed that at the pressure range of 20 to 80 mm. the percentages of water loss and the mortality are directly related. However, he made the significant observation that at the very low pressures of 2 to 3 mm., water loss was greatly reduced and there was no mortality.

Narayanan and Bhambhani (1956) found that adults of *Tribolium castaneum* (Hbst.) were all killed when exposed to a low pressure of 45 - 50 mm. for 135 minutes at 35° C. Loss of weight of the insects averaged 49.3 per cent. For shorter periods under the same conditions both mortality and loss of weight were progressively less. In the same treatments, mature grubs of *Trogoderma granarium* Everts showed only 36 per cent mortality for the longest exposure of 135 minutes, and loss of weight was correspondingly less. These authors suggested the use of reduced pressures alone as a means to control insects such as *T. castaneum* in infested commodities, but did not discuss the possible effects on the results if the humidity of the environment were influenced by the moisture content of the material.

Apart from the actual mortality of insects produced by exposure to vacuum, with attendant water losses, it is remarkable that very little attention has been paid to the effects of reduced pressures alone on insect respiration. Woodworth (1932 and 1936) measured the carbon dioxide produced by honey bees at pressures between 50 and 700 mm. Hg. at 10° and 25° C. He found that at pressures below 600 mm. the insects at first responded by increasing the output of carbon dioxide. He referred to this as "the period of irritation". After some time the insects returned to the normal rate of respiration. These results were explained by Woodworth as being due to acclimatization, similar to that shown by mountaineers who, after an interval of discomfort in breathing, finally adapt themselves to high altitudes.

Effect of Fumigants on Insects Applied at Reduced Pressures

Vacuum fumigation was used for some time in practice before any scientific study was made of the response of insects to the combined action of poison gases and reduced pressures. Cotton (1932) studied the effect of ethylene oxide, methyl chloroacetate, carbon disulphide, and ethylene dichloride on *Ephestia kuehniella* Zell., *S. oryza*, and *T. confusum* at a range of reduced pressures from 25 to 1 inch Hg. pressure. The mortalities recorded varied inversely with the pressure. Cotton concluded that oxygen deficiency was the factor responsible for the greater effectiveness of the fumigants as the pressures were decreased. This deduction was open to the objection that the lower pressures themselves may have been responsible for the differences in mortality observed. Subsequently Cotton, Wagner and Young (1937) reported two further experiments with ethylene oxide as the fumigant in which the oxygen tension in the chamber containing the insects was held at 1.77 per cent in one test and at 7.26 per cent in the other. In both tests the total pressure of the system was the same, the difference in the composition of the gases being made up by the additional atmospheric nitrogen which was present in the first test. In the first experiment mortality of the insects was complete, in the second all the insects survived. These workers therefore contended that the further experiments confirmed the conclusion that, from a physiological standpoint, the oxygen deficiency attendant on vacuum fumigation rendered insects more susceptible to the action of the fumigants with which they worked. From this study they also concluded that the lower the pressure at which the work was done, the greater would be the mortality of the insects. In these two studies by Cotton and his co-workers no attention was paid to the role of moisture in vacuum fumigation.

Moore and Carpenter (1938) investigated the effect of different air pressures on adults of T. confusum and S. oryza during fumigation with hydrogen cyanide (hereafter referred to as HCN). The exposure periods of 10 minutes were very short compared with the three or four hours usual in practical fumigations. With T. confusum the results were similar to those reported by Cotton, namely that mortality varied inversely as the pressure, the highest mortalities being achieved at 1 - 2 mm. pressure. However, this study revealed an important variation in the response of S. oryza. With this species the mortality did not vary inversely as the pressure, the highest mortality being recorded at about 70 mm. pressure.

Salmond (1953) compared two methods of vacuum fumigation with HCN against the adults of S. granarius and S. oryza. In the first method the vacuum was sustained during the exposure period at the initial pressure attained after the introduction of the fumigant. In the second atmospheric pressure was restored in the evacuated chamber by the re-introduction of air immediately after the fumigant was introduced. Pressures, dosage, and exposure period were all varied in an incomplete block arrangement known as a Youden square. It was found that restoration of air ("Preliminary pressure reduction") caused less mortality than fumigation at sustained-vacuum.

Narayanan and Bhambhani (1957) used carbon disulphide against _T. castaneum_ and _T. granarium_ in vacuum fumigation experiments at pressures of 140, 300, and 500 mm. and at atmospheric pressure. They found that the median lethal dose in milligrams per litre varied directly as the pressure. It should be noted at this point that the lowest pressure used by these authors was a comparatively high one for vacuum fumigation. Also, they did not attempt to relate their own results at reduced pressure alone (Narayanan and Bhambhani, 1956) to the possible combined effects of the fumigant and the reduced pressure.

Use of Vacuum Fumigation Against Insects in or on Material

The presence of infested material in the vacuum fumigation system must obviously modify the effectiveness of any given dosage of fumigant applied by any method. This effectiveness must be judged by the ability of the fumigant to reach every part of the material and to bring about the desired mortality of the insects, including those in the free space of the chamber or on the surface of the commodity should any move out of the material during the treatment.

Following the introduction of vacuum fumigation as a practical method of disinfestation, variations in the technique were recommended and carried into practice. These modifications were based on the interpretation by different workers of data derived either from insect mortality or from the results of gas sampling at different points in a loaded chamber undergoing fumigation. As already stated, the variations are concerned with manipulations of the pressure of the system at different times during the progress of the treatment. The criteria of effectiveness included one or more of the following:-

(a) Increased insect mortalities, with every part of the system accounted for.

(b) Better penetration of the fumigants towards the centre of packages as revealed by chemical analysis.

(c) Even distribution of the fumigant concentrations throughout the system.

The different techniques are here considered in the historical order of their adoption. Because the terms suggested by Page _et al_.(1953) are fully descriptive, they are used as titles in the following list:-

1. _Vacuum fumigation with atmospheric pressure restored immediately after dosage_.-This has been known in North America as the "released vacuum" or dissipated vacuum" method. Both these terms are not scientifically descriptive; nevertheless, "released vacuum" is concise and is generally accepted as a designation of this particular technique. This method was extensively adopted in the United States, for the vacuum fumigation of baled cotton imported into that country, as described in some detail in the U.S.D.A. Federal Horticultural Board Service and Regulatory Announcements (1915). The writer has, however, been unable to trace any report of scientific work which would support this method. In the early paper of Sasscer and Hawkins (1915), describing the use of HCN for the fumigation of seed, it is specifically stated that the vacuum was sustained after the introduction of the fumigant. In the report of Mackie (1916)

on the vacuum fumigation of cigars with carbon disulphide to control the tobacco beetle, after the gas was introduced the "vacuum was reduced to 16 inches", presumably by the re-introduction of atmospheric air. However, Mackie gives no experimental data to support this technique.

2. Vacuum fumigation with simultaneous introduction of air and fumigant.-This is "La méthode des introductions simultanées" developed by Lepigre (1934 and 1949). This technique consists not in following the fumigant with a stream of air, but of allowing the fumigant in a gaseous form to be mixed evenly with the air as it flows into the vacuum chamber, so that a constant proportion of fumigant to air is maintained until the entire dosage has been introduced. This method has been widely adopted in France and in the territories of the French Union. Special gasometers are needed to hold the fumigants in gaseous form so that they may be mixed in the correct proportions with the inflowing air.

It is claimed by Lepigre that this method ensures the even distribution of the fumigant throughout every part of the system, the correct proportion being carried in by the air as it flows into the evacuated spaces in the commodity.

3. Sustained-vacuum fumigation.-In this technique, after the introduction of the fumigant, no air is deliberately introduced and the system remains at reduced pressure which may sometimes be slightly increased by leakage of air into the chamber, a condition which is unavoidable under commercial conditions. Atmospheric pressure is not restored until the completion of the desired fumigation period. The possible advantages of this method were first suggested by the findings of Cotton (1932), discussed above, that oxygen deficiency contributed towards the mortality of some species of insects exposed to fumigants. Subsequently, a number

of workers found this method more effective than "released vacuum" against a wide range of insects in a number of different commodities when HCN, ethylene oxide or methyl bromide were used (Crumb and Chamberlin, 1933; Young et al., 1935; Lindgren, 1936; Johnson et al., 1938; Monro, 1941). Recently Brown and Heuser (1935[a & b]), working with HCN, ethylene oxide and methyl bromide, have found the sustained-vacuum more effective than method 2, that of Lepigre.

4. Vacuum fumigation with atmospheric pressure restored after a period.-In their work on the penetration of methyl bromide, as revealed by chemical analysis, into boxes of dates, bagged wheatfeed and whalemeat meal, Brown and Heuser (1953[a & b] and 1956) proposed an index of fumigation effectiveness called a "penetration factor". This factor is calculated by expressing the concentration x time product found at any given point in the system as a percentage of the product of the nominal concentration multiplied by the selected time. They also experimented with a new technique, sustaining the vacuum for part of the exposure and then restoring atmospheric pressure for the balance of the time before the end of the treatment. In all their experiments with methyl bromide on the above products the penetration factors were higher than with methods 1, 2, and 3, when the average was taken of sampling points at the centre of the material and 3 inches deep within it. If the production of high concentrations of fumigant within the material is the end desired, the new method constitutes a notable advance in vacuum fumigation technique. However, it should be pointed out that, because the observations were based entirely on chemical analysis, no information was given on the relative effectiveness of the old and new techniques against insects.

The Role of Sorption in Vacuum Fumigation

Sorption of the fumigant by the load is a factor of prime importance in any fumigation. The initial dosage (usually referred to as the nominal concentration) is progressively reduced by the sorption of the fumigant gas by the material in the chamber. The sorbed gas is removed from active participation in the process, and is not available for poisoning the insects. Important factors affecting the amount and rate of sorption are the chemical and physical properties of the fumigant, the nature of the commodity, and the temperature of the system. Moisture content of a certain material may influence the amount of a given fumigant sorbed by it (Somade, 1955).

The problem of the loss of fumigant concentrations to the load in the chamber is an important study and has been the subject of many investigations and a considerable amount of speculation. The subject has been reviewed by Page and Blackith (1954).

In a study of the biological aspects of fumigation, such as the present, the investigator is principally interested in the concentrations of fumigant available for acting on the insects during the period of exposure. Therefore, no review of this somewhat controversial matter of fumigant sorption is included in this thesis.

The Problem of Residual Vapours

At the conclusion of a vacuum fumigation treatment it is necessary to remove as much as possible of the poison gas both from the chamber and from the fumigated material itself. This procedure is imperative in order to protect the employees working in the chamber, or handling the fumigated material subsequent to treatment. Furthermore, if no attempt is made to remove the fumigant from within the treated material, the residual vapours may continue to react with the material for some time after the actual treatment is completed. This post-fumigation reaction may be of importance with fumigants such as methyl bromide, which may react chemically with certain products to create permanent residues.

The air-washing, consisting of restoration of air followed by evacuation, may be repeated a number of times. With materials which sorb fumigants strongly this process may have to be continued at length before the concentrations in the centre of a package reach a low level. Monro and King (1954), in the field trial of the vacuum fumigation of jute with methyl bromide, observed that concentrations in the centre of the bale were temporarily increased each time atmospheric pressure was restored in the chamber during the air-washing process. This effect appeared to be due to the fact that the inrush of air drove the vapours from all parts of the system back into the centres of the bales.

This internal high concentration may actually work to some advantage by leaving a reservoir of fumigant which may continue to diffuse throughout the material and overcome those insects not already killed during the treatment itself. This is known as the post-fumigation effect, and its value has been noted with hydrocyanic acid gas on cotton by Johnson <u>et al</u>. (1938) and on tobacco by Livingstone and Reed (1940).

In the treatment of foodstuffs such residual vapours may be of critical importance. In the fumigation with methyl bromide of walnut meats in double-walled cellophane bags Gerhardt <u>et al</u>. (1951) compared sustained-vacuum, released-vacuum, and atmospheric treatments. After the two vacuum treatments, which were much more effective than the atmospheric, it was found by chemical analysis that residues of bromine in the nuts, formed by reaction of methyl bromide, increased steadily up to 21 days if the nuts were left undisturbed in the unopened fumigated bags. Methyl bromide, left in the bags after the fumigation, continued to exert a post-fumigation effect which, from the point of view of residue formation, was not a desirable result of the treatment itself.

In investigations of suitable vacuum fumigation techniques for foodstuffs, therefore, attention should be paid not only to those which bring about satisfactory insect mortality, but also to those which leave the residual vapours so situated that they may be removed from the material as rapidly as possible after the termination of the definitive exposure period.

EXPERIMENTAL APPROACH

With the techniques now available, fumigation research may be carried out by two principal methods, bioassay or chemical analysis. In bioassay comparative effectiveness of different treatments is judged by the mortality of the insects, which is determined either from those naturally infesting the material, if sufficient numbers are present, or from specially reared test insects placed in suitable containers in different parts of the fumigation system. In chemical analysis gas samples are extracted from selected points at intervals of time during the treatment. These samples are then subjected to chemical or physical analysis to make quantitative determinations of the gas concentrations present at the particular points at the selected times.

Obviously, if there is already a population of insects present in the material under natural conditions of infestation the bioassay will provide a reliable guide to the effectiveness of the treatment applied. Under laboratory conditions it is difficult to maintain natural populations infesting bulk material in the stable condition required for an extended investigation to be made. On the other hand, populations of insects reared in the laboratory under closely controlled conditions can provide material for comparable studies as long as the investigation continues. There is the possible objection to the use of test insects that by placing them in test containers inside the material a purely artificial environment is created, and the results obtained provide no guidance as to what might be expected under natural conditions. However, as the present study will show, there is no solid ground for the objection if the experiments are properly designed and if, in the progress of the work, it is shown that the methods of handling the insects do allow valid conclusions to be drawn from the results obtained.

There is also one theoretical objection to the use of chemical analysis. No matter how small a sample is taken, its removal upsets to some degree the micro-environment at the sampling point, especially when sampling is done within material. Repetition of sampling at a given point is necessary if a satisfactory curve is to be drawn to demonstrate the flow of gas concentrations. Even if sampling is done at wide intervals of time, repetition can only lead to instability of the air-gas system at such a point. On the other hand, a group of test insects in a suitable container will remain in position without disturbing the gas concentrations surrounding it and the mortality response will show the effectiveness, at a given point, of the concentration x time product brought to bear upon the insects during the exposure.

Brown (1944) rightly contends that no proper understanding of the action of a certain fumigant upon a certain commodity can be attained without the use of chemical analysis. He also suggests that the inherent variability of biological material makes test insects unsuitable media for studying the action of fumigants on the materials themselves. In his opinion studies of the action of fumigants upon insects should be confined to the determination of the concentrations of a fumigant required to kill certain species or stages of insects under closely controlled laboratory conditions in which the insects are exposed to the fumigant alone in the absence of the material. This data could then be related to information on fumigant concentrations obtained by chemical analysis during the treatment of the material concerned. On the other hand, Schmidt (1948) used populations of the adults of S. oryza for quantitative measurements of fumigant concentrations in the soil, and showed that this insect demonstrates great sensitivity to small differences in concentration of the different gases employed. He followed the pattern of diffusion of fumigants with considerable accuracy on the basis of the mortalities observed in the different positions.

In the present study the emphasis has been on the biological aspects of the problem of the vacuum fumigation of jute. Therefore, insects have been extensively used. Also, whenever considered necessary for the investigation, analysis by physical and chemical means has been employed. In order to prevent the interference of gas sampling with the reaction of the test insects, chemical analysis and bioassay have not been used in the same experiment, unless otherwise mentioned.

MATERIAL

Insects

The principal insect species used in this study was the cadelle

Tenebroides mauritanicus (L.). For the investigation of the penetration of the fumigant into the jute bales, mature (fourth instar) larvae of this species were used. In the study of the intrinsic toxicity of the fumigant at different pressures and humidities these larvae and adults were used.

T. mauritanicus was chosen for this work for several reasons:-

(a) It was the insect used in the original field trial which prompted this investigation.

(b) Of all species of insects so far investigated it is the most resistant to methyl bromide (Monro et al., 1956). Thus the study could be made at higher concentrations close to those used in actual practice.

(c) A technique has been developed by Bond and Monro (1954) for the mass rearing of this insect under uniform conditions. The populations so produced are suitable for bioassay purposes, and in the present study were found to give responses of satisfactory uniformity as shown by the statistical analysis employed.

T. mauritanicus is a member of the family Ostomatidae of the Order Coleoptera. It is now a cosmopolitan species of some economic importance as a pest of grains. According to Hatch (1942) in Europe the insect lives outdoors under bark and in rotten wood where it is predatory on xylophagous insects; of the 152 species of the genus Tenebroides known by 1910 this was the only one reported as being of economic importance to man.

The family Ostomatidae (Ganglbauer 1899) is placed by Crowson (1955) in the Superfamily Cleroidea of the Suborder Polyphaga in the Order Coleoptera.

The larvae and adults of T. mauritanicus, used in large numbers in the present study, were fed on wholemeal flour in quart sealers with screened tops. In the rearing room the temperature was held closely at the range 25 ± 1 degrees C., and the humidity at 70% R.H., throughout the entire investigation. The actual technique of rearing is described in detail by Bond and Monro (1954).

In a limited series of experiments several other species of insects in various stages were fumigated at low pressures in order to compare their reactions with those of *T. mauritanicus*. These insects are listed in the appropriate section dealing with this particular study.

Jute

For the study of the penetration of the fumigant into material, bales of jute bags were used for the following reasons:-

(a) This was the commodity used in the field trial.

(b) The material is non-perishable over extended periods of time. For instance, it has been possible to aerate the material completely between tests, so that there are no residual vapours of methyl bromide remaining after 3 or 4 days, if the bales are stored in a well ventilated space at temperatures above 20° C. Thus, it has been possible to use nine bales repeatedly during this study.

(c) The material is markedly hygroscopic and the moisture content becomes uniform throughout the bale, if the humidity of the space surrounding it is held at a constant level. The ability to maintain the bales at a constant moisture content has proved to be very helpful in the present study.

(d) Jute is highly sorptive, and as sorption is an important factor modifying fumigant penetration, this material is suitable as a medium for a study of the reaction of insects inside material.

(e) Bales of this material lend themselves to a manipulation used in this work, whereby the test insects may be introduced into any desired point inside, fumigated, and subsequently removed without disturbing the pack of the bale.

The jute bags used were of uniform weight and texture*. They were all purchased as cleaned used bags from a single dealer, and had been used previously to hold poultry feed, the product of one manufacturer. When in equilibrium at 70% R.H. and 25° C. the weight of cloth in a bale containing 300 bags was 172 pounds. Thus, the average weight of a single bag would be 9.2 ounces (261 grams) under the same conditions. Individual bags were approximately 28 inches long and 20 inches wide.

Fumigant

The methyl bromide used throughout this investigation was purchased from the manufacturer as 100 per cent pure. It was delivered in metal cylinders containing approximately ten pounds of fumigant.

Important physical properties of methyl bromide (CH_3Br) which relate to its use as a fumigant as given by Brown (1954) are:-

Boiling point	3.6° C. (38.5° F.)
Specific gravity of liquid at 0° C.	1.732
Vapour density at 20° C. (Air = 1)	3.27

*A representative sample of 12 pieces of jute from the collection of bags was submitted to the British Jute Trade Research Association, Dundee, Scotland. The material was appraised as follows:-

Cloth weight (oz./40 in.)	9.6
Threads/in. warp	11.7
Threads/in. weft	12.1
Warp yarn count (lb./sp.)	8.9
Weft yarn count (lb./sp.)	9.0

A trade description of this material is "a 10 oz./40 in. Hessian 11 porter 12 shots/in."

Although methyl bromide has been stated in a number of reference books to be only very slightly soluble in water, a careful determination by Haight (1951) has shown that at 25° C. the solubility is 1.341 g. per 100 g. of water.

Simmons and Wolfhard (1956) have demonstrated that in all proportions in air methyl bromide is non-flammable; it is, in fact, also used widely as a fire extinguisher.

METHODS

In this section a description will be given of the main equipment and principal methods used in this study. As the experiments are reported in succeeding sections, additional details of special techniques or apparatus will be given as required.

Fumigation Apparatus

The apparatus used was designed by the writer for investigating a wide range of fumigation problems, and a detailed description has been published by Monro and Buckland (1956). At present it will only be necessary to describe those features which apply more particularly to vacuum fumigation. Most of the apparatus, except the control panel and the vacuum pump, is housed in an insulated room (hereinafter referred to as the "main room") in which temperature and humidity can be held within close limits. This room may be ventilated, if necessary, through a special duct in the ceiling which contains an exhaust fan. By means of a timing mechanism this fan can be operated at intervals, as required.

Fumigation chambers.-Inside the main room are seven cylindrical steel chambers with walls 3/16 inch thick, (Fig. 1). Six of these chambers have a cubic capacity of 525 litres and the seventh has a capacity of 900 litres.

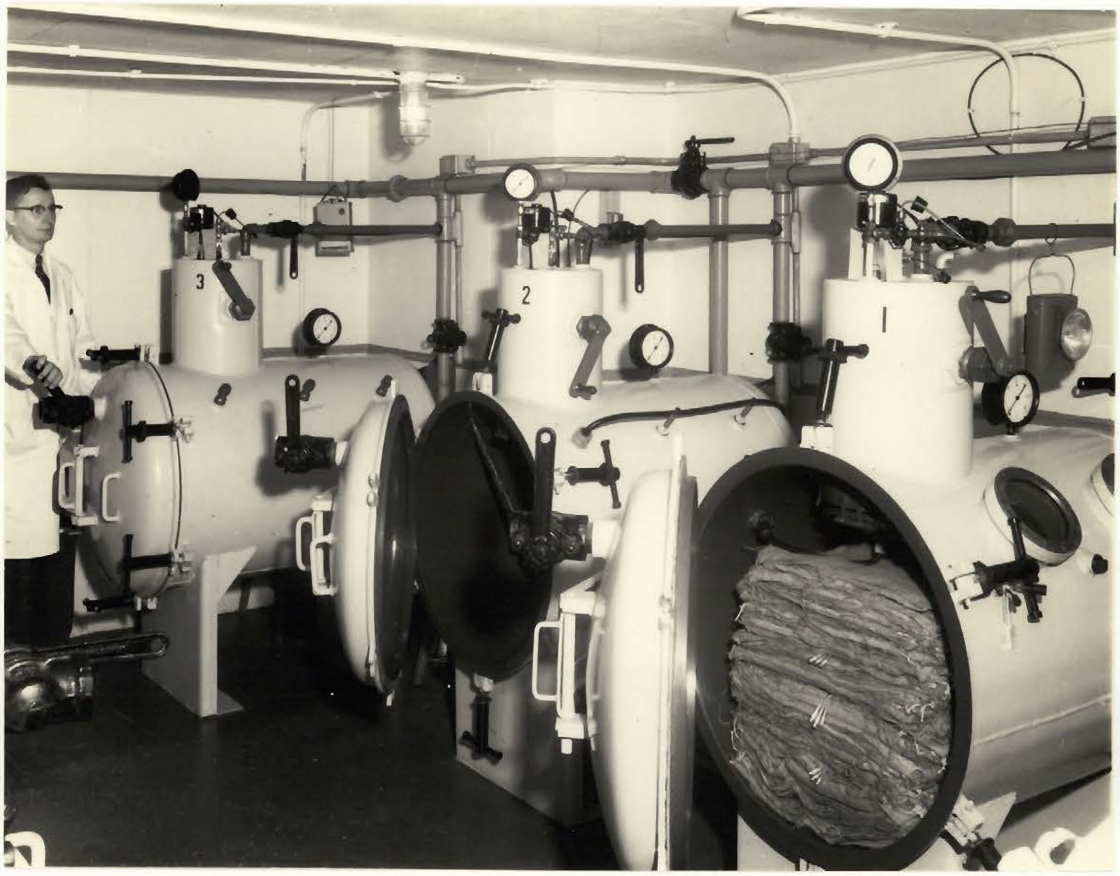

Figure 1. Part of fumigation installation inside insulated room, showing bale of jute bags in a 525 litre chamber.

The entire inside of each chamber is painted with red primer, applied in a bakelite vehicle, covered with two coats of clear varnish. The door of each chamber has a gasket of soft rubber 3/16 inch thick and 1.5 inches wide. The inside of the door has a flanged rim protruding into the chamber when it is closed.

The detailed construction of each of the six smaller chambers is illustrated in Fig. 2. Only one chamber is equipped with the two glass observation ports shown. The dispenser, so called, was originally incorporated in each of the smaller chambers for special manipulations for volatilising liquids in atmospheric fumigation studies. In the present investigation the dropping plate mechanism was not used and the dropping plate itself was left fully open. In all the vacuum fumigations in these chambers, liquid methyl bromide was drawn into the evacuated chamber via the dispenser through the suction tube and onto the surface of the evaporating dish, which was slightly warmed by a small 100 watt heater. In this way the fumigant was volatilized immediately and diffused into the main chamber. The seventh, larger, chamber is not equipped with a dispenser and the liquid methyl bromide was drawn in through a tube onto the surface of an enamel tray over which it spread and evaporated quickly.

On the walls of all seven chambers there are six threaded one inch pipe inlets. Copper tubes running through pipe plugs screwed into the inlets are used to withdraw for analysis samples of the fumigant-air mixture from different parts of the free air space, and from within the commodity, as desired.

<u>Vacuum pump</u>.-The pump used for evacuating the chambers, and for aerating them after the completion of the fumigation period, is situated outside the insulated main room. It is a two stage air cooled vacuum

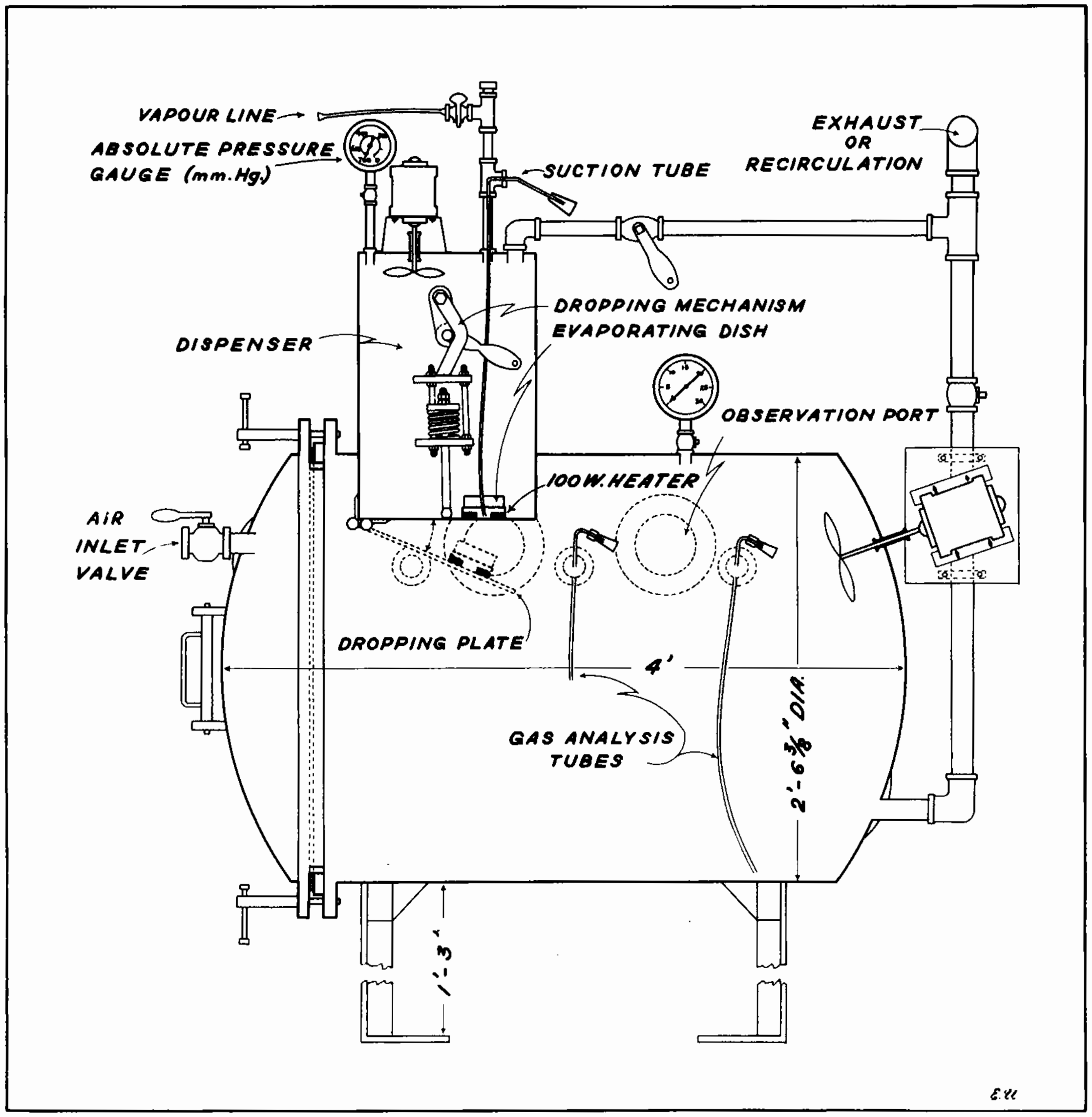

Figure 2. Diagram of fumigation chamber of 525 litre capacity.

pump driven by a 5 h.p. electric motor. This pump has a capacity of 49.5 cubic feet per minute at atmospheric pressure. The lowest pressure attainable in the system of chambers is approximately 8 mm. As this pressure is well below those used in commercial vacuum fumigation chambers it was not considered necessary, in the present investigation, to experiment with pressures lower than those attainable in this system.

Measurement of pressure.-For the measurement of pressure during the experiments, mercury manometers and dial gauges (as illustrated in Fig. 2) were used. The dial gauges* used for the measurement of low pressures were of the aneroid type and barometrically compensated, the aneroid being inside the vacuum system and protected from barometric variations. In the range 0 - 40 mm. pressure these gauges can be read accurately to 0.2 mm. They were checked regularly against a closed arm mercury manometer.

Leakage into steel chambers.-Before each set of experiments the seven steel chambers were checked for leaks. Four of the chambers, including the large one, showed very little leakage. Careful checking of this leakage in the four chambers showed that even when the pressures inside the chambers were reduced to 10 mm. the increase in pressure due to leakage rarely exceeded a rate of 0.5 mm. per hour. However, it must be pointed out that the pressure of the system always increased to some extent in all experiments when a vacuum was created, for two reasons. In the first place, evacuation of a chamber brought about a rapid decrease in temperature inside the chamber. As the result of heat transfer through the chamber walls the original temperature was restored quickly, but this rise was accompanied by a proportionate increase in pressure, according to the gas laws. Secondly,

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*Edwards type CG-1 dial gauge, manufactured by W. Edwards & Co. (London) Ltd., London S.E. 26, England.

when insects or jute were present in the chamber, moisture was given off after the vacuum was created, giving a measurable increase in pressure. This was especially noticeable at low pressures below 50 mm. when, as will be seen, the insects lost a high percentage of their moisture content.

A number of preliminary observations were made on the effect of temperature adjustment on the pressure in empty chambers. It was found that the increases in pressure which could be attributed to temperature adjustment agreed with the theoretical values calculated from the ideal gas laws. The changes were small. For instance, when the original temperature in the large 900 litre chamber was 25° C., a reduction to 60 mm. resulted in a final adjustment to 61.5 mm.

<u>Temperature Regulation</u>

The temperature in the main room can be regulated very closely from 0° C. to 40° C. by means of the specially designed control system. No attempt is made to control the temperatures in the individual chambers. Instead, the entire set of chambers is at the temperature of the main room. Transfer of heat through the steel walls of the fumigation chambers is rapid. In all the experiments, either with insects alone or with bales, the original temperature in the free space of the chambers was restored to 25 ± 1° C within 15 minutes of pressure reduction.

That temperature control may be maintained satisfactorily within the chambers evacuated as low as 8 mm. pressure is in confirmation of the theory that the thermal conductivity of any gas or mixture of gases is independent of the pressure until very low pressures are reached (Dushman, 1949). Dickins (1934) has shown experimentally that by reducing the pressure of air from normal atmospheric to 11 mm. the thermal conductivity is only slightly reduced.

Regulation of Humidity

Humidity in the main room can be held at any desired point of relative humidity between 50 and 100 per cent by means of a control system already described (Monro and Buckland, 1956). When required, water from a reservoir is blown out by compressed air through a fine spray nozzle. In this thesis the humidity conditions prevailing within the chambers at all pressures are expressed in terms of percentage relative humidity (per cent R.H.). This method of expression is not only convenient, it is also theoretically correct. The maximum weight of water vapour which can be present as such in a given space depends only on the temperature, and is not affected by the pressure of the system (in the order of normal atmospheric pressure or less) or by the presence or absence of other gases (Penman, 1955). The value "percentage relative humidity" is an expression of the ratio of the weight of moisture actually present in the space to the weight at saturation for the particular temperature which, in this investigation, was 25° C. throughout.

Evacuation to a certain low pressure resulted in the removal of a proportionate amount of the water vapour from the chamber. The theoretical relative humidity following evacuation of a chamber with a known initial R.H. can be calculated for each pressure below atmospheric by simple proportion. Thus, after evacuation to 15 mm. of a chamber with 50% R.H. at normal atmospheric pressure the relative humidity should be 1%, and at 100 mm., 6.6%. The actual R.H. prevailing in evacuated chambers was checked by the methods described below. Actual and theoretical values for R.H. were found to be close. The presence of insects in the chambers caused the humidity to rise slowly during all vacuum treatments. In none of the experiments with bare insects did this increase exceed 5% R.H. for the three hour exposure.

In the experiments designated as "dry" the chambers were at 50% R.H.

before they were evacuated. In the experiments at 70% R.H., immediately after evacuation, a weighed amount of distilled water at approximately 95° C. was drawn into the chamber through a spray nozzle. The amount of water required to produce the desired relative humidity at a given pressure was determined by experiment, after preliminary calculations. In all treatments at this humidity, in which fumigant was also used, the water required for humidity control was introduced immediately after evacuation of the chamber, before the introduction of the fumigant.

In the saturated vapour experiments distilled water at approximately 95° C. was placed on flat pans inside the chambers before they were evacuated. The amount of water was in excess of that required, to allow for the loss of vapour during the evacuation.

The actual humidities prevailing in the three ranges of humidity described above were checked for each experiment by means of recording hair hygrometers. These hygrometers were calibrated before each daily run of experiments to record accurately at the particular ranges of humidity expected. Also the humidities in the chambers and the performance of the hygrometers were checked periodically by the use of cobalt thiocyanate papers and a Lovibond Comparator, according to the method of Solomon (1957).

Dispensing the Fumigant

Several ways of applying the required dosages of methyl bromide were tried out before the following method was chosen and used throughout the work.

A steel cylinder of 2 litre capacity, without a siphon, was inverted and connected to a copper tube. The copper tube was wound into a number of coils leading through a metal can containing dry ice. The tube passed through the floor of the can to a standard ¼ inch needle valve. By adjusting the amount of dry ice in the can the methyl bromide could be kept as a liquid at 0° C. By means of the valve the liquid was measured into graduated glass centrifuge tubes. The graduated tubes were calibrated with distilled water to give a reading accurate to 0.1 ml. at 10° C.

The centrifuge tube containing the required amount of methyl bromide was then fitted to a rubber stopper through which ran a copper tube leading into the evacuated chamber. The liquid was thus drawn into the evaporating dish as already described. As soon as the centrifuge tube was empty it was removed and the open end of the copper tube was closed by a small rubber stopper. With practice, the process of connecting and disconnecting the centrifuge tube could be carried through with an increase of not more than 0.5 mm. pressure in the evacuated chamber.

Designation of Fumigation Pressures

In this entire text the statement of the pressure prevailing during a given treatment is that of the mean of the two readings made at the beginning and the end of the period of exposure to reduced pressures. The "beginning" reading is that reading made immediately after the completion of all initial adjustments to the pressure, including addition of fumigant or moisture, whenever one or both were added.

Exposure Period

Except where otherwise stated, the standard period of exposure to the fumigant was three hours throughout the entire investigation. This was counted from the time that the introduction of the fumigant was completed to the time that air-washing began. In experiments without fumigant, the period of three hours was counted from the time of establishment of the required moisture or pressure conditions until normal atmospheric conditions were restored.

Aeration of Chambers

At the conclusion of the fumigation treatment as much as possible of the methyl bromide gas was removed by air-washing. If atmospheric pressure within the chamber had been reached at the end of the experiment, the vacuum pump was started and the large air inlet valve on the door of the chamber (see Fig. 2) was opened. Air was then pumped through the chamber, the pressure being maintained at 700 mm. as shown on the large gauge, until it was safe for the operators to remove the bales or fumigated insects. The actual time of aeration varied according to the type of experiment. When bales were present aeration was longer than for a chamber containing only bare insects. However, to ensure consistency, the same aeration time was maintained throughout a series of comparable treatments.

Throughout this investigation the safety of the operators was ensured by a regular routine of checks with a "halide lamp"* which indicates, by different colour reactions in the flame, concentrations of methyl bromide in air down to 30 parts per million. At no time was any operator breathing air which gave indication of methyl bromide on the halide lamp. Under the conditions of this work, in which the operators were exposed to fumigated material for only a few minutes each day, momentary exposure to concentrations below 30 p.p.m. of methyl bromide would not constitute a hazard to health. The American Conference of Government Industrial Hygienists (1957) laid down for methyl bromide a recommended value of 20 p.p.m.

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*Two commercially available halide lamps were used, both of approximately the same sensitivity:

(1) "Prest-O-Lite" brand, which burns acetylene, obtainable from Dominion Oxygen Co. and agencies.

(2) "Bernz-O-Matic" brand, which burns propane, manufactured by Otto Burns Co. Inc., Rochester, N.Y., and obtainable through Canadian agents.

in air as the maximum average atmospheric concentration of this gas to which workers may be exposed continuously for an eight-hour working day without injury to health.

Handling of Insects

The mature (fourth instar) larvae of T. mauritanicus, which were used in greatest numbers, were exposed to the fumigant on plastic trays or in metal capsules.

For the tests of intrinsic toxicity, in which the insects were exposed bare, the trays shown in Fig. 3 were used. The base of the tray is a sheet of clear plastic, 3/16 inch thick, $5\frac{1}{2}$ x $6\frac{1}{2}$ inches in area. On it are securely glued 30 glass rings, each 7/8 inch internal diameter and 5/8 inch high. The glass cells, open at the top, formed by the rings are necessary because both adults and larvae of T. mauritanicus are cannibalistic when in confined quarters. Also, this arrangement facilitated post-fumigation feeding, observation, and mortality counts, each tray containing one replicate of 30 insects.

The larvae were removed from the culture of wholemeal flour by a sieve, and individuals from the population were selected for maturity and vigour. They were placed in the empty rings on the trays and kept overnight at 25° C. and 70% R.H. without food. The following morning the trays were moved into the main fumigation room and placed in the chambers for fumigation. At the completion of the treatment the trays were held in the main room at the same temperature and humidity, still without food, so as to permit aeration of the fumigant from the bodies of the insects. The following morning they were returned to the culture room and a small amount of wholemeal flour was placed in each ring.

For the experiments on mortality within the bales and the free space

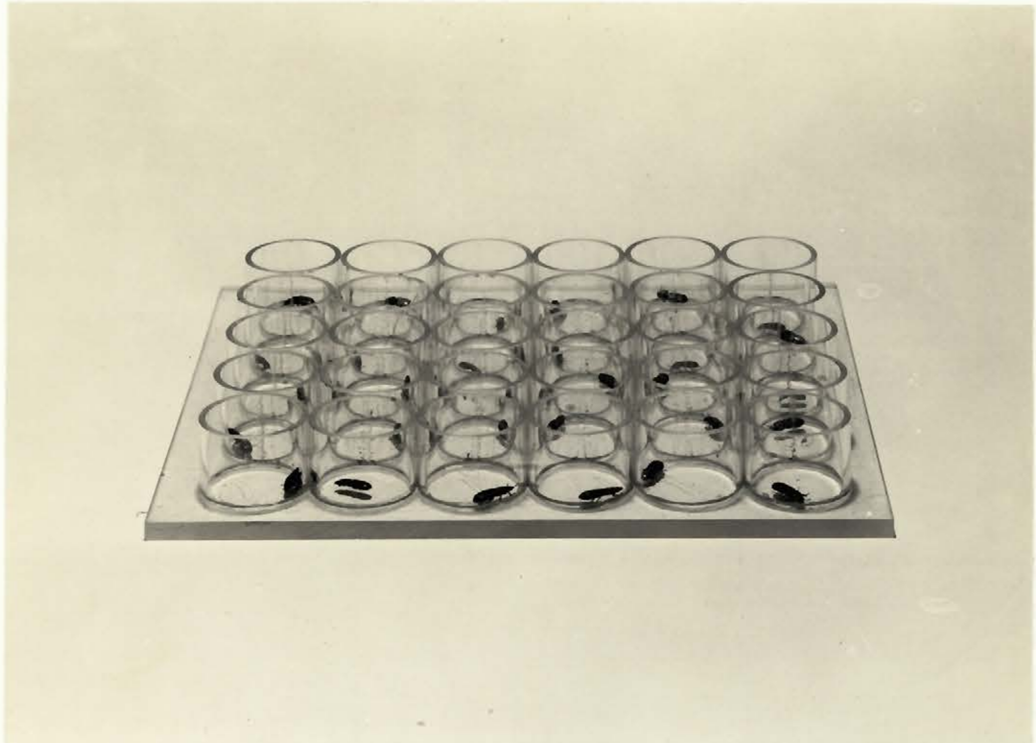

Figure 3. Plastic trays with glass rings, containing adults of T. mauritanicus.

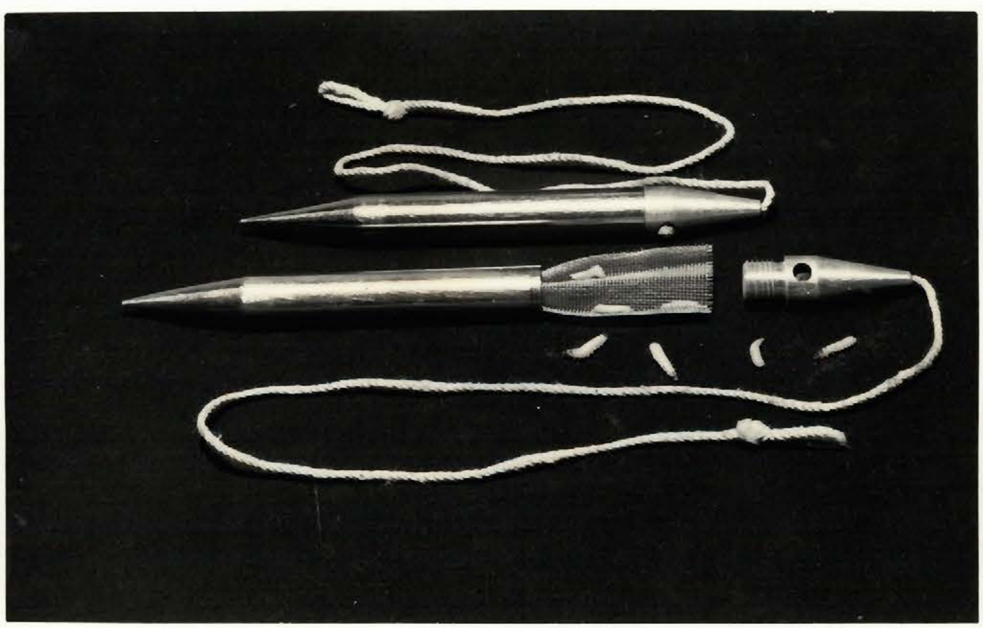

Figure 4. Metal capsules used for exposing insects to fumigation inside bales of jute bags, showing method of placing larvae of T. mauritanicus.

surrounding them, the insects, after being sieved and sorted as above, were placed ten at a time in the metal capsules* illustrated in Fig. 4. Inside each capsule a piece of 28 mesh to the inch plastic screening was kept in four folds. These folds helped to keep the larvae separated so that they would not fight. The capsules containing the larvae were placed as described below, in the bales or in the free space of the chamber, the night preceding an experiment. When the fumigation was finished and the preliminary aeration completed the capsules were removed from the bales and from the chambers, and the insects were taken immediately from the capsules and placed individually in the glass rings on a plastic tray. From this point on the manipulation was the same as for the insects exposed bare.

Adults of T. mauritanicus were handled in the same way as the larvae. The other insect species investigated were placed in petri dishes the night before treatment and exposed bare. The day after treatment all stages were given their normal food.

Determination of Insect Mortality

Most of the insects used in the fumigation tests, including T. mauritanicus larvae and adults, were examined and counted for mortality one week after exposure. Larvae of Tenebrio molitor L. which, before dying, remained moribund for a longer period than the other insects, were counted after two weeks. For adults or larvae the individual was counted as alive if, at the time of examination, it showed any signs of movement with or without stimulation. Stimulation was exposure to warmth from a 60 watt lamp followed, if necessary, by light prodding with a needle. Each day

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*Manufactured especially for fumigation tests by H.T. McGill, Houston 3, Texas, U.S.A.

Figure 5. Bale of jute bags used in fumigation experiments, showing metal capsules containing insects ready for drawing into position within the bales.

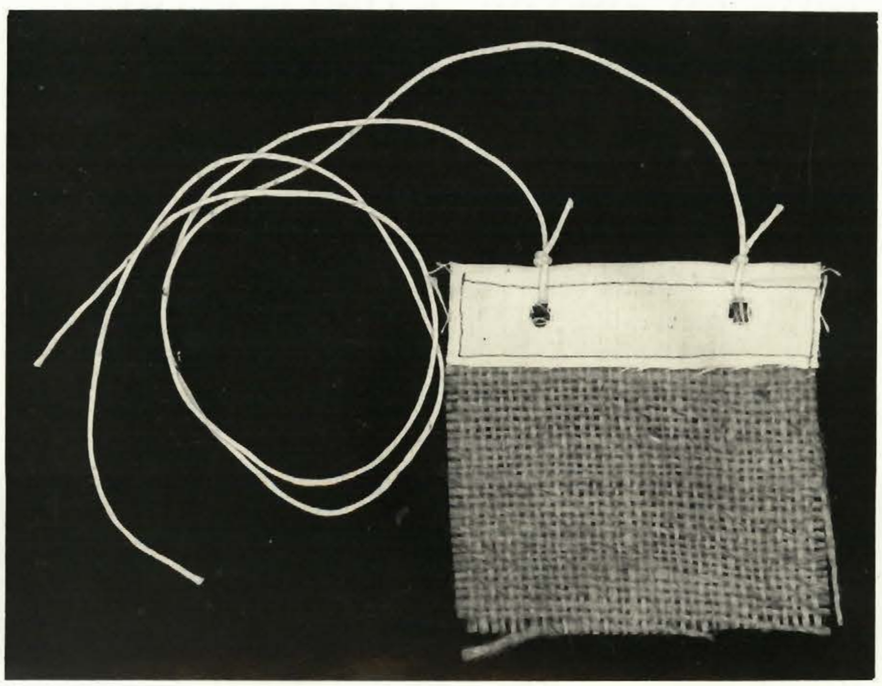


Figure 6. Jute square used for checking moisture content of jute within bale.

of fumigation a tray or dish of non-fumigated controls was prepared. The insects were put through the same manipulations as the treated ones, except the actual fumigation. At the time of counting, corrections for mortality in the controls was made by the method of Abbott (1925) using a table of values prepared by Healy (1952).

Preparation of Bales

The bales of jute, each containing 300 bags, were made up by means of a hand operated baler. While the bales were being made up the insect test capsules, together with the string and wire "pullers", were placed in the desired positions as described below. The bales were then pressed and each bound with four strands of wire.

The metal test capsules, three to a lot, were placed during packing of the bale at the exact centre of the bale (150 bags above and below) and at two intermediate positions, one 75 bags from the top and the other 75 bags from the bottom of the bale. The two latter positions, while nearer to the surface in one dimension, were again at the centre in the two other dimensions. When the bales were compressed the capsules in the two outer positions were 5 inches from the central lot. The bales were thus 20 inches high when compressed, and they were approximately 28 inches long and 20 inches wide.

As already mentioned, each test capsule had a puller at each end; at one end the puller was a string, at the other a wire. The string extended through the bale, between the bags at the appropriate levels, and came out at the opposite end. It was thus possible to draw the capsule inside the bale before fumigation (see Fig. 5) and to pull it out afterwards. Each string was carefully dyed at the exact place so that when the dyed mark was at the edge of the bale the capsule was at the centre of the bale on the appropriate horizontal plane.

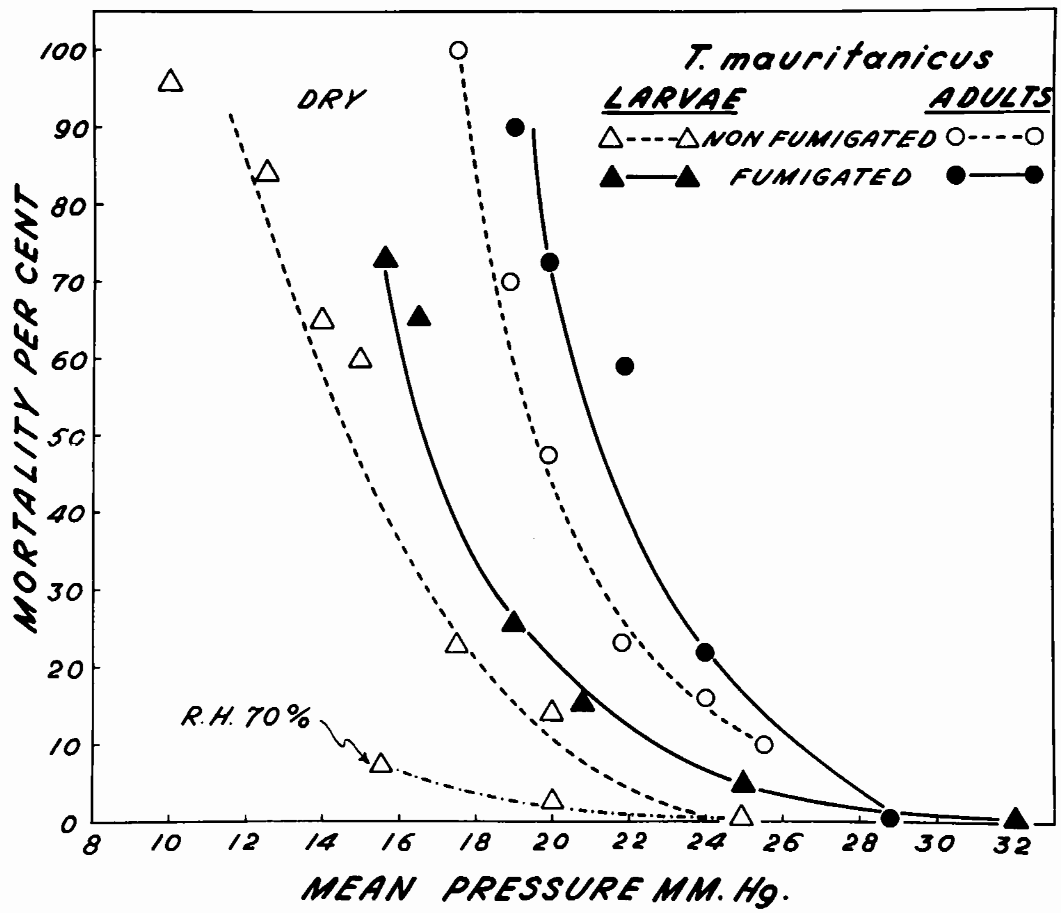


Figure 7. Effect of reduced pressures for three hours exposure, with or without fumigant (CH_3Br 17.3 mg./l.), on mortality of larvae and adults of T. mauritanicus. Relative humidity in dry experiments ranged from 0.7 to 2.0%. Larvae were also exposed at R.H. 70%.

It was thus possible to insert and remove the test insects without disturbing the bales.

Aeration of Bales

On the completion of a fumigation of a given set of bags, and after removal of the insects from the metal capsules, the bales were kept in the main fumigation room overnight. During this time the room was ventilated as already described. The following morning the bales were placed in a warm, ventilated passage outside the laboratory and kept there for 3 or 4 days. At the end of this time no trace of free methyl bromide was detectable by chemical analysis at any point within the bales, and they were ready for use in the experiments again. It was thus arranged that there was an interval of at least one week between fumigations of a given bale.

RESPONSE OF *T. mauritanicus* TO REDUCED PRESSURES

As already suggested, no proper understanding of the effect of vacuum fumigation on insects can be attained without a study of their responses to a range of low pressures in the absence of the fumigant. The responses of mature larvae and adults were investigated under both dry and moist conditions. The adults used had emerged from pupae two weeks before each experiment. After being cleaned with a camel hair brush thirty each of larvae and of male and female adults were weighed individually to the nearest 0.1 mg. and then placed in the rings on the plastic trays already described. The trays containing the weighed insects were placed in the large 900 litre chamber. With them were placed additional trays of insects to make up a total for each treatment of 6 replicates of 30 larvae each and 4 replicates of 30 adults. Larvae and adults were exposed in separate treatments.

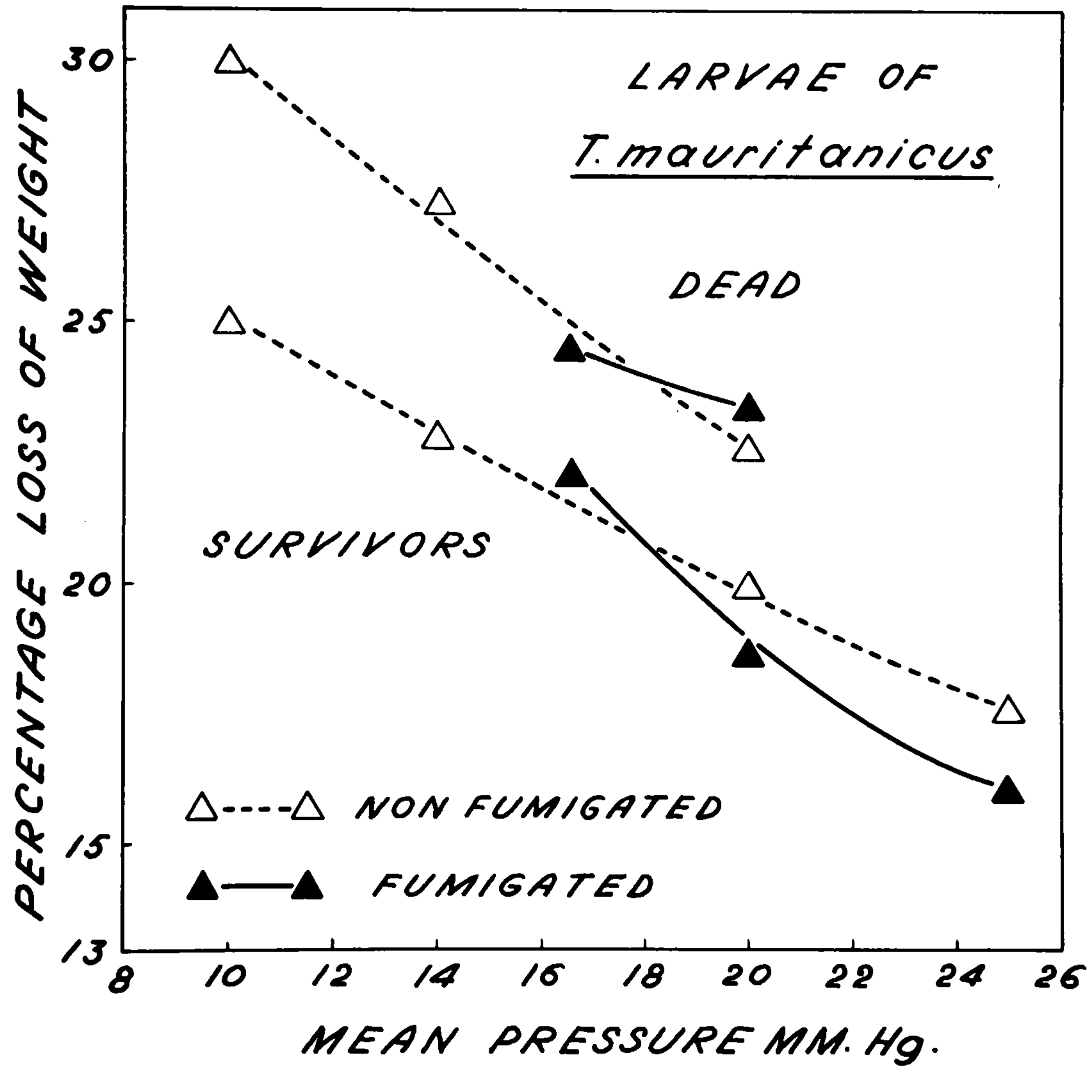


Figure 8. Effect of reduced pressures for three hours exposure, with or without fumigant (CH_3Br 17.3 mg./l.), on percentage loss of weight of dead and surviving larvae of T. mauritanicus, at relative humidity range of 0.7 to 2.0%.

Although this 900 litre chamber is extremely large, considering the size and number of the insects used in these tests, there was an advantage in this instance because the water vapour lost by the insects had very little effect on the humidity of the chamber. It is possible that some of the effects noted in these experiments would not have been observed in a smaller container in which the humidity would have risen appreciably during the exposure of the insects. Bhambhani (1956) found in his experiments with Sitophilus sp. that, in the small glass tubes he used, the amount of water lost from his insects was sufficient to build up a humidity which suppressed further water loss.

The insects were subjected to a range of low pressures by evacuating the air from the chamber until the desired vacuum was reached. In most of the experiments the atmosphere was dry (R.H. approximately 0.7 to 2.5%). Three experiments were also done with the R.H. regulated at 70 per cent in the manner already described. The insects were left exposed to the vacuum for three hours, during which time the pressure rose by 3 mm. for the reasons already given. Air was then re-introduced slowly into the chamber until atmospheric pressure was restored, and the insects were then immediately weighed again to determine the net water loss. The insects were then given a small amount of wholemeal flour and held for one week, after which time a mortality count was made. The observations on mortality are shown in Fig. 7, and the changes in weight (expressed as percentage loss) in Figs. 8 (larvae) and 9 (adults). It is shown that the larvae are able to survive lower pressures than the adults, and that at pressures at which some or all of the larvae survived, and all the adults died, the percentage loss of water from the larvae was less than from the adults.

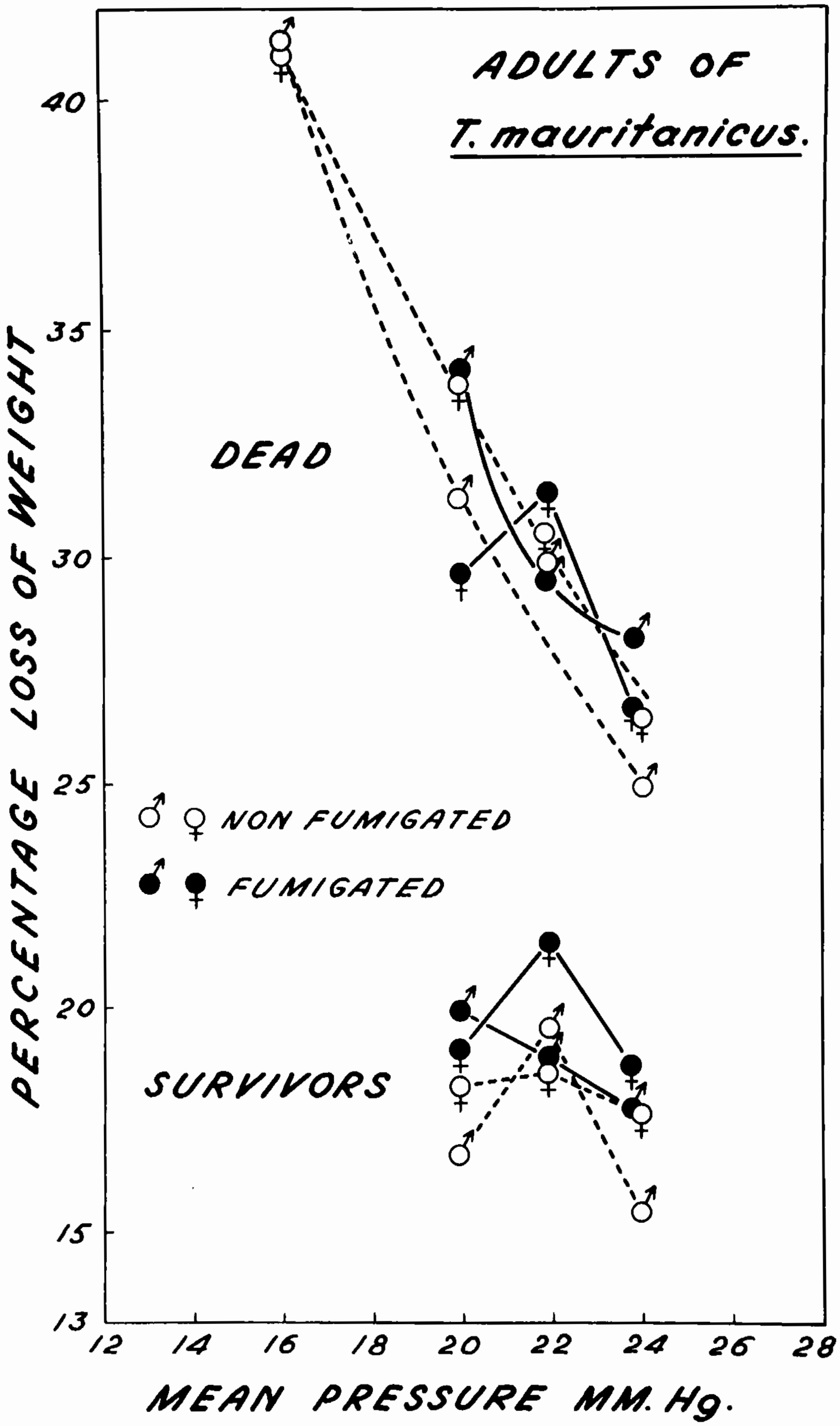


Figure 9. Effect of reduced pressures for three hours exposure, with or without fumigant (CH_3Br 17.3 mg./l.), on percentage loss of weight of dead and surviving adults of T. mauritanicus at relative humidity range of 0.7 to 2.0%.

A noteworthy result with the adults was the great difference in water loss between the survivors and those that succumbed. It was not possible to experiment with the adults in a dry atmosphere over a wide range of pressures, because at 16 mm. all died and at 22 mm. 90 per cent survived. Larval susceptibility in the absence of the fumigant began at 20 mm. and at 10 mm. 30 per cent survived.

Included in Figs. 7, 8, and 9 are results with larvae and adults fumigated with methyl bromide in the same range of reduced pressures. The dosage of methyl bromide of 17.3 mg./l. was chosen because it was toxic at optimum fumigating pressures to a large portion of the population of both larvae and adults (see Figs. 11 and 12). The mortalities for the particular stages at the various pressures are close both for fumigated and non-fumigated lots, and the percentage weight losses are similar under both conditions. These facts give evidence that the mechanism which is preventing water loss in certain individuals, and thus permitting their survival, is the same as that which enables them to survive the exposure to the fumigant. It appeared that this mechanism might lie in some response of the spiracular-tracheal system.

The main site of water loss was determined in this way. Forty mature cadelle larvae were chilled for 24 hours at 3° C. After this time they were quiescent and were removed from the refrigerator. Twenty of the larvae were carefully painted with orange shellac, using a fine camel hair brush, along one side of the body so that all spiracles were covered. They were then returned to the refrigerator and on the following day were turned over and shellacked in the same way on the opposite side. Great care was taken to ensure that the mouth and anus were kept clear of shellac. The larvae were then returned to the refrigerator for another 24 hours. During these manipulations the remaining 20 larvae of the collection were

TABLE 1. LOSS OF WEIGHT OF LARVAE OF T. mauritanicus EXPOSED TO REDUCED AND NORMAL PRESSURES AT 10°C. FOR 16 HOURS

Collection	Weight in Milligrams		
	Before	After	Net Loss
Ten shellacked larvae exposed for 16 hours to reduced pressure (9.7 - 22 mm.)	44.4	43.0	1.4
	50.6	49.9	0.7
	41.9	41.6	0.3
	42.5	41.0	1.5
	33.0	31.4	1.6
	47.8	47.5	0.3
	33.0	32.4	0.6
	35.3	34.3	1.0
	33.3	32.8	0.5
	42.5	40.5	2.0
Means	40.43	39.44	.99
Ten normal unpainted larvae exposed 16 hours to reduced pressure (9.7 - 22 mm.)	28.1	22.6	5.5
	29.8	22.6	7.2
	26.4	21.2	5.2
	31.6	27.6	4.0
	45.5	33.5	12.0
	43.2	34.8	8.4
	36.6	27.3	9.3
	34.8	26.7	8.1
	36.1	28.2	7.9
	33.9	26.4	7.5
Means	34.60	27.09	7.51
Means of ten shellacked larvae kept in room without exposure to reduced pressure	40.95	40.72	.23
Means of ten normal unpainted larvae kept in room without exposure to low pressure	34.15	33.85	.30

Comparison by F test of net loss of weight, following exposure to reduced pressures, of shellacked and normal unpainted larvae.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F.05	F.01
Between treatments	1	212.55	212.55	79.31**	4.41	8.28
Error	18	48.27	2.68			
Total	19	260.82				

**Highly significant difference between treatments.

removed and put back in the refrigerator at the corresponding times, but they were not shellacked. The following afternoon all the larvae were weighed to 0.1 mg. and placed in the main fumigation room which was held at 10° C. Ten each of shellacked and the non-shellacked larvae were placed in the large 900 litre chamber and the pressure was reduced to 9.7 mm. The following morning, after a lapse of 16 hours, during which time the pressure in the chamber had increased to 22 mm., atmospheric pressure was restored. All the larvae were weighed immediately, including the shellacked and non-shellacked ones which had been held all night at 10° C. but not subjected to the vacuum. In this experiment the shellac added considerably to the weight of the larvae. Therefore, the net loss in weight in milligrams was the result analysed. The results of this experiment are given in Table I. The difference between the larvae with their spiracles closed by the shellac and those left unpainted is statistically highly significant. This evidence therefore supports the view that when these insects are exposed to low pressures, loss of moisture occurs mainly through the spiracles.

Behaviour of Insects at Reduced Pressures

The visible reactions of the larvae and adults of T. mauritanicus, mature larvae of Tenebrio molitor L., and adults of Tribolium confusum Duv. were observed through the two portholes in one of the 525 litre chambers (Fig. 2). The insects were placed in lots of ten or more in petri dishes, which were supported close to the glass. The insects were exposed for three hours, both with and without fumigant, to a range of pressures below 100 mm. under dry conditions and also with the humidity established at 70% R.H. The observations are summarised in Table 2. The activity of all species and stages appears to be altered mainly by varia-

TABLE 2. VISIBLE RESPONSE TO REDUCED PRESSURES OF VARIOUS SPECIES OF INSECTS EXPOSED FOR THREE HOURS

Temperature 25° C. methyl bromide (CH_3Br), when introduced, at dosage 17.5 mg./l.

Description of Environment	INSECT SPECIES AND STAGE			
	Tenebroides mauritanicus (L.) Mature Larva	*Tenebroides mauritanicus* (L.) Adult	*Tribolium confusum* Duv. Adult	*Tenebrio molitor* L. Mature Larva
Dry (1-6% R.H.) No fumigant				
A. 25 mm.	Strongly irritated during pressure reduction; motionless during exposure; fully active after pressure restored.	Motionless during exposure; very little activity after restoration.	Motionless; no recovery.	Motionless; fully active after restoration
B. 50 mm.	Irritated during reduction; sluggish during exposure; fully active after restoration.	Same as larva.	Motionless; some recovery of activity after restoration.	Same as A.
C. 100 mm.	Irritated during reduction; normal activity during exposure and afterwards.	Same as larva.	Active, but less than normal.	Normal activity.
Dry (1-6% R.H.) with CH_3Br				
D. 50 mm.	Sluggish, same generally as B.	Same as larva.	Same as A.	Motionless, fully active after restoration.
E. 100 mm.	Same generally as C at start; signs of poisoning towards end.	Same as larva at beginning; all on backs and poisoned at end.	Sluggish, but motionless at end.	Motionless, symptoms of poisoning at end.
Moist (70% R.H.) No fumigant				
F. 25 mm.	Same as A.	Same as larva.	Same as A.	Same as A.
G. 50 mm.	Same as B.	Same as larva.	Motionless; some activity at end.	Same as A.
Moist (70% R.H.) with CH_3Br				
H. 50 mm.	Sluggish, but signs throughout of more irritation than in B & E; symptoms of poisoning towards end.	Same as larva at beginning; motionless at end (poisoned).	Same as A.	Motionless, little activity after restoration, apparently all poisoned.

tions in the pressure at which they are exposed. The other factors of moisture and methyl bromide, separately or in combination, appear to alter the reaction to pressure to only a limited extent.

At 100 mm. both stages of T. mauritanicus and the larvae of T. molitor appeared to be as fully active and normal in their movements as at atmospheric pressure. The activity of adults of T. confusum appeared to be reduced. Below 100 mm. activity was reduced in all species and they became completely motionless at 25 mm. At 25 mm., both in dry and moist conditions, T. confusum failed to recover. The complete mortality of this species at pressures below 35 mm. was confirmed in subsequent experiments (see Fig. 14).

INTRINSIC TOXICITY OF METHYL BROMIDE TO T. mauritanicus AND OTHER INSECTS AT REDUCED PRESSURES

Preliminary Experiments

Before starting the study of the intrinsic toxicity of the fumigant to the insects exposed bare in the chambers, preliminary experiments were undertaken to test the validity of the methods.

Spatial distribution of mortalities.-It was necessary to determine if mortalities of replicates were evenly distributed when the insects were placed at different positions throughout a chamber, and if so, to see if differences between treatments could be demonstrated as statistically significant.

The first experiments, the results of which are given in Table 3, were done under conditions of complete moisture saturation because it was necessary to determine if the introduction of the water contributed to variation. Two doses at mean pressures of 100 mm. and one at 25 mm. were chosen. The

TABLE 3. PRELIMINARY TEST FOR DISTRIBUTION OF MORTALITIES IN EMPTY CHAMBERS

Mature larvae *T. mauritanicus*, 30 per replicate. Sustained vacuum at 25°C. Exposure 3 hours. R.H. approx. 100%

Position of Replicates	Percentage Mortality		
	CH_3Br 11.5 mg./l. @ 100 mm.	CH_3Br 15.4 mg./l. @ 100 mm.	CH_3Br 17.3 mg./l. @ 25 mm.
Front of Chamber			
24 in. from floor (Top)	60	97	70
18 in. from floor	47	100	90
12 in. from floor	52	90	87
6 in. from floor	47	100	72
Back of Chamber			
24 in. from floor (Top)	44	100	87
18 in. from floor	57	100	69
12 in. from floor	47	97	73
6 in. from floor	47	100	62
On Floor			
A - Front	63	100	79
B	37	97	81
C	43	100	77
D	50	100	80
E	43	100	77
F - Back	70	97	83

ANALYSIS OF VARIANCE
(after angular transformation of percentages)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F.05	F.01
Between treatments	2	11604.6894	5802.3447	156.417**	3.80	6.70
Between positions	13	284.3809	21.8755	< 1	3.80	6.70
Error	26	964.4829	37.0954			
Total	41	12853.5532				

**Highly significant difference between treatments.
No significant variations in positions within treatments.

plastic trays, each holding 30 larvae in the individual glass cells, were placed by means of retort stands at the front and rear of the 900 litre chamber at four heights above the floor of 6, 12, 18 and 24 inches. As the internal diameter of this chamber is 29 inches, the upper tray was therefore 5 inches from the top. Also, six trays were placed in line on the floor of the chamber, from front to back. The statistical analysis accompanying Table 3 shows that the differences between treatments were highly significant, but between the different positions the very low F number indicates that these did not contribute significantly to variations in the results. In the next experiment (Table 4) the chamber was dry, (R.H. 7-12%), no water being added. Trays were at three heights of 12, 18 and 24 inches on the retort stands at the front and rear of the chamber. On the floor, three trays were placed together at the front and three at the back. The analysis of variance shows that there were no significant differences between positions or replicates, and that position in the chamber did not contribute to variation in the results.

In the experiments with moisture saturation (Table 3) the results within one treatment were sufficiently homogeneous to permit comparison between treatments. Also when the chamber was dry (Table 4) there were no significant differences between positions or replicates. Thus, the degree of humidity in the chambers, represented by the two extremes of very dry and moisture saturated, did not contribute to variations in the results by affecting conditions within individual treatments.

In most of the experiments with the bare insects plastic trays or petri dishes containing the test lots were placed on the floor of the chamber, this being the most convenient method of handling them. The previous experiment showed that vertical arrangement did not affect homogeneity, and it was now necessary to examine the distribution of results

TABLE 4. PRELIMINARY TEST FOR DISTRIBUTION OF MORTALITIES IN EMPTY CHAMBER

Mature larvae *T. mauritanicus*, 30 per replicate. Sustained vacuum at 100 mm. pressure and 25° C. R.H. 7-12% CH_3Br 17.3 mg./l. for 3 hours.

Position	Percentage Mortality		
	Replicates		
	Height 12 in.	Height 18 in.	Height 24 in.
Front stand	53	57	63
Rear stand	70	50	60
		On floor	
Chamber floor, front	57	71	67
Chamber floor, rear	80	70	70

ANALYSIS OF VARIANCE

(After angular transformation of percentages)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	F.05	F.01
Between positions	3	8.9192	2.9731	< 1	19.16	99.17
Between replicates	2	161.4610	80.7305	3.35	19.16	99.17
Replicates x positions (interaction)	6	144.4104	24.0684	4.01	5.14	10.92
Total	13	314.7906				

No significant difference between positions or replicates.

TABLE 5. PRELIMINARY TEST FOR DISTRIBUTION OF MORTALITIES ON FLOOR OF EMPTY CHAMBER

Mature larvae *T. mauritanicus*, 30 per replicate. Sustained vacuum at 100 mm. pressure and 25° C. R.H. 100% approx. CH_3Br 9.6 mg./l. for 3 hours.

Position	Percentage Mortality		
	Replicates		
Front	14	33	20
Centre	13	33	27
Rear	27	30	22

ANALYSIS OF VARIANCE

(After angular transformation of percentages)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	F.01	F.05
Between positions	2	13.2665	6.6332	0.4096	99.00	19.00
Between replicates	2	141.4346	70.7173	4.3669	99.00	19.00
Replicates x positions (interaction)	4	64.7751	16.1938	4.0484	18.00	6.94
Total	8	219.4762				

No significant difference between positions or replicates.

when all the material was on the floor. In Table 5 it is shown that with a total of 9 trays distributed in three stations on the floor of the 900 litre chamber, there was no statistically significant variation due to stations or replicates.

Placing insects in metal capsules.-In the experiments on the mortalities of the insects within the bales, to be described later, the larvae were placed in the metal capsules already described. Two experiments, one under vacuum at 100 mm. and one at atmospheric pressure, were made to compare the mortalities obtained in the capsules and on the trays over a range of dosages of methyl bromide. These tests also afforded information both on the validity of the experimental methods and on the relative effectiveness of vacuum and atmospheric fumigations in bare chambers. At each dosage mature larvae were placed in the cells on the plastic trays, in four replicates of 30 each. These were distributed evenly on the floor of a 525 litre chamber. Larvae were also put 10 at a time in the metal capsules. Twelve capsules were hung from the roof of the same chamber in four evenly distributed stations, each station comprising three capsules and thus 30 larvae. The vacuum fumigation was done at a mean pressure of 100 mm. The results are shown graphically in Fig. 10 and were evaluated statistically by the method of Wilcoxon and Litchfield (1949).

When placed in capsules the larvae responded to a range of doses of the fumigant at both pressures so as to yield uniform results. In the experiments under vacuum the larvae were more susceptible when placed in the capsules, but at atmospheric pressure there was no significant difference between the responses in trays or in capsules. There was no significant deviation from parallelism among all four lines in Fig. 10.

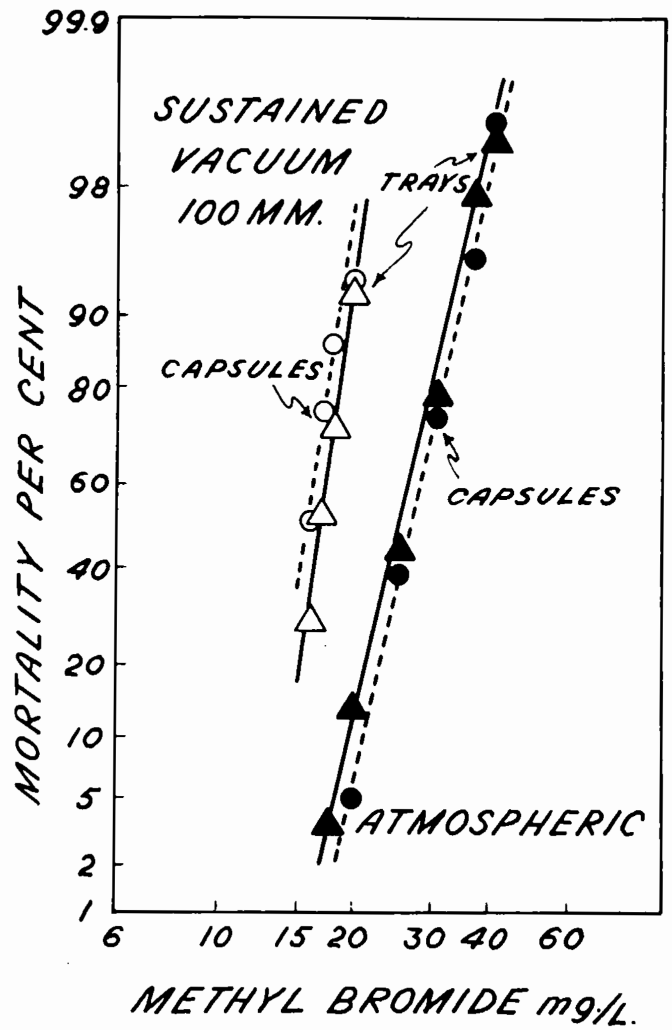


Figure 10. Mortality of mature larvae of T. mauritanicus at sustained-vacuum and atmospheric fumigations with methyl bromide for three hours. Insects placed in glass cells on plastic trays or in metal testing capsules. Each point represents the mortality of 120 insects.

	Treatment	LD_{50} (with 95 per cent confidence intervals).	Efficiency Ratio (with 95 per cent confidence intervals).	
A.	Sustained-vacuum at 100 mm. - capsules	15.9 (15.1-16.9)	Versus B	1.07* (1.07-1.07)
B.	Sustained-vacuum at 100 mm. - trays	17.0 (17.0-17.0)	Versus C	1.47* (1.45-1.48)
C.	Atmospheric fumigation - trays	25.0 (24.0-26.0)	Versus D	1.08 (0.98-1.19)
D.	Atmospheric fumigation - capsules	27.0 (25.2-28.9)		

*Significant P = 0.05

Effect of Different Pressures on Toxicity of Fumigant to *T. mauritanicus*

Because the mature larvae were used in all the experiments with the bales of jute, they were made the principal subjects of this part of the study.

The larvae were fumigated in the plastic trays in six replicates of 30 each placed on the floor of the chambers. Four to six fumigations in separate chambers were made each day. In the range of experiments under dry conditions it was necessary to spread the experiments over three different days, pressures representative of the full range being chosen for each day. With the larvae, the effect of the different pressures was tested under dry (1 - 20% R.H.) moist (70% R.H.) and moisture saturated (approximately 100% R.H.) conditions. Because increase in the humidity of the atmosphere of the experiments made the insects more susceptible to the fumigant at any given pressure the dosages used were as follows: 17.3 mg./l. at 1 - 20% R.H.; 15.4 mg./l. at 70% R.H.; and 13.5 mg./l. at 100% R.H. These dosages, which would yield the best information for each moisture series over the range of pressures, were selected following preliminary experiments. The results with the larvae are shown graphically in Fig. 11. The lower limit of pressures at which experiments could be done at 70% and 100% R.H. was, of course, imposed by the pressure of the water vapour present. (At 25° C. the vapour pressure of water is 23.76 mm.). In order to indicate the fiducial limits of each point on the curve the sample standard deviation for the six replicates is indicated by the vertical lines.

There are two significant considerations arising from the interpretation of the curves shown for the larvae. First, the interaction of pressure and mortality is not direct. In the experiments at low humidity the

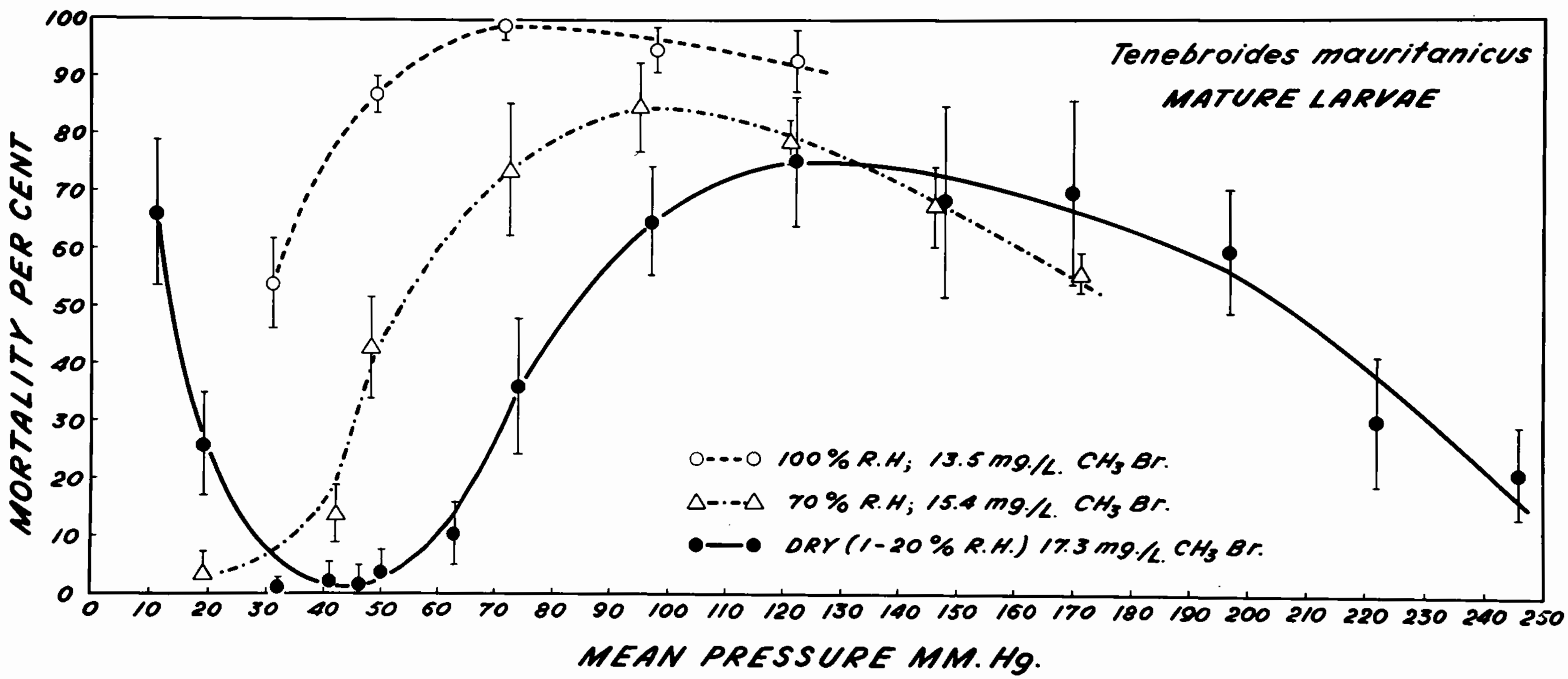


Figure 11. Mortality of larvae of T. mauritanicus exposed to sustained-vacuum fumigation with methyl bromide over a range of reduced pressures in three humidity environments. The vertical lines are the sample standard deviations from the mean percentage mortality at each point.

curve for pressures below 40 mm. follows very closely that for mortality due to dessication as disclosed previously in Fig. 7. At low humidity and at 70% R.H. there is a range of pressures at which mortality approaches zero. As the pressure is increased from this range there is a curvilinear increase in mortality to a peak which moves to the right as humidity is decreased. This peak of maximum mortality then falls away as the pressures are increased again.

Secondly, the curves for mortality at each of the humidity ranges indicate that the response to different pressures is similar throughout. At each humidity the mortality curves are similar after they leave the region of lowest mortality, between 20 and 60 mm. pressure, and approach the peak of highest mortality. From this it may be deduced that they demonstrate an effect which is basically independent of humidity. These pressures for optimum mortality were: in a dry chamber (1 - 20% R.H.), 120 mm.; at 70% R.H., 95 mm.; at moisture saturation (100% R.H.), 70 mm. The results of the experiments on the larvae, as shown in Fig. 11, were conclusive in demonstrating a variation in mortality response of the insects exposed at different pressures but at only one dosage. In view of the importance of these findings, both in theory and practice, it was considered advisable to confirm them by means of a three point assay with graded concentrations. Accordingly, assays were made at 35 and 100 mm. pressure under dry conditions and at 70% R.H. Also included in this series were tests of three manipulations of the pressure system which were later found to be of importance in the fumigation of the bales. Four replicates of 30 larvae each were exposed for each point in the assay.

The results of these tests are shown in Table 6, in which pertinent comparisons are made between different techniques by means of an "efficiency

TABLE 6. COMPARISON OF VACUUM FUMIGATION TECHNIQUES AGAINST BARE LARVAE OF *T. mauritanicus*

Dosage of methyl bromide in milligrams per litre for three hours at 25° C.

Line	Description of Technique	LD_{50} (with 95 per cent confidence intervals)	Efficiency Ratio (with 95 per cent confidence intervals)	
A	Sustained-vacuum, mean pressure 100 mm. R.H. 70%	14.5 (14.2-14.8)	VERSUS B	1.18* (1.11-1.24)
			VERSUS E	1.38* (1.31-1.45)
B	Sustained-vacuum mean pressure 100 mm. Dry (R.H. 5-8%)	17.1 (16.8-17.4)	VERSUS F	1.69* (1.60-1.78)
C	Gradual restoration to atmospheric pressure from 25 mm. Dry (R.H. at beginning 1%, at end 40%)	17 (16.6-17.4)	VERSUS B	1.01 (0.96-1.06)
			VERSUS D	1.04 (0.99-1.09)
D	Atmospheric pressure restored after 45 mins. at 100 mm. pressure. Dry (R.H. at beginning 1%, at end 40%)	17.7 (17.4-18.0)		
E	Sustained-vacuum mean pressure 35 mm. R.H. 70%	20.0 (19.4-20.6)	VERSUS F	1.45* (1.4-1.5)
F	Sustained-vacuum mean pressure 35 mm. Dry (R.H. 2-5%)	29.0 (28.4-29.6)		

*Significant difference between treatments at P = 0.05

There was no significant difference between treatments B, C, and D ratio", which is the ratio between the LD 50s. These values and their confidence intervals were derived by the method of Litchfield and Wilcoxon (1949), the efficiency ratio as used here being the same as the "potency ratio" of these authors. It is seen that, under dry conditions, at 35 mm. the dose of methyl bromide for LD 50 is 1.7 times that required at 100 mm. pressure. At 70% R.H. the ratio at the same two pressures is 1.4.

At this stage, also, it was considered advisable to make a determination by chemical means of the fumigant concentrations acting on the insects at these two operating pressures of 35 and 100 mm. Using the method of chemical analysis described later, my assistant T. Dumas determined the concentrations of methyl bromide in the free air space of the chamber in the region of the trays of insects. Samples were taken at intervals of 5, 15, 30, 60, 90, 120, and 180 minutes after the introduction of the fumigant. As a further check on the effect of the gas concentrations, mature larvae of *T. mauritanicus* in trays were exposed in 6 replicates of 30 each, in the usual manner. The results of these tests are summarized in Table 7.

TABLE 7. METHYL BROMIDE CONCENTRATIONS ACTING ON BARE LARVAE OF *T. mauritanicus* AT TWO PRESSURES

Dosage CH_3Br applied 17.5 mg./l. for 3 hours at 25° C. Insects exposed in 6 replicates of 30 each.

Mean Pressure in Millimetres	Concentration CH_3Br mg./l.		Larval Mortality Per Cent	
	Mean	Standard Deviation	Mean	Standard Deviation
35	16.2	0.5	5.0	4.2
100	16.3	0.4	56.0	11.3

At these two pressures equilibrium in concentration was achieved very rapidly, apparently within the first five minutes. This is shown by the small deviations in fumigant concentration determined at both pressures. The differences in insect mortality are in good agreement with the findings recorded in Fig. 11 and Table 6.

Adults of T. mauritanicus, in lots of 4 replicates of 30 insects each, were treated with methyl bromide over a range of pressures, at low humidity only. The results are shown graphically in Fig. 12. The mortality curve is similar to that for the larvae at the same range of low humidities.

Effect of Different Pressures on Toxicity to Other Insect Species

The implications of the results with T. mauritanicus were of such importance, both in theory and practice, that it was considered advisable to make a limited investigation to see if similar results may be expected with other species of insects. If the response of T. mauritanicus is unique, the study with this insect would not be complete if this singularity were not demonstrated. If, however, other insects or stages of insects respond in the same or similar ways, all these findings become of wider importance. Accordingly a series of experiments, at low humidity only, were made with mature larvae of Tenebrio molitor L. and adults of Tribolium confusum Duv. The first insect was chosen because it is highly resistant to dessication; the second because it was the species used in some of the original investigations on vacuum fumigation, as mentioned in the introductory review.

T. molitor was exposed to methyl bromide over a range of low pressures. In this work two cultures of this insect (A and C), obtained from different sources, were used. The larvae of C were exposed in 6

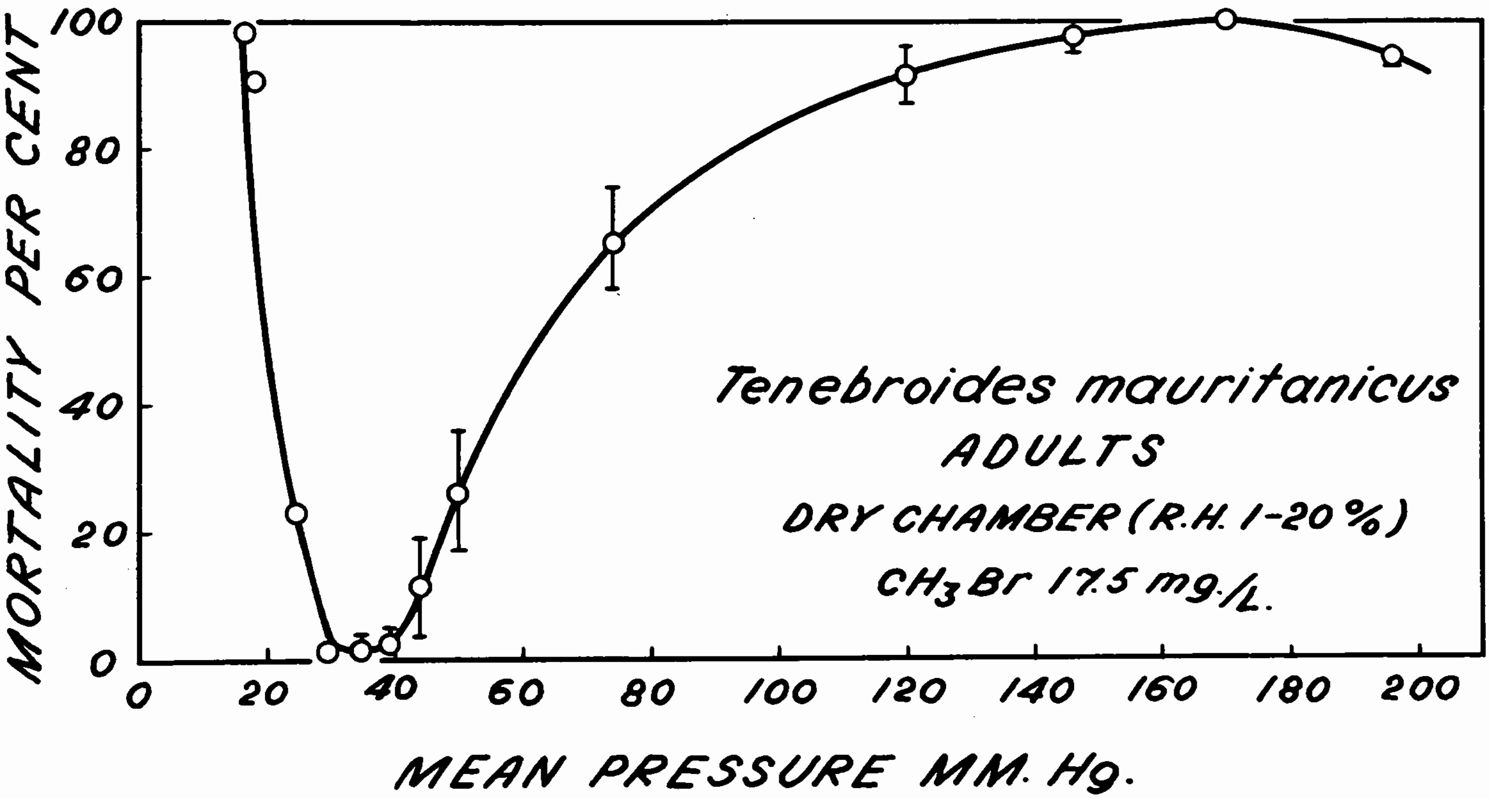


Figure 12. Mortality of adults of T. mauritanicus exposed to sustained-vacuum fumigation for three hours at 25° C. with methyl bromide over a range of reduced pressures in dry environments (R.H. 1-20%). The vertical lines are the sample standard deviations from the mean percentage mortality at each point.

replicates of 30 each to methyl bromide at the dosage of 11.5 mg./l. Also, larvae of A and C were exposed in single lots of 30 each to dosages of 9.6 mg./l. and 13.5 mg./l. respectively. The results are shown in Fig. 13. It is seen that despite the differing susceptibilities of the insects from the two cultures the trend of all three curves is the same. At very low pressures from 15 to 30 mm. mortality is near zero. From 30 to 100 mm. the mortality increases to a peak between 90 and 100 mm. At pressures above 100 mm. the mortality starts to fall away. As was shown for this insect in Table 2, the visible activity is normal at 100 mm., but the insects are motionless at 25 and 50 mm. pressure. The adults of T. confusum were exposed in six replicates of 30 insects each to treatments with the same dosage of methyl bromide over a wide range of pressures, and also to reduced pressures in the absence of the fumigant under dry conditions. Fig. 14 shows the results of this study. It is seen that the curve for mortality in the absence of the fumigant falls away very rapidly from 95 per cent at 50 mm. to zero at 70 mm. In the presence of the fumigant there is also a low point in the mortality curve in the region of 70 - 80 mm., after which the mortality rises again. Here again it is noteworthy that mortality is still rising at 100 mm., at which pressure it was shown (Table 2) that there was visible activity, but that this activity appeared to be less than normal. The significance of these findings will be dealt with fully in the Discussion.

MOISTURE CONDITIONS IN BALES OF JUTE

The experiments with the bare larvae of T. mauritanicus revealed the important influence of humidity on the toxicity of methyl bromide to these insects. The moisture conditions in the jute surrounding the insects

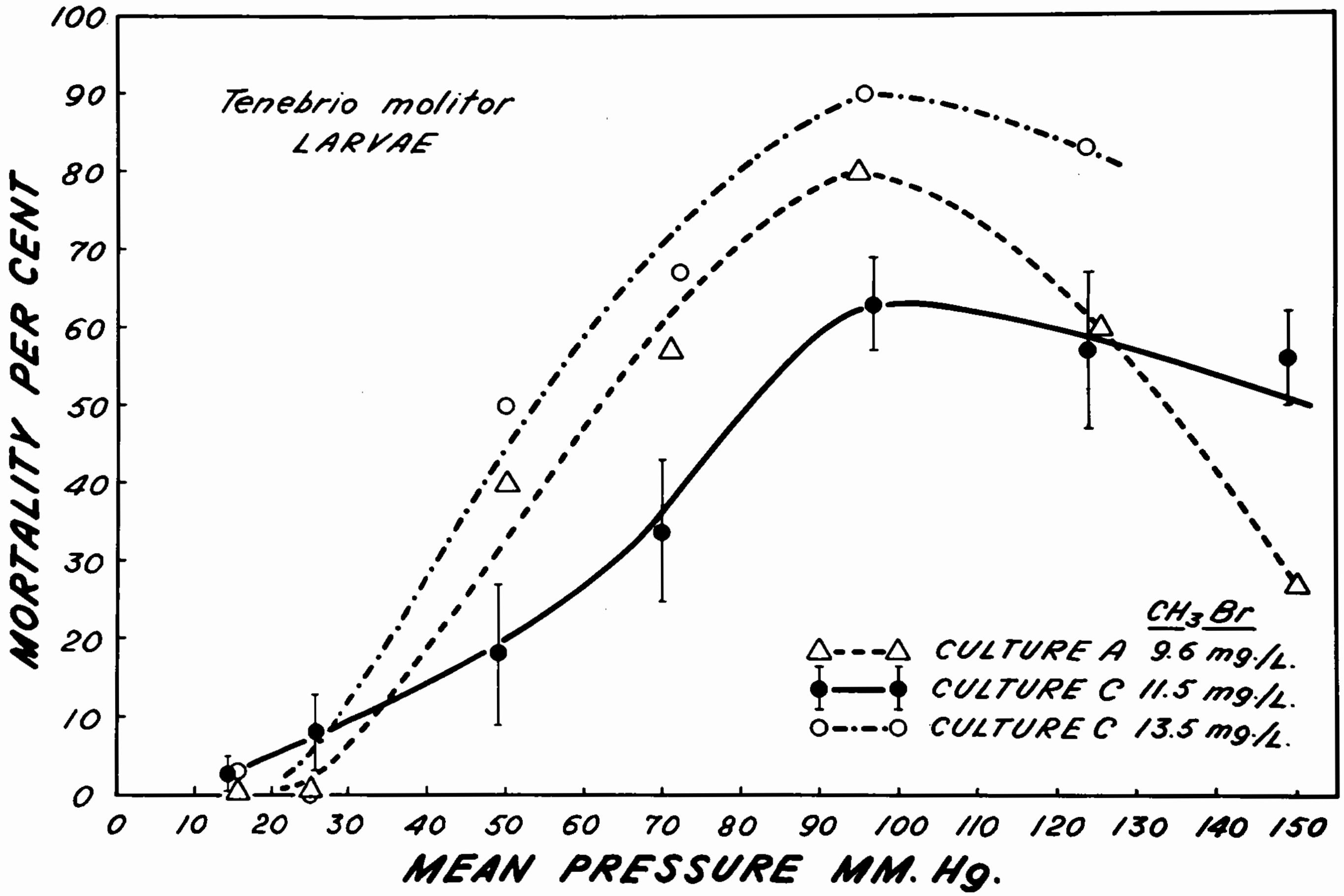


Figure 13. Mortality of larvae of T. molitor exposed to sustained-vacuum fumigation with methyl bromide for three hours over a range of pressures in dry environments (R.H. 1-20%). The vertical line at each point for culture C represents the sample standard deviation.

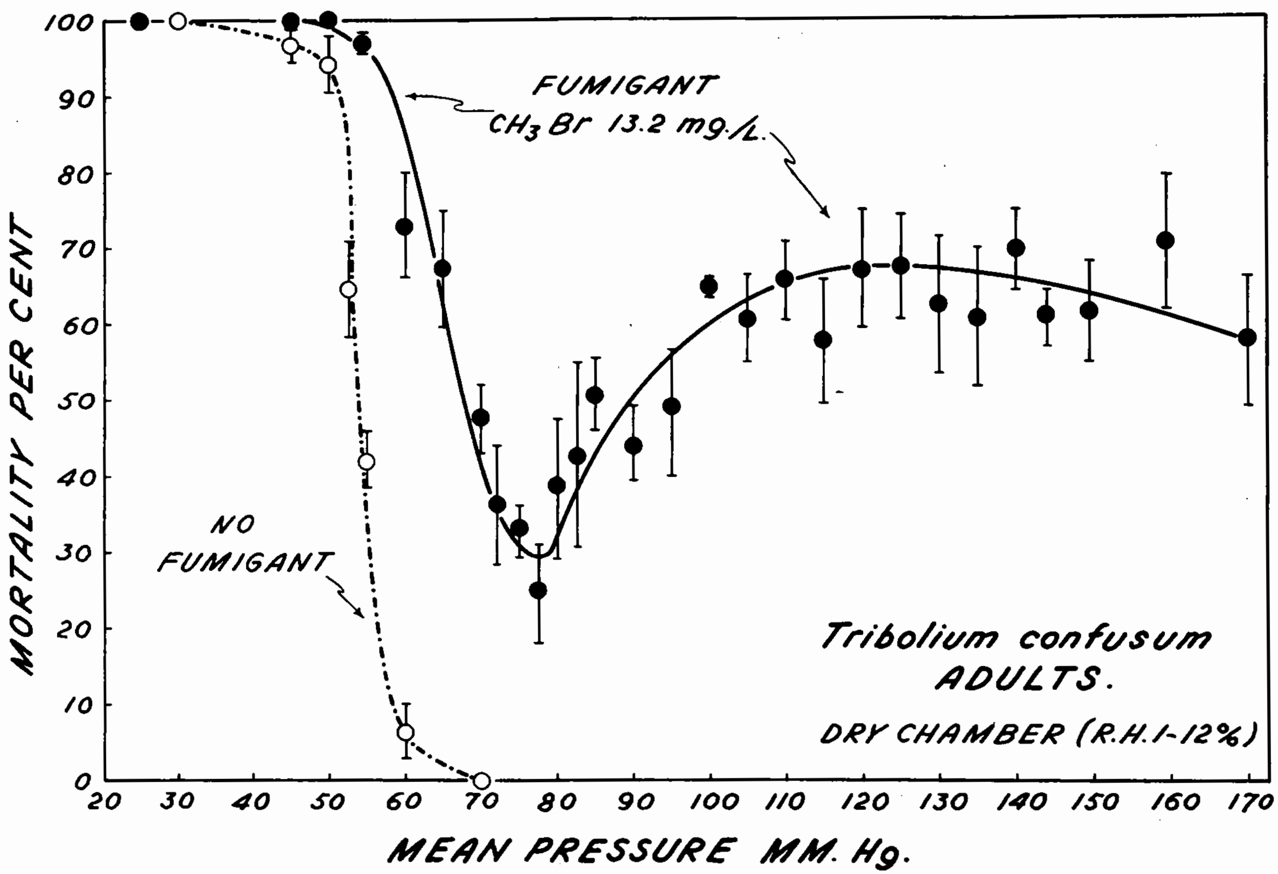


Figure 14. Mortality response of adults of T. confusum at reduced pressures, with or without fumigant, in a dry environment. The vertical lines are the sample standard deviations from the mean percentage mortality at each point.

could therefore be expected to influence the results of the fumigations.

According to Parsons (1953) jute, in common with other textile fibres, absorbs moisture vapour from the atmosphere until a dynamic equilibrium is reached. It may also be assumed from the statement by this authority that if the jute is moved from one atmosphere at a higher humidity to another at a lower humidity, the fibre will lose moisture and come into equilibrium with the new environment. Clearly, the moisture content of the jute would vary according to the conditions under which it was stored before fumigation. Furthermore, a most important point, under certain conditions variations in moisture content could be expected at different positions or depths in a given bale.

Before beginning the study of the response of the insects inside the commodity, therefore, a study was made of moisture conditions under two conditions of storage.

Use of Small Jute Squares as Indicators

For this study a number of pieces of jute 3 x 4 inches were cut out from clean bags representative of the whole collection used in this work. At one end a strip of bleached cotton was sewn (Fig. 6) and a piece of string run through two holes punched in the cotton. A collection of 42 of these were hung in a laboratory room at 22° C. and 45% R.H. for one week. At the end of this time the moisture content of 6 of the pieces selected at random from the collection was determined by oven drying at 105° C. for 16 hours, and was found to be 8.2% on a dry weight basis. The remaining 36 pieces were placed at different depths in a bale of 300 bags while it was being reassembled after storage for 2 months at 25° C. and 70% R.H. Moisture content determinations were made from pieces of bag removed from the depths corresponding to those at which the pieces were to be placed, and the mean was 13.4% dry weight basis. This figure agrees closely with that of 13.2% which is shown for this humidity on a chart published by Powrie and Speakman (1943) based on observations with Chandpur jute cloth.

After reassembly the bale was pressed, bound with wire, and replaced in the same storage under the same environmental conditions. After one and two weeks a piece of jute was pulled from each position and the mean moisture content for the bale determined as 13.1 and 13.4 per cent respectively; thus the dry jute pieces came into equilibrium with the surrounding jute bags in the bale within two weeks.

Moisture Changes in Bales

Two experiments were designed to demonstrate the rate at which moisture changes took place at different depths in bales of jute bags. The results of these two experiments are given in Table 8.

Moist to dry.-300 bags were suspended for 24 hours in a moist room at 25° C. and 70% R.H. Moisture determinations of samples of jute from these bags showed that the mean moisture content was 12.6% dry weight basis. A bale was made up from these bags and a collection of the small jute squares already described was placed in them according to the arrangement outlined in Table 8. This bale was then kept for nearly 4 months in a laboratory room at 22° C., with a relative humidity ranging from 50 to 55 per cent.

The pieces of jute were removed, by means of the attached strings, at intervals as shown in the Table. It will be noted that the jute near the surface of the bale, 0.25 inches from the top, lost moisture quite rapidly. Deeper in the bale the moisture was lost slowly, but apparently equilibrium was being approached after 84 days.

Dry to moist.-A similar experiment was done by placing the pieces of

TABLE 8. MOISTURE CHANGES IN JUTE BALES AFTER REMOVAL TO NEW HUMIDITY ENVIRONMENT

As shown by removal of small jute squares from various positions at different times.

Small jute squares		Percent moisture content (Dry Weight Basis)							
General Position	Distance from Surface of Bale	Days after Removal to New Environment 12	17	24	31	42	56	84	112
		A. MOIST TO DRY (R.H. 70% to 50%)							
Horizontal layer near middle of bale	2-5 in.	12.3	12.3	11.2	11.1	-	-	9.9	
	5-8 in.			12.7	12.9	11.3	10.7		
	8-11 in.			12.6	12.9	-	-	10.3	
	12-18 in. (centre)				13.2	12.0	11.3	10.1	
Vertical array from top to middle of bale at centre of bags	0.25 in.	10.0	9.8	-	9.0	-	-	8.5	
	1.25 in.		10.4	10.4	-	-	9.8	9.1	
	2.5 in.		11.4	10.5	-	10.4	-	9.3	
	5.0 in.			11.5	11.7	-	10.4	9.2	
	7.5 in.			11.9	-	11.6	-	9.8	9.4
	10 in.				12.9	11.5	10.9	-	9.7
		B. DRY TO MOIST (R.H. 50% to 70%)							
Horizontal layer near middle of bale	2-5 in.	10.8	10.5	11.6	-	11.5	-	-	13.1
	5-8 in.		10.6	-	11.3	11.1	11.3		
	8-11 in.		10.2	-	11.3	-	-	-	12.2
	12-18 in. (centre)				11.1	11.4	11.1	-	12.1
Vertical array from top to middle of bale at centre of bags	0.25 in.	12.6	12.4	-	12.3	-	-	13.3	
	1.25 in.	11.6	-	12.6	-	-	12.0	-	14.2
	2.5 in.		11.3	11.9	-	11.7	-	12.8	
	5.0 in.			11.7	11.7	-	11.7	-	13.1
	7.5 in.				11.5	11.3	-	12.6	12.1
	10 in.				11.4	11.1	11.3	-	12.4

jute, which had been kept spread out at 22° C. and 55% R.H. for a week, in a bale made up of bags which had been kept for several months at the same temperature and humidity. The moisture content of the jute in this bale was determined to be 10.1%, agreeing closely with the figure of 10% given by Powrie and Speakman (1943) for Chandpur jute cloth in equilibrium at 55% R.H. This bale was then stored in a room with the climate closely controlled at 25° C. and 70% R.H. The pieces of jute were withdrawn as before. Again adjustment of the moisture content within the bale was rapid near the surface, and slow further in.

Moisture conditions likely to be encountered in bales stored in warehouses or similar places may be judged from these experiments. An important consideration is the moisture content of bales stored in a dry warehouse during the winter months after being assembled during the summer and autumn when air humidities are high. Under these conditions the jute at the outside of the bales would dry rapidly, while the material at the centre might retain a higher moisture content for a long time.

VACUUM FUMIGATION OF BALES OF JUTE BAGS CONTAINING LARVAE OF *T. mauritanicus*

The next stage of the investigation was the study of the response of the mature larvae to vacuum fumigation with methyl bromide when placed in or on the bales of jute.

In all these experiments the metal capsules, each containing 10 insects, were placed three together in the positions described in Table 9, and illustrated diagramatically in Figs. 21 and 22.

All the test capsules placed on or in the bales, regardless of their vertical arrangement, were at the approximate centre of the bale in the horizontal plane.

TABLE 9. DESIGNATION AND DESCRIPTION OF POSITIONS OF TEST CAPSULES CONTAINING *T. mauritanicus* LARVAE IN BALES OF JUTE DURING FUMIGATION

Thirty larvae at each position

Positions B - G inclusive lay at the centre of the bale in the horizontal plane.

Designation	Description of Positions
A	In upper free space of chamber 6 inches away from bale.
B	Lying on top of bale (at centre in horizontal plane) with no jute fabric above it.
C	Inside top bag, with one thickness of jute fabric above it.
D	Five inches from top surface of bale, thus 5 inches from middle, 75 bags above it and 225 below.
E	At middle of bale, 150 bags above and below, 10 inches from top and bottom surfaces.
F	Five inches from lower surface, 5 inches from middle, 225 bags above and 75 below.
G	Lying on floor of chamber, underneath centre of lowest bag in bale.
H	In lower free space of chamber, 6 inches away from bale.

In the long series of experiments with the insects in the jute, at least four and sometimes six bales were exposed on a single day to a graduated series of pressures or dosages or, for comparison of techniques, to a number of different technical manipulations using the same dosage. Thus, full advantage was taken of the range of chambers available to ensure that the insect material, all assembled and randomised on a single day, was homogeneous as far as age was concerned.

For the analysis of the results of most of the individual experiments on the fumigation of the bales, the data for percentage mortality have been grouped under two headings: (1) the mean for the whole system (free air space, periphery of the bale, and the three central positions), based on 240 insects; (2) the figure for the three central positions based on 90 insects. The large amount of data accumulated has called for condensation, and it is thought that this is the best way to do it. In any kind of fumigation the desirable end is to obtain complete mortality of the offending organism throughout the entire fumigation system, including the surface of the commodity and anywhere in the free space. Also, one subject of prime interest and importance is the ability of the fumigant to penetrate within the material. The three central positions in these experimental bales represent loci well within the material and the mortality, at these points, has provided information on the penetration factor.

Preliminary Experiments

Before the main investigation began some preliminary tests were made to obtain basic information on the experimental conditions as they affected the responses of the insects.

Effect of vacuum alone.-Information was needed on the response of the

TABLE 10. EFFECT OF REDUCED PRESSURE ALONE, WITHOUT FUMIGANT, ON LARVAE OF *T. mauritanicus* IN OR ON SIX BALES OF JUTE AND IN FREE AIR SPACE OF CHAMBER

Temperature 25°C.; exposure 3 hours; 30 larvae per position.

Position	PERCENTAGE MORTALITY						
	Mean Pressure 15 mm.						Mean Pressure 30 mm.
	I	II	III	IV	V	Mean	VI
A. Upper free space	64	-	46	55	-		17
B. On top of bale	54	68	48	48	47	53	4
C. Inside top bag	63	60	64	52	57	59	5
D. Five inches from top surface	33	23	32	7	26	24	0
E. Centre of bale	18	33	15	27	7	20	7
F. Five inches from lower surface	23	23	10	35	31	24	4
G. Underneath bale	63	67	70	76	78	71	30
H. Lower free space	59	-	37	46	-		42
Mean of positions B,C,G	60	65	60	55	61	60	
Rank	(7.5)	(10)	(7.5)	(6)	(9)		
Mean of positions D,E,F	25	26	19	23	21	23	
Rank	(4)	(5)	(1)	(3)	(2)		

The difference in percentage mortalities between the three positions towards the centre (D,E,F) and those on or near the surface (B,C,G) is highly significant, $t = -3.6^{**}$.

insects inside the bales when subjected to vacuum alone. This need was stressed by the experiments on the bare insects, already described, in which mortalities were recorded at very low pressures in the absence of the fumigant.

In a series of five experiments insects in five different bales, which had been stored for 4 months at 22° C. and 50% R.H., were subjected to a mean pressure of 15 mm. for 3 hours. The bales were comparatively dry, with a mean moisture content of 9% dry weight basis. The results of these experiments are shown in Table 10. For comparison, the results of a single test done at 30 mm. pressure, also without fumigant, are included. The results of the experiments at 15 mm. were analysed by a rank method described by Oakland (1952). There is a highly significant difference between the positions well within the bale and those at or near the surface. These differences may be ascribed to the fact that, as already shown, the moisture content of the bales stored under these conditions would be higher towards the centre, and the insects at the three inner positions, which suffered the least mortality, would thus be in an environment of higher humidity than those near the surface or in the free air space. At 30 mm. pressure the differences are not so great. These results may be compared with those previously obtained with bare insects at different pressure in dry and moist air (70% R.H.) as shown in Fig. 7.

Validity of results.- In the introduction it was pointed out that the technique of placing capsules containing test insects in commodities undergoing fumigation was open to objection, if only on theoretical grounds. In this study the capsules were drawn into the bales of jute by pieces of string. Here, then, it was necessary to show that the fumigant would reach the insects by the process of diffusion through the material, and that it would not travel along any channels artificially created by the movement of the capsules in and out of the bales. If, in a certain experiment, it could be shown that mortalities of the insects were higher at the periphery of a bale and became progressively lower as the centre was approached there would be direct evidence that the fumigant, in order to reach the insects, had to overcome the obstacle presented by the body of the material.

As will be shown later, in the vacuum fumigations of bales of jute the fumigant diffused very rapidly towards the middle of the bales. At reduced pressures, therefore, it might be difficult to determine the route followed by the fumigant. It was decided to use fumigation at atmospheric pressure to study the pattern of mortalities in relation to depth within the bales. Fig. 15 illustrates the results of these experiments done at atmospheric pressure with 18 hours exposure on bales of 9% moisture content. At the smaller dosages the mortalities were very low at the centre of the bale, and increased as the surface was approached. This relation of mortalities to depth was maintained as the dosage was increased. Not shown by the graph is the fact that mortalities in the free air space were complete at all dosages. From these results it may be concluded that by placing the capsules containing the larvae within the bales, in the manner described, the mortality of the insects could be used as an indication of the diffusion of the fumigant through the bulk of the bale itself, rather than along any abnormal paths which might have been brought about by the technique of the experiment.

Effect of moisture content.-Before beginning the main study with the jute preliminary information was required on how the mortalities of the

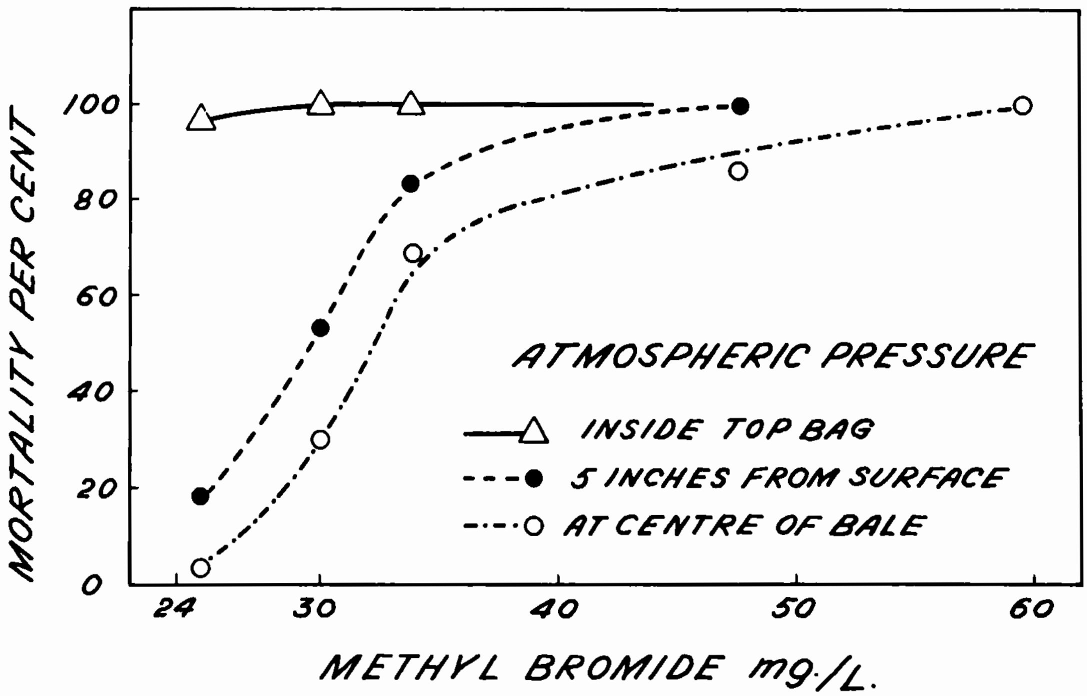
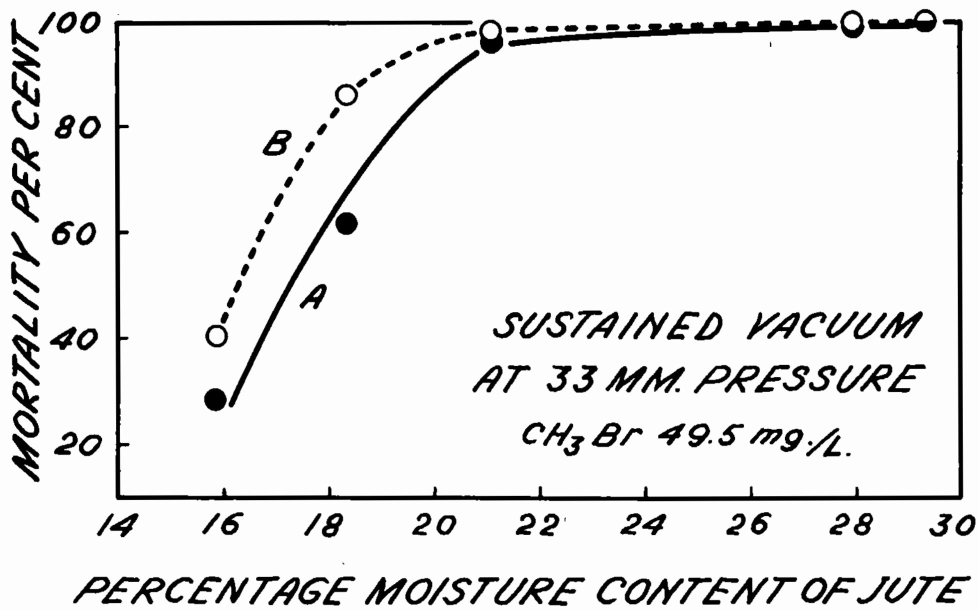


Figure 15. Mortality of mature larvae of <u>T. mauritanicus</u> at different depths in bales exposed to fumigation at atmospheric pressure with methyl bromide for 18 hours at 25° C. Each point represents the results with 30 larvae (3 capsules of ten).



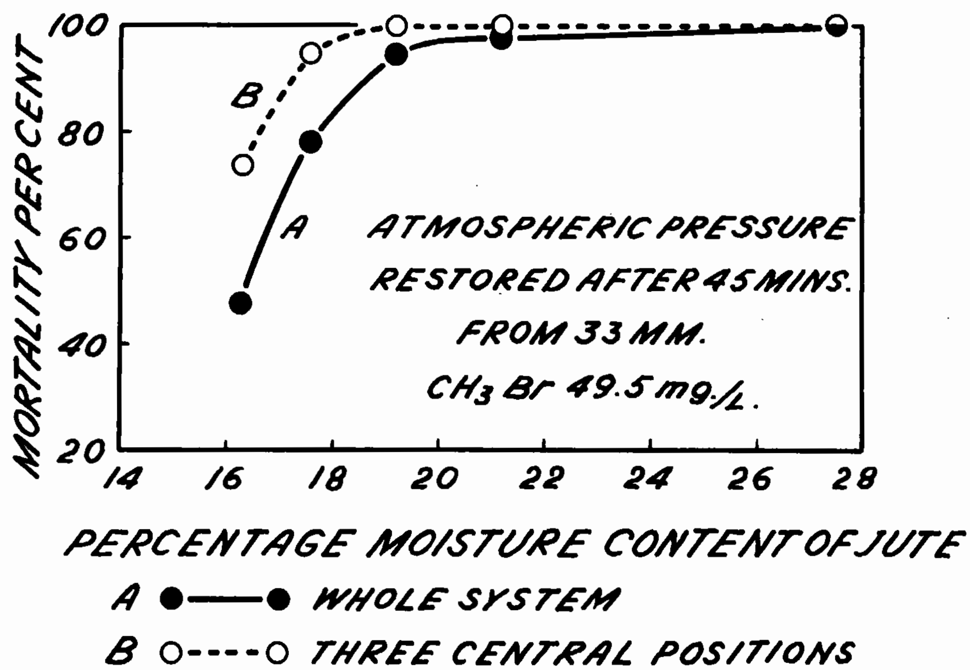


Figure 16. Effect of moisture content of bales of jute on toxicity of methyl bromide to T. mauritanicus in two techniques of vacuum fumigation.

fumigated insects were influenced by the moisture content of the jute in the bales.

A collection of 320 bags, suspended individually from clothes hangers, was kept in a tiled laboratory room at approximately 100% R.H. and 25° C. for 24 hours. The bags were then taken down and one 3 x 3 inch sample piece taken from each of 20 bags from different positions in the room. The remaining 300 bags were assembled in one bale. Fumigations of this bale were done in a sustained vacuum at 33 mm., and in vacuum at 33 mm. with atmospheric pressure restored after 45 minutes. Between fumigations the bale was stored in a room at 22° C. and 50% R.H. and during this time the average moisture content (dry weight basis) fell from 29.3 to 15.8 per cent. The results of these fumigations are shown in Fig. 16. The mortality of the insects at the dosage employed fell off very rapidly as the moisture content fell below 21%. Mortality was consistently lower in all treatments at the periphery of the load, and in the free space, than at the three central positions.

These experiments showed that the moisture content of the jute has an important influence on the effectiveness of the treatments. The steepness of the curves below the point of 95% mortality emphasizes the fact that small changes in moisture content may bring about appreciable changes in mortality. In designing the experiments on the vacuum fumigation of the bales, therefore, provision was made for comparing the effectiveness of the various treatments at two ranges of moisture content (dry weight basis) one at 9 - 10 per cent and another at a higher range of 13 - 14 per cent. These ranges have some practical basis, as the first corresponds to the equilibrium moisture content when bales are stored at 50 - 55% R.H. and the second when the material is kept at 70% R.H. As already pointed out, the first condition might prevail when the material is stored in a dry warehouse. The second condition is likely to be encountered during handling and storage during the summer and early autumn. For example, the moisture content on dry weight basis of bales received from the dealer during the summer and autumn of 1956, determined from 12 samples taken from positions throughout each bale, were as follows:-

2 bales received July 19, 1956, mean moisture content	13.49%
1 bale received Sept.17, 1956, mean moisture content	13.58%
1 bale received Oct. 30, 1956, mean moisture content	12.57%

Hereafter, in this thesis, the bales at the lower range are referred to as those "of low moisture content"; those at the higher range are referred to as "moist bales".

Sustained-Vacuum Fumigation

The results from the fumigation of the bare insects showed significant changes in mortality as the pressures were varied. Therefore, the first work with sustained-vacuum fumigation of the bales was a study of the effect of different pressures between 25 and 225 mm.

Bales of low moisture content.-For the bales of low moisture content two dosages (49.5 and 59.4 mg./l.) were used, found by preliminary experiments to give mortalities in the 50 per cent and 95 per cent ranges, respectively, at the optimum pressure. Because the results with the higher dosage of 59.4 mg./l. of methyl bromide gave results which were of theoretical significance, and also obviously of practical importance, a second series was run to confirm the findings. The results of these experiments are shown in Fig. 17. The optimum pressure for complete mortality in all parts of the system appears to lie between 100 and 125 mm. The curves for the second series with the dosage of 59.4 mg./l. and the one series with 49.5 mg./l. indicate that

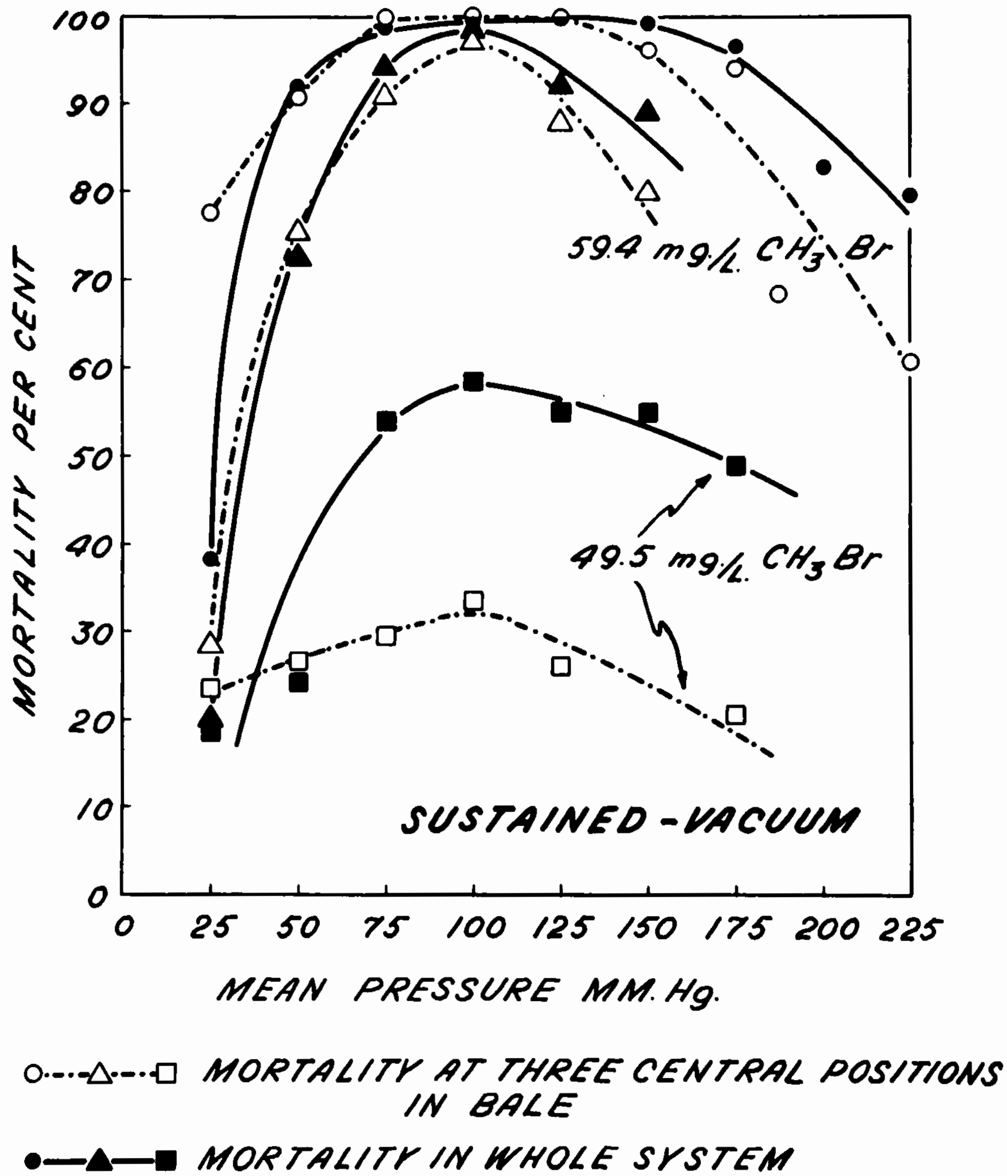


Figure 17. Effect of pressure on mortality of mature larvae of T. mauritanicus exposed at different positions in or near bales of jute bags at low moisture content in sustained-vacuum fumigation with methyl bromide for 3 hours at 25° C. Moisture content of bales 9-10% dry weight basis.

the optimum pressure lies very close to 100 mm. The curve at the higher dosage rises very steeply from 25 mm. and falls away more gradually from 100 mm. to 225 mm. In comparing this curve with those obtained with the bare insects (Fig. 11), the shape of the curve and the position of the optimum is most similar to that for the bare larvae exposed at 70% R.H. This may be accounted for by the fact that the bales, even at this low moisture content, would still supply moisture to the air, and, hence, the insects in the bales were exposed to a comparatively moist rather than a very dry environment.

Moist bales.-Experiments were also made at a range of pressures from 25 to 150 mm. with bales of higher moisture content. The mortality curves (Fig. 18) are again similar to those for the bare insects at 70% R.H., and the optimum pressure is 100 mm.

Distribution of mortalities.-It was mentioned in the introduction of this thesis that in the field trial it was observed that mortalities were higher near the centre of the bales, but lower at the periphery and in the free air space. It was necessary to establish whether this distribution was an artifact, or whether it actually represented a true picture of mortality conditions to be expected as a result of the treatment.

Six bales of low moisture content were fumigated, at a pressure lower than the optimum, in sustained-vacuum. The lower pressure was chosen because it was believed that the explanation of the results of the field trials could be demonstrated better at lower pressures, below the optimum of 100 mm. In each of the six experiments the initial pressure of the system was 15 mm. After the introduction of the fumigant the mean operating pressure during treatment was 24.5 mm. The significance of these pressure differences will be discussed below. The results of these experiments are

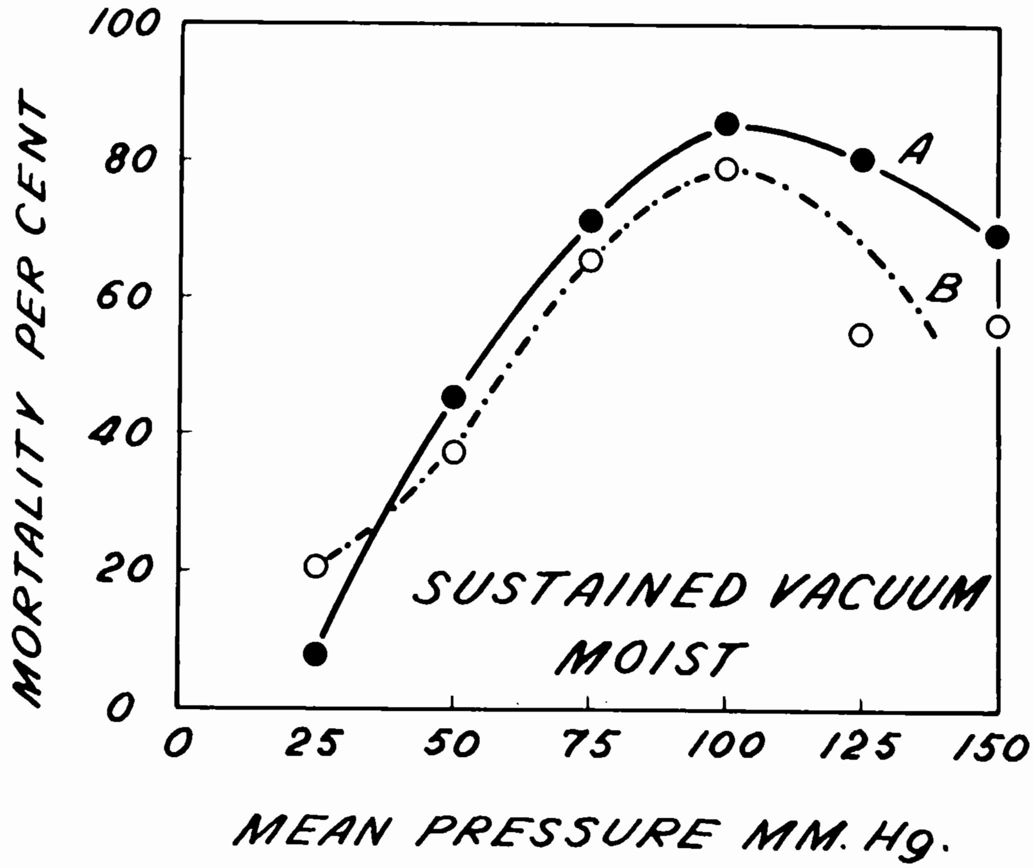


Figure 18. Effect of pressure on mortality of mature larvae of T. mauritanicus exposed at different positions in bales of moist jute bags (13-14% moisture content on dry weight basis) in sustained-vacuum fumigation with methyl bromide at 36.3 mg./l. for 3 hours at 25° C. Broken line shows mortality at three central positions and black line for mortality in whole system.

shown in Table 11. The difference between the central positions D,E,F, and the outer positions A,B,C,G,H, was analysed statistically by the rank method of Oakland (1952) and found to be highly significant. These findings of higher mortality towards the centre of the bales confirmed the observations of the field trials. In view of the results already reported on the distribution of moisture contents in bales, and on the effect of humidity on the mortality of the larvae, the higher mortality in the centre may be ascribed to the effect of higher humidity in this region.

Another mechanism can be suggested to explain the higher mortalities near the centre of the bales fumigated at pressures below the optimum. This is that a slight leakage of air into the chamber would create a pressure differential within the system so that throughout the exposure the fumigant would tend to flow towards the centre of the bale, and methyl bromide concentrations would be higher at this position. This suggestion was tested while the 6 experiments described in the foregoing section were being carried out. These treatments were made in a chamber in which leakage had been cut to a minimum. After the introduction of the fumigant the pressure rose to 27 mm. and then fell slowly during the 3 hour exposure to 22 mm. As will be demonstrated later, in the section dealing with the results of chemical analysis of fumigant concentrations, this fall in pressure is due to progressive sorption of the fumigant by the jute. In these particular experiments, pressure differences caused by any leaks that may have occurred were masked by the sorption effect. Therefore there was no progressive increase of pressure throughout the system which could bring about increasingly higher concentrations towards the centre of the bale. These findings are also confirmed by the results of the chemical analysis (Fig. 26, Charts I and IV), which are described and discussed in a following section.

TABLE 11. DISTRIBUTION OF MORTALITIES IN SIX BALES OF JUTE BAGS FUMIGATED AT SUSTAINED-VACUUM

Mature larvae T. mauritanicus 30 per position. Mean pressure 24.5 mm. Dosage CH_3Br 69.2 mg./l. for 3 hrs. at 25°C.

Position	PERCENTAGE MORTALITY						
	I	II	III	IV	V	VI	Mean
A. Upper free space	50	11	50	73	29	25	40
B. On top of bale	56	29	59	68	30	18	43
C. Inside top bag	32	30	79	63	14	27	41
D. Five inches from top surface	86	93	92	84	69	59	80
E. Centre of bale	63	93	96	100	68	80	83
F. Five inches from lower surface	82	90	87	88	70	81	83
G. Underneath bale	47	70	85	80	38	24	57
H. Lower free space	30	47	54	30	27	19	34
Mean of positions A,B,C,G,H	43	37	65	63	28	23	43
Rank	(9)	(10)	(7)	(8)	(11)	(12)	
Mean of positions D,E,F	76	92	91	91	69	74	82
Rank	(4)	(1)	(2.5)	(2.5)	(6)	(5)	

The difference in mean percentage mortalities between the three positions towards the centre (D,E,F) and those on or near the surface (A,B,C,G,H) is highly significant,

$t = -2.8^{**}$.

Vacuum Fumigation with Atmospheric Pressure Restored After a Period

In the technique of restoring atmospheric pressure after some time of exposure at reduced pressure, as suggested by Brown and Heuser, there are two interacting variables which must be studied before optimum conditions for this method can be defined. These variables are (1) the best time interval before atmospheric pressure is restored after the initial exposure at reduced pressure and (2) the optimum pressure for the initial exposure before restoration. Preliminary experiments at 33 and 100 mm. with both moist bales and bales of low moisture content showed that at dosages of methyl bromide causing high mortalities throughout the system of 90 per cent or better, the best interval of time for preliminary vacuum was 45 minutes. It will be realized that a final determination of the best interval would in itself entail an extensive investigation in which the variables of initial pressure, fumigant dosage, interval time, and the moisture content of the jute would have to be incorporated in a factorial experiment. This problem was not pursued further in the present study because the interval of 45 minutes appeared satisfactory and gave a better over-all mortality than sustained-vacuum, and also because the discovery of the method of gradual restoration, described later, gave promise of a more effective method for vacuum fumigation of baled jute.

Optimum mean pressure.-With the acceptance of 45 minutes as the time interval before restoration of atmospheric pressure, the optimum pressure for the preliminary vacuum was investigated with bales of low moisture content. The results are shown in Fig. 19. This chart may be compared with that in Fig. 17, which gives the results of the sustained-vacuum treatments. With both techniques the optimum pressure was in the region

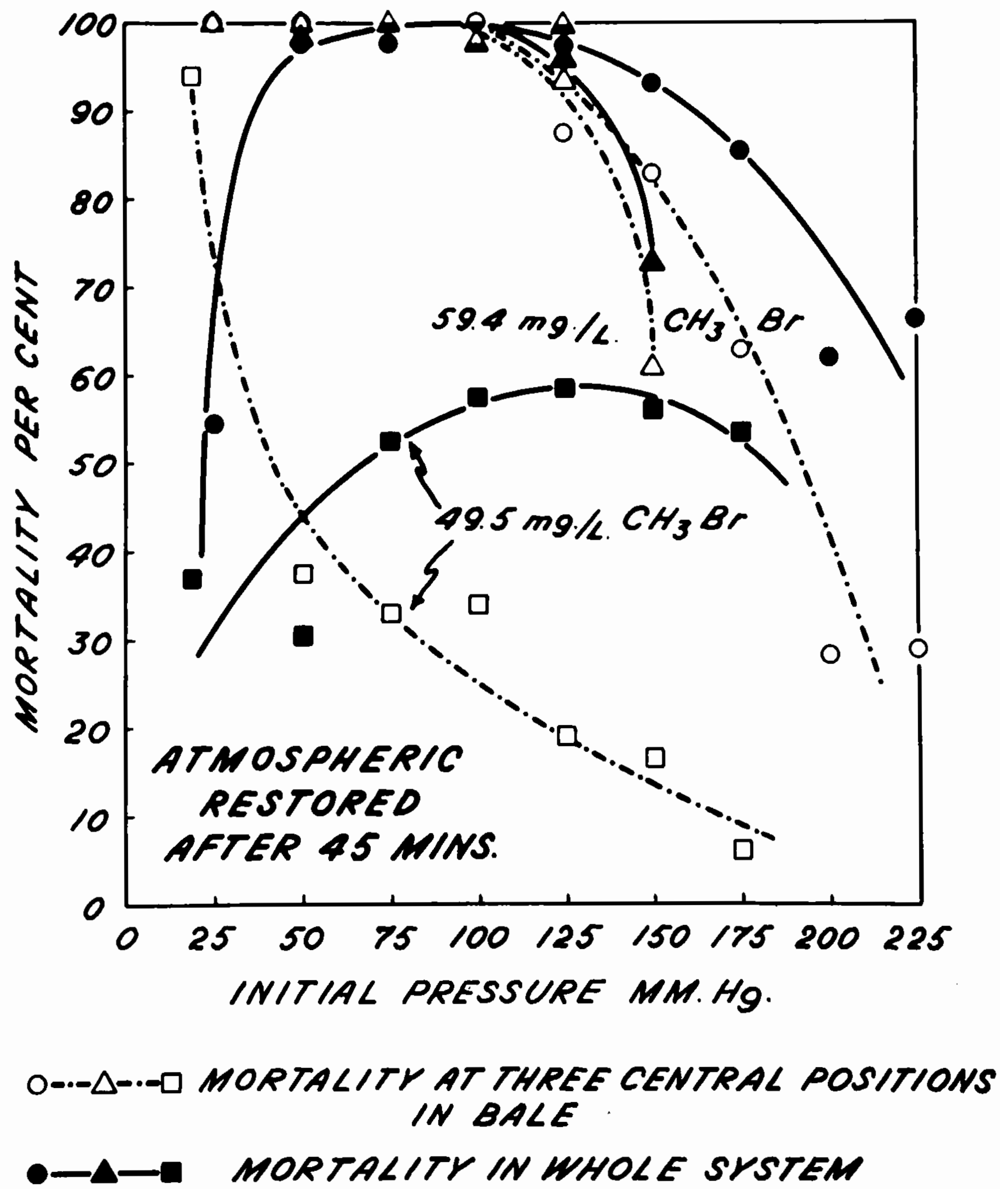


Figure 19. Effect of initial reduced pressure in fumigations with atmospheric pressure restored after 45 minutes. Mortality of mature larvae T. mauritanicus in different positions in or near bales of low moisture content (9-10% dry weight basis) exposed to methyl bromide for 3 hours at 25° C.

of 100 mm. when dosages producing high mortalities are compared. At lower dosages, a remarkable feature is the rapid falling away, in the atmospheric restored technique, of mortalities at the three central positions as the pressures are increased.

Rapidity of restoration.-In the technique of Brown and Heuser atmospheric pressure was restored rapidly. A possible variation of this is to restore atmospheric pressure gradually after the initial period of sustained-vacuum, timing the attainment of atmospheric pressure to coincide with the end of the treatment. Four experiments, each at a different dosage of fumigant, were made with bales of low moisture content to study this method. The results are illustrated in Fig. 22. This variation was not as effective as the rapid restoration method, but was slightly more effective than the sustained-vacuum, in all parts of the system.

Gradual Restoration of Atmospheric Pressure

There is one variation in the technique of restoration of atmospheric pressure which, to the writer's knowledge, has not previously been investigated. That is to draw a high initial vacuum on the system and then gradually to restore atmospheric pressure, over an extended period. This method was studied because it was thought that it would bring about a more even distribution of the fumigant throughout the system. Again there were two interacting variables to resolve, the optimum for initial pressure and for length of time of restoration. Because a high initial vacuum would provide a greater pressure differential it was a reasonable course to investigate first the optimum time, using an initial vacuum of 25 mm. In these experiments the air was restored through two copper tubes of 2 mm. internal diameter, one with the outlet at the top of the chamber, and the other at the bottom. The required restoration times were achieved

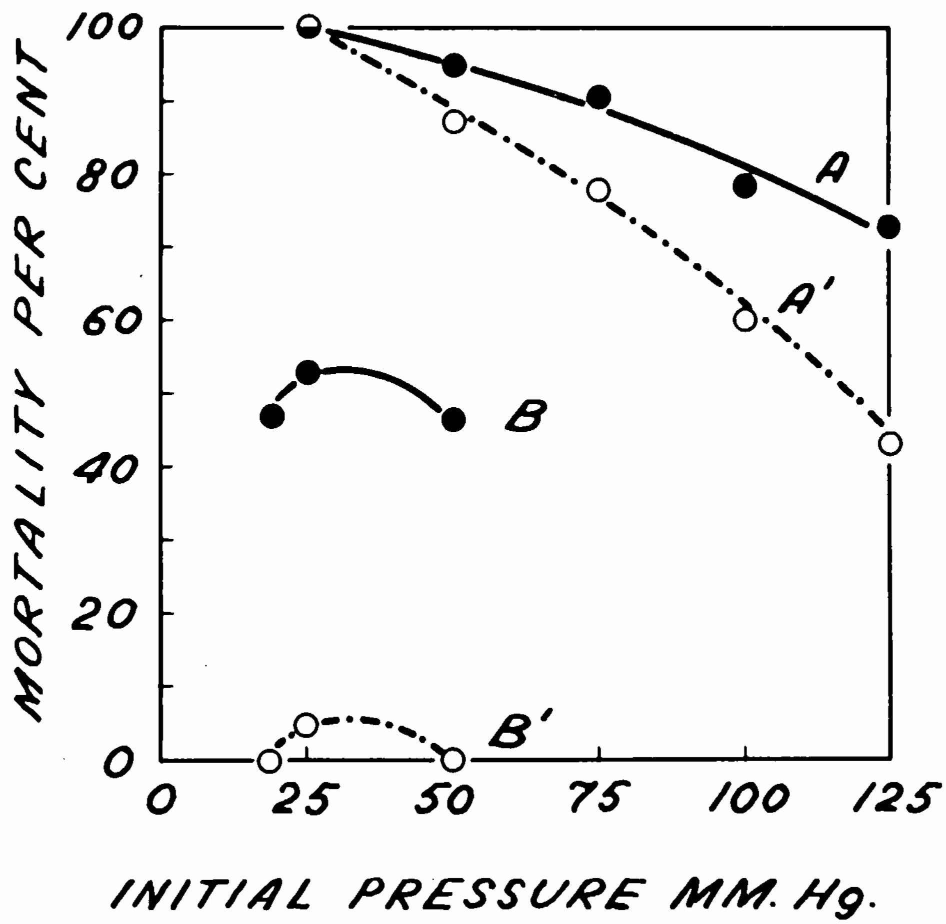


Figure 20. Mortalities of mature larvae of T. mauritanicus exposed to methyl bromide 59.4 mg./l. for total exposure of three hours at 25° C. in bales of low moisture content (9-10% dry weight basis).

Curves A, A[1] effect of initial pressure of fumigation system before beginning of gradual restoration of atmospheric pressure over a period of 2 hours.

Curves B, B[1] effect of initial pressure in the method of simultaneous introduction.

In both pairs of curves, broken line is for three central positions in the bale, black line for the whole system.

by regulating the diameter of the tubes at the inlet. Preliminary experiments indicated that a period of two hours for the restoration of atmospheric pressure gave the highest mortalities throughout the whole system. Here again an extensive investigation, with all variables taken into account, would be needed to find the best length of time for restoration under various conditions. With this period of two hours experiments summarized in Fig. 20 showed that, with bales of low moisture content, of those initial pressures tested, 25 mm. gave the highest mortalities in all parts of the system.

It was at once obvious that this new method of gradual restoration of atmospheric pressure was very effective under the condition of these experiments, and might be tested in wider applications for certain commodities or species of insects. Following the scheme of descriptive terms for vacuum fumigation suggested by Page <u>et al</u>. (1953) this technique may be referred to as "Vacuum fumigation with atmospheric pressure restored gradually after dosage".

<u>Immediate Restoration</u>

Experiments on the bales showed that this technique, sometimes referred to as the vacuum "released" or "dissipated" method, produced erratic results. In these tests, after the initial vacuum was produced, the atmospheric pressure was restored immediately and rapidly by opening the inlet valve (Fig. 2) at the front of the chamber and allowing air to flow in over a period of 2.5 minutes. A preliminary series of trials indicated that an initial pressure of 50 mm. was optimum for the vacuum produced before the introduction of the fumigant and the restoration of atmospheric pressure. Although 25 mm. of initial pressure provided a high pressure differential, it was seen that complete mortality was produced at the centre of the bale at the expense of the whole system.

A series of three trials shown graphically in Fig. 21, comparing this method with others, showed that it was less effective than all other methods investigated, except the technique of simultaneous introduction.

Simultaneous Introduction

Experiments with this method were designed to simulate as closely as possible the actual physical conditions of the technique as described by Lepigre (1949). As mentioned previously, in practice this method is carried out by the use of a gasometer in which the fumigant is stored as a gas, and from which it flows at the beginning of the treatment, being mixed in correct proportions in the air as it flows into the evacuated fumigation chamber. In the present experiments a gasometer was not used, but the manipulation described below produced the conditions required for introduction of the gas-air mixture into the fumigation chamber, according to this method. A bale of low moisture content, and the usual array of test insects, was loaded into one of the 525 litre chambers. The remaining 5 of the 525 litre chambers and the larger chamber of 900 litres were used as fumigant reservoirs. After they were all evacuated to 10 mm. pressure a pre-determined weight of methyl bromide was introduced into the large chamber and allowed to come to equilibrium throughout the 6 chambers comprising the reservoir. Atmospheric pressure was then restored in the reservoir chambers and all the available circulating fans turned on in each of them. After 10 minutes, readings for methyl bromide concentration in 3 of the reservoir chambers were taken by means of a Gow-Mac thermal conductivity analyser*. These readings showed that the

*Gow-Mac Instrument Co., Newark 5, N.J., U.S.A. The use of this method for determining concentrations of methyl bromide gas was first reported by Phillips and Bulger (1953). The application of the method for methyl bromide analysis in the experimental chambers was described by Monro and Buckland (1956).

fumigant was evenly distributed throughout the reservoir system. (Readings in the 3 chambers were 70.3, 70.9, and 70.8 mg./l. of methyl bromide respectively).

Now that the fumigant-air mixture was evenly distributed and ready for entrainment, the chamber containing the bale was evacuated to the required initial pressure. By means of appropriate valves, the chamber was then disconnected from the pump and the fumigant-air mixture was allowed to flow in from the reservoirs. Equilibrium of pressure at 700 mm. was reached throughout the entire system in 2.5 minutes, and the fumigation chamber was closed off from the rest of the system. Air was next introduced directly into the fumigation chamber to bring it to atmospheric pressure for the 3 hour exposure period.

Mortality results with this method were poor. A series of 3 experiments, as shown in Fig. 20, indicated that there might be an optimum initial pressure of 25 mm. before introduction of the air-gas mixture into the fumigation chamber. Comparison with other techniques (Fig. 21) showed that, under the conditions of this investigation, it gave the lowest mortalities for the whole system of all methods studied. Mortalities on the three central positions in the bales were all zero or near to zero. Some mortalities in the free space and at the periphery of the bales were high, but this was obviously due to the fact that penetration was poor and therefore high concentrations were left in the free space. These results with the insects may also be compared with the chemical analysis illustrated in Fig. 28 (Chart VII).

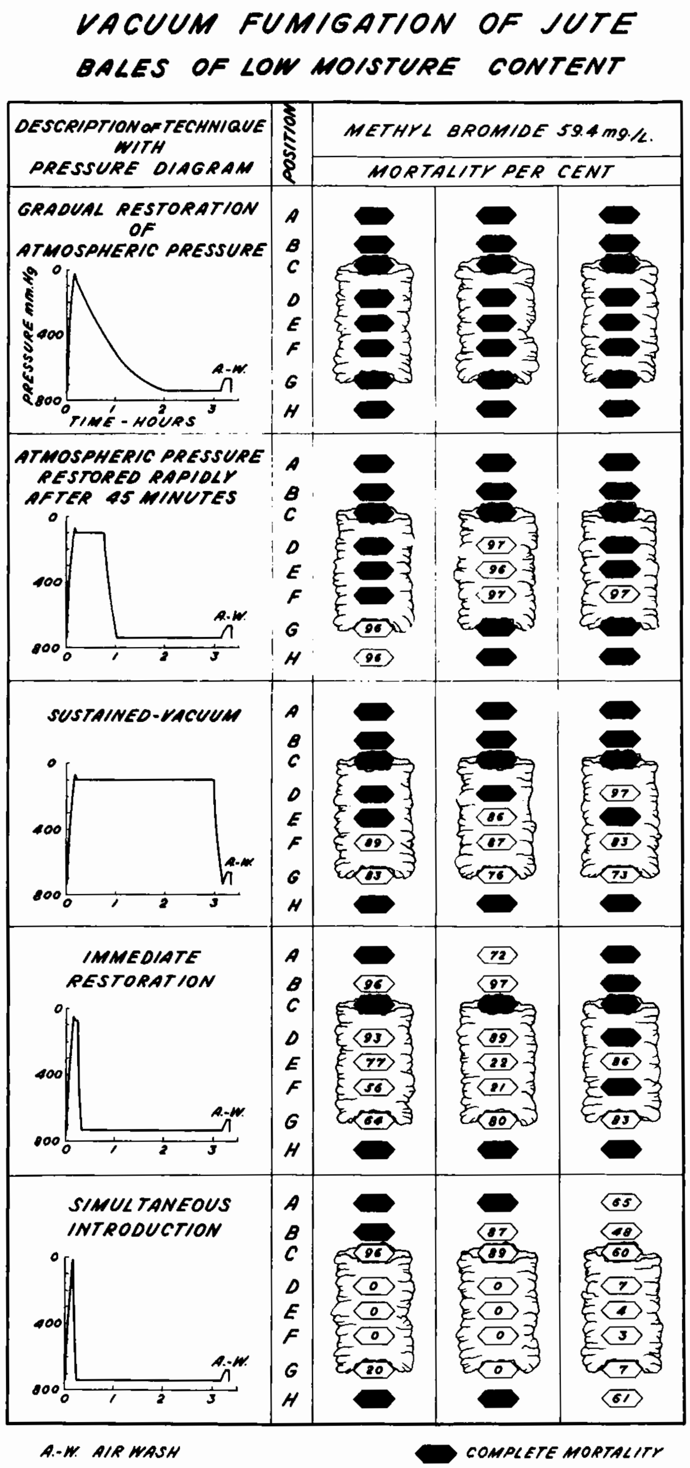


Figure 21. Diagrammatic comparison of mortalities of <u>T. mauritanicus</u> larvae in five techniques of vacuum fumigation of bales of moisture content 9-10%, dry weight basis. Temperature 25° C.

DIRECT COMPARISON OF VACUUM FUMIGATION TECHNIQUES

The apparent optimum conditions were now established for some variations in the technique of vacuum fumigation as applied to bales of jute. The next step was to make direct comparisons of the efficiency of these variations.

Bales of Low Moisture Content

The first experiments, the results of which are shown in Fig. 21 consisted of 3 replicated treatments with each of the variations, all at the same dosage of 59.4 mg./l. This treatment gave complete mortality only in the method of gradual restoration.

The 3 most effective methods were then tested in a series of experiments at graduated dosages, as it was considered that tests at different fumigant concentrations would be the best way to confirm the previous findings. Included in this series, but not shown in the statistical analysis, was the technique of gradual restoration of atmospheric pressure after an initial exposure to 100 mm. pressure for 45 minutes. The results are presented graphically in Fig. 22. A statistical analysis by the method of Litchfield and Wilcoxon (1949), is shown graphically in Fig. 23, and summarized in Table 12.

The method of gradual restoration of atmospheric pressure for two hours after a high initial vacuum was significantly more effective than any of the other treatments for both groupings of positions. The close proximity of lines A and A^1 in Fig. 23 emphasizes the even distribution, in this method, of mortalities throughout the whole system. The technique of rapid restoration after 45 minutes was more effective than the sustained-vacuum, but in both methods there was uneven distribution, as the mortalities were higher throughout the entire system than at the central positions.

VACUUM FUMIGATION OF JUTE BALES OF LOW MOISTURE CONTENT

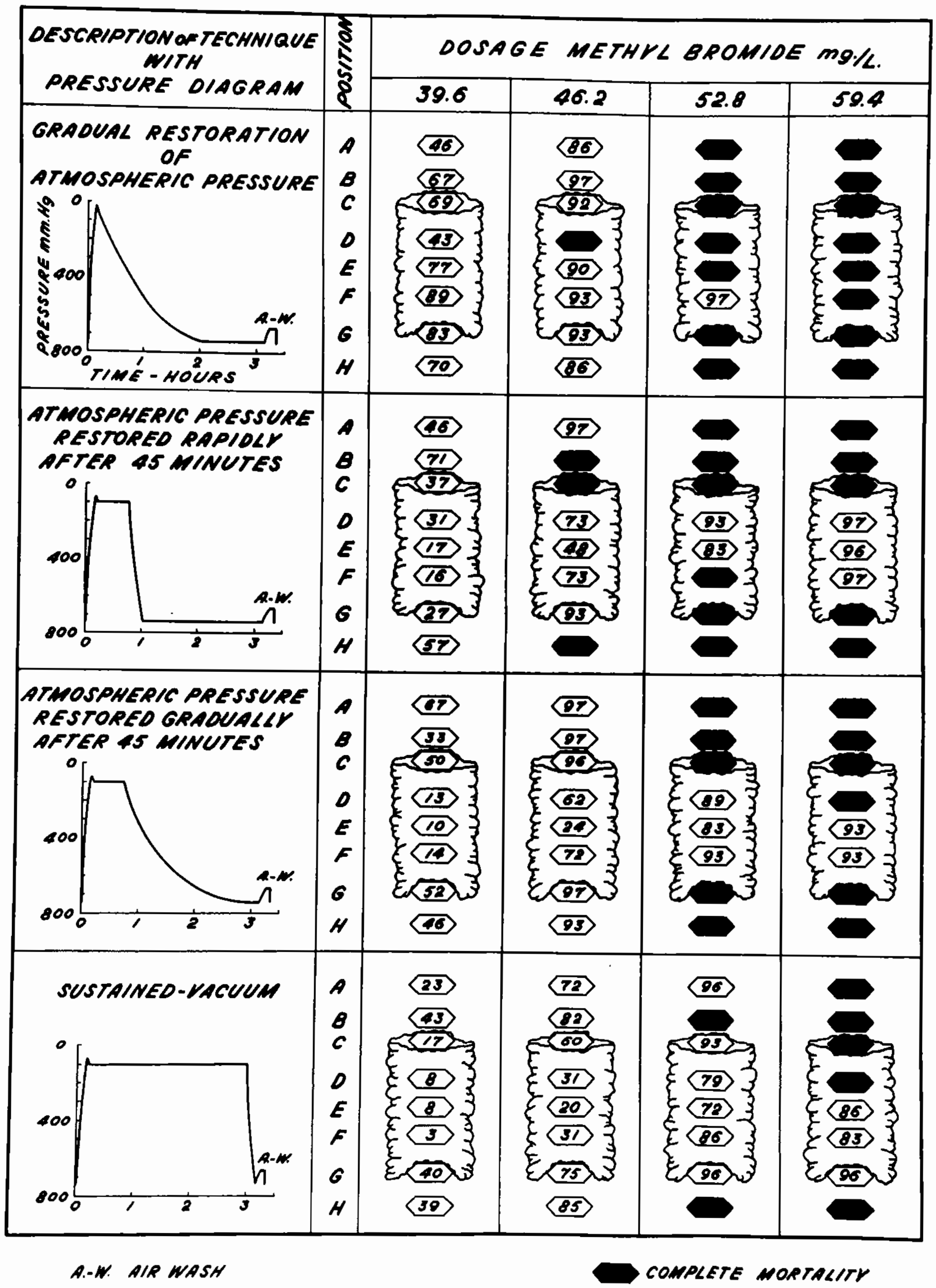


Figure 22. Diagrammatic comparison of mortalities of T. mauritanicus larvae in four techniques of vacuum fumigation of bales of moisture content 9-10%, dry weight basis. Temperature 25° C. The figures in the panels refer to percentage mortality at that position.

See Fig. 23 for logarithmic plot of results and Table 12 for summary of statistical analysis.

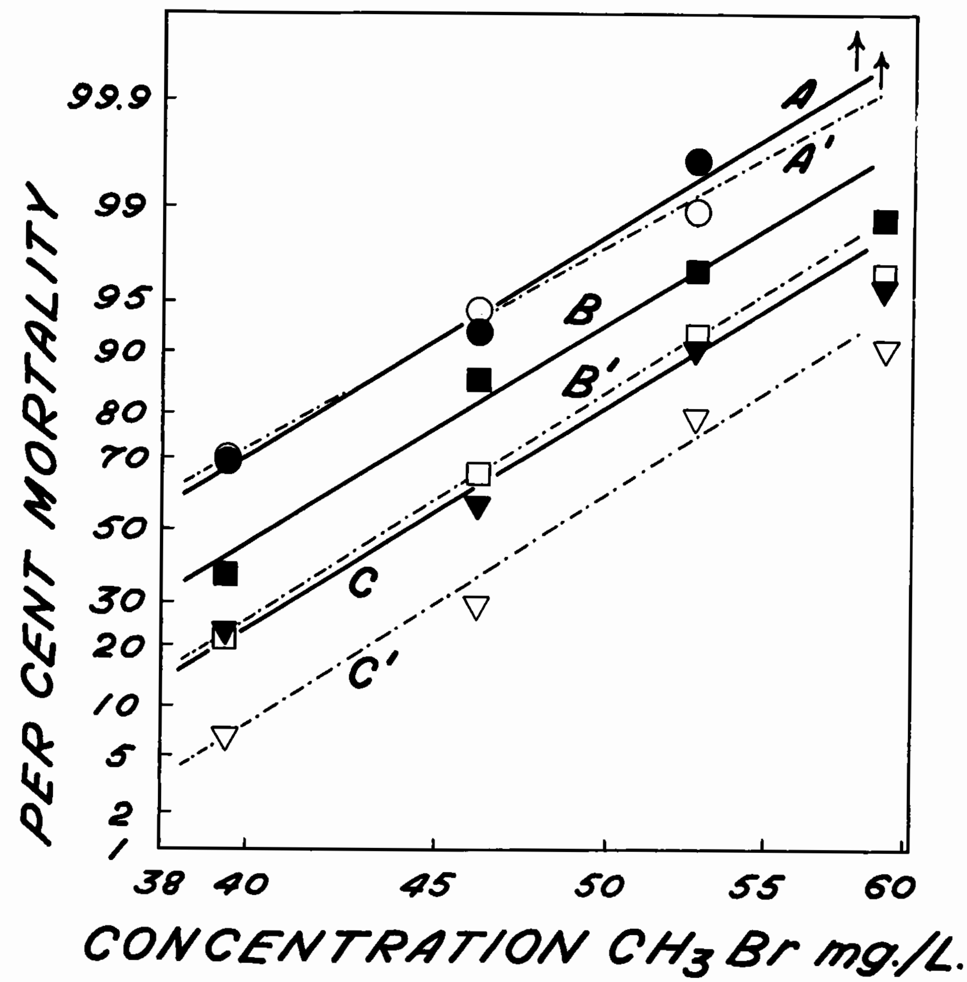


Figure 23. Efficiency of techniques of vacuum fumigation against T. mauritanicus for three hours at 25° C. of bales of jute bags, as shown by dosage response curves on a logarithmic plot. Data of Figure 22. For each technique the broken line is that for the mortality at the three central positions, the black line that for the mortality throughout the whole system. Moisture content 9-10% dry weight basis.

A,A[1]. Slow restoration of atmospheric pressure after initial vacuum of 25 mm.

B,B[1]. Restoration of atmospheric pressure quickly after initial sustained-vacuum of 100 mm. for 45 minutes.

C,C[1]. Sustained vacuum at 100 mm. Hg. for entire exposure.

For summary of statistical analysis see Table 12.

TABLE 12. COMPARISON OF VACUUM FUMIGATION TECHNIQUES AGAINST LARVAE OF *T. mauritanicus* IN BALES OF LOW MOISTURE CONTENT

Statistical analysis of data presented graphically in Figs. 22 and 23. Dosage of methyl bromide in milligrams per litre for three hours at 25° C.

Line in Fig.	Description of Technique	Position of Insects in Chambers	LD_{50} (with 95 per cent confidence intervals)	Efficiency Ratio (with 95 per cent confidence intervals)	Deviation from parallelism of regression lines P = 0.05
A	Gradual restoration of atmospheric pressure from 25 mm.	Whole system	37.2 (36.5 - 37.9)	VERSUS B = 1.09* (1.04 - 1.44)	Not significant
A[1]	"	Three central positions of bale	36.6 (35.1 - 38.3)	VERSUS B[1] = 1.20* (1.16 - 1.23)	"
B	Atmospheric pressure restored after 45 mins. at 100 mm.	Whole system	40.6 (39.7 - 41.5)	VERSUS C = 1.09* (1.07 - 1.11)	"
B[1]	"	Three central positions of bale	43.8 (42.6 - 45.1)	VERSUS C[1] = 1.10* (1.03 - 1.18)	"
C	Sustained-vacuum	Whole system	44.3 (43.3 - 45.2)	VERSUS C[1] = 1.09* (1.07 - 1.11)	"
C[1]	"	Three central positions of bale	48.3 (47.0 - 49.7)		

*Significantly more efficient, P = 0.05.

Moist Bales

Bales of 13.5% moisture content (dry weight basis) were also subjected to the three most effective techniques. This time only a range of 3 dosages was employed because after these were tested it was clear that there were no significant differences between these methods when bales are moist. An exception, within one treatment but not for the treatment as a whole, was the result for the three central positions in the method of gradual restoration (Line A^1, Table 13). The efficiency ratio for this group was significantly higher than that for the corresponding central positions in sustained-vacuum fumigation (Line C^1). The results of this series of tests are shown graphically in Fig. 24. The statistical analysis is summarized in Table 13.

INTERACTION OF FUMIGANT AND COMMODITY

A proper interpretation of the reaction of the insects in the bales of jute to the various treatments applied cannot be made unless there is knowledge of the fumigant concentrations present in the different parts of the system. This information was obtained by means of the analysis of gas samples removed at intervals of time during the treatments from the free air space and from the centre of the bales. The chemical analyses described in this section were made by T. Dumas, Assistant Chemist in my Section.

Method of Chemical Analysis

Samples of the fumigant-air mixture were extracted at intervals from the sampling points, through copper tubing of 0.15 mm. I.D., by means of round bottom flasks of 40 ml. capacity. The flasks were first evacuated to 0.2 mm. pressure and then connected to the outlet of the copper tubing

VACUUM FUMIGATION OF JUTE
MOIST BALES

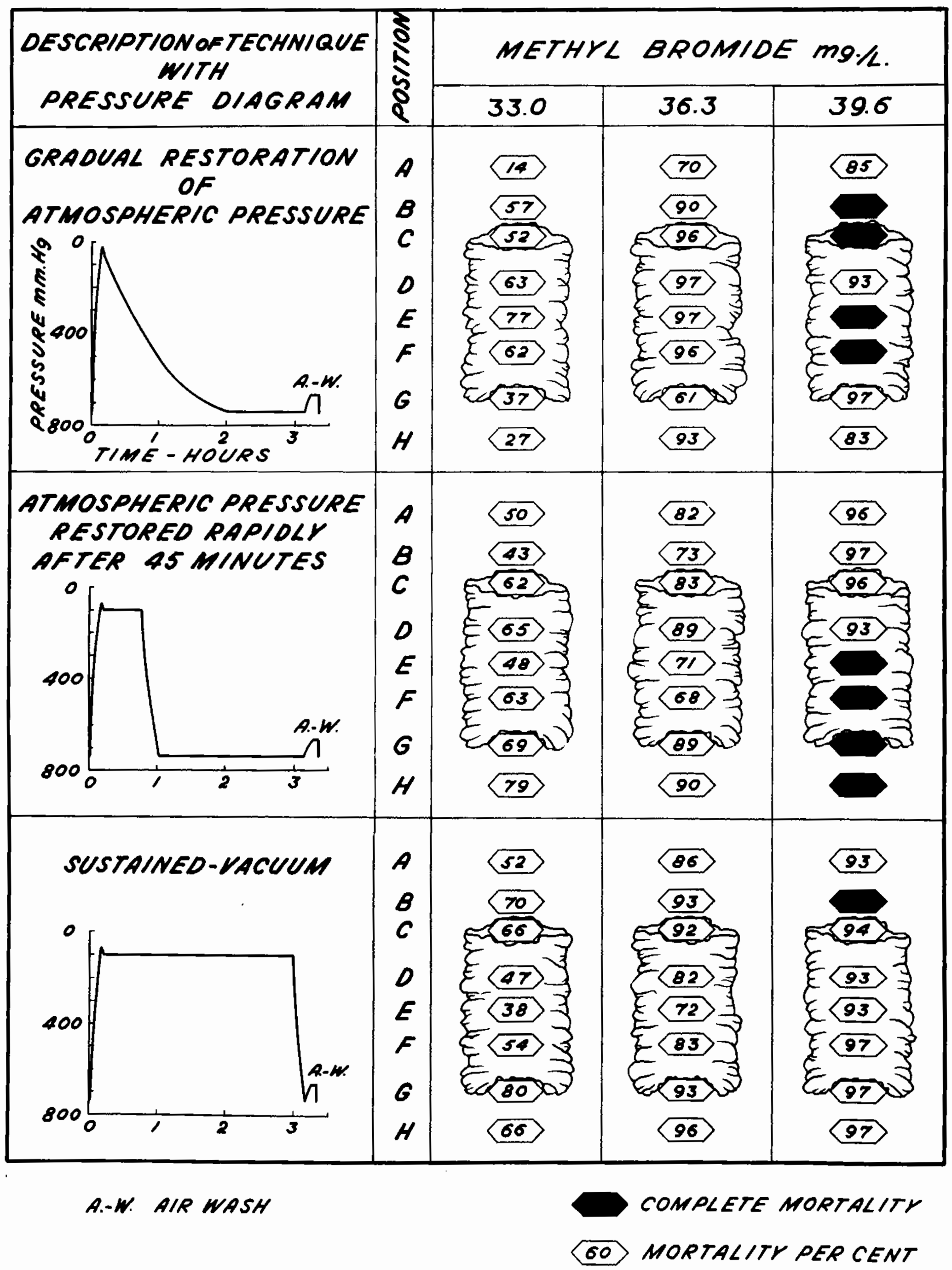


Figure 24. Diagrammatic comparison of three techniques against T. mauritanicus with bales of moisture content 13-14%. dry weight basis.

See Table 13 for summary of statistical analysis.

TABLE 13. COMPARISON OF VACUUM FUMIGATION TECHNIQUES AGAINST LARVAE OF *T. mauritanicus* IN MOIST BALES

Statistical analysis of data presented graphically in Fig. 24. Dosage of methyl bromide in milligrams per litre for three hours at 25° C.

Line in Fig.	Description of Technique	Position of Insects in Chamber	LD_{50} (with 95 per cent confidence intervals)	Efficiency Ratio (with 95 per cent confidence intervals)	Deviation from Parallelism of Regression Lines P = 0.05
A	Gradual restoration of atmospheric pressure from 25 mm.	Whole system	33.1 (32.4-33.8)	VERSUS B = 1.05 (1.0-1.1)	Not significant
				VERSUS C = 1.03 (0.99-1.08)	Not significant
A^1	"	Three central positions of bale	31.0 (30.1-31.9)	VERSUS B^1 = 1.03 (0.98-1.09)	Significant
				VERSUS C^1 = 1.08* (1.03-1.13)	Not significant
B	Atmospheric pressure restored after 45 mins. at 100 mm.	Whole system	31.5 (30.8-32.2)	VERSUS C = 1.02 (0.97-1.08)	Not significant
B^1	"	Three central positions of bale	32.0 31.1-32.9)	VERSUS C^1 = 1.05 (1.0-1.1)	Significant
C	Sustained-vacuum at 100 mm.	Whole system	32.0 (31.3-32.7)		
C^1	"	Three central positions of bale	33.5 (32.7-34.3)		

*Significant difference, P = 0.05

on the outside of the chamber. The glass stopcock on the flask was first opened and then the metal petcock on the copper tube. As the flask was always at a much lower pressure than the fumigation system, a true sample of the fumigant-air mixture was drawn into the flask. An interval of 30 seconds was allowed for establishment of equilibrium between chamber and flask. The cocks were then closed and the flask removed. Next 0.5 ml. of a solution of alcoholic potassium hydroxide (4% KOH in CH_3OH) was drawn into the flask. The flask was then clamped and placed in an oven at 40° C. for three hours. This process converted the methyl bromide to inorganic bromide. The solution was next neutralized by nitric acid (HNO_3). The flask and contents were then thoroughly rinsed with a ground solution, the most suitable, as found by preliminary experiment, being 0.5 molar perchloric acid ($HClO_4$) in 80% ethyl alcohol. Titration to determine the inorganic bromide content of each sample was done by means of a Fisher coulomatic titrator, which works on the principle of the electrolytic generation of silver ions, with a method developed by Latimer and Dumas (1958).

Concentrations in Different Methods

A study was made of the concentration pattern in the free space and in the centre of the bale in the principal techniques of treatment. The results are illustrated graphically in Figs. 25 to 28 and some comments are made below under the headings of the various methods.

For the purpose of analysing the results of the chemical analysis, and for comparing the different techniques, three indices have been employed - the "mean concentration", the "penetration factor", and the "distribution ratio".

The mean concentration has been found suitable for comparing concentrations prevailing in various parts of the fumigating systems (see Fig. 26, IV). This index is obtained after the concentration x time (c x t) product has been calculated for each selected position. The (c x t) product is obtained by measuring the area under a given concentration curve and expressing this in the appropriate units, which in this study would be milligrams per litre hours. The mean concentration is calculated by dividing the c x t product by the exposure period, in this work always 3 hours. The resultant value is in the original units of milligrams per litre.

The penetration factor of Brown and Heuser has already been described and defined (page 12). This factor is useful for demonstrating the degree to which the fumigant has succeeded in reaching a certain point within the commodity, in the present instance the centre of the bale.

The distribution ratio is the ratio of the value of pairs of mean concentrations which may be selected for demonstrating how evenly the fumigant has been distributed throughout the system during the period of treatment. It has already (page 10) been suggested that the attainment of even distribution is a desirable aim in this work, and this point is taken up more fully in the discussion. The distribution ratio in Table 14 is based on the two values available, those for the free space and the centre of the bale. For the purpose of this Table the first value has been divided by the second. The nearer the ratio is to unity the more even the distribution at the selected points.

<u>Sustained-vacuum</u>.--In Charts I, II, and III of Figs. 25 and 26 are shown the results obtained by chemical analysis of the concentrations at three pressures in bales of two moisture contents of 9.5 and 13.4 per cent dry weight basis. Chart IV of Fig. 26 summarizes the data for the

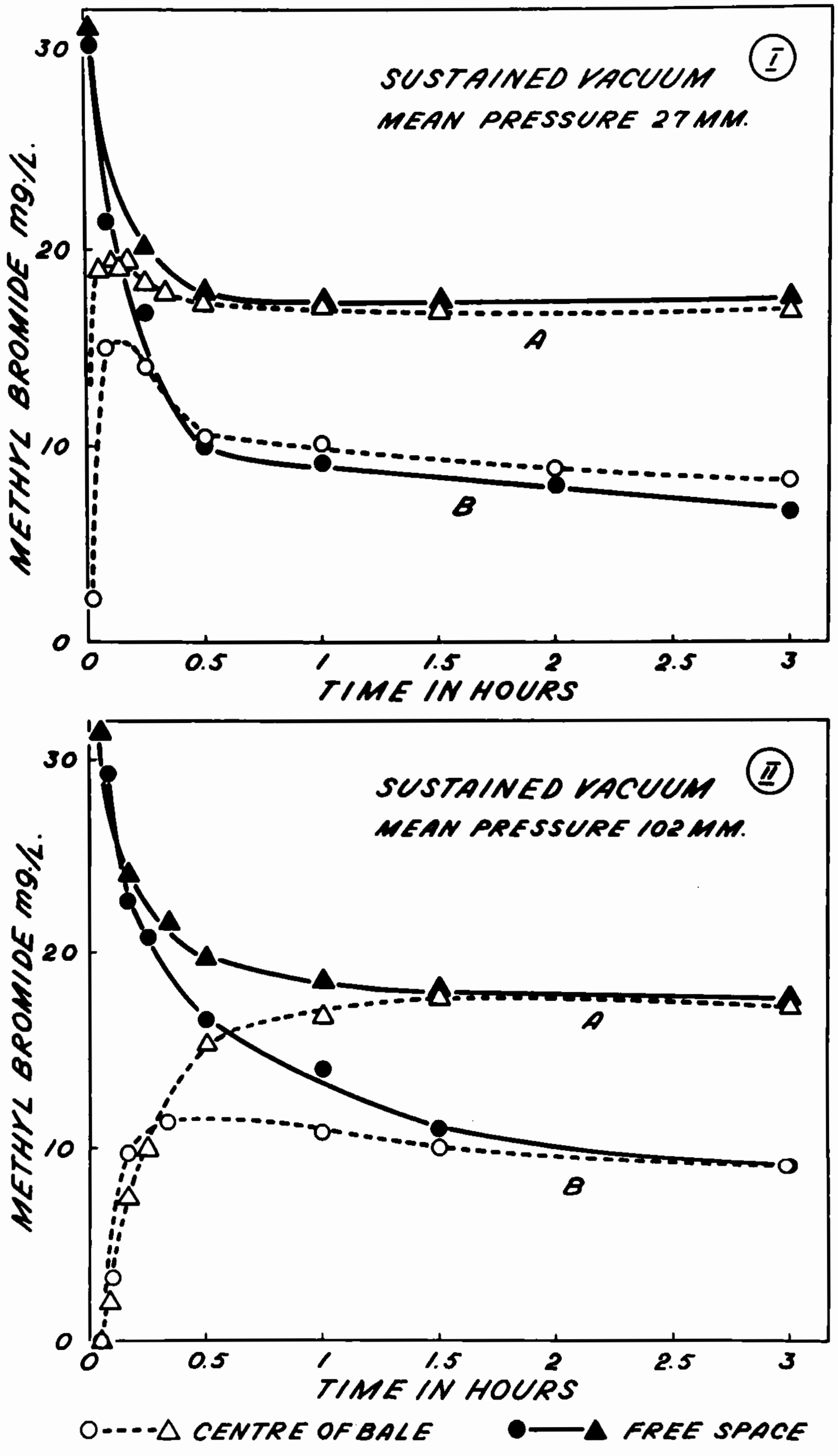


Figure 25. Methyl bromide concentrations in sustained-vacuum fumigation of bales of jute bags. In both charts (I and II) curves A are for moist bales (moisture content 13-14%) and B for those at low moisture content (8-10%) both on dry weight basis. Original dosage (nominal concentration) methyl bromide 39.6 mg./l. for three hours at 25° C.

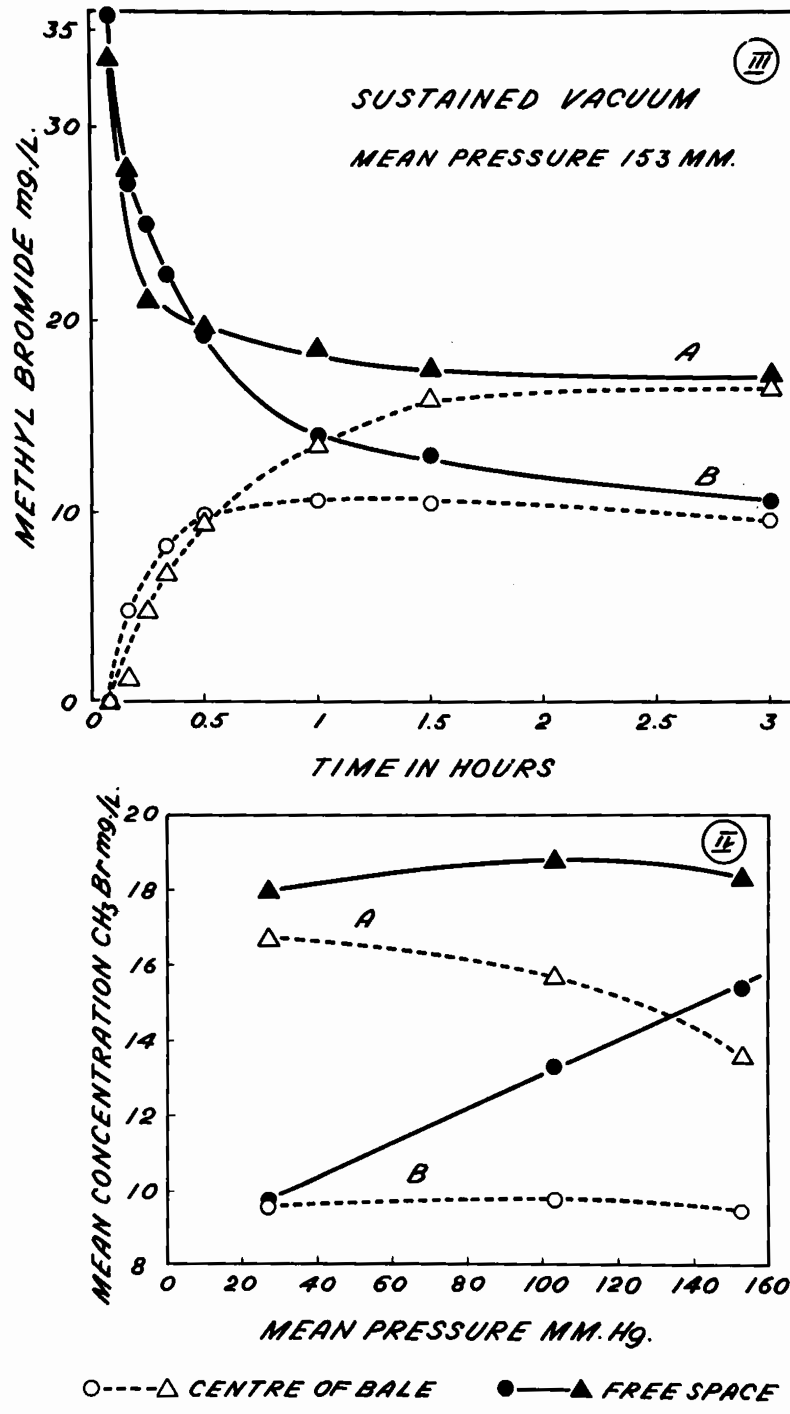


Figure 26. Chart III. Methyl bromide concentrations in sustained-vacuum fumigation of bales at 153 mm. pressure, all other conditions as in Charts I and II of Fig. 25.

Chart IV. Summary of mean concentrations of methyl bromide from charts I, II, and III. Lines A in moist bale, lines B in bales of low moisture content.

mean concentrations at all sampling points in all treatments. The dosage applied was the same in all treatments.

Examination of Charts I, II, and III, illustrating the flow of gas concentrations, leads to the following main conclusions:-

1. The lower the pressure of the system, the more rapid the diffusion of fumigant towards the centre of the bale.

2. At all three pressures the fumigant concentrations in the moist bales were higher than in the dry bales at the corresponding pressure.

3. In the dry bales, loss of fumigant concentrations from the free space was greater as the pressure was reduced. This is forcibly demonstrated by line B in Chart IV of Fig. 26. This line, for the concentration in the free space, rises steeply with increase in pressure.

4. The mean concentrations at the centre of the bale for each set of moisture contents is fairly uniform at all three pressures. In view of the results with the insects, this phenomenon is extremely important and will be dealt with in the Discussion. The indices set out in Table 14 show that for the three pressures at which sustained-vacuum was studied the penetration factors are very close in value. However, the distribution ratios of 0.98 and 0.93 for the treatments at 27 mm. indicate more even distribution than at the two other pressures. The good distribution at 27 mm. in sustained-vacuum is of little value when the mortality of _T. mauritanicus_ is considered, because this pressure is in the zone of high resistance of this insect.

Atmospheric pressure restored after 45 minutes.- Fig. 27, Chart VI, shows the gas concentration diagram for this treatment. Analysis of fumigant concentrations was done only with a bale of low moisture content. The penetration factor was higher than for all three sustained-vacuum treatments

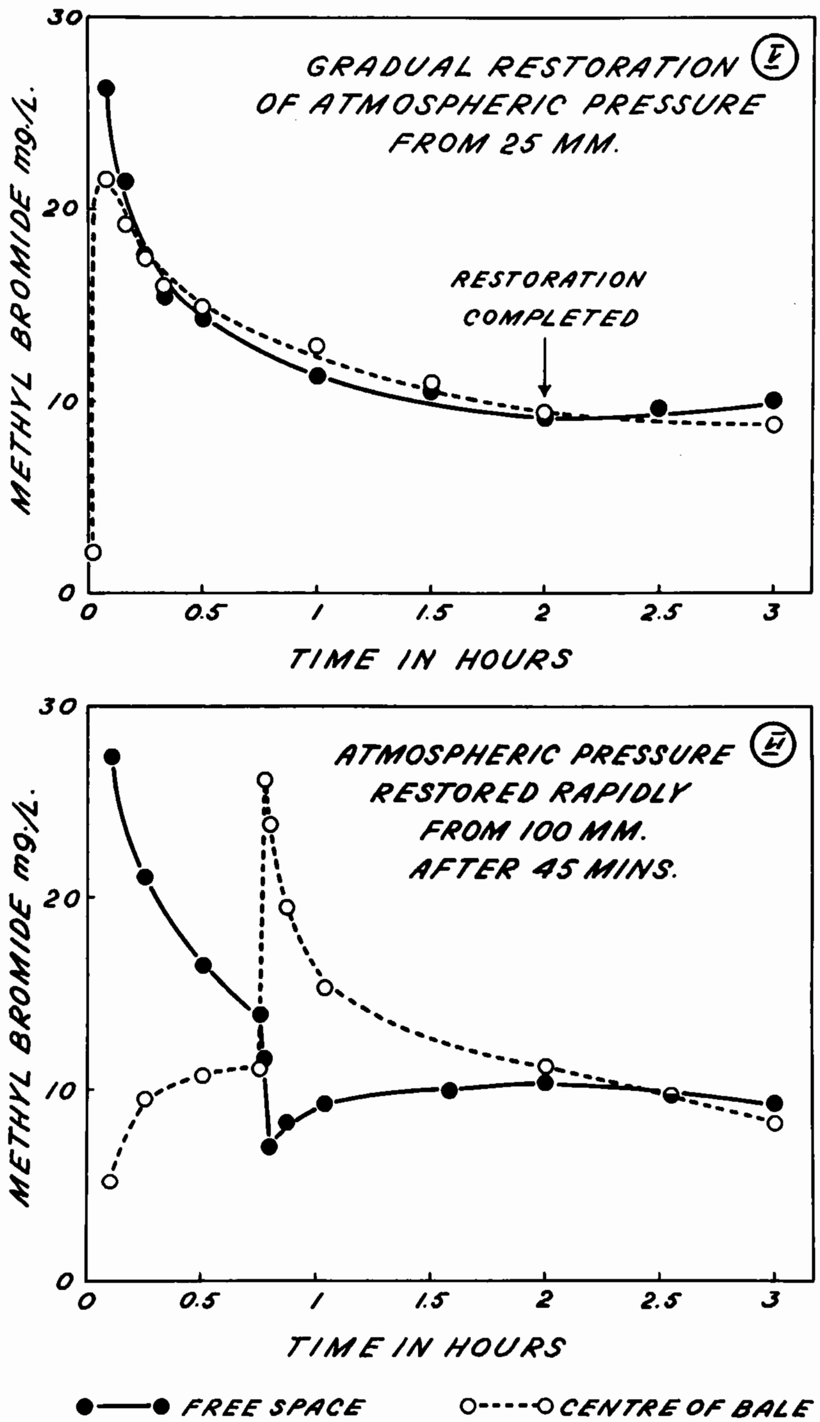


Figure 27. Methyl bromide concentrations in vacuum fumigation of jute bags with atmospheric pressure restored. Bales of low moisture content (9.6 to 10.1% dry weight basis). Nominal concentration 39.6 mg./l. for three hours at 25° C.

at the same dosage, and distribution was also better than with the sustained-vacuum technique in the optimum range (104 mm.).

<u>Gradual restoration of atmospheric pressure</u>.- The flow of fumigant concentrations in this technique, for a bale of low moisture only, is illustrated in Fig. 27, Chart V. It is seen that very soon after the beginning of the fumigation the curves for concentrations in the free space and at the centre of the bale are very close throughout the exposure period. The summary of indices in Table 14 also shows that this technique had the best penetration factor for the low moisture content bales, and that the distribution ratio of 0.98 is near to the ideal of unity. This distribution ratio exceeded all others except that for sustained-vacuum at 27 mm., with which it was equal.

<u>Simultaneous introduction of air and fumigant</u>.-This was studied for gas concentrations with a moist bale and with an initial dosage greater than with the other treatments. The concentration diagram is given in Fig. 28, and the indices are summarized in Table 14. Despite the higher dosage this method had the lowest mean concentration of all treatments, regardless of the bale moisture contents. Both the penetration factor of 13 and the distribution ratio of 0.27 were the lowest of all treatments studied. Comparatively high fumigant concentrations were present in the free space. This chemical analysis confirmed the findings with the insects (Fig. 21), showing that poor penetration of the fumigant led to mortalities in the region of zero near the centre of the bale.

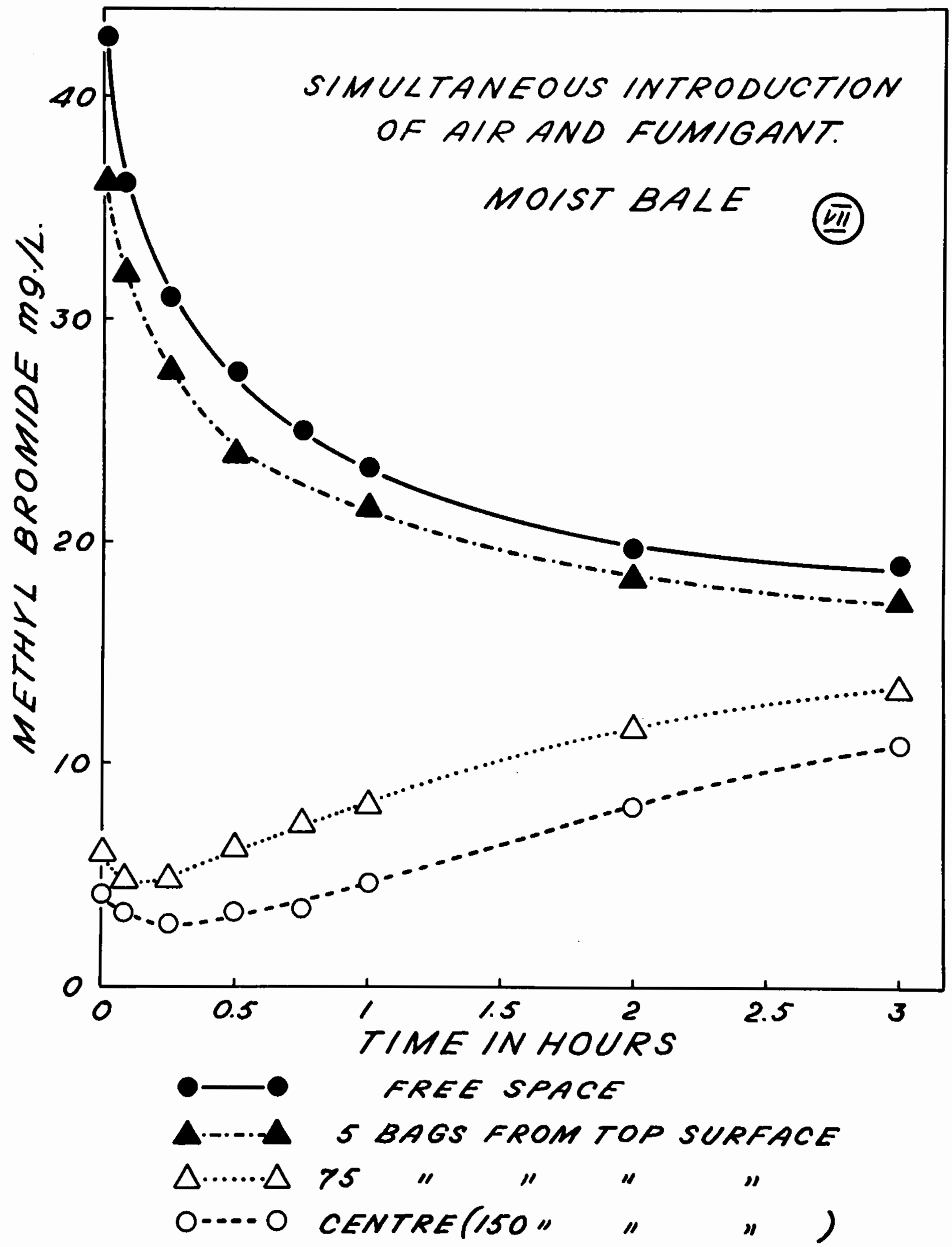


Figure 28. Methyl bromide concentrations in vacuum fumigation of jute bag with simultaneous introduction of air and fumigant. Bale with moisture content 13.4% dry weight basis. Nominal concentration 48 mg./l. for three hours at 25° C.

TABLE 14. SUMMARY OF INDICES OF METHYL BROMIDE CONCENTRATIONS FOUND DURING VACUUM FUMIGATION OF BALES OF JUTE BAGS

Exposure period 3 hours. Temperature 25° C.

Figure Number	Description of Technique	Percentage Moisture Content of Bale (Dry Weight Basis)	Nominal Concentration CH_3Br mg./l.	Mean Concentration Free Space	Mean Concentration Centre	Penetration Factor	Distribution Ratio
	BALES OF LOW MOISTURE CONTENT						
25 (I)	Sustained-vacuum at 27 mm.	8.4	39.6	9.8	9.6	24	.98
25 (II)	Sustained-vacuum at 104 mm.	8.6	39.6	13.3	9.8	25	.74
26 (III)	Sustained-vacuum at 153 mm.	8.7	39.6	15.4	9.5	24	.62
27 (VI)	Atmospheric pressure restored after 45 mins. at 100 mm.	10.1	39.6	13.1	11.5	29	.88
27 (V)	Gradual restoration of atmospheric pressure for 2 hours from 25 mm.	9.6	39.6	12.3	12.1	31	.98
	MOIST BALES						
25 (I)	Sustained-vacuum at 27 mm.	14.0	39.6	18.0	16.8	42	.93
25 (II)	Sustained-vacuum at 104 mm.	13.5	39.6	18.7	15.7	40	.84
26 (III)	Sustained-vacuum at 153 mm.	14.9	39.6	18.4	13.6	34	.74
28 (VII)	Simultaneous introduction of air and fumigant from 50 mm.	13.4	48	23.2	6.3	13	.27

DISCUSSION

The most important conclusion arising from this investigation is that the response of an insect to a constant dose of poison gas does not necessarily vary directly with reductions in the pressure of the environment in which the gas is applied.

Response of Bare Insects

It will be seen from Figs. 11 and 12 that the mortality curve obtained with larvae and adults of *T. mauritanicus* exposed to methyl bromide over a range of reduced pressures exhibits four distinct zones of response which may be distinguished, and defined, as follows:

1. Zone of dessication.-At a higher range of vacuums used in this work, approximately between 15 and 30 mm., the mortality of both stages in a dry chamber increases rapidly as the pressure is reduced. This mortality has been shown to be related to water loss, and this region may therefore be called the "zone of dessication". The curves for mortality with or without fumigant (Fig. 7) are very close, and the part played by the fumigant is small compared with the effect of dessication. It has also been demonstrated that this loss of water in larvae may take place principally through the spiracles. In adults there is a very effective control of water loss among surviving individuals exposed between 20 and 24 mm.

2. Zone of high resistance.-Between approximately 20 and 50 mm. there is a "zone of high resistance" in which the mortality in a dry environment is in the region of zero at dosages which cause high mortality at higher pressure. Owing to the fact that loss of water is comparatively small at these pressures it has not been possible, so far, to relate this effect to any spiracular mechanism. On the other

hand, there is no doubt that the response connected with resistance in this zone is partly independent of humidity because even when the atmosphere is saturated with moisture vapour there is a falling off of larval mortality in this range of pressures. At 70% R.H. the mortality curve is closely parallel to that for the insects exposed in the dry environment.

This is also the zone in which a progressive lowering of visible activity was observed as the pressure was reduced from 50 mm. even in a humid environment (see Table 2). From 50 mm. pressure upwards activity was resumed. At pressures of 50 to 100 mm. the mortality of the larvae appears to be more related to visible activity than to the humidity of the environment.

3. Zone of susceptibility.--At the three ranges of humidity in which the responses were investigated there is a zone of susceptibility of the insects to the fumigant lying between 50 and 175 mm. The optimum varies according to the humidity, but appears to lie between 75 and 150 mm.

At all three humidities the mortality curve rises very steeply from the low point at the end of the zone of high resistance. At the other side of the peak, however, the curve falls away more gradually. As recorded in Table 2, visible activity of *T. mauritanicus* appears to be normal at 100 mm.

4. Zone of declining susceptibility.--As the pressures are increased above 150 mm. the mortality of the insects declines gradually. Pressures above 250 mm. were not investigated, but at this point there begins an approach to the mortality response found at atmospheric pressure, at which a higher dose is required to bring

about a given mortality in three hours of exposure (Fig. 10).

It should be pointed out that, although the response which makes the insects more difficult to kill in the zone of resistance is able to operate even at moisture saturation, as far as actual comparative mortalities are concerned the insects are more susceptible at high than at low humidities at any of the pressures investigated. This is clear from Fig. 11 and Table 6. The mortalities were higher at higher humidity even though the doses were lowered as the humidity was increased.

It has also been shown that two other insect species, represented by the mature larvae of T. molitor and the adults of T. confusum, give a complex response to methyl bromide as the pressure is progressively reduced. The response of T. molitor (Fig. 13) is similar to that of T. mauritanicus, except that at the range of pressures used, down to 15 mm., a zone of dessication was not reached.

The results with T. confusum (Fig. 14) show a zone of resistance near 75 mm. pressure, but the differences due to pressure are not so strongly marked with this insect.

The mortality responses of T. confusum with and without fumigant in the pressure range of 50 to 100 mm. were very carefully studied in the present work. Between the narrow limits of 55 and 70 mm. the fall in mortality for both fumigated and non-fumigated adults of T. confusum is very rapid. It was only by very careful choice of dosage that it was possible to demonstrate the low point and subsequent rise in the curve for fumigated insects. It is easy to understand why with T. confusum previous workers misinterpreted the rapid fall in mortality as the pressure was reduced in this range, and did not distinguish between the effects due to dessication and those due to fumigant toxicity.

These findings may be contrasted with the hypothesis of Cotton (1932 and 1937) that oxygen deficiency, and not the reduction in pressure itself, is the factor in vacuum fumigation that makes insects more susceptible to the action of certain fumigants. Cotton did not use methyl bromide in any of his experiments, so no direct comparison may be made between the results of this study and his. However, the statement of Cotton was clearly intended as a generalization of this hypothesis. Certainly with the three insects studied here, the concept of oxygen deficiency could not account for the high mortalities at the optimum pressures, at which there would be more oxygen present than at lower, and less effective, pressures.

The evidence presented in this paper indicates that the lowered mortalities in the zone of resistance are connected either with a response to pressure reduction itself, or with pressure reduction in combination with the action of the fumigant. It is significant that the lowered mortalities of all three insects in the zone of resistance occur when visible activity is very low or non-existent.

It must be pointed out that there is no evidence from this work that rules out oxygen deficiency as one possible factor. However, if it is a factor, its effect on <u>T. mauritanicus</u> is masked at very low pressures by the effect of dessication, and at higher pressures by an increased susceptibility which is associated in some way with increased activity at these pressures.

Carpenter and Moore (1938) found that some species of insects, exposed to HCN for the very short period of ten minutes, gave curvilinear responses with optimum mortalities at 30, 60, or 90 mm. and low kills at 2 mm. Exceptions were <u>T. confusum</u> and <u>R. dominica</u>, in which the mortality appeared to vary inversely as the pressure. These authors did not investigate, or discuss, the role of humidity or the bearing that dessication of the insects might have on their results. Neither did they demonstrate a multi-peaked curve with any of the insects they studied. However, they made the very pertinent observation, which is confirmed by the present study, that some species of insects are more easily killed in vacuum fumigation at pressures at which they are active, this activity usually occurring at pressures of 60 mm. and above. The high mortality of T. confusum adults at very low pressures was also attributed by these authors, as it was by Cotton, to the effect of the fumigant, whereas the writer has shown that in the critical range of 50 to 70 mm. pressure for three hours exposure the mortality curves for fumigated and non-fumigated insects are very close.

The work of Bhambhani (1956) on the two weevils S. granarius and S. oryza, also exposed to reduced pressures without fumigant, revealed a linear increase of the responses of water loss and mortality with increase of exposure period, decrease of pressure, and decrease of relative humidity at pressures between 20 and 80 mm. However, at 3 - 4 mm. the mortality was zero at a four hour exposure period which in the previously mentioned pressure range had caused high mortalities. Also, at the very low pressures the percentage water loss was greatly reduced. Therefore, with the two weevils there was a pressure range at which the weevils were able to withstand the dessicating effects of reduced pressures, an effect similar to that exhibited in the present study by the survivors among the adult cadelles between pressures of 20 and 24 mm.

Response of Insects in Bales

The responses of the larvae of T. mauritanicus, in, on, or near the bales of jute during the vacuum fumigation with methyl bromide can be explained from the results with the bare insects. The most significant finding is that in the fumigation of jute bags in bales the mortality of *T. mauritanicus* was influenced more by the environmental conditions of pressure and humidity than by the depth of the insects within the bales. However, as has already been pointed out, jute cloth is open in texture and fumigants may penetrate more quickly into a mass of this fabric than into certain other materials. Nevertheless, even if, with other materials, penetration is more of a problem, the factors of pressure and humidity would still have an important bearing on the response of the insects.

Furthermore, the chemical analysis showed (Fig. 26, Chart IV) that the mean concentrations at the centre of the bales of low moisture content were almost the same for the same nominal dosage at three widely separated operating pressures (27, 102, and 153 mm.) whereas the mortalities at the centre varied according to the pressures (Fig. 17 shows mortalities in the top curve of 23, 98, and 100 per cent respectively at the above-mentioned pressures).

Thus the work with the bales confirmed that with the bare insects by demonstrating the presence of an optimum range of fumigating pressures, and by showing that this optimum range prevailed at different moisture contents of the material.

Efficiency of methods.-The study of the comparative efficiency of different methods, related to the manipulation of the pressure system, was useful because it emphasized the theoretical and practical significance of the findings with the bare insects. With the bales of low moisture content sustained-vacuum was not the most efficient method, as it ranked third behind two methods employing the principle of restoration of atmospheric pressure. The greater effectiveness of the restoration treatments is correlated with better distribution of effective fumigant concentrations throughout the system. The "new" technique of gradual restoration of atmospheric pressure, after an initial high vacuum, is of interest because it constitutes a final realization of the concept which may have prompted the introduction of the immediate restoration and simultaneous introduction techniques, namely that of using the pressure differential at the beginning of the treatment as a source of energy to drive effective concentrations of fumigant towards the centre of a commodity. This technique was markedly superior only at low moisture contents. Its value in practice can be determined only by trial. However, it is another method which could be tested out for dealing with specific field problems.

In the meantime, two features of this technique are worth mentioning:

1. It may with certain materials, as it did with jute, lead to a more even distribution of the fumigant throughout the system. This would mean in practice that an economy in the use of the fumigant might be effected. Apart from the saving in fumigant itself, such economy would reduce the amount of residue formed in a given commodity. At present government authorities are laying down residue tolerances for fumigated materials, some of which allow only small margins of safety when effective dosages are used. A technique which reduces residue would be acceptable under these conditions.
2. In the sustained-vacuum technique, and also in that of the rapid restoration of atmospheric pressure after a period, the fumigant is carried by an onrush of air to the centre of the commodity. In the first method this occurs at the beginning of air-washing, in the second after the initial period under vacuum at the time of restoration of atmospheric pressure. It is shown (Fig. 27) that this results in the production of an abnormally high concentration

of fumigant at or near the centre of the commodity for some length of time. This may bring about a higher residue in material near the centre of a bale or package of material. In the method of gradual restoration the vapours are evenly distributed almost from the start of the treatment and they can be removed in the same proportions when air-washing begins.

Explanation of Field Results

The attempt to find a complete and satisfactory explanation for the observations in the field trials led to a very comprehensive investigation. This embraced a study of pertinent aspects of the response of insects to reduced pressures and of conditions in bales of jute bags which influenced the results of the treatments.

From the observations made in this study an adequate explanation can be given for the fact that mortalities of the insects in the trial were higher at the centre of the bale than at the periphery or in the free space of the chamber. These results were simulated in the laboratory and it was shown that the differences can be ascribed to the presence of higher humidities at the centre of the bale than at the outside or in the free air.

In fumigation research contact with field conditions should be maintained, for such contact may prove fruitful in opening up areas of rewarding investigation.

Practical Implications

This work shows that in vacuum fumigation with methyl bromide great care must be exercised in the selection of the pressures at which the system is held during the actual exposure period. Even massive doses may not be sufficient to counteract the effect of fumigating an intrinsically resistant insect, or stage of an insect, at a pressure or humidity at which greater concentrations are needed for kill than at optimum conditions. This means that careful preliminary investigations are always needed to deal with specific problems. Following these, the results of commercial treatments must be constantly checked. Special attention should be paid to variations in moisture content of different packages of the commodities treated.

Although this study was done with only one fumigant, methyl bromide, it is believed that the results presented may prove of guidance for dealing with problems in which other fumigants, commodities, and insect species are investigated.

SUMMARY

Mature larvae and adults of Tenebroides mauritanicus L. were exposed to methyl bromide fumigation at reduced pressures between 15 and 225 mm. Hg. When mortality is plotted against pressure the resultant curve shows four zones of varying susceptibility:

1. A zone of dessication, between 15 and 30 mm., in which mortalities appear to be due more to loss of water from the insects than to the effect of the fumigant.
2. A zone of high resistance, between 30 and 50 mm., in which mortality is low. This resistance is independent of humidity and appears to be connected with the lowering of visible activity of the insects at these pressures.
3. A zone of susceptibility, between 50 and 175 mm.
4. A zone of declining susceptibility, from 175 mm. up to normal atmospheric pressure.

Although the response in the zone of high resistance appears to be intrinsically independent of humidity, progressively lower doses of fumigant

are required to produce the same mortalities as the humidity is increased.

Experiments with two other species of insects, mature larvae of *Tenebrio molitor* L. and adults of *Tribolium confusum* Duv., indicate that similar curves of response may be demonstrated, although these may differ considerably in detail.

The findings with the larvae of *T. mauritanicus* were applied to a study of the relationship between the responses of this insect and conditions prevailing inside bales of jute bags during vacuum fumigation by various techniques. It is shown that the principal modifying factors, at a constant temperature of 25° C., are the mean pressures during fumigation and the moisture content of the jute.

The technique of sustained-vacuum fumigation was compared with other methods, all based upon some manipulation of the pressure during the exposure period. It was shown that, when the bales were at a comparatively low moisture content of 9 - 10 per cent (dry weight basis), better and more evenly distributed kills could be effected by two methods in which atmospheric pressure was restored after the initial reduction of pressure and the introduction of the fumigant. At higher moisture contents in the jute, of 13 - 14 per cent, differences between the techniques were not marked.

Studies were made by chemical analysis of the patterns of diffusion and concentration of the fumigant within the bales. These analyses showed that with the same initial dosage of methyl bromide in sustained-vacuum fumigations, the operating pressures of 25, 100, and 150 mm. did not affect significantly the mean concentrations at the centres of the bales. With the same initial dosages, concentrations were higher inside moist bales than inside bales of low moisture content.

The chemical analysis also confirmed the work with the insects by showing that, in bales of low moisture content, the two most effective methods of those tested embodied the technique of restoring atmospheric pressure some time during the exposure period.

With bales of low moisture content the insecticidal results and chemical analysis both showed that, for even distribution of high mortality and effective penetration of methyl bromide, the most effective method was that of gradual restoration of atmospheric pressure, from initial high vacuum, during two of the three hours of the fumigation period.

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