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YUN-PEI SUN

INTRODUCTION

OUR KNOWLEDGE of methods of combating insects that infest stored products has developed rapidly in the last decade along lines of damage to the product, prevention of losses, and destruction of the pests involved. Part of the progress in stored-product insect control is due to the development of new fumigants. Considering these insecticides in relation to the many kinds of stored products and the variety of insects infesting them, one realizes how much needs to be known about their comparative toxicity and adaptability. Most attention has been paid to such factors as temperature, fumigation methods, losses of fumigant during fumigation, action of fumigants upon materials, treatment of results, and methods of expressing toxicity. In spite of numerous discoveries there are many points upon which there still is no general agreement. In fact, significant variations in the results obtained by any one investigator indicate that a more refined technique is necessary to obtain precise results in toxicological work with fumigants. For example, there is the varied opinion on the effect of relative humidity upon the results of fumigation. As can be seen in some of the work with *Tribolium confusum* Duv. complete contradictions may occur under conditions when no difficulty would be expected. While studying the effect of carbon disulfide on the various stages of the insect, Cotton (1932) found that adults were more susceptible than larvae. Lindgren (1935), on the contrary, discovered that larvae were more susceptible than adults. Such differences are not uncommon in the literature. They may have resulted not alone from variations in conditions during fumigation, as is too often thought, but as well from variations in conditions before or after fumigation.

Some have considered that because of the accuracy with which the concentration of fumigants can be controlled, the investigation of such materials is a simple task. Nothing could be farther from the truth, for it is at least as complex as, if not more so than, the study of contact and stomach insecticides. It is the purpose of this bulletin to point out some of the important factors affecting insect fumigation as shown by results obtained with standard cultures of insects used under standard conditions.

It is a common belief that laboratory results are not comparable with results obtained under practical conditions and hardly ever convertible into practical recommendations. This belief cannot be attributed to differences in fundamental principles, but instead is due largely to a lack of sufficient knowledge of the many factors involved in the practical problem. A thorough study of these factors is not only of considerable academic interest but can lead to the improvement of practical application as well. The author hopes that his results may help to clarify some controversial points and stimulate further investigation.[1]

METHODS OF REARING INSECTS FOR FUMIGATION EXPERIMENTS

Stock cultures of adults of the confused flour beetle, *Tribolium confusum* Duv., were maintained in wide-mouth, pint fruit jars containing 40 grams of whole wheat flour that had been passed through a 50-mesh sieve. About 100 beetles were kept in each jar.

Eggs were obtained for new cultures and for fumigation by sifting the stock cultures through a 20-mesh brass sieve to remove the adults, and then removing the eggs from the flour by sifting them through a 50-mesh silk bolting cloth. By sifting the stock cultures daily, eggs less than one day old could be obtained in large numbers. They were kept in a desiccator under standard conditions of temperature (25° C. ± 0.1°) and relative humidity (60 ± 3 per cent) ready for use.

Cultures of larvae of any desired age were secured by separating eggs from stock cultures at 3-day intervals. Eggs no more than 3 days old were kept at standard conditions without flour until larvae began to hatch. The young larvae were separated from unhatched eggs every 24 hours by pouring the eggs and larvae

[1] Extensive discussions of the literature bearing on this subject were deleted from the manuscript to conserve paper, but the complete bibliography is presented. The literature review is available in the original thesis deposited in the library of the University of Minnesota.

back and forth from one paper to another. The larvae that clung to the paper could be jarred into culture dishes containing food. Petri dishes 3.5 inches in diameter containing 20 grams of whole wheat flour were used in rearing larvae. The population density was maintained at about 20 larvae per square inch of surface area or at about 10 individuals per gram of flour. This is equivalent to about 200 larvae in each dish.

Owing to the difficulty of obtaining large numbers of pupae of sufficiently uniform age in whole wheat flour, patent flour fortified with 5 per cent dry powdered yeast[2] was used instead. Larvae 25 $\pm$ 0.5 days old were separated from larvae cultures and transferred to small salve-tin covers with a thin layer of food. If the original culture was not too crowded, most of the larvae thus removed became pupae within about 3 days. The pupae could be removed from the covers daily without injury from sifting or other rough handling.

To obtain adults of any desired age, eggs no more than 3 days old were placed with flour in pint jars, and this culture left undisturbed until 10 days after the appearance of the first adult. At that time beetles were removed from the culture and placed in fresh jars with flour enough to make about 20 individuals per square inch of flour surface or 5 per gram of flour. This density is equivalent to about 200 adults in each jar. When still less variation in adult age was desirable, young adults were separated from pupae every 24 hours. When in some experiments these cultures of adults of known age were maintained for several months, the beetles were transferred to new flour each month.

The general stock cultures of *T. confusum* adults were reared in whole wheat flour under room conditions (24°-27° C. and 40-75 per cent relative humidity).

Standard cultures of the granary weevil, *Sitophilus granarius* (L.), and the rice weevil, *S. oryza* (L.), were reared in pint jars covered with cloth under the same conditions as *T. confusum*, except that each pint jar contained 100 grams of wheat. About 200-300 adults were allowed to remain in the wheat and lay eggs there for 3 to 4 weeks before they were removed. When the new generation of adults emerged, they were sifted out every day through a 10-mesh brass sieve and kept at standard conditions. Since the new weevils sifted out each day were not necessarily those which emerged from pupae that day, the age of weevils

[2] Brewers' dried yeast powder, strain G, manufactured by Anheuser-Busch, Inc., St. Louis, Missouri.

was not exact but an approximation. This is also true for Mexican bean weevils. The wheat was changed once every 4 weeks to avoid mixing weevils of different ages. In feeding experiments corn was used at 200 grams per jar for comparison with wheat. In general, the population density of adult weevils was maintained at about two individuals per gram of wheat, but in no case was it greater than four per gram.

S. granarius eggs no more than one day old were obtained by leaving the weevils in empty jars without food. The adults were separated from eggs by sifting with a 20-mesh sieve.

The adults of the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.), were reared with chili beans under the same conditions as weevils in wheat.

All the food materials were sterilized at 70° C. for about 8 hours. After cooling they were brought to moisture content near equilibrium with 60 per cent relative humidity.

Some book lice and grain mites were found in the cultures; however, they did not do any noticeable damage.

These standard cultures of test insects were reared under standard conditions of temperature and moisture. The temperature of the rearing cabinet was kept at 25° C. $\pm$ 0.1° with the aid of a mercury-toluene thermostat. All thermometers used in temperature-controlled cabinets were corrected to within 0.05° C. accuracy by comparison with a standard thermometer. The standard relative humidity was 60 $\pm$ 3 per cent; it was regulated by placing a pan of water in each rearing cabinet. In order to overcome the variation of relative humidity caused by changes in the outside atmosphere it was necessary to make certain adjustments. The most important of these were regulation of the area of exposed water surface, position of the water pan in relation to an air-circulating fan, and the temperature of water in the cooling coils. The latter proved to be the most efficient means of making an adjustment. Control of relative humidity at 60 per cent was accomplished successfully for a full year in a basement laboratory where the variation of relative humidity was from 75 per cent in the summer to 10 per cent in the winter. Moisture conditions in the rearing cabinets were measured with a hair hygrometer which had been standardized in a desiccator over a sulfuric acid solution in equilibrium with air at 60 per cent relative humidity.

The air in the cabinets was circulated constantly by an 8-inch fan. Several jars of a 25 per cent sodium hydroxide solution were placed in the cabinets to absorb carbon dioxide. This precaution

was essential in a fairly airtight cabinet containing a large number of cultures, because the accumulation of carbon dioxide caused a decreased rate of development and variations in the results of fumigation tests.

METHODS OF CONDUCTING FUMIGATION TESTS

A study of reports of fumigation tests shows that the most important variable factors are the kind of apparatus, the exposure time, and the temperature existing in the fumigation chamber. In the following discussion the standard procedure used in the present study will be described.

Apparatus

The type of fumigation chamber used has varied widely as to size, shape, and type of connections. However, such chambers may be classified into two main groups. In the first group the fumigant is introduced into a glass flask, bell jar, or other container. There is no continuous movement of the fumigant through the apparatus. In the second group the required concentration of gas is maintained in the fumigation chamber by continuous flow of the gas through the apparatus. The structure of such chambers is necessarily much more complex than in the first group.

Of the many types of apparatus and procedures, that developed by Strand (1930) has been followed by a great number of recent toxicologists. By this method, tests are run in 6.4-liter flasks for 5 hours at 25° C. In certain cases it may not be practical to follow this standard procedure because of a difference in the purpose of a given study, or an extended range of toxicity, or a desire to simulate certain environmental conditions. However, it is the author's opinion that one such standard method should be followed as closely as possible so that the results of many independent studies will be comparable.

Measurement of Liquid Fumigants

In both general types of fumigation tests mentioned above, a small error in the measurement of the fumigant is unavoidable, though the actual deviation is seldom determined. Inasmuch as the error of measurement contributes to the total difference be-

tween the specified and the actual amount of fumigant used, this point will be briefly discussed. A special treatment of fumigant losses will be included in another section.

The measurement of small volumes of liquid fumigants, especially the most toxic ones, involves some error in the actual concentration of the fumigant in a flask. The following methods of introducing a small amount of liquid fumigant into a large flask were found to be convenient and subject to a minimum loss of fumigant. A small quantity, 0.1 ml. or more, can be measured accurately enough for fumigation tests and introduced into a 6.4-liter flask by using a microburette or a micropipette graduated into divisions of 0.01 ml. The error of measurement is about $\pm$ 0.001 ml., which represents less than 1 per cent of the volume. A pipette is much better than a burette because in the latter a small amount of stopcock grease passes along with the fumigant. An accumulation of the grease not only makes washing of fumigation flasks more difficult but increases the amount of fumigant sorbed by the apparatus. The usual difficulty of manipulating a pipette can be overcome by covering the forefinger with a thin smear of stopcock grease, and then moving the forefinger back and forth over the upper end of the pipette to allow flow amounting to a fraction of a division. Smaller volumes can be measured in capillary tubes with an inside diameter of 1 mm. After the tube is drawn to a fine point at one end a narrow strip of graph paper, having each division equal to 1/20 inch, can be glued to one side. This measuring tube can be observed easily and used like an ordinary micropipette. The volume is determined by drawing clean mercury into the capillary tube and weighing the amount needed to fill it. Afterward the value of each division on the graph paper is calculated by using the weight of the mercury and the length of the tube. In one case a division equaled 1.52 cubic mm., with an average error of only 0.3 per cent. Smaller volumes can be measured by drawing a tube of even smaller diameter from a capillary tube if a uniform bore can be produced.

Control of Temperature and Relative Humidity during Fumigation

Because of the generally recognized importance of controlling temperature during fumigation tests, attention was given to the temperature within fumigation flasks. It is often desirable to run a test at a temperature different from that in the laboratory, a procedure which necessitates bringing the fumigation flask into

temperature equilibrium with a temperature-controlled cabinet. Experiments have been carried out with a 6.4-liter, pyrex Erlenmeyer flask with an average thickness of 2.3 mm. A thermometer was suspended in the center of the flask with its bulb about 2 inches from the bottom. The flask was held at one temperature until equilibrium had been reached and was transferred quickly to another air temperature for further observation. Direct readings of the temperature were taken again at intervals until equilibrium was reached. The readings were recorded at the time of each observation and were plotted as shown in figure 1. The temperature gradients used varied from 5° to 15° C., and the time required to reach temperature equilibrium in the flask ranged from 25 to 50 minutes. This information was used in setting a time schedule for the standard fumigation procedure described later.

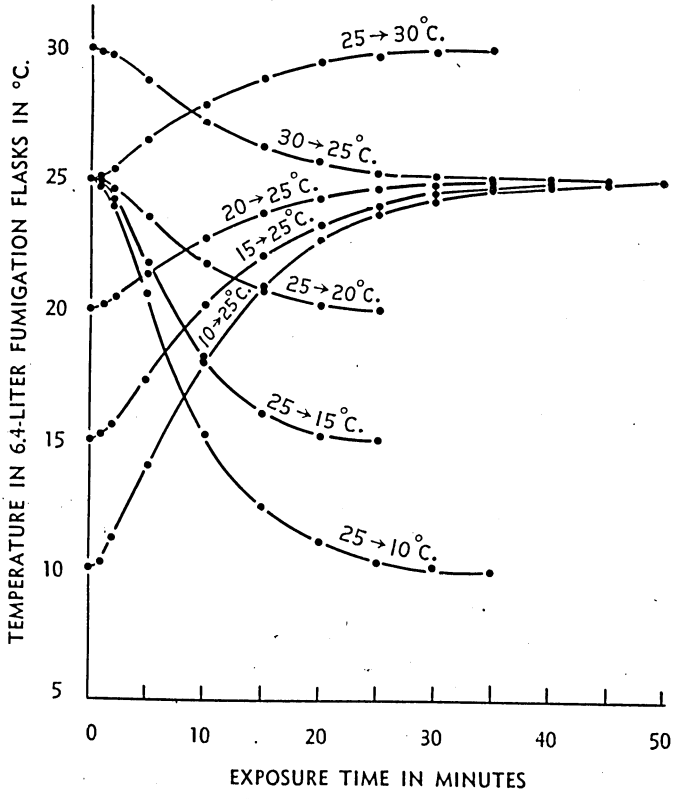

FIG. 1. The time required for the temperature in fumigation flasks to come into equilibrium with that of constant temperature cabinets

Any desired humidity can be obtained in a fumigation flask by passing air through sulfuric acid solutions of the proper concentrations. However, this method is slow and indirect. For the various needs in this study several methods were tried and their efficiency in regulating the relative humidity in 6.4-liter flasks determined.

The relative humidity was maintained at any desired percentage in a small room, and air was blown into the flask with a hand vacuum cleaner by means of a rubber hose connected in place of of the dust bag, the other end extending into the flask. This method could be used at relative humidities in the median range. If the desired relative humidity was too high to be produced in a room without too much difficulty, a given amount of water equal to the difference in weight between the required and initial water content of the air was added with a microburette. A similar method can be used to calculate the amount of water necessary to produce a required relative humidity at any desired higher temperature.

Another simple method of obtaining a relative humidity higher than that of the surrounding air is a modification of the vacuum cleaner method described above. The inlet end was covered with several layers of wet cloth so that air drawn through the system would pick up moisture from the cloth. By varying the amount of water, the number of layers of cloth, and the position of the cloth at the inlet, one could obtain various percentages of relative humidity in a flask in a few minutes. It was possible to produce any desired relative humidity from a little above that in the atmosphere of the room to near saturation, even when the original relative humidity of the room atmosphere was as low as 15 per cent. Too much moisture in the cloth must be avoided or water droplets will be carried over into the flask. For low humidity, drying agents such as lime, calcium chloride, etc., may be wrapped in a dry cloth and substituted for the wet one. This method has the advantage that the relative humidity can be measured directly at the mouth of the flask with wet and dry bulb thermometers.

Under many circumstances it is impossible to measure relative humidity with a sling psychrometer or use wet and dry bulb thermometers in a current of air because of limited working space. A weighing technique that proved accurate and convenient was developed during the course of this study. Two weighing bottles of the same size but containing different known concentrations of sulfuric acid were weighed and then placed in an atmosphere where the relative humidity was unknown. After a suitable length

of time they were removed simultaneously and weighed again. The increase or decrease in weight expressed in milligrams was plotted as shown in figure 2. The intersection of the line connecting the two weights, or an extension of it, with the abscissa gives directly the relative humidity in percentage. The advantage of this method over the usual weighing techniques results from the use of two bottles, one with sulfuric acid at a concentration which is in equilibrium with air having a high relative humidity, and the other in equilibrium with air having a low relative humidity. The result of this arrangement is a loss from one solution equivalent to the gain of water in the other, if the unknown relative humidity lies equally between the two values, and consequently less disturbance of the moisture conditions in the container. This

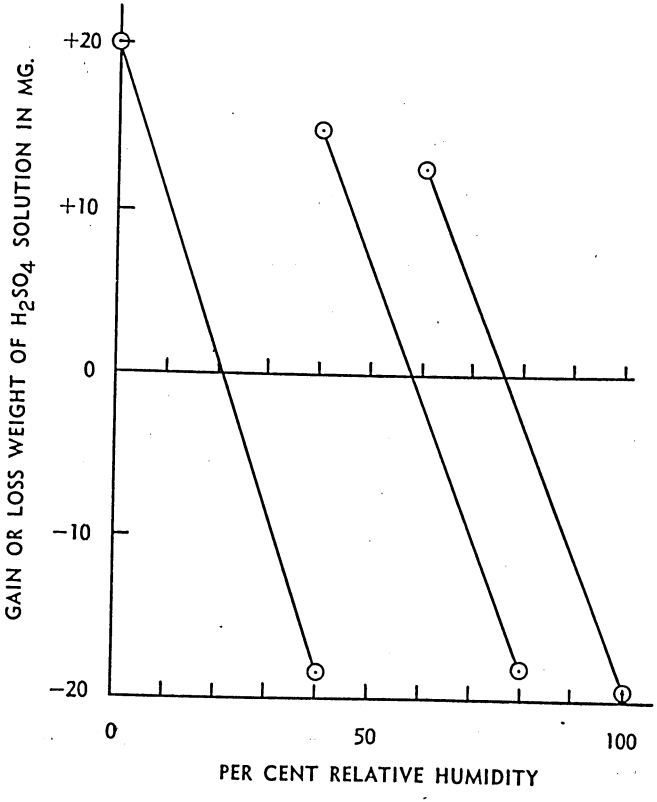

FIG. 2. Graphic determination of relative humidity by the weighing method

Table 1. Determination of Relative Humidity in Quart Fruit Jars by Weighing Method at 25 °C.

Weighing bottle with H_2SO_4 solution of low R.H.		Weighing bottle with H_2SO_4 solution of high R.H.		R.H. determined by graphic method	Known R.H. of H_2SO_4	Difference from actual R.H.
Known R.H.	Increase in weight	Known R.H.	Decrease in weight			
Per cent	Mg.	Per cent	Mg.	Per cent	Per cent	Per cent
0	+20.1	40	−18.4	20.8	20	+0.8
40	+15.0	80	−18.1	58.2	60	−1.8
60	+12.7	100	−19.4	76.0	80	−4.0

is the ideal arrangement, but satisfactory measurements can be obtained by using two different sulfuric acid solutions of any strength.

Experiments were carried out in quart fruit jars containing sulfuric acid solutions of known relative humidities. The results (table 1) agreed to within an average of 2 per cent of the actual humidity. In quart jars, disturbance is likely to be serious during the introduction of weighing bottles. Because of this the results are slightly high when the humidity in the jar is lower and slightly low when the humidity is higher than that of the air.

Usually fumigants are introduced into fumigation flasks at room temperature and humidity. When this is done and the test carried out at a temperature above or below room temperature, there is a change in the relative humidity in the flask related to the temperature difference. For example, if the room temperature is 25° C. and the relative humidity 56 per cent and then the temperature is changed to 15° C., the air becomes saturated with water vapor. The relative humidity would be reduced to 33 per cent if the temperature of the flask were raised to 35° C. In addition, the air pressure in a flask will be increased at higher temperatures and decreased at lower temperatures. For these reasons it is very essential that the required conditions be obtained before insects are introduced into a toxic vapor. This is especially true in short-time fumigation tests.

Number of Individuals and Method of Selecting Insects for Fumigation Tests

It is very important for toxicologists to know how many individuals should be used to determine each point on a toxicity curve and produce a definite, continuous trend without using unnecessarily large numbers. There are so many variables associated with the susceptibility of insects to fumigation that it is usually neces-

sary to determine the number of insects required for a valid test by experimentation in each case. The results of observations on hundreds of fumigations indicate that greater variations occur when eggs, nearly mature larvae, pupae, and young adults are used. During these ages and stages not only more individuals are needed but more replications of tests are required. The approximate number of individuals which should be used can be determined statistically at each stage for each species.

Another source of variation in fumigation results lies in the selection of samples to be used in tests. Even among standard cultures the results may vary from day to day and with insects taken from one part of a culture or another. When small differences in treatment, such as the effect of humidity after fumigation, are being compared, it is absolutely necessary to select insects from the same culture or from mixtures of several cultures to reduce the inherent biological variation. In this study samples were drawn by transferring a few insects from one culture or a mixture of several cultures with a small brush, the process being repeated until a sample of sufficient size was obtained. When this technique was used, the variation in mortality of adults of *S. granarius,* fumigated with 33 mg. per liter of carbon disulfide, was only 4.3 per cent from the mean of 13 independent fumigations. When fumigation was carried out in the same flask, with insects from the same culture, the variation could be held down to indicate a 5 per cent difference due to treatment. The actual number of individuals used in each test described in this study is included with other data in the tables.

Standard Method of Fumigation

Standard fumigations were carried out in 6.4-liter Erlenmeyer flasks at 25° C. ± 0.1° and 60 ± 3 per cent relative humidity. The fumigation flask was first brought to the required humidity (see humidity control). To minimize the loss without using any special device, fumigants were added from a micropipette (see measurement of liquid) which extended down through a small hole in a paper cover for several inches below the top of the flask. No carbon disulfide odor was noticed at the mouth of the flask. Then the flask was stoppered tightly with a heavily waxed rubber stopper, shaken thoroughly with a piece of screen wire as a stirrer, and placed at the fumigation temperature for about half an hour. After the temperature reached an equilibrium, the insects were introduced into the flask in small glass baskets (for all stages of

T. confusum) or in wire cages (for all adult weevils). Although it required only about 1-2 seconds to open and close the flask a certain loss of carbon disulfide was noticed by its odor. After a 5-hour fumigation at 25° C. ± 0.1° the insects were transferred from the toxic vapor to a small salve tin (for all stages of *T. confusum*) or to a new cage (for all adult weevils) with food, kept at 25° C. ± 0.1° and 60 ± 3 per cent relative humidity, and counted at both 2 ± 0.1 and 10 ± 0.2 days after fumigation. All fumigation treatments referred to as standard in this bulletin were carried out under the above conditions, and 2-day mortality was used in the discussion of results unless otherwise stated.

Methods of Observing Mortality

Many methods of determining the mortality of insects after fumigation have been suggested in the literature. This problem will be discussed more fully later. The standard method used in this study was to remove first those insects which showed movement. Then the quiet adults were prodded gently with the forefinger and larvae moved with a camel's-hair brush. After removing those which moved after prodding, the remainder were subjected to heat and light at a distance of about one inch away from a 60-watt light for about 20-30 seconds. The mortality of eggs after fumigation was determined by the difference between the total number of eggs and the number of larvae successful in emerging from the eggs. Observations were made every day after fumigation until there was no more hatching. The mortality of pupae was determined by the subtraction of either normal adults alone or the total of normal and abnormal adults from the number of pupae used. Those adults which showed full growth of wings were called normal, while those which varied from individuals having a free-moving adult head and small wing pads to nearly complete wing growth were called abnormal.

Methods of Expressing the Toxicity of Fumigants

The selection of a method of expressing the toxicity of insecticides is quite a problem in itself. For practical purposes LC_{95}, LC_{99} (fumigant concentration required to produce 95 or 99 per cent mortality), or minimal lethal concentration, should be used to describe the toxicity of a fumigant; however, not much difference can be found at the upper end of the curve when two fumigants are not widely different in toxicity. For this reason M.L.C. or LC_{50} (fumigant concentration required to produce 50 per cent

mortality) is generally used to compare the toxicity of two or more fumigants in the laboratory. Before discussing the whole toxicity curve, the methods should be reviewed of calculating the mortality caused by a single fumigation, which forms one of the points on the curve. In the calculation of per cent mortality there are two different methods. Some have suggested that a control is not necessary, especially if the natural mortality is low. However, Abbott's (1925) formula has been generally adopted.

$$\text{Corrected per cent mortality} = \frac{x\text{-}y}{x}100 \qquad (1)$$

Where x is the per cent of living in the check and y that in the treated lot. For the convenience of calculation this formula may be transferred into the following form (Moore and Bliss, 1942):

$$\text{Corrected per cent mortality} = \frac{P_t\text{-}P_w}{100\text{-}P_w}100 \qquad (2)$$

Where P_t is the per cent dead in the treated plot and P_w is the per cent dead in the check.

At low concentrations of hydrogen cyanide Gough (1939) found that more eggs of *T. confusum* sometimes survived among those fumigated than among the controls. Instead of correcting for the per cent survival of controls in each batch of eggs, he decided to use the average percentage of survival when computing the net kill in each test. The same result was found in this study when *T. confusum* eggs were fumigated with low concentrations of carbon disulfide. By correcting the results with the modified Abbott's formula (equation 2), the percentage of unhatched eggs was a negative value, which was not reasonable. The higher rate of hatching of fumigated eggs may be attributed to the "stimulation" of the chemicals. Again, insects from a poor culture or given a special treatment may show a high mortality in the checks. It is rather doubtful that such results corrected with Abbott's formula are comparable with those from a healthy culture. In one experiment *S. granarius* adults (0-1 day old) were kept with food in airtight and open jars covered with cloth. After 28 days the natural mortality of weevils in the airtight jar was 48.0 per cent and in the open jar only 2.0 per cent. It seems that the weevils in the open jar were much healthier than those in the airtight one. One would expect a great difference in their susceptibility to fumigants. However, the mortalities after fumigating with 30 and 35 mg. per liter of carbon disulfide were 48.4 and 92.0 per cent for weevils from the airtight jar and 36.6 and 88.2 per cent for those from the open jar, respectively. These differ-

ences in mortality could be expected from the fumigation results of different cultures kept under the same conditions. From the above examples, therefore, it can be seen that it is not wise to stress the correction formula of Abbott too much, especially if a good standard culture is not at hand.

Returning to the discussion of methods of expressing the toxic effect of fumigants, a review of the literature indicates that there are varied opinions. From the results of various authors it appears that there is no constant relationship between the concentrations of different fumigants to give 50 and 95 or 99 per cent kills for various species of insects. Therefore, it is worth while to list LC_{95} or $LC_{99\text{-}100}$ with LC_{50} in a toxicological study.

For its practical and theoretical interest, as well as for the comparison of results from different experiments, the author suggests further the use of LC_{50}, LC_{95}, and V_t both for 2-day and 10-day mortalities. This gives not only a greater range of comparison but also a correct view of the rate of increasing toxicity. The latter factor is easily confused by observation when two mortality curves are given on different scales. With the above data, the general shape of a curve can be reproduced without the original data.

The aim of toxicologists to reproduce their results from time to time is not easily accomplished. Tattersfield and Martin (1935) have stressed the importance of carrying out comparative toxicity tests at not too widely separated intervals of time, though they were more particularly concerned with plant-feeding insects. Shepard *et al.* (1937) were able to reproduce, after an interval of time, their original results of fumigation experiments on *T. confusum*. Richardson and Casanges (1942a) also found that chemicals tested in 1939 and in 1941 gave similar results. However, in many cases the results are not comparable. This can be attributed to both the variation of fumigation technique and the differences in insect cultures. In this project many factors have been investigated and controlled in rearing as well as in fumigation. Biological variation seems to be a more important factor than fumigation technique in introducing experimental errors.

To be sure of the value of M.L.C. for any fumigant at any stage of a species, the test should be repeated several times so as to determine the statistical significance of the value as well as its possible variation. To repeat the determination of an entire toxicity curve requires a great deal of time and materials. It is suggested here that with the repeated use of a dosage at or near M.L.C. for successive fumigation of insects from different cultures

the mortality will fall near the 50 per cent point. The corresponding M.L.C. of each result can be calculated from the following equation:

$$\text{M.L.C.} = C + \frac{50\text{-}x}{V_t} \qquad (3)$$

Where C is the concentration of a fumigant in milligrams per liter, giving a mortality of x per cent. V_t, $(=\frac{a}{b})$, the rate of increasing toxicity, is expressed by the straight part of the curve in per cent of mortality per milligram of concentration. This has the same value all through the straight part of the curve. Finally, a is the per cent of mortality, and b is the concentration of the fumigant in milligrams per liter.

For example, the M.L.C. of *T. confusum* eggs (5 ± 0.5 days old) is 146.2 mg. per liter (figure 3). From the toxicity curve two points, at 20 and 80 per cent mortality, were taken on the straight part of the curve and from them lines drawn perpendicular to both axes. Four intersecting points were obtained on the axes. As shown in figure 3, $a = 80 - 20 = 60$ and $b = 158.0 - 134.1 = 23.9$. Therefore, V_t was equal to $60 \div 23.9$ or 2.51. In repeating the fumigation with 150 mg. per liter of carbon disulfide the corrected per cent of mortality was 56.9 (point p). These values can be substituted in the above equation,

$$\begin{aligned}\text{M.L.C.} &= 150 + \frac{50\text{-}56.9}{2.51} \\ &= 150 - 2.8 \\ &= 147.2 \text{ mg./l.}\end{aligned}$$

In this calculation no point should be considered if its corresponding mortality lies beyond that part of the curve which is a straight line.

Graphical solution can also be obtained by drawing a line through the point p parallel to the straight-line portion of the curve. The intersection between the line cd and 50 per cent mortality is the M.L.C., 147.5.

The above two methods have the advantage of making it possible to obtain more data on M.L.C. without repeating the whole curve (table 2).

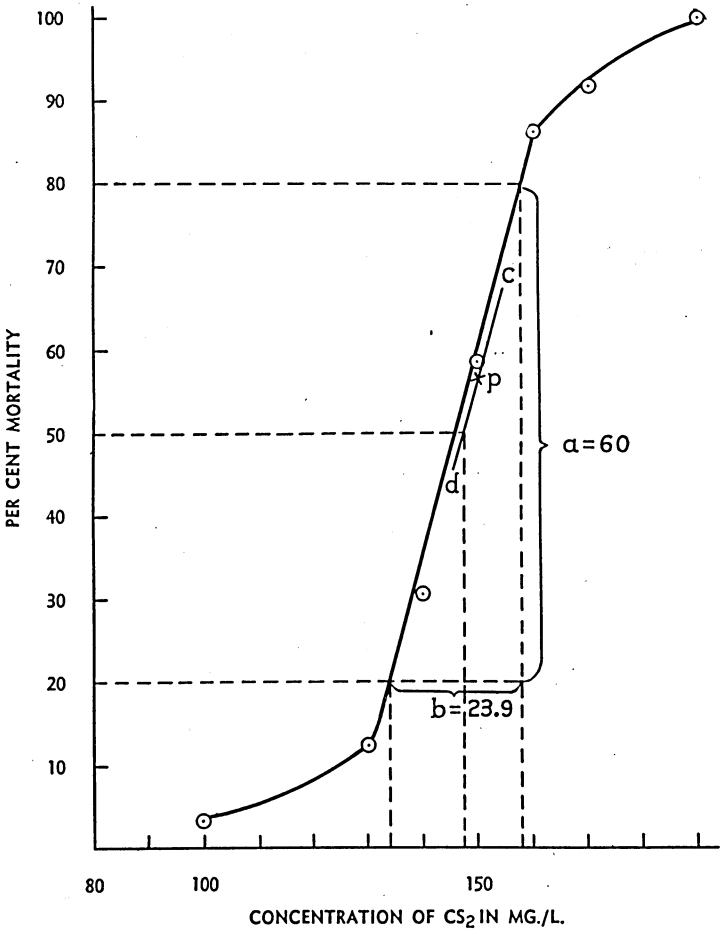

FIG. 3. Graphical and mathematical derivation of M.L.C. from a single datum within the range of the straight-line portion of a toxicity curve: *T. confusum* eggs (5 ± 0.5 days old) fumigated with carbon disulfide

Reliability of Fumigation Tests

Even when the important factors in fumigation are taken into careful account, there is more or less variation from treatment to treatment. This is due not only to biological variation but to physical factors as well. After hundreds of fumigations it has been found that the reliability of the results is related to species, stage, age, concentration of fumigant, exposure time, method of counting mortality, and other factors.

T. confusum adults vary more in the degree of recovery when the mortality count is made 10 days after fumigation than after

Table 2. Average, Calculated M.L.C.'s of Carbon Disulfide for Eggs, Larvae, and Adults of *T. confusum* of Various Ages

Age, days	Total number of insects	Average, calculated M.L.C. of five tests mg./l.	Age, days	Total number of insects	Average, calculated M.L.C. of five tests mg./l.
A. Eggs			B. Larvae—continued		
1 ± 0.5	793	220.6	28 ± 0.5	395	47.3
3 ± 0.5	612	169.1	35 ± 0.5	362	50.4
5 ± 0.5	788	146.8	C. Adults		
7 ± 0.5	638	95.5	1 ± 0.5	290	128.3
B. Larvae			4 ± 0.5	290	97.6
1 ± 0.5	517	66.2	7 ± 0.5	289	108.7
7 ± 0.5	538	24.5	14 ± 0.5	295	90.3
14 ± 0.5	461	27.0	21 ± 0.5	277	81.4
21 ± 0.5	440	39.6	28 ± 0.5	342	77.7
			35 ± 0.5	285	78.7

only 2 days. In addition, the mortality of *T. confusum* is not so easily determined as that of *S. granarius,* especially at the median lethal concentration. For these reasons the results of fumigation are more easily reproduced for *S. granarius,* although much smaller concentrations of carbon disulfide are used.

Among the various stages of *T. confusum* more variation is likely during the egg stage, because a slight change in incubation temperature will cause a great deal of difference in resistance to a fumigant. During the egg stage, susceptibility varies most near the time of hatching. For the eggs of *T. confusum* it is not as easy to reproduce results at the seventh day as with younger eggs.

If there is no great difference between two species used in an investigation of susceptibility to a certain fumigant, more uniform results can be obtained from time to time with the species that requires the higher concentration of fumigant. Owing to the great susceptibility of *Z. subfasciatus* adults to carbon disulfide the variation of results is unbelievable even though the death end point is sharp and easy to observe.

In the study of the relation between the length of time of fumigation and the concentration of fumigant more variation may be expected when insects are exposed to very high concentrations and short exposures or to very low concentrations and long exposures.

The method of counting, the length of time after fumigation when counting is done, the conditions after fumigation, etc., affect not only the per cent of mortality but also the degree of variation. In general, mortality figures taken 2 days after fumigation are easier to reproduce than those taken 10 days after fumigation, especially in the case of *T. confusum* adults.

EFFECTS OF PREFUMIGATION CONDITIONS ON SUSCEPTIBILITY OF INSECTS DURING FUMIGATION

The prefumigation conditions include those factors, such as temperature, humidity, kind of food, population density, starvation, etc., which may act on insects before fumigation. Because it was thought that these factors might produce physiological effects which would persist through the fumigation period a number of experiments were run to test this idea.

Respiration of Insects

It is generally believed that the toxicity of a fumigant depends mainly, if not completely, upon the rate of respiration. Temperature, oxygen, and carbon dioxide, three important factors affecting the rate of respiration, also affect the degree of toxicity. With theoretical support this idea has been proved indirectly, namely, that the susceptibility of an insect is closely correlated with its rate of respiration in air. Much attention has been paid to the opening or closing of spiracles, rate of movement of respiratory processes, and rate of respiration in air, but little is known about respiration during fumigation. It is known that physical factors not only affect the respiration but also the absorption, adsorption, diffusion, permeation, volatility, chemical reaction, water loss, etc. The above factors more or less affect the toxicity of fumigants to insects.

A method for determining insect respiration during fumigation has not been found in the literature. However, Carpenter and Moore (1938) determined the amount of fumigant retained by insects during fumigation. This indicates a combined effect of all factors, including respiration. Lindgren (1935) determined the rate of respiration per gram of insects in 48 hours. Apparently the result was an average rate of respiration during that period. A more uniform rate of respiration would be expected when the insects are kept with food. However, the low rate of respiration when without food must be an average of the gradation from the normal rate to the low rate after they were deprived of food. At a temperature of 35° C. and with wheat having a moisture content of 17.4 per cent, the concentration of carbon dioxide in the chamber was slightly over 9 per cent, by calculation. This apparently affected the rate of respiration, because Dendy and Elkington (1920) have shown that all *S. oryza* placed in an atmosphere con-

taining 23.2 per cent of carbon dioxide became motionless in 18 to 43 hours. Other methods, such as the use of Warburg's or Barcroft's respirometer, give results in a comparatively shorter time. However, none of the above methods show the actual rate of respiration during fumigation.

Effects of Stage, Age, Sex, and Species

The susceptibility of different stages of insects to fumigants has often been compared without any indication of their ages. The stages used in toxicological tests are more frequently adults or larvae and less often eggs or pupae. When the comparative susceptibility of one species to another is mentioned, usually only one stage is referred to; but for practical fumigation it is necessary to consider that all stages are present in all ages. Gough (1939) determined the relative resistance for all stages of *T. confusum* to hydrogen cyanide, using only a few selected ages. However, a full investigation of any one species of insect has not been found in a survey of the literature. Nevertheless, it is important to know the susceptibility during the whole life cycle of some important species both for its academic interest as well as for its practical importance. *T. confusum* was selected for such a study. In addition, *S. granarius* and *S. oryza* adults were also used for comparison.

The close dependence between rate of metabolism and susceptibility is supported by indirect evidence for the different stages of insect, as pointed out by Cotton (1932). The order of metabolic rate, adult⟶larva⟶pupa, has been paralleled by a similar order of susceptibility. However, Lindgren (1935) found that, if the egg can be neglected, the order of resistance to all these fumigants is pupa, adult, and larva. The order of metabolism is

Larva⟶adult⟶pupa⟶egg (without flour)
Old adult⟶larva⟶young adult⟶pupa⟶egg (with flour)

With the exception of the egg it appears that the stage with the highest metabolism is least resistant. As to *T. confusum* alone, wide differences in the order of susceptibility were found in the literature (table 3) not only because of differences among authors but also because of the action of different fumigants. Other factors, such as age, criterion of death, etc., may also play an important role. The order of resistance of various stages of *T. confusum* to several fumigants is shown in table 3.

Differences of susceptibility of one stage to different fumigants or of various stages to the same fumigant, as well as differences in susceptibility due to age during any stage and differences be-

Table 3. Order of Resistance of Various Stages of *T. confusum* to Several Fumigants

Fumigants	Order of resistance (Starting from the most resistant stage) 1	2	3	4	Author
Carbon disulfide	Pupa	Larva	Adult		Cotton (1932)
Carbon disulfide (at low R.H.)	Egg	Pupa	Adult	Larva	Lindgren (1935)
Carbon disulfide (at high R.H.)	Pupa	Egg	Adult	Larva	Lindgren (1935)
Carbon disulfide (at 60 per cent R.H.)	Egg	Pupa	Adult	Larva	Original
Chloropicrin	Egg	Pupa	Adult	Larva	Lindgren (1935)
Ethylene oxide	Pupa	Adult	Larva	Egg	Lindgren (1935)
Hydrogen cyanide	Pupa	Adult	Larva	Egg	Gough (1939)
Mercury vapor	Adult	Egg			Gough ((1938b)
Methyl bromide	Adult	Egg			Fisk & Shepard (1938)
Chloropicrin	Egg	Adult			Lindgren (1931)
Ethylene oxide	Adult (young)	Egg			Lindgren (1931)

tween species, were found in the literature. An extensive review of these aspects of the fumigation testing problem will be found in the original thesis, Sun (1943).

In the study described in this section[3] all the stages of *T. confusum* and adults of *S. granarius* and *S. oryza* were reared and fumigated under standard conditions. The points in each curve were not obtained on the same day nor from the same culture so that the final curve will not represent results from a single culture. In addition, the fumigation of the eggs, larvae, and adults of *T. confusum* was repeated near M.L.C. five times. The average of the results, as calculated by equation 3, will approach the true value.

The average M.L.C.'s of carbon disulfide for 1, 3, 5, and 7-day-old eggs (about 150 eggs were used for each test) were 220.6, 169.1, 146.8, and 95.5 mg. per liter, respectively. This indicates that the susceptibility of *T. confusum* eggs to carbon disulfide increased consistently with age from 1 to 7 days (figure 4). This was apparently related to the stage of embryonic development. By using the modified Abbott's formula (equation 2) at low concentrations, negative results were obtained. This may be due to some stimulative action of carbon disulfide which helped the larvae during hatching, for the unhatched larvae were mostly well developed, but were prevented from emerging by mechanical difficulties. Like the adult stage, the eggs of *S. granarius* are more susceptible to carbon disulfide than those of *T. confusum*.

[3] For this study and the following experiments, many tables were omitted from the text, owing to the limit of space. Readers who are interested in the details of these results should consult the original thesis (Sun, 1943).

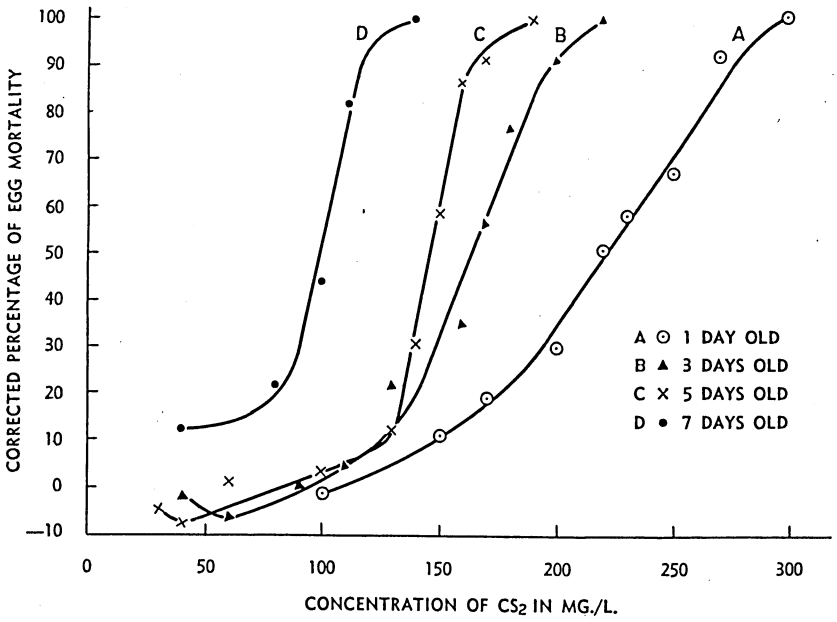

FIG. 4. Mortality curves for *T. confusum* eggs of various ages exposed to carbon disulfide

For various insect eggs, Fink (1925), Melvin (1928), Bodine (1929), and Burkholder (1934) showed that there was an early "formative" period, characterized by a low respiratory rate, and a late period, in which the metabolism increased up to the time of hatching. With this explanation the increase in susceptibility of *T. confusum* eggs to carbon disulfide may be due to the increase of respiration. In the case of eggs, an increase of respiration indicates a corresponding increase of permeation of the egg chorion.

The susceptibility of larvae of various ages to carbon disulfide was different from that of eggs. Their susceptibility increased from 1 day to 7 days. There was no appreciable change from 7 to 14 days. After that their susceptibility decreased as the age increased (figure 5). The average M.L.C.'s of carbon disulfide for 1, 7, 14, 21, 28, and 35-day-old larvae (about 90 larvae were used for each test) were 66.2, 24.5, 27.0, 39.6, 47.3, and 50.4 mg. per liter, respectively. The relation of the weight of larvae to both their age and M.L.C. is shown in figure 6. As the age increased, the larval weight increased slowly; however, the susceptibility of larvae decreased rapidly until near 14 days. After that both curves run upward side by side and then deviate more and more as the larval weight increases. The weight of larvae is increased in ac-

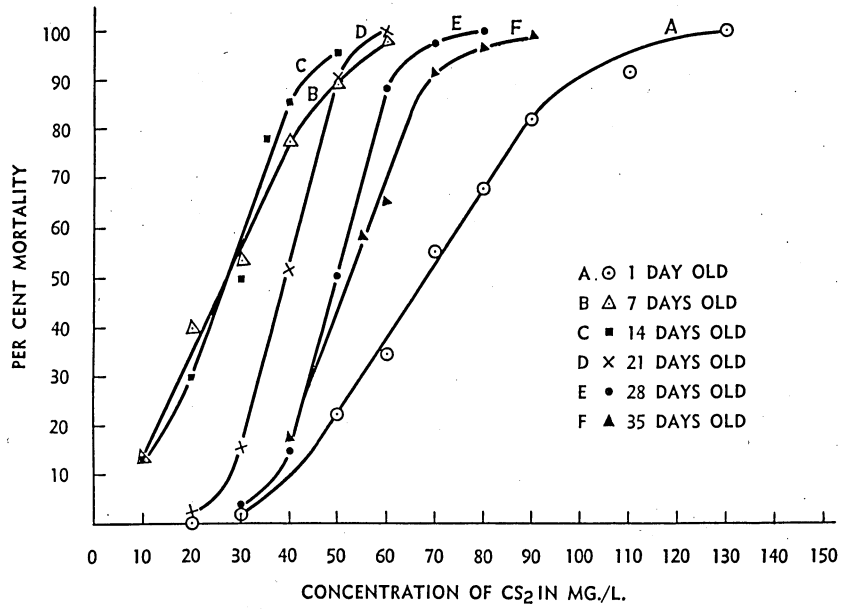

FIG. 5. Mortality curves for *T. confusum* larvae of various ages exposed to carbon disulfide under standard conditions (2-day mortality)

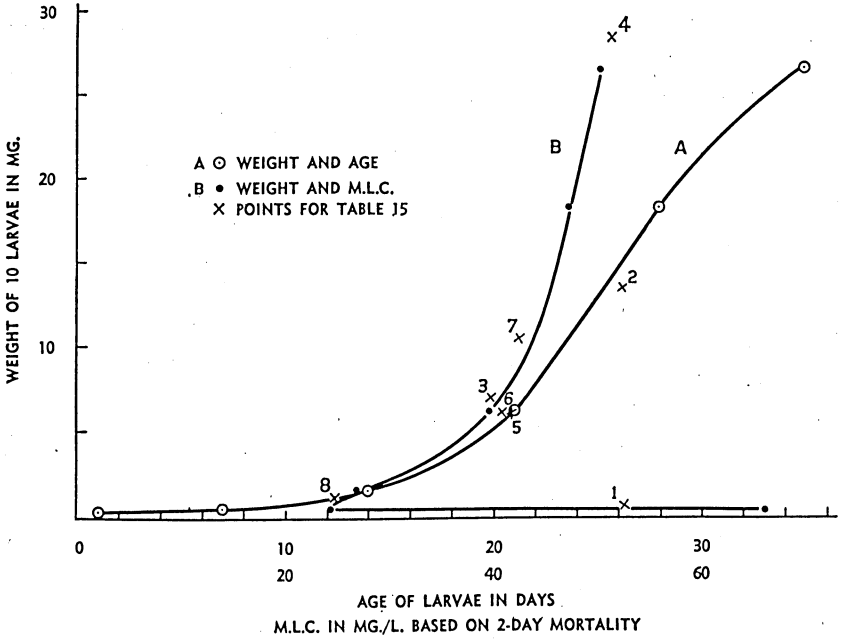

FIG. 6. The relation of the weight of *T. confusum* larvae to their age and the toxicity of carbon disulfide

cordance with the increase of metabolism, which in turn indicates the rate of respiration. During the first two weeks the larvae may increase their rate of metabolism without much increase of weight. It was found by Lindgren (1935) that young *T. confusum* adults, which were more resistant to fumigant than older adults, had a low respiration rate. The same thing may have occurred in the larval stage.

In the experiments performed, pupae 1, 3, 5, 7, and 9 days old were fumigated with carbon disulfide. About 50 pupae were used in each fumigation test. For pupae 1, 3, 5, 7, and 9 days old the M.L.C.'s of carbon disulfide were 127.7, 119.4, 178.0, 174.0, and 119.4 mg. per liter for no emergence and 95.0, 75.3, 92.5, 135.1, and 64.3 mg. per liter for abnormal emergence respectively. The results are shown in table 6 and in figures 7 and 8. The pupae resistance to carbon disulfide differs greatly when the M.L.C.'s are compared on the basis of abnormal emergence or no emergence. During the whole pupal stage their resistance to carbon disulfide is a reverse order of the V-shaped curve of pupal respiration. When one age of pupae is compared with a certain age of eggs the pupal resistance can be either greater or less than that of the eggs. This can cause contradictory results in literature as to their order of resistance. However, the average value of M.L.C.'s of carbon disulfide for eggs of various ages is slightly greater than that of pupae.

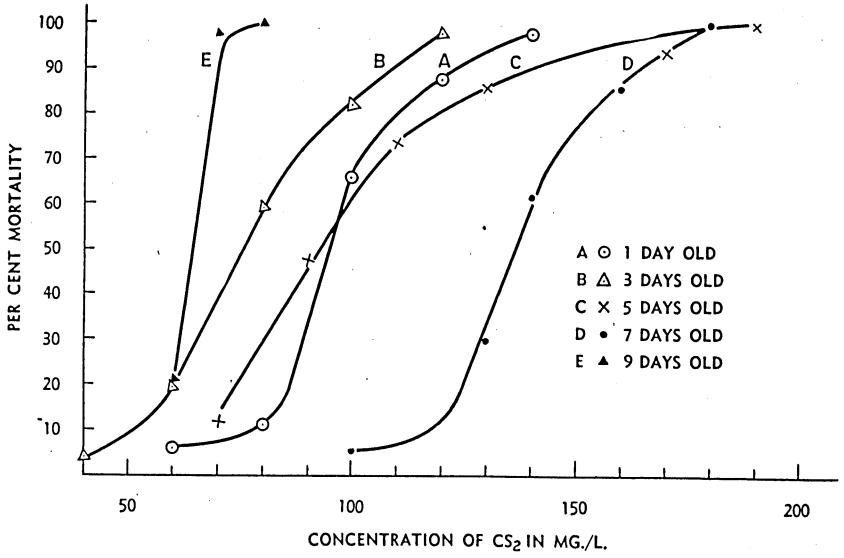

FIG. 7. Mortality curves for *T. confusum* pupae reared in patent flour with 5 per cent yeast and fumigated in carbon disulfide (abnormal emergence)

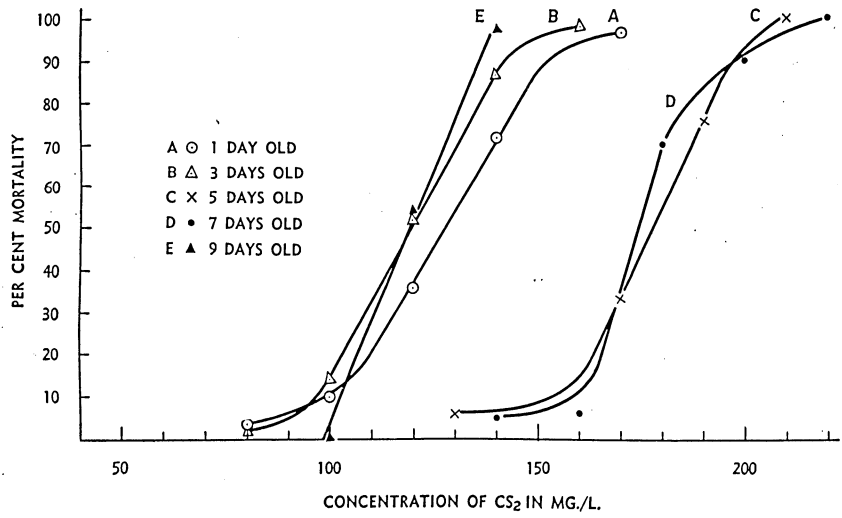

FIG. 8. Mortality curves for *T. confusum* pupae reared in patent flour with 5 per cent yeast and fumigated in carbon disulfide (no emergence)

T. confusum adults from 1 day to 24 weeks old were fumigated with carbon disulfide. The average M.L.C.'s of carbon disulfide for 1, 4, 7, 14, 21, 28, and 35-day-old adults were 128.3, 97.6, 108.7, 90.3, 81.4, 77.7, and 78.7 mg. per liter respectively. For older adults their susceptibility to carbon disulfide changed only slightly, because the M.L.C.'s for 8, 12, 16, 20, and 24-week-old adults were 74.0, 70.2, 68.4, 65.0, and 69.3 mg. per liter, respectively. The average number of adults used in each fumigation test was about 60.

Adults of *S. granarius* and *S. oryza* of various ages were fumigated under the same conditions as were *T. confusum* adults. The average M.L.C.'s of carbon disulfide for 1, 4, 7, 14, 21, 28, 35, 56, 84, 112, and 140-day-old adults of *S. granarius* were 40.4, 37.2, 35.3, 32.1, 32.2, 32.9, 33.1, 33.4, 32.4, 31.9, and 28.8 mg. per liter, respectively, and those for 1, 4, 7, 14, 21, 28, 35, 56, and 84-day-old adults of *S. oryza* were 29.0, 25.1, 20.8, 20.5, 21.7, 22.2, 20.7, 21.0, and 19.8 mg. per liter, respectively.

In general, the older *T. confusum* adults were more susceptible to the fumigant than the young ones (figure 9). However, adults 7 days old became more resistant than those 4 days old. With the exception of this reversal resistance the same general trend was followed also by adult weevils when M.L.C.'s were compared

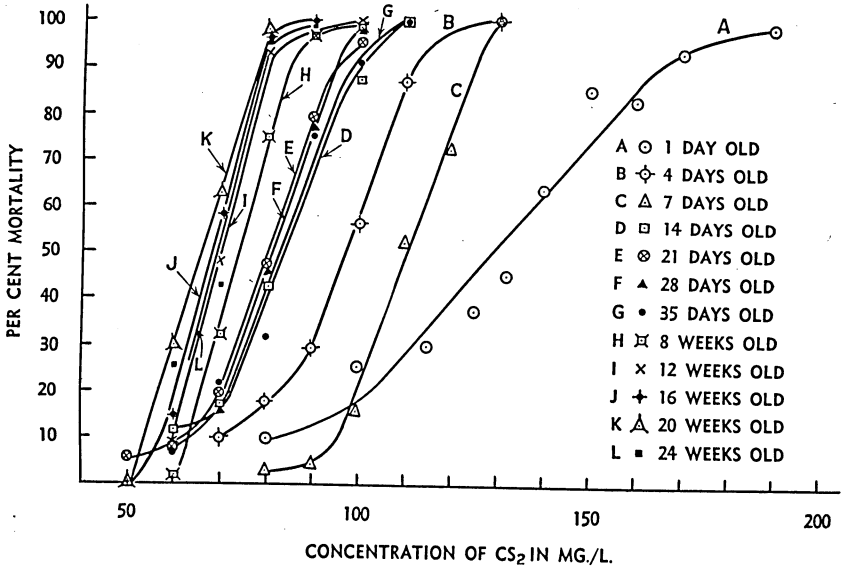

FIG. 9. Mortality curves for *T. confusum* adults of various ages exposed to carbon disulfide

(figures 10, 11, and 13). The determination of their respiration may give some light as to the reason for this difference. The greatest difference in the susceptibility of all three species was found during the first 2 weeks. After that their susceptibility increased constantly but slowly.

The recovery of *T. confusum* adults was considerable but varied greatly. Two-day mortality counts gave more consistent results than 10-day mortality counts, although it was not easy to determine the criterion of death 2 days after fumigation. From 2 to 10 days after fumigation, recovery of *T. confusum* adults was often found. The mortality of *S. oryza* adults was almost constant from 2 to 10 days after fumigation. *S. granarius* adults died gradually 2 days after fumigation. When both weevils were compared, the difference in susceptibility to carbon disulfide was much less at 10 days than at 2 days after fumigation.

The M.L.C.'s of carbon disulfide for various stages and ages of *T. confusum* are shown in figure 12, and for various ages of *T. confusum, S. granarius,* and *S. oryza* adults are shown in figure 13.

The differences in weight and rate of respiration between two sexes of the same species have been mentioned often in the literature, yet not much is known about their differences in suscepti-

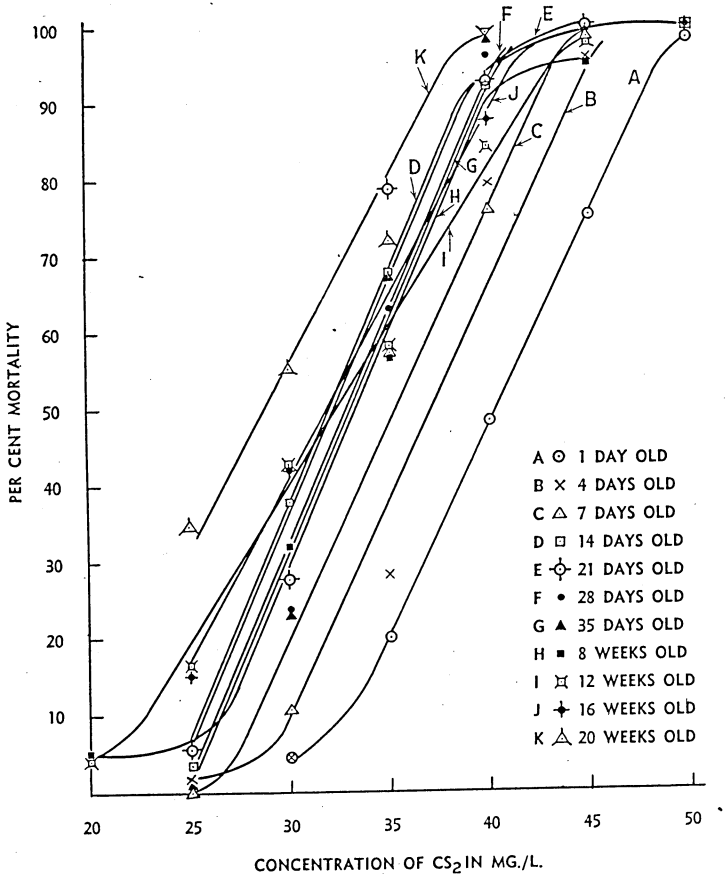

FIG. 10. Mortality curves for *S. granarius* adults of various ages exposed to carbon disulfide

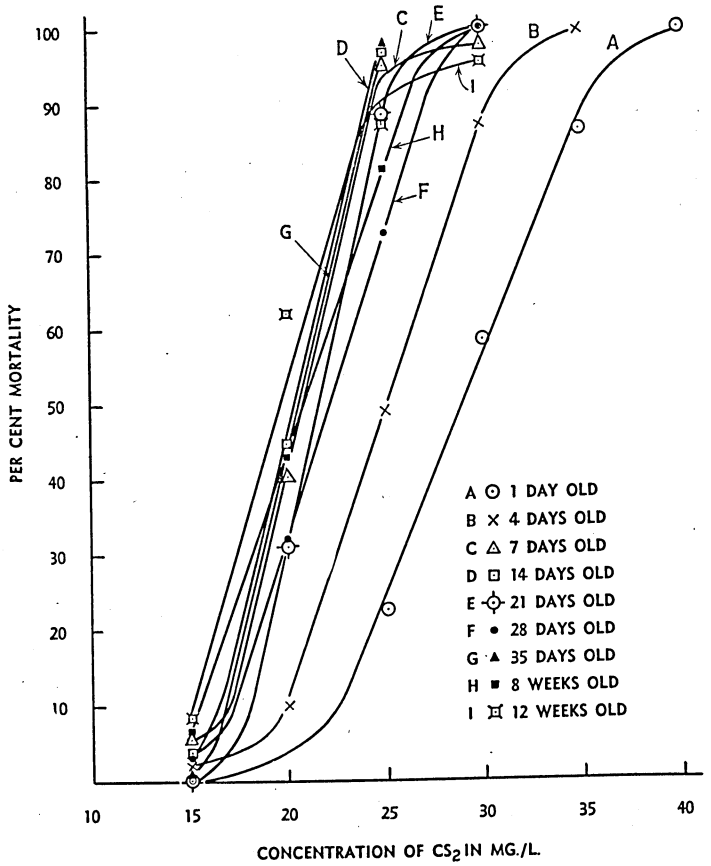

FIG. 11. Mortality curves for *S. oryza adults* of various ages exposed to carbon disulfide

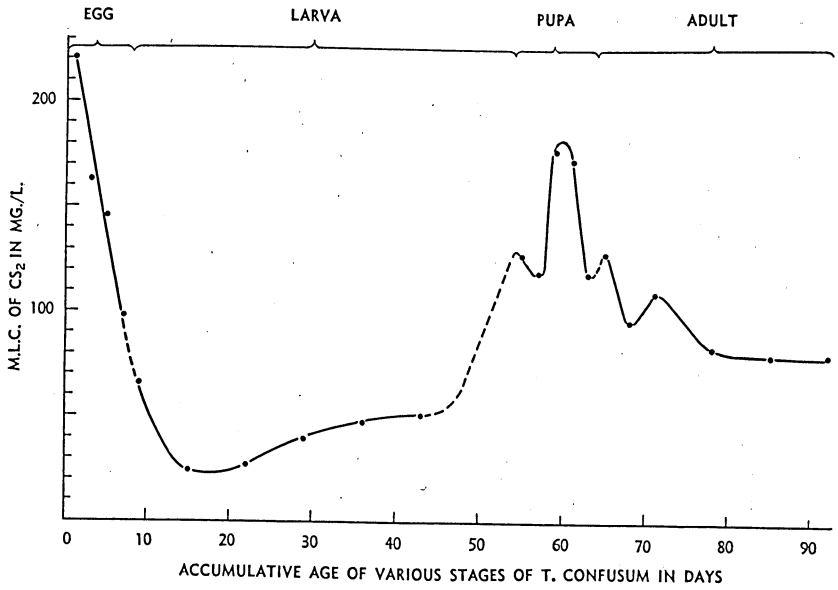

FIG. 12. M.L.C. of carbon disulfide for *T. confusum* at various stages and ages

bility to fumigants. Table 4 shows almost no difference in susceptibility between male and female adults of *Z. subfasciatus* to carbon disulfide. When these results are expressed as mortality curves they lie almost on the same track. By repeating the fumigation four times with 18 mg. per liter of carbon disulfide the males gave an average mortality of 39.8 per cent, the females, 35.0 per cent. The difference was very slight when their M.L.C.'s were compared. On the other hand, the females of *T. confusum* were slightly more susceptible than males (table 5). The mortality of the former was 66.4 per cent and the latter 53.2 per cent when fumigated with 80 mg. per liter of carbon disulfide. In general, the differences of susceptibility between sexes of these

Table 4. Susceptibility of Male and Female Adults of *Z. subfasciatus* (10 ± 1 Days Old) to Carbon Disulfide

Sex	Concentration of CS_2 mg./l.	Total number of insects	Per cent mortality (2 days after fumigation)	Sex	Concentration of CS_2 mg./l.	Total number of insects	Per cent mortality (2 days after fumigation)
Males	15	144	2.1	Females	15	139	2.2
	18	137	46.0		18	104	37.5
	20	177	71.2		20	145	69.0
	25	115	99.1		25	103	98.1

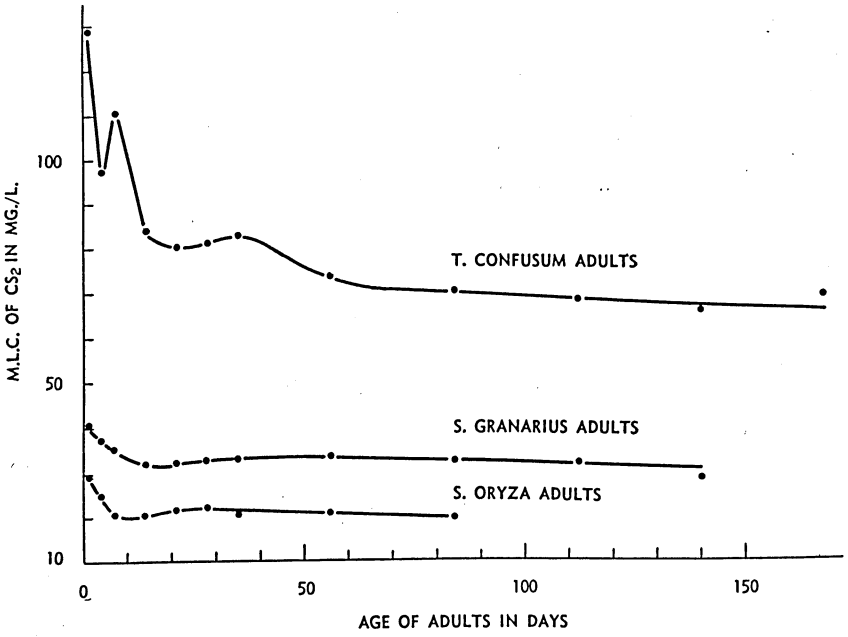

FIG. 13. M.L.C. of carbon disulfide for adults of *T. confusum*, *S. granarius*, and *S. oryza* at various ages (2-day mortality)

two species were not of great enough significance to demand much attention in toxicological studies, especially when the sex ratio is almost one.

The average values of calculated M.L.C.'s of carbon disulfide (using equation 3) for eggs, larvae, and adults of *T. confusum* of various ages are shown in table 2. This not only shows the usefulness of the equation to calculate the M.L.C., but also gives more reliable results for each age.

Figure 12 indicates the relation between the age of *T. confusum* of all stages and their corresponding M.L.C.'s of carbon disulfide.

Table 5. Average Susceptibility of Male and Female Adults of *T. confusum* and *Z. subfasciatus* to Carbon Disulfide

Species	Sex	Concentration of CS_2 mg./l.	Total number of insects	Number of tests	Average per cent mortality (2 days after fumigation)	Average calculated M.L.C. mg./l.
T. confusum	Male	80	268	4	53.2	78.9
	Female	80	259	4	66.4	74.6
Z. subfasciatus	Male	18	450	4	39.8	18.7
	Female	18	432	4	35.0	19.1

The curve is composed of four parts representing egg, larval, pupal, and adult stages. The average time required for the development of each stage, as determined with 32 individuals in a separate rearing experiment, was 8.2 days for eggs, 46.4 days for larvae, and 10.1 days for pupae. Between parts of the curve the broken lines, which represent different stages, indicate the possible trend connecting the ends of two successive stages.

In the discussion of the whole life cycle of *T. confusum* it is shown in figure 12 that the resistance of eggs decreased greatly and constantly as the embryos developed. The same rate of decreasing resistance appears to hold true for the first week of the larval stage. After that the larval resistance increases slowly but steadily. There is a tremendous increase of resistance from larval to pupal stages. During the pupal stage their resistance decreases slightly from the first to the third day but increases greatly from the third through the fifth day. After they are 7 days old their resistance decreases sharply again. The whole curve of the pupal stage is symmetrical and in a reverse order to the V-shaped curve of pupal respiration. This again indicates a close correlation between resistance of insects to fumigants and their rate of respiration. In the adult stage there is a peculiar decrease of resistance at the age of 4 days. The adults beyond 2 weeks old show less change in resistance.

The relation between M.L.C.'s of carbon disulfide for the adults of *T. confusum, S. granarius,* and *S. oryza* and their ages from 1 day to 24, 20, and 12 weeks respectively is shown in figure 13. For all three species there is more variation in resistance among younger adults than in older adults. However, the variation is greater for *T. confusum* than for granary weevils. In general, their susceptibility to carbon disulfide decreased as their age increased. In planning research on fumigation it is often useful to know the age range of insects that shows the least variation in resistance to fumigants. This can be selected from curves (figures 12 and 13).

Table 6. Median Lethal Concentration (LC_{50}), 95 Per Cent Lethal Concentration (LC_{95}), and Rate of Toxicity (V_t) of Carbon Disulfide to *T. confusum* (All Stages), *S. granarius* (Adults), *S. oryza* (Adults), and *Z. subfasciatus* (Adults) at Various Ages

Age*	2 days after fumigation			10 days after fumigation		
	LC_{50} mg./l.	LC_{95} mg./l.	V_t %/mg.	LC_{50} mg./l.	LC_{95} mg./l.	V_t %/mg.
A. *T. confusum* eggs	(Based on unhatched eggs)					
1 ± 0.5d	221.0	284.0	0.74			
3 ± 0.5d	163.5	207.0	1.28			
5 ± 0.5d	146.0	175.0	2.55			
7 ± 0.5d	98.5	123.0	2.27			

Table 6. Median Lethal Concentration (LC_{50}), 95 Per Cent Lethal Concentration (LC_{95}), and Rate of Toxicity (V_t) of Carbon Disulfide to *T. confusum* (All Stages), *S. granarius* (Adults), *S. oryza* (Adults), and *Z. subfasciatus* (Adults) at Various Ages (Continued)

Age*	2 days after fumigation			10 days after fumigation		
	LC_{50} mg./l.	LC_{95} mg./l.	V_t %/mg.	LC_{50} mg./l.	LC_{95} mg./l.	V_t %/mg.
B. *T. confusum* larvae						
1 ± 0.5d	66.2	110.0	1.52	42.4	59.0	3.57
7 ± 0.5d	24.5	56.4	2.11	20.7	48.0	2.66
14 ± 0.5d	27.0	49.5	2.78	21.7	42.5	2.56
21 ± 0.5d	39.6	52.0	3.75	33.8	47.3	4.45
28 ± 0.5d	47.3	65.4	3.66	46.4	71.0	2.14
35 ± 0.5d	50.4	75.4	2.68	49.0	80.6	1.46
C. *T. confusum* pupae	(Based on no emergence)			(Based on abnormal emergence)		
1 ± 0.5d	127.7	162.0	1.69	95.0	132.2	3.41
3 ± 0.5d	119.4	149.6	1.82	75.3	115.5	1.98
5 ± 0.5d	178.0	202.0	2.11	92.5	155.0	1.63
7 ± 0.5d	174.0	207.5	3.46	136.1	171.8	2.65
9 ± 0.5d	119.4	138.0	2.40	64.3	70.5	7.15
D. *T. confusum* adults						
1 ± 0.5d	129.0	173.2	1.16	128.0	187.0	1.16
4 ± 0.5d	97.3	115.0	2.88	95.0	126.0	1.82
7 ± 0.5d	110.5	126.3	2.82	108.2	153.0	2.14
14 ± 0.5d	84.0	104.1	2.76	78.0	.....	2.31
21 ± 0.5d	80.4	100.0	2.97	78.8	95.6	2.90
28 ± 0.5d	81.4	96.8	3.02	78.0	97.3	2.36
35 ± 0.5d	83.1	102.7	2.78	79.8	.....	2.44
8w ± 0.5d	74.0	87.8	3.77	82.6	.....	2.42
12w ± 0.5d	70.2	83.8	4.00	82.4	.....	1.76
16w ± 0.5d	68.4	79.9	3.87	75.8	96.0	2.72
20w ± 0.5d	66.0	79.3	3.34	68.2	81.0	3.53
24w ± 0.5d	69.3	80.8	3.97	68.0	80.5	3.57
E. *S. granarius* adults						
1 ± 0.5d	40.4	48.5	5.52	21.4	29.8	8.80
4 ± 0.5d	37.2	45.1	5.62	25.7	32.7	7.85
7 ± 0.5d	35.3	43.3	5.70	26.8	31.3	12.5
14 ± 0.5d	32.1	40.7	6.10	27.4	32.7	15.1
21 ± 0.5d	32.2	40.5	6.07	27.4	35.6	8.25
28 ± 0.5d	32.9	40.5	6.10	28.7	37.5	9.00
35 ± 0.5d	33.1	40.8	6.10	29.7	36.2	7.91
8w ± 0.5d	33.4	43.9	6.07	25.9	36.3	4.57
12w ± 0.5d	32.4	43.2	4.15	22.1	33.0	4.40
16w ± 0.5d	31.9	41.5	4.80	23.5	36.5	4.45
20w ± 0.5d	28.8	38.0	4.91	21.6	32.0	4.88
F. *S. oryza* adults						
1 ± 0.5d	29.0	36.7	6.36	24.5	31.4	6.56
4 ± 0.5d	25.1	31.8	7.61	22.7	30.5	5.84
7 ± 0.5d	20.8	26.0	10.8	19.7	24.9	8.51
14 ± 0.5d	20.5	24.8	10.5	20.0	24.9	9.20
21 ± 0.5d	21.7	26.1	11.4	20.0	26.1	7.34
28 ± 0.5d	22.2	28.0	8.08	20.0	26.7	6.68
35 ± 0.5d	20.7	24.9	10.5	19.6	24.5	9.10
8w ± 0.5d	21.0	27.0	7.41	15.4	24.4	7.75
12w ± 0.5d	19.8	29.2	8.70	16.3	22.2	8.70
G. *Z. subfasciatus* adults						
10 ± 1d (Male)	18.7	22.1	16.5			
10 ± 1d (Female)	19.1	22.9	14.3			

* d = day; w = week.

Effect of Nutrition

Another factor which may account for some of the differences in fumigation results is the quality and quantity of food supplied prior to fumigation. Quayle (1920) said that it is an established fact that scale insects on citrus fruit are much more difficult to kill than those on the leaves or branches. The resistance to fumigation is evidently related to food supply in this case. Likewise, Richardson and Casanges (1942a) found that the resistance of the green peach aphid, *Myzus persicae,* to nicotine fumigation varied greatly when the insects were reared on three different host plants.

Investigations of this problem are much easier to make on stored-product insects because their food can be varied and also standardized very easily. *T. confusum* larvae and adults and the adults of *S. granarius* were used in a study of the nutritive effect. The foods used for *T. confusum* larvae were corn starch, corn starch with 5 per cent yeast, patent flour, patent flour with 5 per cent yeast, enriched flour, whole wheat flour, whole wheat flour with 5 per cent yeast, and tankage. All food materials were prepared for use by sifting them through a 50-mesh sieve. One-day-old larvae were separated from egg cultures and then reared on the different foods under standard conditions of temperature and moisture. An average of about 80 larvae was used in each fumigation test for the results in figure 14. Weevils were reared on wheat and corn by standard method.

The *Tribolium* larvae and adults reared on different foods were fumigated separately with carbon disulfide. *S. granarius* adults from corn and wheat were fumigated in the same flask at the same time at each concentration of fumigant to eliminate variations due to treatment. A very small difference in the results with weevils from the two foods made this procedure necessary.

The results of this study on larvae are presented in table 7. The data included are the median lethal concentration of fumigant (LC_{50}), the 95 per cent lethal concentration (LC_{95}), and the rate of toxicity expressed as percentage mortality per milligram per liter of fumigant (V_t).

From the results of eight different combinations of food it is significantly indicated that both rate of growth and susceptibility of larvae to carbon disulfide vary greatly with the nature of food. Pure starch or a heavy protein food, like tankage, is not suitable for the development of *T. confusum* larvae. Enriched flour is only

Table 7. LC_{50}, LC_{05}, and V_t of Carbon Disulfide to *T. confusum* Larvae (21 ± 0.5 Days Old) Reared on Various Kinds of Food

Food	Average weight of 10 individuals mg.	2 days after fumigation			10 days after fumigation		
		LC_{50} mg./l.	LC_{05} mg./l.	V_t %/mg.	LC_{50} mg./l.	LC_{05} mg./l.	V_t %/mg.
1. Corn starch	0.54	52.5	68.0	3.00	37.5	64.2	2.45
2. Corn starch with 5 per cent yeast	13.6	52.4	68.0	5.40	44.6	.....	4.60
3. Patent flour	7.1	39.8	57.1	2.60	36.5	56.7	2.67
4. Patent flour with 5 per cent yeast	28.4	51.5	61.8	4.92	64.0	.....	4.54
5. Enriched flour	8.1	41.4	58.5	3.10	36.5	56.7	2.67
6. Whole wheat flour	6.3	40.7	54.3	4.11	34.7	47.2	6.17
7. Whole wheat flour with 5 per cent yeast	10.6	42.6	60.4	4.48	33.6	58.8	4.00
8. Tankage	1.2	24.7	31.7	7.00	21.7	30.2	5.42

slightly better than the patent flour, while the sample of whole wheat flour used was even less favorable to growth than patent flour (table 7). However, another sample of whole wheat flour that was different in appearance from the rest and was not used in the experiments was more favorable for development than patent flour. In each case the addition of 5 per cent of dry powdered yeast increased the rate of growth tremendously, especially in the combinations with corn starch and patent flour. The average weight of 10 larvae (21 ± 0.5 days old) reared from each kind of food is shown in table 7. The weight ratio between those larvae reared from corn starch and patent flour with 5 per cent yeast was 1:52.6.

The susceptibility of larvae reared from different food media is not easily explained individually on the basis of food composition. However, with the exception of patent flour with yeast the curves in figure 14 can be generally divided into three groups. Those larvae reared in starch were most resistant to carbon disulfide, those reared in a heavy protein food, tankage, were least resistant, and those reared in foods with both carbohydrates and proteins stood midway.

Since larvae differ greatly in size it may be incorrect to explain susceptibility differences in terms of food composition alone. Figure 6 shows the relations between the age of larvae and their weight and also between the M.L.C. and their weight. By plotting the data representing weight of larvae and M.L.C., taken from table 7, on the curve for weight and M.L.C. (figure 6) it was found that with the exception of corn starch with yeast the points follow

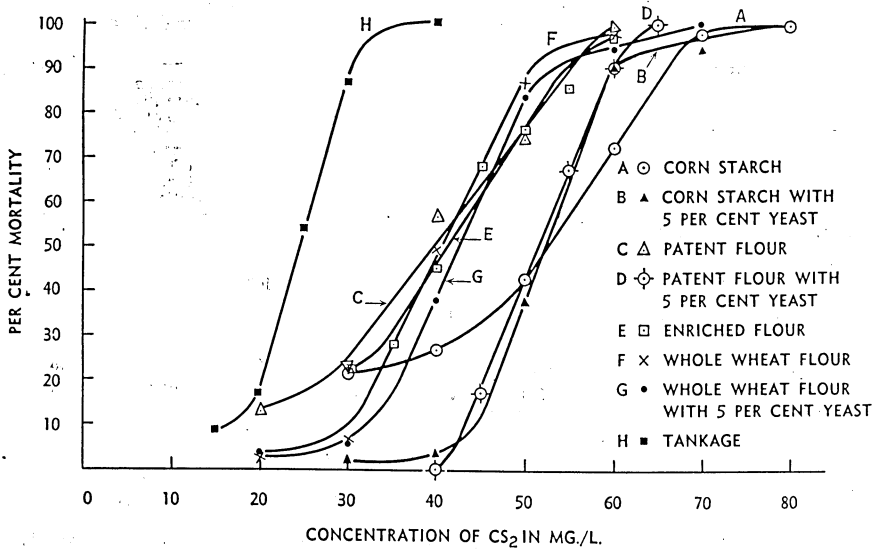

FIG. 14. Mortality curves for *T. confusum* larvae reared on various kinds of food and fumigated with carbon disulfide (2-day mortality)

closely the original curve. It appears that in general the susceptibility of larvae is closely correlated with their weight, which in turn indicates their rate of metabolism.

The results of experiments with adults of *S. granarius* and *T. confusum* are presented in tables 8 and 9. A relationship between their weight and toxicity also exists. *T. confusum* adults from patent flour with yeast are slightly heavier than those from whole wheat flour, and *S. granarius* adults from corn are slightly heavier than those from wheat. The heavier insects are also the more susceptible to carbon disulfide. The increase of mortality which corresponds to their body weight may be caused by a slight increase of respiration in the more nutritious food.

Table 8. Effect of Food on the Susceptibility of *S. granarius* Adults (28 ± 4 Days Old in Corn and 28 ± 1 Days Old in Wheat) to Carbon Disulfide

Food	Concentration of CS_2 mg./l.	Total number of insects	Per cent mortality	
			2 days after fumigation	10 days after fumigation
Corn	25	68	13.2	26.5
	30	70	38.6	65.7
	35	91	83.5	96.7
	40	78	98.7	98.7
Wheat	25	72	1.4	6.9
	30	94	36.2	57.5
	35	74	74.3	89.2
	40	85	98.8	98.8

Table 9. The Relation of Weight and Susceptibility of *T. confusum* and *S. granarius* Adults to Different Kinds of Food

Food	Average weight of 10 individuals mg.	Concentration of CS_2 mg./l.	Number of tests	Total number of insects	Average percentage of mortality (2 days after fumigation)	Calculated average M.L.C. mg./l.
A. *T. confusum* adults (28 days old)						
Patent flour with 5 per cent yeast	23.5	81	7	406	70.3	74.3
Whole wheat flour	21.5	81	5	342	60.0	77.7
B. *S. granarius* adults (28 days old)						
Corn	29.9	33	5	495	69.1	30.9
Wheat	26.0	33	5	509	63.6	31.5

Population Density Effect

The density of animal populations has long been studied as affecting to a considerable extent nutrition, oviposition, migration, longevity, etc. However, there has been very little study as to its effect on the results of fumigation. Although in the past there have been no definite statements about the density of insect stock cultures used in fumigation, it is likely that the actual density of population varied greatly from author to author. Before the standardization of the cultures in the writer's experiments it was noticed that *T. confusum* larvae with a high population density developed less rapidly than those with a lower density. A very striking retardation of development of larvae was observed in a closed can containing many separated insect cultures which had neither an adequate supply of oxygen nor any ventilation to remove carbon dioxide. Certainly, it would be expected that a variable population density could complicate the prefumigation conditions in many ways.

Young adults of *S. granarius* not more than 1 day old were separated into half-pint jars at densities of 50, 100, 200, and 300 individuals per 30 grams of wheat. They were kept at standard temperature and humidity until 28 ± 0.5 days old. Groups from the different densities were fumigated simultaneously in the same flask at a concentration of 30 mg. per liter of carbon disulfide. The natural mortality due to difference of density of population was negligible.

It is interesting to note that the 2-day mortality of the weevils significantly decreased as the density of population increased (table 10). At 50 weevils per 30 grams of wheat the mortality was 55.1 per cent; however, it decreased to 25.6 per cent at a density of

Table 10. Effect of Population Density on the Susceptibility of *S. granarius* Adults to Carbon Disulfide

Population density, number of weevils in 30 g. of wheat	Per cent mortality before fumigation	Total number of insects	Average per cent mortality in 2 tests	
			2 days after fumigation	10 days after fumigation
50	0	146	55.1	72.5
100	0.5	192	38.2	63.0
200	0	192	30.1	51.1
300	0	272	25.6	50.2

300 individuals. However, Yust and Howard (1942) found that mortality of scales on the half-infested lemon was slightly lower than that with double population, when fumigated with cyanide.

Effect of Rearing Temperature

Since insects have a higher rate of respiration at higher temperatures, this effect may persist for a while even when they are removed to a different temperature. If this is true it is incorrect to compare the results of fumigation of insects reared at different temperatures, even though they were subsequently treated exactly the same during and after fumigation.

T. confusum adults reared at 25°, 30°, and 35° C. and 60 per cent relative humidity were fumigated with carbon disulfide at various concentrations. An average of about 60 beetles was used in each fumigation test. It was found that the higher the rearing temperature, the greater was their susceptibility (figure 15). The M.L.C.'s of carbon disulfide are 81.5, 73.7, and 48.8 mg. per liter for the beetles reared at 25°, 30°, and 35° C. respectively. This shows significant differences caused by different rearing temperatures. In another experiment young adults from 30° and 35° C. cultures were transferred to 25° C. and 4 weeks later they were fumigated in the same flask together with insects reared at 25° C. The difference of mortality in the three groups was slight both at 2 and 10 days after fumigation (table 11). Such differences may

Table 11. Effect of Rearing Temperature Previous to Adult Stage on the Susceptibility of *T. confusum* Adults (28 ± 5 Days Old) to Carbon Disulfide (80 mg. per liter)

Rearing temperature before adult stage °C.	Rearing temperature during adult stage °C.	Total number of insects	Average per cent mortality of 3 tests	
			2 days after fumigation	10 days after fumigation
25	25	222	64.3	40.8
30	25	194	54.7	46.4
35	25	219	68.4	53.9

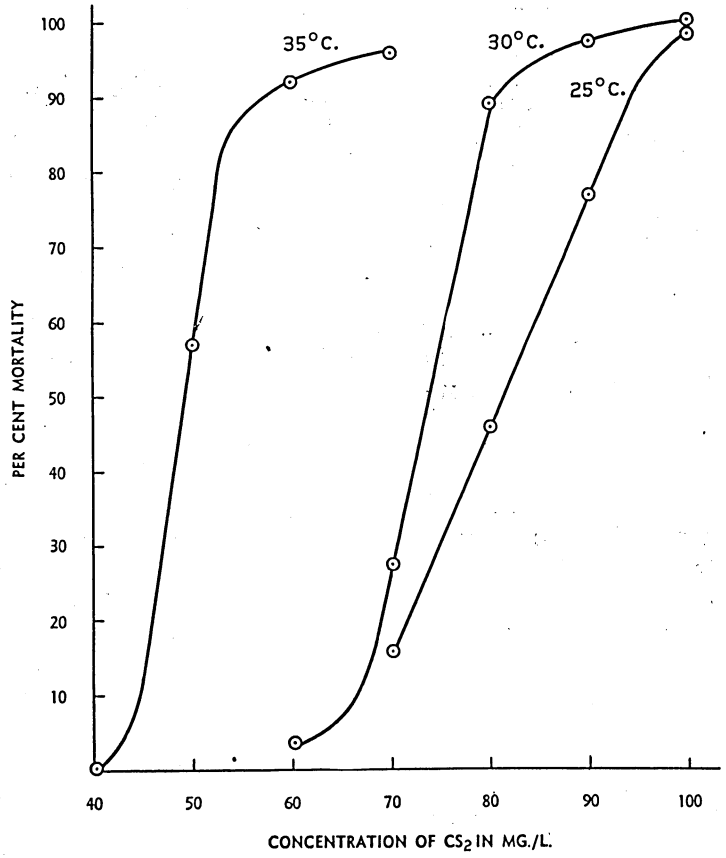

FIG. 15. Mortality curves for *T. confusum* adults reared at 25°, 30°, and 35° C. and fumigated with carbon disulfide at 25° C.

be found even in cultures kept constantly at 25° C. This suggests that previous temperature during development does not affect the results of subsequent fumigation if sufficient time is allowed for them to condition. Since *T. confusum* develops much more rapidly at 30° and 35° C. than at 25° C. it is more convenient for the toxicologists to obtain testing animals, as described above, in a shorter time at higher temperatures without losing accuracy.

Both *T. confusum* and *S. granarius* adults were used in the temperature preconditioning experiments. In order to observe the effect of different periods of preconditioning, *S. granarius* adults from the same culture were preconditioned with food at 15° and 35° C. for 0, 1, 2, 4, 6, and 8 days over a saturated solution of sodium chloride in quart jars. A small cup of sodium hydroxide

solution was included to remove carbon dioxide. After preconditioning, the insects were removed from the quart jars into the fumigation flask in about 15 minutes. Only those which would walk after stimulation (being blown on) were used in the fumigation. The natural mortality due to preconditioning was very small within 8 days. Those weevils preconditioned at 15° C. and 35° C. were fumigated with 35 and 30 mg. per liter of carbon disulfide respectively under standard conditions. For each temperature those preconditioned for 0, 2, and 6 days were fumigated in one flask and those for 1, 4, and 8 days in another flask. Each series of experiments was repeated once with insects from a different culture. An average of 50 beetles or 100 weevils was used for each test.

When preconditioned at 15° and 35° C. the mortality of both groups decreased as the number of days increased.

Effect of Preconditioning Temperature

It has been agreed that the temperature during fumigation is so important that it must be controlled very rigidly; however, the temperature at which cultures are held before fumigation has not attracted so much attention. Many experiments have been made with the cultures reared at room temperature. In the ordinary room the temperature variation may be several degrees above or below the required one, especially during the summer season. This natural change of temperature is accompanied by a fluctuation of humidity. The latter effect is shown in figure 18. The increase or decrease of temperature not only will affect the activity and respiration of insects but may seriously change other physiological conditions. The artificial simulation of such changes of temperature is called temperature preconditioning.

Most experiments in temperature preconditioning in relation to fumigation have been done on scale insects, fumigated with cyanide. In general, greater kills were obtained when the insects were preconditioned at lower temperatures before normal fumigation. The general type of the 2-day mortality curves (figure 16) is very similar; however, that of the 10-day mortality curves is somewhat different. The 2-day mortality of those weevils preconditioned at 35° C. decreased more than 40 per cent with the first 2 days of preconditioning. Then it decreased more slowly. The decrease of mortality for those preconditioned at 15° C. for the same period of time was only about 28 per cent. With a preconditioning period of 8 days both gave a decrease of about 52 per cent mortality in comparison with those without preconditioning.

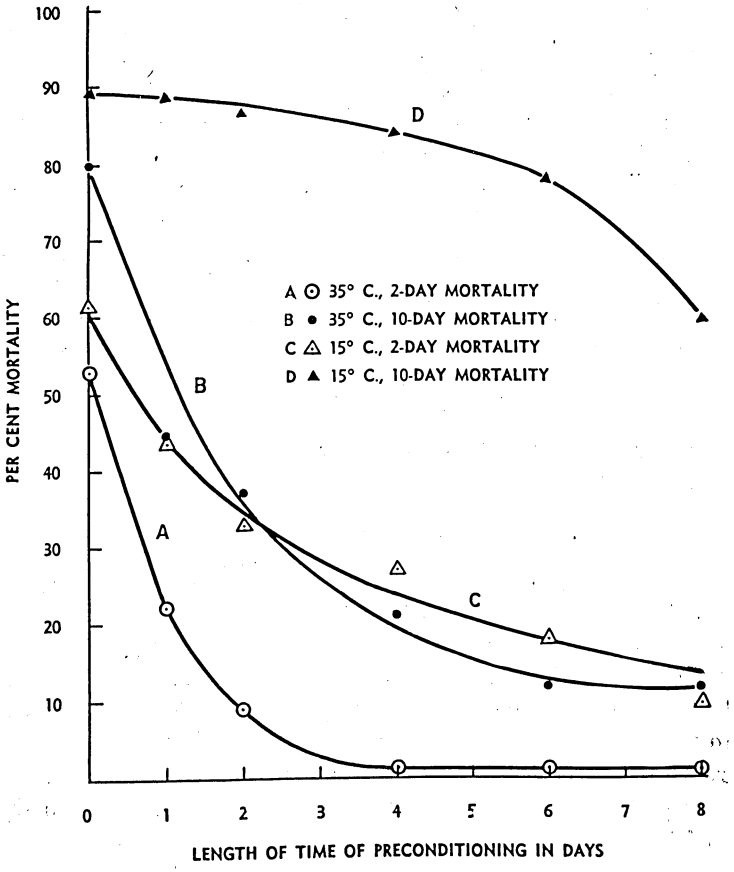

FIG. 16. Effect of the length of time of preconditioning at 15° and 35° C. on the susceptibility of *S. granarius* adults to carbon disulfide under standard conditions

Other series of experiments were performed by preconditioning at 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. for 3 days over saturated sodium chloride solution and subsequently treated under the same conditions as when the time of preconditioning was varied, except that *T. confusum* adults were fumigated 30 minutes after being removed from the cabinets instead of 15 minutes. *S. granarius* adults preconditioned at lower temperatures recovered as soon as they were returned to the room temperature; however, *T. confusum* adults took a longer time (about 5 minutes) to recover from preconditioning at 5° C. Immediately after preconditioning *T. confusum* adults became more active as the preconditioning temperature increased, while *S. granarius* adults were much less active at high temperatures (30° and 35° C.) In both

cases the percentage of natural mortality due to preconditioning was small. *S. granarius* was fumigated with 35 mg. per liter of carbon disulfide and *T. confusum* with 150 mg. per liter of carbon tetrachloride under standard conditions. The mortalities shown in two separate experiments after the same treatment differed from each other only by a few per cent.

Striking differences resulted when insects were preconditioned for 3 days at various temperatures, as shown in figure 17. It is interesting to note that the 2-day mortality curve of *S. granarius* differs from that of *T. confusum*. The former shows a minimum at 15° C. and a maximum at about 25° C., while the latter gives about the same results from 5° to 15° C. and then increases steadily to 35° C. However, these curves have one portion, from 15° to 25° C., in common. The deviation above 25° C. may be due to the decrease

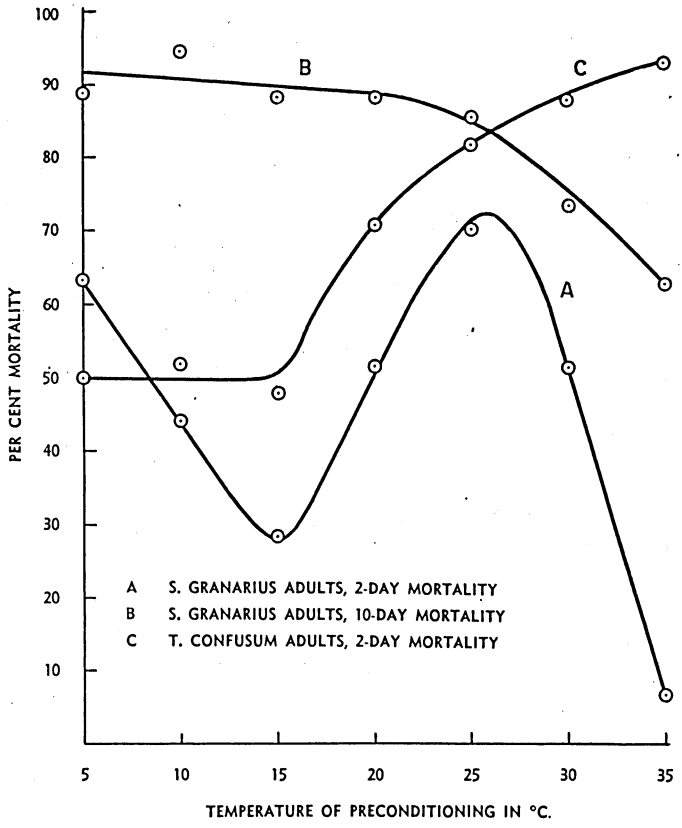

FIG. 17. Effect of preconditioning temperature (for 3 days) on the susceptibility of *S. granarius* and *T. confusum* adults to carbon disulfide and carbon tetrachloride respectively under standard conditions

of activity of *S. granarius* and the continuous increase of activity of *T. confusum.* For the most part the 10-day mortality curve of *S. granarius* runs opposite to the 2-day mortality curve of *T. confusum.*

There has been some experimental work done which has demonstrated that physiological changes do take place during temperature preconditioning which may persist for some time (Ryan, 1941). However, these changes have not been associated directly with corresponding changes in the susceptibility of insects to fumigants. The study of Sumner and Doudoroff (1938) on the acclimatization of fishes to heat showed that fish acclimatized to 30° C. were more resistant to a solution of potassium cyanide at room temperature than those preconditioned at 10° C. They explained the greater resistance after exposure to 30° C. in two ways: (1) a regulative decline in the initially much increased respiratory metabolism; and (2) another change of an unknown nature, by which the threshold of susceptibility to an unfavorable temperature is raised. Behre (1918) found something similar in her studies with *Planaria.* When they were brought from medium to high temperatures they had a greater rate of carbon dioxide production, and were more susceptible to cyanide poisoning.

In studying heat resistance of blowflies, Fraenkel and Hopf (1940) indicated that the higher the breeding temperature the greater is the degree of saturation of the phosphatides, e.g., lecithin. They believed that this, rather than the difference in cell permeability, explains the increase of the resistance to heat after acclimatization. Heilbrunn (1937) and Belehradek (1935) pointed out that acclimatization to heat could result from the production of fats having successively higher melting points. Because of the great variation in the course of susceptibility of different species of insects and the observed variation in activity, the writer believes that the variation of the susceptibility of insects is not due to a single factor but to a combination of factors, such as cell permeability, rate of respiration, and insect vitality.

The effect of temperature preconditioning of insect eggs as shown by Lindgren and Dickson (1941) may not be due only to preconditioning. It is more complex than that for other stages because slight differences in age at a critical period mean considerable difference in egg susceptibility. It follows then that when eggs are preconditioned at high temperatures it is difficult to determine whether differences in susceptibility are due more to the difference in the stage of embryonic development or to the preconditioning effect.

Effect of Preconditioning Humidity

Relative humidity has been considered a factor of less importance than temperature. For this reason there are not many well-organized data on humidity, and especially few on the preconditioning humidity. The air humidity varies greatly from season to season and also from a dry day to a rainy day. In the insecticide laboratory of the University of Minnesota the relative humidity varies from 10 per cent in the winter to 75 per cent during the summer. A sudden change of 30 per cent relative humidity has been observed during a rapid change of weather. In the stock culture containing a comparatively large volume of wheat and a great number of weevils the humidity will increase steadily to saturation near the lower part of the culture. It seems that little is known about the significance of such changes of humidity.

S. granarius adults were preconditioned at 25° C. and 30 per cent relative humidity with food for 0, 1, 2, 4, and 6 days. An average of about 90 weevils was used in each test. The natural mortality due to preconditioning was low except after 6 days (44.5 per cent). After preconditioning, the weevils which could move about after stimulation by being blown on were fumigated following a 15-minute period of separation, under standard procedure with 33 mg. per liter of carbon disulfide. The average results of two tests are shown in figure 18. It is clearly shown in figure 18A that the mortality increases very rapidly during the first day of preconditioning and then slowly but steadily with a maximum difference of about 26 per cent from 0 to 6 days. Ten-day mortality shows the same tendency but less difference.

In another series of experiments the weevils were preconditioned with food at relative humidities of 0, 20, 40, 60, 80, and 100 per cent at 25° C. for 3 days. The natural mortality was high (18.9 per cent) when preconditioned over concentrated sulfuric acid. The weevils were removed after various treatments and fumigated with 33 mg. per liter of carbon disulfide under the same conditions as described for different periods of preconditioning. As shown in figure 18B, the 2-day mortality decreased first and then increased again as the humidities increased from 0 to 100 per cent. The maximum difference was about 30 per cent and the minimum mortality was at about 40 per cent relative humidity. Ten-day mortalities showed the same tendency but differed in degree.

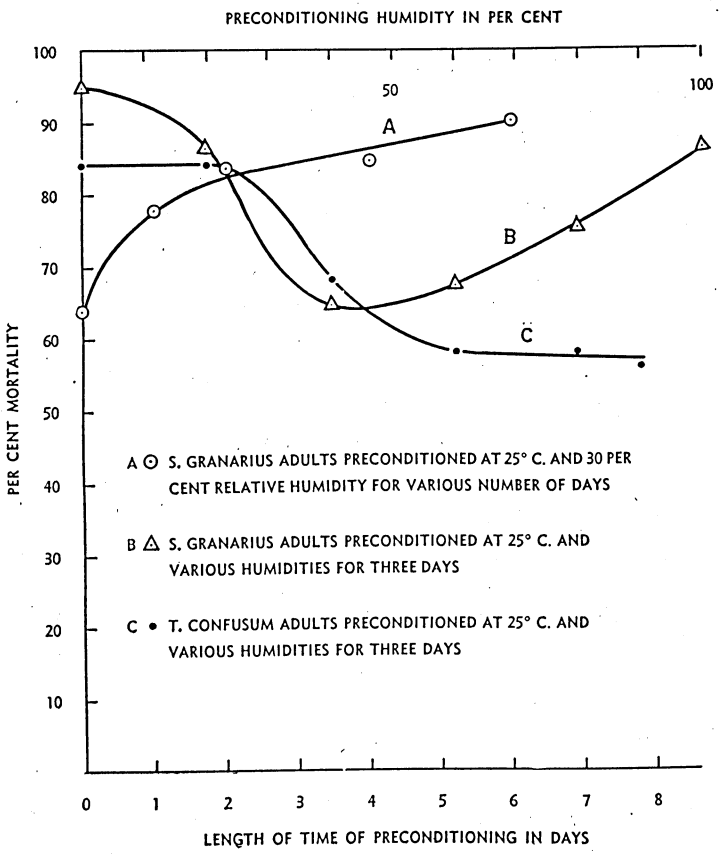

FIG. 18. Effect of preconditioning humidity on the susceptibility of *S. granarius* and *T. confusum* adults fumigated with carbon disulfide and carbon tetrachloride respectively

After preconditioning, the weevils from the treatment of below 40 per cent relative humidity were less active, especially those preconditioned at 0 per cent.

Twenty-five adults of *T. confusum* from the general culture were preconditioned for each of two tests over a thin layer of flour for 3 days at 25° C. and various humidities. They were more sluggish after being preconditioned at high humidities, especially at 80 and 90 per cent. Natural mortality due to preconditioning was nil at lower humidities and only about 1 per cent at high humidities. After normal fumigation with 150 mg. per liter of carbon tetrachloride under standard conditions the end point of mortality was more definite as the humidity of preconditioning decreased.

From the results in figure 18C, the greatest rate of change of mortality due to preconditioning was between 20 and 60 per cent relative humidity. Less change in mortality occurred beyond this range than was found with *S. granarius*.

Effect of Starvation before Fumigation

Starvation, a factor which may be related to the kind of food used, the temperature, population density, and methods of handling cultures, also affects the results of fumigation tests. In studying this effect, about 80 adults of *S. granarius* from standard cultures were starved for 0, 1, 2, 4, 6, and 8 days at 25° C. and 60 per cent relative humidity. Then they were transferred and fumigated in two flasks, as described in the temperature preconditioning experiment, with 30 mg. per liter of carbon disulfide under standard conditions. It was noticed that the activity of the weevils decreased as the time of starvation increased. The natural mortality due to starvation was low within 4 days; however, it increased rapidly after 4 days. The results of starvation before fumigation (figure 19A) show that the susceptibility of the weevils decreased slightly after the first day of starvation and then increased the second day. After that the mortality was almost constant up to 8 days.

In addition 50 adults of *T. confusum* from the general culture were starved in each of two tests for 0, 1/4, 1, 2, 3, 4, 5, and 6 days at 25° C. and 75 per cent relative humidity before standard fumigation with carbon tetrachloride (150 mg. per liter). Their loss of weight was almost directly proportional to the time of starvation. The natural mortality due to starvation was low within a period of 6 days. The results of fumigation of *T. confusum* adults after starvation were somewhat different from those of the *S. granarius* adults (figure 19). The mortality of the beetles increased steadily with the time of starvation.

During starvation two important factors—metabolism and vitality—will operate at the same time. The decrease of metabolism which will undoubtedly occur after the start of starvation will cause a corresponding decrease of respiration. In turn, less fumigant will be taken in as a result of decreased respiratory movement. Eventually, starvation may cause a slight lowering of the mortality so far as metabolism is concerned. On the other hand, starvation will decrease the vitality of the insects and, as a result, decrease their resistance to fumigants. The balance of these

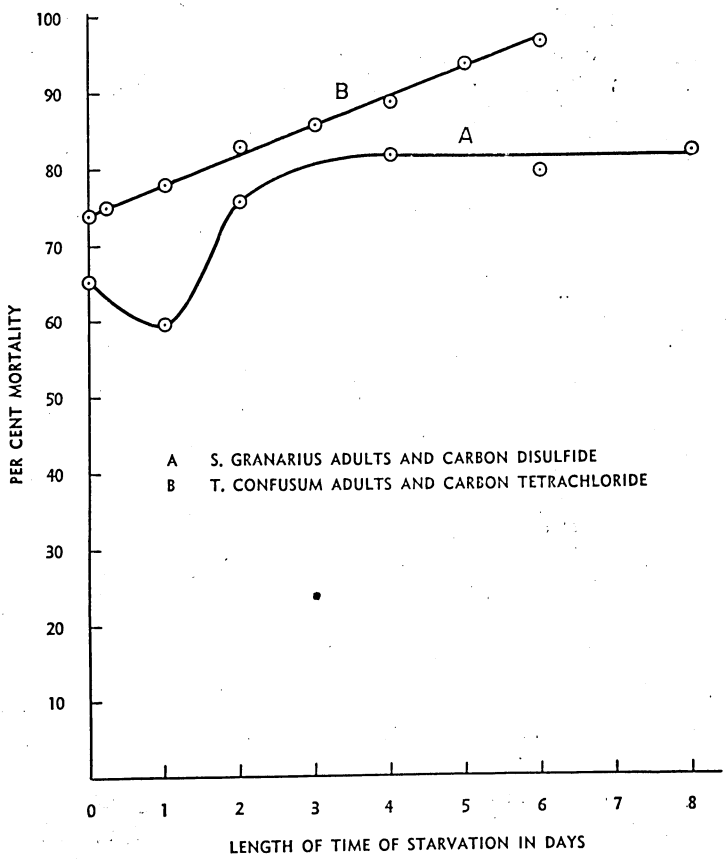

FIG. 19. Effect of starvation on the susceptibility of *T. confusum* adults to carbon tetrachloride and *S. granarius* adults to carbon disulfide after normal fumigation

two factors will determine the course of the curve. The depression of the curve of *S. granarius* is likely due to the change of the dominant factor from metabolism to vitality. In the case of *T. confusum* adults the decrease of vitality seems to be more important than the effect of the rate of metabolism.

FACTORS AFFECTING MORTALITY OF INSECTS DURING FUMIGATION

Toxicologists have considered most seriously the conditions during fumigation. Naturally, these factors are more closely related to the susceptibility of insects than prefumigation and postfumigation conditions. Among these factors, temperature, ex-

posure time, and concentration of fumigant were most extensively studied. In this study humidity, sublethal fumigation, sorption, penetration, and distribution of fumigant were considered also.

Concentration and Distribution of Fumigants in a Fumigation Vessel

Both in practical and laboratory fumigation uniform distribution of fumigants is usually very important. The uniformity of distribution depends upon the method of introducing fumigants, the readiness of volatilization, the efficiency of stirring, the tightness of the container, the sorption power of the fumigated materials, etc. A constant, measured concentration of gas surrounding the experimental insects seems to be maintained with difficulty. In large fumigation tents or chambers it was often found that the concentration was not uniform and fell considerably during the experiment through leakage and sorption. In empty, small glass vessels leakage and sorption are not as serious as in large chambers; however, the difficulty of measuring an accurate volume is increased for very toxic substances.

In the absence of circulation the concentration of the fumigants, especially those heavier than air, varies at different layers. This variation is generally not determined. In a larger fumigation chamber, like the fumigation tent for citrus trees, the concentration at a definite spot can be sampled and analyzed without much disturbance. In a small fumigation flask it can hardly be done without a serious disturbance of the adjacent concentration. A biological method for the determination of concentrations in various layers has been successfully developed, and used in this paper, in order to determine how much error may result when the fumigant is undisturbed after introduction.

A known amount of fumigant was introduced into a 6.4-liter Erlenmeyer flask which was then thoroughly shaken after the complete evaporation of the fumigant. A uniform concentration of fumigant was built up in the flask. Ten adults of *T. confusum,* reared from a general culture, were introduced into each flask in a small glass basket. As soon as they were in the toxic vapor they changed from their normal walking to running. Then the movement of their legs became abnormal and out of control, yet they still could walk. After that they began to struggle, as if they wished to walk about but were unable to do so. This was termed "locomotory paralysis." The time was recorded when half of the insects showed such a paralysis. For each concentration 10 dupli-

cates were carried out. By varying the concentration of a fumigant a series of data on the time when 50 per cent locomotory paralysis occurred was obtained. Curves in figure 20 show the relationship between concentration of fumigant and time for 50 per cent locomotory paralysis due to carbon tetrachloride, chloropicrin, carbon disulfide, and ethyl formate. From these curves the apparent concentration which produces 50 per cent locomotory paralysis at equal intervals of time can be obtained. Among these four fumigants carbon tetrachloride gave the best end point. At concentrations of and below 100 mg. per liter the end point of carbon tetrachloride was less sensitive.

To determine the unknown concentration at a certain layer of the flask, carbon tetrachloride, chloropicrin, carbon disulfide, and ethyl formate were introduced in two ways into 6.4-liter flasks at a concentration of 200, 60, 220, and 140 mg. per liter, respectively. In the first method each fumigant was delivered into and spread on the bottom of the flask without stirring or shaking. In the second method the fumigant was allowed to run down and spread over the wall of the flask. Two baskets, each containing 10 *T. confusum* adults, were introduced into each flask, one at a height of 1 inch and the other at 8 inches from the bottom, immediately after the spreading of the fumigant. The results are shown in table 12. The equivalent concentration of each fumigant at any

Table 12. Estimation of the Concentration of Fumigants at Different Layers of a 6.4-Liter Erlenmeyer Flask, Using *T. confusum* Adults as Testing Animals

Fumigant	Distance from the bottom of flask	Observed time for 50 per cent locomotory paralysis	Equivalent concentration reading from figure 20	Predetermined concentration	Difference from predetermined concentration
	Inches	Minutes	Mg./l.	Mg./l.	Mg./l.
Method I (spreading over bottom, without stirring)					
1. Carbon tetrachloride	1	1.84	390	200	+190
	8	29.22	127		− 73
2. Chloropicrin	1	0.78	67	60	+ 7
	8	30.63	32		− 28
3. Carbon disulfide	1	2.01	432	220	+212
	8	18.19	118		−102
4. Ethyl formate	1	2.92	280	140	+140
	8	20.88	74		− 66
Method II (spreading over the side wall of the flask, without stirring)					
1. Carbon tetrachloride	1	6.88	197	200	− 3
	8	8.05	182		− 18
2. Carbon disulfide	1	4.42	280	220	+ 60
	8	10.09	163		− 57

level was obtained by finding the value of observed time for 50 per cent locomotory paralysis on the X-axis of the same fumigant in figure 20, and then reading the corresponding value of the concentration on the Y-axis.

By spreading the fumigant on the bottom of a flask (method I) the concentration of all four fumigants was much greater at 1 inch than at 8 inches. The ratios of the equivalent concentrations at 1 inch and 8 inches from the bottom of the flask are 3.1, 2.1, 3.7, and 3.8 for carbon tetrachloride, chloropicrin, carbon disulfide, and ethyl formate respectively. Only carbon tetrachloride and carbon disulfide were used in method II. For both of them there was less variation in concentration at 1 inch and 8 inches and their concentration ratios are 1.1 and 1.7 instead of 3.1 and 3.7.

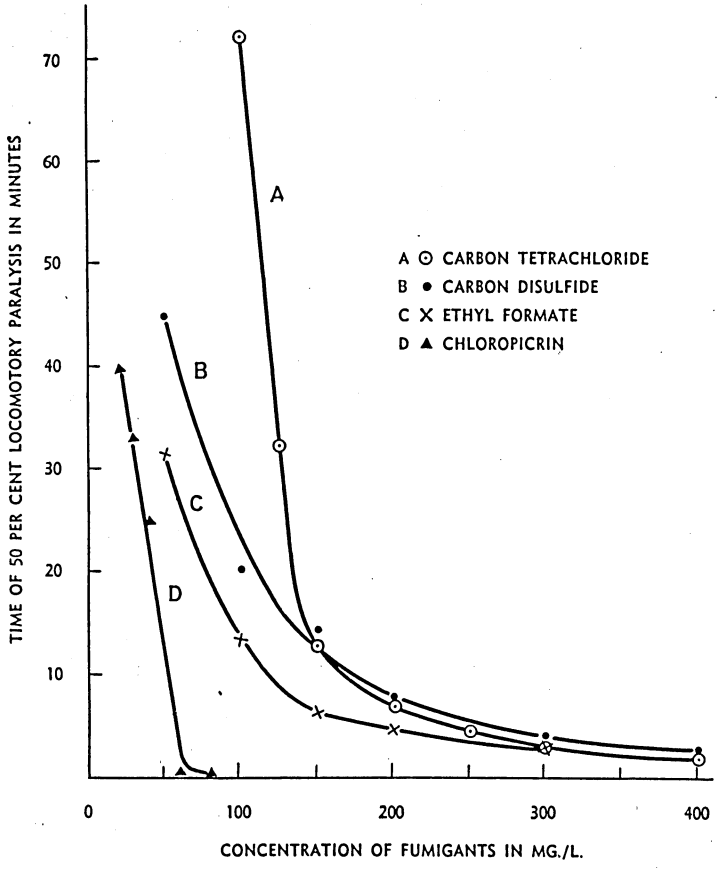

FIG. 20. Effect of various concentrations of four fumigants on the time of 50 per cent locomotory paralysis of *T. confusum* adults at 25° C.

When carbon tetrachloride was delivered rapidly from a two-milliliter pipette by moving it around the side of the flask, the insects at 1 inch high were paralyzed after a slightly shorter period of time than those at 8 inches. If delivered slowly the result was reversed. This indicated that the actual concentrations at various layers were affected by the rate of pouring the fumigant along the side of the flask. Since more loss of carbon tetrachloride was noticed by its odor during slow delivery, for these experiments the fumigants were delivered as fast as possible to make an accurate reading.

The difference in time of 50 per cent locomotory paralysis at two layers for these four fumigants is a more or less straight-line relationship to their molecular weight. This indicates that the heavier the vapor, the slower the rate of diffusion.

The curves in figure 20 indicate not only the concentrations of various layers at a certain moment but also the period of activity of tested insects at a certain concentration. More curves of this type for various fumigants and other species of insects are necessary to further the explanation of insect susceptibility in relation to the active respiration period in certain toxic vapors.

It has been shown clearly that nonuniform distribution of fumigants in 6.4 liter flasks will cause a tremendous variation of the concentration of fumigants at different layers (table 12). The variation is much less when the fumigants are spread along the side wall of the flask than when spread over the bottom. Naturally the toxic vapor will be gradually mixed with the air by its own diffusion power. In the course of a 5-hour fumigation the results will not differ as much as in the time required for 50 per cent locomotory paralysis.

Experiments have been carried out on the fumigation of *T. confusum* adults with carbon tetrachloride (200 mg. per liter) at 1 inch and 8 inches above the bottom of the flask by spreading the fumigant over the bottom (figure 21). In a series of fumigation results those fumigated at 1 inch always gave a significantly higher kill than at 8 inches; however, these differences are smaller after 12 hours of fumigation. The difference in mortality, an average of 19 per cent from 1 to 12 hours of fumigation, is much less than the difference in the time for complete locomotory paralysis, 3 minutes at 1 inch and 60 minutes at 8 inches.

When carbon tetrachloride (200 mg. per liter) was spread over the side wall of the flask the time for complete locomotory paralysis was 15 to 17 minutes at 8 inches and 7 to 9 minutes at 1 inch; however, the percentage mortality after 1-hour fumigation was

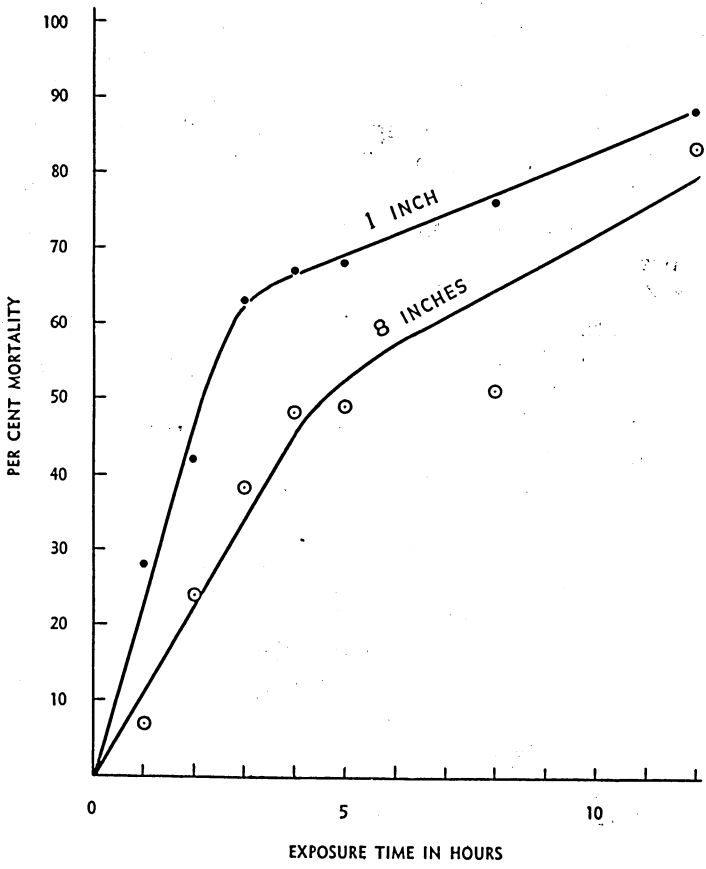

FIG. 21. Effect of nonuniform distribution of carbon tetrachloride (200 mg./l.) on the mortality of *T. confusum* adults after fumigation for various lengths of time

the same (16 per cent). By introducing insects after a uniform stirring of carbon tetrachloride in the flask it took 10 to 11 minutes for complete locomotory paralysis and produced an average mortality of 14 per cent.

Effect of Exposure Time and Concentration of Fumigation

The factor of time in relation to the toxicity of fumigants is so obvious that it does not need much explanation. The difficulty of measuring exposure time is due to the fact that the insects cannot be exposed to the full concentration of a fumigant instantaneously. This problem is more acute in practical fumigation. From the calculations based on the assumption that $CT = K$, Busvine (1938)

found that with Bovingdon's apparatus (1934) there is a definite lag, as measured by chemical determination, before full experimental concentration is attained. By integrating, the effect of the lag may be estimated as being equivalent to a complete hiatus of 1.6 minutes. However, in a 5-hour fumigation with moderate concentrations this lag, as explained later, may be negligible.

In a study of fumigation time Swain (1918) found that there was no practical difference in the efficiency of killing scale insects between 45, 60, and 90 minutes in experimental fumigations, or 45, 50, and 55 minutes in commercial fumigations. Direct comparisons in citrus orchard fumigations against red and black scales have revealed no significant differences in kills resulting from exposures of 25 to 30 minutes as against exposures of 45 minutes (Kirkpatrick, 1939).

In practical fumigation the situation is different from experimental studies where the fumigation may be completed with the concentration remaining uniform throughout the experimental period. As indicated by Pratt *et al.* (1931) and others, the concentration of hydrogen cyanide both in a tent and in a fumigatorium reaches its maximum within 5 minutes, and the concentration is very low after 40 minutes, especially when the mortality is compared at or above 90 per cent. There would be hardly any difference to be observed at low concentrations or where there was high mortality after prolonged fumigation.

The method of fumigation used in this paper is to expose the tested insects to the full desired concentration immediately. In studying the influence of the time factor nine different concentrations of carbon disulfide were used to fumigate *T. confusum* larvae, with an average of about 60 individuals in each test. Each concentration was combined with several variations of exposure time. The results are shown in figure 22.

The importance of exposure time increased as the concentration of fumigant increased. By figuring their comparative degree of importance the mortality of larvae fumigated at 10, 20, 30, 40, 70, 100, 120, 150, and 200 mg. per liter of carbon disulfide increased or decreased 1.4, 3.6, 4.4, 6.3, 8.9, 10.9, 13.7, 18.9, and 26.4 per cent respectively during a 10-minute period along the straight-line portion of the curves (figure 22).

Various attempts have been made to find a definite relationship between exposure time, T, and concentration of fumigant, C. Among the earlier investigators (Quayle and Knight, 1921; Knight, 1925; Brinley and Baker, 1927; and Peters and Ganter, 1935), the equation, $CT = K$ (constant), was used. However, this

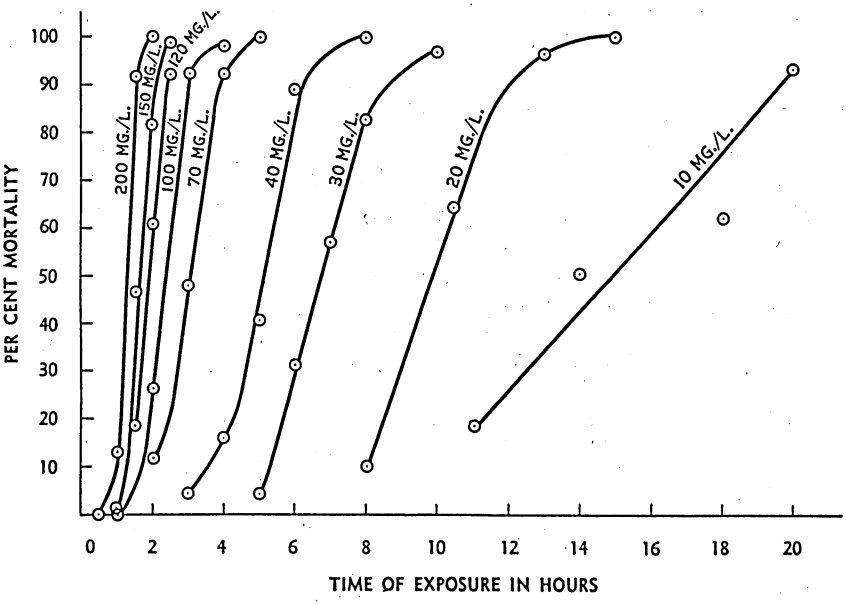

FIG. 22. Toxicity curves indicating the relation between concentration of carbon disulfide and exposure time for *T. confusum* larvae

simple equation can only be true within a limited range of time and concentration. Potter (1937) found that the time-concentration product of "Etox" (i.e., ethylene oxide and 10 per cent carbon dioxide) when used against *Plodia interpunctella* was a constant within a certain limit. Richardson and Casanges (1942a) found that nicotine vapor concentration affected efficiency more than did exposure time. Moore (1936) quoted and recalculated a large quantity of data from various papers to find the relation between concentration and time. He found that their relation does not always hold by the simple equation: $CT = K$. When the relationship of concentration and length of exposure was of unequal value the exponent of one or the other became greater or less than one. Their relation could be represented by a general equation: $CT^x = K$. By calculation with the regression equation ($C^n t = W$) Busvine (1938) obtained $C^{1.51}\ t = 40.6$ for *Tribolium castaneum*, $C^{1.18}\ t = 19.3$ for *Sitophilus granarius*, $C^{1.00}\ t = 12.6$ for *S. oryza*, and $C^{1.05}\ t = 26.8$ for *Cimex lectularius*. He applied the exponent to the concentration of fumigant instead of to exposure time. Bliss (1940) proved that Ostwald's equation (Ostwald and Dernoscheck, 1910) $(C - C_o)^n t = k$, is suitable for a large majority of cases which dealt with exposure time and concentration of fumi-

gant. English (1943) indicated that the product of concentration (D) and exposure (E) was found to be an exponential function of temperature (T, in °F.), and the relationship is approximated by the equation $DE = 40.23e^{-0.03T}$.

In the study of the relation between exposure time and the concentration of a fumigant, *T. confusum* larvae (21 ± 0.5 days old), *S. granarius* adults (28 ± 1 days old), and *S. oryza* adults (28 ± 1 days old) were fumigated with carbon disulfide under standard conditions of temperature and humidity. The results are shown in figures 22 and 23. Table 13 and figure 24 show the relation between time, T, and its corresponding M.L.C., and also between 1/T and M.L.C. The former relation is a parabola while

Table 13. The Effect of Exposure Time on the M.L.C. of Carbon Disulfide to *T. confusum* Larvae, *S. granarius*, and *S. oryza* Adults

	Exposure time, T	$\frac{1}{T}$	M.L.C. (based on 2-day mortality)	x value of equation $CT^x = K$ calculated from two successive values
	Hours		Mg./l.	
T. confusum larvae	1.27	0.787	200	
				1.19
	1.61	0.621	150	
				1.43
	1.88	0.532	120	
				0.79
	2.37	0.422	100	
				1.45
	3.04	0.329	70	
				1.08
	5.10	0.196	40	
				1.03
	6.73	0.149	30	
				1.07
	9.83	0.102	20	
				1.68
	14.88	0.067	10	
				1.21
S. granarius adults	1.5	0.667	92.9	
				1.07
	2.5	0.400	53.9	
				0.69
	5.0	0.200	33.3	
				0.57
	10.0	0.100	22.3	
				0.75
S. oryza adults	1.5	0.667	56.7	
				0.81
	2.5	0.400	37.3	
				0.74
	5.0	0.200	22.3	
				0.82
	10.0	0.100	12.7	
				0.79

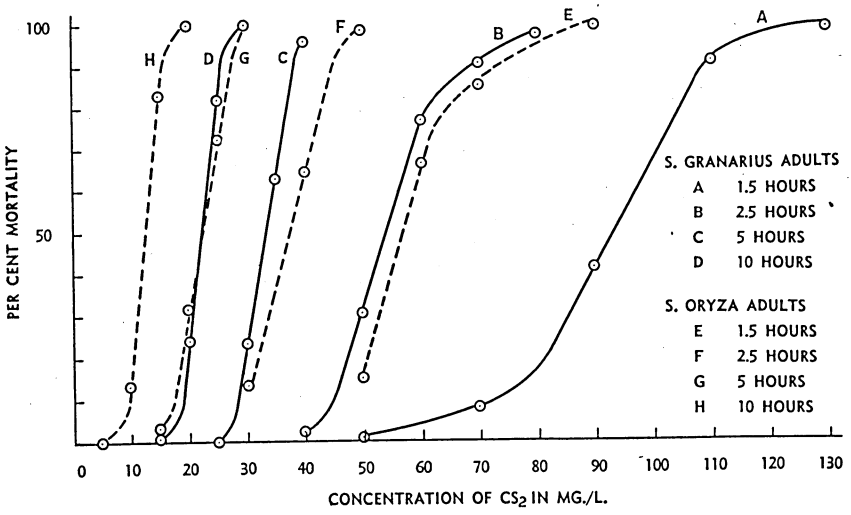

FIG. 23. Mortality curves of *S. granarius* and *S. oryza* adults fumigated with carbon disulfide with various lengths of exposure time

the latter is, in general, a straight line passing through the origin. The parabolic function of the curves in figure 24 verifies Knight's (1925) statement: "There is a minimum concentration below which no kill is effected regardless of length of exposure, and vice versa, a minimum exposure below which no concentration however high will effect a kill." The straight-line relationship between 1/T and M.L.C. can be used for the predetermination of M.L.C. at an unknown exposure time or to determine the exposure time at a given concentration.

By using the simple equation $CT = K$, the K values of *T. confusum* larvae decrease and those of the weevils increase as the exposure time increases. When two successive values were calculated according to the modified formula $CT^x = K$, the value of the exponent x still varied to a certain extent (table 13). Greater differences can be expected to occur at the extremes of concentration and exposure time. The exponent values of ^{T} obtained by Moore (1936) from various papers are calculated from a few data which do not show as much variation as they would if more data were available. For example, the exponent values of ^{T} calculated from the data on *S. oryza* found in the work of Shepard and Lindgren (1934) seem to have been obtained from the concentrations producing 100 per cent mortality. However, some of the values calculated from the concentrations producing 50 per cent mor-

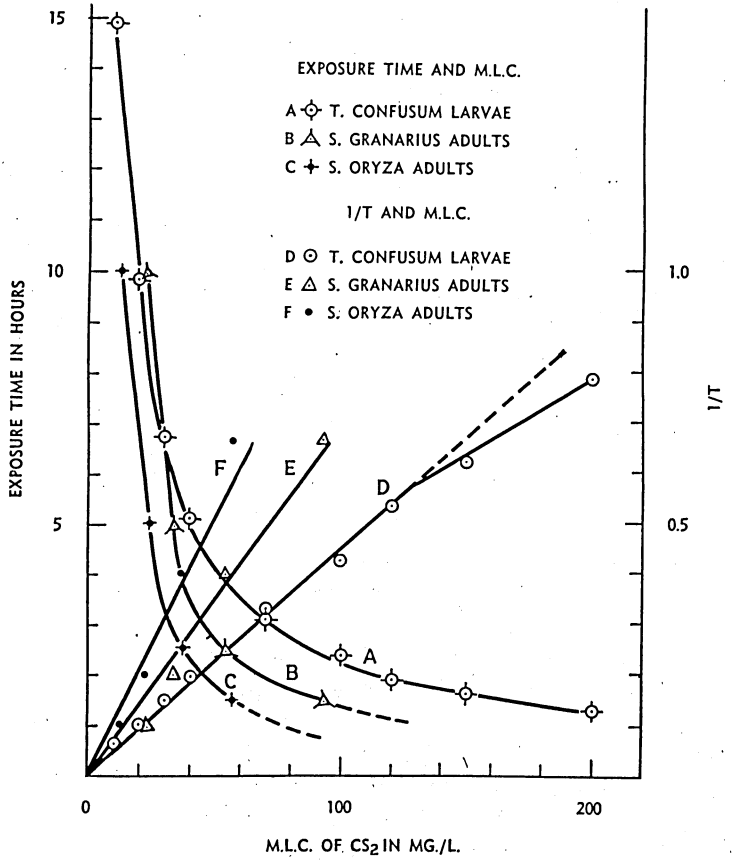

FIG. 24. The parabolic relation between exposure time, T, and M.L.C., and the straight-line relation between 1/T and M.L.C.

tality are quite different from those obtained from the concentrations producing 100 per cent mortality. It appears that the modified formula is also only true to a certain limit.

In order to provide the basis for a better understanding between the relation of concentration of a fumigant and the exposure time it is necessary to reveal the nature of the insect reaction to fumigants. When the insects in an active stage, such as adults, are introduced into a fumigant they are first excited for a while and then become quiet because of toxic action. This involves a large amount of toxic vapor entering the insect body through active spiracle movement at the very beginning. The time of such an increase of activity, as indicated in figure 20, varies with the nature and concentration of the fumigant. On the other hand, it

takes a certain interval of time for the toxic vapor to diffuse into the vital center to produce injury. The intensity of these two factors, if there are no other factors, and the percentage of time occupied by each during fumigation would cause a complex relationship between concentration of fumigant and exposure time. The deviations of curves in figure 24 from a straight line may be caused by the action of the above factors.

Without regard to the respiration effect, which may be smaller in case of larvae, the concentrations of carbon disulfide and the respective time necessary to produce 50 per cent mortality of *T. confusum* larvae can be calculated by the equation:

$$C\ (T - x) = K \qquad (4)$$

where C is the concentration of carbon disulfide; T, the time of exposure to produce 50 per cent mortality; x, the time required for the fumigant to diffuse into the vital center before building up a constant flow of fumigant; and K, a constant. From calculation on the basis of 40 mg. per liter the average value of x is 0.33 hour. By substituting each pair of values of concentration and time in the above equation the calculated values of K are 190, 192, 191, 190, 204, 186, 192, and 188 at 20, 30, 40, 70, 100, 120, 150, and 200 mg. per liter concentration respectively. The average value of K is 191.6, and the equation for *T. confusum* larvae is

$$C\ (T - 0.33) = 191.6 \qquad (5)$$

The results of calculation did deviate more at a concentration of 10 mg. per liter, and this value was not included in the average values of x and K given above.

Effect of Temperature during Fumigation

It has been generally recognized that the rate of metabolism and consequently the respiratory activity of insects is affected very greatly by temperature. This relationship has been experimentally proved by various authors (Raffy, 1934; Kozhanchikov, 1934; Crescitelli, 1935; Koidsumi, 1935; and Lindgren, 1935) on many species of insects. Respiratory activity increases with an increase of temperature only to a certain limit and then decreases.

Since respiration greatly influences the rate at which insects take up fumigant vapor and is itself greatly affected by temperature, it follows that temperature is highly important in determining the effectiveness of a fumigant. As a general trend the toxicity of a fumigant will increase with an increase of temperature. Lindgren (1935) took into consideration the respiration of insects

at various temperatures in the study of fumigants. He concluded that the rate of respiration was important but not the only factor to take into consideration when studying susceptibility of insects to fumigants.

Besides respiratory activity, a rise of temperature will increase the rate of diffusion of gases, decrease sorption, increase the reactivity of chemicals, increase volatility, and affect the vigor of insects. In addition extreme temperatures may affect the permeation of the membrane. The above actions will be reversed, in general, if the temperature is lowered.

Among the studies of the temperature effect on the toxicity of a fumigant, Shepard *et al.* (1937) made a more thorough investigation of the effect of temperature upon median lethal concentrations of carbon disulfide, ethylene dichloride, and chloropicrin on *T. confusum* adults. It is interesting to note that they found the same peak at about 10° C., which corresponds to the optimum temperature in longevity experiments (figure 27).

The writer performed the same experiment over a range of temperatures from 0° to 35° C. with carbon disulfide, using eggs of *T. confusum*. Standard cultures of eggs 7 ± 0.5 days old were fumigated at various temperatures and 75 ± 5 per cent relative humidity (except at 0° C.). An average of about 120 eggs was used in each test. The correct percentage of unhatched eggs was calculated by equation (2) from checks which were treated at the corresponding temperature for 5 hours. It is clearly shown that the effectiveness of carbon disulfide decreased as the temperature decreased until 5° C. and then it increased at 0° C. (figure 25). The rate of toxicity, V_t, also decreased with the temperature and then increased below 10° C. The M.L.C.'s of carbon disulfide for *T. confusum* eggs fumigated at 0°, 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. were 201, 242, 221, 181, 138, 88.5, 37.5, and 21.2 mg. mg. per liter respectively. When the M.L.C.'s for the eggs are plotted against temperature (figure 26) and compared with the results for adults (Shepard *et al.*, 1937) it may be seen that a straight-line relationship holds true from 10° to 30° C. for eggs but from 15° to 35° C. for adults. These two curves are similar in general shape except that a particularly large amount of carbon disulfide is required for adults when the temperature changes from 15° to 10° C. This may be explained by a difference in their method of respiration. In the adult stage the fumigant penetrates into the insect body through the spiracles by the aid of respiratory movements, while in the egg stage the toxic vapor can get into the insect only by diffusion through the egg chorion. Below 15° C.

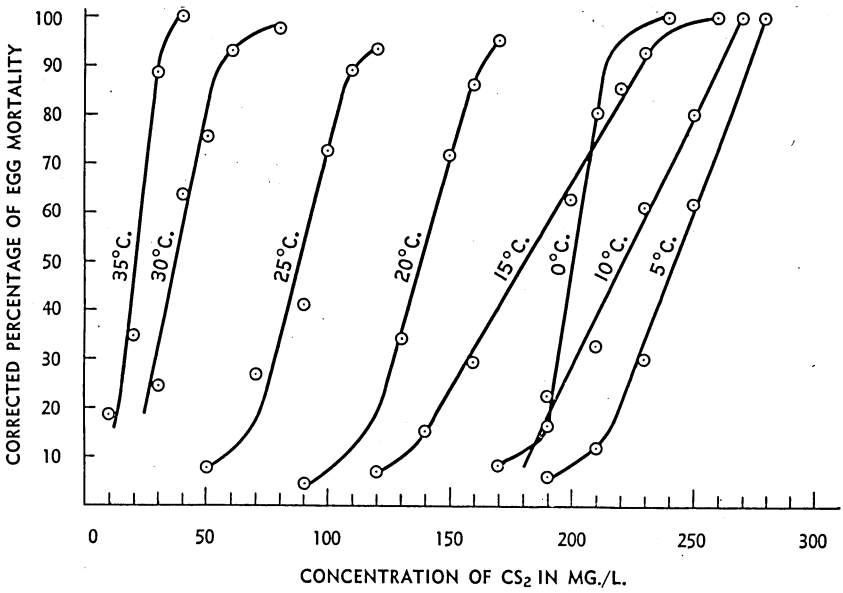

FIG. 25. Mortality curves for *T. confusum* eggs fumigated with carbon disulfide under standard conditions but at various temperatures

the adults are almost motionless, but the temperature is not low enough to affect the vigor of the adults so that a sudden increase of M.L.C. is shown in the curve. The curve for M.L.C. of eggs shows a straight-line relationship between the rate of permeation and temperature, while that of the adults shows a straight-line relationship mainly between activity and temperature.

According to the literature the increase of effectiveness of a fumigant may be explained in several ways. Moore (1936) stated that the mortality of an insect may be greater at lower temperatures which favor the absorption of the gas rather than higher ones which favor their chemical and physiological action. Peters (1936) found that the resistance of the granary weevil to hydrogen cyanide was lowered at a temperature of 5° C., owing to the loss of the ability to effect a defense reaction which occurs at higher temperatures. Peters and Ganter (1935) pointed out that at 0° C. lower concentrations of hydrogen cyanide were required to kill the granary weevil than at 17° C., giving as the reason the physiological condition of the insects was very different at the two temperatures. Shepard *et al.* (1937) showed that at temperatures below 10° C. the M.L.C. of a fumigant was rapidly reduced, probably because of the combined toxic effects of the fumigant and of

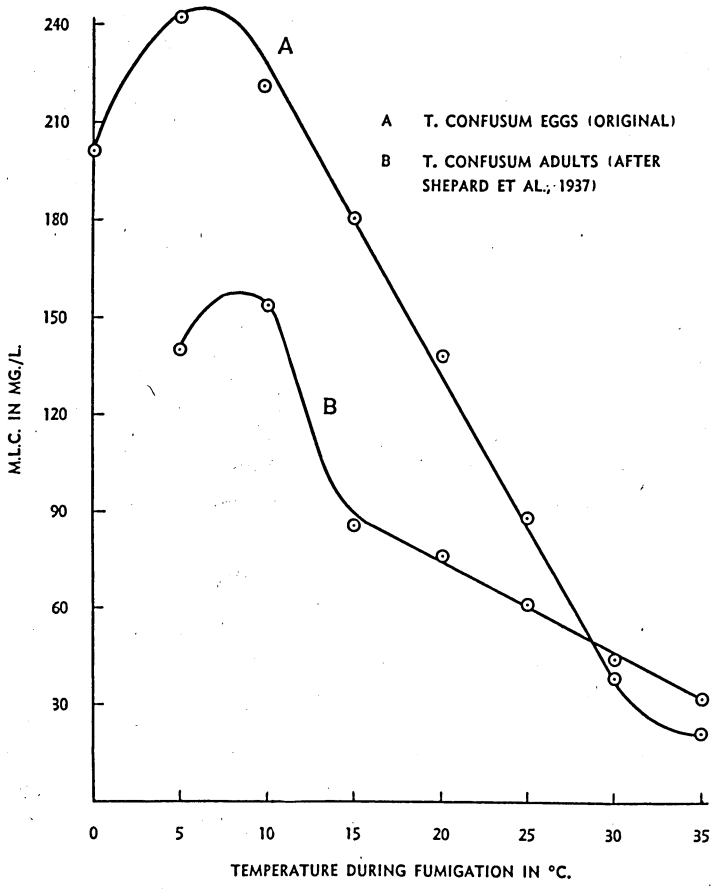

FIG. 26. Effect of temperature upon median lethal concentration of carbon disulfide for eggs and adults of *T. confusum*

the low temperature. In regard to the nature of the toxicity of fumigants, Peters (1938) stated that the effect of ethylene oxide, which is a cell poison, is accelerated by an increase in temperature; the effect of hydrogen cyanide, a respiratory poison, is reduced by high temperature owing to a respiratory effect. On the other hand, methallyl-chloride, an irritant poison, is as effective at a low temperature as at a high one.

As to chemical and physical laws the action of a fumigant should have a definite relationship to temperatures from 0° to 35° C., provided the concentration of the fumigant is not above saturation. Therefore, chemical reaction, diffusion, and sorption should persist in their normal relationship even when the tem-

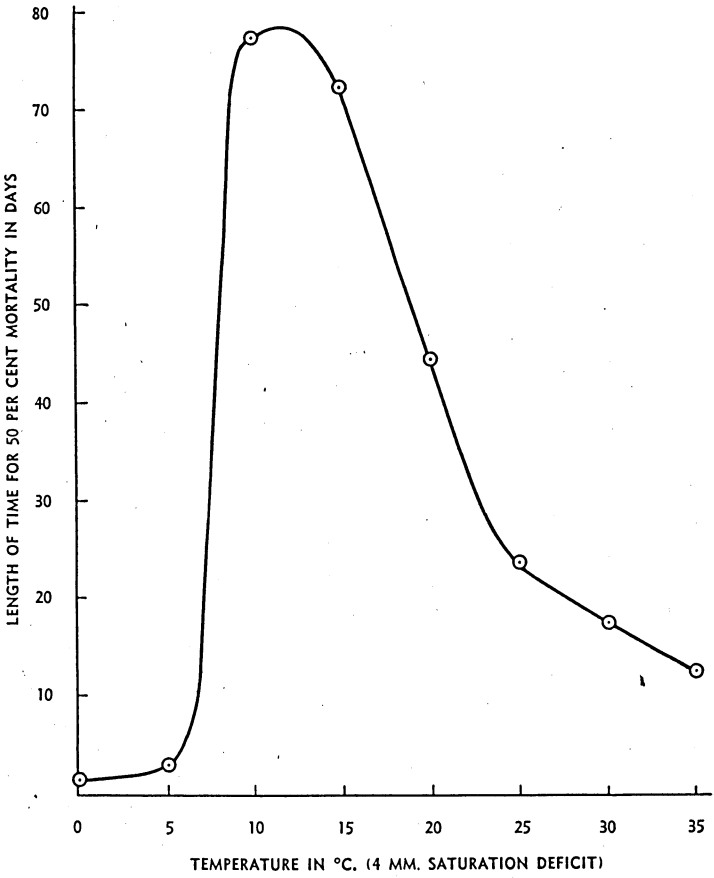

FIG. 27. Effect of temperature on the longevity (50 per cent mortality) of *T. confusum* adults kept without food

perature is as low as 5° or 0° C. The probable reason for increasing toxicity at lower temperatures is more biological than chemical or physical. In the case of water-soluble fumigants, like hydrogen cyanide, a saturated humidity at lower temperatures should be avoided in order to eliminate the local high concentration of fumigant around the insects. There has been no satisfactory explanation as to the actual effect of lower temperature on insects during fumigation. From the results of longevity experiments (figure 27) it may be seen that the survival time of *T. confusum* adult is greatly decreased from 10° C. to 5° C. Since the insects lose a great deal of water before death it appears that water permeability through insect membranes may be greater at 5° C. than at 10° C. This may be true also for fumigants. All the three fumi-

gants used for *T. confusum* adults (Shepard *et al.*, 1937) and carbon disulfide for eggs (figure 26) show a peak at about 10° C. These data, together with the preceding reason, can explain the specific nature of the membrane permeability of this species. In the adult stage spiracle movement is the primary cause of the penetration of a fumigant into the insect body; however, the subsequent diffusion through the tracheae, tracheoles, and the cell membrane cannot be neglected.

Effect of Humidity during Fumigation

The effect of relative humidity during fumigation on the susceptibility of insects is reported in the literature for quite a few species of insects and fumigants. Conflicting results were found not only in different species of insects but also in the same species fumigated with different fumigants and different stages of the same species fumigated with the same fumigant. Very little has been suggested to explain such differences of results.

In this study eggs (5 ± 0.5 days old) and larvae (21 ± 0.5 days old) of *T. confusum* reared under standard conditions were fumigated at several different humidities. The average number of insects used in each test was about 170 for eggs and about 80 for larvae. It is shown in figure 28 that the effect of relative humidity during fumigation of *T. confusum* eggs with carbon disulfide is significant. The median lethal concentrations at 15, 30, 60, and 100 per cent relative humidity are 136.0, 141.1, 146.6, and 125.5 mg. per liter respectively. It appears that the susceptibility of eggs is greater at either extremes of humidity, especially the highest one. In order to confirm the difference of humidity effect during fumigation, eggs (5 ± 0.5 days old) from the same culture were fumigated four times with 145 mg. per liter of carbon disulfide by the standard method at 15 and 100 per cent relative humidity. The average mortality at 100 per cent relative humidity was 74.0 per cent, while that at 15 per cent was only 38.3 per cent. In the case of *T. confusum* larvae (figure 28) their mortalities at 30, 60, and 100 per cent relative humidity were very similar. Their respective median lethal concentrations were 39.7, 39.2, and 40.0 mg. per liter of carbon disulfide. These differences are within experimental error.

It has been suggested that certain insects may show a greater rate of metabolism in dry air than in moist air at the same temperature. If this statement is true, certain species may require more oxygen in dry air. This, in turn, will cause more fumigant

to be taken into the insects and will produce a higher mortality than in moist but otherwise like conditions.

Buxton (1930) obtained results which indicated that, at 23° C., the meal worm used up its reserves more rapidly in dry air than in moist, and kept the ratio of water to dry matter constant. Mellanby (1932) made numerous experiments and found that the utilization of reserves was governed by temperature and less affected by humidity. Experiments with other insects, including bedbugs (Mellanby, 1935), clothes moths (Mellanby, 1934), and adult (Nash, 1936) and pupal tsetse flies (Buxton and Lewis, 1934), all showed that the rate of metabolism was governed by temperature and unaffected by changes in humidity. If these facts are true, humidity will not affect fumigation results through spiracle respiration.

In spite of numerous results indicating the negligible effect of humidity on the rate of metabolism as well as on fumigation, a number of authors, such as Neifert and Garrison (1920), Strand (1930), Lehman (1933), Parkin and Busvine (1937), and Gough (1939), carried out their fumigation at controlled humidity.

From the results in this study as well as the general trend in the literature it seems that humidity during fumigation affected least those insects in stages where respiration is active, such as larvae and adults, but produced considerable effect on the inactive stages, such as eggs and pupae. During the active stages a period of 5-hour fumigation may be too short to produce a substantial effect even if the rate of metabolism is slightly changed. However, in the inactive stages the entrance of fumigant depends entirely upon its diffusion through the outside chorion, which may change in permeability at different relative humidities.

Effect of Sublethal Fumigation

The discovery of "protective stupefaction" of insects acting against a sublethal concentration (or dose) of fumigant was first discovered among scale insects fumigated with hydrogen cyanide. The fact that exposure of scale insects to sublethal concentrations preceding the regular fumigation will increase the resistance of the scales has been supported by many authors.

The idea of protective stupefaction has been extended to stored-product insects, such as the granary weevil. Mackie and Carter (1937) stated that during the fumigation of large masses of grain or other such products, very low concentrations of gas first reach the insects which are at a distance from the point of

application. This low concentration of fumigant is not sufficiently strong to kill the insects but causes a suspension of normal respiration, thus protecting them against further action of the fumigant. That the phenomenon actually occurred with *S. granarius,* but not with *T. confusum* or *Hippodamia convergens,* was proved in fumigation experiments by Lindgren (1938). Peters (1936) explained that protective stupefaction in the case of *S. granarius* against hydrogen cyanide was due to cessation of mechanical ventilation so that cyanide could enter by diffusion. Hardman and Craig (1941) proved that the relative ability to maintain closure of the spiracles explains the difference in resistance to hydrogen cyanide of resistant and nonresistant red scales. The same mechanism may also operate during protective stupefaction.

In testing the prefumigation effect of carbon disulfide on *S. granarius* adults, sublethal (5, 10, and 15 mg. per liter), median lethal (33 mg. per liter), and lethal (60 mg. per liter) concentrations were used at equal or different exposure times before normal fumigation. Standard cultures of 28 ± 1.5-day-old adults were used. In order to avoid any difference among the cultures they were mixed up before they were used for the test in a series of experiments. All the insects were treated under standard conditions in one flask during sublethal fumigation; however, they were treated with the standard concentration in different flasks. Checks were fumigated at frequent intervals with the experimental lots. It was found that the process of fumigation was so well standardized that the difference between flasks was almost negligible.

During sublethal fumigation with carbon disulfide at all the concentrations shown in table 14 the weevils became much more active than normal. There was no indication that they fell into protective stupefaction. It is not easy to tell whether scale insects undergo protective stupefaction or not; however, it is very easy to observe the weevils. Such an observation has not been described in those papers in which the idea of protective stupefaction for granary weevils was accepted. After sublethal fumigation the weevils continued to be active, although the rate of their activity decreased somewhat. It is recognized that the responses might be different if fumigants other than carbon disulfide were used.

The mortality of weevils that were fumigated at 15, 33, and 60 mg. per liter of carbon disulfide for 25, 11, and 6 minutes respectively increased at first and then decreased rapidly after subsequent normal fumigation (33 mg. per liter) as the time of recov-

Table 14. Effect of Sublethal Fumigation with Carbon Disulfide on the Results after Fumigating *S. granarius* Adults (28 ± 1.5 Days Old) by the Standard Method

CS_2 concentration of sublethal dose	Duration of sublethal fumigation	Time of recovery	Total number of insects	Average per cent mortality of 2 tests (2 days after fumigation)
Mg./l.	Minutes	Hours		
5	25	0.05	162	54.5
		1	145	62.8
		3	162	56.4
		5	156	58.1
		Check	302	56.4
10	25	0.05	177	62.4
		1	133	52.6
		3	142	56.2
		5	136	54.4
		Check	302	56.4
15	25	0.05	154	73.7
		1	149	57.2
		3	160	41.9
		5	160	36.3
		7	184	38.4
		10	208	48.4
		14	162	55.5
		18	163	65.7
		23	178	63.6
		Check	415	60.4
33	11	0.05	181	66.6
		1	186	58.4
		3	137	38.1
		5	166	42.7
		7	161	49.4
		10	139	57.0
		14	154	61.8
		18	152	62.5
		23	170	63.0
		Check	359	53.2
60	6	0.05	201	64.6
		1	194	51.9
		3	136	35.4
		5	131	42.7
		7	150	51.3
		10	181	59.2
		14	164	55.6
		18	167	62.8
		23	149	62.5
		Check	359	53.2

ery increased from 0 to 3 or 5 hours. After that the mortality increased gradually until their susceptibility was about normal. Those fumigated at 5 and 10 mg. per liter of carbon disulfide for 25 minutes did not change their susceptibility very much when they were subsequently fumigated after 0 to 5 hours of recovery (table 14, figure 29).

The reaction of a fumigant upon insects is caused both by its concentration and the time of exposure, if temperature and other

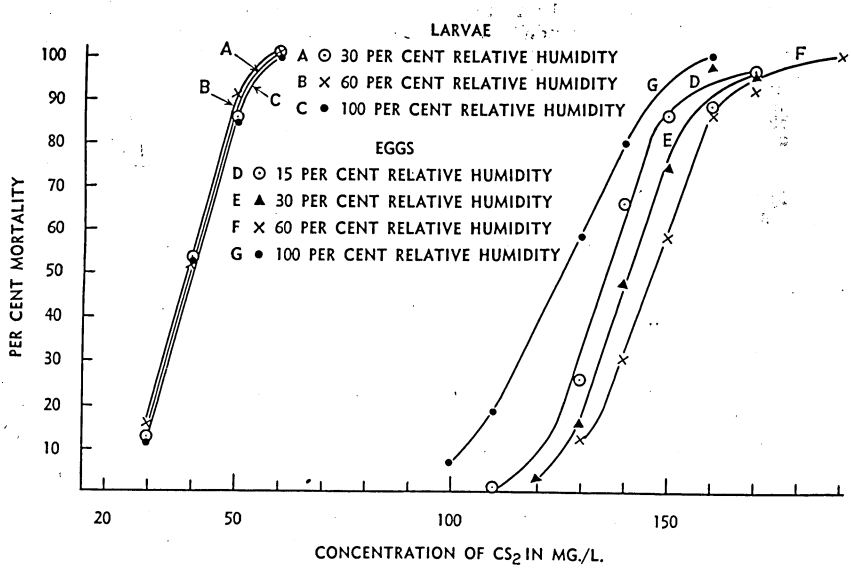

FIG. 28. Effect of relative humidity during fumigation on the susceptibility of *T. confusum* eggs (5 ± 0.5 days old) and larvae (21 ± 0.5 days old) to carbon disulfide

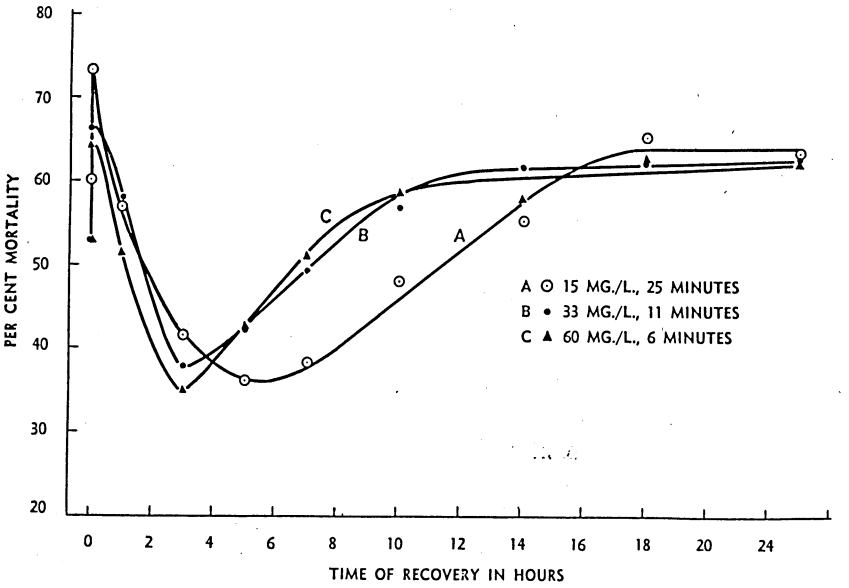

FIG. 29. Effect of time of recovery after sublethal fumigation with carbon disulfide upon the mortality of *S. granarius* adults after standard fumigation (2-day mortality)

factors are the same. Therefore, the common terms, such as sublethal dose, sublethal concentration, and sublethal charge, are inadequate to indicate a time factor. In the above experiments sublethal, median lethal, and lethal concentrations have been used; however, with the adjustment of time the products of concentration and time are about the same. Hence it would be more proper to call such a treatment sublethal fumigation. The actual amount of fumigant during sublethal fumigation is called a sublethal dose.

Sorption

The molecules of some gases show a strong tendency to adhere to the surface of solids and liquids, resulting in a high concentration of the gas at the point of contact. This phenomenon is called adsorption. In many cases adsorption is accompanied by absorption. The combined effect of both adsorption and absorption is called sorption. The amount of sorption is determined by temperature, humidity, pressure, amount of free moisture, concentration of gas, and the nature of sorbing and sorbed materials.

In practical fumigation the sorption capacity of most products, which is often very great, must be satisfied before a constant concentration can be maintained in the surrounding atmosphere. In laboratory tests there is also some chance of sorption effects, hence we should not only know the relative toxicity of a fumigant for the insects but the sorptive capacity of the products as well.

In order to know the amount of fumigant sorbed by materials used in laboratory fumigation tests, such materials as glass, silk bolting cloth, paper, thread, galvanized screen cloth, cork, beeswax, and rubber stoppers were tested. They were exposed in the vapor of carbon tetrachloride and carbon disulfide at 150 and 60 mg. per liter respectively. In most cases their sorption powers per 10 square inches were comparatively small and negligible (table 15). However, the capacity of rubber stoppers was surprisingly high (152 mg. per 10 square inches for CCl_4) in the sorption of fumigants. The sorption power of beeswax was much lower than rubber but slightly higher than cork, so that it is advantageous to cover a rubber stopper with beeswax but not to cover the cork.

The fumigation flasks used in this study were washed with household cleanser after every three fumigations; the rubber stoppers with an area of less than 1 square inch exposed to the toxic vapor were heavily waxed after each fumigation; and the fumigant was measured from a micropipette instead of a burette. These precautions should eliminate most factors in the sorption of fumigant vapor with the exception of insect cages used for

Table 15. Amount of Carbon Disulfide and Carbon Tetrachloride Sorbed by Various Substances During 5-Hour Fumigation at 25 °C.

Materials	Area exposed to fumigant (sq. in.)	Amount of sorption, mg. per 10 sq. in.	
		CCl_4 (150 mg./l.)	CS_2 60 mg./l.
Glass	25	0.04	nil
Silk bolting cloth (64-mesh)	12	0.08	nil
Paper (index card)	20	0.1	nil
White thread	20 feet	0 .02 per foot	nil
Galvanized screen cloth (16-mesh)	32	0.25	nil
Cork	13.34	3.5	nil
Beeswax	15.5	7.5	2.2
Rubber stopper	11.5	152.0	13.0

weevils. The determination of the rate of sorption of four insect cages in carbon tetrachloride (150 mg. per liter) at standard temperature and humidity was done by weighing the cages before and after each exposure in the fumigant in a confined container. The increase in weight was the weight of sorbed fumigant. The rate of desorption was measured by successive weighing of the cages after 5 hours exposure in the fumigant. Both the rate of sorption and desorption were great during the first few hours and then slowed down (figure 30). Eventually an equilibrium should

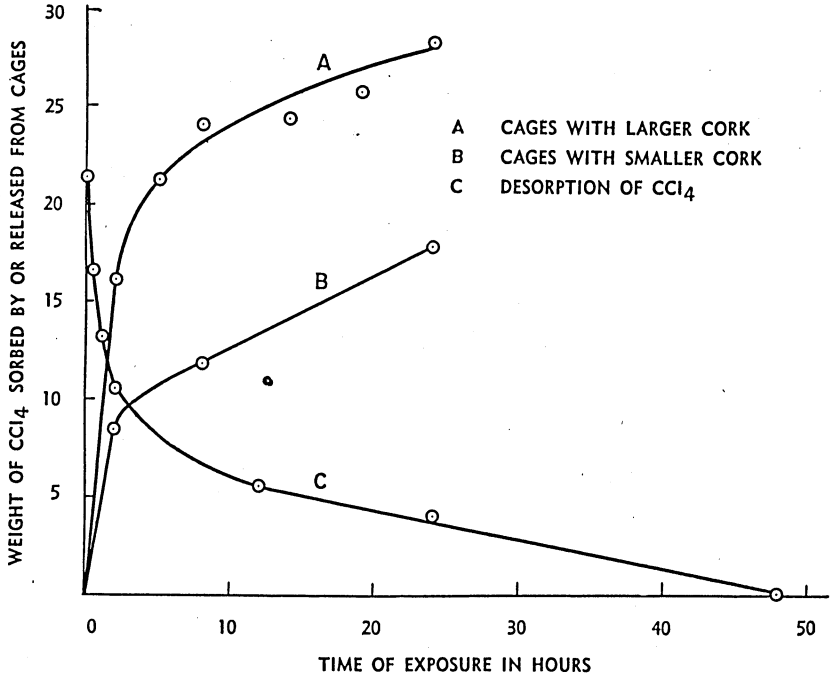

FIG. 30. The rate of sorption and desorption of carbon tetrachloride (150 mg./l.) by four insect cages under standard temperature and relative humidity

be reached in sorption, although it was not observed in the experimental period. Cages with larger corks sorbed much more fumigant than those with smaller corks because of greater exposed wax surface. Corks used in standard fumigation were not waxed.

Weight of sorption and sorption ratio of various fumigants were determined in the laboratory under standard conditions. Eight grams of patent flour were weighed into a weighing bottle 3 centimeters in diameter and 6 centimeters high. The bottle was tapped slightly so that the flour reached a height of 1 inch. One bottle was placed in each of the fumigation flasks, which contained 200 mg. per liter of one of the fumigants. After 5 hours the bottle was taken out and weighed with a cover. The increase in weight after the correction of check value was the weight of fumigant sorbed by the flour. The sorption ratios were obtained by taking carbon disulfide as unity. Table 16 shows the sorbed weight, sorption ratio, and boiling point of tested fumigants.

The general trend of sorption ratio of fumigants followed the order of their boiling points, except methyl acetate which deviated to a greater degree and ethylene dichloride to a lesser degree. In general, the higher the boiling point of the fumigant, the greater was the weight of sorption. In regard to their solubility in water it was found that methyl acetate was very soluble in water and ethylene dichloride was slightly soluble, while other fumigants were almost insoluble. This deviation was also observed in the experiments of Fisk and Shepard (1938) in the determination of absorption ratios from M.L.C.'s. Ethylene oxide and methyl formate, which are very soluble in water, also gave greater ratios. However, differences were not shown in some other water-soluble fumigants. This may be due to their comparison to their own values rather than to some standard fumigant. More study is necessary to make a final conclusion.

Table 16. Sorption of Various Fumigants by Patent Flour after Five Hours Exposure under Standard Conditions

Fumigants	Boiling point °C.	Corrected weight of sorption after five hours (mg.)	Sorption ratio
Carbon disulfide	46	10.9	1
Methyl acetate	57.5	68.5	6.3
Carbon tetrachloride	76.7	14.7	1.3
Ethylene dichloride	84	41.0	3.8
Trichlorethylene	87.1	25.6	2.3
Propylene dichloride	96	34.1	3.1
Chloropicrin	112	78.3	7.2
Tetrachlorethylene	120	113.7	10.4

Penetration

The discussion of penetration in this section will include both diffusion and permeation in relation to load and insect. In practical fumigation, penetration of a fumigant through a commodity is chiefly affected by sorption. Both factors more or less directly or indirectly depend upon the molecular weight and concentration of the gas or vapor, upon the size of the openings through which it must pass, upon nature and water content of the commodity, upon temperature of fumigation, upon the distance of diffusion, etc. The rate of diffusion of a fumigant is the driving force causing penetration, while the sorption power of the commodity provides resistance to its penetration. Therefore, any fumigant will penetrate bulk commodities if a sufficient quantity is used to satisfy the sorption requirements. Finely divided products such as flour are, in general, more difficult to penetrate by most gases than granulated substances such as wheat, even if the composition is the same, because the flour particles have a very large area of surface exposed to the fumigant for sorption but very little space for diffusion.

It is a common belief that heavier vapor, like carbon disulfide, sinks and permeates more easily to the bottom if it is supplied from the top. For example, Moore (1918a) infers that the vapor of chloropicrin should "diffuse" downward through grain more quickly than the vapor of carbon disulfide, because the vapor of the former is about twice as heavy as that of the latter. Such a conception was based more on the sinking power due to their own weight rather than on true diffusion.

Theoretically, the gases of small molecular weight diffuse more rapidly than others. However, Cotton (1941) pointed out that other factors appeared to exert a greater influence than molecular weight, for methyl bromide with a molecular weight of 95 and carbon disulfide with a molecular weight of 76 both diffused through bulk commodities more rapidly than hydrogen cyanide, which has a molecular weight of but 27. In this case the water-soluble property of hydrogen cyanide may be the reason.

Strand (1927) was the first to measure penetration in an extensive series of controlled experiments. Contrary to the general belief, even heavy fumigants such as carbon disulfide and chloropicrin did not necessarily sink very far down in a mass of grain. Adsorption by the top layer of grain prevented their rapid downward movement. More recently Cotton (cited by Shepard, 1939)

has shown the downward penetration of grain by a fumigant is not limited so seriously if the grain temperature is high enough.

The penetration of carbon disulfide, methyl acetate, carbon tetrachloride, ethylene dichloride, trichlorethylene, propylene dichloride, chloropicrin, and tetrachlorethylene through patent flour at standard temperature and moisture was determined by the weighing method. The diagram of the apparatus is shown in figure 31. A 6.4-liter fumigation flask was connected by a tube (1.6 cm. diameter and 8.5 cm. long) to a weighing bottle which was half filled with active carbon. Nine grams of patent flour were put into the connecting tube so that it occupied a 3-inch length. Both ends of the flour were held in place by a piece of bolting cloth and a piece of galvanized screen cloth. Each fumigant was measured into one flask at a concentration of 200 mg. per liter. After a thorough shaking, the flask was connected to the system as shown in figure 31. The gain in weight in the weighing bottle with active carbon in a 24-hour period was the apparent weight of fumigant passing through the test material. Such values were corrected with a check to give the final data. The weight of each fumigant passing through the flour in this arbitrary determination and its ratio with carbon disulfide as unity are listed in table 17. With the exception of methyl acetate it is shown that both the weight of fumigant and penetration ratio decrease with an increase of boiling point. In this series of fumigants it appears that the higher the sorption ratio of a fumigant, the smaller will be the amount of vapor that will penetrate through patent flour. Chloropicrin, which is considered to be good in its ability to penetrate,

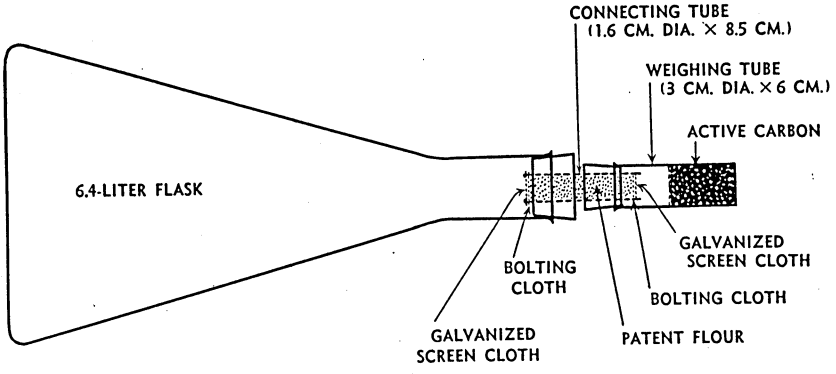

FIG. 31. Diagram of apparatus for the determination of relative penetration of fumigants

Table 17. Penetration of Various Fumigants Through Patent Flour after 24 Hours Exposure under Standard Conditions

Fumigants	Boiling point °C.	Corrected weight of fumigant penetrated through patent flour after 24 hours (mg.)	Penetration ratio
Carbon disulfide	46	154.6	1.00
Methyl acetate	57.5	108.2	0.70
Carbon tetrachloride	76.7	119.7	0.78
Ethylene dichloride	84	111.3	0.72
Trichlorethylene	87.1	95.8	0.62
Propylene dichloride	96	94.8	0.61
Chloropicrin	112	65.1	0.42
Tetrachlorethylene	120	57.6	0.37

was found poor in this respect in this study. This misleading concept may arise from the fact that the odor of chloropicrin can be easily detected at very low concentrations.

In the case of diffusion and permeation of fumigants into the insect body, the situation is much more complex and extremely difficult to study in living animals. However, a theoretical hypothesis and experiments *in vitro* may help to a certain extent to explain what would happen *in vivo*.

In 1866 Graham (1866) studied the diffusion of gases through rubber. He regarded the permeation process as solution, diffusion, and re-evaporation of the diffusion gas, a viewpoint which in essential details is held today. There are many hypotheses of the mechanism responsible for the permeability of membranes. Among these the sieve hypothesis, solution hypothesis, and electrical hypothesis are most important. In fumigation of eggs in various experiments the solution hypothesis seems the most reasonable.

Solution hypothesis: "Substances that dissolve fats should readily pass through a membrane made of fat. Thus thought Overton, but the membrane that he postulated was of lipoids and not true fats, and all the fat solvents with which he experimented (alcohol, ether, chloroform, etc.) are also water soluble. They would, therefore, also enter were the membrane an aqueous one but perhaps not so rapidly as when passing through a layer of fat. In either case, the entrance would be by solution. It should be pointed out that Overton qualified his hypothesis by the condition that only such fat solvents can enter as are soluble both in lipoid and in water—a fact often neglected" (Seifriz, 1936, p. 284). The solution theory has been supported by many later experimental results. For example, with the permeability constants of Dewar it was found that the permeation velocities bore no simple rela-

tionship to molecular size or mass, the larger molecules of ethyl chloride (Sager, 1937) being transmitted 10 times as fast as the smaller molecules of methyl chloride. The solubility of the diffusing substance in the membrane is one important factor in diffusion. Payne and Gardner (1937) also proved that the permeability of a membrane to a vapor depended upon the solvent capacity of the membrane for the vapor.

Although very few papers have been published on the diffusion and permeation of fumigants through the insect body, the contact insecticides have been studied more extensively. In many phases the properties of liquids are comparable with those of gases. Thorpe (1928) found that carbon dioxide was eliminated largely through the cuticula of many small, thin-skinned insects. Alexandrov (1935) found a selective permeability of insect integument which favored the undissociated organic compounds. Owing to the presence of the extremely thin lipoid-containing epicuticle (Wigglesworth, 1939), fat-soluble compounds or fat solvents had a more rapid action as insecticides. These factors may also play an important role in the toxicity of fumigants.

The influence of temperature upon permeability constants is twofold: first, the effect upon the diffusion constant; and secondly, the effect upon the sorption coefficient. In general, the permeability constants of the least permeable membranes are highly sensitive to temperature changes, but those of porous membranes are independent or slightly dependent upon temperature.

The interpretation of results in terms of penetration or permeation can be found here and there in this study. The differences in resistance of various stages of *T. confusum* can be satisfactorily explained by the two methods by which fumigants entered the insects. In the case of adults and larvae, spiracle respiration and subsequent diffusion were predominant factors, while permeation is the only way in which a fumigant can get into the eggs. Actually eggs are not more resistant, or even less resistant, than adults and larvae. For some fumigants, such as ethylene oxide, hydrogen cyanide, and methyl bromide, eggs were less resistant than adults or other stages. Ethylene oxide and hydrogen cyanide are very soluble in water, while methyl bromide is slightly soluble. Other fumigants, such as chloropicrin and carbon disulfide, that are insoluble in water are more toxic to adults than eggs or pupae. It seems that the water-soluble fumigants can penetrate through the egg chorion more readily than water-insoluble fumigants; of course, the former should be soluble in a lipoid too. This conclusion seems to fit Overton's solution hypothesis, as described above.

The effect of temperature during fumigation is mainly due to a change of permeation for eggs and to an increase of spiracle respiration for adults. The results of such differences are shown in figure 26. Barrer (1937 and 1939) showed that the permeability constants of membranes increased with temperature. His experiments *in vitro* may explain the effect of temperature during fumigation *in vivo*.

In the experiments on the effect of relative humidity during fumigation it has been found that *T. confusum* eggs were much more susceptible to carbon disulfide at high humidity (100 per cent) than at a medium humidity (60 per cent or lower). However, the susceptibility of larvae to the same fumigant was almost unaffected at various humidities (figure 28). This difference may be due to the fact that the permeability of egg chorion is increased at a high humidity. On the other hand, the change of permeability of the larval skin does not affect the final result to an appreciable degree, for respiration and diffusion of a fumigant through the spiracles are more important. It was observed by Alexejev and Matalski (1927) that, while desiccated cellulose membranes were impermeable to air, the same membranes after sorbing water became permeable to air. Although there is no direct experimentation to prove this point, the same may be true in the permeation of an egg chorion.

On the theory that the water-soluble solvents penetrate into eggs most readily, methyl and ethyl alcohols were used in the fumigation of eggs. The latter fumigant was used by Lehman (1933) to dilute some very toxic fumigants. However, the author found that a concentration of 60 mg. per liter was a lethal concentration for *T. confusum* eggs (5 ± 0.5 days old). Various concentrations of ethyl alcohol were used either alone or in combination with 100 mg. per liter of carbon disulfide in the fumigation of the eggs. Practically no differences were found in three combinations, whether carbon disulfide was added or not. It seemed that the egg chorion showed some selectivity in regard to its permeability to fumigants. At that concentration carbon disulfide may not be able to enter the egg in an appreciable amount to cause a combined effect. However, the addition of carbon tetrachloride did increase the toxicity of organic compounds (Richardson, 1943) when the active stage of the insects was used. This again shows the difference between permeation for eggs and spiracle respiration and diffusion for adults. On the contrary, Jefferson (1943) concluded that in no case did the addition of carbon tetrachloride give an increase in toxicity.

Boiling Point and Toxicity of Fumigants

It has been mentioned in the last two sections that the boiling point of fumigants appears to have a certain relationship with sorption and penetration of fumigants. Such functions may, in turn, affect the toxicity of fumigants. In the literature the relation between the boiling point and the toxicity of insecticides has long been suggested and discussed, not only for fumigants but also for contact insecticides. The increase of toxicity of fumigants as the boiling point rises, followed by a sharp decline beyond a critical boiling point (250° C.), was early noticed by Moore (1917a) and Tattersfield and Roberts (1920). Beyond 250° C. the compounds are usually so slightly volatile that not enough of the chemical will evaporate to be effective. However, Neifert *et al.* (1925) found that there was no constant relationship between the boiling point and the lethal concentrations of certain compounds. Cupples *et al.* (1936) pointed out that the toxicity of the thiocyanates gave no evidence to show that their toxicity increased with the molecular weight or boiling point; in fact, the lower members of the series seemed to be most effective.

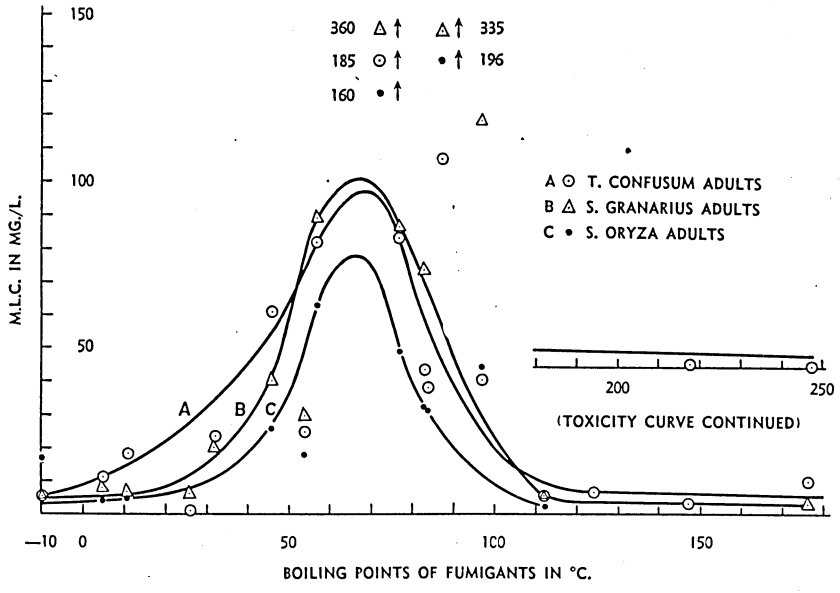

FIG. 32. The relation between the boiling point and the toxicity of fumigants

The boiling point of fumigants determines the amount of sorption during fumigation and the rate of release of toxic vapor after fumigation, but this is not the only factor which determines the toxicity of a fumigant. When a small number of fumigants is considered, the effect of boiling point is easily overshadowed by other factors.

A collection of published and original data on three species of stored-product insects is listed in table 18 in the order of increasing boiling point. It is hard to interpret the results from the table, but they become clear when they are plotted, as they are in figure 32 on page 77.

The general relation between the boiling point and toxicity is shown by the M.L.C.'s of 20 fumigants for 3 species of insects. The fumigants with either low or high boiling points are more toxic than those within the middle range (at about 55° to 80° C.). Very toxic fumigants are found with extremely low or extremely high boiling points. The values of those fumigants, such as carbon tetrachloride and ethylene dichloride, that show a delayed toxicity effect are often far off from the general trend.

Table 18. The Relation of Boiling Point of Fumigants to Their Toxicity to Three Species of Stored-Product Insects

Fumigant	Boiling point	M.L.C. in mg./l.			Author
		Tribolium confusum	*Sitophilus granarius*	*Sitophilus oryza*	
Sulfur dioxide	—10.0	5.7	5.7	17	Shepard *et al.* (1937)
Methyl bromide	4.5	11.2	7.4	4.0	Shepard *et al.* (1937)
Ethylene oxide	14	18	5.6	5.7	Shepard *et al.* (1937)
Hydrocyanic acid	26.1	0.6	5.8	.....	Shepard *et al.* (1937)
Methyl formate	32.3	23.5	20	.....	Shepard *et al.* (1937)
Carbon disulfide	46.2	61.0	40	26	Shepard *et al.* (1937)
Ethyl formate	54.3	24.5	29	17.5	Shepard *et al.* (1937)
Methyl acetate	57.5	82	88	63	Shepard *et al.* (1937)
Carbon tetrachloride	76	185	360	160	Shepard *et al.* (1937)
Ethyl acetate	77	83	86	49	Shepard *et al.* (1937)
Tert.-butyl alcohol	82.9	43	73	32	Shepard *et al.* (1937)
Ethylene dichloride	84	37.5	138	31	Shepard *et al.* (1937)
Trichlorethylene	87	108	335	196	Shepard *et al.* (1937)
Propylene dichloride	96.8	40	118	44	Shepard *et al.* (1937)
Chloropicrin	112	3.1	.....	.....	Original
Ethide (1, 1-dichloro-1-nitroethane)	124	7.2	.....	.....	Original
Paradichlorobenzene	173	2.4	.....	.....	Calculated from Lehman's (1930) data
Furoyl chloride	176	9	2.6	.....	Shepard *et al.* (1937)
Naphthalene	218	1.1	.....	.....	Calculated from Lehman's (1930) data
Nicotine	247.3	0.16	.....	.....	Richardson and Busbey (1937)

EFFECTS OF POSTFUMIGATION CONDITIONS ON MORTALITY OF INSECTS AFTER FUMIGATION

Some toxicologists have made an attempt to control such factors as temperature and relative humidity during the critical period of recovery after fumigation. However, very few data are found in the literature, with the exception of the results of some studies on the amount of time which should elapse before mortality counts are made. That this important question should have received so little attention is surprising, particularly when one considers the precautions taken during fumigation and the difficulties encountered in setting up criteria of death.

In this section various factors, such as time, temperature, humidity, and starvation, will be evaluated with regard to their significance in fumigation results.

Effect of Postfumigation Temperature

Temperature after fumigation may not seem important to toxicologists; however, some variations of temperature can be expected in the laboratory and more will occur in the field. For example, Moore (1933) noticed that very low temperatures (38° F.) shortly following fumigation with hydrogen cyanide will tend to reduce the kill of red scales more than at 48° F. Yust and Howard (1942) found that an increase of 18° F. in the temperature after fumigation favored increased mortality of scale insects.

In order to avoid the possible effect of other factors as much as possible, all the insects used in each series of experiments were obtained from the same culture, fumigated under the standard conditions in the same flask, and carefully sampled after fumigation. Each sample of insects containing 50 individuals was then placed at several temperatures over a saturated sodium chloride solution.

The curves in figure 33 show that the mortality of *T. confusum* (adults and larvae) and *S. granarius* (adults) at 2 days after fumigation is a V-shaped curve from 5° to 35° C. The main difference between the adults of *S. granarius* and *T. confusum* is the shifting of the results of the former to the left side with a minimum of about 5 degrees lower than that of *T. confusum.* The mortality curve of the larvae of *T. confusum* is similar to that of the adult, with the minimum mortality at a temperature of 25° C.

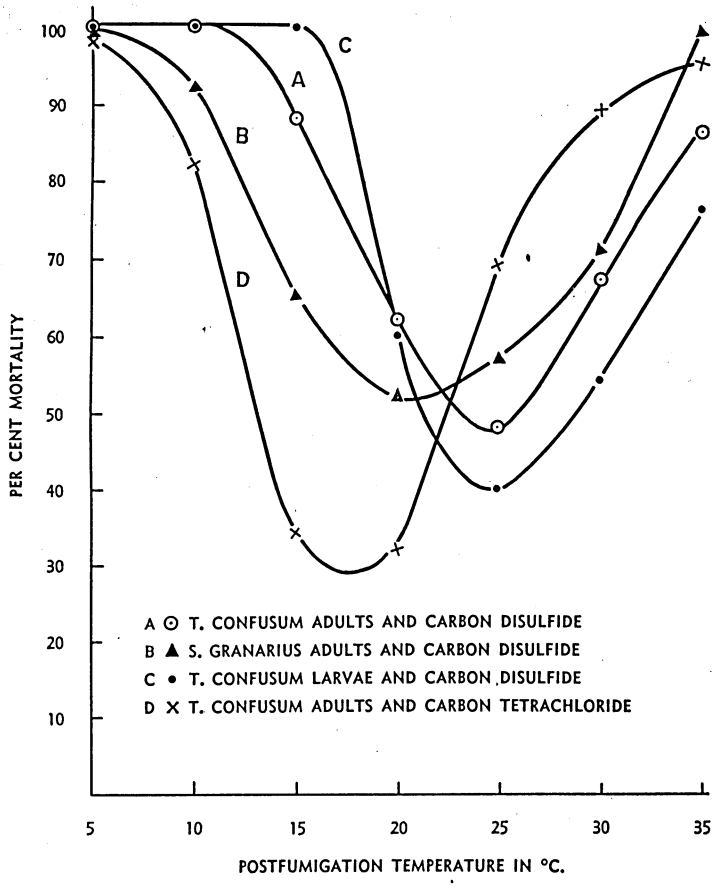

FIG. 33. Effect of postfumigation temperature upon the mortality of *T. confusum* and *S. granarius* to carbon disulfide and carbon tetrachloride

It is appropriate to discuss here the factors which cause such a variation after fumigation. As soon as the insects were removed from the vapor of a fumigant three main factors were in operation: chemical action of fumigant on the insects, desorption of fumigant vapor, and the recovery of the insects. These three will in combination determine the final mortality. At lower temperatures, from 5° to 15° C., there was no appreciable cold injury to *T. confusum* and *S. granarius* within 2 days (Sun, unpublished data). The chemical reaction of fumigant upon an insect is decreased as the temperature decreases; however, a low rate of desorption and prolonged action of the fumigants in the body of the insect must be considered. The combined action at 5° to 15° C., as indicated by a great increase in mortality (figure 33), shows

clearly that the lowering of chemical action is comparatively less important than the other two factors.

At higher temperatures, from 30° to 35° C., the condition is more favorable for the elimination of carbon disulfide through desorption, diffusion, and respiration. However, the temperature is so high that the chemical action of carbon disulfide upon insects may kill them in a relatively shorter time before any sufficient amount can be eliminated through the above-mentioned functions. This may explain why the curves rise again when the temperature is above 25° C.

At moderate temperatures, from 20° to 25° C., the chemical action is not as fast as that at higher temperatures, while desorption, diffusion, and respiration processes are fast enough to eliminate toxic vapor before the chemical reaction has killed the insects. The combination of the three factors is least harmful to *S. granarius* at 20° C. and *T. confusum* at 25° C.

Carbon tetrachloride acted similarly to carbon disulfide when used on *T. confusum* adults in experiments with various temperatures after fumigation. The main difference was that the temperature of minimum mortality shifted from 25° to 18° C. At lower temperatures the dead *T. confusum* adults do not dry up or change appreciably in color after two days, while those at higher temperatures are dry and darkened in color.

In practical fumigation if the temperature can be kept lower or higher than room temperature after fumigation the results should be better than at normal room temperature. For the same percentage of kill less fumigant can be used, or for the same amount of fumigant the insect mortality will be higher.

Effect of Postfumigation Humidity

The effect of relative humidity after fumigation has been almost unexplored, even though relative humidity varies in the laboratory from about 10 per cent in the winter to 75 per cent or more in the summer. Such a wide range of variation ought to have some influence on insects after fumigation.

The experiments were conducted in the same way as those described for postfumigation temperature with 50 individuals for each of the four tests. After fumigation with 33 mg. per liter of carbon disulfide, *S. granarius* adults were sampled and removed to 0, 20, 40, 60, 80, and 100 per cent relative humidities at 25° C. Four series of the same treatments were made. The average values of mortality are shown in figure 34.

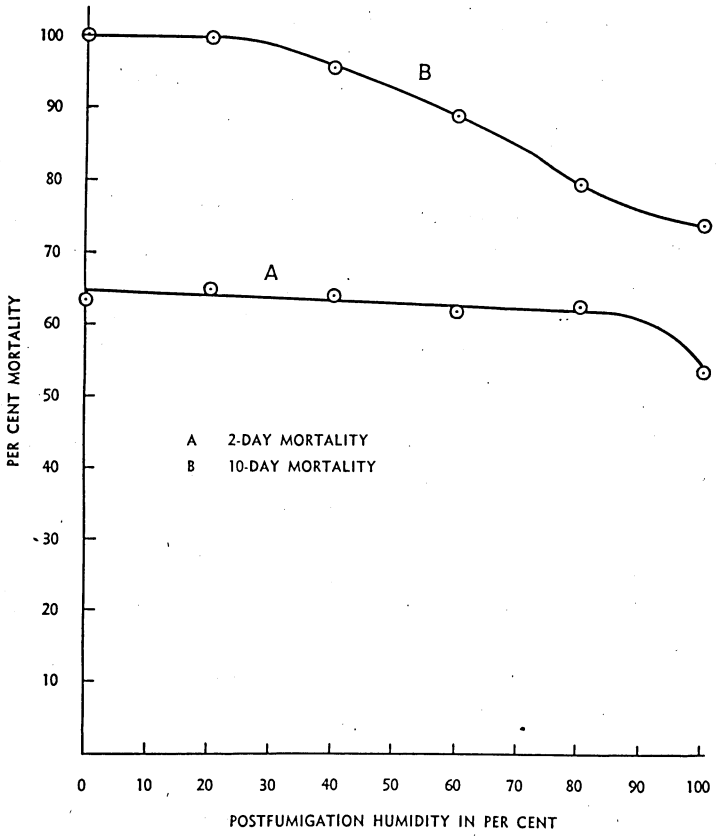

FIG. 34. Effect of postfumigation humidity upon the mortality of *S. granarius* adults fumigated with carbon disulfide (33 mg./l.)

With the exception of a mortality at 100 per cent relative humidity, the 2-day mortalities do not show much difference. However, mortality decreased gradually as the relative humidity increased at 10 days after fumigation. This difference may be explained by the increased chance for desiccation of the injured insects at low humidities over the longer period of time.

Effect of Starvation after Fumigation

The experiment on starvation after fumigation was carried out by fumigating *S. granarius* adults (28 ± 0.1 days old) in one flask at a concentration of 30 mg. per liter of carbon disulfide. After fumigation the insects were carefully divided into eight groups. Half of them were fed with wheat while the other half were starved during the period of counting.

Table 19. Effect of Starvation after Fumigation on the Susceptibility of *S. granarius* Adults to Carbon Disulfide

Treatment after fumigation	Total number of insects	Average per cent mortality of four tests at various days after fumigation				
		2 days	4 days	7 days	10 days	13 days
With wheat	200	46.5	58.5	67.5	75.0	77.0
Without wheat	200	46.0	56.0	87.5	96.0	100.0

It is shown (table 19) that within the first 4 days the results were nearly the same whether the insects were fed or not. During that period they might not have recovered sufficiently to care for food. After the fourth day food became important, as indicated by the increasing difference in mortality. For mortality counts made 2 days after fumigation no food is necessary for *S. granarius* adults. However, for those made at a longer time after fumigation, such as 10 days, food is absolutely essential. This may be true for many other species, especially those which recover rather quickly after fumigation.

Effect of Length of Time and Methods of Determining Mortality on Results of Insect Fumigation

Even when the same standards of temperature, time, and other factors are held constant during fumigation the length of time between the fumigation and the determination of mortality will modify the results. The time, as suggested by various authors, varies from a few hours to 60 days.

The criterion of death varies from author to author. This factor seems to have been overlooked by many investigators, for few statements pertaining to it can be found in the literature. Some of these statements are presented in abbreviated form in table 20. The criterion of death, together with the time of counting mortality after fumigation, changes to a great extent the degree of toxicity of a fumigant.

Different reasons have been given for using a certain criterion of death. The criterion of death used by Richardson and Haas (1932) and Jones (1938) was the inability of the insect to walk on a flat surface, as experience showed that in general those which could not walk nearly always failed to recover. Shepard and Buzicky (1939) stated that those insects showing any movement visible to the naked eye have been counted as alive on the chance that they might live. For this reason the mortality figures reported in terms of dosage were believed to be on the safe side in being high rather than low.

Table 20. Various Criteria of Death Used in Fumigation Experiments

Criterion of death	Time between fumigation and determination of mortality (days)	Author
No motion	2-3	Schoene (1913) and Hamlin and Reed (1927)
Unable to walk	1	Lehman (1930)
No movement when prodded	10	Lehman (1933)
Unable to walk on a flat surface	1-3	Richardson and Haas (1932) and Jones (1938)
No visible movement to naked eye	2	Fisk and Shepard (1938) and Shepard and Buzicky (1939)
Count hatching after 15 days with flour	15	Lindgren (1931)
Remove larvae from eggs as soon as hatched	8-9	Lindgren and Shepard (1932)
Remove larvae daily from eggs until no more hatching		Original
No visible movement when prodded and under light	2 and 10	Original
No metamorphosis or incomplete metamorphosis for pupae		Original

Various criteria of death in toxicological tests have been suggested (table 20). Differences in the number of days after fumigation when the count is made plus differences in the criteria of death cause much variation in mortality reports. It is interesting to note how much variation there is when different criteria of death are applied to two different species or different stages, and ages of the same species after a variable number of days.

Adults (28 ± 0.5 days old) and larvae (21 ± 0.5 days old) of *T. confusum* and young (1 ± 0.5 days old) and older (28 ± 0.5 days old) adults of *S. granarius* were fumigated under standard conditions at concentrations of 80, 40, 33, and 40 mg. per liter of carbon disulfide respectively. In each treatment two 50-individual lots were used. The counts were made at 1, 2, 4, 7, and 10 days after fumigation with four different criteria of death. These criteria were as follows: (1) Unable to walk—those insects which could not walk forward or backward were called dead. The time should be long enough to make sure that capable ones are allowed to show their ability to walk on a piece of paper. (2) No visible movement—no visible movement of any part of the body to the naked eye after a sufficient time of observation. (3) No visible movement when prodded. This is the same as (2) except that the insects without movement were prodded slightly with a finger tip (for adult beetles) or a camel's-hair brush (for larvae). (4) No visible movement when prodded and held under light (standard method). After the treatment of (3) those insects which did not

show any movement were placed about 1 inch away from a 60-watt light for 20 to 30 seconds. The combined action of light and heat activated some of them which did not show any movement when prodded. The so-called movement should be a motion to and fro of head, legs, or antennae. A single movement in one direction which may be a relaxation after death was not considered as indicative of life. *T. confusum* adults, especially young ones, were more sensitive to prodding than to light while, on the contrary, *S. granarius* adults were more sensitive to light and heat.

In every case (figures 35 and 36) the mortality decreased from criterion 1 to 4. The difference was greatest at the end of the first day and then decreased. At 10 or 13 days after fumigation the results were almost the same under all the four methods of counting

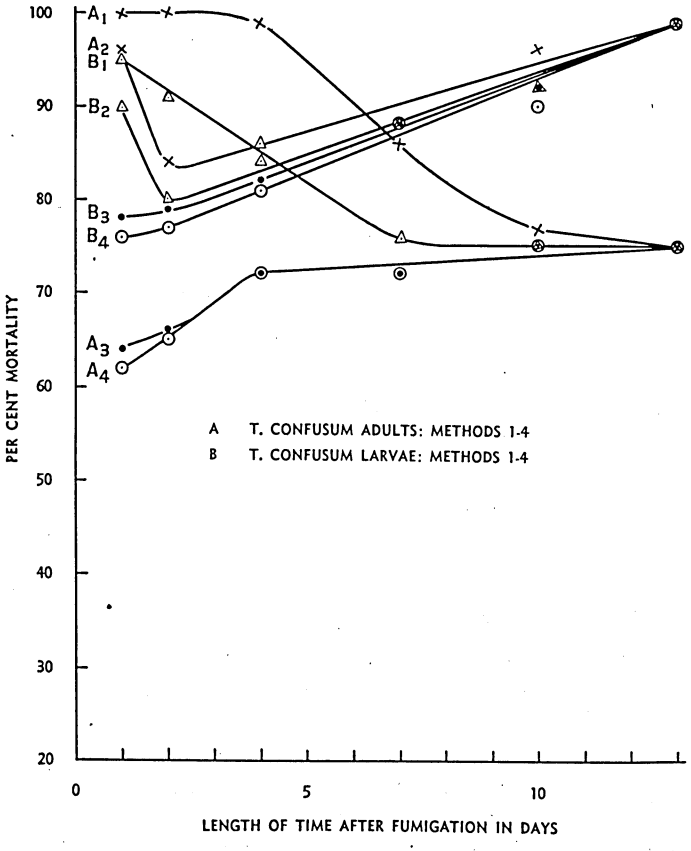

FIG. 35. Mortality curves for adults (28 ± 5 days old) and larvae (21 ± 0.5 days old) of *T. confusum* determined at various lengths of time after fumigation and by using different criteria of death

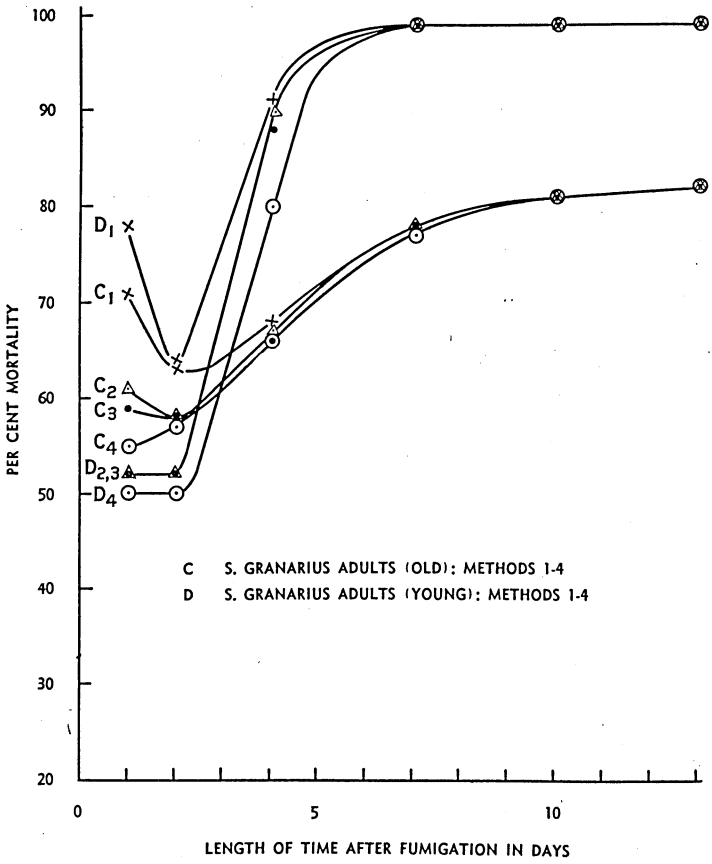

FIG. 36. Mortality curves for young (1 ± 0.5 days old) and old (28 ± 0.5 days old) adults of *S. granarius* determined at various lengths of time after fumigation and by using different criteria of death

mortality. In the above experiments the insects reacted differently from one species, age, and stage to another. Immediately after fumigation all adults and larvae of *T. confusum* were motionless but quite a few adults of *S. granarius* moved their legs. During the 10-day period after fumigation some of the *T. confusum* adults shifted from one classification to another, i.e., some of those previously classified as dead recovered and others died after apparent recovery. By examining the results of using the standard method (No. 4) of counting mortality it may be seen that the mortality of *T. confusum* adults increased slowly during the first 4 days and then gradually became almost constant. In the case of older adults of *S. granarius* the mortality increased almost regularly through a 13-day period. The mortality of young weevils was

constant between 1 and 2 days and then increased rapidly until the seventh day. The mortality of *T. confusum* larvae increased slowly but continuously through a 13-day period. In no case did they react in the same way, so that it is hardly possible to draw any general conclusion regarding the mortality after fumigation. It appears, however, to be desirable to include both 2-day and 10-day mortality figures when one describes the susceptibility of insects to fumigants.

Diagrammatic Arrangement of Various Factors in Relation to Toxicity of Fumigants

As a result of this study a larger number of factors than is usually realized has been shown to cause the marked variations common in fumigations. In each case they were studied individually by keeping other factors constant. One can imagine the complexity of the problem of fumigation when these factors are acting together. For example, an increase of temperature (figure 37) will cause a certain increase of respiration, rate of diffusion, permeation of insect membrane, etc., and at the same time cause a decrease of sorption. Figure 37 shows diagrammatically the qualitative relationship among the factors and their counter and parallel effects on the susceptibility of insects. Solid lines indicate a positive relationship; broken lines represent a negative one; and dotted lines show an uncertain relationship. In many cases these relationships can be true only to a certain limit. The relative importance of the factors is represented by the comparative size of the circle. For the details of their interactions one must refer to the original literature. (See figure 37 on page 88.)

SUMMARY AND CONCLUSIONS

Extensive studies were made to determine some of the important factors which should be controlled before, during, and after fumigation tests with insects. Among the factors investigated were temperature, relative humidity, starvation, sublethal fumigation nutrition, and population density. All of these factors produced important or interesting effects on the results of fumigation, and some, particularly postfumigation conditions, have received little attention before. As far as possible one factor was varied while others were held constant. To eliminate other possible sources of error a standard procedure for rearing cultures

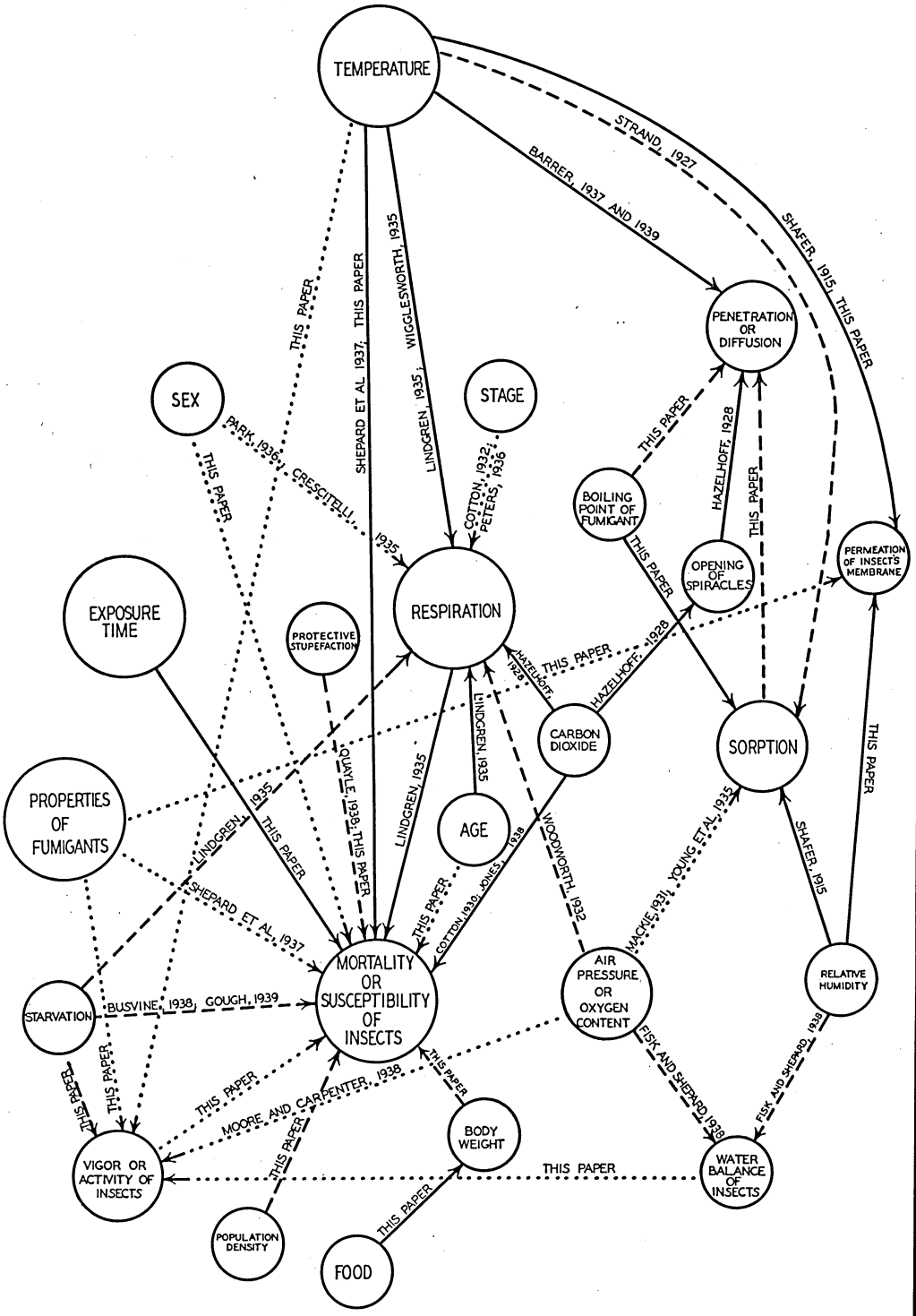

FIG. 37. Diagram showing the relation of various factors and their counter and parallel effects on the susceptibility of insects to fumigants

and fumigating test animals was used. Standard cultures were maintained at 25° C. ± 0.1° and 60 ± 3 per cent relative humidity with excessive carbon dioxide removed from the cabinet. Standard fumigation tests were carried out at 25° C. ± 0.1° and 60 ± 3 per cent relative humidity with a 5-hour exposure.

Complete mortality curves for the entire life cycle of the confused flour beetle, *Tribolium confusum* Duv.—from 1-day-old eggs to 24-week-old adults—were obtained by fumigating with carbon disulfide. From this experiment it was learned that the resistance of eggs decreased greatly and constantly as the embryos developed. The same rate of decreasing resistance appears to hold true for the first week of the larval stage. After that the larval resistance increased slowly but steadily. There was a tremendous increase of resistance from larval to pupal stages. During the pupal stage resistance decreased slightly from the first to the third day but increased greatly from the third through the fifth day. After pupae were 7 days old their resistance decreased sharply again. The whole curve of the pupal stage is symmetrical and in a reverse order to the V-shaped curve of pupal respiration. This indicates a close correlation between resistance of insects to fumigants and their rate of respiration. In the adult stage there was a peculiar decrease of resistance at the age of 4 days. Adults more than 2 weeks old showed less change in resistance. The resistance of the adults of the granary weevil, *Sitophilus granarius* (L.), and the rice weevil, *S. oryza* (L.), to carbon disulfide was quite similar to that of *T. confusum,* with the exception that in the case of the weevils there was no depression in the curve at an age of 4 days. In general, their susceptibility to carbon disulfide decreased as their age increased. One can take advantage of these results in planning laboratory research on the toxicity of fumigants by selecting a stage or age which will show the least variation in resistance. Furthermore, the results emphasize the importance of having such information so that fumigants may be used at concentrations which will kill the most resistant stages.

T. confusum larvae and adults and the adults of *S. granarius* were used in a study of the nutritive effect. From the results of eight different combinations of food it was indicated significantly that both the rate of growth and the susceptibility of *T. confusum* larvae to carbon disulfide varied greatly with the nature of the food. When larvae were fumigated the observed differences of susceptibility to carbon disulfide could be explained either on the basis of corresponding differences in the nature of the food or in the larval weight. Those larvae reared in starch were most re-

sistant to carbon disulfide, those reared in a heavy protein food, tankage, were least resistant, and those reared in foods with both carbohydrates and proteins stood midway. From graphic interpretation on the basis of larval weight, it appears that in general the larval susceptibility is closely correlated with their weight without taking into consideration the composition of food. The differences in results after the fumigation of the adults of *T. confusum* and *S. granarius* reared on different kinds of food were not as great as those for larvae. This may be due to the smaller differences in their body weight.

Of all the factors, temperature produced the most striking differences in fumigation results. *T. confusum* adults reared at high temperatures (30° and 35° C.) were more susceptible to carbon disulfide than those reared at 25° C. If those reared at 30° and 35° C. were transferred at adult stage to 25° C. for 4 weeks before fumigation their susceptibility to carbon disulfide was nearly the same as for those reared constantly at 25° C. This suggests that the temperature during the development of egg and larval stages does not affect the results of subsequent fumigation if sufficient time is allowed for the adults to be conditioned. Because of this, toxicologists can obtain test animals in a short time by using high rearing temperatures, and by conditioning still have results comparable to those from cultures reared at lower constant temperatures.

Preconditioning of both the adults of *T. confusum* and *S. granarius* at different temperatures for a few days before fumigation will increase or decrease their mortality even when they were reared at a constant temperature. For *T. confusum* adults the mortality after standard fumigation was almost the same whether they were preconditioned at 5°, 10°, or 15° C. for 3 days. At higher temperatures their mortality increased rapidly with an increase of the preconditioning temperature. In the case of *S. granarius* adults, when the temperature of preconditioning increased, their mortality decreased at first, then increased, and finally decreased again.

The preconditioning effect of relative humidity on fumigation results was not as great as that of temperature. For *S. granarius* adults both low and high preconditioning humidities were harmful, while *T. confusum* adults were less affected at high than at low preconditioning humidities.

Nonuniform distribution of fumigants in a 6.4-liter flask caused a tremendous variation of the concentration of fumigants at different layers. These differences were measured indirectly by the time required for 50 per cent locomotory paralysis of *T. confu-*

sum adults. In addition actual differences in mortality were found at different layers during the fumigation of *T. confusum* adults with carbon tetrachloride when the fumigant was not well mixed.

The susceptibility of *T. confusum* eggs to carbon disulfide was decreased as the temperature during fumigation decreased down to 5° C. and then it increased at 0° C. A straight-line relationship between temperature of fumigation and M.L.C. of carbon disulfide holds true from 10° to 30° C. for eggs. The probable reason for increasing toxicity at lower temperatures is more biological than chemical or physical.

Relative humidity during fumigation affected least those insects in an active stage, such as larvae, and showed the greatest effect on the inactive egg stage. During the active stages a 5-hour fumigation period was too short to produce a substantial effect even if the rate of metabolism were changed slightly. However, in the inactive stages the entrance of a fumigant depends entirely upon its diffusion through the outside chorion which may change in permeability at different relative humidities.

The term "sublethal concentration or dose" associated with the so-called "protective stupefaction" of insects has been misused in the literature. It should be called sublethal fumigation since sublethal, median lethal, and lethal concentrations of carbon disulfide, when used with different periods of exposure time before normal fumigation, all produced similar effects.

The general trend of sorption ratios of eight fumigants followed the order of their boiling points, except for methyl acetate which deviated to a greater degree, and ethylene dichloride to a less degree. In general, the higher the boiling point of the fumigant, the greater was the weight of sorption. In regard to their solubility in water it was found that methyl acetate was very soluble in water and ethylene dichloride was slightly soluble, while other fumigants were almost insoluble.

An arbitrary method was given for the determination of the relative penetration of eight fumigants through flour. In this group of fumigants it appears that the higher the boiling point or the higher the sorption ratio of a fumigant, the smaller will be the amount of vapor that will penetrate through patent flour. Chloropicrin, which is usually considered to penetrate well, was found poor in this respect. This mistaken idea may arise from the fact that the odor of chloropicrin can be detected easily at very low concentrations.

The general relation between the boiling point and toxicity is shown in a diagrammatic representation of 20 fumigants and 3

species of insects. The fumigants with either low or high boiling points are more toxic than those within the middle range (about 55° to 80° C.). Very toxic fumigants are found with extremely low or extremely high boiling points. The values of those fumigants which show a delayed kill are often far off from the general trend.

Postfumigation temperature has not received much attention from toxicologists previous to this study. By placing the insects at various temperatures, 5° to 35° C., after fumigation it was found that the mortality curves for the larvae and adults of *T. confusum* as well as for *S. granarius* adults were V-shaped. In general, the mortality was high at 5° C., then decreased to a minimum at about 20° to 25° C. Mortality increased again above 25° C. This may be explained as the combined effect of chemical action of fumigants on the insects, desorption of fumigant vapor, and the activity of the insects.

A great variation in mortality was observed when different criteria of death were used, and results differed also depending upon the time after fumigation when mortality counts were made. Four criteria of death were used: (1) unable to walk, (2) no visible movement, (3) no visible movement when prodded, and (4) no visible movement when prodded and exposed under light. In every case the mortality of *T. confusum* and *S. granarius* decreased from criterion 1 to 4. The difference was great at the end of the first day and then decreased. At 10 or 13 days after fumigation the results were almost the same under all the four methods of counting mortality. The variation of mortality during the 13-day period differed from one species, age, and stage to another. Because of these differences it was impossible to draw any general conclusion regarding the course of mortality after fumigation. It appears, however, to be desirable to include both the 2-day and 10-day mortality figures when describing the susceptibility of insects to fumigants.

In the study of the relation between the concentration of a fumigant and the exposure time it was found that the following equation holds true for a wide range of concentrations (20 to 200 mg. per liter) of carbon disulfide against *T. confusum* larvae:

$$C\ (T - x) = K$$

where C is the concentration of carbon disulfide; T, the exposure time necessary to produce 50 per cent mortality; x, the time required for the fumigant to diffuse into the vital center before building up a constant flow of fumigant; and K, a constant. The

calculated values of K are 190, 192, 191, 190, 204, 186, 192, and 188 at 20, 30, 40, 70, 100, 120, 150, and 200 mg. per liter concentration respectively. The average value of K is 191.6. The significance of the value "x" to the formula is understood when one considers the importance of the lag in time between the first exposure to a toxic vapor and the time when the insect receives the full concentration.

It has been found that with the repetition of a dosage at or near M.L.C. for successive fumigations of insects from different cultures the mortality will fall near the 50 per cent point. The corresponding M.L.C. of each result can be calculated from the following equation:

$$\text{M.L.C.} = C + \frac{50 - x}{V_t}$$

where C is the concentration of a fumigant in mg. per liter, giving a mortality of x per cent. V_t $(=\frac{a}{b})$, the rate of increasing toxicity, is expressed by the straight part of the curve in per cent of mortality per milligram of concentration. Finally "a" is the per cent of mortality and "b" is the concentration of the fumigant in mg. per liter. The same result may be obtained by graphical solution.

Other factors that received some attention and that produced some effect on fumigation results were population density, starvation before and after fumigation, the time of exposure, and post-fumigation humidity. Of these factors differences in population density produced the most surprising effect, for mortality after fumigation was greater as the density of the culture decreased from 300 to 50 weevils in 30 grams of wheat. The other factors listed were of minor interest.

As a result of this study a larger number of factors than are usually considered have been shown to cause the marked variations common in fumigation results. One can imagine the complexity of the problem of fumigation when these factors are acting together. A diagrammatic arrangement was made to indicate the qualitative relationship among the factors and their counter and parallel effects on the susceptibility of insects.

The several factors that have been shown to have such a marked effect on the results of fumigation should always be carefully considered. Most of them must be controlled when fumigants are being compared in the laboratory, and some can be manipulated to ensure a more successful and economical control by fumigation in the practical field.

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