COMPARATIVE TOXICITY OF THREE FUMIGANTS TO THE CONFUSED FLOUR BEETLE (*TRIBOLIUM CONFUSUM* DUV.) AND THE RUST-RED FLOUR BEETLE (*T. CASTANEUM* HBST.)

by

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INTRODUCTION

The control of insect damage has been for many years a difficult problem facing flour millers all over the world. The cost of the damage by these insects has been conservatively estimated to be about $300,000,000 annually. Consequently, much experimental work has been done in an effort to develop insecticides, especially those of the class known as fumigants, for use against these insects. Many chemical compounds and mixtures have been investigated in this regard, especially in more recent years. However, investigators are still searching for new and better fumigants.

Insects of the genus Tribolium have become one of the standard insects used in comparing the relative toxicity of these prospective fumigants. This genus contains several species, two of which are small reddish-brown beetles which at the present time comprise a large percentage of all insects inhabiting flour mills in this region. These flour beetles have long been known to be among the most serious pests of flour, meal and other cereal products. Numerous references in the literature give ample evidence of their importance as destroyers of these foodstuffs.

One of these flour beetles used extensively in toxicity experiments has been <u>Tribolium confusum</u> Duv., the confused flour beetle. <u>Tribolium castaneum</u> Hbst., the rust-red flour beetle, has also been used to some extent. However, there is very little data on the comparative behavior of these two insects to fumigants.

During the winter of 1937-38, the author investigated the effects of an increase in temperature on the toxicity of certain fumigants to the confused flour beetle, <u>Tribolium confusum</u> Duv. While conducting tests with carbon bisulphide against this species, the insect culture was accidently depleted of adults. Upon replenishing the supply from miscellaneous insect cultures about the laboratory, a population was built up that was more resistant to the effects of the fumigant than the previous culture. These insects were identified by the author and found to be composed almost entirely of the rust-red flour beetle, <u>T. castaneum</u> Hbst.

At the time, cultures of both species were being reared at room temperature (about 70°F.). Therefore, a series of fumigations was conducted at this temperature to study the effects of the fumigant on these two species. One fumigant, carbon bisulphide, was used in these preliminary tests which indicated that a difference in susceptibility did exist.

These preliminary results caused the author to undertake further tests to determine more about this apparent difference in the toxicity of fumigants to these two closely-related species of flour beetles.

The author has noticed that these two species are often found living together. Frequently a culture that is predominately composed of the confused flour beetle will, after a few months, become greatly infiltrated with the rust-red flour beetle.

If a consistent difference in susceptibility were found, it would help to explain some of the diversity of data recorded in the literature. Numerous workers have conducted tests of fumigants with the flour beetles although throughout the published results of these experiments no mention can be found of their method of identifying the insects used. While a culture of _T. confusum_ may be pure at the beginning of a series of experiments, it could easily become contaminated with _T. castaneum_ unknown to the investigator. A more satisfactory method is to identify each individual insect as it is used.

MATERIALS

Chemicals

In investigating this problem three fumigants, carbon bisulphide, chloropicrin and ethylene dichloride were used. The first and third of these chemicals are highly inflammable and must be mixed with other compounds for industrial use as fumigants. However, in these tests the materials used were undiluted. The other fumigant, chloropicrin, was a world war gas. These chemicals have been used extensively as standard or check fumigants in toxicity tests of prospective fumigants.

Carbon bisulphide is a colorless, volatile liquid which boils at 114.8°F. The vapors of this liquid when mixed with air in amounts from 1 to 50 per cent by volume, form an explosive mixture. In the following experiments the technical grade of carbon bisulphide was used.

Ethylene dichloride is a colorless liquid with an odor similar to that of chloroform. It has a boiling point of 183.2°F. and evaporates on exposure to air. Although the vapors of ethylene dichloride are somewhat inflammable, this difficulty has been overcome in commercial fumigation by adding one part of carbon tetrachloride to three parts of ethylene dichloride.

Chloropicrin, which is a slightly yellowish liquid, is a little more than one and a half times as heavy as water. It has a boiling point of 233.6°F. and forms a mixture with air that is 1.14 times as heavy as air. This gas is non-inflammable and non-explosive. It is highly toxic to insects and man and has a strong lachrymatory effect and irritates the respiratory passages of man. The two latter characteristics are considered by many as a safety precaution, since one is unlikely to attempt an entrance into a building filled with this gas without a mask. Although this material has a high toxicity to insects, it does have the disadvantage of being adsorbed and absorbed in large quantities by any material with which it comes in contact.

Insects

Both the eggs and the adults of the confused flour beetle and the rust-red flour beetle were used in the experiment. Both of these destructive flour insects are small reddish-brown beetles of about 3.5 mm. in length with striated elytra. In each species the sexes are identical externally and cannot be differentiated until the genitalia are exerted by a slight pressure on the abdomen (Good, 1936). Both species have well developed wings, but only T. castaneum has been observed to use them.

These beetles are so similar in size, color, shape of body, and habits that they are easily confused, even by experienced entomologists. The morphological characters used in distinguishing the species is discussed in the Review of Literature.

Apparatus

All of the fumigations with the exception of one series were conducted in six-liter Erlenmeyer flasks. This one series of tests was conducted in 20-liter flasks due to the difficulty in measuring the very small dosages used.

The flasks were sealed with tight-fitting rubber stoppers through which were placed two glass stopcocks for use in introducing the fumigating material.

Glass vials, 20 mm. X 70 mm. with bottoms replaced by a piece of 10-XX silk bolting cloth, served as insect cages in the flask-fumigatorium.

A series of glass pipettes, calibrated to the tip, were used in measuring the fumigants all of which were liquids at room temperature. These pipettes were of various capacities. The largest was of 10 cc. capacity caliberated into 0.1 of one cc., while the smallest was of 0.2 cc. capacity caliberated in 0.001 of a cc.

All of the rearing and fumigating was done in a home-constructed constant temperature cabinet. A thermostat was set so the temperature fluctuated between 82° and 86°F. The heating unit consisted of four 60-watt electric light bulbs placed at one end of each cabinet behind baffle plates. A small electric fan circulated the air when the bulbs were lighted.

A vacuum pump was used to draw a partial vacuum in each flask-fumigatorium preparatory to applying the dosage.

A home-constructed mercury manometer was used to measure the amount of vacuum drawn in each flask. This manometer was an upright wooden frame to which had been attached a piece of glass tubing. The bottom end of this vertical tube was submerged in a small bottle of mercury. The wooden upright was marked off in inches so that the amount of vacuum could be measured.

A small air compressor was used to good advantage as an aid in blowing the gases from the flasks following a fumigation.

METHODS

The following technique was employed in securing the data of this thesis. For at least an hour before each series of fumigations, the flasks were kept in the cabinet to allow them to warm up to the fumigation temperature of

82° to 86° F. The relative humidity, although not actively controlled, ranged from 35 to 45 per cent.

To secure insects for the tests, a portion of the culture was sifted through 4-XX bolting cloth in order to separate the beetles from the flour of the culture. For removing the eggs, a 10-XX cloth was required.

The insects were transferred from the cloth sieve to a square aluminum cake pan, one edge of which was raised with a block. The beetles would slide to the lower edge of the pan where they could be easily collected free of all debris. Thirty insects were swept into each vial cage by means of a camel's hair brush.

Eggs were taken from cultures made up for that purpose. These cultures were pint jars containing very finely sifted flour and a large number of adult beetles. In counting out the eggs, the top of a petri dish was used much in the same manner as the cake pan for the adults. The oval eggs would roll off the tilted top, leaving the bits of flour and debris that were less rounded.

After the insects were introduced into their vial cages a series of fumigations was begun. Each vial cage was suspended by a cord into the center of a flask after which a rubber stopper was tightly fitted into the flask opening.

The hose of the vacuum pump was then fitted to one stopcock and the hose of the mercury manometer to the other stopcock. The pump was started and when the manometer showed a vacuum of between eight and nine inches, both stopcocks were closed. Only a few seconds were needed to measure and apply the proper dosage through one of the stopcocks. After the fumigant was applied, the time, the dosage, and other pertinent information were marked on each bottle with a colored wax pencil. From the time a bottle was taken from the cabinet until it was returned with the proper dosage, approximately two minutes elapsed. In the series, dosages were stair-stepped so as to produce an increasing percentage of mortality from the lowest to the highest dosage.

The fumigations were conducted for three definite time periods, one-hour, three-hours, and 24-hours. Both eggs and adults of the confused flour beetle and of the rust-red flour beetle were tested for each of these periods using the three fumigants, ethylene dichloride, carbon bisulphide, and chloropicrin.

In each of the vial cages, approximately 30 insects were used. Through statistical treatment of their data, previous investigators have established this number as the most satisfactory for fumigation experiments.

To reduce the probability of error in measuring the dosages, both species were fumigated at one time in each flask.

At the end of a fumigation period the flasks were taken from the cabinet and the vial-cages recovered. The insects were transferred to petri dishes, separate dishes being used for each species at each dosage. At this time a microscopic examination was made to determine if all the insects in one petri dish were of the same species. When strays of the other species were found, which was not infrequent, they were discarded.

At this time a count of the mortality was made. The dead were easily distinguished from the living. In determining the percentage mortality, each insect was touched with a small camel's hair brush. Such treatment caused live insects to move some part of their body. Dead insects were usually found with legs outstretched, whereas those that still retained life were found with legs tightly folded against the body. When the beetles had been dead for a few hours the underside of the thorax was blackened.

After a count of the mortality was made, the insects were placed in the temperature cabinet. Twenty-four hours later, the insects were again examined for a final determination of the mortality. In no case when the insects were held for later counting was a lower percentage of mortality found. Eggs were examined daily until it was certain that no more would hatch. At first, counts were made for 25 days, but it was found that there was no hatching after the 12th day. For the majority, 14 daily counts were made.

In the 24-hour tests with chloropicrin, the dosages were so small that it was necessary to use 20-liter bottles as fumigatoriums, so that these dosages could be measured. However, the procedure was the same regardless of the size of the bottle.

REVIEW OF LITERATURE

Economic Importance

Cotton and Good (1937) estimated that the damage caused by insects to stored grain and cereal products in the United States amounts to at least $300,000,000 annually. These authors list nearly 200 different insects and closely related Arthropods recorded as damaging this type of foodstuff. However, they consider only ten of these as major pests. Good (1936) states that _Tribolium confusum_ and _Tribolium castaneum_ are by far the most abundant and destructive insects of flour. Of the ten major pests listed by Cotton and Good (1937), _T. confusum_ is noted as, "probably the worst insect pest of flour mills in the United States today". _Tribolium castaneum_, is noted as, "second only to *T. confusum* as a pest to flour mills in the United States".

Further evidence of the importance of these two beetles is supplied by Good (1937) who states:

> In the course of studies relating to insects inhabiting flour mills, it was found that 84.65 per cent of all insects present in samples of flour from the elevator boots of 17 mills in Kansas, Missouri, and Oklahoma were these two species of *Tribolium*.

Both of these beetles are cosmopolitan in distribution (Cotton and Good, 1937).

These insects make their home in flour, broken grain, and other cereal products. In rearing large numbers of *T. confusum* Payne (1925) found that the flour used acquired a pinkish tinge and had a distinct pungent odor. It also showed an exceedingly low viscosity as compared with normal flour and its elasticity was markedly affected. On being exposed to the air, the flour did not recover its viscosity or elasticity and turned a light brown color. Payne also found that flour infested with adults was more affected than that infested with larvae. This author explained that the beetles give off a characteristic secretion, soluble in water which may be responsible for the effect. Flour so damaged is unsuitable for human consumption.

Whether or not insects of the genus *Tribolium* are capable of injuring undamaged grain has been a controversial subject. Burkhardt (1922) says:

> *Tribolium castaneum* which plays a subordinate roll in Germany as a pest of stored flour and grain does attack entire grains, contrary to the accepted view that only broken ones are affected. Injury by the larvae and adults is limited to the germ of the grain and has been observed in rye and wheat.

Bertrand, Borcq-Rousseu, and Dassonville (1919) state that this beetle attacks a variety of cereals but only those grains that have been already infested by weevils. Good (1936) states, "the species of *Tribolium* cannot feed on entire undamaged grain because their mandibles are not strong enough to chew through the tough outer coating". Practically all lots of grain, however, contain a certain percentage of broken kernels.

Distinguishing Characteristics

The taxonomic characters of *Tribolium* species are so constant that once they are known, there is no doubt as to the identity of specimens.

Good (1933) lists the characters most useful in distinguishing the two species:

> In *T. castaneum* the width of each eye is approximately equal to the distance separating them on the under side of the head. In *T. confusum* the eyes, viewed from below, appear small, the width of each eye being approximately only one-third that of the distance separating them. This character can be used for the identification of living specimens.

The character usually given, the shape of the antennae, was not employed extensively by Good because of the difficulty of its use on living specimens. However, he describes it:

> In T. castaneum the last three joints of the antennae are enlarged and are about the same size. Whereas, in T. confusum there is a gradual enlargement from the base to the tip of the antennae.

Good suggests a trait useful in field identification. When placed on a flat surface under a strong light, T. castaneum often attempts to fly, and sometimes makes short flights of a few feet, while T. confusum never attempts to fly.

Good measured adults of these two species. He found that T. castaneum averaged .15 mm. shorter and .04 mm. narrower (through the thorax) than T. confusum.

Place of Origin of Genus

In discussing the place of origin of the genus, Good (1936) says:

> As with most stored product insects the question of the place of origin of Tribolium is very difficult to solve because their distribution by commerce has long since made them cosmopolitan.

From quoted references Good concludes that the probable origin of this genus was somewhere in the general region comprising India, southwestern Asia, and the eastern Mediterranean lands.

Bionomics

The eggs of both species are oblong shaped, averaging about .6 mm. in length. They are whitish or colorless and are placed directly in the flour or other foodstuff in which the adult is living. Good (1936) found that although the number of larval instars ranged from five to eleven, the usual number was seven or eight. He also found that the larval period ranged from 22 to over 100 days, according to temperature and food. The duration of the pupal period averaged between seven and eight days. Felt (1923) found that the entire life cycle was usually completed in from 50 to 90 days.

The adult life of these beetles is among the longest recorded for the stored products insects. In tests conducted by Good (1936) seven adults lived more than two years and eleven months. Six of these lived more than three years. In another series by the same author, 14 individuals lived more than two years. Good found also that males of T. confusum may be fertile even when they are three years or more of age. He was unable to get females to lay fertile eggs when more than one year and 94 days old.

Good also notes that T. castaneum seems to be less resistant to heat than T. confusum. In a later paper Good

(1937) says that the larval period of *T. confusum* is somewhat longer than for *T. castaneum*, and that the former species lives longer than the latter.

Influence of Environmental Factors

Some work has been done on the effects of temperature on these beetles. Park (1935b) found that when a *Tribolium* culture was accidentally exposed to a temperature of 39°C. for a short time, the larvae were unusually active but completed their development after the culture cooled. The apparently normal adults were paired, but only three or four out of 50 oviposited in 25 days. He suggests that fecundity was affected by the high temperatures. Voute (1936) found a somewhat similar result while working with the rice weevil. He found that larvae from apparently normal adults reared at 31° and 33°C. died before pupation when reared at the same temperatures. It has been reported that, with humidities up to 90 per cent, development of the confused flour beetle is more rapid and larger insects are produced. The larger size is due to real growth and not to increased water content. Mikulski (1936) found that the range of survival in both eggs and pupae was wider at alternating than at constant temperatures, though the maximum was about 88 per cent, never 100.

Several authors have contributed facts pertaining to the effect of conditioned flour on Tribolium populations. Park (1932, 1934, 1935a, and 1937), who has worked for some time on this problem says:

> It appears that the modification of its environment by Tribolium living in flour is largely responsible for the invariable reduction in populations, which is probably induced primarily by the decrease of the fecundity of the females and an increase in the larval period and in larval mortality.

His data show that in general the rate of egg production varies in inverse ratio with the conditioned flour content of the median. The fertility, on the other hand, was not appreciably altered by such differential conditions. Chapman (1928, 1933, and 1934) found that under constant environmental conditions Tribolium populations rise to the saturation point independently of the size of the environment and size of the initial population. He also found that insects of this genus go through a definite reproductive cycle, reaching a high in the amount of egg production at one period of the cycle and a low of egg production at a different period.

Control Methods

Use of Temperature. The application of high temperature has long been recognized as an effective control for T. confusum and T. castaneum. Developmental work along the line of heat sterilization of flour mills was conducted at the Kansas State College from 1910 to 1913 (Dean, 1913).

Felt (1923) in writing of the confused flour beetle says, "Heat is considered the most satisfactory method of killing this beetle for which a temperature of 120°F. is necessary".

In 1936, Oosthuizen conducted a series of experiments on the effect of heat on _T. confusum_. This author found that beetles exposed to 114.8°F. for two to three hours were all killed. This was also true for all the stages except the pupae, which required four hours at this temperature. It is that author's opinion that in general, dry heat appears to be more effective in killing _T. confusum_ than is a rather moist heat but that a saturated atmosphere is the most effective. (Oosthuizen, 1936).

During sterilization of large buildings by heat, the lowest temperatures are found at the surface of concrete floors because of the stratification of heated air. Pepper and Strand (1935) found that the temperature in their sterilization chamber reached 120°F. in two hours at a distance of one-half inch above the floor. However, at the surface of the floor, eight hours was required to reach this temperature. These authors point out that the greatest height of adults of _T. confusum_ ranged from 0.015 to 0.023 inch when placed on a cool surface and 0.023 to 0.031 inch on a heated surface. They conclude that the beetles are considerably below any point the temperature of which can be measured by the ordinary mercury thermometer. It is necessary, therefore, to maintain the lethal temperature for several hours after 120°F. is recorded by thermometers lying on the floor.

Some work has also been done on the effect of low temperatures on the confused flour beetle. Nagel and Shepard (1934) found that an exposure for 25 days to 44.6°F. or for 24 hours to 21.2°F. gave 100 per cent mortality to all stages.

Use of Chemicals. Probably the greatest amount of control work done on these insects has been in the development of fumigants. One of the earliest fumigants to be used successfully was carbon bisulphide. This fumigant was used more than 60 years ago, and aside from its inflammable nature, it is still considered a very good fumigant. Investigators have continued the search for the ideal insect fumigant, particularly during the past few years. Neifert, Cook, Roark, et. al. (1925) investigated 105 organic chemicals as to their insecticidal value. These chemicals were hydrocarbons, bromides, chlorides, fluorides, iodides, alcohols and phenols, aldyhides, esters, ethers, chlorohydrins, sulphur compounds and nitrogenous compounds. They found a series of compounds which in their estimation warranted further tests. The authors considered that a fumigant composed of 40 volumes of ethyl acetate and 60 volumes of carbon tetrachloride was probably the most effective fumigant tested.

In a later paper, Cotton and Roark (1928a) give data showing the value of certain alkyl and alkylene formates as insect fumigants. These compounds were found to be very toxic to the rice weevil and did not affect the germination of grain. Although these compounds are inflammable, this fault can be reduced for most of them by the addition of carbon tetrachloride. Jones (1935) reported that the addition of small quantities of methyl formate to carbon dioxide made a mixture highly toxic to _T. confusum_. He secured a 100 per cent kill of all stages within six hours by using an atmosphere containing 50 per cent carbon dioxide to which had been added 10 mgm. of methyl formate.

In 1928 Roark and Cotton investigated the insecticidal action of some esters of halogenated fatty acids. They found that from the standpoints of toxicity to insects, availability, cost and freedom from fire hazard, methyl, isopropyl, and ethyl monochloroacetates appeared to be the most promising. Unfortunately, the monochloroacetates were found to injure the germination of wheat.(Roark,Cotton,1928).

A mixture containing three parts of carbon tetrachloride and one part of ethylene dichloride advocated by Cotton and Roark was used by Hoyt (1928) and found to be very satisfactory for insects in shelled peanuts, upholstered furniture and for _T. confusum_ in flour.

Strand (1927) conducted preliminary experiments using chloropicrin for control of _T. confusum_. He found that one pound of chloropicrin at 68°F. is equal in toxicity for this insect to 10 pounds of carbon bisulphide or 80 pounds of carbon tetrachloride.

While working with certain aliphatic compounds, Cotton and Roark (1928b) found that the vapors of ethylene oxide are highly toxic to insects. Comparative tests indicated that ethylene oxide is slightly more toxic to pests of stored products than carbon bisulphide and about 30 times more toxic than carbon tetrachloride.

Shepard, Lindgren, and Thomas (1937) conducted tests of 16 fumigants then in commercial use against _T. confusum_, _Sitophilus granarius_, and _S. oryzae_ L. The data indicated that the order of toxicity of the fumigants may differ greatly for species showing considerable structural differences. These authors also conducted tests of 15 new compounds, chiefly chlorinated chemicals. Some were found to be more toxic than chloropicrin. The relative susceptibility of six species of Coleoptera to three fumigants was examined. There appeared to be a fairly consistent correlation between susceptibility and the requirement of a high temperature for development.

In view of considerable variation in results of experiments on the relative toxicity of various fumigants to different stages of T. confusum, Lindgren and Shepard (1932) conducted tests with ethylene oxide, carbon bisulphide and chloropicrin to determine the effect of humidity on their toxicity. They found that the toxicity to the adults was unaffected by ordinary variations in relative humidity, but dry air conditions reduced materially the effect of chloropicrin and carbon bisulphide on the eggs. Contrary to the generally accepted view that the egg is a particularly resistant stage, ethylene oxide appeared to be nine times as toxic to the eggs as to the adults.

Cotton (1932) reported on the relation of respiratory metabolism of insects to their susceptibility to fumigants. He confirmed the findings of other workers that the rate of metabolism varies with the rate of the insect's development. The adult has the highest metabolism and also is the most susceptible to the fumigants, followed in order by the larva and pupa. Cotton used this principle to advantage by adding carbon dioxide to fumigation atmospheres, thereby increasing the rate of insect metabolism and heightening the efficiency of the fumigant. The metabolic rates have also been shown by this author to be widely divergent in different species of the same genus. This may account for the fact that different species of a genus vary markedly in their susceptibility to fumigants.

Lindgren (1936) studied the relationship of respiration to the susceptibility of insects to fumigants. His conclusions were that the rate of respiration is not the only factor to take into consideration. Within a given stage any extrinsic factor, that may tend to increase the rate of metabolism of that stage, may also tend to increase the susceptibility of it to fumigants. Three environmental factors that are known to increase the rate of metabolism are, an increase in temperature, a decrease in oxygen or an increase in carbon dioxide.

Strand (1930) determined the median lethal dosage of several fumigants to _T. confusum_. Among the fumigants tested were chloropicrin, carbon bisulphide, and ethylene dichloride. The period of fumigation was five hours and the median lethal dosage for chloropicrin was 4.7 mg/l, that for carbon bisulphide was 60.85 mg/l, and that for ethylene dichloride was 33 mg/l.

Shepard, Lindgren, and Thomas (1936) studied the comparative toxicity of two fumigants to _T. confusum_ and _T. castaneum_. These tests were conducted at 25°C. for a period of five hours. The authors determined the median lethal dose of chloropicrin and carbon bisulphide. Both fumigants proved to be more toxic to _T. castaneum_ than to _T. confusum_. The median lethal dose of chloropicrin for the rust-red flour beetle was 2.4 mg/l, whereas that for the confused flour beetle was 4.6 mg/l, a difference of 2.2 mg/l. For carbon bisulphide, the median lethal dose was 28 mg/l for the rust-red beetle and 61 mg/l for _T. confusum_, a difference of 33 mg/l.

Some other experimenters have worked along this line of comparative resistance of closely related species to fumigants with other insects. Gortner, (1913) found that _Tenebrio molitor_ L. was more resistant to high concentrations of carbon dioxide than was _Tenebrio obscurus_ F. However, the reverse was true at high temperatures.

Cotton (1932) determined the minimum lethal dosage of ethylene dichloride at 75°F. to be 9 mg/l for _Sitophilus oryzae_ and 13.5 mg/l for _S. granarius_. These tests were conducted for a three-hour period.

Fisk and Shepard (1938) found that the median lethal dosage of methyl bromide to _S. granarius_ was 5.5 mg/l and to S. oryzae was 4 mg/1.

Lindgren (1936) determined the median lethal dosage of three fumigants to T. confusum, S. oryzae, and S. granarius. For carbon bisulphide, the median lethal dosages were calculated at 63 mg/1, 24 mg/1, and 40 mg/1, respectively. For chloropicrin the median lethal dosages were 4.4 mg/1, 2.0 mg/1, and 5.6 mg/1. For ethylene oxide, the dosages were 18.0 mg/1, 5.7 mg/1, and 5.6 mg/1.

Shepard, Lindgren, and Thomas (1937) determined the dosages of 13 fumigants required to kill 99 per cent of S. granarius and S. oryzae. Sitophilus oryzae was the most susceptible to all of the fumigants with the exception of sulphur dioxide. The dosages necessary to produce 99 per cent mortality in S. granarius and S. oryzae were, for chloropicrin 21 mg/1 and 15.2 mg/1; for carbon bisulphide, 66 mg/1 and 40 mg/1; and for ethylene dichloride, 246 mg/1 and 137 mg/1, respectively.

EXPERIMENTAL DATA

Results of Fumigation Tests Against the Adults

A study of the data secured from the experimental work indicated that a real difference in susceptibility exists between the adults of these two species. However, this difference in susceptibility is not the same with all fumigants. For example, the adults of T. confusum were more resistant to carbon bisulphide than were the adults of T. castaneum. The same thing was found in the case of chloropicrin, but the reverse was true for ethylene dichloride. When T. castaneum was fumigated with ethylene dichloride, its resistance was particularly prominent for the longer time periods.

Carbon Bisulphide. Using the minimum lethal dosage, T. confusum and T. castaneum are about equally susceptible to carbon bisulphide in the one-hour fumigation tests. However, curves drawn to include the mortality data at the lower dosages suggest that T. confusum might be slightly more resistant although the author believes this difference to be insignificant. Figure 1 is a curve interpretation of these data.

A teneral adult of T. castaneum was alive at the end of a one-hour fumigation using 2.7 c.c. Another teneral adult survived a 2.6 dosage. These insects were eliminated from the data, however, because they were in the transition period between the pupal and adult stages and therefore, not comparable with the other insects in the test.

In the experiments with carbon bisulphide in the three-hour period there was no doubt as to which species of the flour beetle was more resistant. (Table 2 and Fig. 2).

Table 1. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a one-hour period.*

Expt. No.	Date	Minimum lethal dose			T. confusum		T. castaneum	
		Total cc.	cc/l	Mg/l	No.	% kill	No.	% kill
38-12	11- 6-38	3.0	0.500	630.5	38	100	32	100
38-12	11- 6-38	2.9	0.483	609.06	31	100	31	100
38-12	11- 6-38	2.8	0.466	587.6	35	100	32	100
38-12	11- 6-38	2.7	0.450	567.5	30	100	30	100
38-17	12- 3-38	2.7	0.450	567.5	26	100	30	100
38-17	12- 3-38	2.6	0.433	546.0	32	100	27	100
38-12	11- 6-38	2.6	0.433	546.0	31	100	22	100
38-12	11- 6-38	2.5	0.416	524.6	30	100	32	100
38-17	12- 3-38	2.5	0.416	524.6	30	100	30	100
38-17	12- 3-38	2.4	0.400	504.4	34	100	30	100
38-17	12- 3-38	2.3	0.383	482.9	29	100	31	100
38-17	12- 3-38	2.2	0.366	461.5	29	100	31	96
38-35	12-30-38	2.0	0.333	419.9	34	100	31	100
38-35	12-30-38	1.8	0.300	378.3	34	100	31	100
38-30	12-21-38	1.8	0.300	378.3	35	100	--	--
38-30	12-21-38	1.8	0.266	335.4	41	100	--	--
38-35	12-30-38	1.6	0.266	335.4	33	100	30	100
38-35	12-30-38	1.4	0.233	293.8	33	97	28	96
38-30	12-21-38	1.4	0.233	293.8	34	100	--	--
38-30	12-21-38	1.2	0.200	252.2	35	91	--	--
38-35	12-30-38	1.2	0.200	252.2	34	88	32	84
38-35	12-30-38	1.0	0.166	209.3	32	15	29	69
38-30	12-21-38	1.0	0.166	209.3	35	26	--	--
38-30	12-21-38	0.8	0.133	167.7	35	14	--	--
38-49	3- 9-39	0.7	0.116	146.28			30	16.6
38-49	3- 9-39	0.5	0.083	104.66			32	0
38-49	3- 9-39	0.3	0.050	63.05			33	0

*Unless otherwise noted, these tests were all conducted in six-liter Erlenmeyer flasks at a temperature between 82° and 86°F.

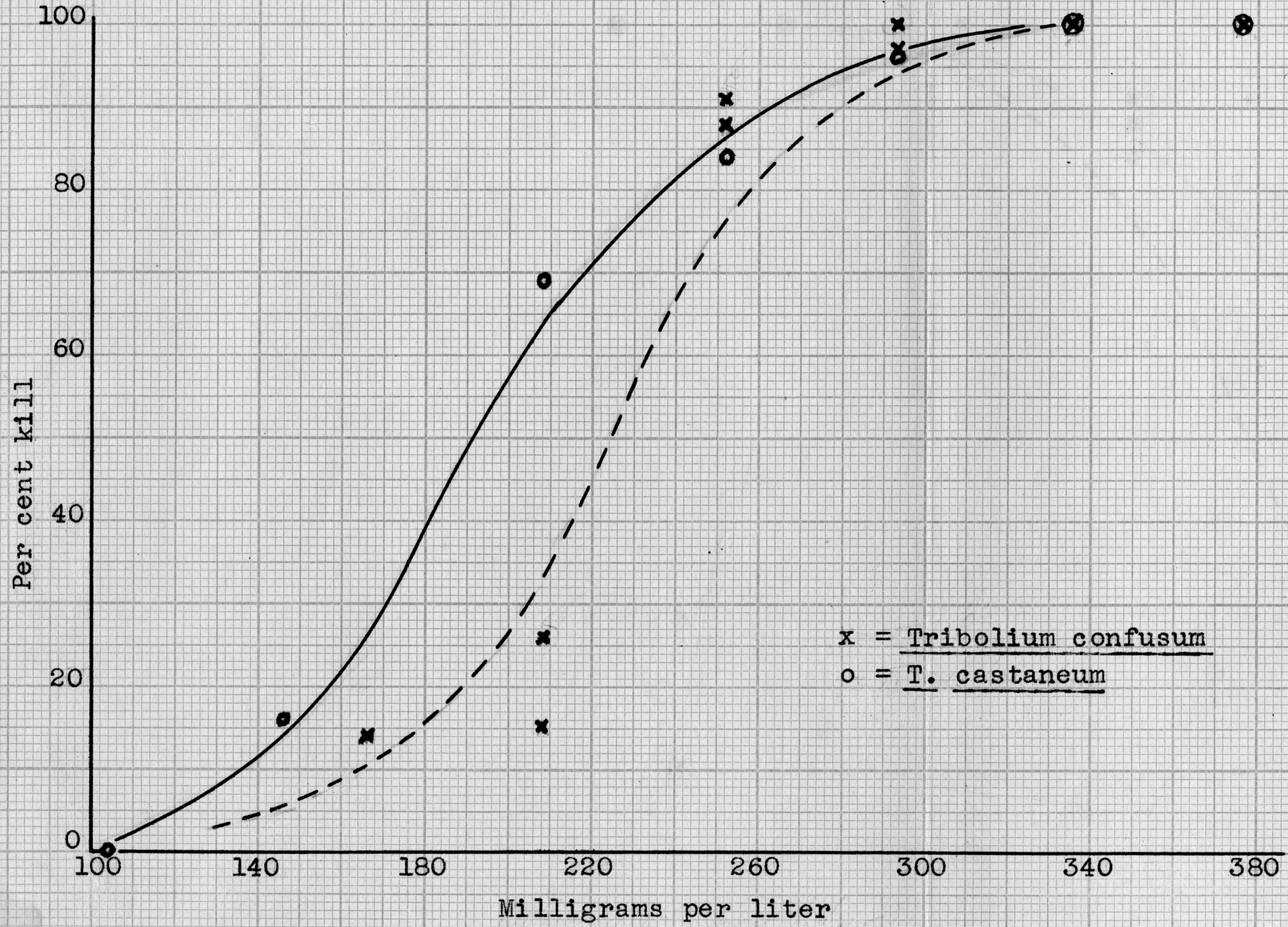

Fig. 1. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a one-hour period.

Table 2. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a three-hour period.

Expt. No.	Date	Minimum lethal dose Total: cc.	cc/l	Mg/l	Tribolium confusum No.	% Kill	Tribolium castaneum No.	% Kill	Remarks
38-20	12-11-38	1.6	0.266	335.4	30	100	--	--	Examined after 48 hrs.
38-20	12-11-38	1.5	0.250	315.3	33	100	--	--	" " "
38-20	12-11-38	1.4	0.233	293.8	32	100	--	--	" " "
38-36	12-31-38	1.4	0.233	293.8	37	100	31	100	
38-20	12-11-38	1.3	0.216	272.4	31	100	--	--	" " "
38-20	12-11-38	1.2	0.200	252.2	32	100	--	--	" " "
38-36	12-31-38	1.2	0.200	252.2	33	100	31	100	
38-20	12-11-38	1.1	0.183	230.8	33	100	--	--	" " "
38-36	12-31-38	1.0	0.166	209.3	33	100	30	100	
38-26	12-18-38	1.0	0.166	209.3	32	100	--	--	
38-26	12-18-38	0.8	0.133	167.7	31	100	--	--	
38-38	1- 2-39	0.8	0.133	167.7	32	100	31	100	Nearly last of castaneum culture
38-38	1- 2-39	0.7	0.116	146.3	31	100	31	100	
38-26	12-18-38	0.7	0.116	146.3	31	100	--	--	
38-36	12-31-38	0.7	0.116	146.3	33	100	30	100	
38-36	12-31-38	0.6	0.100	126.1	33	100	31	100	
38-26	12-18-38	0.6	0.100	126.1	31	97	--	--	
38-38	1- 2-39	0.6	0.100	126.1	29	100	31	100	
38-38	1- 2-39	0.5	0.083	104.7	32	100	32	100	
38-26	12-18-38	0.5	0.083	104.7	28	82	--	--	
38-36	12-31-38	0.5	0.083	104.7	32	94-	32	100	
38-38	1- 2-39	0.4	0.066	83.2	31	45	29	100	
38-38	1- 2-39	0.3	0.050	63.1	32	3	31	87	
38-26	12-18-38	0.3	0.050	63.1	31	13	--	--	Confusum move
38-46	2-22-39	0.3	0.050	63.1	30	0	54	83.3	slowly
38-46	2-22-39	0.25	0.041	51.7	31	0	40	80.5	Confusum very active
38-46	2-22-39	0.20	0.033	41.6	34	0	38	31.6	" "
38-46	2-22-39	0.15	0.025	31.5	31	0	30	0	" "
38-46	2-22-39	0.10	0.015	20.2	30	0	33	0	" "
38-46	2-22-39	0.05	0.008	10.1	28	0	45	0	

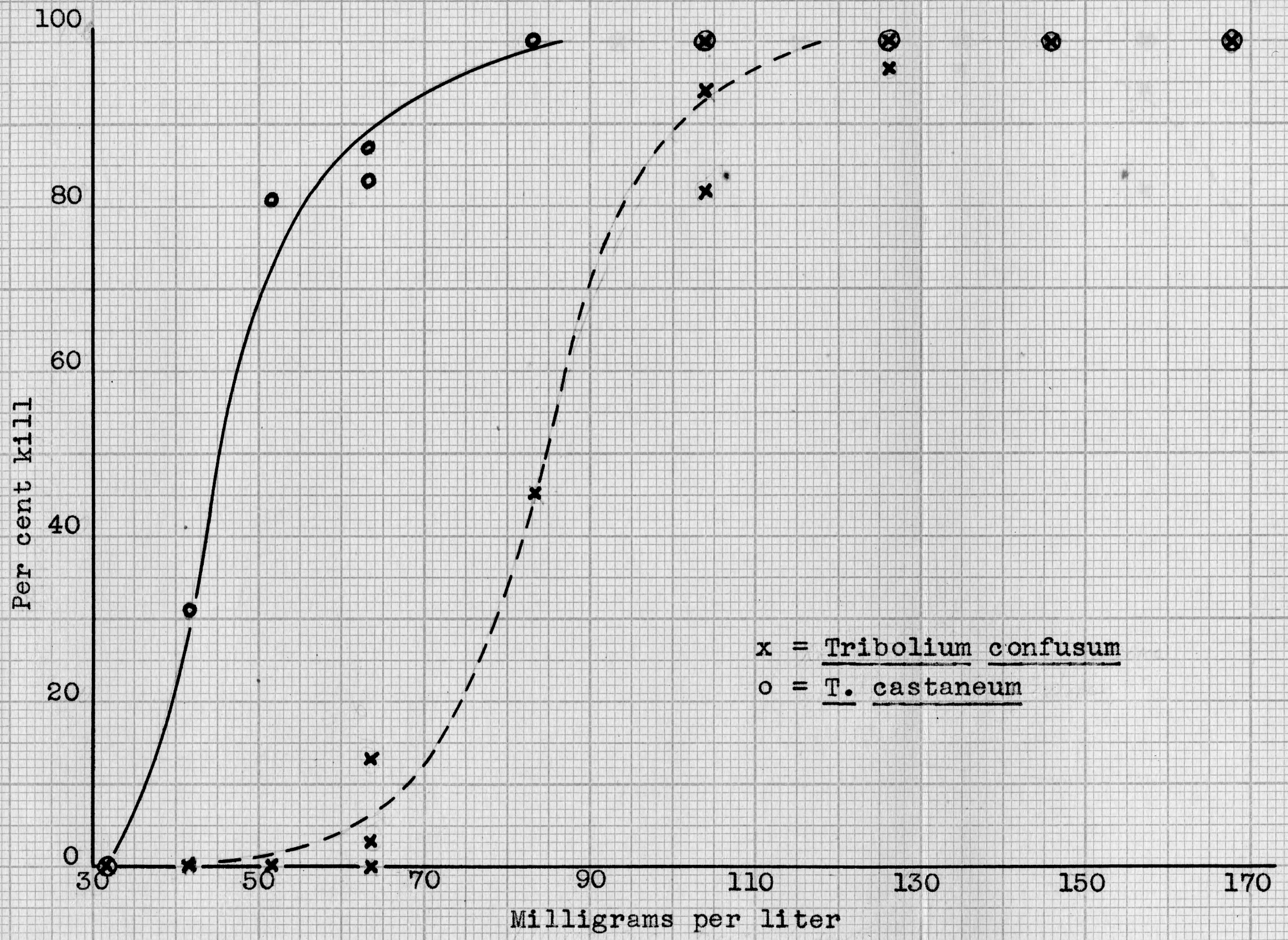

Fig. 2. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a three-hour period.

In this experiment T. confusum was more resistant at every dosage than T. castaneum. The curve interpretation shows that the differences in susceptibility at all dosages were far greater than was found for this fumigant in the one-hour period. In one test only 3 per cent of T. confusum were killed while 87 per cent of T. castaneum were killed. (Table 2, dosage 63.1 mg/l). In another test at a higher dosage the mortality of T. confusum was 45 per cent, whereas that of T. castaneum was 100 per cent. (Table 2, dosage 83.2 mg/l).

The data for the 24-hour tests with carbon bisulphide showed a trend similar to those of the three-hour tests. (Table 3 and Fig. 3). At every dosage T. confusum was the more resistant species. The difference in susceptibility here was about the same amount as was found in the three-hour tests. Table 3 shows that in one test there was 100 per cent mortality of T. castaneum but only 66 per cent mortality of T. confusum. (Table 3, dosage 13.87 mg/l).

Chloropicrin. In the experiments with chloropicrin T. confusum was found to be the more resistant species as indicated in Tables 4, 5, and 6. In the one-hour period 79 per cent of the rust-red flour beetles were killed, whereas none of the confused flour beetles were killed. (Table 4, dosage 9.81 mg/l). These results are shown in Figure 4.

Table 3. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a 24-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	T. confusum: No.	% Kill	T. castaneum: No.	% Kill
38-37	1- 1-39	0.090	0.015	18.19	32	100	31	100
38-23	12-15-38	0.090	0.015	18.19	25	100	--	--
38-23	12-15-38	0.085	0.014	17.65	31	100	--	--
38-37	1- 1-39	0.085	0.014	17.65	31	100	31	100
38- 1	9-22-38	0.085	0.014	17.65	34	100	31	100
38- 1	9-22-38	0.080	0.013	16.39	34	98	31	100
38-37	1- 1-39	0.080	0.013	16.39	30	100	29	100
38-23	12-15-38	0.080	0.013	16.39	30	100	--	--
38-23	12-15-38	0.075	0.012	15.13	31	100	--	--
38- 1	9-22-38	0.075	0.012	15.13	30	90	35	100
38-37	1- 1-39	0.075	0.012	15.13	31	90	31	100
38-37	1- 1-39	0.070	0.011	13.87	30	66	30	100
38-23	12-15-38	0.070	0.011	13.87	31	77	--	--
38- 1	9-22-38	0.070	0.011	13.87	29	76	32	97
38- 1	9-22-38	0.065	0.0108	13.24	32	75	30	80
38-37	1- 1-39	0.065	0.0108	13.24	31	48	30	83
38-23	12-15-38	0.065	0.0108	13.24	32	72	--	--
38- 1	9-22-38	0.060	0.010	12.61	29	72	31	84
38-61	3-24-39	0.054	0.009	11.35	30	30	30	100
38-61	3-24-39	0.032	0.0053	6.68	30	0	28	50
38-61	3-24-39	0.028	0.0046	5.80	20	0	30	7

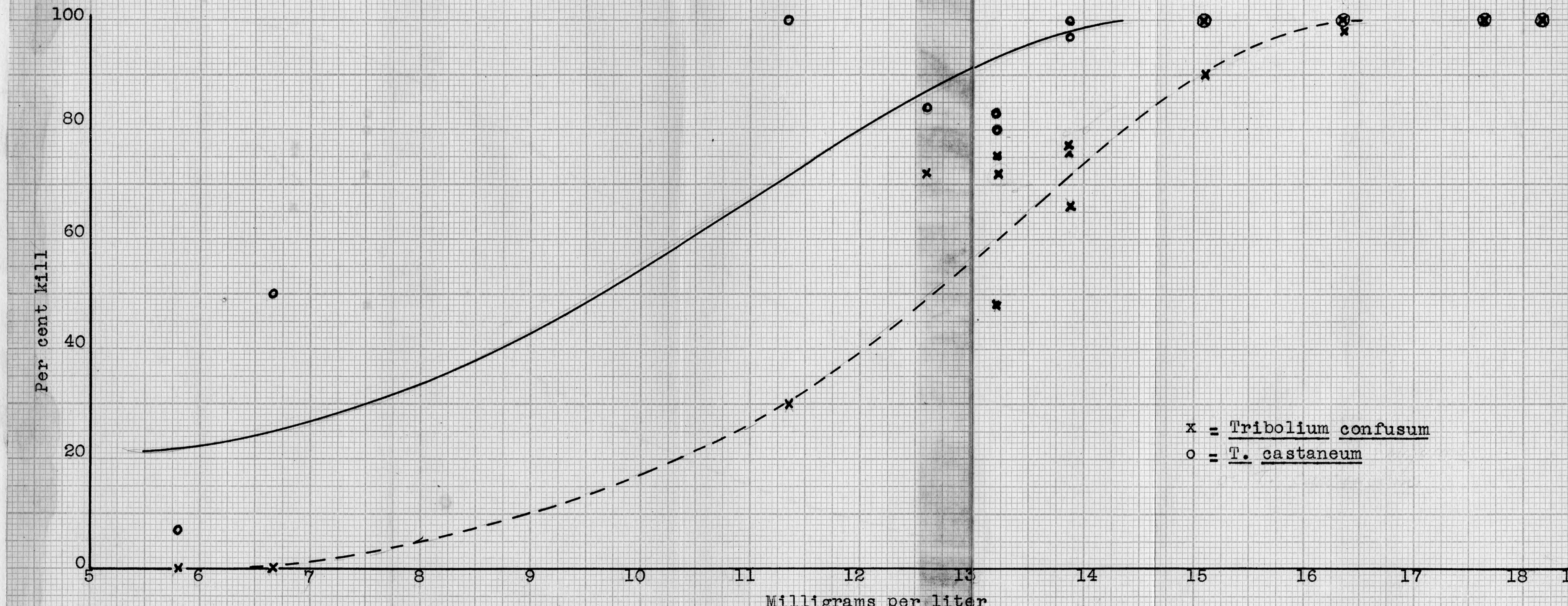

Fig. 3. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a 24-hour period.

Table 4. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to chloropicrin for a one-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	T. confusum: No.	% kill	T. castaneum: No.	% kill
38-59	3-24-39	0.075	0.0125	21.15	32	94	30	100
38-32	12-22-38	0.075	0.0125	21.15	33	91	--	--
38-32	12-22-38	0.070	0.0116	19.63	33	80	--	--
38-32	12-22-38	0.065	0.0108	18.27	34	76	--	--
38-32	12-22-38	0.060	0.0100	16.92	36	67	--	--
38-32	12-22-38	0.055	0.0091	15.40	34	53	--	--
38-59	3-24-39	0.055	0.0091	15.40	32	72	30	100
38-28	12-20-38	0.052	0.0086	14.55	31	61	--	--
38-28	12-20-38	0.051	0.0085	14.38	32	69	--	--
38-28	12-20-38	0.050	0.0083	14.04	31	68	--	--
38-32	12-22-38	0.050	0.0083	14.04	33	36	--	--
38-28	12-20-38	0.049	0.0081	13.71	32	56	--	--
38-28	12-20-38	0.048	0.0080	13.54	54	57	--	--
38-28	12-20-38	0.047	0.0078	13.20	34	56	--	--
38-59	3-24-39	0.035	0.0058	9.81	30	0	32	79

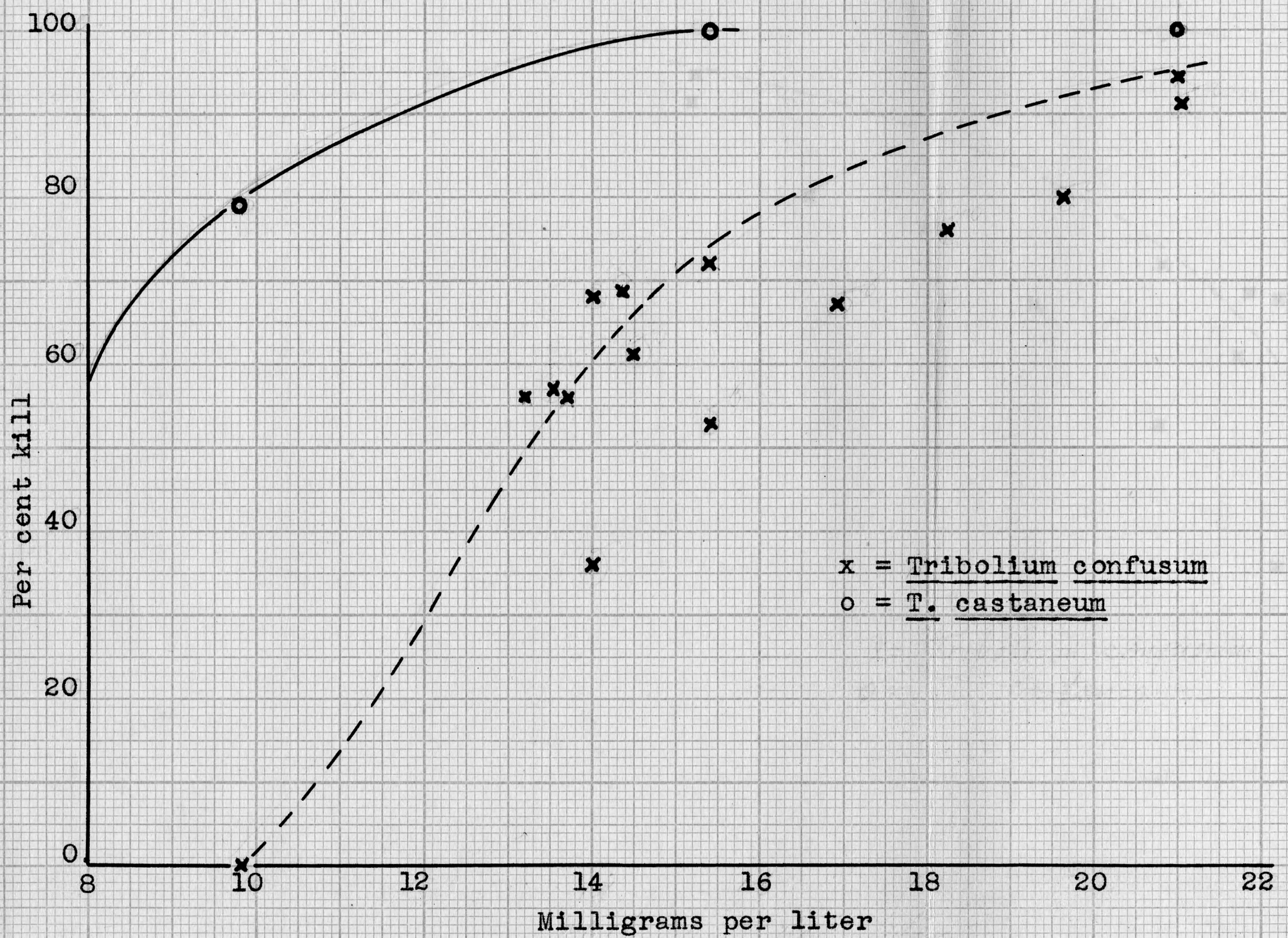

Fig. 4. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to chloropicrin for a one-hour period.

Table 5. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to chloropicrin for a three-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	*T. confusum*: No.	% kill	*T. castaneum*: No.	% kill
38-50	3-10-39	0.020	0.0033	5.58	31	58	28	100
38-25	12-17-38	0.020	0.0033	5.58	34	100	--	--
38-25	12-17-38	0.018	0.0030	5.08	31	61	--	--
38-53	3-23-39	0.018	0.0030	5.08	30	50	30	100
38-25	12-17-38	0.016	0.0026	4.40	32	38	--	--
38-25	12-17-38	0.015	0.0025	4.23	34	9	--	--
38-50	3-10-39	0.015	0.0025	4.23	33	30	32	100
38-25	12-17-38	0.014	0.0023	3.89	29	0	--	--
38-53	3-23-39	0.013	0.0021	3.55	30	43	31	100
38-25	12-17-38	0.012	0.0020	3.38	31	3	--	--
38-50	3-10-39	0.010	0.0016	2.71	26	12	31	100
38-53	3-23-39	0.008	0.0013	2.20	28	4	40	97

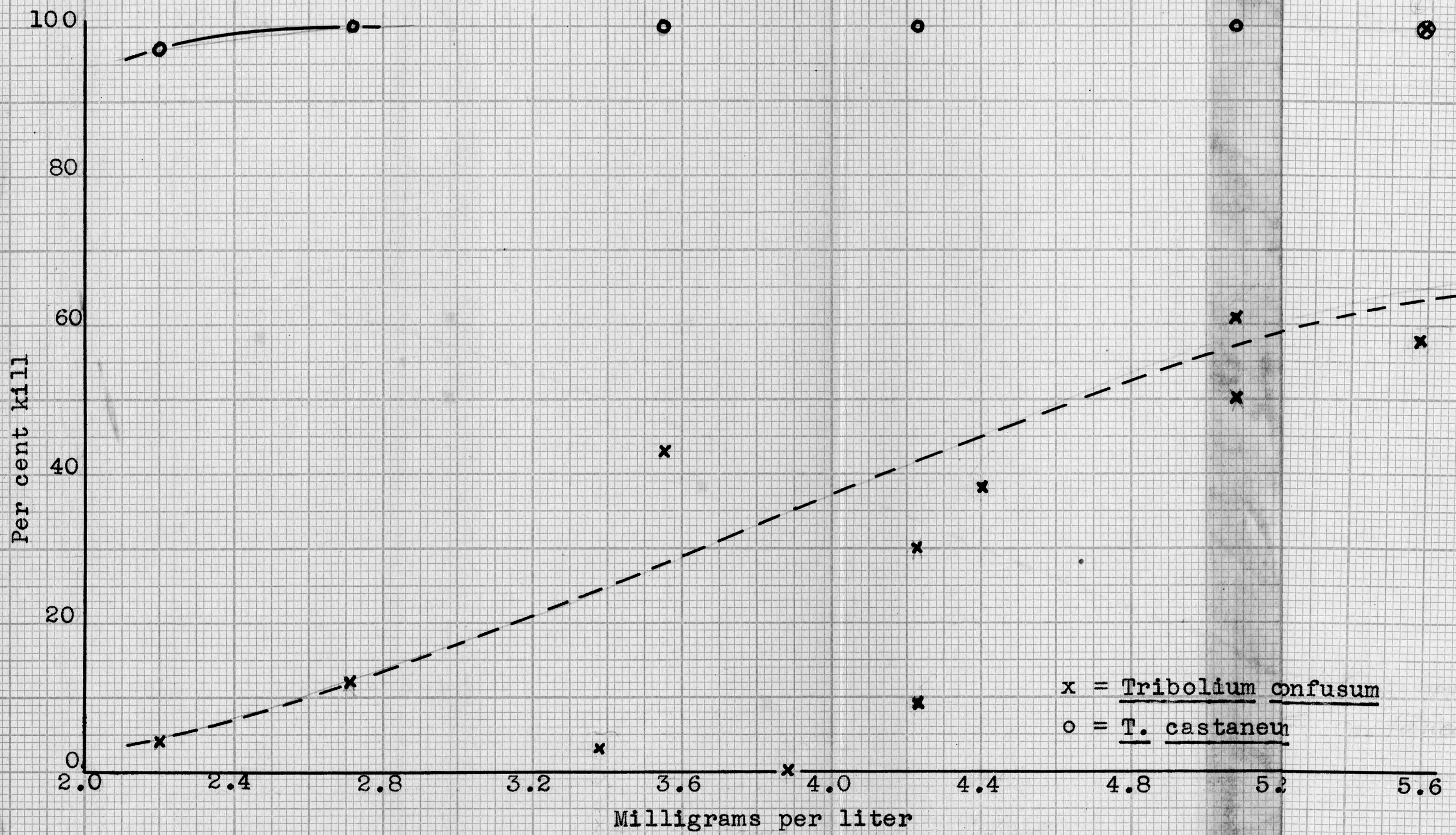

Fig. 5. Showing comparative susceptibility of adults of Tribolium confusum Du. and T. castaneum Hbst. to chloropicrin for a three-hour period.

Table 6. Tests of the comparative susceptibility of adults of _Tribolium confusum_ Duv. and _T. castaneum_ Hbst. to chloropicrin for a 24-hour period.

Expt. No.	Date	Minimum lethal dose			T. confusum		T. castaneum	
		Total	cc/l	Mg/l	No.	% kill	No.	% kill
38-45	2-21-39	0.018	0.0009	1.52	31	87	31	100
38-22	12-13-38	0.018	0.0009	1.52	43	81	--	--
38-22	12-13-38	0.016	0.0008	1.35	64	69	--	--
38-45	2-21-39	0.016	0.0008	1.35	32	53	31	100
38-45	2-21-39	0.014	0.0007	1.18	33	56	32	100
38-22	12-13-38	0.014	0.0007	1.18	30	30	--	--
38-22	12-13-38	0.012	0.0006	1.02	32	22	--	--
38-51	3-20-38	0.012	0.0006	1.02	31	6	31	90
38-51	3-20-38	0.010	0.0005	0.85	31	22	31	100
38-45	2-21-39	0.010	0.0005	0.85	33	10	32	88
38-51	3-20-39	0.008	0.0004	0.68	31	16	30	100
38-51	3-20-39	0.006	0.0003	0.51	32	3	31	35
38-63	3-25-39	0.006	0.0003	0.51	30	0	30	30
38-63	3-25-39	0.005	0.00025	0.42	28	0	22	0

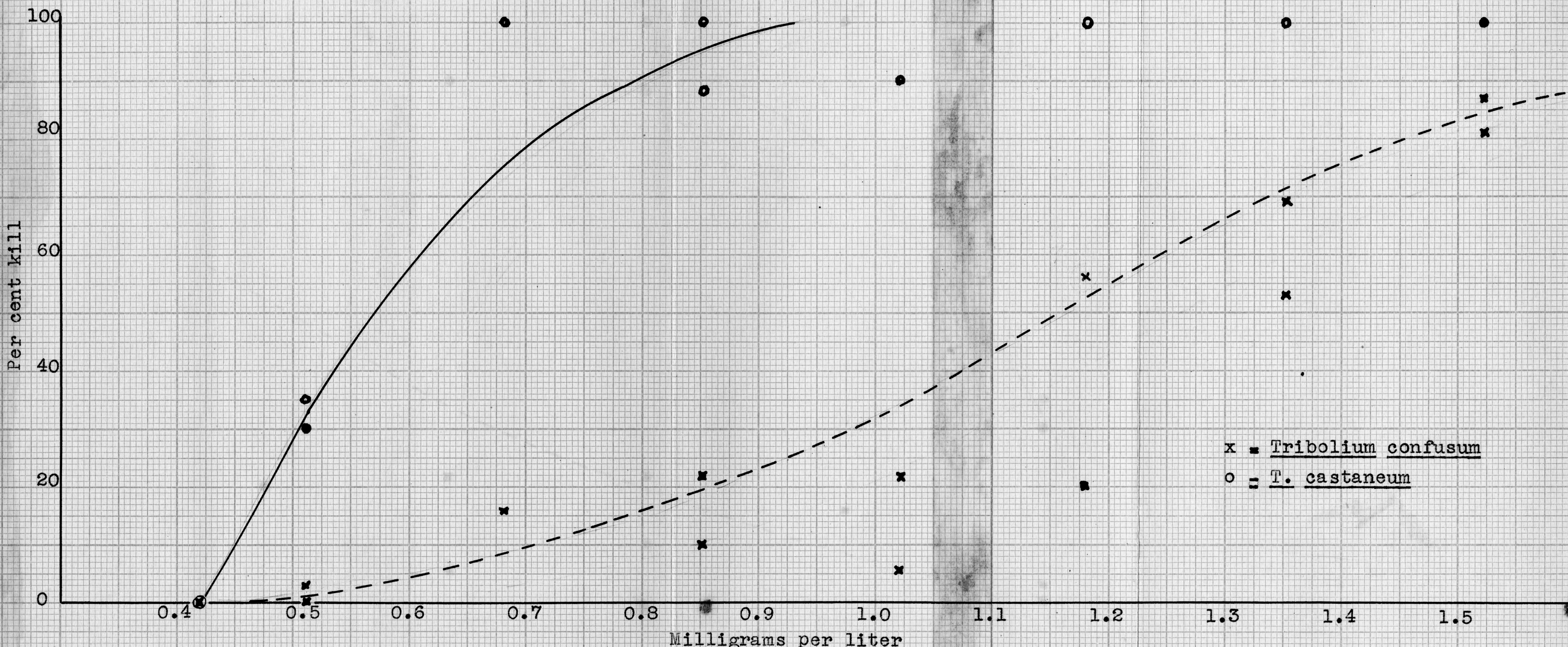

Fig. 6. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to chloropicrin for a 24-hour period.

In one test of the three-hour experiments the mortality of *T. castaneum* was 100 per cent whereas that of *T. confusum* was only 12 per cent. (Table 5, dosage 2.71 mg/l). In one test for the 24-hour period, *T. confusum* had a mortality of only 16 per cent whereas *T. castaneum* had a mortality of 100 per cent. (Table 6, dosage .68 mg/l). This is also shown in Figure 6.

Ethylene dichloride. Tests with ethylene dichloride showed that adults of the rust-red flour beetle were more resistant than those of the confused flour beetle when exposed to this fumigating material.

For the one-hour exposure, the minimum lethal dosage of this material would indicate that *T. confusum* was about equally susceptible to *T. castaneum*. Mortality percentages at the lower concentration, however, indicated that *T. confusum* was somewhat less resistant at sub-lethal dosages. These curves lay sufficiently distant at the sub-lethal dosages to show that *T. confusum* was slightly more susceptible. This is shown in Table 7 and Figure 7.

For the longer fumigation periods, *T. castaneum* was very definitely the more resistant species. The curves for the 24-hour period were much farther apart than were those for the three-hour period. These curves are shown in Figures 8 and 9.

Table 7. Tests of the comparative susceptibility of adults of <u>Tribolium confusum</u> Duv. and <u>T. castaneum</u> Hbst. to ethylene dichloride for a one-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	T. confusum No.	% kill	T. castaneum No.	% kill
38-19	12- 4-38	2.2	0.366	460.06	36	100	34	100
38-19	12- 4-38	2.1	0.350	439.95	35	100	30	100
38-19	12- 4-38	2.0	0.333	418.58	31	100	31	100
38-24	12-16-38	2.0	0.333	418.58	29	100	--	--
38-19	12- 4-38	1.9	0.310	389.67	31	100	34	100
38-19	12- 4-38	1.8	0.300	377.10	30	100	30	100
28-24	12-16-38	1.8	0.300	377.10	32	100	--	--
38-24	12-16-38	1.6	0.266	334.36	29	100	--	--
38-24	12-16-38	1.4	0.233	292.88	30	100	--	--
38-24	12-16-38	1.2	0.200	251.40	30	100	--	--
38-19	12- 4-38	1.2	0.200	251.40	30	100	30	100
38-24	12-16-38	1.0	0.166	208.66	32	91	--	--
38-56	3-24-39	1.0	0.166	208.66	31	100	30	100
38-56	3-24-39	0.8	0.133	167.18	31	97	30	100
38-48	3- 9-39	0.6	0.100	125.70	37	94	32	62
38-56	3-24-39	0.5	0.083	104.33	30	97	31	45
38-48	3- 9-39	0.4	0.066	82.97	30	57	32	19
38-48	3- 9-39	0.2	0.033	41.48	32	3	36	0

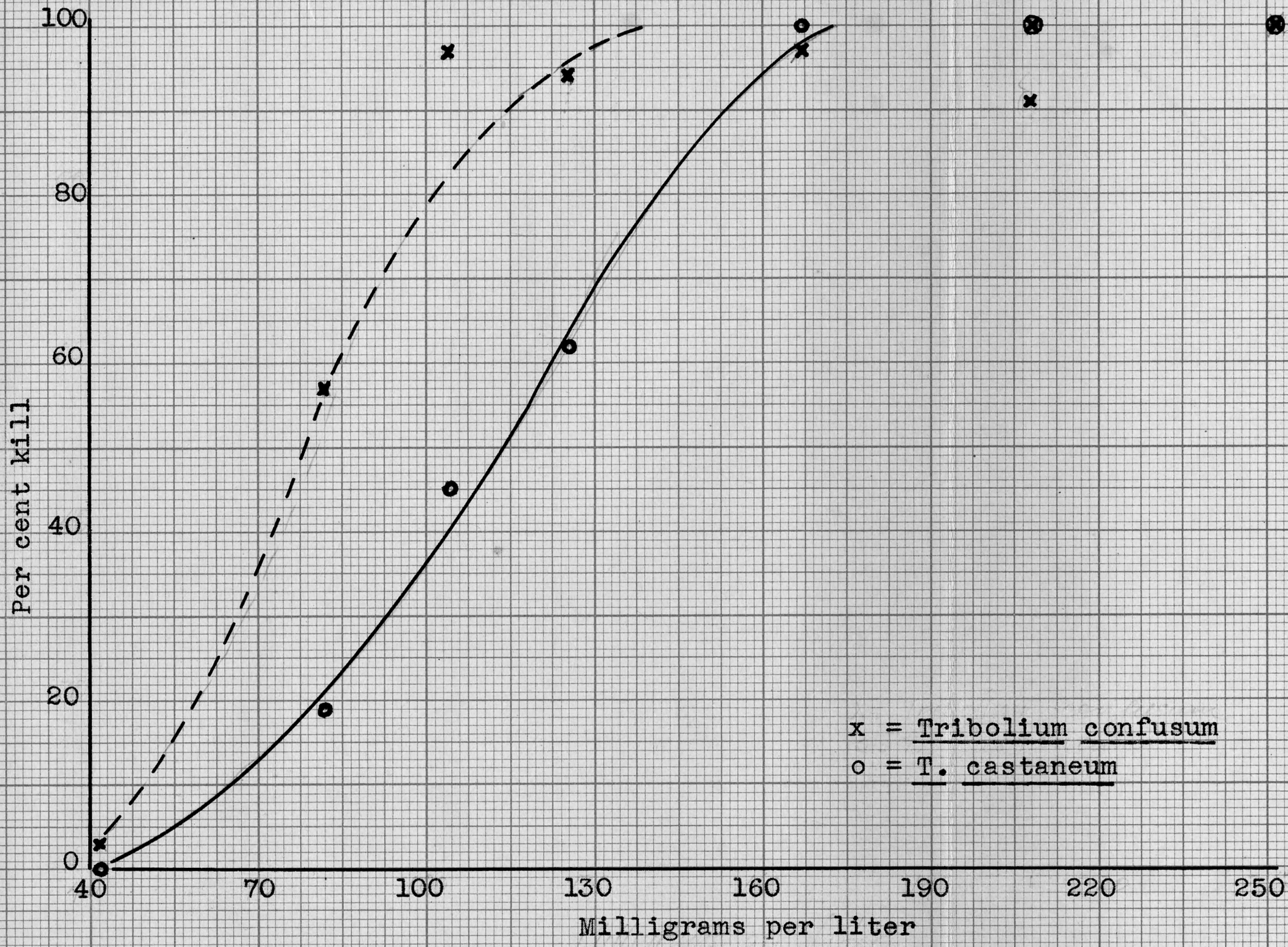

Fig. 7. Showing comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a one-hour period.

Table 8. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a three-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/1	Mg/1	T. confusum: No.	% kill	T. castaneum: No.	% kill
38-57	3-24-39	0.50	0.083	104.33	29	100	30	97
38-54	3-23-39	0.46	0.076	95.53	30	100	--	--
38-39	1- 3-39	0.46	0.076	95.53	31	100	--	--
38-39	1- 3-39	0.43	0.071	89.25	30	100	31	87
38-39	1- 3-39	0.40	0.066	82.97	32	100	32	94
38-54	3-23-39	0.40	0.066	82.97	--	--	31	97
38-33	12-29-38	0.40	0.066	82.97	35	100	--	--
38-33	12-29-38	0.38	0.063	79.19	35	98	--	--
38-54	3-23-39	0.38	0.063	79.19	31	100	--	--
38-39	1- 3-39	0.38	0.063	79.19	31	100	31	80
38-39	1- 3-39	0.35	0.058	72.91	29	93	32	56
38-33	12-29-38	0.35	0.058	72.91	36	100	--	--
38-31	12-21-38	0.34	0.056	70-39	35	94	--	--
38-57	3-24-39	0.34	0.056	70.39	29	100	30	97
38-39	1- 3-39	0.32	0.053	66.62	31	100	31	26
38-33	12-29-38	0.32	0.053	66.62	37	100	--	--
38-31	12-21-38	0.32	0.053	66.62	50	100	--	--
38-31	12-21-38	0.30	0.050	62.85	34	76	--	--
38-57	3-24-39	0.30	0.050	62.85	31	97	31	77
38-21	12-11-38	0.30	0.050	62.85	31	97	--	--
38-33	12-29-38	0.29	0.048		36	78	--	--
38-21	12-11-38	0.29	0.048		33	97	--	--
38-31	12-21-38	0.28	0.046	60.34	34	85	--	--
38-21	12-11-38	0.27	0.045	56.57	34	97	--	--
38-21	12-11-38	0.26	0.043	54.05	36	100	--	--
38-33	12-29-38	0.26	0.043	54.05	37	60	--	--
38-31	12-21-38	0.26	0.043	54.05	34	74	--	--
38-21	13-11-38	0.25	0.041	51.54	34	88	--	--
38-31	12-21-38	0.24	0.040	50.28	34	24	--	--

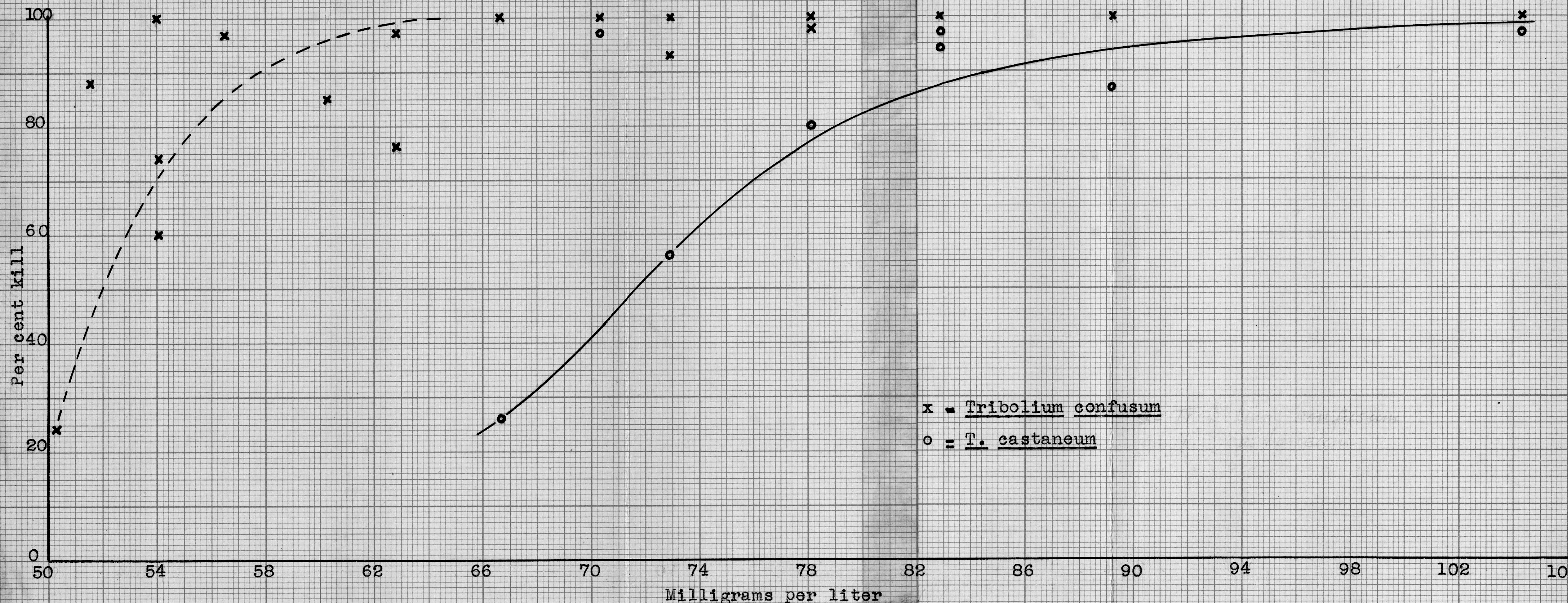

Fig. 8. Showing comparative susceptibility of adults of _Tribolium confusum_ Duv. and _T. castaneum_ Hbst. to ethylene dichloride for a three-hour period.

Table 9. Tests of the comparative susceptibility of adults of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a 24-hour period.

Expt. No.	Date	Minimum lethal dose Total cc.	cc/l	Mg/l	T. confusum No.	T. confusum % kill	T. castaneum No.	T. castaneum % kill
38-52	3-21-39	0.30	0.050	62.85	31	100	30	100
38-52	3-21-39	0.25	0.041	51.54	32	100	30	100
38-52	3-21-39	0.20	0.033	41.48	30	100	31	100
38-58	3-24-39	0.20	0.033	41.48	26	100	31	100
38-58	3-24-39	0.15	0.025	31.43	29	100	29	41
38-47	3-25-39	0.14	0.023	28.91	29	100	32	21.6
38-58	3-24-39	0.12	0.020	25.14	28	100	30	20
38-34	12-29-38	0.115	0.0191	24.01	36	95	--	--
38-34	12-29-38	0.11	0.0183	23.00	34	100	--	--
38-27	12-18-38	0.11	0.0183	23.00	28	100	--	--
38-27	12-18-38	0.105	0.0175	22.00	29	97	--	--
38-34	12-29-38	0.105	0.0175	22.00	35	100	--	--
38-34	12-29-38	0.100	0.0166	20.86	32	100	--	--
38-27	12-18-38	0.100	0.0166	20.86	33	97	--	--
38-47	2-25-39	0.100	0.0166	20.86	31	97	34	3
38-27	12-18-38	0.095	0.0158	19.86	32	97	--	--
38-34	12-29-38	0.095	0.0158	19.86	35	97	--	--
38-16	11-16-38	0.091	0.0151	18.98	54	92	29	10
38-16	11-16-38	0.090	0.0150	18.85	63	100	29	7
38-34	12-29-38	0.090	0.0150	18.85	35	94	--	--
38-27	12-18-38	0.090	0.0150	18.85	33	100	--	--
38-16	11-16-38	0.089	0.0148	18.60	50	96	30	6
38-16	11-16-38	0.088	0.0146	18.35	54	99	30	0
38-16	11-16-38	0.087	0.0145	18.23	52	100	26	0
38-16	11-16-38	0.086	0.0143	17.98	43	98	30	10
38-27	12-18-38	0.085	0.0141	17.72	35	100	--	--

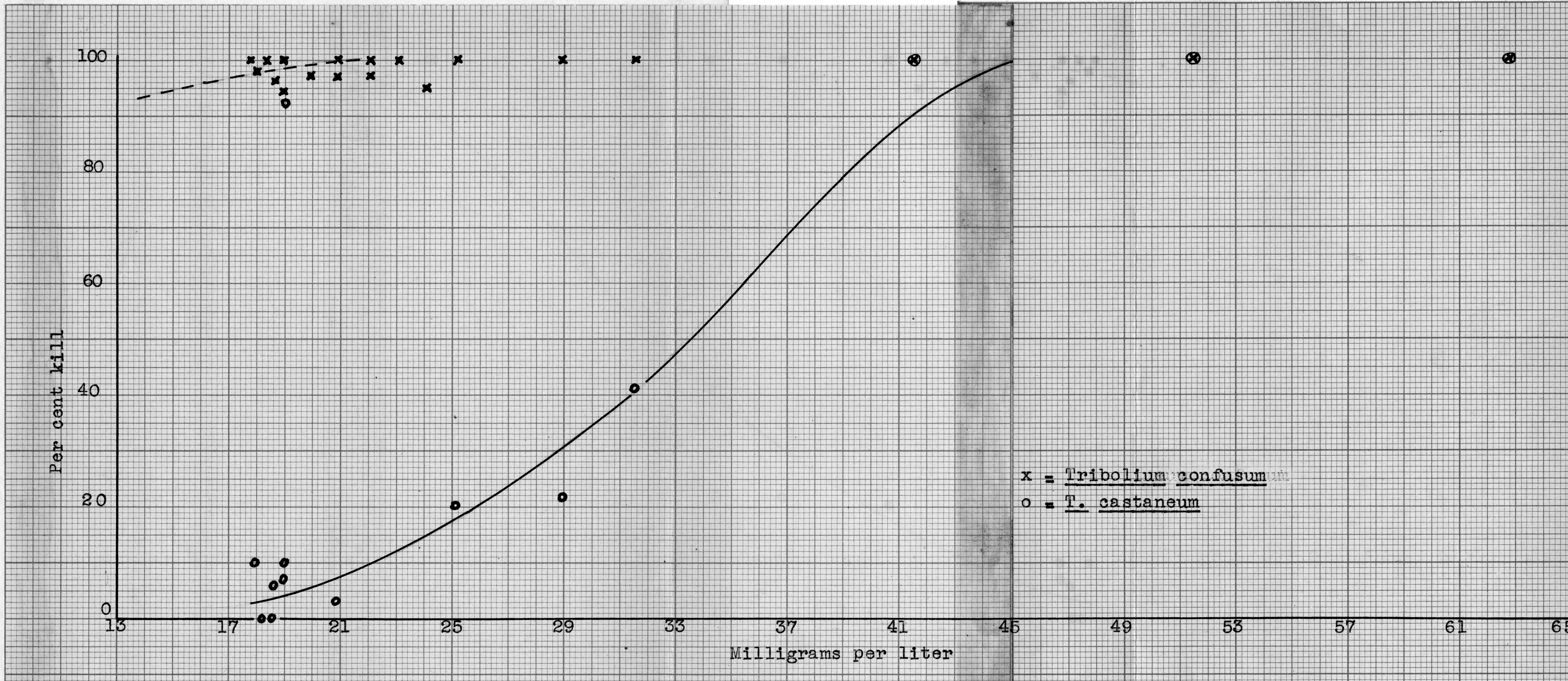

Fig. 9. Showing comparative susceptibility of adults of Tribolium confusum Duv. and T. castaneum Hbst. to ethylene dichloride for a 24-hour period.

Results of Fumigation Tests Against the Eggs

A series of tests were conducted using the three fumigants against eggs of T. confusum and T. castaneum. It was found that the difference in susceptibility of the eggs of these beetles to the fumigants was a very difficult matter to determine with accuracy. To secure a true picture of comparable susceptibility, one should conduct many more tests than the author was able to in the limited time available. One of the greatest variants in this case was the percentage mortality of untreated eggs. In the check vials used by the author in every test, the mortality ranged from 0 to 65 per cent. Such a difference in percentage mortality of untreated eggs unquestionably indicated that the results obtained did not present a true picture of relative susceptibility.

Other unusual facts were noted when the data were studied. In several instances there was a lower percentage mortality among the fumigated eggs than among the corresponding unfumigated eggs. The reason for such results is not known. The fumigant might have acted as a stimulating agent on the eggs although it seemed more probable that the fumigant acted as a sterilizer in killing some disease or fungi responsible for the high percentage of mortality in the checks.

Although the data suggested that T. castaneum was the more resistant species to ethylene dichloride, the author believed the results insignificant. The data varied greatly and the differences between the two species were not very large.

In the one-hour tests there was only one instance in which T. confusum proved to be the more resistant species. In all other tests T. castaneum proved to be slightly more resistant as can be seen by the data of Table 10.

The data for the three-hour fumigation period again showed only one test in which T. confusum was the more resistant.

Fewer tests were conducted with the eggs for the 24-hour period, however, here again a slight difference showed up. In every case T. castaneum was slightly more resistant.

The data secured from tests of carbon bisulphide against the eggs of the flour beetles were very indefinite as far as determining differences in susceptibility between them. In some of the tests T. confusum was found to be more resistant, while in other tests ran in the same series but with slightly different dosage of fumigant, T. castaneum proved to be the more resistant. Curves for this data are

Table 10. Tests of the comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a one-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	Minimum lethal dose: cc/l	Minimum lethal dose: Mg/l	T. confusum: No.	T. confusum: % kill	T. castaneum: No.	T. castaneum: % kill
38-64	3-26-39	3.7	0.616	774.31	30	100	30	83
38-64	3-26-39	3.7	0.616	774.31	30	100	30	80
38-64	3-26-39	2.4	0.400	502.8	30	100	30	80
38- 8	10-30-38	2.4	0.400	502.8	30	40	30	50
38-14	11- 9-38	2.4	0.400	502.8	33	100	33	96
38-14	11- 9-38	2.4	0.400	502.8	33	57	33	40
38-14	11- 9-38	2.3	0.383	481.43	33	82	33	50
38-14	11- 9-38	2.3	0.383	481.43	--	--	33	53
38- 8	10-30-38	2.3	0.383	481.43	30	100	30	66
38- 8	10-30-38	2.2	0.366	460.06	30	100	30	90
38-14	11- 9-38	2.2	0.366	460.06	33	90	33	60
38-14	11- 9-38	2.1	0.350	439.95	33	70	33	33
38- 8	10-30-38	2.1	0.350	439.95	30	73	30	60
38- 8	10-30-38	2.0	0.333	418.88	30	83	30	60
38- 8	10-30-38	1.9	0.310	389.67	30	96	30	73
Check								
38- 8	10-30-38	--	--	--	30	50	30	57 Age 2 da.
38-14	11- 9-38	--	--	--	33	6	33	3
38-64	3-26-39	--	--	--	30	33	30	10

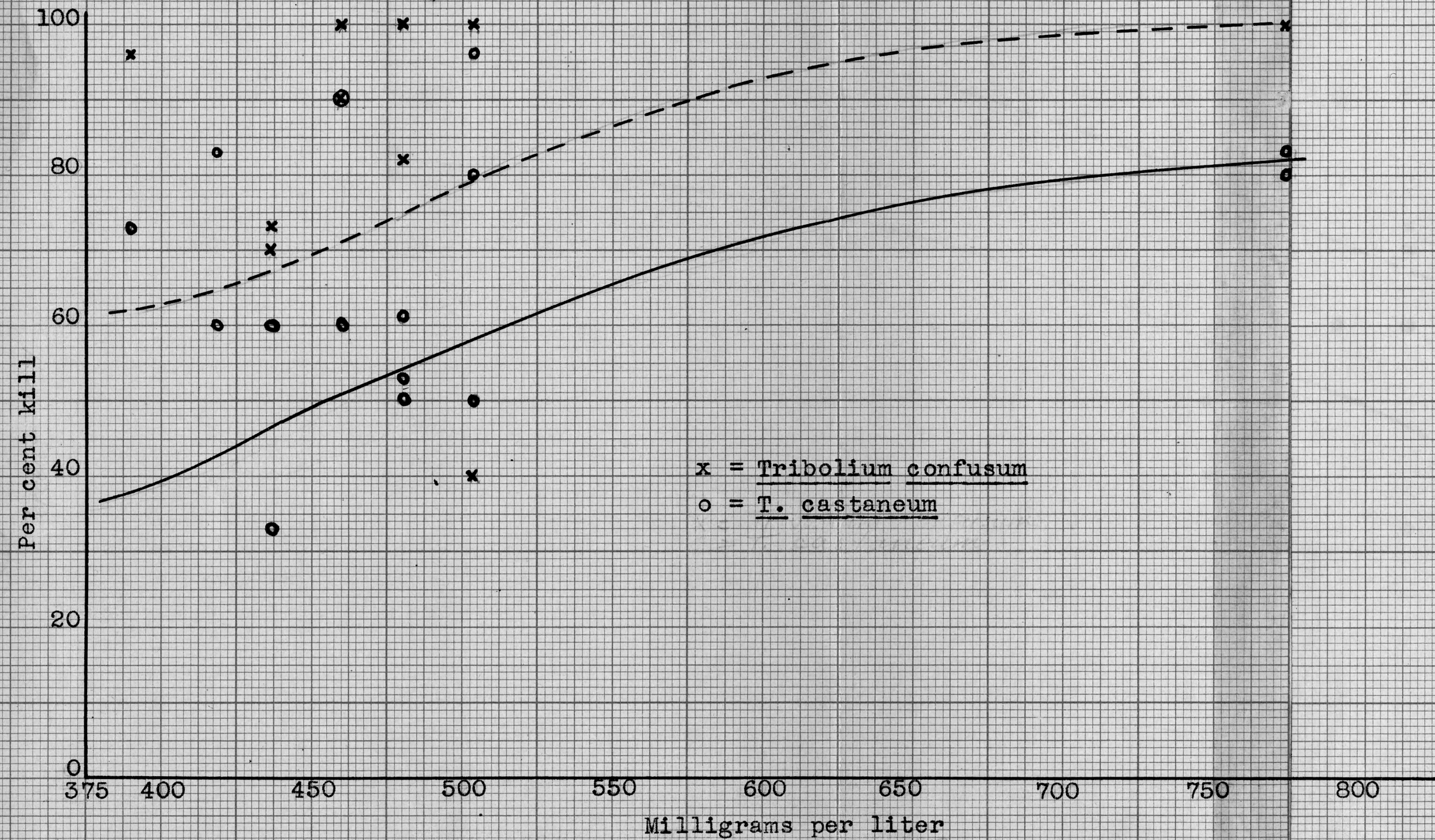

Fig. 10. Showing comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a one-hour period.

Table 11. Tests of the comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a three-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	T. confusum: No.	% kill	T. castaneum: No.	% kill	Remarks
38-29	12-21-38	0.95	0.158	198.61	35	100	37	89	
38-41	1- 4-39	0.95	0.158	198.61	30	100	30	88	
38-41	1- 4-39	0.90	0.150	188.53	30	100	30	66	
38-29	12-21-38	0.90	0.150	188.53	35	100	37	78	
38-13	11- 7-38	0.87	0.145	183.52	30	93	30	70	
38-13	11- 7-38	0.85	0.141	177.24	30	97	30	73	
38-29	12-21-38	0.85	0.141	177.24	35	100	37	81	
38-41	1- 4-38	0.85	0.141	177.24	30	93	30	83	
38-13	11- 7-38	0.83	0.138	173.47	30	63	30	100	
38-13	11- 7-38	0.81	0.135	169.70	30	97	30	47	
38-41	1- 4-39	0.80	0.133	167.18	30	100	30	50	
38-29	12-21-38	0.80	0.133	167.18	35	97	37	81	
38-13	11- 7-38	0.79	0.131	164.67	30	60	30	47	
38-13	11- 7-38	0.77	0.128	160.90	30	80	30	27	
38-41	1- 4-39	0.75	0.125	157.13	30	93	30	60	
Check									Age
38-29	12-21-38	--	--	--	35	0	37	0	3 days old
38-41	1- 4-39	--	--	--	30	0	30	16	2 days old
38-13	11- 7-38	--	--	--	30	3	30	17	2 days old

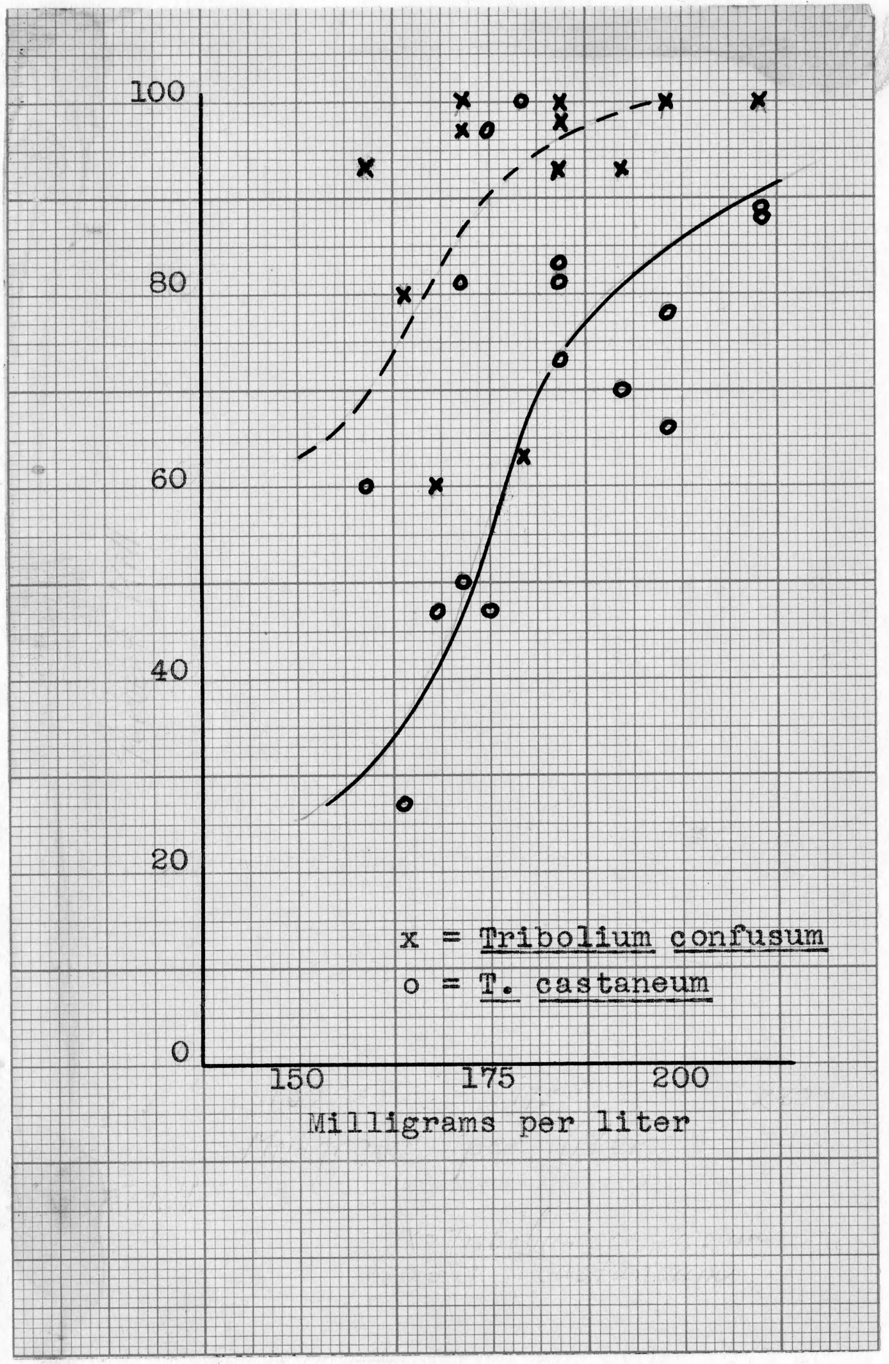

Fig. 11. Showing comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a three-hour period.

Table 12. Tests of the comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to ethylene dichloride for a 24-hour period.

Expt. No.	Date	Minimum lethal dose Total cc.	cc/l	Mg/l	T. confusum No.	% kill	T. castaneum No.	% kill
38-62	3-25-39	0.18	0.030	37.71	32	100	32	100
38-62	3-25-39	0.15	0.025	31.42	32	97	32	44
38-62	3-25-39	0.10	0.016	20.21	32	81	32	56
38-15	11-11-38	0.10	0.016	20.21	33	71	35	23
38-15	11-11-38	0.09	0.015	18.86	33	71	35	34
38-15	11-11-38	0.08	0.013	16.34	33	82	35	46
38-15	11-11-38	0.07	0.011	13.83	33	39	35	6
38-15	11-11-38	0.06	0.010	12.57	33	32	35	11
Check								
38-15	11-11-38	--	--	--	38	0	35	65
38-62	3-25-39	--	--	--	32	6	32	0

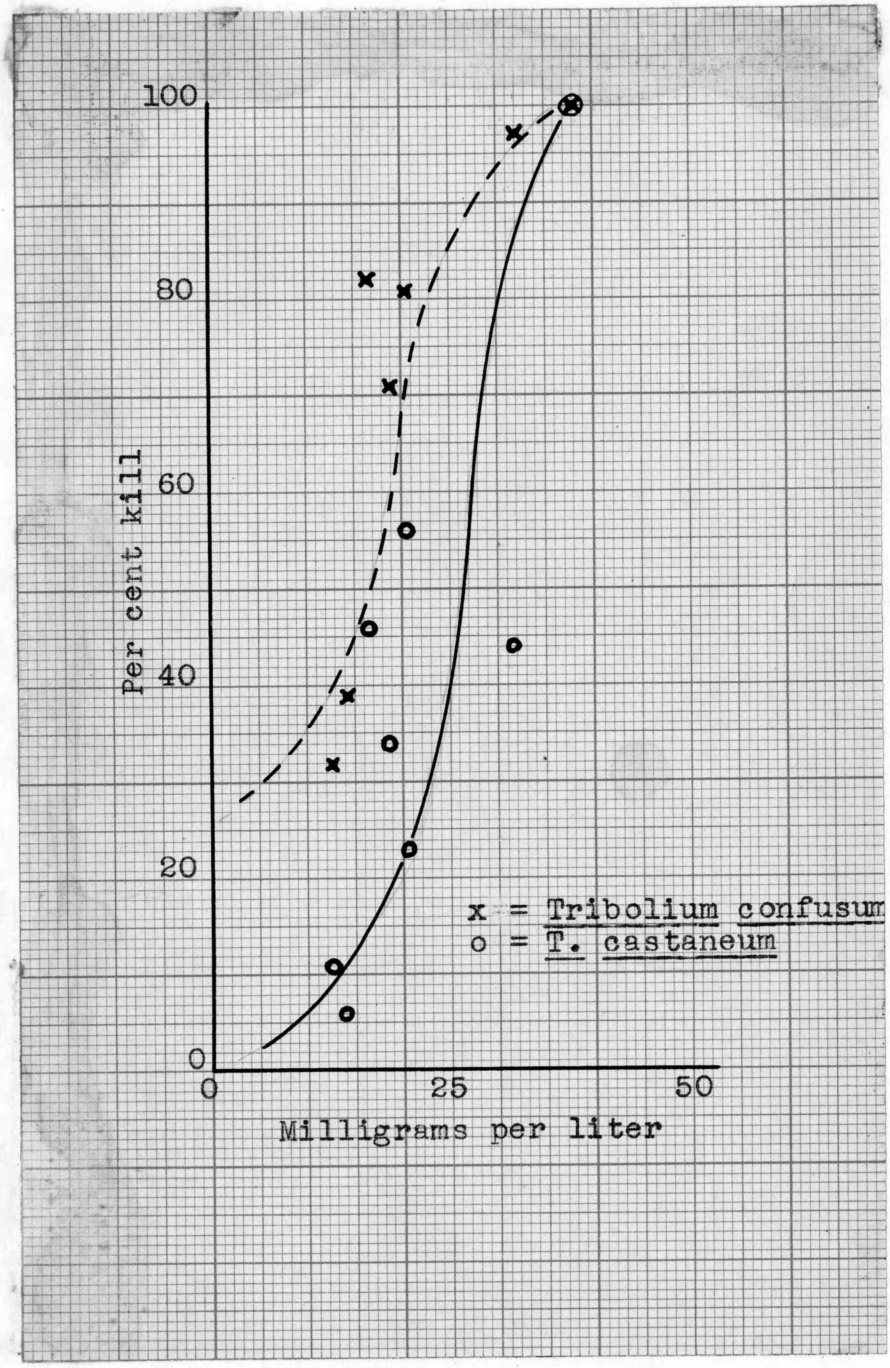

Fig. 12. Showing comparative susceptibility of eggs of Tribolium confusum Duv. and T. castaneum Hbst. to ethylene dichloride for a 24-hour period.

shown in Figure 13. They lay fairly far distant at the lower mortality percentages, but at about 85 per cent mortality they meet and cross slightly. By this curve interpretation it was indicated that T. castaneum was slightly more resistant at the lower dosages but not so at the higher dosages.

This same type of curves resulted from the interpretation of data for the three-hour test against the eggs. These are shown in Figure 14. Here again T. castaneum was evidently more resistant at the lower dosages. The curves for the two species meet at a point representing approximately 70 per cent mortality. From here on, T. confusum was the more resistant species, although the curves representing the two species were rather close together.

Tests were conducted against the eggs with carbon bisulphide for the 24-hour period and also with chloropicrin for the three time periods. However, due to the work being done in a period when the percentage fertility of the eggs was very low, these data were too meager to attempt to draw any conclusions from, therefore it has been omitted from this thesis.

Table 13. Tests of the comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a one-hour period.

Expt. No.	Date	Minimum lethal dose: Total cc.	cc/l	Mg/l	*T. confusum* No.	% kill	*T. castaneum* No.	% kill	Remarks
38- 4	10- 6-38	2.9	0.483	609.1	30	100	--	--	
38- 4	10- 6-38	2.8	0.466	587.6	30	100	30	100	
38- 6	10-16-38	2.8	0.466	587.6	30	100	30	100	
38- 6	10-16-38	2.8	0.466	587.6	30	100	30	100	
38- 6	10-16-38	2.7	0.450	567.5	30	100*	30	100*	*Very few
38- 6	10-16-38	2.7	0.450	567.5	30	100	30	100	eggs after
38- 4	10- 6-38	2.7	0.450	567.5	30	100	30	100	fumigation
38- 4	10- 6-38	2.6	0.433	546.0	30	93	30	93	
38- 6	10-16-38	2.6	0.433	546.0	30	80	30	73	
38- 6	10-16-38	2.6	0.433	546.0	30	100	30	100	
38- 4	10- 6-38	2.5	0.416	524.6	30	77	30	90	
38- 4	10- 6-38	2.4	0.400	504.4	30	83	30	60	
38-55	3-23-39	2.2	0.366	461.53	30	83	30	76	
38-55	3-23-39	2.0	0.333	419.91	30	60	30	70	
38-55	3-23-39	1.8	0.300	378.30	30	20	30	43	
Check									Age
38- 4	10- 6-38	--	--	--	30	3	30	27	2 days old
38- 6	10-16-38	--	--	--	30	43	30	0	2 days old
38-55	3-23-39	--	--	--	30	33	30	3	2 days old

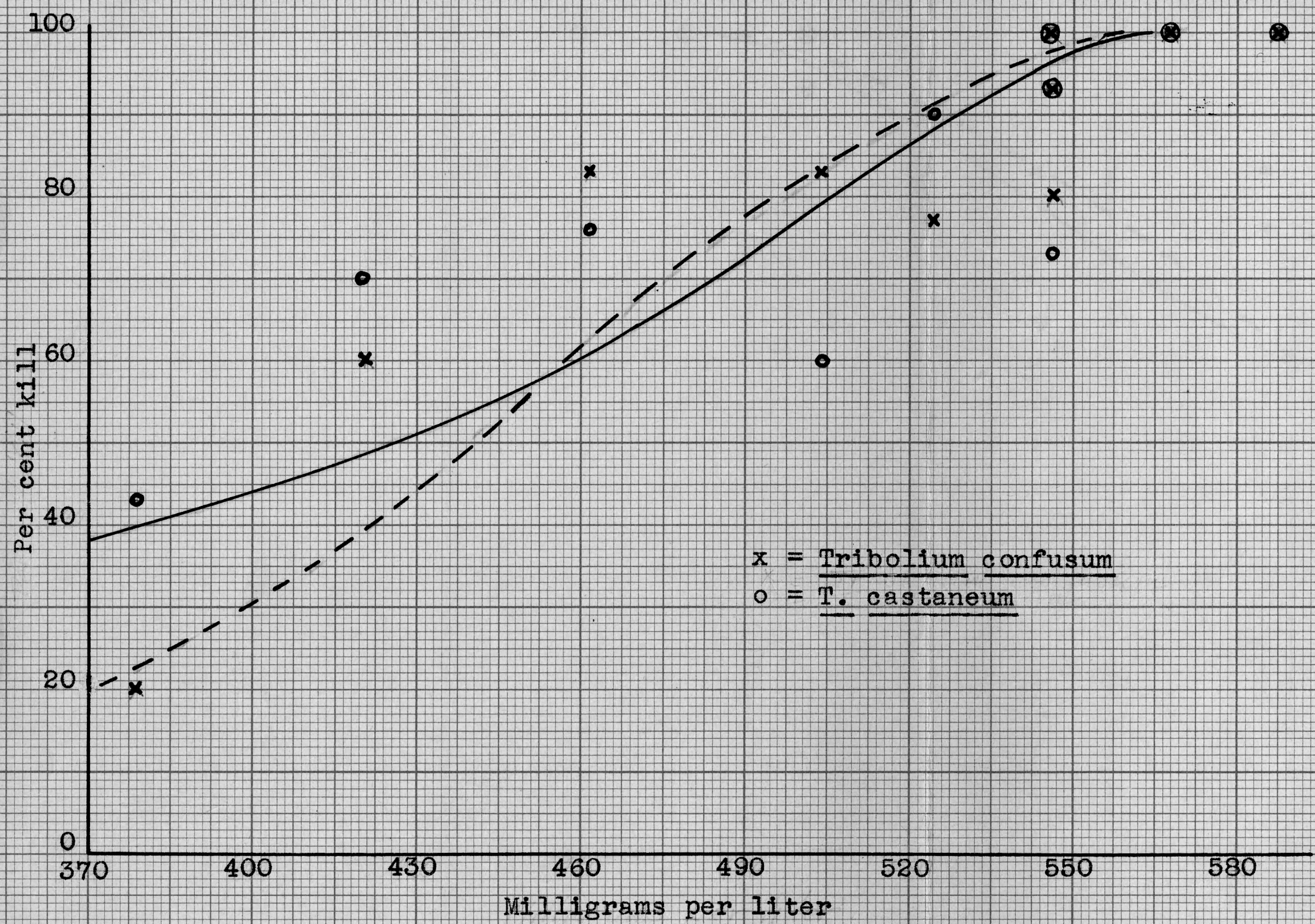

Fig. 13. Showing comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a one-hour period.

Table 14. Tests of the comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a three-hour period.

Expt. No.	Date	Minimum lethal dose: Total: cc.	cc/l	Mg/l	T. confusum: No.	% kill	T. castaneum: No.	% kill	Remarks
38- 3	9-30-38	2.2	0.366	423.7	30	100	30	100	
38- 3	9-30-38	2.0	0.333	419.91	30	100	30	100	
38- 3	9-30-38	1.8	0.300	378.3	30	100	30	100	
38- 3	9-30-38	1.6	0.266	335.43	30	100	30	100	
38- 7	10-19-38	1.5	0.250	315.25	30	96	30	100	
38- 7	10-19-38	1.4	0.233	293.81	30	100	30	100	
38- 3	9-30-38	1.4	0.233	293.81	30	100	30	100	
38- 7	10-19-38	1.3	0.215	272.38	30	76	30	100	
38- 7	10-19-38	1.2	0.200	252.20	30	90	30	91	
38- 3	9-30-38	1.2	0.200	252.20	30	87	30	90	
38- 7	10-19-38	1.1	0.183	230.76	30	77	30	91	
38-60	3-24-39	1.0	0.166	209.33	30	93	30	91	
38-60	3-24-39	0.8	0.133	167.71	30	63	30	46	
38-60	3-24-39	0.6	0.100	126.10	30	60	30	20	
Check:									Age
38- 3	9-30-38	--	--	--	30	0	30	37	3 days old
38- 7	10-19-38	--	--	--	30	3	30	20	2 days old

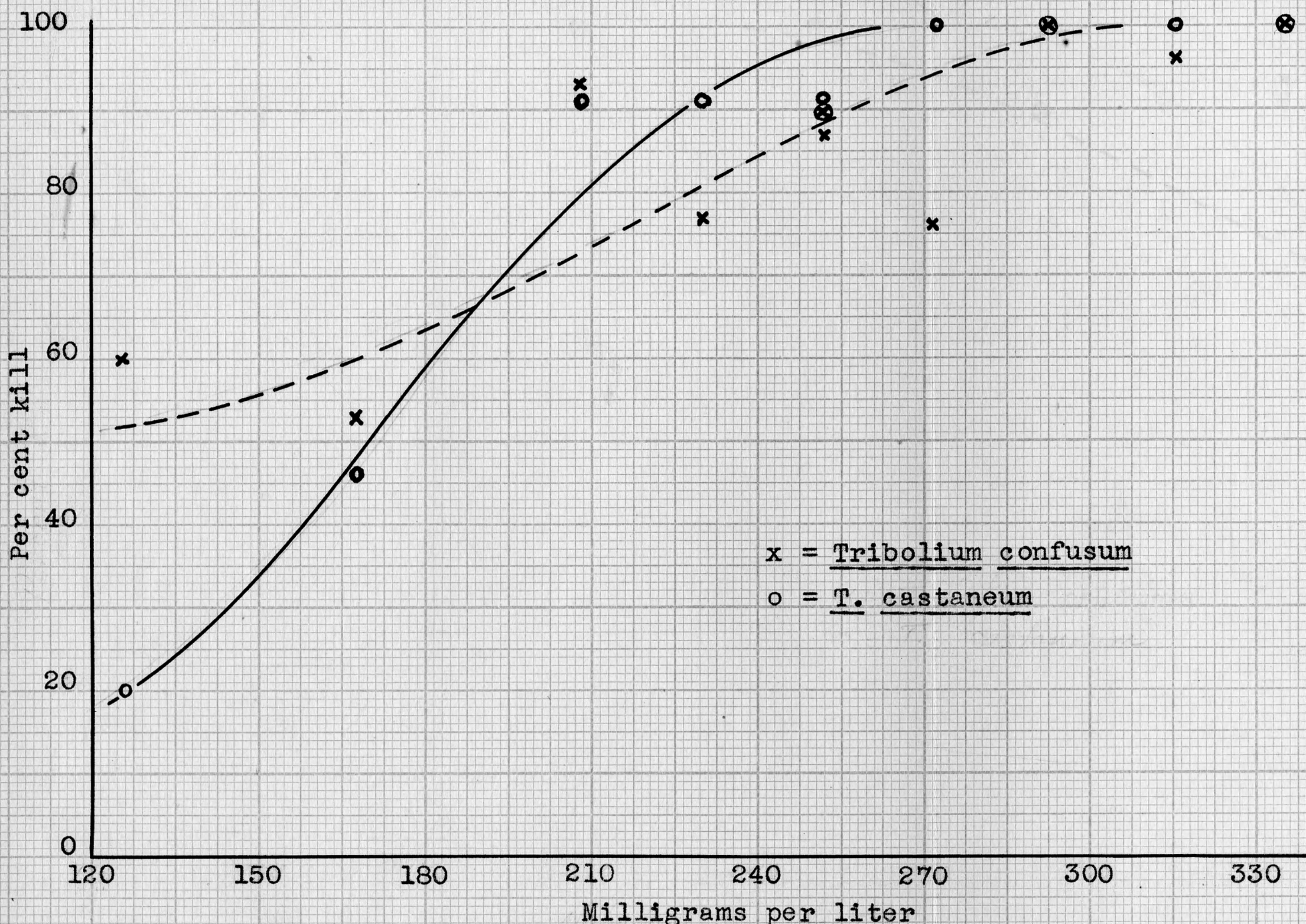

Fig. 14. Showing comparative susceptibility of eggs of *Tribolium confusum* Duv. and *T. castaneum* Hbst. to carbon bisulphide for a three-hour period.

DISCUSSION

Individual Variations as a Factor of Susceptibility

Variations in susceptibility to the fumigants by individuals of the same species were very noticeable throughout the experiment. A group of more resistant insects used in a single test would cause the percentage mortality of that test to vary widely from the general trend of the experiment. In most duplicate tests, the variations were within fairly close limits, although in one case (Table 4, dosage 13.24 mg/l) there was a variance of as much as 27 per cent in one species while the other species was almost constant. Since both species were fumigated in each of the two tests, this would indicate that the variation was due to individuality and not to external factors of dosage or temperature.

Oosthuizen (1936) noted that the range of individual resistance to high temperature within a given population of *T. confusum* was considerable. He said, "at 44°C. the most susceptible adults succumb in three or four hours, whereas some resistant individuals can stand 12 hours of exposure".

This author also determined the length of time required to kill 50 per cent of the four stages of *T. confusum* at different temperatures. He found that the adult stage was the most susceptible. Following in order of their susceptibility were larval, egg, and pupal stages, the latter being the most resistant.

It has been generally accepted that the egg is a particularly resistant stage of insects, however, Lindgren and Shepard (1932) found that ethylene oxide was nine times as toxic to the eggs as to the adults.

A reason for this difference in susceptibility of a life stage was explained (Cotton, 1932) as a difference in respiratory metabolism. This same factor may account for individual variations in susceptibility within any given stage as was found in the data of this experiment and in those of Oosthuizen.

The Fumigant as a Factor of Susceptibility

The data of this investigation showed that T. confusum was the more resistant of the two species when subjected to fumigation by carbon bisulphide and chloropicrin while it was the more susceptible when ethylene dichloride was used. Similar results have been found by other investigators of fumigants.

Shepard, Lindgren, and Thomas (1937) found this effect with the rice weevil and granary weevil (Sitophilus spp.).

When sulphur dioxide was used, S. granarius was the more susceptible, although for 12 other fumigants, S. oryzae was the more susceptible.

The data of these authors and of those of Lindgren (1936) showed that the amount of difference in susceptibility depends on the fumigant. The data of Shepard, Lindgren, and Thomas are based on a 99 per cent kill. The difference in the dosage necessary to kill S. granarius over S. oryzae was 5.8 mg/l for chloropicrin, 26 mg/l for carbon bisulphide, and 109 mg/l for ethylene dichloride.

SUMMARY

The comparative susceptibility of Tribolium confusum Duv., the confused flour beetle, and Tribolium castaneum Hbst., the rust-red flour beetle, to carbon bisulphide, ethylene dichloride, and chloropicrin was investigated.

It was found that when ethylene dichloride was used as the fumigant, the adults of T. confusum were the more susceptible of the two species, although for the one-hour exposure period the difference was not significant. When chloropicrin and carbon bisulphide were used as the fumigant, the adults of T. castaneum were the more susceptible.

Accurate information regarding the comparative susceptibility of the eggs of these two species was a difficult

matter to obtain due to the large variance in the percentage fertility. However, the data indicated that the eggs of T. confusum were equally susceptible to ethylene dichloride with those of T. castaneum. For carbon bisulphide, the data were extremely variable. For the lower dosages, the curves indicated that eggs of T. confusum were the more susceptible, but for the higher dosages as indicated by the curves, those of T. castaneum were slightly the more susceptible.

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