THE OVICIDAL EFFECT OF FUMIGANTS ON STORED GRAIN INSECT EGGS

by

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INTRODUCTION AND REVIEW OF LITERATURE

In a world with an ever increasing population and a constantly rising standard of living, man has need for all the food he can produce. Under such conditions the problem of food storage assumes great importance. Many foods, especially grains, are stored for varying lengths of time before use and surpluses must be stored for leaner years. With ideal conditions grain can be stored for long periods without deterioration. However, ideal conditions are seldom present, so a continuous battle is waged between man and the insects which attack stored grain. Spoilage, on the other hand, has been greatly reduced by the use of modern storage equipment. The problem of grain damaging insects is far from solved; their control is essential if stored grain is to be available when needed.

The damage by grain infesting insects is not restricted to dollars and cents, but also must include untold suffering of people. When huge quantities of grain in storage are spoiled by insects, mold, heating and other causes, famine may result.

In 1947 it was found that insects destroy at least 5 percent of the world production of cereal grains. A survey in 1947 by the United Nations indicated that at least five percent of the world's production of cereal grain is destroyed annually by grain damaging insects. In 29 countries the total loss of cereals was 25,750,000 tons, of which 50 percent could be attributed to insects. In the United States alone it is estimated that as a result of their feeding activities, their presence in grain and cereal products, and the cost of methods employed to destroy them, grain infesting insects exact a yearly toll of at least $300,000,000 (Cotton, 1952).

Methods Used to Determine Infestation

The earliest method of determining infestation consisted of examining the grain and noting the presence of crawling or flying insects, or by sifting a sample of grain through a screen which would separate the grain from the insects. These procedures did not give accurate estimates of infestations because several species deposited eggs inside the kernel and lived their entire immature stages inside the kernel.

The important methods employed today to determine internal or "hidden infestation" include: the iodine test, the gentian violet test (Goossens, 1949), the acid fuchsin stain test (Frankenfeld, 1948), the berberine sulphate stain test (Milner, 1950), the sodium hydroxide test (Apt, 1950), the ultra-violet lamp, the x-ray (Katz, 1950), flotation method by using ferric nitrate as the flotation medium (Apt, 1952), the spectrophotometric method (Potter, 1952), sound amplification (Adams, 1953), the projection of grain into the air (Katz, 1954) and separation of the infested grain with a simple blowing device (Milner, 1953). An apparatus employing mirrors has been developed by White (1949) which exposes all sides of the kernels at the same time, thus showing any visible damage readily.

Each of the above mentioned methods has advantages and disadvantages. The iodine test results in staining small sections of the endosperm which were chipped out in the handling process as well as the gelatinous plug. Certain methods are cheap and easily performed while others are satisfactory but time consuming. The x-ray method is gaining in popularity, its chief disadvantage being the high cost of the machine. Of all the methods mentioned, the x-ray is the most reliable and exact in determining internal infestation.

History of Fumigation

Fumigation was first practiced when burning incense or aromatic substances was used in the religious ceremonies of primitive man. It was thought to counteract the disagreeable odors arising from the slaughter and burning of animals offered in sacrifice and to impart a pleasing odor to the sacrifice and to mystify and exert a benign psychological influence over the religious devotees. Later it was used in churches to purify the air in time of public sickness and to dispel the foulness caused by large congregations or poisonous gases arising from poorly constructed burial vaults under the church floors. The fumes of burning sulphur were commonly used during the 12th century B.C. to disinfect homes and in the treatment of various diseases (Cotton, 1952).

Though smoke and gases have been used since early times for medicinal and destructive purposes, it was not until 1880 that they were used to any extent as insecticides. Carbon disulphide was first used as a fumigant in 1854, but its use was not extensive until 1879. The insecticidal use of hydrocyanic acid gas began in 1886, of chloropicrin in 1907. Carbon tetrachloride was used in 1910 for fumigating nursery stocks, later for stored products. Methyl and ethyl formates were first used in 1925; methyl and ethyl acetates, 1925; ethylene and propylene dichlorides, 1927; ethylene oxide, 1927; tert butyl alcohol, 1929; and methyl bromide in 1932. Others which have been used include: trichloroethylene, furoyl chloride, formaldehyde, propylene oxide, acetyl chloride, propionyl chloride, thionyl chloride, a b - dichloroethyl ether, b b dichloroethyl ether, chloromethyl ether, methylene chloride, tetrachloroethylene, chloroform, methyl thiocynate, ethyl thioacetate.

The Problem

This thesis problem was chosen to determine whether insect eggs can be destroyed with reasonable dosages of fumigants. If so, it is possible that some species can be more easily eliminated by treating for eggs than for adults, pupae or larvae.

Eggs deposited on the surface or among the kernels can probably be killed with smaller dosages of fumigant than those eggs which are inserted inside of the kernel. Seemingly it would be possible to eliminate the insect eggs by applying a suitable fumigant. Before this solution can be applied, there are several problems to be solved. It must be determined whether it is cheaper to kill the eggs than to kill the adults or the larvae; how much of the fumigant must be used; for what period of exposure; and what effect the fumigant will have on the grain itself. As time prevented the solution of all these problems, the phase selected concerned the fumigation of the eggs of three grain damaging insects which oviposit outside the kernels.

Biology of the Insect Egg

An insect egg normally is enclosed by two envelopes, the chorion and the vitalline membrane (Wigglesworth, 1939). As the egg develops, the chorion becomes separated into four layers. At this point another layer may be added with its primary function being to cement the shell to the surface on which it is deposited (Beament, 1946b).

The chorion, which Beament defines as "that part of the extra oocytic material which is secreted by the follicle cells of the ovary", can be broken up into four groups:

(1) The resistant endochorion (protein with granulate material) freely

permeable to water soluble molecules of comparatively large size, but impermeable to oils.

(2) The amber layer (lipidized protein) impermeable to all but very small particles, and somewhat resistant even to the passage of water and uniatomic ions.

(3) The soft exochorion (protein) as in (1).

(4) The exochorion (lipoprotein or chorionin), despite partial penetration by pore canals, this layer allows only very small particles to pass through it (Beament, 1947).

The vitalline membrane is formed or arises by the condensation of the outermost layer of the ovum (Wigglesworth, 1945). It is a homogenous layer, some 2 microns thick, and stains heavily in the water-soluble protein stain, ninhydrine. It is not, therefore, a waterproofing layer. It does not contain any polyphenol or oil at any stage, and consequently does not contribute to any of the processes of endochorion formation (Beament, 1946b).

Studies have shown the presence of minute openings in the chorion and the vitalline membrane called micropyles. The number of these openings varies with each egg according to the species, but an average would be approximately 15 micropyles per egg. The true micropyles run from a funnel shaped orifice in the rim of the shell, through the chorion. The distal portion of the tube, or outer micropyle, has a diameter of approximately 2 microns and its walls consist of the more lipophilic chorionin. The inner micropyle, with a diameter of approximately 0.5 microns is lined with tanned protein. The total length of the tube varies with each species, but averages about 20 microns. The pseudomicropyles lie entirely within the chorion. These vary from about 12 microns to 20 microns in length and 2 microns in diameter, but have a group of pore canals leading from the outer end to the

surface of the shell. The inner ends lie in the resistant endochorion layer, thus, with the exception of the innermost micron, the tube has a comparatively lipophilic surface. An interesting fact about the micropyles is that as the female producing the egg ages, the number of micropyles decreases (Beament, 1947).

O'Kane and Baker, Kuenen and Beament (Beament, 1947) demonstrated that the micropyles are the main gateway into the insect egg for certain toxic oils. Penetration through the chorion is secondary. O'Kane and Baker in 1934 and 1935 (Beament, 1947) while investigating the penetration of oils into various insect eggs, immersed the eggs in liquids containing dyes. These were sectioned and studied later. A similar method was used in 1946 by Kuenen, who crushed the eggs after applying the oils. In both cases the eggs were immersed in the oils for periods exceeding 72 hours. This may have resulted in the oil stains penetrating through a preferential site of entry where it could diffuse through the yolk - even into the shell layers from the interior. In 1948 Beament experimented with pieces of shells, using a varied range of materials, e.g., oils, acids and salt baths, with widely different properties, and was only able to penetrate the shell through the micropyles.

MATERIALS AND METHODS

The test insects used in this study were selected from the more destructive stored grain insects. They are: Angoumois grain moth, Sitotroga cerealella Oliv., Indian-meal moth, Plodia interpunctella Hbn., and the confused flour beetle, Tribolium confusum J. duV. These species deposit their egg externally on or near the grain.

The insects used in this experiment came from reared cultures maintained under controlled conditions at the U.S.D.A. Stored Product Insect Section

Laboratory, at Manhatten, Kansas.

Maintenance of Stock Culture

Stock cultures were reared in a constant-temperature rearing room at 80° F. with a relative humidity of 70 to 80 percent. Corn of 12 to 14 percent moisture was the food medium. The grain moisture was determined with a Steinlite moisture tester. Distilled water was used when moisture was added to the corn. Whole kernels of corn were used in culturing the Angoumois grain moth; cracked corn was used for the Indian-meal moth. The corn was stored in wide mouthed quart jars, and two-quart jars, capped with metal lids. A hole in the center of each lid was covered by a fine mesh wire screen, allowing aeration. The jars were half filled with grain as shown in Plate I, Fig. 1, and were kept on racks in the rearing room. New cultures were started every four months or sooner, depending upon the condition of the grain.

Indian-meal moths reared in blue tinted jars produced more fertile eggs than those reared in the clear glass jars.

Confused flour beetles were reared on a mixture of shorts and enriched patent flour.

All grain used in these experiments was sifted and subjected to low refrigerator temperatures for one week to kill any insects present.

Handling of Insects to Obtain the Eggs

A bulb type aspirator was used to collect confused flour beetles, after which they were placed in eight ounce salve tins with patent flour. A collecting cage (Plate III, Fig. 2) similar to one constructed by L. O. Warren (Warren, 1954) was built to collect the moths. It was 16 inches square; the bottom, sides and back were of one-fourth inch plywood; the top and front were

EXPLANATION OF PLATE I

Fig. 1. Cultures in 1 and 2 quart jars used for rearing experimental insects.

Fig. 2. 20-liter fumigation bottle with egg fumigating baskets ready for fumigation.

PLATE I

Fig. 1

Fig. 2

EXPLANATION OF PLATE II

Fig. 1. Indian-meal moth eggs magnified 20X.

Fig. 2. Angoumois grain moth eggs magnified 15X.

PLATE II

Fig. 1

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Oviposition cage used to collect Angoumois grain moth and Indian-meal moth eggs.

Fig. 2. Writer using transfer cage to transfer adult moths from stock culture to oviposition cage.

PLATE III

Fig. 1

Fig. 2

of double-weight window glass, with the front piece sliding up and down behind retaining wood strips to open and close the cage. On each side two holes eight inches in diameter were cut, around which a muslin sleeve was attached to enable the collection of the moths, using an electrically operated aspirator. A bulb type aspirator was impractical because of its manual type suction. The rapid movements of the moths made it difficult to operate with the aspirator in one hand and the collecting jar in the other.

Fumigants Used

Four fumigants were used so that their physical and toxic properties might be compared. They are: carbon disulphide, boiling point of 46.3° C., molecular weight 76.13, specific gravity 0.797 and vapor density 2.64; ethylene dichloride, boiling point 83.7° C., molecular weight 98.97, specific weight 2.180 and vapor density 3.42; methyl bromide, melting point of 4.6° C., molecular weight 94.95, and vapor density 3.27; carbon tetrachloride, boiling point of 76.8° C., molecular weight 153.84, and specific weight 1.595 (Lange, 1944).

Fumigation Chambers

Tests were conducted in empty 20-liter glass bottles. These were closed with tight fitting rubber stoppers, through each of which fitted a glass delivery tube with a glass stopcock extending to the bottom. The lots of insect eggs were placed in wire baskets and suspended into the bottle (Plate I, Fig. 2). The fumigant was applied by reducing the pressure in the bottle to about 18 pounds per square inch and using the resulting partial vacuum to draw in the fumigant from the pipette. The pressure was then restored to normal, where it remained throughout the exposure periods.

Baskets for Fumigating Eggs

Baskets for exposing eggs to fumigants were made of 10X copper screen measuring 1-1/2 inches high and 3/4 inch in diameter. Each basket has a small wire handle. A cotton string with three paper clips attached, three inches apart, held the baskets while the eggs were undergoing fumigation. Each bottle contained six baskets (Plate I, Fig. 2).

Collecting the Eggs

A special oviposition cage was used to collect Angoumois grain moth and Indian-meal moth eggs. (These eggs are shown in Plate II, Figs. 1 and 2, respectively.) The cage was made from glass tubing, six inches long and 1-11/16 inches in diameter (outside measurements) with the ends sealed off by tight fitting cardboard caps. Cardboard strips described by Simmons and Ellington (1933) were used to collect Angoumois grain moth eggs (Plate III, Fig. 1). Black photographic paper was placed at each end of the tube for oviposition by Indian-meal moths. A cardboard strip was placed in the tube for the moths to walk. After several unsuccessful attempts to collect Indian-meal moth eggs, a few kernels of corn and a small glass vial with wet cotton were placed in the bottom of the cage. This stimulated the females and resulted in the deposition of many eggs.

Eggs were collected every 24 hours after which the adults were placed in fresh supplies of corn to produce new cultures. The eggs were handled with a number 2 camel hair brush.

To collect confused flour beetle eggs, the 24-hour infested flour was sifted through a 10XX wire screen which retained the eggs. These eggs were also handled with a number 2 camel hair brush. It was difficult to distinguish flour beetle eggs from fecal pellets as they look alike when covered with

flour (Plate V, Fig. 1)

Sexing

Adults of the three species were not sexed so that large numbers were required to assure satisfactory reproduction.

Microscopic Equipment

A Spencer binocular microscope with a 9X magnification was used to examine the eggs before and after fumigation as shown in Plate IV. The microscope was used also in making egg counts, in separating the eggs (mainly Angoumois grain moth), and in searching for the Harvestor mite Pediculoides ventricosis (Newport) which was an egg and larval predator.

Hatching the Eggs

Newly hatched larvae of Indian-meal moths and confused flour beetles were cannibalistic. This necessitated handling of eggs separately after fumigation exposures. Each egg (except Angoumois) after being fumigated was placed in one-half of a number 3 gelatin capsule. Each half of the capsule was then placed in holes in a 12-1/2 x 11-1/2 x 1 inch wooden rack. The rack was made by drilling a series of 1/4 inch holes evenly over the entire board. The rack with the capsules and eggs (shown in Plate V, Fig. 2) was then placed in the rearing room and examined daily. The Angoumois grain moth eggs were kept in small 2-1/4 x 2-1/4 x 1-1/2 inch plastic boxes for hatching purposes in the same rearing room.

Freezing Equipment

Tests were conducted to determine the effects of low temperatures on the

EXPLANATION OF PLATE IV

The writer inspecting eggs using a Spencer binocular microscope with 9X magnification.

PLATE IV

EXPLANATION OF PLATE V

Fig. 1. Confused flour beetle eggs magnified 15X.

Fig. 2. Wooden rack with gelatin capsules for holding insect eggs separately during incubation and observation period.

PLATE V

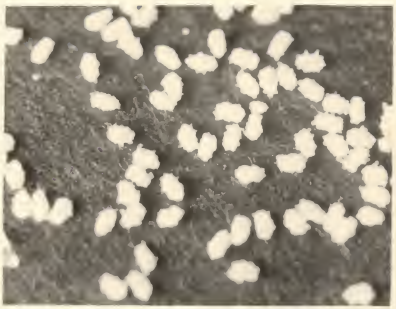

Fig. 1

Fig. 2

eggs of the three species. A 12 cubic foot Philco deep freeze and a 6 cubic foot General Electric refrigerator were used in this experiment to provide temperatures of 2° F. and 39° F.

Controls

Twenty eggs were set aside from each group of eggs fumigated as controls. These were examined daily and the number of eggs hatching recorded.

Determination of Results

Eggs of the species tested in these experiments were examined daily but the final count was not made until the 15th day. All control eggs hatched between 4 and 11 days. Eggs which failed to hatch by the 15th day were counted as dead. The percentage of eggs hatching after fumigation was compared with the controls and plotted on graphs. The five replicates were averaged and used as plotting points.

Methods of Counting

Twenty eggs of each species were used for each replicate making a total of 100 eggs per test. The extent of hatching of Angoumois grain moth and Indian-meal moth eggs was determined by counting the empty transparent shells. Hatching of confused flour beetle eggs was determined by counting live larvae.

Length of Exposure

Exposures to the fumigants of 10, 15, 20, 24 and 30 hours were selected for this study. Twenty-four hours is the average exposure time in the fumigation of stored grain.

PRESENTATION AND DISCUSSION OF DATA

Effects of Low Temperature

Angoumois grain moth eggs were subjected to temperatures of 2° F. and 39° F. for periods of one to eight hours (Plate VI, Fig. 1) and permitted a 14-day incubation period at the end of which the number of hatched eggs was recorded and the percentages plotted.

No eggs were observed to hatch following a 2° F. exposure of four hours; whereas a one-hour exposure at this temperature resulted in a 65 percent hatch. At 39° F. an eight-hour exposure inhibited all hatching while a one-hour exposure at this temperature yielded a 100 percent hatch. Ninety-six percent of the control eggs hatched. It can be determined therefore that the LE_{50} (lethal exposure to effect a 50 percent hatch) is two hours at 2° F. and 3.3 hours at 39° F. (Plate VI, Fig. 1).

Comparable studies were made on Indian-meal moth eggs. The eggs were exposed from one to five hours to 2° F. and 39° F. (Plate VI, Fig. 2). Following a four-hour exposure at 2° F., no hatching was observed; a one-hour exposure at this temperature resulted in a 52 percent hatch. At 39° F., a five-hour exposure totally inhibited hatching while a one-hour exposure yielded a 65 percent hatch. Thus, the LE_{50} at 2° F. is 1.6 hours and at 39° F. is 2.5 hours (Plate VI, Fig. 2). Ninety-six percent of the untreated eggs hatched.

Confused flour beetle eggs were studied in a similar manner. These eggs were exposed one to six hours to 2° F. and 39° F. temperatures (Plate VI, Fig. 3). At 2° F. an exposure of five hours inhibited hatching of all eggs, but a one-hour exposure at this temperature resulted in a 17 percent hatch. At 39° F., a six-hour exposure totally inhibited all hatching while a one-hour exposure yielded a 47 percent hatch. Thus, the LE_{50} at 2° F. is much less than

EXPLANATION OF PLATE VI

Fig. 1. The percentage of Angoumois grain moth eggs hatching at various time exposures using temperatures of 2° F. and 39° F.

Fig. 2. The percentage of Indian-meal moth eggs hatching at various time exposures using temperatures of 2° F. and 39° F.

Fig. 3. The percentage of confused flour beetle eggs hatching at various time exposures using temperatures of 2° F. and 39° F.

PLATE VI

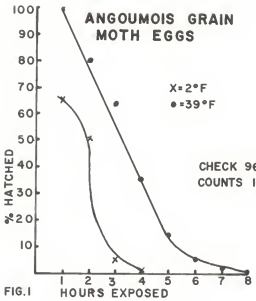

FIG.1

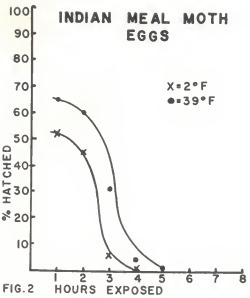

FIG.2

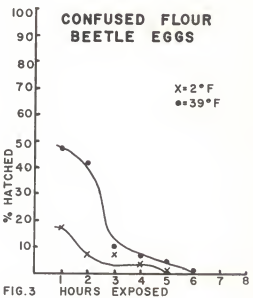

FIG.3

one hour, while at 39° F. it is 0.9 of an hour (Plate VI, Fig. 3). Ninety-six percent of the control eggs hatched.

Ethylene Dichloride

Eggs of Angoumois grain moth, confused flour beetle and Indian-meal moth were exposed 10 to 30 hours to ethylene dichloride at the following dosages expressed in mg/lt: 12.57, 25.14, 37.71, 50.28 and 62.85 (Plate VII, Figs. 1, 2,3,4,5).

Fumigation with 12.57 mg/lt: (1) 10-hour exposure - no eggs of Indian-meal moth were observed to hatch; 82 percent of the Angoumois grain moth eggs and 100 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure - no Indian-meal moth eggs were observed to hatch; 81 percent of the Angoumois grain moth eggs and 85 percent of the confused flour beetle eggs hatched.

Fumigation with 25.14 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs hatched; 83 percent of the Angoumois grain moth eggs hatched; 98 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure - no Indian-meal moth eggs hatched; 60 percent of the Angoumois grain moth eggs and 87 percent of the confused flour beetle eggs hatched.

Fumigation with 37.71 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs hatched; 81 percent of the Angoumois grain moth eggs and 97 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure - no Indian-meal moth eggs hatched; 47 percent of the Angoumois grain moth eggs and 41 percent of the confused flour beetle eggs hatched.

Fumigation with 50.28 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs were observed to hatch; 81 percent of the Angoumois grain moth eggs and 91 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure -

EXPLANATION OF PLATE VII

Fig. 1. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 12.57 mg/lt of ethylene dichloride.

Fig. 2. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 26.14 mg/lt of ethylene dichloride.

Fig. 3. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 37.71 mg/lt of ethylene dichloride.

Fig. 4. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 50.23 mg/lt of ethylene dichloride.

Fig. 5. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 62.85 mg/lt of ethylene dichloride.

ETHYLENE DICHLORIDE

▲ = ANGOUMOIS GRAIN MOTH EGGS
● = INDIAN-MEAL MOTH EGGS
■ = CONFUSED FLOUR BEETLE EGGS

CHECK 98% HATCH AT 80°F
COUNTS 14 DAYS AFTER EXPOSURE

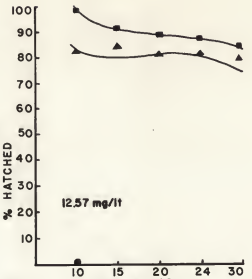

FIG.1

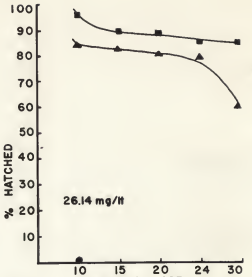

FIG.2

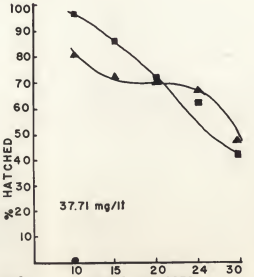

FIG.3

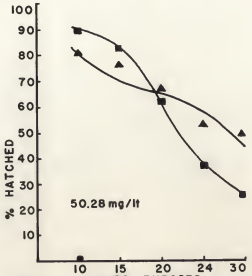

FIG.4

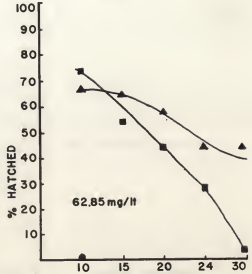

FIG.5

no Indian-meal moth eggs hatched; 50 percent of the Angoumois grain moth eggs and 27 percent of the confused flour beetle eggs hatched.

Fumigation with 62.85 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs were observed to hatch; 68 percent of the Angoumois grain moth eggs and 75 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure - no Indian-meal moth eggs hatched; 46 percent of the Angoumois grain moth eggs and 1 percent of the confused flour beetle eggs hatched.

Discussion

The results of this experiment demonstrate that Indian-meal moth eggs are very susceptible and Angoumois grain moth and confused flour beetle eggs are appreciably less susceptible to ethylene dichloride vapors at dosages between 12.57 and 62.85 mg/lt.

At dosages lower than 37.71 mg/lt, ethylene dichloride is less toxic to confused flour beetle eggs than Angoumois grain moth eggs. In dosages greater than 37.71 mg/lt, it is less toxic to Angoumois grain moth eggs over a longer exposure period than to confused flour beetle eggs.

The highest dosage of 62.85 mg/lt did not kill 100 percent of either the confused flour beetle eggs or the Angoumois grain moth eggs.

Indian-meal moth eggs were extremely sensitive to ethylene dichloride and failed to hatch when exposed to low dosages and short exposures of time. When calculated from data shown on Plate VII, Fig. 4, the LE_{50} at dosages of 50.28 mg/lt for Angoumois grain moth eggs is 30 hours; confused flour beetle eggs 25 hours.

Carbon Tetrachloride

Eggs of Angoumois grain moth, Indian-meal moth and confused flour beetle

were exposed 10 to 30 hours to vapors of carbon tetrachloride at the following dosages expressed in mg/lt: 398.85, 478.62, 558.39, 638.16 and 807.93 (Plate VIII, Figs. 1,2,3,4,5).

Fumigation with 398.85 mg/lt: (1) 10-hour exposure - 94 percent of the Angoumois grain moth eggs hatched; 100 percent of the confused flour beetle and Indian-meal moth eggs hatched. (2) 30-hour exposure - 23 percent of the Angoumois grain moth eggs hatched; 15 percent of the confused flour beetle eggs and 26 percent of the Indian-meal moth eggs hatched.

Fumigation with 478.62 mg/lt: (1) 10-hour exposure - 93 percent of the Angoumois grain moth eggs hatched; 93 percent and 91 percent hatched for the confused flour beetle and Indian-meal moth eggs, respectively. (2) 30-hour exposure - 18 percent of the Angoumois grain moth eggs hatched; 11 percent of the confused flour beetle eggs and 21 percent of the Indian-meal moth eggs hatched.

Fumigation with 558.39 mg/lt: (1) 10-hour exposure - 74 percent of the Angoumois grain moth eggs hatched; 73 percent of the confused flour beetle eggs and 41 percent of the Indian-meal moth eggs hatched. (2) 30-hour exposure - no hatching of Angoumois grain moth and Indian-meal moth eggs; 3 percent of confused flour beetle eggs hatched.

Fumigation with 638.16 mg/lt: (1) 10-hour exposure - 20 percent of the Angoumois grain moth eggs hatched; 67 percent and 21 percent of confused flour beetle and Indian-meal moth eggs, respectively, hatched. (2) 30-hour exposure - none hatched.

Fumigation with 807.93 mg/lt: (1) 10-hour exposure - 48 percent of the Angoumois grain moth eggs hatched; 58 percent and 11 percent of the confused flour beetle and Indian-meal moth eggs, respectively, hatched. (2) 30-hour exposure - none hatched.

EXPLANATION OF PLATE VIII

Fig. 1. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 398.85 mg/lt carbon tetrachloride.

Fig. 2. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 478.62 mg/lt carbon tetrachloride.

Fig. 3. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 558.39 mg/lt carbon tetrachloride.

Fig. 4. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 638.16 mg/lt carbon tetrachloride.

Fig. 5. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 807.93 mg/lt carbon tetrachloride.

CARBON TETRACHLORIDE

▲ = ANGOUMOIS GRAIN MOTH EGGS
● = INDIAN-MEAL MOTH EGGS
■ = CONFUSED FLOUR BEETLE EGGS

CHECK 100% HATCH AT 80° F
COUNTS 14 DAYS AFTER EXPOSURE

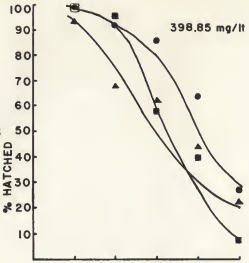

FIG.1

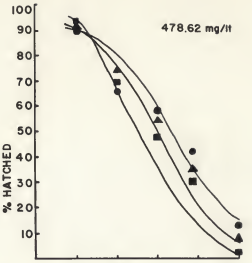

FIG.2

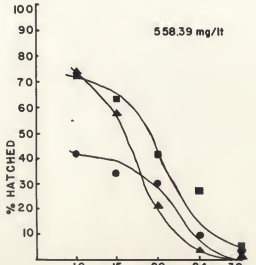

FIG.3

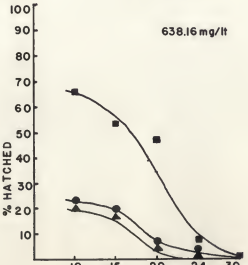

FIG.4

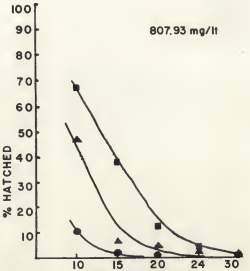

FIG.5

Discussion

Eggs of confused flour beetle, Angoumois grain moth, and Indian-meal moth were about equally resistant to carbon tetrachloride in dosages under 500 mg/lt. At higher dosages, confused flour beetle eggs were most resistant.

A dosage of 638.16 mg/lt of carbon tetrachloride with a 30-hour exposure totally inhibits the hatching of eggs of the three species.

Data shown on Plate VIII, Fig. 1, indicate that the LE_{50} for Angoumois grain moth, Indian-meal moth and confused flour beetle eggs was 21 hours, 24 hours, 19.5 hours, respectively.

Carbon Disulfide

Eggs of Angoumois grain moth, Indian-meal moth and confused flour beetle were exposed 10 to 30 hours to carbon disulfide at the following dosages expressed in mg/lt: 12.63, 31.57, 50.52, 63.15 and 126.30 (Plate IX, Figs. 1, 2,3,4,5).

Fumigation with 12.62 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs hatched; 72 percent of Angoumois grain moth eggs hatched; 100 percent of confused flour beetle eggs hatched. (2) 30-hour exposure - no eggs of Indian-meal moth and Angoumois grain moth hatched; 39 percent of confused flour beetle eggs hatched.

Fumigation with 31.57 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs were observed to hatch; 38 percent of Angoumois grain moth eggs and 93 percent of the confused flour beetle eggs hatched. (2) 30-hour exposure - no eggs of Indian-meal moth and Angoumois grain moth and 15 percent of confused flour beetle eggs hatched.

Fumigation with 50.52 mg/lt: (1) 10-hour exposure - no Indian-meal moth

EXPLANATION OF PLATE IX

Fig. 1. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 12.63 mg/lt carbon disulfide.

Fig. 2. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 31.57 mg/lt carbon disulfide.

Fig. 3. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 50.52 mg/lt carbon disulfide.

Fig. 4. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 63.15 mg/lt carbon disulfide.

Fig. 5. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 126.30 mg/lt carbon disulfide.

CARBON DISULFIDE

▲=ANGOUMOIS GRAIN MOTH EGGS
●=INDIAN-MEAL MOTH EGGS
■=CONFUSED FLOUR BEETLE EGGS

CHECK 100% HATCH AT 80° F
COUNTS 14 DAYS AFTER EXPOSURE

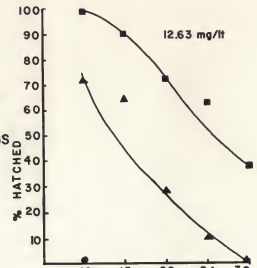

FIG.1

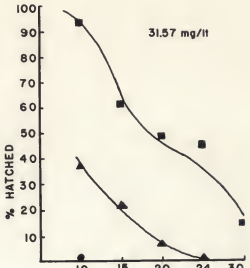

FIG.2

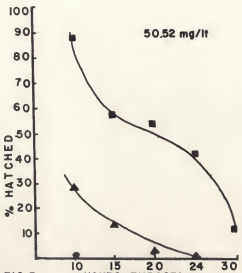

FIG.3

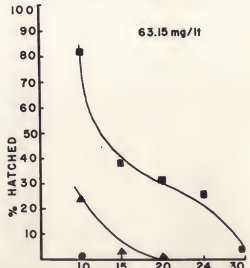

FIG.4

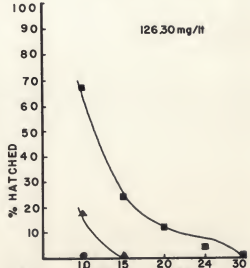

FIG.5

eggs hatched; 88 and 29 percent of confused flour beetle and Angoumois grain moth eggs, respectively, hatched. (2) 30-hour exposure - no Indian-meal moth or Angoumois grain moth eggs hatched; 11 percent of confused flour beetle eggs hatched.

Fumigation with 63.15 mg/lt: (1) 10-hour exposure - no eggs of the Indian-meal moth hatched; 25 and 82 percent of the Angoumois grain moth and confused flour beetle eggs, respectively, hatched. (2) 30-hour exposure - no Indian-meal moth or Angoumois grain moth eggs hatched; 4 percent of confused flour beetle eggs hatched.

Fumigation with 126.30 mg/lt: (1) 10-hour exposure - no Indian-meal moth eggs hatched; 18 percent of the Angoumois grain moth eggs hatched; 67 percent of confused flour beetle eggs hatched. (2) 30-hour exposure - none of the eggs hatched.

Discussion

Indian-meal moth eggs are extremely sensitive to carbon disulfide and are easily prevented from hatching by low dosages and short exposures.

Confused flour beetle eggs were more resistant than the Angoumois grain moth eggs and required a dosage of 126.30 mg/lt for a 30-hour exposure before all eggs failed to hatch. At this dosage Angoumois grain moth eggs were twice as susceptible to the carbon disulfide as confused flour beetle eggs.

Data in Plate IX, Fig. 1, indicate that at dosages of 12.63 mg/lt, the LE_{50} for confused flour beetle eggs is 27 hours and for Angoumois grain moth eggs, 17 hours.

Methyl Bromide

Eggs of Angoumois grain moth, Indian-meal moth and confused flour beetle

were exposed from 10 to 30 hours to methyl bromide at the following dosages expressed in mg/lt: 1.732 and 3.464 (Plate X, Figs. 1,2).

Fumigation with 1.732 mg/lt: (1) 10-hour exposure - 100 percent of confused flour beetle eggs hatched; 90 percent of Angoumois grain moth eggs and 61 percent of Indian-meal moth eggs hatched. (2) 30-hour exposure - 73 percent of the confused flour beetle eggs hatched; 15 percent of the Indian-meal moth eggs and 38 percent of the Angoumois grain moth eggs hatched.

Fumigation with 3.464 mg/lt: (1) 10-hour exposure - all eggs failed to hatch. (2) 30-hour exposure - all eggs failed to hatch.

Discussion

The above tests indicate that methyl bromide is less toxic at dosages of 1.732 mg/lt to confused flour beetle eggs than Indian-meal moth or Angoumois grain moth eggs.

Dosages between 1.732 mg/lt and 3.464 mg/lt of methyl bromide inhibited all hatching at 10 hours or more exposure.

Data in Plate X, Fig. 1, indicate that at dosages of 1.732 mg/lt, the LE_{50} for Angoumois grain moth eggs is 27.5 hours and for Indian-meal moth eggs 15 hours. Incomplete data do not permit the calculation of the LE_{50} for the confused flour beetle eggs.

SUMMARY

In controlled tests, the ovicidal effect of four fumigants and two low temperature exposures on Angoumois grain moth, Indian-meal moth and confused flour beetle eggs has been studied.

1. Ethylene dichloride was highly effective as an ovicide against Indian-meal moth eggs at dosages of 12.57 mg/lt for 10-hour exposures. It was not

EXPLANATION OF PLATE X

Fig. 1. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 1.732 mg/lt methyl bromide.

Fig. 2. The percentage of Angoumois grain moth, Indian-meal moth and confused flour beetle eggs hatching after 10, 15, 20, 24, and 30-hour exposures to 3.464 mg/lt methyl bromide.

METHYL BROMIDE

▲ = ANGOUMOIS GRAIN MOTH EGGS
● = INDIAN-MEAL MOTH EGGS
■ = CONFUSED FLOUR BEETLE EGGS

CHECK 96% HATCH AT 80°F
COUNTS 14 DAYS AFTER EXPOSURE

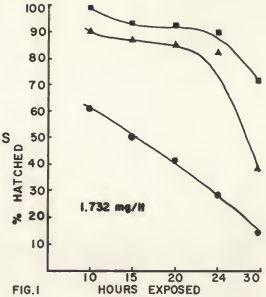

FIG.1

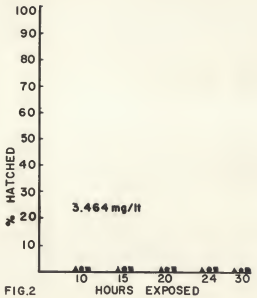

FIG.2

effective against Angoumois grain moth and confused flour beetle eggs at dosages of 62.85 mg/lt for 30-hour exposures.

2. Carbon tetrachloride was not effective as an ovicide at dosages of 398.35 mg/lt and short exposures for all species of eggs tested. Dosages of 807.93 mg/lt and exposures of from 20 to 30 hours were necessary before these eggs fail to hatch.

3. Carbon disulfide was highly effective as an ovicide against Indian-meal moth eggs. Administered in both low dosages for a 30-hour exposure and high dosages for 15-hour exposures, this fumigant totally inhibited the hatching of Angoumois grain moth eggs. It is ovicidal for confused flour beetle eggs when administered in large dosages over a 30-hour exposure period.

4. Methyl bromide is highly ovicidal for all species of eggs tested at dosages of 3.464 mg/lt but not at dosages of 1.732 mg/lt.

5. In low temperature tests, all eggs of the three species studied failed to hatch following an eight-hour exposure at 39° F. and a five-hour exposure at 2° F. On the basis of these studies, it is recommended that further tests be conducted to evaluate the practicality of fumigation of stored grain insect eggs from a viewpoint of cost, damage to grain, and methods of application.

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THE OVICIDAL EFFECT OF FUMIGANTS ON STORED GRAIN INSECT EGGS

by

VICTOR EUGENE ADLER

B. S., Memphis State College, 1950

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

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Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1956

The damage to stored grain by insects is of world-wide economic significance. A United Nation's survey in 1947 revealed that in 29 countries the total loss of cereals was 25,750,000 tons, of which 50 percent could be attributed to insects.

Current methods of detecting insect infestation include chemical, physical and mechanical tests. Among control measures, fumigation is being increasingly recognized for its insecticidal effectiveness.

The ovicidal effect of fumigants on certain stored grain insect eggs is the subject of this thesis. Its practical significance lies in the assumption that if all insect eggs can be destroyed with reasonable dosages of fumigants, new generations can be more easily eliminated by treating the infested grain for eggs than for adults, pupae or larvae.

Eggs of Angoumois grain moths, Indian-meal moths and the confused flour beetles were exposed to vapors of ethylene dichloride, carbon tetrachloride, carbon disulfide and methyl bromide for from 10 to 30 hours. New procedures and equipment for collection and handling of insect eggs were devised prior to the fumigation experiment. A special transfer and oviposition cage was constructed for handling the moths and collecting the eggs. Small wire baskets were built to hold the eggs suspended within a 20 liter bottle which served as fumigation chamber. The bottle was closed with a tight fitting rubber stopper through which fitted a glass delivery tube, with a glass stopcock extending to the bottom. The fumigant was applied by reducing the pressure in the bottle to approximately 18 pounds per square inch and using the resulting partial vacuum to draw in the fumigant from the pipette. The pressure was then restored to normal, where it remained throughout the exposure periods. After the exposure period each confused flour beetle and Indian-meal moth egg was placed in a gelatinous capsule and set in a wooden rack. The rack was retained in the

rearing room for a 14-day incubation period.

Eggs of all species tested were examined daily, a final count being made on the 15th day. The number of eggs hatching after fumigation was compared with the untreated eggs and then plotted on graphs. One hundred eggs of each species tested were used. Hatching of Angoumois grain moth and Indian-meal moth eggs was determined by counting empty transparent shells; hatching of confused flour beetle eggs by counting live larvae. For control and test purposes Angoumois grain moth, Indian-meal moth and confused flour beetle eggs were exposed to temperatures of 2° F. and 39° F. for periods of one to eight hours. They were examined after a 14-day incubation period and the number of hatched eggs recorded and graphed.

Results of this study indicate that: (1) Ethylene dichloride is highly effective as an ovicide against Indian-meal moth eggs at dosages of 12.57 mg/lt. It is not effective against Angoumois grain moth and confused flour beetle eggs at dosages of 62.85 mg/lt for 30-hour exposures. (2) Carbon tetrachloride is not effective as an ovicide at dosages of 478.62 mg/lt for 10-hour exposures for all species of eggs tested. Dosages greater than 807.93 mg/lt for 30-hour exposures are necessary to inhibit hatching of all three species tested. (3) Carbon disulfide is highly effective as an ovicide against Indian-meal moth eggs. Administered in a dosage of 12.63 mg/lt for a 30-hour exposure and 126.30 mg/lt for a 15-hour exposure, this fumigant totally inhibited the hatching of Angoumois grain moth eggs. It is ovicidal for confused flour beetle eggs when administered in dosages of 126.30 mg/lt over a 30-hour exposure period. (4) Methyl bromide is highly ovicidal for all three species of eggs tested in dosages of 3.464 mg/lt. It is not ovicidal at a dosage of 1.732 mg/lt when administered to these species. (5) In temperature control tests eggs of the three species studied failed to hatch following an eight-hour exposure at 39° F.

No eggs of these species were observed to hatch following a five-hour exposure at 2° F.

On the basis of these findings, it is recommended that further studies be made to evaluate the practicality of fumigation of stored grain insect eggs from a viewpoint of cost, damage to grain and methods of application.