FACTORS AFFECTING THE EVALUATION
AND USE OF GRAIN PROTECTANTS
FOR INSECT CONTROL

by

ARCHIE TUNNOCK, JR.

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1957

APPROVED:

[redacted]

Associate Professor of Entomology

In Charge of Major

[redacted]

Chairman of Department of Entomology

[redacted]

Chairman of School of Science Graduate Committee

[redacted]

Dean of Graduate School

Date thesis is presented May 26, 1956

Typed by Jolan Eross

ACKNOWLEDGMENTS

The writer wishes to thank Dr. Knud G. Swenson, Associate Professor, Oregon State College, for his assistance and advice during the work on this thesis.

The writer also wishes to thank Dr. Roger G. Peterson, Agricultural Experiment Station Statistician, Oregon State College, for his help in outlining and explaining the statistics used in this study.

The writer is very grateful to his wife, Carolyn H. Tunnock, for her aid in some of the labor envolved while gathering data.

LIST OF TABLES

LIST OF TABLES (CONTINUED)

TABLE OF CONTENTS

FACTORS AFFECTING THE EVALUATION AND USE OF GRAIN PROTECTANTS FOR INSECT CONTROL

INTRODUCTION

Insect infestations and damage to stored grain are prevented or controlled by such methods as drying the grain before storage, sanitary measures, residual sprays, fumigation, and chemical protectants. Protectants are usually applied to grain during the storage process. Chemically inert dusts were used in earlier attempts to protect grain from insect damage. The dusts were effective because they caused breaks in the insect's cuticle, and as a result, the insect would die of excessive evaporation of body moisture (8, pp. 629-639). Inert dusts that have been used successfully include finely divided silica gel, rock phosphates, precipitated chalk, magnesium oxide, and aluminum oxide (8, pp. 629-639).

At present, laboratory evaluation of grain protectants consists of applying insecticides to pint or quart lots of grain. The insecticides are applied to the grain as a spray or incorporated as a dust. The treated grain is placed in jars and periodically exposed to insects.

This thesis is concerned with the evaluation of several insecticides in the laboratory. Their properties indicate that they may be able to protect stored grain from insect damage for 6 months or over. It would also be interesting to see how the treatment of pint or quart lots of grain compared with a method that dealt with the treatment of bushel lots of grain. The latter method would be treated to more closely approximate field conditions. The residual action of the insecticides, applied to the larger bulks of grain, would be tested over a long period.

The comparison of results from the two ways of evaluating grain protectants may aid in the development of a laboratory method that will give a truer picture of the potentialities of an insecticide when it is applied to stored grain in the field.

REVIEW OF LITERATURE

The only material in current usage as a grain protectant in the United States is Pyrenone, the principle active ingredients of which are pyrethrins and piperonyl butoxide.

Watts and Berlin (30, pp. 371-373) using Sitophilus oryzae as a test insect, evaluated piperonyl butoxide alone, pyrethrins alone, and combinations of both. These workers concluded that synergism between piperonyl butoxide and pyrethrins was very evident and operated over a wide range of ratios.

Previous work was done in Oregon with Pyrenone in 1952 by Tanner (29, pp. 1-17). He showed that a dust, applied at the rate of 78.8 pounds per 100 bushels, protected stored wheat in bins against insect infestation for 8 months. Wilbur (31, pp. 913-920), using Pyrenone at the same rate, applied it to wheat in several farm bins in Kansas and reported outstanding protective value against weevils.

A pyrethrins-piperonyl butoxide dust was applied at the rate of one pound to 10 bushels of shelled or shucked ear corn. The corn had a low-moisture content and was already lightly infested by rice weevils. The dust prevented weevil damage for 9 months. Sprays of this material were ineffective in preventing damage to shucked corn (9, pp. 1105-1107).

Another factor of importance in the evaluation of Pyrenone is the repellent action of pyrethrum to some insects. After a few days, pyrethrum in a clay diluent coated on paper bags or cartons was not toxic enough to kill *Tribolium* spp. (15, pp. 1104-1107). It was noticed, though, that a long-lasting repellency was present. To test this repellency, pyrethrins and piperonyl butoxide in a 1-10 ratio was applied to corn and whole black pepper. The insecticide residue was expressed as parts per million of pyrethrins. After exposure periods of from 3-24 hours in an apparatus that was developed to test this repellent action, results indicated that adult *Tribolium confusum* were repelled and could detect the difference between 0.1 and 0.3 p.p.m. pyrethrins on black pepper and between 0.25 and 0.37 p.p.m. pyrethrins on corn.

Schroeder (25, pp. 25-27) studied, under laboratory conditions, some factors that might influence the effectiveness of piperonyl butoxide-pyrethrins combinations. Lots of shelled corn were adjusted to different levels of moisture content. These lots were dusted with various dosages of Pyrenone. There was a reduction in mortality of rice weevils placed in treated corn, when the moisture content of the grain was more than 13 per cent. Wilbur (32, pp. 121-125) had previously treated wheat with a piperonyl butoxide-pyrethrins combination. He noted that survival of rice weevils improved with each successive rise in moisture content above 11 per cent. Schroeder dusted corn and wheat with Pyrenone to test its residual effects. A higher dosage was dusted on the wheat. After 90 days, rice weevils were exposed to the treated grains for 7 days. The protectant on corn caused 85 per cent mortality, but on wheat only 30 per cent occurred. Rice weevils 1-5 weeks old were placed in corn treated with Pyrenone. At the end of 7 days only 66 per cent of the week-old weevils were dead, while 92 per cent of the 5 week-old weevils had died.

There is a possibility that Sitophilus granarius could become resistant to a mixture of pyrethrins and piperonyl butoxide. Mathlein (17, pp. 1-20) found that weevils resistant to DDT were also much more resistant to a mixture of pyrethrins and piperonyl butoxide than was a control group which had not been exposed to DDT.

Early in 1943 the gamma isomer of BHC (lindane) was isolated and found to be more toxic to granary weevils than any substance previously tested by the Imperial Chemical Industries (26, pp. 314-319). In the United States, lindane has not as yet been accepted as a protectant as grain intended for human consumption. It is being used for this purpose on a small scale in the United Kingdom, India and a few other countries.

In a paper by Fiedler (10, p. 120) it was stated, "medical authorities in the United Kingdom consider that they are justified in recommending 2.5 p.p.m. lindane as a maximum permissible concentration in foodstuffs ready for direct consumption."

Laboratory tests were made against granary weevils using wheat dusted with lindane diluted with kaolin, and with pyrethrins (3, pp. 731-732). After 16 weeks, granary weevils were exposed to the treated wheat for 10 days. Lindane at 1.0 p.p.m. caused 86 per cent mortality, while 27 p.p.m. pyrethrins only caused 3 per cent mortality. Chao and DeLong (5, pp. 908-910) found that a Pyrenone dust containing 0.16 p.p.m. pyrethrins and 1.6 p.p.m. piperonyl butoxide killed no granary weevils in 2 weeks, but 50 per cent kill was obtained with 0.16 p.p.m. lindane. After 3 months, time-mortality curves showed that the toxicity of lindane at 4 p.p.m. dropped rapidly, but Pyrenone, 4 p.p.m. pyrethrins and 40 p.p.m. piperonyl butoxide, retained most of its toxicity. At the end of 6 months, no emerging adult granary weevils were found in lots of wheat that had been treated with lindane at 0.8 p.p.m. or with 4 p.p.m. pyrethrins and 40 p.p.m. piperonyl butoxide.

Lindane dust at one p.p.m. was completely effective for 16 months in protecting 200 pound bags of treated corn from infestation by rice weevils. Some of the bags of corn had infestations of weevils previous to treatment. The dust was also effective by controlling any adults that emerged during the 16 months (23, pp. 295-311).

An interesting method of controlling pests of barley and wheat in the field and in storage was experimented with by Hidetsugu (13, pp. 93-99). He sprayed and dusted both crops with gamma BHC during the period from heading to maturity. Sitophilus sasakii, introduced 45 days after harvest into huskless barley, succumbed almost totally within a week. The reproductive rate was very low, but when the insects were introduced into wheat, mortality was less and reproduction higher.

Laboratory tests were carried out (10, pp. 115-122) in South Africa to test the assumption that less lindane is needed in warm climates than in temperate climates for complete control of grain pests. At a rate of 2 p.p.m., 60 per cent mortality was obtained at 16°C, 83 per cent at 21°C, and 93 per cent mortality of granary weevils at 24°C. It was thought that the fumigant action of the lindane dust was the main factor responsible for killing the weevils. Further discussion on the results from the fumigant action of lindane in this paper and the following (18, pp. 137-139), (20, pp. 639-646), and (27, pp. 596-601) will be taken up under a section dealing with the fumigant properties of lindane.

Wolfram (33, pp. 73-75) tested the residual toxicity of a 0.65 per cent lindane dust. He mixed it at a rate of 0.1 per cent with wheat, into which granary and rice weevils had been introduced 8 days previously. The wheat was cleaned 7-10 days later and all weevils were found dead. Fourteen weeks later the wheat was cleaned and washed again. No adult weevils had developed from eggs which might have been laid in the kernels. Granary weevils, 14-28 days old, were subsequently enclosed with samples of the treated wheat. After 19 days, 89.7-100 per cent mortality was attained. These results show that the toxicity of the lindane was still high after 15 weeks, and not much had been removed by cleaning and washing.

Lindane in xylol was impregnated into crushed corn cobs and applied at the rate of 3 grams of technical lindane per hundredweight. This gave complete protection to rough rice and shucked corn exposed to a heavy insect infestation for 11 months (11, pp. 771-774).

One of the latest insecticides to be experimented with as a grain protectant is the organic phosphate, chlorthion. It has proven toxic to most stored grain pests and is relatively non-toxic to mammals. The LD 50 of chlorthion to rats was found to be 1500 mg/kg when taken orally.

Lindgren et al. (16, pp. 705-706), in preliminary tests, treated 250-gram samples of wheat with chlorthion dust. Initially after dusting, rice and granary weevils were exposed to the treated wheat for 10 days. At 4 p.p.m., 100 per cent kill of rice and granary weevils was attained. At 2 p.p.m., 100 per cent of the rice weevils were killed and only 77 per cent of the granary weevils.

MATERIALS AND METHODS

TEST INSECTS

The test insects used in this work were the granary weevil, *Sitophilus granarius* Linne., and the confused flour beetle, *Tribolium confusum* Duval.

The life cycle and degree of infestation of both test insects is dependent on temperature, relative humidity, moisture content of the grain, and the type of medium upon which they are feeding. The confused flour beetle has developed from egg to adult in 37 days (22, p. 43). The entire life cycle of the granary weevil may be passed in from 4 to 7 weeks (19, p. 796).

These particular insects were used in this experiment because: they are both pests of stored grain, they can be reared easily in the laboratory in large quantities, and because they show a difference in tolerance to most insecticides employed as stored grain protectants (4, p. 706).

Several media are used to rear flour beetles. Peterson (24, p. 45) states that flour beetles reproduce and grow well in a mixture of 95 per cent finely ground dog food and 5 per cent dry powdered brewers' yeast. One quart of whole wheat flour to one tablespoonful of brewers' yeast was also recommended. The writer found that 2 parts by volume of meal-type dry food to 1 of unsifted whole wheat flour was easy to prepare and gave very satisfactory results. Colonies were reared in $1\frac{1}{2}$ quart Pyrex caserole dishes with tight fitting lids. This allowed easy access to adult beetles. The caserole dishes were filled about three-quarters full of medium. When starting a new colony, a piece of burlap was placed on top of the medium. It was noticed from previous work that the pupae were found underneath and in the burlap. After adults started emerging, the burlap was removed and a piece of paper toweling was put in its place. All that was necessary to remove adults from the dishes was to lift out the toweling, which usually had adults clinging to it, and brush them off into a pan. Excess adults were removed at 2-week intervals to keep the colony vigorous.

Peterson (24, p.45) suggests that granary weevils be reared on whole wheat or shelled corn of 14-15 per cent moisture content. Whole kernelled white wheat was adopted for rearing. Gallon jars were three-quarters filled with the wheat before introducing adult weevils for breeding purposes. A large hole was cut in each lid and fine-mesh brass straining cloth was soldered over it, to allow air to enter. About every two weeks, or when emerging weevils were getting too numerous in a jar, they were sifted out of the wheat and discarded. The weevils were sifted through an 8-mesh aluminum sieve which retained the wheat.

The test insects were reared in a room that was kept between 75-80°F and had a relative humidity of about 50 per cent.

INSECTICIDES EVALUATED

The insecticides evaluated were all in the form of emulsifiable concentrates. Pyrenone has as its principle active ingredients pyrethrins and piperonyl butoxide. The formulation, pyrenone 10-1, contains 1.18 per cent pyrethrins and 11.8 per cent piperonyl butoxide by weight. However, the remainder of the ingredients also have insecticidal properties and the formulation is designated as having 100 per cent active ingredients. The insecticides evaluated are listed in Table 1. Chemical names of the active ingredients of these insecticides are given by Haller (12, pp. 112-114).

TABLE 1. INSECTICIDES EVALUATED FOR GRAIN PROTECTANT PROPERTIES

Insecticides	Active Ingredients Per Gallon		Manufacturer
	Percent	Pounds	
Lindane	20	1.6	Ethyl corp.
Lindane-Aroclor*	20 20	1.6 1.6	Ethyl corp.
Pyrenone 10-1	100	7.03	U.S. Industrial Chem.
Piperonyl butoxide	11.8	.832	
Pyrethrins	1.18	.083	
Diazinon	25	2.0	Geigy Co. Inc.
Malathion	57	5.0	American Cynamid Co.
Chlorthion	44	4.0	Chemagro Corp.

* Aroclor is a trade name of a resinous chlorinated polyphenyl which is combined with lindane to extend the residual action of lindane.

APPLICATIONS OF INSECTICIDES

The method of applying an insecticide to a pint lot of wheat involved spreading the wheat out evenly in a 9 x 12 inch wooden retaining frame placed on a piece of wax paper. The insecticidal solutions were prepared by the dilution of emulsifiable concentrates obtained from the manufacturers. The desired amount of insecticide was applied to the grain in 10 cc of water per pint, by means of an atomizer.

After the wheat had dried it was divided into ½-pint lots and each lot was placed in a wide mouth, Mason-type, pint jar. The two ½-pint lots, of the originally treated pint of wheat, were considered a replicate for a particular dosage. Fine mesh brass strainer cloth was soldered over a hole cut in the jar lids for ventilation.

The method adapted for treating bushel lots of wheat consisted of spreading out 8 bushels of white wheat on a wooden floor in about a ½-inch layer. The insecticidal emulsion was sprayed on the wheat with a 3-gallon, pressure type sprayer. Howe et al. (14, pp. 1-34) used this method to spray bean seeds. The sprayed wheat was allowed to dry for about 2 hours and then 2-bushel lots of it were placed in small wooden bins. The wooden bins were made of 5/8 inch interior plywood, and were built to form a cube, 18 inches on a side. The division of the original 8 bushels of wheat into 2-bushel lots gave 4 replicates per treatment. The replicates were then arranged in randomized blocks, in an attempt to equalize variations due to differences in temperature that might occur within the room where they were stored.

RECORDING AND ANALYZING DATA

The adult test insects were usually exposed to treated wheat for 7 days. At the end of the exposure period, the insects were sifted out of the wheat and the numbers dead and live recorded. In preliminary tests, with pint lots of wheat, any insects showing signs of life were returned to the same wheat and exposed for a further period. Both species of test insects feigned death when disturbed. In order to separate living from dead beetles, it was necessary to inspect the insects with a reading glass of high magnification and probe each one. The probe used was a piece of fine glass tubing which had been slightly rounded on the end. An insect was recorded as alive if it moved any of its appendages when probed.

Abbott's formula (1, p. 266) was used to correct for natural mortality, which was determined from the per cent dead in the checks. With the use of this formula, results are no longer expressed in per cent mortality but in per cent control. The formula can be illustrated as follows:

$$\frac{x - y}{x} \times 100 = \text{Per cent control.}$$

x = per cent living in checks.

y = per cent living in treatments.

x-y = per cent killed by the treatment.

Statistical analysis was used to analyze the data, on lindane and chlorthion applied to pint and bushel lots of wheat, for the control of *T. confusum*. The data was arranged in a split-plot design (6, p. 218), which had the main effects confounded. A factorial analysis was carried out because of this arrangement of data. Factor A consisted of 8 treatments combining the effects of the two insecticides, the two dosage levels, and the two evaluation methods. The plots were split into 6 sub-plots by the effects of the 6 months and were considered under factor B.

The results of the evaluation methods were recorded in per cent control, and because percentages, to be used as observations, do not lend themselves to statistical analysis, the per cent control for each replicate was transformed into a corresponding angle by the formula $\arcsin \sqrt{\text{percentage}}$ (28, p. 449).

RESULTS

PRELIMINARY EVALUATION OF INSECTICIDES

Before comparative tests could be made between insecticides or between lots of wheat of different sizes, it was necessary to find the amounts of toxicant needed to give results which could be used for comparative purposes. In other words, it was desired that the dosages applied would give some degree of mortality below 100 per cent.

Preliminary tests, using pint lots of wheat, were carried out with the insecticides listed earlier. Each pint of wheat was prepared as described previously and was divided into two ½-pint lots and placed in jars. The two ½-pint lots of a treated pint of wheat were considered a replicate for a particular dosage. Fifty *Tribolium confusum* were placed in each ½-pint lot immediately after the wheat had dried. Mortality counts were made at the end of a 7 day exposure period, and any insects still alive were returned to the wheat. Fourteen days later, or 21 days after treatment, mortality counts were made on the remaining beetles. The infested jars of wheat were placed in a cabinet kept at 78-81°F and 50 per cent relative humidity for the 21 days.

Granary weevils were not used in these preliminary tests. The writer found that granary weevils were much more susceptible to Pyrenone and lindane than the confused flour beetles. Therefore, it

TABLE 2. CONTROL OF TRIBOLIUM CONFUSUM IN HALF-PINT LOTS OF WHEAT TREATED WITH SIX INSECTICIDES

Insecticide	Mg Per Pint of Wheat	Average Per Cent Control		Number of Replicates For Each Dosage
		7 Days	21 Days	
Pyrenone	8.23	1.5	4.1	2
	41.13	4.4	26.1	3
	82.26	5.2	59.8	3
	205.65	15.0	91.4	4
	411.30	62.4	96.7	5
	575.82	96.0	99.5	2
	822.60	95.0	96.8	2
	1645.20	100.0	-	1
Lindane	.19	2.0	27.1	3
	1.92	36.3	69.2	5
	4.68	99.6	100.0	3
	9.35	100.0	-	4
	18.70	100.0	-	2
Lindane-Aroclor	.19	2.0	3.1	2
	1.92	54.3	99.3	3
	4.68	100.0	-	2
	9.35	100.0	-	2
	18.70	100.0	-	1
Diazinon	.24	1.6	2.1	2
	1.19	100.0	-	1
	5.99	100.0	-	1
Chlorthion	.48	94.0	95.0	1
	2.39	100.0	-	1
	11.98	100.0	-	1
Malathion	.59	3.0	3.2	1
	2.99	100.0	-	1
	14.98	100.0	-	1

was assumed that any dosage of insecticide that would kill flour beetles would be more than enough for control of weevils. Table 2 summarizes the data obtained from preliminary tests with Pyrenone and several other insecticides. The amounts of the insecticides per pint of wheat, required to obtain 100 per cent control of T. confusum in 7 days, were as follows:

Pyrenone	822.60 - 1645.20	milligrams
Lindane	4.68 - 9.35	"
Lindane-Aroclor	1.92 - 4.68	"
Diazinon	0.24 - 1.19	"
Chlorthion	0.48 - 2.39	"
Malathion	0.59 - 2.99	"

In order to obtain a more critical comparison of the insecticides, they were applied to pint lots of wheat at approximately equivalent dosages. Because the insecticides were in the form of emulsifiable concentrates, it was more convenient to adjust to equivalent dosages by volume than by weight. The insecticides were applied at the rate of 0.002 cc actual toxicant in 10 cc of water per pint of wheat. The actual amounts of insecticides in milligrams, per pint lot of wheat, are as follows:

Pyrenone	1.6	milligrams
Lindane	1.9	"
Diazinon	1.9	"
Chlorthion	2.4	"
Malathion	2.1	"

The procedure for treating the pint lots of wheat was the same as described previously. A series of these five materials, applied at 0.002 cc actual per pint, was replicated five times. The moisture content of the wheat varied from about 13-14 per cent before it was sprayed, and was determined by a Tag-Heppenstall Moisture Meter. Fifty T. confusum were added to each ½-pint lot immediately after treatment. Mortality counts were made at the end of 7, 28, and in a few replicates, 49 days. The method of evaluating differed in these tests from the previous ones. At the end of 7 days, both dead and live insects were removed from the wheat. Fourteen days later, fresh flour beetles were added, and 7 days later mortality counts were made and all insects removed again. For the 49 days period, new insects were added on the 42 day after treatment.

An average per cent control for the five replicates was computed for each insecticide, and the standard deviations from the mean found. Table 3 summarizes these results for the 3 exposure periods.

TABLE 3. CONTROL OF TRIBOLIUM CONFUSUM WITH SEVERAL INSECTICIDES APPLIED AT THE RATE OF 0.002 cc ACTUAL TOXICANT PER PINT OF WHEAT

Insecticide	Average Per Cent Control		
	7 days	28 days	49 days*
Lindane	50 ± 12.4	1.1 ± 1.2	0 ± 0
Pyrenone	0.4 ± 0.2	0 ± 0	- -
Diazinon	90.4 ± 6.2	15.4 ± 11.2	1.3 ± 1.3
Malathion	75.1 ± 11.5	9.8 ± 8.4	1.0 ± .9
Chlorthion	84.0 ± 7.6	55.4 ± 11.2	7.3 ± 6.3

* Average per cent control computed from 3 replicates.

It can be seen in Table 3 that Diazinon was the most toxic insecticide to T. confusum at the end of 7 days. Chlorthion gave the highest per cent control for the 28 day period and at the end of 49 days. Lindane and Pyrenone at this dosage were not highly toxic to the confused flour beetle.

Lindane, Pyrenone, and chlorthion were chosen for further evaluation as grain protectants in tests with pint and bushel lots of wheat. Pyrenone was chosen because it was already recommended as a commercial treatment for stored grain. Lindane had been investigated by other workers and showed promise of long residual action. Chlorthion gave good results in preliminary tests and appeared to be the best of the new insecticides having a low mammalian toxicity.

TESTING EVALUATION METHODS

Trial One

Various dosages of lindane and Pyrenone were made up and sprayed on 8-bushel lots of wheat. The dosages were based on preliminary experiments using wheat sprayed in pint volumes. The dosages of lindane and Pyrenone decided upon, expressed in milligrams per bushel, are as follows:

Pyrenone	5,265 mg
Pyrenone	39,593 "
Lindane	120 "
Lindane	598 "
Lindane-Aroclor	120 "

TABLE 4. CONTROL OF TRIBOLIUM CONFUSUM IN PINT SUBSAMPLES OF WHEAT TAKEN FROM BINS TO CHECK ON DISTRIBUTION OF TOXICANTS*

Treatments Mg Per Bushel	Treatments 37 Days Old. Insects Then Exposed 32 Days.	Treatments 83 Days Old. Insects Then Exposed 14 Days.	Treatments 106 Days Old. Insects Then Exposed 30 Days.
Pyrenone 5,265	39.3 ± 13.3		end
Pyrenone 39,593	81.3 ± 4.9		62.2 ± 8.4
Lindane 120	84.0 ± 6.1		35.6 ± 13.2
Lindane 598	100.0 ± 0	80.9 ± 12.5	99.5 ± 0.6
Lindane-Aroclor 120	77.6 ± 11.3		27.8 ± 6.8
Checks Per Cent Alive	98.6 ± 1.3	98.4 ± 1.0	99.7 ± 0.6

* Average per cent control for 4 replicates or 16 pint jars (25 T. confusum per jar).

The desired amount of insecticide for each treatment was mixed in 5½ pints of water, and this volume was applied to an 8-bushel lot. A check was prepared by spraying 8 bushels of wheat with an equal amount of water. The same volume of water was applied to all 8-bushel lots, so that the moisture content of the treated wheat would not be raised above that of the check. The moisture content of the wheat was about 14 per cent before it was sprayed. Each 8-bushel lot of treated wheat was split up into 2-bushel lots and placed in wooden bins as described on page 12.

To check uniformity of insecticide application, 4 pint subsamples were taken from each of the 2-bushel lots. These subsamples were taken from different areas within the two bushels of wheat. Test insects were added, to the bins and to the subsamples in pint jars, 5 weeks after the wheat had been sprayed. Five hundred T. confusum and 500 weevils (mixed rice and granary) of mixed ages and sexes were added to each bin, and 25 of each per jar. Mixed species of weevils were used, because the original colony of granary weevils had become contaminated with rice weevils. At this time, there were not enough granary weevils in pure cultures to obtain the large numbers needed. The room, in which the bins and pint subsamples were stored, varied in temperature from 60-75°F, the average being around 70°F.

The control of T. confusum, in the pint subsamples removed from the bins to check on uniformity of insecticide application, is presented in Table 4. A 100 per cent kill of weevils was obtained in all treatments up to the end of the experiment. This data was not included in Table 4.

The standard deviation of the average per cent control for the 4 replicates per treatment was computed. The deviations ranged from ± 6.1 to ± 13.3 the first time T. confusum were exposed to the wheat in the jars. Because of these rather high standard deviations, the differences between per cent T. confusum alive in the pint subsamples for each treatment, were subjected to the analysis of the variance. There was no significant difference in per cent alive between replicates for any treatment except Pyrenone at 5,265 mg per bushel. It should be noted, that the standard deviation of the average per cent control for the 4 replicates, was the highest for this treatment. The analysis of the variance also indicated that there was no significant difference in per cent alive between pint subsamples within any treatment. The per cent alive was used as the statistic in computation because it was recorded for each subsample, while the per cent control was not.

These results, it was felt, were indications of fairly uniform application of insecticides on 8-bushel lots of wheat.

Before the insects were added to the 2-bushel lots of wheat, the bins were covered with a plastic screening called lumite. Half-inch wooden strips were tacked on top of the lumite around the edges of the bins to seal them. To infest the bins with insects, a 1 x 1 inch V-shaped cut was made in the center of the screening. After the insects were poured through, the cut was sealed with wide strips of adhesive tape. A quarter inch plywood cover was placed on top of the screening to keep out the light and slow down the loss of moisture from the surface of the wheat. These two factors can affect the residual life of an insecticide. The lumite covers were removed later on when new sampling methods of the treated wheat in the bins were developed.

A month after the insects were added to the bins, mortality counts were made. The first method of sampling consisted of taking 5 samples from the top 6-8 inches of each bin. The samples were removed by a regulation grain probe thrust through the hole cut in the lumite. The probe was sectioned into compartments and only the wheat from the first two was used as a sample. The amount of wheat from these two compartments was about 100 cc. Five samples made up about a pint. The number of dead and live insects of each species was recorded for each sample. The number of insects recovered by this sampling method was too few to permit any conclusions.

A few days later, a new sampling method was employed. The lumite screen covers were removed and 4 pints of wheat were taken from each bin. Each pint of wheat was sifted and counts made on the dead and live insects present. The screen covers were tacked back on to retain any live insects. Computation of means for each treatment showed that the mean number of insects recovered again varied considerably with no relationship to the expected relative efficiency of the insecticides. This was true of the number of dead insects recovered as well as of the total (dead and live) number of confused flour beetles and weevils.

TABLE 5. DISTRIBUTION OF SITOPHILUS SPP. AND TRIBOLIUM CONFUSUM IN TWO-BUSHEL LOTS OF WHEAT *

Treatment Mg Per Bushel	Vertical Strata	Replicate A Sitophilus Alive	Replicate A Sitophilus Dead	Replicate A Tribolium Alive	Replicate A Tribolium Dead	Replicate B Sitophilus Alive	Replicate B Sitophilus Dead	Replicate B Tribolium Alive	Replicate B Tribolium Dead
1. Pyrenone	1st 3 inches	0	526	326	119	3	544	370	71
	2nd 3 inches	0	33	51	19	1	38	12	4
	3rd 3 inches	0	1	3	9	0	0	3	5
	4th 3 inches	0	0	6	2	0	3	4	0
	Total	0	560	386	149	4	585	389	80
2. Pyrenone 39,593	1st 3 inches	0	501	3	526	0	543	5	374
	2nd 3 inches	0	7	0	14	0	6	0	2
	3rd 3 inches	0	2	0	1	0	1	0	1
	4th 3 inches	0	1	0	0	0	0	0	1
	Total	0	511	3	541	0	550	5	378
3. Lindane 120	1st 3 inches	0	588	20	335	0	351	46	71
	2nd 3 inches	0	34	6	107	0	32	5	22
	3rd 3 inches	0	1	1	42	0	1	2	12
	4th 3 inches	0	0	4	15	0	0	1	8
	Total	0	623	31	499	0	384	54	113
4. ** Lindane 598	1st 3 inches	0	1461	84	1202	0	1420	184	1122
	2nd 3 inches	0	27	16	152	0	72	22	145
	3rd 3 inches	0	10	3	38	0	5	3	14
	4th 3 inches	0	2	0	5	0	3	0	10
	Total	0	1500	103	1397	0	1500	209	1291
5. Lindane-Aroclor 120	1st 3 inches	0	416	12	236	0	310	179	158
	2nd 3 inches	0	40	10	115	0	19	14	33
	3rd 3 inches	0	1	1	48	0	1	3	12
	4th 3 inches	0	0	2	25	0	0	4	22
	Total	0	457	25	424	0	330	200	225
6. Checks	1st 3 inches	29	129	470	5	86	64	372	5
	2nd 3 inches	40	30	82	2	106	23	21	0
	3rd 3 inches	126	12	3	0	158	19	2	0
	4th 3 inches	165	10	3	2	140	15	4	0
	Total	360	181	558	9	490	121	399	5

* Insects were in treatments 1, 2, 3, 5, and 6, 52 days before counts were made.

**Insects were in treatment 4 for 60 days. At the end of 46 days another 1000 granary weevils and 100 confused flour beetles had been added.

Two months after the insects had been introduced into the bins, all the wheat from bins A and B for each treatment was sifted and all the insects counted. This was done to see if the treatments had any effect on the distribution of the insects in the wheat. The average height of the wheat in the bins was 12 inches. The wheat in each bin was divided into four, 3-inch layers. Each layer was carefully removed and all the insects were sifted out and recorded.

The results (Table 5) show no difference between treatments in the vertical distribution of the insects in the wheat. In the Check it was found that most of the weevils were in the bottom half (6 inches) of the wheat. Table 5 also shows that all the weevils were dead in the treated grain and few had gotten below the top 3 inches. T. confusum were found mostly in the top 3 inches and very few were below 6 inches. While removing the wheat for sifting, it was noticed that Tribolium confusum had a definite tendency to be gregarious in the corners and along the sides of the bins. The sifting of the wheat also showed that Tribolium confusum distributed themselves throughout the grain in a way that would make obtaining adequate estimates of their control impractical either by systematic or random sampling. The same problems would be faced when trying to obtain data on the weevils, for as the toxic effects of the protectants grew less, the weevils would work toward the bottom.

Other factors that would make sampling from the bins difficult and impractical would be the addition of fresh insects after most of the first batch were killed. Mortality counts of these additional groups would be complicated by the presence of the dead insects from the previous groups.

From sifting the grain, data on the effectiveness of the treatments were obtained. All the weevils were dead in the treated wheat, so that 100 per cent control of weevils was achieved for at least 5 weeks. Weevils were added to wheat treated with lindane at 598 mg per bushel, 14 days before the wheat was sifted. At the time of sifting all the weevils were dead, so this dosage gave complete control of weevils for 60 days. A high degree of control of Tribolium confusum was obtained at the same time with all materials except the lower dosage of Pyrenone and the lindane-Aroclor combination.

Previous inadequate sampling methods and the information on uniformity of distribution of the insecticides, showed the need for a new sampling technique of the wheat in the bins.

Treatments with lindane-Aroclor at 120 mg per bushel, and Pyrenone at 5,265 mg per bushel were discarded, because they were giving low control of Tribolium confusum. The wheat in the check bins was also discarded for it was heavily infested with weevils.

The new method adapted for testing the treated wheat consisted of taking 4 pint subsamples at monthly intervals from each bin.

The subsamples, in this case, were not randomized but were taken from approximately each corner of a bin, 6-8 inches deep. Fifty Tribolium confusum were then placed in each pint jar and left for 7 days before mortality counts were made. This procedure eliminated the previous sampling difficulties, and the only disadvantage that might arise would be more or less underrating any material, such as lindane, which had any fumigant action. The removal of subsamples every month would undoubtedly dissipate some of the gas or vapour concentration which may have accumulated in the larger bulk.

The results from infesting pint subsamples at monthly intervals with Tribolium confusum (Table 6) indicated that lindane at 120 mg and Pyrenone at 39,593 mg per bushel did not give very good control past 4 months. Lindane at 598 mg per bushel gave fair control of Tribolium confusum up to 5 months by this evaluation method.

The toxic effects of the insecticides became too low to give practical kills of Tribolium confusum so all the treatments were discarded. Before the bins were emptied, 6 pint subsamples were removed from each bin, making a total of 24 subsamples for each treatment. At monthly intervals, one subsample from each bin or 4 subsamples per treatment, was infested with fifty, 7 day-old granary weevils. The moisture content of the treated grain and of the checks was determined at this time to make sure it was high enough (11 per cent) for reproduction to take place. Mortality counts were made at the end of 7 days and all weevils removed. After the weevils were removed, the subsamples were held for 2-3 months and then examined for newly emerging adults. Emergence indicated that eggs laid in the kernels were not affected by the insecticide at that time, and that development could take place inside the kernels, although, the outside was coated with insecticide.

TABLE 6. CONTROL OF TRIBOLIUM CONFUSUM IN PINT SUBSAMPLES REMOVED AT MONTHLY INTERVALS FROM TWO-BUSHEL LOTS OF WHEAT

Treatment	Average Per Cent Control	
Mg Per Bushel	End of Five Months	End of Six Months
Lindane 120	0.13 ± 0.1	-
Lindane 598	46.5 ± 15.5	7.4 ± 2.6
Pyrenone 39,593	1.1 ± 0.6	-
Check Per Cent Alive	100 ± 0	100 ± 0

Table 7 shows that no weevils emerged in any of the treatments. The data indicates that the 3 treatments gave complete protection from infestations of granary weevils for 12 months. The reason for the absence of newly emerged adults in the checks at the end of 12 months was because the moisture content of the wheat used was only about 9.6 per cent. Granary weevils need at least 11 per cent moisture to oviposit in wheat. Occasional dead weevils were found in the treatments. This was attributed to poor sifting at the end of the 7 day exposure period.

TABLE 7. DEVELOPMENT OF GRANARY WEEVILS FROM EGGS LAID IN PINT SUBSAMPLES REMOVED FROM TWO-BUSHEL LOTS OF WHEAT

Treatment	Age of Treatment When Weevils Were Introduced (months):					
Mg Per Bushel	7	8	9	10	11	12
Lindane 120	No	No	No	No	No	No
Lindane 598	No	No	No	No	No	No
Pyrenone 39,592	No	No	No	No	No	No
Checks	Yes	Yes	Yes	Yes	Yes	No

The average per cent control of granary weevils (Table 8) placed in the pint subsamples once a month seemed to increase rather than to decrease as the treatments aged. A plausible reason for this increase was that from 7-9 months the pint subsamples, when infested, were kept in the same room as the wheat in the bins. The temperatures in this room, at that time, varied a great deal from month to month and were usually around 60°F. After the 10 month point, the infested subsamples were held in a temperature-controlled cabinet, at around 80°F, for the 7 day exposure period. This was done to try to reduce the variations due to fluctuations in temperature.

Lindane at 598 mg per bushel consistantly gave a very high degree of control of granary weevils during the 7-12 month period.

Although, Pyrenone at 39,593 mg per bushel gave good control of granary weevils for 12 months, it only gave an average of 1.1 per cent control of T. confusum at the end of 5 months. A high degree of control of both pests is to be desired for at least 6 months in the field. In the light of these facts, the dosage of Pyrenone used for further evaluation work would have to be doubled to 79,186 mg per bushel. At this dosage, Pyrenone could hardly be compared with lindane even at 1,196 mg per bushel. These reasons weighed heavily against the use of Pyrenone emulsifiable concentrate in further evaluation methods of grain protectants. It was concluded that Pyrenone was inferior to lindane in respect to its residual effects against T. confusum and its toxicity to S. granarius and T. confusum.

TABLE 8. CONTROL OF GRANARY WEEVILS IN PINT SUBSAMPLES REMOVED FROM TWO-BUSHEL LOTS OF WHEAT

Treatment	Average Per Cent Control at End of (Months):					
Mg Per Bushel	7	8	9	10	11	12
Lindane 120	62.5±5.3	36.3±2.5	86.9±3.5	99.0±1.2	97.5±3.8	99.4±1.4
Pyrenone 39,593	91.5±1.7	81.2±7.7	97.0±2.6	99.5±1.4	99.0±2.0	100
Lindane 598	98.0±2.8	83.5±3.0	99.5±1.4	100	100	100
Checks Per Cent Alive	100	100	100	100	100	80.0±9.8

Trial Two

Fresh 8-bushel lots of wheat were treated, as in Trial One, with lindane at 598 mg and 1,196 mg per bushel. Chlorthion replaced Pyrenone and was applied at 307 mg and 614 mg per bushel.

Four pint subsamples were again removed from each bin each month and infested with 50 Tribolium confusum. This time, the surface of the wheat was divided into 9 equal areas, and the 4 areas to be sampled were picked from a randomized table each month. Pint lots of wheat were prepared as on page 12 and divided into ½-pint lots. The amount of insecticide sprayed on each pint lot was computed from the theoretical amount on a pint of wheat in a 2-bushel lot. Four ½-pint lots were assigned to each bin and each lot was infested with 50 Tribolium confusum every month. The results from the treatment of pint lots of wheat were to be compared with those from the treatment of bushel lots.

At the end of 4 months, 50 granary weevils were also added along with the confused flour beetles to wheat taken from the bins. After the 7 day exposure period both insects were sifted out, and the wheat replaced in respective jars. At the end of 2-3 months, these jars were inspected for adult weevils that might have developed from any eggs laid during the period of infestation.

The treated wheat in the bins was left in the same room as before. The jars of wheat from both methods of evaluation were placed in a temperature-controlled cabinet at around 80°F with near 50 per cent relative humidity for the 7 day exposure period. After counts had been made, all the jars were returned to the tops of their related bins.

An analysis of the variance (Table 14) was computed for the data recorded up to the end of 6 months for lindane and chlorthion sprayed on pint and bushel lots of wheat, at two dosage levels. The method of analysis has been discussed on page 14. The analysis only covers control of Tribolium confusum.

There was no difference between replicates, which indicated that the insecticides were distributed fairly uniformly throughout the wheat, and the conditions of the insects were fairly homogeneous.

Lindane gave higher controls of Tribolium confusum than chlorthion (Table 14 A) at both dosages. Both insecticides gave the most control at their highest dosage.

There was significance between evaluating methods (Table 15 G). The pint-lot method gave the higher control for 6 months at both dosage levels of each insecticide. The differences between the two methods were the same at both dosage levels. Chlorthion at both dosage levels gave the higher control of Tribolium confusum by the pint-lot method, while the opposite occurred with lindane, which gave the higher kill in the bushel lots.

The rate of control decreased as the treatments aged (Table 15 D), which was to be expected. There was interaction between the insecticides and the months, and this indicated that the differences between lindane and chlorthion were not constant from month to month.

During the second and sixth month, chlorthion gave higher control than lindane. An analysis of Table 15 E showed that there was interaction between evaluating methods and months. The bushel lots gave a higher kill during the first month only. The differences between the low and high dosage levels for both insecticides fluctuated from month to month (Table 14 B). No conclusions could be drawn on whether the low or high dosage levels of the insecticides would decrease in toxicity at an even rate.

When looking at the tables dealing with months x insecticides, months x levels, and months x methods, it can be observed that lindane, the bushel-lot method, and the high dosage level, decreased in toxicity at a more even rate than chlorthion, the pint-lot method, and the low dosage level. Table 14 C shows that there was interaction between months, insecticides, and levels. It can be surmised, from this interaction, that the differences in control between dosage levels for each insecticide, and the differences in control between insecticides changed from month to month.

The average per cent controls of Tribolium confusum for the 4 replicates in the treatment combinations, later than 6 months, are recorded in Table 9. At the end of these later periods, lindane applied to bushel lots of wheat still gave the higher control. The average per cent control by chlorthion increased some when the final counts were made at the end of 7 months.

Table 10 shows the average per cent control (4 replicates) of granary weevils in pint subsamples removed from 2-bushel lots of

Percent control?

TABLE 9. CONTROL OF *TRIBOLIUM CONFUSUM* BY LINDANE AND CHLORTHION APPLIED TO PINT AND BUSHEL LOTS OF WHEAT AT TWO DOSAGE LEVELS

Months	Pint-Lots Method				Bushel-Lots Method			
	Dosages of Lindane		Dosages of Chlorthion		Dosages of Lindane		Dosages of Chlorthion	
	Mg Per Pint		Mg Per Pint		Mg Per [redacted] BUSHEL		Mg Per [redacted] BUSHEL	
	9.4 Mg	18.7 Mg	4.8 Mg	9.6 Mg	598 Mg	1.196 Mg	307 Mg	614 Mg
1	99.0%	99.1	85.8	88.9	100.0	99.0	89.9	87.4
2	90.8	99.9	98.1	99.6	95.4	87.5	85.9	98.6
3	84.6	96.8	93.3	98.6	97.9	96.5	63.8	81.4
4	66.9	99.2	91.2	99.2	83.1	99.9	33.5	52.8
5	47.6	61.3	70.2	92.9	78.1	93.9	15.2	27.9
6	10.6	85.2	21.5	67.8	41.2	97.3	8.8	71.9
7	7.0	71.5	59.5	83.3	41.7	97.9		
8	6.9	82.1			31.6	96.0		
9		27.8			67.9	89.9		
10					6.3			
11					19.0			

A.T.

wheat. The wheat had been sprayed with lindane or chlorthion 4 months previous to the addition of weevils. Lindane at 598 mg and 1,196 mg per bushel gave a 100 per cent kill of granary weevils for 9 months. Neither dosage of chlorthion gave a 100 per cent kill at any time for all 4 replicates.

TABLE 10. CONTROL OF GRANARY WEEVILS IN PINT SUBSAMPLES REMOVED FROM TWO-BUSHEL LOTS OF WHEAT (TRIAL TWO)

Treatment Mg Per Bushel	Average Per Cent Control At The End of (Months)							
	4	5	6	7	8	9	10	11
Lindane 598	100	100	100	100	100	100	100	100
Lindane 1,196	100	100	100	100	100	100		

	Average Per Cent Control At The End of (Months)			
	4	5	6	7
Chlorthion 307	89.1 ± 5.8	73.4 ± 7.1	79.4 ± 9.6	72.4 ± 6.3
Chlorthion 614	99.7 ± 0.3	96.7 ± 1.4	99.6 ± 0.5	99.0 ± 0.1

Lindane seems more toxic to granary weevils than chlorthion when applied to bushel lots of wheat. Lindane at 598 mg gave higher kills than chlorthion at 614 mg per bushel (Table 10).

After the granary weevils had been removed at the end of every monthly test period, the subsamples were placed on their respective bins. Then, 2-3 months later they were checked for emerging adults.

Table 11 presents data on the results in each replicate at the end of those periods. No living or emerging adult weevils were found in wheat treated with lindane, but the related checks contained many healthy beetles. Emergence was more evident in wheat treated with chlorthion at 307 mg per bushel than double this dosage. Lindane at both dosages eliminated any development of eggs that might have been laid in the subsamples during the 7 day exposure period to adult granary weevils. Chlorthion gave no protection to the wheat at the dosages applied. Occasional dead weevils were found in the lindane treated subsamples. These were probably due to poor sifting.

TABLE 11. DEVELOPMENT OF GRANARY WEEVILS FROM EGGS LAID IN PINT SUBSAMPLES REMOVED FROM TWO-BUSHEL LOTS OF WHEAT (TRIAL TWO)

Treatment Mg Per Bushel	Rep.	Age of Treatment When Weevils Were Introduced (Months). 4	5	6	7	8	9	10	11
Lindane 598	A	no	no	no	no	no	no	no	no
	B	no	no	no	no	no	no	no	no
	C	no	no	no	no	no	no	no	no
	D	no	no	no	no	no	no	no	no
Checks	A	yes	yes	yes	yes	yes	yes	yes	yes
	B	yes	yes	yes	yes	yes	yes	yes	yes
	C	yes	yes	yes	yes	yes	yes	yes	yes
	D	yes	yes	yes	yes	yes	yes	yes	yes
Lindane 1,196	A	no	no	no	no	no	no		
	B	no	no	no	no	no	no		
	C	no	no	no	no	no	no		
	D	no	no	no	no	no	no		
Checks	A	yes	yes	yes	yes	yes	yes		
	B	yes	yes	yes	yes	yes	yes		
	C	yes	yes	yes	yes	yes	yes		
	D	yes	yes	yes	yes	yes	yes		
Chlorthion 614	A	yes	yes	yes	yes				
	B	yes	yes	yes	no				
	C	yes	no	yes	no				
	D	yes	yes	yes	nc				
Chlorthion 307	A	yes	yes	yes	yes				
	B	yes	yes	yes	yes				
	C	yes	yes	yes	yes				
	D	yes	yes	yes	yes				
Checks For Both Levels of Chlorthion	A	yes	yes	yes	yes				
	B	yes	yes	yes	yes				
	C	yes	yes	yes	yes				
	D	yes	yes	yes	yes				

FUMIGANT ACTION OF LINDANE

Experiments have shown that lindane acts as a fumigant under certain conditions, but has a relatively low vapor pressure compared to other fumigants. In 1949 Smallman (27, pp. 596-601) found that because of this slow volatilization process, lindane did not prove to be a good spot fumigant when tested in elevator boots.

Conditions in stored grain seem to be right for the vapors of lindane to play a part in the control of insects. The vapor pressure, although low, has been known to increase at least 4.5 times with a 10°C rise in temperature (2, p. 54). Through correspondence with another worker, Fiedler (10, p. 118) was told that lindane residues placed on filter paper or frosted glass were not capable of causing a 100 per cent kill of Sitophilus spp. within 5 days after they had been in direct contact with the residues for 24 hours. The same dosage on grain gave complete mortality within 24 hours. A strong fumigant action must have been taking place within the treated grain. Fiedler concluded from further studies that the mortality rate of weevils is dependent on amount of lindane per unit air, and the temperature.

A lot of work has been done with lindane in the United Kingdom and it has been found (21, pp.104-111) that the vapors of lindane have made interpretation of results from laboratory tests very difficult. It was almost impossible to parallel conditions of exposure and variable ventilation which would occur in the field.

McIntosh (18, pp. 137-139) performed simple laboratory fumigation tests with lindane on Oryzaephilus surinamensis and Tribolium castaneum, at 30°C and 11°C. He killed Oryzaephilus surinamensis faster at 11°C than at 30°C, but killed Tribolium castaneum faster at 30°C than at 11°C.

Wheat, after storage for 6 months in an atmosphere saturated with the vapors of lindane, retained sufficient sorbed insecticide to control adult granary weevils (20, pp. 639-646).

To test the effectiveness of any fumigant action going on within the treated wheat placed in the bins, it was necessary to devise some simple sampling method. Before any fumigant action of lindane in the bins was examined, a test was performed to see if enough toxic vapors were given off at the dosages applied to kill both insects. The method of preparing this test follows: Salve tins, 1 inch high, and 2½ inches in diameter had fine-mesh, brass, straining cloth soldered on in place of their cellulose lids. In each of the tins were placed, 15 cc of untreated wheat, 50 confused flour beetles, and 50 granary weevils. Lindane at 18.7 mg in 10 cc of water was sprayed on each of 5 pints of sifted wheat. About 4½ pints of the treated wheat were placed in a deep glass dish. This filled the dish to within a ½ an inch from its lip. The salve tins were sunk into the wheat until the wheat came up to the brass screening. No wheat was allowed to cover the tops of the tins. A check was prepared by the same procedure with untreated wheat sprayed with 10 cc of water. A flat piece of glass laid on top of the dishes formed a tight cover.

The tins were left in the treatment and check for 7 days before mortality counts were made. The tins were washed, and fresh wheat and test insects were added. They were then placed back in the dishes as before for another 7 days. Temperatures ranged from 78° - 80°F during the 7 day exposure periods. Mortality counts for the first week showed 100 per cent kill of both insects, and these results were repeated one week later.

This brief experiment indicated that the fumigant action of lindane, at 18.7 mg per pint, in a closed container was toxic enough to kill both species of insects.

To sample the fumigant action of lindane at various dosages on 2-bushel lots of wheat placed in bins, 15 more salve tins were prepared as described previously. Fifteen cc of untreated wheat was again placed in each, along with 25 confused flour beetles and 25 granary weevils. The assumption was that if the insects could not come in contact with the treated wheat while in the tins, any found dead at the end of 7 days would most likely have been killed by lindane vapors coming down through the brass straining cloth.

The tins were placed in the treated 2-bushel lots, 2 days after the wheat had been sprayed. Three infested salve tins were placed in each of the 4 bins comprizing a treatment - one tin was placed on the bottom, another in the middle, and the third, about 2 inches under the surface of the wheat. One bin of untreated wheat was used for the check. The degree of fumigant action was then sampled once every month after the initial test.

The control of confused flour beetles and granary weevils, by the fumigant action of lindane at 598 mg and 1196 mg per bushel, can be seen in Table 12. The raw data showed that there was no appreciable difference in per cent control between the different depths at which the salve tins were sunk in the wheat. It was expected that the highest mortality would be found in the tins near the surface because of the vapors rising.

The per cent control of both insects in the treatments was quite variable between replicate bins for some months. This is indicated by high standard deviations. Lindane at 1,196 mg per bushel exhibits a gradual decrease in control, without too many erratic fluctuations, from month to month. While lindane at 598 mg per bushel gradually decreases in toxicity, more fluctuations, probably due to the lower dosage, are present.

For the 2 months that 100 per cent control of granary weevils was obtained, the high percentage of mortality in the checks should be taken into account.

The fumigant action of lindane seems to increase with an increase in dosage, because higher control of both insects occurred at the greater dosage level of the insecticide. Some of the fluctuations in control from month to month were probably due to changes in temperature. During the nine months observations were made, temperatures ranged from 60-75°F.

TABLE 12. CONTROL OF CONFUSED FLOUR BEETLES AND GRANARY WEEVILS PLACED IN TWO BUSHEL LOTS OF WHEAT TO TEST THE FUMIGANT ACTION OF LINDANE

Lindane Mg Per Bushel	Control of Flour Beetles in Four Bins At The End of (Months):								
	1	2	3	4	5	6	7	8	9
598 Mg	65.6 ± 16	10.5 ± 9.0	50.0 ± 16	3.8 ± 2.0	11.8 ± 11	0.7 ± 1.3	0		
Mortality In Checks	0	0	0	1.3	1.3	0	0		
1,196 Mg	81.6 ± 6.7	52.4 ± 14	36.0 ± 14	15.0 ± 7.1	3.6 ± 1.9	1.3 ± 1.9	0.6 ± 0.7		
Mortality In Checks	0	1.3	0	0	0	1.3	0		
	Control of Granary Weevils								
598 Mg	100	76.3 ± 5.3	100	68.0 ± 21	83.3 ± 8.4	72.2 ± 7.0	19.7 ± 7.0	94.7 ± 4.3	76.8 ± 13
Mortality In Checks	46.9	0	21.3	5.3	1.3	4.0	0	0	6.7
1,196 Mg	96.9 ± 2.5	90.6 ± 4.3	98.4 ± 1.8	89.9 ± 5.1	98.1 ± 1.7	89.6 ± 4.1	34.4 ± 11	93.7 ± 4.3	74.6 ± 19
Mortality In Checks		0	1.3	0	0	0	0	0	5.3

DISCUSSION

The main factors covered in these experiments, that would affect the evaluation of grain protectants in the laboratory, were the size of the lots of grain to be treated, and the methods of sampling the toxic action of the insecticides after they were applied to the grain.

In Trial Two, the pint-lot method gave the higher per cent control of T. confusum. This high rate of control was correlated with chlorthion, which continually caused high mortalities at both dosage levels by this method. Lindane gave higher control by the bushel-lots method. The fumigant action of lindane may have played a part here. A uniform application of lindane on the larger quantities of wheat may not have been as important, due to the ability of its vapors to permeate throughout the bulk. A certain amount of recrystallization might have taken place on unsprayed kernels, or the wheat in the subsamples was able to give off enough vapor in 7 days to add to the kill by contact. The lindane applied to pint lots might have broken down faster because the lots were sifted every month. Also, there was probably less vapor present because of the smaller amount of wheat per jar. With chlorthion, a more uniform application may be needed on larger quantities of wheat to insure kill by contact. A more uniform coverage of the individual kernels is probably obtained when pint lots are sprayed.

Table 14 A shows that for 6 months lindane gave a higher control of *T. confusum* than chlorthion. This was the result of combining and analyzing data gathered for the two insecticides applied to different amounts of wheat at two dosage levels. The statistics are misleading because the amounts of toxicant at each dosage level were not the same for both insecticides. Table 2 shows that chlorthion gave higher control of *T. confusum* than lindane, with about half as much insecticide. Table 3 indicates almost double the control by chlorthion at the same dosage as lindane. Table 9 gives evidence that chlorthion at 4.8 mg per pint gave higher controls every month than lindane at 9.4 mg per pint.

Experiments with the fumigant action of lindane illustrated that the vapors played an important role in the control of *T. confusum* and *S. granarius* in 2-bushel lots of wheat. Not much reliability can be placed on the sampling method used. Other factors that could have contributed to the mortalities of both insects were not ruled out. A certain amount of toxic dust could have filtered through the brass cloth and aided in killing.

Factors that favor the bushel-lots method of evaluation are: The larger bulk of treated grain in a storage unit, 128 pints compared to a $\frac{1}{2}$ pint for the pint-lot method, resembles more closely conditions found in the field. Another factor is that the moisture content of the grain in the bins is more stable. A low moisture content usually results in higher control and stops egg laying. Bushel lots of treated wheat supply more material to work with and fresh subsamples can be used each month. Fumigant action, if present, can be detected more easily. Test insects that lay eggs in the kernels of grain can be used, for the subsamples can be discarded or put aside.

Some bad features of the bushel lots are that they take up a great deal of space, although, 24 bins when pushed together only take up 9 x 6 feet of floor space. This would allow 5 different treatments plus a check, replicated 4 times, to be evaluated. The bushel-lots method is more expensive and takes longer to set up. Once prepared, it does not require much more time to evaluate treatments than the pint-lot method. By subjecting subsamples to a higher temperature than the wheat in the bins, a difference in per cent mortality might occur. This would not give a true picture of what was happening in the bins.

Pint lots of grain are easily treated in the laboratory within a short time. Many treatments can be evaluated at once, for jars do not require as much space as bins. Also, experiments can be replicated more often because of less expense, less time, and less space. With the pint-lot method it is possible to underrate insecticides, such as lindane, which posses fumigant action. The smaller amount of grain also would lose moisture faster from continued sifting. If the residual action of an insecticide was to be tested against insects that laid eggs in the kernels of grain, many extra replicates of a treatment would have to be prepared at the same time. Exposed samples would have to be discarded when they became contaminated with emerging adults.

Within the limits of this experiment, no conclusion can be drawn on what method of evaluation was the most informative. If lindane and chlorthion had both given higher controls by one method, a conclusion might have been reached. There was not enough difference between the results obtained by the treatment of pint lots of wheat and the treatment of bushel lots, to recommend the use of the bushel-lots method of evaluation. It is reasonable to expect, though, that large amounts of grain treated in the field will not have insecticides applied uniformly throughout their bulks. The writer feels that the evaluation of grain protectants sprayed on bushel lots would give a more realistic picture of what the protectants would be able to accomplish outside the laboratory. If work was to be continued with the bushel-lots method of evaluation, it might be possible to devise a better sampling method of the treated grain in the bins. A method is needed that exposes the insects to conditions within the bins.

Other factors that affect the evaluation of grain protectants were taken into account during the experiment. These factors were: The moisture content of the grain, temperatures of the grain, ages and sexes of insects, susceptibility of different species of insects to insecticides used, and age of treatments.

An example of temperatures probably being the major cause of a difference between results, can be illustrated when Tables 6 and 9 are compared. Lindane, at 598 mg per bushel, in Trial One (Table 6), gave an average of 46 and 7 per cent control of _T. confusum_ at the end of 5 and 6 months respectively. Lindane, at the same dosage, in Trial Two (Table 9) gave 78 and 41 per cent control at the end of 5 and 6 months. The infested subsamples in Trial Two were kept at a temperature of 80°F for 7 days, instead of about 65°F, as in Trial One.

The susceptibility of different age groups or sexes of both species of insects were not subjected to specific tests with the insecticides evaluated. Other workers (32, pp. 121-125) have shown that these factors can cause variability in results. Marked fluctuations in per cent control of *T. confusum* (Table 9), as the treatments aged, are probably due mostly to the differences in ages of the insects from month to month.

SUMMARY

At present, laboratory evaluation of grain protectants consists of applying insecticides to pint or quart lots of grain. The treated grain is placed in jars and periodically exposed to insects. Treatment of bushel lots of grain was considered because it was felt that this process would more closely approximate field conditions.

To compare several emulsifiable concentrates, preliminary tests, using pint lots of wheat, were carried out. The insecticides tested were: Diazinon, lindane, Pyrenone, lindane-Aroclor, molathion and chlorthion. The pints of treated wheat were split into ½-pint lots and infested with Tribolium confusum for 7 day exposure periods. Diazinon gave the highest control of T. confusum at the end of 7 days and chlorthion gave the highest at the end of 49 days. Pyrenone and lindane had already been investigated as grain protectants by other workers and showed prospects of having long residual effects. Chlorthion proved to be the most promising of the 3 newer insecticides with potentialities of being used as grain protectants. For these reasons chlorthion, lindane, and Pyrenone were chosen for further evaluation by treatment of pint and bushel lots of grain.

Various dosages of lindane and Pyrenone were applied to 8-bushel lots of wheat, which were later split up into 2-bushel lots and placed in wooden bins. Weevils and confused flour beetles were placed directly in the bins. Methods of sampling the toxic action of the insecticides on the wheat in the bins were inadequate at

first. The number of dead and live insects recovered showed no relationship to the expected relative efficiency of the insecticides. Sifting the wheat out of the bins by layers revealed no difference between treatments in the vertical distribution of the insects. Most of the dead insects were in the top 3 inches. The confused flour beetles distributed themselves throughout the wheat in a way that would make obtaining adequate estimates of their control impractical. Pint subsamples removed at monthly intervals from the bins, and then infested with insects, proved to be the easiest and most significant method of evaluation.

Results from the removal of pint subsamples showed that lindane, at 598 mg per bushel, gave 46 per cent control of T. confusum at the end of 5 months, and 100 per cent control of Sitophilus granarius for 12 months. Pyrenone, at 39,593 mg per bushel, only gave 1.1 per cent control of T. confusum at the end of 5 months, but gave almost 100 per cent control of S. granarius for 12 months. It was concluded that Pyrenone was inferior to lindane in respect to its residual effects against T. confusum and its toxicity to both insects.

Fresh 8-bushel lots of wheat were treated with two dosages of lindane and chlorthion. A series of pint lots were treated with equivalent dosages. The results from the treatment of pint lots were to be compared with the corresponding bushel lots. Pint subsamples were removed at random from the wheat in the bins at monthly intervals. The subsamples and ½-pint lots were infested with T. confusum and placed in a temperature controlled cabinet for 7 days at 80°F.

At the end of 4 months, granary weevils were also placed in the pint subsamples. The subsamples were retained for 2-3 months and then checked for emerging adult weevils.

The data recorded over a 6 month period for lindane and chlorthion applied to pint and bushel lots of wheat, for the control of *T. confusum*, was arranged in a split-plot design. A factorial analysis was carried out because of the arrangement of data. Analysis showed that there was no difference between the 4 replicates, that lindane gave higher control of *T. confusum* than chlorthion at both dosage levels, and that the pint-lot method gave the higher control for 6 months at both dosage levels for each insecticide. Chlorthion gave the higher control of *T. confusum* by the pint-lot method, while lindane gave the higher kill in the bushel lots. Chlorthion was shown to be more toxic to *T. confusum* than lindane, in all experiments, when applied to pint lots of grain. Lindane, at 598 mg per bushel, gave complete protection from granary weevils to wheat in pint subsamples for 11 months, both times it was applied to 8-bushel lots of wheat. Chlorthion, at 307 and 614 mg per bushel, did not control the weevils 100 per cent. Adult weevils emerged from all the pint subsamples of chlorthion treated wheat that had been previously exposed for 7 days to weevils.

The fumigant action of lindane was tested by placing both species of insects in salve tins that had brass cloth soldered on as lids. Three tins were positioned at different levels in each bin of wheat and left for 7 days. The assumption was that the vapors would go down through the cloth and kill the insects. Results showed that the fumigant action of lindane aided in killing insects within the bins. There was no appreciable difference in per cent control at the different levels within the wheat.

The second time lindane was applied to 8-bushel lots of wheat, at 598 mg per bushel, it gave higher controls of *T. confusum* at the end of 5 and 6 months. This was attributed to a 15°F rise in temperature.

No conclusion could be drawn on what method of evaluation was the most informative, because the insecticides did not give the same rate of control by one method. The differences in control between the two methods were not great enough to justify the use of the bushel-lots method over the pint-lot-method of evaluation.

BIBLIOGRAPHY

1. Abbott, W. S. A method of computing the effectiveness of an insecticide. Journal of economic entomology 46:265-267. 1925.

2. Balson, E. W. Studies in vapour pressure measurements - Part 3. Transactions of the Faraday Society 43:54-60. 1947.

3. Bovington, H. H. S. The persistence of pyrethrin and the gamma isomer of BHC. Nature 163:731-732. 1949.

4. Brown, A. W. A. Insect control by chemicals. New York, John Wiley and Sons, 1951. 817p.

5. Chao, Yung-Chang, and D. M. DeLong. Stability of lindane and Pyrenone impregnated dusts and their relative toxicity to the granary weevil. Journal of economic entomology 46:908-910. 1953.

6. Cochran, W. G. and G. M. Cox. Experimental designs. New York, John Wiley and Sonc, 1950. 454p.

7. Cotton, R. T. Insect pests of stored grain and grain products; identification, habits, and methods of control. Rev. ed. Minneapolis, Burgess, 1950. 244p.

8. Cotton, R. T. and W. Ashby. Insect pests of stored grains and seed. In U. S. Dept. of agriculture. Insects; the yearbook of agriculture, 1952. Washington, U. S. government printing office, 1952. pp. 629-639.

9. Eden, W. G. Control of rice weevils in corn with protectant dusts and sprays. Journal of economic entomology 46:1105-1107. 1953.

10. Fiedler, O. G. H. Lindane dust for grain weevil control. Journal of the entomological society of South Africa 17:115-122. 1954.

11. Floyd, E. H. and C. E. Smith. The effects of pyrethrum and lindane in the protection of corn and rough rice from stored grain insects. Journal of economic entomology 46:771-774. 1953.

12. Haller, H. L. Common names of insecticides for use in the journal of economic entomology. Journal of economic entomology 48:112-114. 1955.

13. Hidetsugu, I. and K. Ozaki. Survival and propagation of rice weevils and Angoumois grain moths on barley and wheat from crops treated with BHC during the period from heading to maturing. Botyu kagaku 18:93-99. 1953. (Abstracted in Chemical abstracts 48: 321e. 1954)

14. Howe, W. L., W. T. Schroeder and K. G. Swenson. Seed treatment for control of seed-corn maggot and seed decay organisms. Geneva, Cornell university, 1952. 34p (New York, Agricultural experiment station. Bulletin no. 752)

15. Laudani, H. and G. R. Swank. A laboratory apparatus for determining repellency of pyrethrum when applied to grain. Journal of economic entomology 47:1104-1107. 1954.

16. Lindgren, D. L., H. E. Krohne, and L. E. Vincent. Malathion and chlorthion for control of insects infesting stored grain. Journal of economic entomology 47:705-706. 1954.

17. Mathlein, R. The development of resistance to DDT in the grain weevil Calandra granaria. Statens Vaxtskyddsanstalt, Meddelande 62:1-20. 1952. (Abstracted in Chemical abstracts 48:7248f. 1954)

18. McIntosh, A. H. Temperature coefficients of insect kill by volatile solid insecticides. Bulletin of entomology research 45:137-139. 1954.

19. Metcalf, C. L. and W. P. Flint. Destructive and useful insects. 2d ed. New York, McGraw-Hill, 1939. 979p.

20. Nasir, M. Responses of pests to fumigation. V. The toxicity of the free and sorbed vapours of BHC and DDT to some insects infecting stored products. Bulletin of entomological research 45:639-646. 1954.

21. Parkin, E. A. Progress in the control of insects infesting stored foods. Annals of applied biology 42:104-111. (Proceedings of the 1954 jubilee meeting)

22. Park, T. Observations on the general biology of the flour beetle, Tribolium confusum. Quarterly review of biology 9:36-53. 1934.

23. Pelley, R. L. and S. Kockum. Experiments in the use of insecticides for the protection of grain in storage. Bulletin of entomological research 45:295-311. 1954.

24. Peterson, A. A manual of antomological techniques. 9th ed. Ann Arbor, Edwards Brothers, 1953. 367p.

25. Schroeder, H. O. Some factors influencing the effectiveness of piperonyl butoxide-pyrethrins combinations for the control of insects in stored grains. Journal of economic entomology 48-25-27. 1955.

26. Slade, R. E. The gamma isomer of hexachlorocyclohexane (Gammexane). An insecticide with outstanding properties. The Hunter memorial lecture to Liverpool section, March 8, 1945. Chemistry and industry 40:314-319. 1945.

27. Smallman, B. N. Residual action of low vapor pressure fumigants. Journal of economic entomology 42:596-601. 1949.

28. Snedecor, G. W. Statistical methods. 4th ed. Ames, The Iowa State College Press, 1950. 485p.

29. Tanner, C.R. Report on the use of wheat protectants as a means of preventing insect infestations in stored wheat. Corvallis, Agricultural research foundation, Oregon agricultural experiment station, In cooperation with U. S. industrial chemicals company, Division of national distillers products corporation, New York, New York, 1953. 17 numb. leaves.

30. Watts, C. N. and F. D. Berlin. Piperonyl butoxide and pyrethrins to control rice weevils. Journal of economic entomology 43:371. 1950.

31. Wilbur, D. A. Effectiveness of dusts containing piperonyl butoxide and pyrethrins in protecting wheat against insects. Journal of economic entomology 45:913-920. 1952.

32. Wilbur, D. A. Effect of the moisture content of wheat on the toxicity of insecticidal dusts containing piperonyl butoxide and pyrethrins. Journal of the Kansas entomological society 24:121-125. 1952.

33. Wolfram, R. Dusting stored grain with contact insecticides. Anzeiger fur Schadlingskunde 25:73-75. 1952. (Abstracted in Review of applied entomology, Ser. A 42:60. 1954.

APPENDIX

TABLE 13. ANALYSIS OF VARIANCE AT THE FIVE PER CENT LEVEL OF SIGNIFICANCE

Variation Due To:	Sum of Squares	D.F.	Mean Square	F
Replications	130.170	3	43.39	N.S.
Insecticides	3846.800	1	3846.80	79.27
Levels	10725.280	1	10725.28	221.00
Insecticides x Levels	4.039	1	4.04	N.S.
Methods	767.160	1	767.16	15.81
Methods x Insecticides	7396.733	1	7396.73	152.42
Methods x Levels	54.709	1	54.71	N.S.
Methods x Levels x Insecticides	679.019	1	679.02	13.99
Error (a)	1019.100	21	48.53	
Whole Plot Total	24623.010	31		
Months	34606.915	5	6921.38	644.69
Months x Insecticides	2564.752	5	512.95	47.78
Months x Levels	5961.871	5	1192.37	111.06
Months x Levels x Insecticides	1831.536	5	366.31	34.12
Months x Methods	866.233	5	173.25	16.14
Months x Methods x Treatments	5622.806	15	374.85	34.92
Error (b)	1288.371	120	10.74	
Total	77365.494	191		

$$\text{Whole Plot Coefficient of Variation} = \frac{(\sqrt{48.53})(192)}{12735.39} = 10.5\%$$

$$\text{Sub Plots Coefficient of Variation} = \frac{(\sqrt{10.736})(192)}{12735.39} = 4.9\%$$

TABLE 14. TABLES OF MEAN PER CENT CONTROLS RETRANSFORMED FROM ARC SIN ANGLES USED IN FACTORIAL ANALYSIS

A

Insecticides x Levels

Dosage Levels	Insectisides: Lindane	Insectisides: Chlorthion	Level Means
Low	80.0	65.8	73.3
High	95.8	87.7	92.2
Insecticide Means	89.2	77.7	

B

Months x Levels

Months	Dosage Levels: Low	Dosage Levels: High	Month Means
1	96.0	95.2	95.6
2	93.6	98.2	96.3
3	87.4	97.3	93.3
4	94.0	97.5	87.5
5	52.9	78.5	66.3
6	19.0	73.7	45.6
Level Means	73.3	92.2	

C

Months x Insecticides x Levels

Insecticides	Dosage Levels	Months 1	2	3	4	5	6	Level Means	Insecticide Means
Lindane	Low	99.8	93.8	92.7	76.5	64.5	24.1	80.0	89.2
	High	99.1	96.5	96.7	99.7	80.5	92.8	95.8	
Chlorthion	Low	88.0	93.5	81.0	65.7	41.0	14.4	65.8	77.7
	High	88.3	99.4	98.0	93.2	76.5	47.8	87.7	
Month Means		95.6	96.3	93.3	87.5	66.3	45.6		

TABLE 15. TABLES OF MEAN PER CENT CONTROL RETRANSFORMED FROM ARC SIN ANGLES USED IN FACTORIAL ANALYSIS (CONTINUED)

D

Months x Insecticides

Months	Insecticides: Lindane	Insecticides: Chlorthion	Month Means
1	99.5	88.1	95.6
2	95.2	97.2	96.3
3	94.9	91.4	93.3
4	92.4	81.5	87.5
5	72.9	59.4	66.3
6	62.0	99.2	45.6
Insecticide Means	89.2	77.7	

E

Months x Methods

Months	Methods: Pint Lots	Methods: Bushel Lots	Month Means
1	94.9	96.2	95.6
2	98.3	93.4	96.3
3	94.6	91.8	93.3
4	92.9	80.7	87.5
5	70.1	62.4	66.3
6	45.5	45.7	45.6
Method Means	86.4	81.2	

F

Methods x Insecticides

Insecticides	Methods: Pint Lots	Methods: Bushel Lots	Insecticide Means
Lindane	84.2	93.3	89.2
Chlorthion	88.4	64.9	77.7
Method Means	86.4	81.2	

G

Methods x Dosage Levels

Dosage Levels	Methods: Pint Lots	Methods: Bushel Lots	Level Means
Low	75.5	70.9	73.3
High	94.4	89.7	92.2
Method Means	86.4	81.2	