determined for mevinphos and Stauffer N-2790 against black carpet beetle larvae, as preliminary tests indicated that these chemicals were relatively ineffective against this species. The dosage (mg/liter) required for the LD_{95}, depending on the species, varied from 0.66 to 32.73 for Zinophos, 1.81 to 4.72 for Ethide, 0.48 to 3.06 for *n*-butyl isothiocyanate, 1.68 to 2.23 for mevinphos, 0.89 to 4.11 for 2-propyn-1-ol, 1.21 to 3.82 for Stauffer N-2790, 0.32 to 4.14 for Virginia-Carolina 3-676, and 2.39 to 4.40 for methyl bromide.

REFERENCES CITED

Sokal, R. R. 1958. Probit analysis on a digital computer. J. Econ. Entomol. 51 (5) : 738–9.

Soles, R. L., and P. K. Harein. 1962. The fumigant toxicity of two new chemicals to stored-product insects. J. Econ. Entomol. 55 (6) : 1014–5.

Whitney, W. K., and P. K. Harein. 1959. Effects of number of test insects, exposure period, and posttreatment interval on reliability of fumigant bioassays. J. Econ. Entomol. 52 (5) : 942–9.

DDT-Metabolism and Excretion in *Coleomegilla maculata* De Geer[1]

YOUSEF H. ATALLAH[2] and W. C. NETTLES, JR.[3]

ABSTRACT

Adults of the lady beetle *Coleomegilla maculata* De Geer, were topically treated with 40 μg of DDT. DDT and DDE were extracted with acetonitrile, partitioned with n-hexane, and determined by gas-liquid chromatography using an electron capture detector. DDT was metabolized to DDE. DDE [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene] probably was the only metabolite of DDT, since most of the applied dose was accounted for as DDT and DDE. In addition to metabolizing DDT to DDE, these beetles resist DDT poisoning by excreting DDT and DDE in the feces and eggs; as much as 5 μg of DDT-DDE were present in an average egg mass and as much as 1.1 μg of DDT-DDE were excreted in the feces per day. For 3–4 days after DDT treatment, feces contained greater amounts of DDT than DDE; after 5 days the quantity of DDE exceeded that of DDT in both feces and eggs. When lady beetles were topically treated with 40 μg of DDT, the dead insects contained about 1.5 to 2 times as much DDT as the surviving individuals. A large part of this difference was probably caused by the metabolism of DDT to DDE, since during the first 5 days after treatment with DDT, the surviving beetles contained more DDE than the dead individuals. Resistance of *C. maculata* to DDT is due at least in part to its ability to metabolize DDT to DDE and to excrete these 2 compounds in feces and eggs.

The lady beetle *Coleomegilla maculata* De Geer, is an important predator of several pests in Louisiana. Since this beetle is highly resistant to DDT, this study was undertaken to determine the resistance mechanism. In many insects DDT is detoxified by metabolism to DDE (1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene).

Reports on the metabolism of DDT to DDE in resistant house flies, *Musca domestica* L., were first published by Sternburg et al. (1950) and Perry and Hoskins (1950). They studied the difference in absorption and metabolism of DDT in resistant and susceptible flies. Sternberg and Kearns (1952) studied the metabolism of DDT in 3 naturally tolerant insects and stated that in the differential grasshopper, *Melanoplus differentialis* (Thomas), large amounts of DDT appeared unchanged in the excreta when DDT was fed to it and smaller amounts of DDE were also excreted. The Mexican bean beetle, *Epilachna varivestis* Mulsant, degraded oral and topical dosages of DDT to DDE and appeared to convert DDE to an unidentified compound (s). Neither DDT nor DDE was excreted. The red-banded leaf roller, *Argyrotaenia velutinana* (Walker), degraded topical and oral dosages of DDT to DDE and both were excreted. Tahori and Hoskins (1953a, b) concluded that in house flies treated with DDT, survival seemed to depend upon reduction in the amount of DDT, and that DDT was metabolized to DDE, which was metabolized in turn to an unknown compound. Other investigations along the same lines proved that different species and even different instars have different mechanisms for the detoxication of DDT. Several unknown metabolites were reported (Brown 1960). Tsukamoto (1959) identified dicofol as a DDT metabolite in DDT-resistant strains of *Drosophila melanogaster* Meigen. He stated also that no DDE was found in the ether extract of resistant strains of *D. melanogaster* reared on media containing DDT; furthermore, these strains did not metabolize DDE. Agosin et al. (1961) found a microsomal enzyme system in the German cockroach, *Blattella germanica* (L.), capable of converting DDT to a dicofol-like product; they found also the enzyme (s) in the house fly and American cockroach, *Periplaneta americana* (L.). In nymphs of *Triatoma infestans* (Klug), 5 DDT metabolites were found by Dinamarca et al. (1962). Abedi et al. (1963) stated that DDE was the only metabolite of DDT in the yellow-fever mosquito, *Aedes aegypti* (L.). Kimura and Brown (1964) concluded that in *A. aegypti* the only DDT metabolite was DDE, except that highly resistant strains produced a small amount of a dicofol-like substance. Agosin et al. (1964) suggested that in addition to the metabolism of DDT to DDE by *T. infestans*, DDT was also metabolized to dicofol, which in turn was metabolized to an unidentified metabolite.

MATERIALS AND METHODS.—Hibernating *C. maculata* adults were collected from the bases of pecan trees in a cotton field near Boyce, La., and placed in ½-gal cylindrical cartons with artificial diet and water tubes (Atallah and Newsom, unpublished data) and held at 27°C under a 14-hr photoperiod provided by a 400-w, Cool White light giving 70 ft-c. *P,p* DDT isomer was prepared by recrystallation from ethanol. Each of 1500 beetles was topically treated on the scutellum with 1 μliter of acetone solution containing

[1] Accepted for publication January 24, 1966.
[2] Department of Entomology, Louisiana State University, Baton Rouge.
[3] Entomology Research Division, Agr. Res. Serv., USDA, Baton Rouge, La.

10 μg of DDT (LD_{30}). Fifteen treated beetles were placed in 1-pint cartons and provided with food and water. Two samples of 20 live beetles were collected at the following intervals after treatment: 0, 0.5, 1.5, 2.5, 3.67, 5.5, 8.5, 10.5, 12.5, 17.5, 24.5, 30.5, and 100 days. Dead beetles and fecal samples also were collected. Eggs were collected from treated, mated females. All samples were placed in 10 ml of acetonitrile and held at −18 ± 2°C until analyzed. Each sample was homogenized in a Virtis® homogenizer for 1 min, filtered under pressure through a fritted glass funnel into a 50-ml graduated cylinder. The homogenizer flask was washed twice each time with 3–5 ml acetonitrile and filtered through the same funnel into the same graduated cylinder. The filtrate was transferred to a 125-ml separatory funnel and 10 ml of n-hexane were measured in the same graduated cylinder used for the filtrate. The hexane was poured into the separatory funnel, then the funnel was shaken vigorously for 3–4 min. One ml of saturated sodium chloride and 60 ml of distilled water were added and mixed gently but thoroughly. The mixture was allowed to separate and the aqueous layer was discarded. The solvent layer was then washed twice with 10 ml of distilled water. The solvent was shaken vigorously after the addition of 1.5–2.0 g of anhydrous sodium sulfate. A gas chromatograph with an electron capture detector and a column of 5% Dow® 11 on acid and base washed Chromport® 60/80 support was used for separation and determination of the chlorinated hydrocarbons present in the extract. For confirmation of the results a column of 15% Dow® 200 and 1.7% Tween® 80 on acid and base washed and silanized Chromport support was used. If the extract had a high concentration of DDT and/or DDE, appropriate dilutions were made (1–2 ng/μliter). The amounts of DDT and DDE were calculated from a calibration curve. By this method about 98–100% of the DDT could be extracted from the acetonitrile solution when no insect specimens were involved. Results are presented as recovery per insect, egg, or larva. For the artificial diet results are presented as per g of diet. The methods of extraction are essentially those of Mills (1961), Johnson (1962), Onley and Mills (1962), Mills et al. (1963), and Blinn and Gunther (1963).

RESULTS.—Table 1 shows the rate of breakdown and excretion of DDT and the excretion of its metabolite DDE in the feces of live adults of *C. maculata*. Each number in the table is an average (per beetle) from 2 samples of 20 beetles each, and 4 portions were analyzed from each sample. The average weight of the beetles was 12.2 mg. The fecal samples were collected at the end of each sampling period, then calculated as the quantity of DDT and DDE excreted per beetle per day during this period. The amounts of DDT and DDE excreted were computed to account for the total excretion of DDT-DDE per beetle during the period preceding the collection of each sample. Therefore, the total amount of DDT-DDE recovered was obtained by adding the quantity of DDT-DDE present in the body to that calculated from the amount previously excreted in the feces.

The data provide strong evidence that DDE is the major DDT metabolite and that it is not further

Table 1.—Recovery of DDT and DDE from adult *C. maculata* surviving topical treatment with 40 μg of DDT/insect. DDE is expressed as DDT equivalents.

Time in days	μg/beetle[a]		μg in feces/beetle/day		Total as DDT[b] μg/beetle	Percent recovery	Ratio of DDT/DDE in body extract	DDT metabolized[b] to DDE	
	DDT	DDE	DDT	DDE				μg	%
			0.00	0.005[c]					
0.02	35.4	2.2			37.6	94.0	16.09	2.20	5.85
			.60	.110					
.50	30.6	5.0			36.0	90.0	6.12	5.06	14.06
			.60	.320					
1.50	20.1	13.2			34.6	86.5	1.52	13.58	39.25
			.50	.430					
2.50	17.7	14.0			33.9	84.8	1.26	14.81	43.69
			.50	.640					
3.67	16.0	16.3			36.0	90.0	0.98	17.87	49.64
			.30	.430					
5.50	10.8	14.9			30.5	76.3	.72	17.21	56.43
			.30	.430					
8.50	9.1	14.7			30.8	77.0	.62	18.30	59.42
			.25	.160					
12.50	7.1	8.7			24.5	61.3	.82	12.94	52.82
			.16	.210					
17.50	2.7	14.6			27.8	69.5	.19	19.89	71.55
			.10	.300					
24.50	2.7	14.6			30.6	76.5	.19	21.99	71.86
			.10	.290					
30.50	2.2	14.0			31.9	79.8	.16	23.13	72.51
			.05[e]	.170[e]					
100.00	0.1	2.1			33.3	83.3	.05	23.13	69.46
			.00[d]	.040					

[a] Determined from 2 samples consisting of 20 beetles/sample; 4 gas chromatographic analyses/sample.
[b] Calculated from amount present in body and previously excreted in feces.
[c] Trace present in feces of the check beetles. See Table 3.
[d] Unmeasurable traces.
[e] Average.

Table 2.—Recovery of DDT and DDE from adults of *C. maculata* which died at different periods after being topically treated with 40 μg of DDT. DDE is expressed as DDT equivalents.

Time in days	μg/beetle[a]		μg in feces/beetle/day		Total as DDT[b] μg/beetle	Percent recovery	Ratio of DDT/DDE in body extract	DDT metabolized[b] to DDE	
	DDT	DDE	DDT	DDE				μg	%
			0.6	0.32					
1.50	23.5	7.4			32.28	80.70	3.18	7.88	24.41
			.5	.55					
3.67	20.3	12.9			36.88	92.20	1.57	14.58	39.53
			.3	.43					
5.50	19.9	15.5			40.34	100.85	1.28	17.94	44.47
			.3	.43					
8.50	17.6	18.0			42.73	106.83	0.98	21.73	50.85
			.3	.20					
10.50	14.4	9.2			31.73	79.33	1.57	13.33	42.01
			.2	.21					
17.50	6.9	14.3			32.20	80.50	0.48	19.90	61.80
			.1	.30					
24.50	4.1	12.3			30.20	75.50	.33	20.00	66.23

[a] Determined from individuals which died at the end of the indicated period, e.g., 8.5 day specimens represent the individuals which died in the period from 5.5 to 8.5 days; each figure determined from 2 samples of 20–50 beetles/sample; 4 chromatographic analyses/sample.
[b] Calculated from amount present in body and previously excreted in feces.

metabolized since the total recovery of DDT was 83.3% 100 days after treatment. At this time 31.1 μg of DDT and DDE (transformed to DDT equivalents) were excreted in the feces. That is to say, during the 100 days following treatment each insect excreted 78% of the original topical application in the feces after metabolizing a large part of the DDT to DDE. The ratio of DDT to DDE excretion in the same period was 11.4 μg of DDT to 21.3 μg of DDE (transformed to DDT equivalents). Traces of 2 unknown compounds in samples collected during the 17½ days following application were detected in the beetle extracts. These unknowns were present also in the DDT used for the topical application. Table 2 shows the fate of DDT in adults of *C. maculata* which died at different periods after being topically treated with 40 μg of DDT. In Table 2 the data showing the amounts of DDT and DDE in the feces were calculated from live individuals, since it obviously was impossible to collect fecal samples from dead individuals. These calculated amounts may be more or less than the actual; however, this was the only way to determine the fate of DDT in the feces of the dead individuals. The fecal samples for each period included feces of some individuals which died later. The data from analyses of extractions of field-collected and laboratory-reared "untreated" adults, food, eggs, larvae, and feces are shown in Table 3.

DISCUSSION.—The resistance of this insect to DDT depends largely on the dehydrochlorination of this compound to DDE and the excretion of DDT and DDE in the feces. DDE appears to be the only DDT metabolite, since 100 days after the original application 83.3% of the original DDT dose could be accounted for. This is almost the same as the recovery rate during the first few days following the original application (76.3–90%, Table 1).

If there were another DDT metabolite which was not detected, the percentage recovery would decrease gradually with time. The same evidence indicates that DDE is the final breakdown product; in other words, DDE is not further metabolized. Apparently the rate of penetration of DDT through the integument is not an important factor in resistance, since

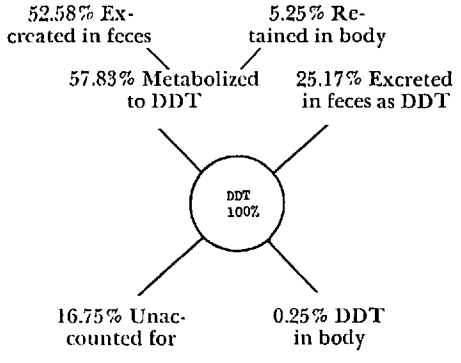
Table 3.—Amounts of DDT and DDE found in untreated field-collected adults, food, eggs, and feces. The data are expressed as μg/beetle, egg, or larva. For the analysis of food the data are presented as μg/g of diet. The avg wt of an egg is 138 μg.

Category	μg[a]/unit		Ratio of DDT/DDE	Ppm
	DDT	DDE		
Field-collected adults	0.0000	1.1000		90.1120
Field-collected adults kept in the lab for 100 days	.0000	0.2700		22.1220
Feces of field-collected adults	.0000	.0055		5.5000
Eggs of field-collected adults	.0000	.0034		24.6370
Food (artificial diet)	.0154	.1286	0.120	0.1286
Laboratory-reared adults	.0000	.0710		5.8190
Feces of lab-reared adults	.0000	.0006		0.6000
Eggs of lab-reared adults	.0000	.0030		21.7390
Larvae of lab-reared adults	.0000	.0049		
Eggs of treated females 5.5 days after treatment	.0050	.2314	.022	1676.8110
Eggs of treated females 10.5 days after treatment	.0000	.5067		3671.7390

[a] DDE is expressed as DDT equivalents.

the disappearance (metabolism and excretion) of DDT was much faster in the live than in moribund individuals (Tables 1 and 2). If resistance were due to slower penetration through the integument, the amount of DDT excreted from the body of live individuals would have been more, at least as much as in the dead individuals.

The following diagram illustrates the fate of a topical treatment of 40 μg DDT after 100 days, when each group of 15 treated beetles was placed in a 1-pint carton and provided with food and water (under such conditions the females are crowded and do not lay eggs). The 16.75% unaccounted for is probably due to (1) the loss of some DDT and DDE during the extraction procedure and/or (2) autohemorrhage (Happ and Eisner 1961) which is a phenomenon exhibited by adults and larvae of this species.



In addition to the excretion of DDT and DDE in the feces, treated females lay eggs containing relatively large amounts of the 2 compounds if the eggs are laid a short time after treatment. However, 10½ days after treatment the eggs contain DDE only. This phenomenon might indicate a high titer of DDT dehydrochlorinase in the gonads, which would agree with the research of Tombes and Forgash (1961), who found that DDT-dehydrochlorinase activity is very high in the gonads of the Mexican bean beetle. Eggs of untreated *C. maculata* females contained 3 ng DDE and no DDT. Eggs laid 5½ days after treatment contained 5 ng DDT and 231 ng DDE/egg (average egg wt 138 μg), while those laid at 10½ days contained 507 ng of DDE/egg and no DDT, Table 3. Since there was an average of 10 eggs/batch and 6 batches/female per month, and DDT treatment increases egg laying (Atallah and Newsom 1965, unpublished), a relatively tremendous amount of DDT and DDE was transferred to the eggs from the laying females (Table 3). Individuals which survived treatment had a faster rate of breakdown of DDT than the individuals which died from the treatment. This fact is clearly shown in Tables 1 and 2 by the faster "disappearance" of DDT and buildup of DDE. DDT was metabolized rapidly to DDE during the 5 days following treatment, then the rate declined gradually. It appears from the data that DDT metabolism reaches an equilibrium at a DDT/DDE ratio of 0.2 or less. Another possibility might be that DDT is tied up at sites where metabolism does not take place. For example, DDT may be concentrated in the fat body where there may be a low titer of DDT dehydrochlorinase. This condition would agree with the finding of Tombes and Forgash (1961) that the enzyme activity is higher in the gonads than in the fat body.

It is of interest to note that the food of the beetles (the artificial diet) contained 128 ng of DDE/g of the artificial diet (0.128 ppm); the amount of DDE was about 10 times that of DDT. However, this did not affect our results since beetles reared on the diet contained 71 ng DDE/beetle and no DDT, and the feces contained 0.6 ng DDE/beetle per day (Table 3). These amounts are not significant when compared with the amounts extracted from the treated beetles and their feces (Tables 1, 2, and 3). The eggs of laboratory-reared beetles contained 3 ng of DDE/egg, not a significant amount when compared with 231 or 507 ng DDE/egg after 5½ and 10½ days, respectively.

When reared in the laboratory (on the artificial diet) an egg, a larva, and an adult contain 3, 4.9, and 71 ng of DDE, respectively, implying that the adults accumulate DDE in their bodies about 23-fold more than that present in the eggs. Based on weight, the food, laboratory-reared adults, and their eggs contain 0.13, 5.82, and 21.74 ppm DDE. Therefore, the adults accumulated about 45 times as much DDE from the diet when it contains 0.13 ppm of DDE. The adults containing 5.82 ppm DDE lay eggs containing 21.74 ppm DDE, implying that DDE is not uniformly distributed in the adult's body and that 1 of the sites for accumulation of DDE is the gonads. However, 5½ days after topical treatment with 40 μg DDT the adults contain 2105 ppm DDT-DDE while their eggs contain 1675 ppm DDT-DDE; 10½ days after treatment the adults contain 1620 ppm DDT-DDE while their eggs contain 3670 ppm. Adults containing 22.1 ppm DDE laid eggs containing 24.6 ppm. It seems that there are certain limits for the accumulation of DDT in the gonads which cannot be exceeded regardless of the amount of DDT and/or DDE in the body. Obviously the amount of DDT-DDE in the body affects the amount of DDE in the gonads.

The quantity of DDE in the body of field-collected adults decreased from 90 ppm to 22 ppm in a 100-day period when fed on the artificial diet. Whether the beetle accumulates DDE from the diet into its body or excretes it depends on the concentration of DDE within its body and in the food. When the bodies contained less than 5.82 ppm DDE they concentrated DDE from a diet containing 0.13 ppm, while on the same diet the DDE level diminished from 90 to 22 ppm in a 100-day period (Table 3).

REFERENCES CITED

Abedi, Z. H., J. R. Duffy, and A. W. A. Brown. 1963. Dehydrochlorination and DDT-resistance in *Aedes aegypti*. J. Econ. Entomol. 56: 511–7.

Agosin, M., D. Michaeli, R. Miskus, S. Nagasawa, and W. M. Hoskins. 1961. A new DDT-metabolizing enzyme in the German cockroach. J. Econ. Entomol. 54: 340–2.

Agosin, M., A. Morello, and N. Scaramelli. 1964. Partial characterization of the in vivo metabolites of DDT-C^{14} in *Triatoma infestans*. J. Econ. Entomol. 57: 974–7.

Blinn, R. C., and F. A. Gunther. 1963. Rapid colorimetric determination of DDT in milk and butter. J. Ass. Offic. Agr. Chem. 46: 191–4.

Brown, A. W. A. 1960. Mechanisms of resistance against insecticides. Annu. Rev. Entomol. 5: 301–26.

Dinamarca, M. L., M. Agosin, and A. Neghme. 1962. The metabolic fate of C^{14}-DDT in *Triatoma infestans*. Exp. Parasitol. 12: 61–72.

Happ, G. M., and T. Eisner. 1961. Hemorrhage in a coccinellid beetle and its repellent effect on ants. Science 138: 329–31.

Johnson, L. 1962. Separation of dieldrin and endrin

from the other chlorinated pesticide residues. J. Ass. Offic. Agr. Chem. 45: 363–5.
Kimura, T., and A. W. A. Brown. 1964. DDT-dehydrochlorinase in *Aedes aegypti*. J. Econ. Entomol. 57: 710–6.
Mills, P. A. 1961. Collaborative study of certain chlorinated organic pesticides in dairy products. J. Ass. Offic. Agr. Chem. 44: 171–7.
Mills, P. A., T. H. Onley, and R. H. Gaither. 1963. Rapid method for chlorinated pesticide residues in nonfatty food. J. Ass. Offic. Agr. Chem. 46: 186–91.
Onley, J. H., and P. A. Mills. 1962. Detection and estimation of chlorinated pesticides in eggs. J. Ass. Offic. Agr. Chem. 45: 983–7.
Perry, A. S., and W. M. Hoskins. 1950. The detoxification of DDT by resistant houseflies and inhibition of this process by piperonyl cyclonene. Science 111: 600–1.
Sternburg, J., and C. W. Kearns. 1952. Metabolic fate of DDT when applied to certain naturally tolerant insects. J. Econ. Entomol. 45: 497–505.
Sternburg, J., C. W. Kearns, and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. J. Econ. Entomol. 43: 214–9.
Tahori, A. S., and W. M. Hoskins. 1953a. The absorption, distribution and metabolism of DDT in DDT-resistant houseflies. J. Econ. Entomol. 46: 302–6.
1953b. The absorption, distribution, and metabolism of DDT in DDT-resistant house flies. J. Econ. Entomol. 46: 829–37.
Tombes, A. S., and A. J. Forgash. 1961. DDT-dehydrochlorinase in the Mexican bean beetle, *Epilachna varivestis* Mulsant. J. Insect Physiol. 7: 216–23.
Tsukamoto, M. 1959. Metabolic fate of DDT in *Drosophila melanogaster*. I. Identification of a non-DDE metabolite. Botyu-Kagaku 24: 141–51.

Relationships of Insects to Hot Spots in Stored Wheat[1]

LLOYD E. EIGHME[2]
Department of Entomology, Oregon State University, Corvallis

ABSTRACT

Four species of insects were introduced singly and in various combinations into areas of fungus heating in stored wheat to determine their ability to induce or promote the spread of a hot spot. The granary weevil, *Sitophilus granarius* (L.), demonstrated the greatest capacity to initiate and promote the spread of hot spots in stored wheat; the lesser grain borer, *Rhyzopertha dominica* (F.) had considerably less. The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst), were ineffectual both in hot-spot initiation and in spread.

Heating in farm-stored grain results in deterioration of the product and the damage is increased by insects that are attracted to the centers of heating, known as hot spots.

Studies in England (Howe 1962) established the fact that insect-initiated hot spots were produced by metabolic heat from the insects, which accumulated more rapidly than it escaped from the immediate environment of the insect population. Christensen and Hodson (1960) showed that moisture produced by weevils in wheat raised the moisture content sufficiently to encourage the growth of storage fungi. When moisture vapor was not allowed to escape rapidly from the grain mass, peripheral portions of the mass showed an increase in moisture content with a corresponding increase in fungus growth, accompanied by heat production. Oxley and Howe (1944) thought the role of metabolic water in most insect infestations was insignificant, but that extreme densities of insects owing to some concentrating factor could raise the moisture content by several percent in 1 generation as a result of an accumulation of metabolic water. Sikorowski (1964) confirmed the attractiveness to insects of moldy wheat in hot spots in a study of food relationships between 3 species of insects and a series of storage fungi. A series of studies has been conducted to discover the insects, mites, and fungi associated with hot spots in wheat by Sinha (1961), Sinha et al. (1962), and Sinha and Wallace (1965).

A series of experiments was conducted in this study to determine the effects of insect infestation on fungus hot spots in stored wheat. These effects varied according to the species of insects present, and to the amount of moisture present.

METHODS AND MATERIALS.—Four species of insects commonly found in stored grain were used in this study. They were the granary weevil, *Sitophilus granarius* (L.); the lesser grain borer, *Rhyzopertha dominica* (F.); the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); and the red flour beetle, *Tribolium castaneum* (Herbst). These species were introduced singly and in various combinations into areas of fungus heating in stored wheat to determine their ability to induce or promote the spread of a hot spot. A series of 18 experiments was conducted in the laboratory on a small scale by the method described previously (Eighme 1964) in which a box containing 1.1 ft^3 of wheat was wired with 12 thermocouples and 4 RH elements connected to meters by a system of switches. In addition to the 18 laboratory experiments, a series of 4 field experiments was conducted on a larger scale with a system of 539 thermocouples and 64 RH elements evenly spaced on a framework 4×4×4 ft and buried in the center of 600 bu of wheat. The thermocouples were spaced horizontally at 6-in. intervals and vertically at 4-in. intervals, forming a grid within the grain mass. The RH elements were spaced horizontally at 12-in. intervals and vertically at 9-in. intervals and were supported by the thermocouple grid (Fig. 1). Temperature readings were taken with a temperature potentiometer con-

[1] Part of a doctoral dissertation completed at Oregon State University under the direction of Dr. G. W. Krantz. Accepted as Technical Paper no. 2033, Oregon State University Agricultural Experiment Station, Corvallis. This research was supported in part by the Federal Cooperative Regional Project WM-16. Accepted for publication January 12, 1966.
[2] Present address: Pacific Union College, Angwin, Calif.