

EFFECT OF INFESTATION STAGE, FORM AND TREATMENT
ON FRAGMENT COUNT IN FLOUR

by

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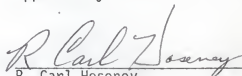
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INTRODUCTION

Even with the most sophisticated techniques used to store cereal grains during the post-harvest period it seems rather impossible to rid the grains completely of insect pests. Insect infestation has primarily been considered a factor in storage losses of grains and their products. It is comparatively recent that stored product insects gained importance as major contaminants and a source of micro-filth in flour and other cereal products.

Growing consumer consciousness of the presence of insect contamination in the final product provides a constant pressure on grain processors to reduce the contamination to an irreducible minimum.

A two phase study of insect fragments in hard red winter wheat flour is reported here. First, the level of fragments contributed by wheat kernels containing different developmental stages of the rice weevil, Sitophilus oryzae (L), was determined. Secondly, the effect of treating rice weevil infested wheat with an insecticide protectant versus a fumigant on insect fragment count in resultant flour was determined.

REVIEW OF LITERATURE

Researchers particularly in the Food and Drug Administration (FDA) have been concerned primarily with problems of adulteration and chemical residues in foods. Micro-analytical and micro-biological methods developed by the FDA provided both law enforcement officials and industry with the tools to detect contamination and adulteration (Troy 1958). The Federal Food, Drug and Cosmetic Act of 1938, sections 402(a) (3) and 402(a) (4) made inspection analyses and use of reliable sanitation techniques necessary to ensure wholesome food products (Harris 1957). This had a strong

impact on food handling and modified the good housekeeping concept. It was recognized that utmost care in the selection of raw materials was essential to prevent contributing filth to the finished product (Lennington 1957). Use of sound raw materials and adoption of good manufacturing practices during production were considered important to comply with the FDA regulations and to satisfy the more conscious consumer (Siegel 1957). USDA, FDA and agricultural universities such as Kansas State University investigated the origin of contaminants (i.e. insect fragments, rodent hair, etc.) in the final product. Harris et al. (1952) studied the relationship between insects detected in wheat and the resultant fragment count in flour. It was a complex relationship influenced by factors such as type of insect, whether alive or dead, type of wheat, the size and flow of the mill and the kind of sieving equipment used.

Farrell and Milner (1952) surveyed 109 flour mills in the United States to study the relationship of insect and rodent contamination in mill mixes and contamination in finished flour. Internal infestation in commercial wheat received at mills was found to be the principal source of insect fragments in flour. Hard red winter wheat showed the highest infestation level.

Wagner (1960) stated that living grain infesting insects would be shredded rather than shattered during milling and would form small dough balls that would tail off in the animal feed fraction. Contrarily, dead and dehydrated insects would be crushed into fine fragments and pass through bolting cloth with flour.

Harris (1950), Heuermann and Kurtz (1955), Jackson et al. (1956, 1958), Kurtz (1956), Heuermann (1957), Ensminger (1958) and Ensminger et al. (1967)

studied the micromorphology of insect fragments for identification as contaminants in the final products. They discussed the characters which distinguish insect fragments from those of plant structure, and tried to establish the morphological relationship of insect parts to the whole. These investigations, particularly with the insects infesting food grains, have been of diagnostic value in sanitation control work.

Burquest (1955) and Liscombe (1962) reported a direct correlation of ash content of mill stream with its insect fragments beyond certain infestation levels. Attempts to produce low fragment flour by diversion of some streams to lower grades resulted in loss of patent flour yield.

MATERIALS AND METHODS

Wheat: Hard red winter wheat was selected for insect fragment count experiments because it constitutes a large volume of the wheat milled for bakers flour where fragments are a major concern. The specific variety of wheat used was Parker (1976 Crop) grown near Alta Vista, Kansas. It was obtained from the Stored Product Insect Laboratory (SPIL), Department of Entomology, where it had been in cold storage at 5°C since the harvest in July 1976.

To assure the wheat was free of prior infestation, a representative sample was drawn and a Boerner sample divider used to take a 100 gram sample for infestation testing. Acid fuchsin stain (Frankenfeld 1948) was used to determine whether the wheat had been subjected to weevil infestation prior to this study. A modification of Frankenfeld's method was used and is described in Appendix A.

To further assure that test wheat was free of internal forms of insect infestation a 100 gram sample was radiographed using a General

Electric Grain Inspection Unit and the radiograph examined for internal forms. The x-ray technique is described in Appendix B.

Insects: The rice weevil, Sitophilus oryzae (L) a typical internal infesting stored grain insect, reared in wheat under controlled conditions ($27 \pm 1^{\circ}\text{C}$, $69 \pm 3\%$ RH) in the SPIL, was used in the work reported here. In addition to adults, early-instar and late-instar larvae were selected as test forms to observe the contribution of each to fragment counts in flour. Kernels with emergence holes were also included for analysis.

Stock cultures of test insects were set up on a weekly basis. Kernels with internal forms and kernels from which adults had emerged were selected from cultures as follows:

Early-instar larvae = 1 week old cultures

Late-instar larvae = 3 week old cultures

Kernels with emergence holes = 6-7 week old cultures

Adult weevils (1-7 days old) were selected from 6-7 week old cultures.

Infested kernels were first segregated by means of egg-plug staining with Acid Fuchsin (Appendix A). Radiographs of internally infested kernels and those with emergence holes were examined to confirm the presence of the desired stage of development (Fig. 1).

Phase I: Effect of Insect Development Stage on Fragment Count in Flour

Level of Infestation. Two levels of infestation were used to determine the fragment contribution of each stage of development. The lower level of infestation consisted of 125 kernels with early-instar larvae, late-instar larvae, kernels from which adults had emerged or 125 adults added to 2500 gram standard test samples. A higher level of infestation was obtained by using 375 individuals instead of the 125. These two

Figure 1: Radiographs of rice weevil,
Sitophilus oryzae (L) stages
in wheat.

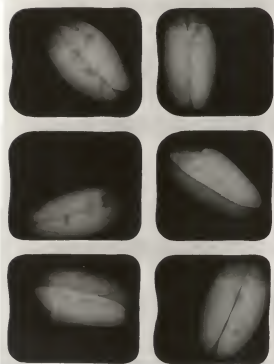
- (A) Early-instar larvae
- (B) Late-instar larvae
- (C) Pre-emerged adults
- (D) kernels with emergence holes



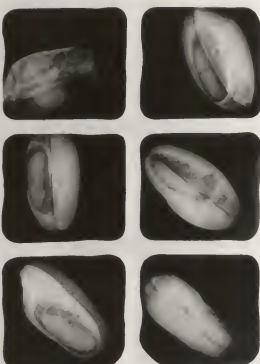
B



D



A



C

levels of infestation were used for both live and dead adult forms of infestation to find the comparative contribution of each to fragment count in the flour. Only dead forms were used in the case of early and late-instar larvae.

Early and late-instar larvae inside of kernels and adult insects were killed in a hot-air oven maintained at $130 \pm 1^{\circ}\text{C}$ for half an hour to provide dead insect forms. After cooling, the desired number of kernels with larval forms or emergence were added to the test samples at 12.07% moisture content and allowed to condition for at least one week prior to tempering for milling. Live or dead adults were added to the samples after tempering and immediately before milling.

Phase II: Effect of Grain Treatment on Fragment Count in Flour

Level of Infestation. To find the relative effect of an insecticide protectant and a fumigant on fragment count in the final product, 4 wheat samples (2500 gram each) at 13.43% moisture content were infested each with kernels carrying live insect forms as follows:

Early-instar larvae = 125 kernels +

Late-instar larvae = 125 kernels +

Adults (pre-emerged) = 125 kernels.

Protectant and Fumigation Treatment. Two such infested samples, in 1-gallon size plastic jars, were treated with an insecticide protectant (Malathion at the rate of 1 pint 57% E.C./1000 Bu). Two other similarly infested samples were fumigated in an air-tight 55 gallon drum using 2 Phostoxin pellets (0.2 gram PH_3 each) for 6 days. All treated samples and two uninfested control samples were placed in a room maintained

at $25 \pm 1^{\circ}\text{C}$ and $70 \pm 3\%$ for 45 days (Figure 2A).

After 45 days, sufficient time for rice weevils to develop and emerge under normal conditions, each sample was sieved and the adult forms that had emerged from the kernels removed and counted. These insects would have been sifted out in the normal cleaning operation in a mill. A Great Western Test Sifter was used for 2 minutes to sieve each sample prior to tempering for milling. A total period of 9 weeks was provided between treatment and milling of wheat samples.

Milling: Prior to milling, moisture content of the wheat samples was determined using a Motomco Moisture Tester and calculated amounts of water added to the samples in a rotary metal drum to bring the moisture content to 16%. After water was added and mixed for 5 minutes samples were placed in plastic bags for tempering over night at room temperature.

A Buhler Automatic Laboratory Mill Model MLU-202 (Fig. 2B) in the Department of Grain Science and Industry, was used to mill all samples. It is a self-contained, easy-to-clean, commonly used experimental mill that accommodates the 2500 grams samples used in this work. The flow sheet of the Buhler Experimental Mill is shown in Figure 3.

A tempered clean-wheat sample (2000 grams) was milled as a warm up operation and the product discarded. Only milled out flour of individual control and infested test wheat samples was retained in identified plastic bags for contaminant analysis.

Between milling of each test sample, the mill was cleaned out by brushing the inside of both break and middling sections of the mill and the disassembled sieves, milling 1000 grams of cleaned, tempered wheat and again brushing the various sections of the mill. This was done to

Figure 2. (A) Containers used for 9 weeks storage of infested hard red winter wheat treated with malathion, Phostoxin or control

(B) Buhler Experimental Mill

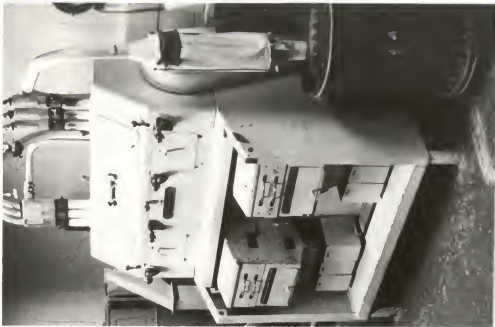
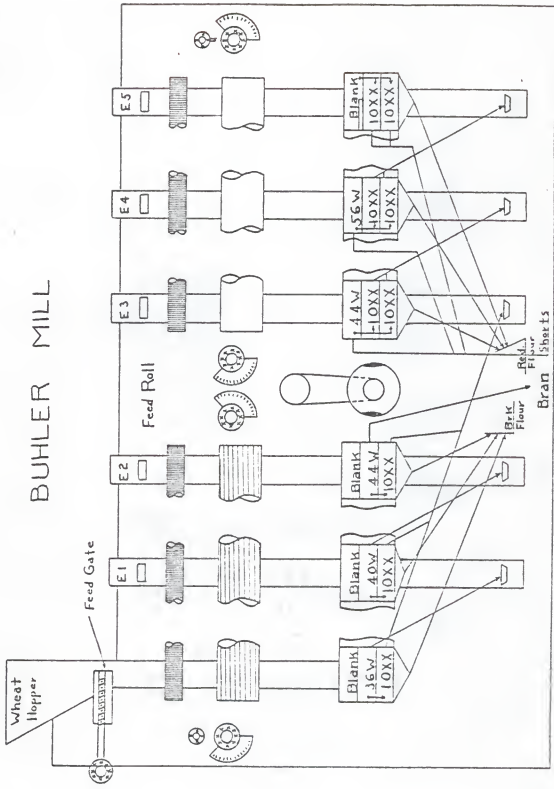
**B****A**

Figure 3. Flow sheet for Buhler experimental mill.

BUHLER MILL



ensure distinction of each test sample during the milling operation.

Contaminant Analysis: A modified AOAC Acid Hydrolysis Method (Pedersen et al. 1974) was used to isolate insect fragments from 50 gram samples of flour. The method is discussed in Appendix C. Contaminant analysis laboratory equipment is shown in Fig. 4A. Samples were run in triplicate. Fragments were collected on 9 cm diameter square-ruled (0.5cm) filter papers and placed in petri dishes.

A wide-field Wild-Heerbrugg binocular stereo-microscope, Model M7A using transmitted light (bright field) from below and reflected light from above was used to examine, identify and count the insect fragments (Figure 4B). Most of the time 26.7 magnification was used with a 10X lens while the range of magnification was from 21 to 31.

Texture, color, shape and recognizable characteristics were used in identifying fragments (Kurtz and Harris 1962). Insect fragments were counted and the number recorded.

Because it was impossible to count the very large number of fragments produced specially by the adult insects at the high levels of infestation, a method of estimating the number of fragments per sample was devised.

Fragments on 9 selected sections measuring 25mm^2 each i.e., 225mm^2 of the total 4359.156mm^2 (area of the filter paper with 174.36 square units) were counted (see Figure 5) and a total fragment count of the sample arrived at by using the formula:

$$\text{Total estimated fragment count} = \frac{\text{actual fragments counted} \times 4359.156}{225}$$

- Figure 4. (A) Contaminant analysis laboratory equipment for isolation of insect fragments from wheat flour samples.
- (B) Wide-field Wild-Heerbrugg binocular stereo-microscope, Model M7A using transmitted light (bright field) from below and reflected light from above used to examine, identify and count insect fragments.



B



A

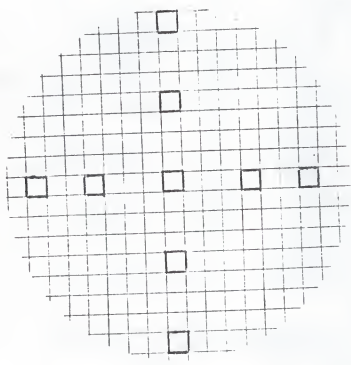


Figure 5. Nine selected sections on filter papers for estimating fragment counts

RESULTS AND DISCUSSION

The hard red winter wheat used throughout these experiments was found to be free from any live or dead infestation, by sieving, magnified visual examination, acid fuchsin staining and an x-ray technique.

Phase I. Effect of Insect Developmental Stage on Fragment Count in Flour

Contaminant Analysis. Contaminant analysis plates on which fragments from 50-gram flour samples were collected were examined microscopically, and fragment counts resulting from different developmental stages of rice weevil are presented in Table 1 and Figure 6.

Control sample plates showed no insect fragments.

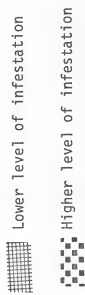
A considerable difference in fragment count between subsamples was observed in almost all samples. It appears that in the samples of flour, there was uneven distribution of insect fragments. Also fragment counts were not proportionately three times greater in samples with the higher level of infestation when compared with the low level. This was especially true in the case of samples infested with adult weevils.

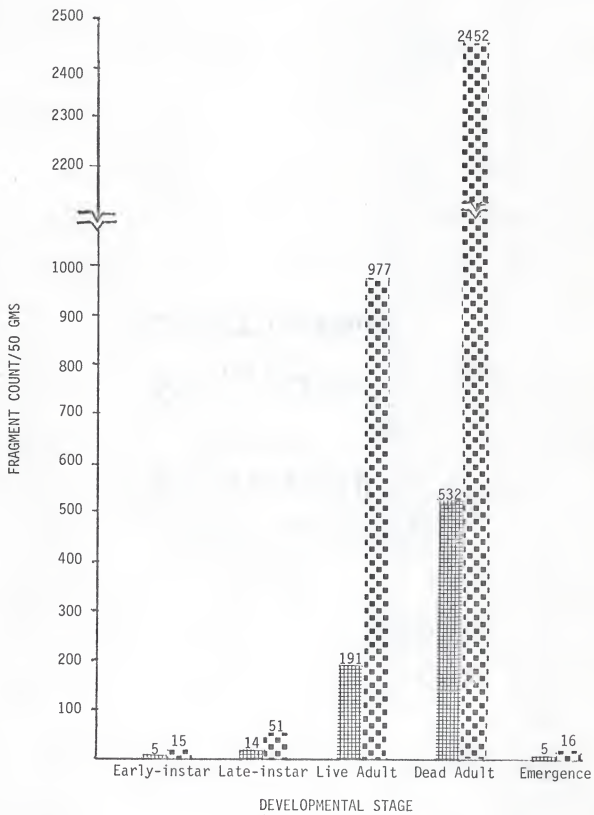
Fragments Contributed by Larvae. Fragment counts for plates prepared from samples containing larvae were much lower than those for adult rice weevils. Early-instar larvae contributed about 1/3 as many fragments as late-instar larvae at both low and high levels of infestation. This could be the result of: (1) more chitinized material as the larvae increased in size through moulting and/or, (2) presence of cast exoskeletons. Observable fragments are most likely to come from the larval head capsule. As the larva advances in its developmental stages, the head capsule increases in size and thickness (Soderstrom, 1960) and thus are likely to produce more fragments.

TABLE 1
 FRAGMENTS COUNT PER 50 GRAM SAMPLE
 OF FLOUR MILLED FROM WHEAT SAMPLES
 CONTAINING VARIOUS STAGES OF SITOPHILUS ORYZAE (L)

Stage		INFESTATION LEVEL	
		Lower Level (125/2500 gram)	Higher Level (375/2500 gram)
Early-Instar Larvae (Dead)	A	8	10
	B	5	23
	C	5	14
	Average	5.3	15.6
Late-Instar Larvae (Dead)	A	9	48
	B	18	49
	C	15	58
	Average	14.0	51
Adult (Live)	A	348	1162
	B	34	792
	C*		*Samples lost
	Average	191	977
Adult (Dead)	A	650	2697
	B	522	2474
	C	425	2185
	Average	532	2452
Emergence	A	3	29
	B	3	11
	C	10	8
	Average	5.3	16

Figure 6. Fragment contribution of developmental stages of Sitophilus oryzae (L).





Harris et al. (1963) indicated that the frons is probably the most readily recognized larval fragment of the weevil and is the precursor of several equally well recognized fragments. The frons and other portions of the larval exoskeleton are weakly sclerotized in the early-instar stage with the frons darkened and minutely granular in late-instar. Harris et al. (1963) also indicated that some weakly sclerotized portions of larval exoskeleton are not generally seen unless the suspect structure is observed at high magnification or carefully oriented under the wide-field microscope. They considered that the degree of flour comminution and the stage of the larval instar determined to a large extent the fragmentation of the frons.

Observations made during this study reveal that the most commonly occurring recognizable fragments are the mandibles and gena. In the samples containing early-instar larvae 75 to 80% of the fragments observed were mandibles. They are easily recognized by their more or less triangular shape with a single, rather blunt apical tooth at the apex. In the late-instar samples both late-instar mandibles and early-instar cast mandibles were observed. The mandibles are heavily sclerotized structures and apparently capable of withstanding the milling process.

As the larva passes through its developmental stages it periodically forms a new exoskeleton to provide for increase in size. The old exoskeleton is cast off in moulting and remains inside the wheat kernel to add to the total fragment potential of the sample. The rice weevil develops through four larval instars.

In the samples with late-instar larvae three times higher number of fragments than early-instar larvae samples are due to the better developed

mouth parts of the larva and due to the exuviae cast off by it on earlier moultings.

Fragment Contribution by Adult Weevils. The adult stage is a potential contributor of many fragments to the product due to its highly chitinized exoskeleton. Elytra and appendages which were vestigial or absent in the larvae are now highly chitinized and pigmented in the adult weevil. The mouth parts are stronger and darkened. A significant rise in the number of well defined fragments in samples infested with adult weevils was noticed when compared to those infested with larvae.

In adult samples, the most common fragments observed were chips of exoskeleton from various unidentifiable parts of the insect body. Those fragments which could be identified as to their location included portions of compound eyes, pitted elytra, segments of antennae and jointed legs.

Since the fragment counts of both low and high level samples was very high an estimate was made by counting the fragments on 9 square units of the filter paper (Figure 5). The fragment counts shown in Table 1 for all samples with live and dead adult weevils at both levels of infestation are based on this method.

Comparison of Alive and Dead Adult Weevils. On the average more fragments (nearly 3 times more) were observed in samples with dead adults as compared to those with live adults. As explained by Wagner (1960), this could be due to live insects (with their body fluids intact) being crushed in processing, forming dough balls with flour and then tailed off with offals. On the other hand, dead insects which are dried and brittle are likely to be shattered into many pieces and result in greater numbers of fragments in the flour.

In the higher level adult samples the number of fragments observed were nearly 4 to 5 times greater than lower level samples. The distribution of fragments in replicate samples showed a relatively wide range i.e., 425 to 650 and 2185 to 2697 fragments respectively for lower level and higher level dead adult samples. The live adult replicate samples showed an even greater range between the 2 replicates available. (Note: The flour samples were lost before the third replicate could be run.)

Significance of Kernels with Emergence Holes. After passing through the developmental stages the adult weevil eats its way out of the kernel. Cast larval exoskeletons produced at moulting can remain inside the deserted, hollowed kernels. On the basis of this, one might expect to find fragments in flour roughly equivalent to those found in late-instar larvae samples. However, results indicate that fragment counts resulting from larval exuviae are approximately the same as those of early instar larvae. It is possible that some of the exuvial material may be lost in cleaning or have been consumed by the larvae during development. In emergence samples both late and early-instar mandibles were found.

Phase II. Effect of Grain Treatment on Fragment Count in Flour

Samples of wheat each weighing 2500 grams and containing a combination of various stages of rice weevil infestation were treated either with an insecticidal protectant (Malathion) or with a fumigant (Phos-toxin). The effect of these treatments on infestation control and insect fragment counts in flour samples is discussed in the following paragraphs.

Variation in Weevil Mortality. Mortality results of live internal infestation in wheat samples when exposed to either treatment were deter-

mined by x-ray and by collecting the dead insects by sieving the wheat samples prior to milling.

Treated samples were x-rayed weekly to follow the progress of infestation control. Observations are recorded in Table 2. See Figure 7.

A greater number of insects died as a result of one week's exposure to phostoxin fumes than observed in malathion treated samples. Two hundred gram samples of the latter showed 8 live forms as compared to just one in the former. After 3 weeks while 5 live forms were still observed in the malathion treated samples, there was none in the phostoxin treated samples. In the next two weekly x-rays no live forms were observed in either treatment. After 6 weeks, only two dead insects were observed inside kernels in the malathion treated samples as compared to 9 in fumigated samples. In weekly radiographs more emerged kernels were observed in malathion treated samples than in fumigated ones.

Table 4 shows the numbers of adult insects removed by sieving prior to milling the samples into flour. Dead adult weevils collected from the malathion treated samples were 166 and 94 as compared to just 1 dead weevil collected from each of the fumigated samples. The number of adult weevils sieved from malathion treated samples indicated that some of the introduced forms completed their development and emerged from the kernels. Weevils that emerged died outside the kernels on coming in contact with the insecticidal protectant. It appears that malathion also proved lethal for some of the internal forms.

The quick action of the fumigant brought about almost complete stoppage of insect activity inside the kernels eliminating the possibility of

TABLE 2
 NUMBER OF KERNELS WITH INSECT FORMS AND
 EMERGENCE HOLES OBSERVED IN WEEKLY X-RAYS OF
 WHEAT SAMPLES PRETREATED WITH MALATHION OR PHOSTOXIN

Weeks	Malathion				Phostoxin			
	Live forms	Dead forms	Total	Emergence	Live forms	Dead forms	Total	Emergence
<u>WHEAT DURING STORAGE</u>								
1	8	0	8	7	1	7	8	0
2	1	0	1	5	1	9	10	0
3	5	0	5	4	0	7	7	1
4	0	1	1	8	0	8	8	0
5	0	1	1	6	0	8	8	1
6	0	2	2	5	0	9	9	0
<u>CLEANED WHEAT SAMPLES BEFORE MILLING (200g)^{1/9}</u>								
9	I	0	0	9	0	12	12	1
	II	0	2	2	10	0	6	6

^{1/9} wk x-ray samples based on 200 gm. All others on 100 gm.

Figure 7. Effect of malathion (A) and Phostoxin (B) treatment on wheat infested with Sitophilus oryzae (L).



B



A

TABLE 3
 FRAGMENT COUNT PER 50 g FLOUR SAMPLE
 MILLED FROM WHEAT SAMPLES^{1/} CONTAINING STAGES OF
 RICE WEEVIL INFESTATION^{2/} AND TREATED WITH MALATHION OR PHOSTOXIN

Sample	Replicate	TREATMENT	
		Malathion	Phostoxin
I	A	98	406
	B	179	402
	AV	138	404
II	A	274	440
	B	194	380
	AV	234	410
I&II	AV	186	

^{1/} Each wheat sample was 2500 g.

^{2/} Each wheat sample contained 125 kernels with live early-instar larvae, 125 kernels with late-instar larvae and 125 kernels with pre-emerged adult rice weevils at the time of treatment

TABLE 4

COMPARISON OF INSECTS REMOVED BY SIEVING, NUMBER OF INTERNAL FORMS AND NUMBER OF INSECT FRAGMENTS FROM MALATHION OF PHOSTOXING TREATED WHEAT FLOUR SAMPLES

<u>Treatment</u>	<u>Sample</u>	<u>Number of Insects removed by sieving (2500 gm)</u>	<u>Number of internal forms on x-ray (200 gm)</u>	<u>Number of insect fragments in 50 g flour sub-samples/Av.</u>	
Malathion	I	166	0	A 98 B 179	138
	II	94	2	A 274 B 194	234
Phostoxin	I	1	12	A 406 B 402	404
	II	1	6	A 440 B 380	410
Control	I	0	0	0	
	II	0	0	0	

lower forms developing further or adults emerging. In such a situation, we can expect more internal infestation to be retained by infested kernels. Such kernels before reaching the break rolls in a standard mill could be broken open by impact action like that of the entoleter. Even this action would have limitations in doing away with the insects thus exposed. All insect material from kernels broken open may not be removed by aspiration. Kernels with early stages may not even be broken open by the impact because they have not been sufficiently weakened by the insect. We can, therefore, expect more insect fragments in flour from fumigated internally infested wheat when compared to that of wheat treated with an insecticidal protectant like malathion with its local and external action to control insect activity in grain.

X-Ray of Treated Cleaned Wheat. Number of internal insect forms observed by x-ray in treated, sieved wheat ready for milling into flour are given in Table 2. Malathion treated wheat had 0 and 2 internal forms in 200 gram samples drawn from such wheat. In fumigated samples, 6 and 12 internal dead forms were counted. Presence of 9 and 10 emergence kernels malathion treated samples, compared to 1 in each fumigated sample, showed that more infestation escaped into the grain mass where it could be removed by cleaning. It is the presence of internal forms in the wheat ready for milling that makes the ultimate difference in insect fragment counts in flour.

Insect Fragment Count. Two 50-gram samples of flour drawn from each lot of milled wheat was acid hydrolysed to collect the insect fragments (see contaminant analysis method in Appendix C). Fragment count results from microscopic examination of filter plates are given in Tables 3 and 4.

On the average, nearly double the number of insect fragments were found in the fumigated samples compared to those treated with malathion. Based on numbers of dead insects retained in fumigated samples, 5 to 10 times more fragments might be expected in the fumigated samples (see Table 4). Possibly, elimination of fragments with offals depending upon the sifting efficiency of the mill and also the size of fragments produced make a difference in the fragments actually collected.

This study is basic to investigations into factors contributing to insect fragments in flour from internally infested hard red winter wheat. Hard red winter wheat flour is commonly used as the major raw material for breadmaking. Some conclusions can be drawn which will create interest in more work on this problem.

Internal infesting insect pests like the rice weevil contribute increased numbers of fragments as their development progresses with maximum numbers added in the adult stage when dead. Measures to minimize dead insects inside grain kernels should minimize fragment counts in flour made from wheat which may have become infested.

Chemical control, a common measure taken to arrest insect activity in bulk grain, needs precision in its application. Timely use of the right chemical is not only necessary for reducing storage losses attributable to insect attack but also for minimizing micro-filth, like insect fragments, in the final product.

The present work provides an insight in choosing the type of chemical control which may have a bearing on the fragment count in the flour produced.

It was not the purpose of this study to determine whether an

insecticidal protectant-type chemical control is better suited than fumigation to keep the losses due to insect attack low. A decision to select the insecticide/fumigant to be used will depend upon the nature of infestation (external or internal), whether an internal infestation has advanced in its development, the proposed length of storage and the intended utilization of the wheat. While a fumigant, when applied with due precautions and in proper dose, may give a complete and instant control of insect activity it may not be helpful in minimizing the fragment count in the product. Fumigation could be a good measure to combat an existing insect infestation in grain meant for seed purposes or for feed manufacturing. For wheat meant for human consumption malathion or other insecticidal protectants could help in keeping down the fragment count in flour specially when it is an important factor in the acceptance of flour.

CONCLUSIONS

1. Early-instar larvae of rice weevil produce comparatively fewer fragments than late-instar larvae.
2. Adults produce maximum numbers of fragments.
3. Live adults add fewer fragments in the milled flour than dead ones.
4. Emergence kernels retain exuvial material of larval moultings and contribute almost as many fragments as early-instar larvae.
5. Protectant treated wheat samples yield fewer fragments than the fumigated ones.

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APPENDICES

- Appendix A. . . . Acid Fuchsin Staining Method
Appendix B. . . . X-Ray Technique
Appendix C. . . . Contamination Analysis

APPENDIX A

ACID FUCHSIN STAINING METHOD

Procedure for Testing Weevil Infestation in Wheat

(A modified FRANKENFELD'S staining method)

Procedure:

1. Prepare the dye solution
 - (a) Weight out 0.5gm acid fuchsin
 - (b) Measure 50.0 cc of glacial acetate acid and 950.0 cc distilled water and mix.
 - (c) This dye solution can be stored for a long time and may be used repeatedly until it becomes murky.
2. Soak grain to be treated for 5 minutes in warm water.
3. Drain off water and cover grain with acid fuchsin solution for 2 to 5 minutes. If left longer the kernels may absorb enough solution to make the identification of the egg-plugs difficult.
4. Pour off dye solution (retain for future use) and wash grain in tap water to remove excess dye.
5. Examine the kernels to locate the gelatinous egg-plugs which stain a deep cherry red. Note that feeding punctures, and mechanical injuries stain a lighter color than the egg-plugs.

APPENDIX B

X-RAY TECHNIQUE

An outline of procedure for use of an X-ray Grain Inspection Unit for detection of internal infestation in grain. A General Electric Grain Inspection Unit is used for this work.

Procedure:

1. Using the Boerner Grain Divider, obtain a uniform sample of 100 grams.
2. Spread the sample, one kernel thick over half of the plastic grain tray. A second sample can be placed on the other half of the tray. Use lead letters to identify the samples.

Note: Kernels may also be radiographed in soda straws, in gelatin capsules, glued to plastic sheets of in grid trays if it is desirable to X-ray the same sample more than once or to be able to recover specific kernels.

3. X-ray film should be handled only under the proper safe light in the dark room and should be placed directly over the grain or in a cassette over the grain.
 - a. Various types of films are available.
Kodak Industril X-ray film, Type M provides good results.
 - b. Exposure time and machine settings for various types of grain using Type M film are:

<u>Grain</u>	<u>KV</u>	<u>MA</u>	<u>Time (Min.)</u>
Wheat	20	5	1.5
Corn	25	5	1
Rice	17	5	2
Oats	20	5	1.5

- c. The above exposures are for grain placed directly on the grain tray. If grain is placed in straws, capsules or on other films it is usually necessary to increase the exposure time.
4. After exposure to the X-ray, the film should be developed, fixed and washed.
 - a. Development usually requires about 5 minutes at 68⁰F.
 - b. The film should be rinsed in water then placed in the fixing solution for 30 minutes.
 - c. After fixing the film should be washed in running water for 30 minutes.
5. X-ray radiographs may be viewed wet, but are generally dried before examination.
 - a. An X-ray viewer provides a uniform light background for examining the X-ray radiograph.
 - b. A low powered hand lens can be used to examine the radiographs for kernels containing internal infestation.
 - c. Various stages of insect development can be determined in radiographs.
6. Examine the radiograph and record (or select) kernels having the desired form (early-instar larva, late-instar larva, adult, or with emergence hole).

APPENDIX C

CONTAMINATION ANALYSIS

Isolation and Detection of Insect Fragments in Wheat Flour---

ACID HYDROLYSIS METHOD

I Materials, reagents and solutions

A. Materials.

1. Balance
2. Beakers - 800 or 1000 ml.
3. Stirring rods - with rubber policemen.
4. Hot Plate.
5. Separatory funnels - 1000 ml. equipped with rubber drain tubes fitted with pinch clamps.
6. Buchner funnel and filtering flask.
7. Petri dishes
8. Ruled analytical filter paper

B. Reagents and solutions

1. Hydrochloric acid solution - 5%
2. Mineral oil
3. Tween 80-alcohol solution (1 part polyoxyethylene sorbitan monooleate to 5 parts 60% alcohol)
4. Naptha gas or any lead-free gasoline
5. Glycerine solution - 1 part glycerine to 1 part water
6. Isopropyl Alcohol - 60%

II Method

A. Proceudre

1. Register the sample in a contaminant analysis logbook and assign a laboratory number to it.

2. Weigh 50 grams of flour into an 800 ml beaker
3. Add, with stirring, about 500 ml of 5% HCL solution
4. Add with stirring, 30 ml of the Tween-80/alcohol solution
5. Add 20 ml of light mineral oil
6. Heat to boiling, with intermittent stirring to prevent the flour from scorching, boil for 6-10 minutes
7. Remove beaker from hot plate and allow to cool. Cold water (about 200 ml) may be added to help cooling
8. Pour 30 ml of kerosene or lead-free gasoline into the 1000 ml separatory funnel
9. Quantitatively transfer the digested sample from the beaker to the separatory funnel. Rinse the beaker and stirring rod with hot water (use 60% isopropyl alcohol for the final rinse)
10. Allow the sample to settle for 30-45 minutes. Drain off the sediment so the oil-gas layer is about 1.5 to 2 inches from the bottom.
11. Fill the separatory funnel with water and let settle for 30-45 min. Drain off the sediment retaining the oil-gas layer in the separatory funnel
12. Filter the material remaining in the funnel through ruled analytical filter paper using the Buchner funnel
13. Transfer the filter paper in the petri dish to which has been added several drops of glycerine solution

III Microscopy

1. Examine filter paper in the petri dish using binocular microscope (at 30X magnification)
2. Record insect fragments observed in the logbook
3. Make notations as to identifiable repeatedly found insect fragments in each sample

EFFECT OF INFESTATION STAGE, FORM AND TREATMENT
ON FRAGMENT COUNT IN FLOUR

by

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An Abstract of

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ABSTRACT

Various developmental stages of the rice weevil, Sitophilus Oryzae (L), a serious internal pest of hard red winter wheat, were added at two levels to infestation-free wheat samples to determine the fragment contribution of each to the flour. Flour samples were acid hydrolysed and fragments collected on filter paper. Fragment counts by microscopic examination revealed that dead adult weevils contributed the maximum fragments at both high and low levels of infestation. Early-instar larvae added the least fragments and late-instar larvae added nearly three times as many fragments. Flour from wheat samples with live adults showed a lower count than with dead adults. Emerged kernels retained some exuviae of larval moultings. They contributed nearly as many fragments as the kernels with early-instar larvae.

As a second phase, the effect of chemical control applied to infested wheat in minimizing fragment counts in the milled flour was tested. Known numbers of kernels containing live early and late-instar larvae and adults of rice weevils were added to hard red winter wheat samples. The effects of malathion (an insecticidal protectant) and Phostoxin (a fumigant) treatments of these samples on fragment counts in flour were compared. Death of insects inside the fumigated kernels resulted in higher fragment counts in the flour. Malathion treatment allowed some insects to emerge and die outside the kernels. This resulted in much lower fragment counts in the flour as dead insects were eliminated from wheat samples by cleaning prior to milling.