THE EFFECTS OF INSECT INFESTATIONS IN WHEAT ON THE ZELENY SEDIMENTATION TESTS

by

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INTRODUCTION AND OBJECTIVES

The Zeleny (1947) sedimentation test has been designed as a simple and rapid estimate of the strength (bread-baking quality) of wheat. Although the test method is simple and quick, it is an empirical test and is subject to several variable factors. With this in mind, together with the importance[1] currently being placed on the Zeleny sedimentation values for wheat, it is necessary to determine the extent, if any, of the effects of insect damage on these values.

The purpose of these experiments was to determine the effects of these variable factors which can affect this test; and to evaluate the effects of various insect infestations on wheat sedimentation values.

LITERATURE REVIEW

Various methods have been designed to evaluate wheat quality, such as the conventional Kjeldahl protein test. These methods do not adequately reflect the bread-baking potentials of wheat that has inferior gluten quality as a result of unfavorable environmental conditions during growth, damage in storage from insects and other causes, or because it is a variety having inherently inferior gluten quality.

Zeleny (1947) devised a simple sedimentation test for estimating the bread-baking and gluten qualities of wheat flour. Further developments and modifications in the sedimentation test for the determination of wheat quality have been described by Pinckney et al. (1957). These modifications make the test applicable to soft as well as hard wheat and provide greater uniformity of interlaboratory results.

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1. Its widespread use is being adopted by the U. S. Department of Agriculture in connection with the wheat loan program, which is "the market for much of the wheat produced in the United States."

Unfortunately, however, the literature does not adequately reflect the many investigations into the applied research, with regard to variable factors which affect these tests.

Linko and Ruei-Chen (1962), investigated the effects of germination, and observed that as germination period increased, the sedimentation value decreased. They further studied the effect of storage at a temperature of 30° to 40° F. on the sedimentation values. Their conclusion was that the sedimentation test has merit in evaluating wheat quality, providing certain limitations are understood and correctly interpreted such as quality of wheat; condition of storage; and most important, but difficult to standardize, the milling process.

Shuey (1961) studies the effects of heat treatment on a single lot of wheat as well as the effects of sprout damage and insecticides. His work showed: (a) sedimentation values can be lowered by excessive heat during drying; (b) fumigation does not appear to have any effect on sedimentation; and (c) sprout damaged wheat in percentages up to 50% does not lower sedimentation values.

Schlesinger (1963) at Enid, Oklahoma observed several lots of wheat under normal elevator storage conditions for one year. Sedimentation values of these lots, which were homogeneous at the outset, were observed to decline gradually during the first five months. Durham and Warren (1962) conducted a study of such factors as ash content of whole wheat and of Tag-Hour, particle size distribution and crude fiber, affecting the determination of wheat sedimentation value. This project was concerned primarily with the determination itself and not with justification for application of sedimentation value.

Sibbitt and Giles (1962) conducted a study on the sedimentation test as an index of hard red spring wheat quality. They concluded that some effects of grinding and other sources of variation, such as when wheat is subjected to successive wetting and drying, commercial drying, and blending of wheat of dissimilar protein content, do affect the statistical accuracy of the test. Therefore, replicated tests are necessary for final determination of sedimentation values.

MATERIALS AND METHODS

Equipment

The following equipment, equivalent to that described by Zeleny (1947) was employed:

1. A motor-driven corrugated steel roll equivalent to Tag-Heppenstall moisture meter rolls for small grain.
2. Sieve, of (100) one hundred mesh (U. S. Standard No. 100 woven wire cloth sieve), eight inches in diameter, and equipped with bottom pan.
3. Several glass-stoppered 100 ml. graduated cylinders having a distance of 180 to 185 millimeters between the zero mark at the bottom and 100 milliliter mark at the top.
4. Stop-watch or interval timer for accurate and precise timing during critical periods.
5. Automatic burrettes for quick and careful measurements of reagents.
6. A cylinder shaker, which is optional, designed as a rack approximately 23 by 12 by 2 inches is pivoted at the center of each end and oscillated through 60°, 30° each side of the horizontal position, at the rate of about forty times per minute. The rack is so designed that eight graduated cylinders may be quickly and securely placed while the mixer is in motion. Power is supplied by an electric motor.
7. Siever shaker.

Reagents

1. Isopropyl alcohol, 99-100%, N. F., or equivalent.

2. Distilled water containing 4 mg. of brom phenol blue per liter.

3. Lactic acid stock solution, which is 250 ml. of U. S. P. 85% lactic acid to one liter with distilled water. Reflux[2] the diluted acid six hours without loss of volume.

4. These reagents should be thoroughly mixed as follows: 180 ml. of lactic acid stock solution, 200 ml. of isopropyl alcohol, 99-100%, with the addition of distilled water to make one liter. The reagent should stand forty eight hours[3] before using and be protected against evaporation.

Kinds of Grain Used

Wheat Triticum aestivum (L.), Ottawa variety, Hard Red Winter, (Class III).

Wheat Triticum aestivum (L.), Pawnee variety, Hard Red Winter, (Class IV).

Wheat Triticum aestivum (L.), Ottawa and Ponca varieties, not classed, because it was of inferior quality, having been exposed to excessive inclement weather conditions in the field after it was reaped.

Test Insects

The insects used in these experiments were those which may be present in the grain.

Cryptolestes pusillus (Schönherr) - the flat grain beetle.

Oryzaephilus surinamensis (L.) - the saw toothed grain beetle.

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2. Concentrated lactic acid normally contains associated molecules which on dilution gradually dissociate in part until a state of equilibrium is reached. Attainment of equilibrium after dilution, which is necessary for consistent sedimentation test results, is greatly hastened by refluxing. The refluxed stock solution should be about 2.78 normal. The mixed reagent is approximately 0.5 normal and contains 20% of isopropyl alcohol.

3. The reagent thus prepared, will be kept indefinitely without change in strength after forty eight hours, and thus give consistent (conditions) results.

Rhizopertha dominica (F.) - the lesser grain borer.

Sitophilus granarius (L.) - the granary weevil.

Sitophilus oryzae (L.) - the rice weevil.

Sitotroga cerealella (Olivo) - the Augoumois grain moth.

Tribolium confusum Jacq. du Val - the confused flour beetle.

Cultures

One hundred adult insects of a particular species were added to one-quart wide mouth mason jars containing 200 g. of wheat. These jars with infested wheat were then placed in the rearing room, at conditions of 80° ± 5°F. and relative humidity of 70 ± 5 per cent. After approximately seven days in the mason jars with the wheat, the adult insects, having oviposited, were removed from the mason jars. This was accomplished by screening the wheat cultures through wire sieves, the size of which depended upon the species of insect. The adult insects were then placed in other samples of wheat in different mason jars to repeat the process. Thus infested wheat was readily available with different stages of larval development.

The criteria used to determine the various larval instars were: (1) age; (2) amount of damage to infested kernels; and (3) head capsule measurements. Therefore, the insects' development can be determined relatively accurately as exemplified in Tables 1 and 2.

Table 1. Larval instars of rice weevil - (Sitoplilus oryzae (L.))

Days From Start of Culture (Infestation)	Stage and Instar
3 - 4	Eggs
8 - 9	First instar larvae
11-12	Second " "
15-16	Third " "
20-21	Fourth " "
25-26	Pupae
27-30	Pre-emergence adult
30 plus	Adult

Table 2. Developmental data of larval instar of confused flour beetle. (Tribolium confusum Jacq. du Val)

Days: Between each stage after infestation	Days: Mean	Stages and Instars
6 - 8	7	Eggs
1½- 3	2	First instar larvae
4 - 8	5	Second " "
4 - 8	6	Third " "
4 -10	8	Fourth " "
4 - 9	6	Fifth " "
4 -16	7	Sixth " "

Based upon the period before examination of the kernels, the actual insect infestation of the wheat samples differed from one group of insects to another. The actual infestation of kernels at the time of testing the different larval stages varied from 30% in some species to 85% in others. This was determined by X-ray analysis as illustrated by Plates I - IV.

Sedimentation Test

The basic procedure of the Sedimentation test as outlined by Zeleny in 1947, and amended by Pinckney et al. in 1957 was followed. During the test procedure, brom phenol blue was added primarily as an indicator. The other reagents are used because some glutelins/proteins are insoluble in distilled water and alcohol solutions but are soluble in dilute alkali or dilute acids such as lactic acid. They have been isolated only from plant seeds, such as glutelin from corn endosperm and glutenin from wheat endosperm.

RESULTS AND DISCUSSION

A standardization test for an original sedimentation value of the different varieties of wheat was obtained for Ottawa wheat 60, Pawnee wheat 34 and mixed wheat 24. Moisture content obtained by the oven moisture analysis method and verified by the "Stenlite electronic tester", at the time of

EXPLANATION OF

PLATES I-IV.

Enlarged prints of radiographs, showing immature development of insect infestation of the rice weevils, *Sitophilus oryzae* (L.). Larval, pupal and pre-emergence stages are visible. Determined by X-ray analysis, followed by appropriate development. Percent infestation can be calculated.

PLATE I

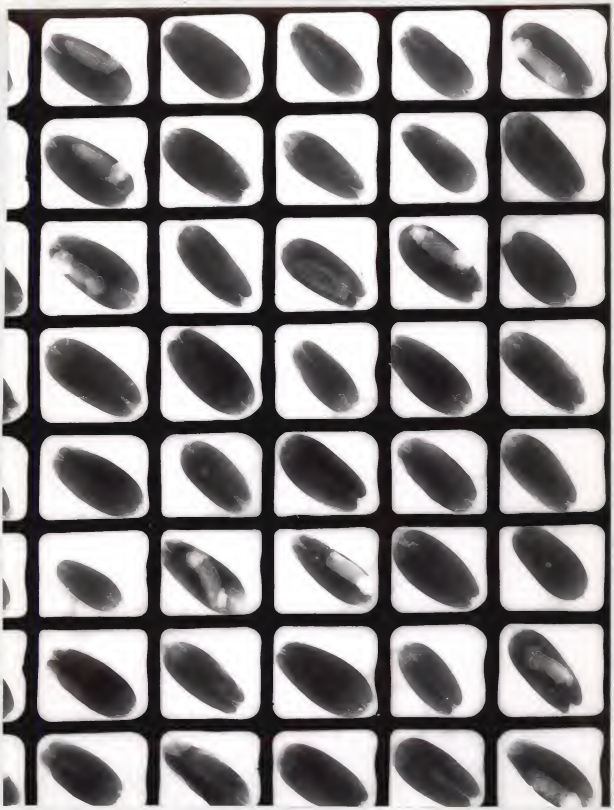


PLATE II

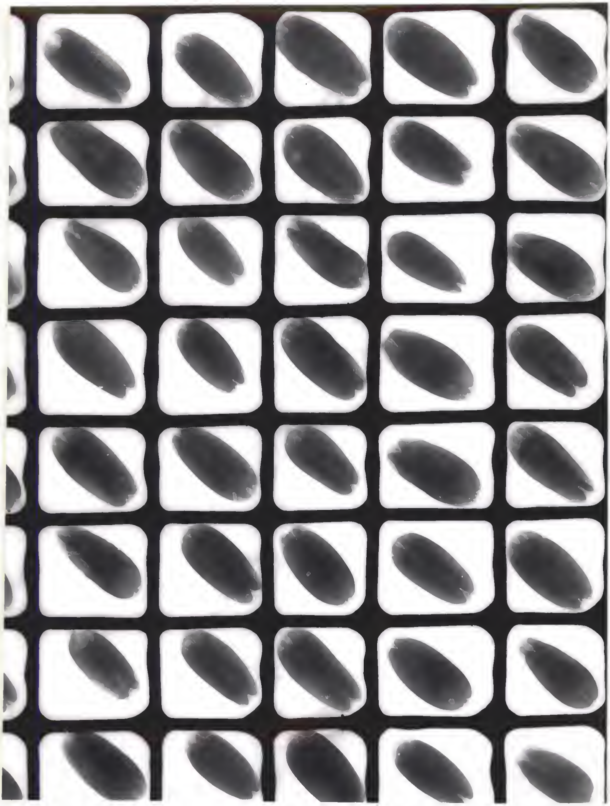


PLATE III

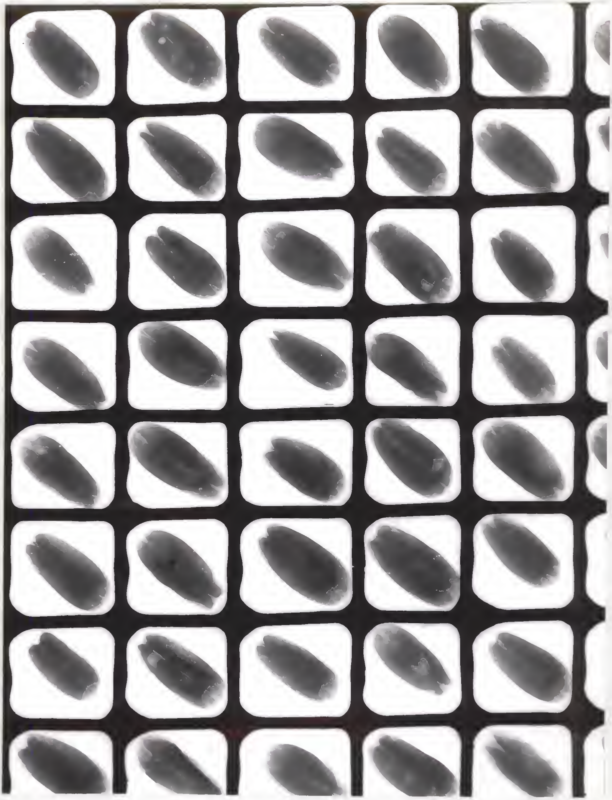
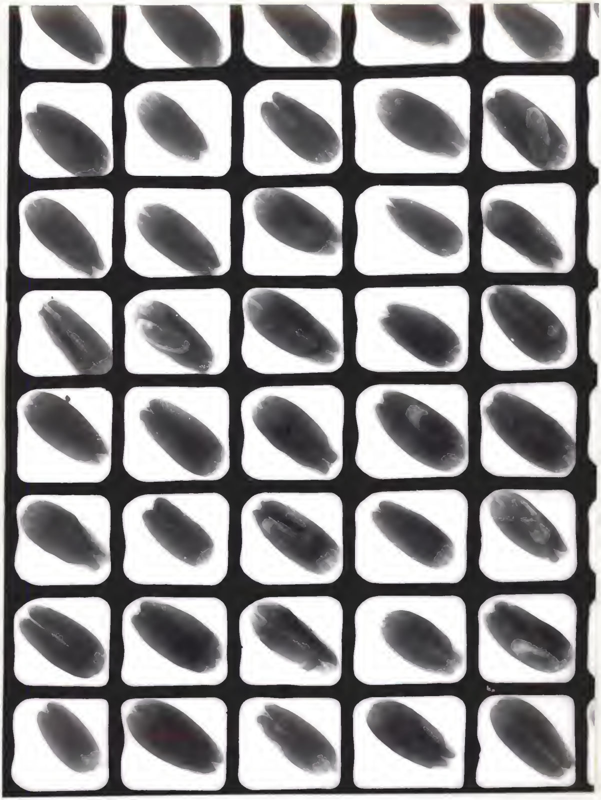


PLATE IV



standardization was 11.5%, 10.8%, and 12.0%, respectively. To obtain the corrected sedimentation value (14% moisture basis), the uncorrected sedimentation value was multiplied by the appropriate factor in Table 3 below:

Table 3. Conversion Factors.

Wheat Moisture %	Factor	Wheat Moisture %	Factor	Wheat Moisture %	Factor
8.0	1.14	11.0	1.00	14.0	1.00
8.5	1.10	11.5	0.99	14.5	1.02
9.0	1.07	12.0	0.98	15.0	1.04
9.5	1.05	12.5	0.98	15.5	1.07
10.0	1.03	13.0	0.98	16.0	1.10
10.5	1.01	13.5	0.99	16.5	1.14

An example of the correction is:

Basic Sedimentation Value	Percent Moisture	Moisture Adjustment Factor	Adj. Sedimentation Value	Corrected Sedimentation Value
63	11.95	0.98	61.74	62.

With the exception of Averages and Control, sedimentation values stated are on a corrected basis.

These data obtained as standardization values correlated with results obtained from similar tests on samples of the same wheat performed by the Department of Flour and Feed Milling Industries, Kansas State University and the United States Department of Agriculture, ASCS Laboratory, Manhattan.

After infestation of the wheat, each level of larval instar infestation was tested, replicated five to six times. The maximum and minimum sedimentation values obtained for each immature and adult stages in a sample of wheat was procured, and an average value determined. Subsequently the effect of these insect stages on the sedimentation value was obtained by finding the difference between the average and control sedimentation values (Table 4).

Table 4. Decrease in Sedimentation values of different varieties of wheat due to insects

Insect Stage	Ottawa Wheat						
	I	II	III	IV	V	VI	VII
Eggs	0.5	4	2	0.5	3.5	1.5	3
1st larval	4	2	1.5	0.5	5	2	2
2nd "	4	4	9.5	2	1	4.5	2
3rd "	3	5	13.5	0.5	1.5	2	4.5
4th "	15.5	4.5	18	1.5	4.5	3	4.5
Pupae	14.5	4.5	9.5	2.5	5.5	5.5	5
Adults	13	13	-	-	2	10	7.5
Adults (dead)[a]	-	15	-	-	-	-	-
	Pawnee Wheat						
	I	II	III	IV	V	VI	VII
Eggs	7	4	2	2.5	-	-	-
1st larval	7	2.5	3	4.5	-	-	-
2nd "	0.5	3.5	1	4.5	-	-	-
3rd "	8.5	4	2.5	3	-	-	-
4th "	8.5	4	3	4.5	-	-	-
Pupae	9	8	1.5	5	-	-	-
Adults	-	6	-	-	-	-	-
Adults (dead)[a]	-	7.5	-	-	-	-	-
	Mixed Wheat						
	I	II	III	IV	V	VI	VII
Eggs	0.5	1	-	-	-	-	-
1st larval	1	2	-	-	-	-	-
2nd "	3.5	2	-	-	-	-	-
3nd "	1.5	1.5	-	-	-	-	-
4th "	3	2	-	-	-	-	-
Pupae	2	2	-	-	-	-	-
Adults	-	2	-	-	-	-	-
Adults (dead)[a]	-	-	-	-	-	-	-

I - S. oryzae; II - T. confusum; III - S. granarius; IV - R. dominica; V - S. cerealella; VI - O. surinamensis; VII - C. pussilus.

a. Adults were oven dried. Dashes (-) indicate that no data were taken.

The moisture content of the infested wheat increased as the insect infestation progressed. This had a marked effect on the sedimentation value, which decreased as the moisture content increased. This was important as the wheat absorbed moisture due to the condition of the rearing room. These comparative sedimentation values are recorded with the conditions in Table 5.

Table 5. Effects of moisture and temperature variation on the sedimentation value of Ottawa wheat.

	Sedimentation Value	Moisture Content
Sample value at the start of experiment "a".	60	11.55
*Sample from the rearing room.[1]	52	13.60
*Sample in laboratory from mason jars with only sieve covers.[2]	54	12.73
*Sample in container outside the rearing room and sealed with masking tape to conserve moisture.[2]	52	12.70

1. Temperature 80° F. and Relative Humidity 70%. 2. Temperature 75° F. and Relative Humidity 40%. * Tested 30 days after "a".

As tests progressed with different larval stages of insects in wheat samples, it was observed that a decrease in sedimentation values occurred. Additional tests were then devised, in which both stored infested wheat and non-infested wheat were to be used, so that immediate and delayed effects could be observed. Tests were conducted after certain period of days had passed in which both the infested and non-infested wheat samples were stored. The resulting sedimentation values differed and these differences are expressed as "net change" due to storage. Table 6 shows the data derived and illustrates that storage over a prolonged period of time affects the sedimentation value of wheat.

Table 6. "Net Change"[a] of sedimentation values in wheat due to storage.

Days from start of Tests	Net Change		
	Ottawa Wheat	Pawnee Wheat	Mixed Wheat
Initial	0	0	0
16	-5	-3	-0.5
35	-9	-4	-1
39	-4	-2	-2
46	-4	-2	-2

a. Values equivalent to the difference between infested and control stored samples.

However, as shown by the results of investigation on the sedimentation tests by Linko and Ruei-Chen (1962), other factors such as a minute variation in moisture and temperature must be taken into account as storage alone will not affect the sedimentation value.

On the other hand, it was further observed and substantiated by X-ray analysis, that different species of insect infestation cause conspicuous ranges on the sedimentation values. This is attributed to percentage development of larval instar within the kernels of the samples to be milled. Since rice weevils lay their eggs directly into the wheat kernels, it causes a much higher, about 50% larval infestation, within the wheat kernels; whereas confused flour beetles laying their eggs scattered in the grain, ensures larval infestation of about 25% within the wheat kernels. The amount of infestation within the kernels apparently has an effect on the tolerance of the endosperm within the wheat kernels. The ensuing tests will then be deviated, since flour of superior quality will not be produced. Graphs in Figs. 1, 2, and 3 present the effects due to different larval instar infestations with different varieties of wheat kernels.

The quality of wheat used in these experiments varied in terms of protein quality determined by their sedimentation value. Based upon a range from about three for very weak to seventy for the strongest wheat, a classification of wheat based on sedimentation values is determined. Thus, the quality wheat

EXPLANATION OF PLATE V.

Fig. 1. Examples of the effects of larval instars of Sitophilus oryzae and Oryzaephilus surinamensis in Ottawa wheat on the Zeleny sedimentation test values.

Fig. 2. Examples of the effects of larval instars of Sitophilus oryzae and Sitophilus granarius in Pawnee wheat on the Zeleny sedimentation test values.

Fig. 3. Examples of the effects of larval instars of Sitophilus oryzae and Tribolium confusum in mixed wheat on the Zeleny sedimentation test values.

PLATE V.

In Ottawa wheat

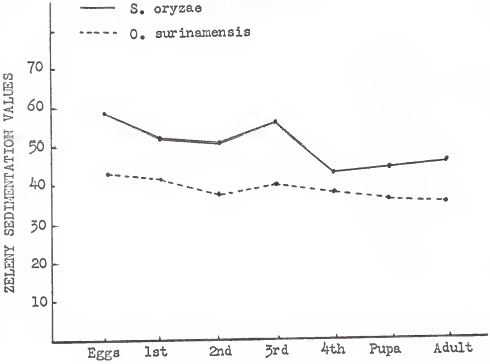


Fig. 1 INSECT STAGES

In Pawnee wheat

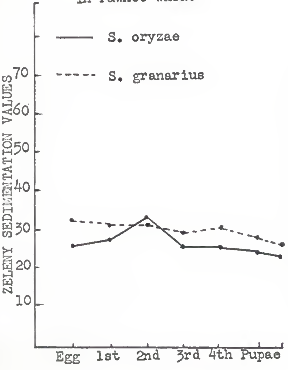


Fig. 2 INSECT STAGES

In Mixed wheat

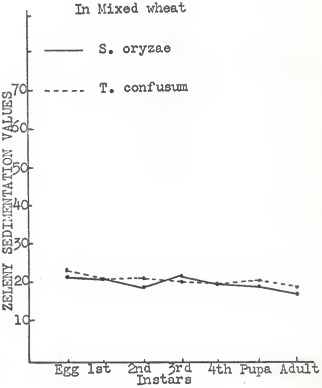


Fig. 3 INSECT STAGES

used in these tests differed. These classes are high (Ottawa), medium (Pawnee), and low (mixed wheat) which suffered gluten injury as a result of unfavorable weather conditions prior to harvest.

These wheat varieties are a good source for contrasting and observing the effects of the same insect species on different qualities wheat. There was a noticeable progressive decrease in sedimentation value from Ottawa through Pawnee to the mixed wheat. This was apparently due to the composition of these wheats, in which there are two unknown major components, an x and y factor. But one of the factors, preferably the x-factor, differs in quantity in each wheat variety, and is apparently the major source from which the insect takes or gains the greater volume of its food supply. The sedimentation values will have a wider range in Ottawa wheat than in the other varieties of of wheat. Therefore, the Ottawa wheat may have a greater quantity of the x-factor than the Pawnee wheat, and the Pawnee wheat more than the mixed wheat. As shown in the graph on Plate VI, Fig. 4, this x-y factor hypothesis is very significant in determining the effects or variation of the sedimentation value as affected by insects and their larval developments.

Insect pests, such as the confused flour beetles, whenever present in numbers, can cause the flour from milled wheat kernels to become grayish and discolored, and will mold more quickly than clean flour. Sometimes the disagreeable, pungent "quinone" odor given off by the scent glands is imparted to wheat which has been infested and which will be ultimately transferred to the flour. Thus, a contrast, which involved two samples of Ottawa wheat infested with these insects in relation with different duration of exposure in the grain, was conducted. The data obtained after the confused flour beetles were removed from the wheat samples for several days, showed a sedimentation value of 51. On the other hand, the wheat sample having the confused flour

EXPLANATION OF PLATE VI.

Fig. 1. Illustration of the effectiveness of insect (Sitophilus oryzae, (L.)) in causing deviations in the Zeleny sedimentation values in the three varieties of wheat tested Ottawa>Pawnee>Mixed wheat.

Hypothesized as an x factor which is nutritional and is in Ottawa>Pawnee> Mixed wheat.

PLATE VI

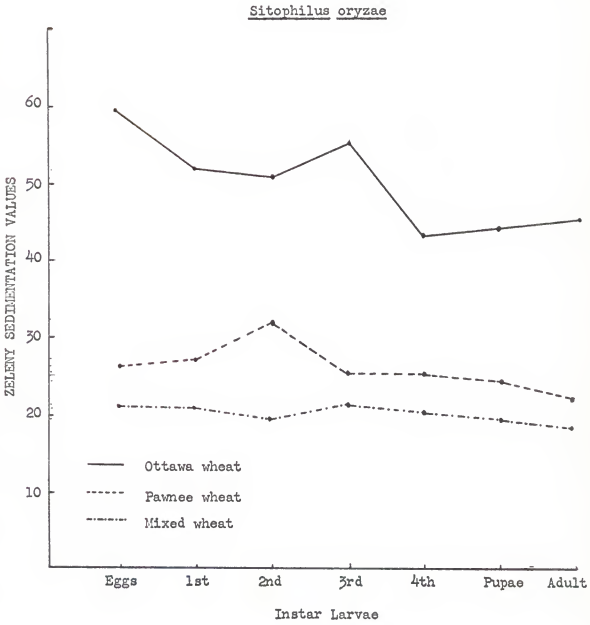


Fig. 4 INSECT STAGES

beetles along with their larval cast skins and frass just prior to being tested, indicated a sedimentation value of 50. Thus, a comparison of results obtained, show that the pungent "quinone" odor given off by the confused flour beetles causes no advertent deviation of the sedimentation values.

Some insects are not a primary pest of stored grain, however, and the adult is apparently unable to survive in sound, uninjured grain. It follows up the attack of the more vigorous grain pests such as the rice weevils. Whereupon, their eventual effects upon the grain and consequently upon the sedimentation value will be lower in comparison to most other grain insects. This is illustrated by the variation of the sedimentation values caused by the flat grain beetle, Cryptolestes pussilus, on Ottawa wheat.

Inasmuch as too much care cannot be undertaken in controlling insect infestation in grain, as well as the necessary precaution to avoid their excessive population increase, experiments to study the apparent deviation, if any, of different quantity and state of a single insect species was arranged. Rice weevils were frozen to death in a deep freeze and placed in a dessicator afterwards, there to remain prior until being placed in the wheat sample to be milled. Figs. 5 and 6, Plate VII, illustrate the deviation of the sedimentation values from an unusually high infestation to a low infestation. With the inclusion of a very high insect infestation, the Tag-Hepenstall moisture meter rolls become clogged and failed to grind the wheat sample. No flour will then be obtained, which signifies that too high an insect infestation in stored grain will be disadvantageous for owners who store wheat and are dependent upon the present loan basis which is influenced by the sedimentation tests.

Live confused flour beetles were also tested in a similar manner, after they were anesthetized with CO_2, previous to their infesting the wheat samples.

EXPLANATION OF PLATE VII.

Fig. 5. An example of the effects of weight (grams) of insects on the Zeleny sedimentation values in Ottawa wheat.

Fig. 6. An example of the effects of different numbers of insects on the Zeleny sedimentation values in Ottawa wheat.

PLATE VII.

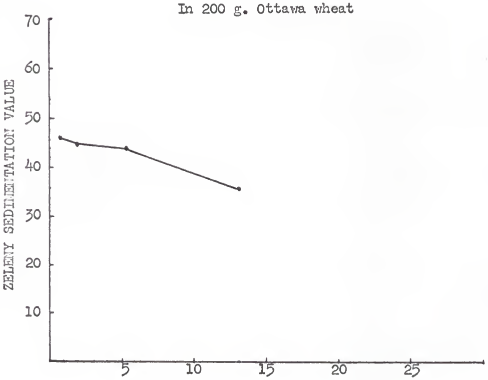


Fig. 5 WEIGHT OF INSECTS (Grams)

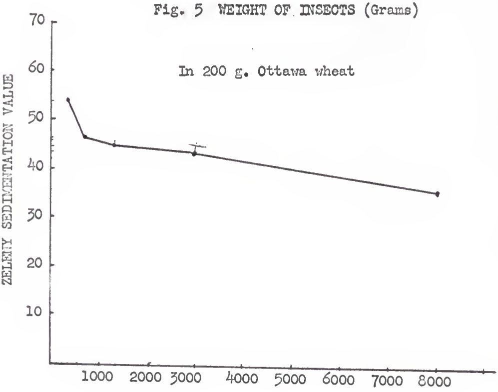


Fig. 6 NUMBER OF INSECTS

The results, with slight deviation, conveyed the same effects as the dead rice weevils.

Insects which feed primarily on the endosperm upon entering a wheat kernel will cause a steady progressive decrease in sedimentation value. But insects such as Sitotroga cerealella, the Angonmois grain moth, which feeds on both the germ and the endosperm, depending on segment of the kernel entered, will cause (See Table 3), uncertain variations in the sedimentation values of wheat.

SUMMARY AND CONCLUSIONS

In considering the variable factors and their limitations, this project has verified the work of others that temperature, moisture and storage do affect the sedimentation tests. It can also be definitely concluded that insects do affect the Zeleny sedimentation tests, but the effectiveness may vary from one variety of wheat to another.

Several factors are responsible for this effect. The chief factor is the amount of insect infestation in the grain, that is, the insect developmental stages and/or the insects infesting the wheat samples do cause a marked variation on the corrected sedimentation values. In addition, the immature stages have different effects on these tests. The least effect is at the egg stage. The most pronounced effects are during the second and fourth larval instar stages, with a progressive increase in effects to the adult stages.

At higher sedimentation values, accuracy becomes moderately less. This may be due to several factors: 1, if the insects start feeding on the germ primarily, the sedimentation value will be greater, whereas feeding on the endosperm primarily the values will be considerably less, apparently because the glutelins and glutenins in the endosperm or an x and y factor are the

EXPLANATION OF FIG. 7

PLATE VIII.

Determination of the least and most effective insect stage in the three varieties of wheat on the Zeleny sedimentation tests.

PLATE VIII.

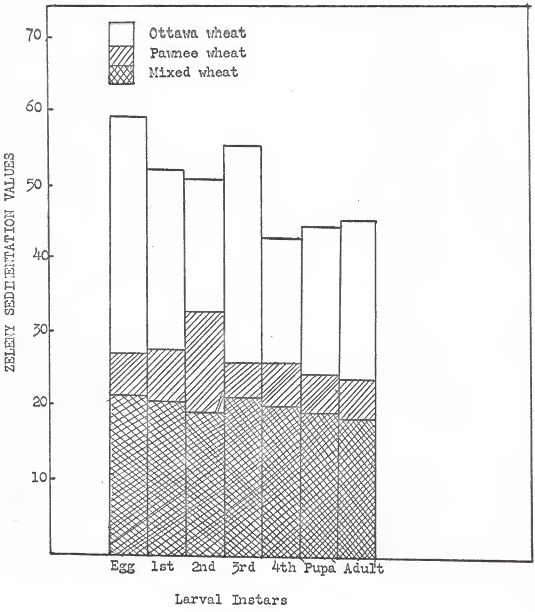


Fig. 7 INSECT STAGES

principal constituents of the grain which influence the sedimentation values; 2, the results obtained from tests of the egg stages of infestation may be tests upon the damaged kernels only, or the tests made with the larval instar stages may be kernels with addition of protein (depending upon insect species and larval stage), thus increasing or decreasing the sedimentation values variably at times.

Pungent "quinone" odors or any other gases as that given off by the confused flour beetle, have no immediate or delayed effects on the sedimentation tests.

Insects which lay their eggs inside the wheat kernels, such as Sitophilus oryzae and Sitophilus granarius, cause a greater effect on the Zeleny sedimentation tests than insects which lay their eggs scattered loosely in the grain such as Tribolium confusum. But, Rhizopertha dominica, although feeding primarily on the flour produced by boring, will not be effective in altering the sedimentation values, unless several damaged kernels are in the sample to be tested.

Therefore, as most of the literature has shown, highly significant correlation can be determined between sedimentation values and bread loaf volumes, baking scores and other recognized qualities of baking strength. It can be assumed that insect infestation in wheat and their effects, can be correlated to the bread-baking quality of wheat.

ACKNOWLEDGMENTS

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THE EFFECTS OF INSECT INFESTATIONS IN WHEAT
ON THE ZELENY SEDIMENTATION TESTS

by

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The Zeleny sedimentation test has been designed as a simple and rapid estimate of the strength (bread-baking quality) of wheat.

The purpose of this study was to evaluate the effect of insect infestations in stored wheat on the Zeleny sedimentation test. Emphasis was placed on: (1) live insects which normally live inside kernels; (2) dead insects which normally are found inside kernels; (3) live insects which normally do not live inside kernels; and (4) dead insects which normally are not found inside kernels.

Tests were conducted at 80°F. $\pm$5°F. and a relative humidity of 70$\pm$5 per cent. Ottawa, Pawnee, and a mixed variety of wheat were used. These wheats were infested with some widespread and prominent stored grain pest species.

The general results of these insect infestations on the Zeleny sedimentation test were a positive effect on the corrected sedimentation values. The egg stage is the least effective stage, while the second and fourth larval instars are the most effective of the instars. There is a general progressively greater effect toward the adult stage.

Thus the insect infestation of wheat on the sedimentation tests, affects the ultimate bread-baking quality of wheat (flour).