FIVE PROPOSED METHODS FOR DETERMINING SMUT CONTENT IN WHEAT[1]

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ABSTRACT

Five simple, uniform, and accurate methods for quantitative determination of the total smut contamination in wheat have been developed; namely: light transmittance, sedimentation, catalase activity, light reflectance, and light absorption. These methods were applied to 138 samples of smutty wheat ranging from 0.5 to 3.0% smut and representing three crop years. The results were compared to smut spore counts based on a reference microscopic method. Correlation coefficients for the relation between test values for all five methods and respective spore counts were highly significant.

Six factors — namely, simplicity, practicability, rapidity, accuracy, precision, and cost of equipment — were considered in evaluating the methods. The sedimentation method would be preferred where low cost of operation is desired. This method could also be used in areas of low smut incidence, where an occasional test for smut may be required. The sedimentation method was used to differentiate between smut and other forms of mold infection in samples of questionable smutty wheat. However, the light-absorption method measuring smut-spore concentration directly on bulk wheat, which takes only 45 seconds per determination, was selected as the most practical and suitable method for routine testing of smut content in wheat where time is a prime factor.

The objective of this research was to develop a uniform, accurate method for determining the total amount of smut in wheat which may be used in routine inspection under the official grain standards of the United States. In selecting the best method the most important considerations are simplicity, practicability, rapidity, accuracy, precision, and cost of equipment.

The present methods for determining the amount of smut in wheat lack accuracy and precision and are slow. Some wheat samples grade smutty on odor alone, some on loose spores adhering to the brush ends of kernels (tagged ends), and some on smut balls alone; some samples grade smutty on a combination of two or three of these criteria. There is need for a uniform, simple, rapid, and more accurate method for quantitative determination of total smut contamination in wheat, including smut balls, broken smut balls, and smut spores.

The search for a method led to several possible approaches. Besides the classic Levi counting-chamber method by Heald (5), the

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following procedures were investigated: 1) analysis of wheat washings or 2) analysis of intact wheat by a light-absorption method. The washings were analyzed by measuring: light transmittance through the spore suspension; volume of sediment; catalase activity; or light reflected from the sediment after it was filtered from the suspension.

Materials and Methods

One hundred and thirty-eight samples obtained from the Pacific Northwest area from the 1954, 1955, and 1956 crops were used. All dockage that could be readily separated from the samples by appropriate sieves was removed. For most accurate results, damaged kernels and foreign material were also removed by hand separation. These samples were graded "smutty" by Government grain supervisors according to the smut dockage method, and the samples were classified into three groups as follows:

Light smut: 0.5 but less than 1.0% smut dockage
Medium smut: 1.0 but less than 2.0% smut dockage
Heavy smut: 2.0% or more smut dockage

Reference Microscopic Method

Cherewick (3) described the use of a wetting agent to aid in removing spores from grain, and the use of a gelatin solution to suspend them so that they would not settle out. In this research, Bactoagar (0.1% solution) was added to the wash water instead of gelatin. A 0.1% solution of a wetting agent (Tween-20, a product of Atlas Powder Co.)[3] was also added to the warm wash water to facilitate removal of spores from the brush ends of the kernels. The presence of wheat starch grains in the filtered wash-water suspensions interfered with smut-spore counts, since the size and shape of the starch grains and colorless, sterile smut spores were frequently similar. Boiling the water suspensions for approximately 30 seconds solubilized the starch grains but left the thick-walled smut spores intact. After cooling to approximately 50°C., about 0.1 g. of Takadiastase was added to eliminate the starch grains.

The microscopic method described below, which includes the above modifications and additions to Cherewick's method, was used as a basis for checking the five proposed methods for determining smut-spore content. The method which was adopted is as follows:

Wash 100 g. of dockage-free wheat with about 110 ml. of warm agar-Tween-water solution (1:1:1000) in an 8-oz. screw-capped square jar for 5 minutes, using a mechanical shaker.

[3] The mention of specific instruments or trade names is made for the purpose of identification and does not imply any endorsement by the United States Government.

Decant suspension through a 70-mesh screen and rinse screen with approximately 10 ml. of warm water.

Bring filtrate and washings (generally about 97 ml.) to the boiling point.

Add approximately 10 mg. of Takadiastase after cooling to approximately 50°C.

Make to 100 ml. in a graduated cylinder and mix.

Remove a drop of this suspension with micropipet, place on a hemocytometer, cover with a cover glass, and count spores.

All spores in all fields of six different mounts were counted and the average spore count per mount was then multiplied by a factor of one-million [100 (ml.) × 10,000 (hemocytometer factor)] to convert the results to the number of spores in the 100-g. sample of wheat.

Light Transmittance Method

Smut-water suspensions can be divided into many degrees of opacity, depending on the concentration of the spores in suspension. Light transmittance of these various suspensions was measured at a wave length of 525 mμ, because greatest differences in light transmittance between smutty and nonsmutty samples were apparent at this wave length. A Bausch & Lomb Spectronic "20" colorimeter was used to measure the light transmittance through the smut-water suspension.

To determine the relation between absorbance and spore count, the "Spectronic 20" was used to estimate the absorbance of suspensions containing different concentrations of pure smut ranging from 2 to 20 mg. per 50 ml. of agar-water solutions. Pure smut spores were prepared by crushing smut balls in a mortar and sieving through a 400-mesh screen. A linear relationship is apparent between the meter readings at 525 mμ and the concentration of pure smut spores (Fig. 1).

The group of 138 samples of smutty wheat was treated in the manner described under "Reference Microscopic Method." Absorbance readings at 525 mμ were made on washings from all of the

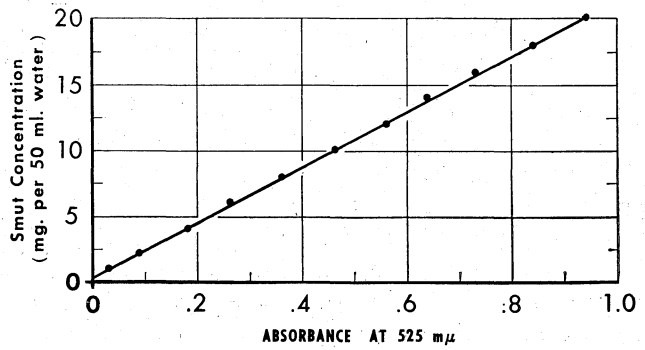

Fig. 1. Relationship between smut-spore concentrations and absorbance at 525 mμ.

samples. The results were statistically analyzed with the spore counts, and a regression equation was derived from the data. Figure 2 shows the regression equation and the straight-line relationship which exists between smut spores in suspension, obtained from washing the 138 samples of wheat, and their respective absorbance readings. The correlation coefficient for the relation between meter readings and microscopic spore counts was +0.81**. The standard error of estimate in determining smut content was 0.21 million spores.

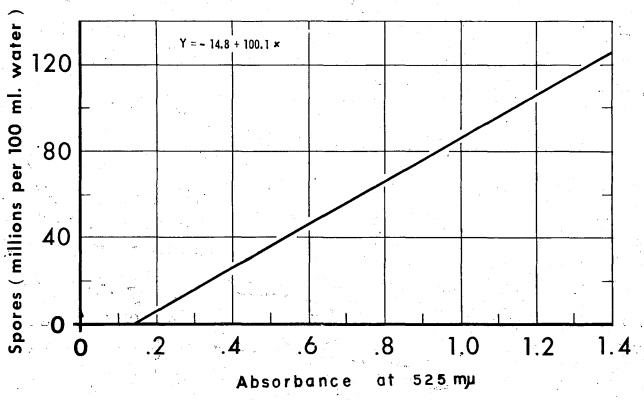

Fig. 2. Relation between smut content (Y) and absorbance (x). Y = predicted value; x = variable which is determined.

Absorbance readings corresponding to smut dockage grades are as follows:

Meter Reading	*Grade*
0.10—0.49	Light smut
0.50—1.00	Medium smut
>1.00	Heavy smut

Sedimentation Method

If it were possible for the smut spores in suspension to coalesce and form a sediment, the amount of smut might be measured directly without using an expensive instrument. Accordingly, efforts were made to find an agent which would cause sedimentation.

Among a large number of chemicals used to determine their suitability to cause spore coalescence such as Hyamine: 10X, 1622, 2389, 3500, also Tetrosan, Cetab, Ceepryn cl. and Nopcocide K, a 10% quaternary ammonium salt solution (Roccal) proved most effective and was selected for use in this method.

The proposed method is as follows:

Weigh 100 g. of wheat into a screw-capped bottle. Add approximately 120 ml. of warm tap water (120°–135°F.) and a drop of Tween-20. Allow to stand for 5 minutes. Shake vigorously for 30 seconds. Replace cap with screw-capped filter and decant into an oil centrifuge tube.

Add approximately 0.6 ml. Roccal with a pipet or graduated medicine dropper, stopper, and mix contents by inverting tube twice.

Allow to stand 10 minutes and read volume of sediment in a tapered centrifuge tube which is graduated from the bottom end.

The rate of smut-spore sedimentation after addition of Roccal is dependent on the temperature of the wash water. When a few drops

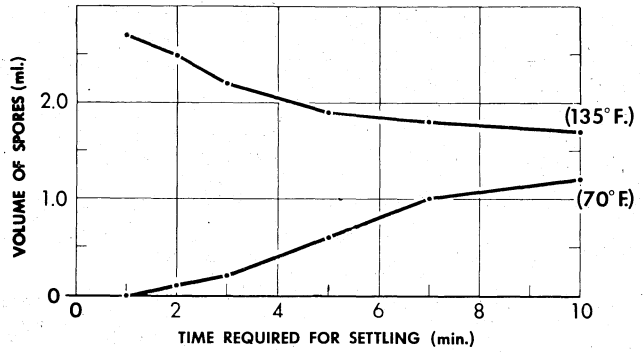

Fig. 3. Sedimentation rate as affected by temperature of suspension.

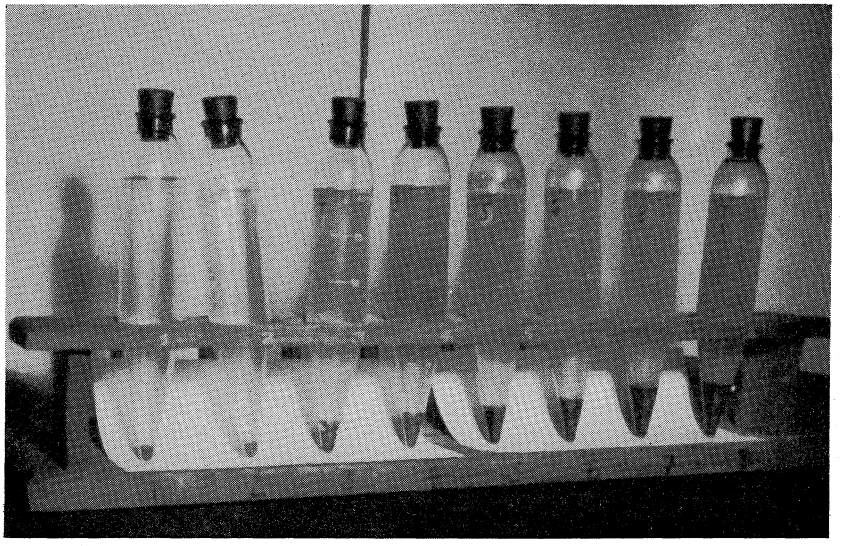

Fig. 4. Smut sediment formed 10 minutes after addition of Roccal. Tubes 1 and 2 represent no smut; 3 and 4, low; 5 and 6, medium; and 7 and 8, heavy smut.

of Roccal are added to a warm-water smut suspension, the spores begin to settle almost immediately. However, when Roccal is added to a cool-water smut suspension, sedimentation is not apparent for the first 2 minutes. Forty percent more smut can be measured at the end of 10 minutes when warm water is used than when cool water is employed for washing (Fig. 3).

In order to determine the optimum quantity of Roccal required and the time for complete sedimentation, two factors were considered: 1) rate of falling of spores, and 2) rate of compaction of the sediment.

On the basis of an experiment with three different volumes of Roccal (0.2, 0.6, and 1.2 ml.), spore falling and compaction were nearly complete at the end of 10 minutes. However, when 0.6 ml. Roccal was used the greatest differences in quantity of sediment were obtained between light and heavy smut samples.

Figure 4 shows the sediment formed 10 minutes after addition of 0.6 ml. Roccal.

Volume of spores corresponding to smut dockage grades is as follows:

Volume of spores	*Grade*
ml.	
0.5–1.49	Light smut
1.5–3.00	Medium smut
> 3.00	Heavy smut

One hundred and eleven or 80.4% of the 138 samples graded by grain supervisors using the smut dockage method fell within the proposed ranges for light, medium, and heavy smut.

Figure 5 represents the regression equation and shows the straight-line relationship that exists between results by the basic microscopic

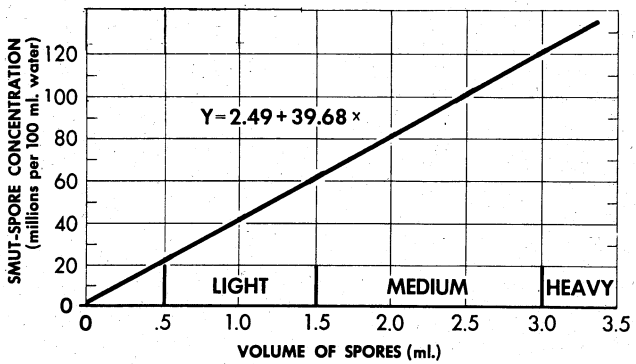

Fig. 5. Relationship between spore counts, volume of spores, and smut grades. Y = predicted value; x = variable which is determined.

and sedimentation methods. The correlation coefficient for this relationship was +0.82**. The standard error of estimate in determining smut content was 0.71 million spores.

Catalase Activity Method

The enzyme catalase is known to occur in nearly all forms of life. According to available reports in the literature, the amount of catalase in wheat decreases during ripening and is generally quite low in the harvested seed. Investigations showed that the catalase activity in smut spores is much greater than that in wheat kernels. Therefore, the catalase activity of smut water suspensions was studied for possible use as an indirect method for determining the amount of smut present in wheat.

Because there was no method for quantitative determination of catalase in smut, the method for determining catalase in beef liver described by Von Euler and Josephson (8) was modified. The amount of hydrogen peroxide that had not been decomposed after reacting with smut catalase under fixed conditions was used as a chemical measure of catalase activity in smutty wheat. The amount of hydrogen peroxide consumed in the reaction with catalase was determined by titration with potassium permanganate in an acid medium.

In the reaction between beef catalase and hydrogen peroxide, the temperature and hydrogen-ion concentration are important factors to be considered, as Morgulis *et al.* (6) have pointed out. Titrations on suspensions having different pH values and at temperatures of 10° and 30°C. indicated that this method could be carried out most satisfactorily at 10° and at a pH of 7.

Pure smut spores were prepared as before by crushing smut balls in a mortar and sieving through a 400-mesh screen. Smut-spore sus-

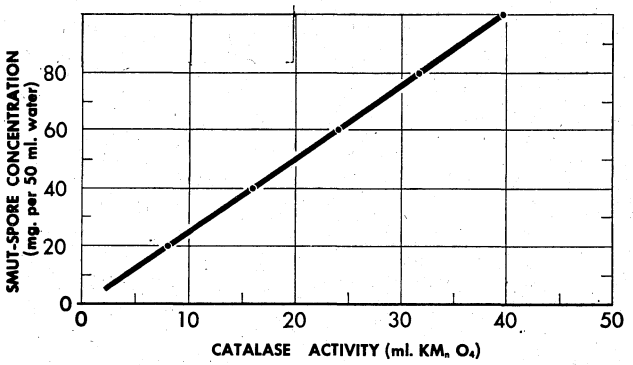

Fig. 6. Catalase activity of pure smut-spore concentration.

cessed compartment of the Agtron reflectance instrument, and the readings were recorded after the meter was standardized with two appropriate color disk standards. After it was determined that pure smut-spore concentrations on filter paper and meter readings are di-

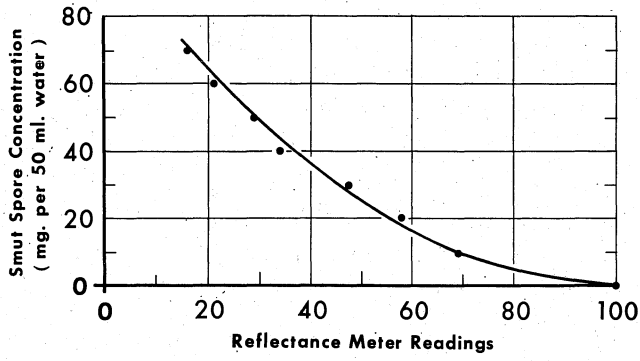

Fig. 8. Relationship between quantity of smut deposits on filter paper and reflectance meter readings.

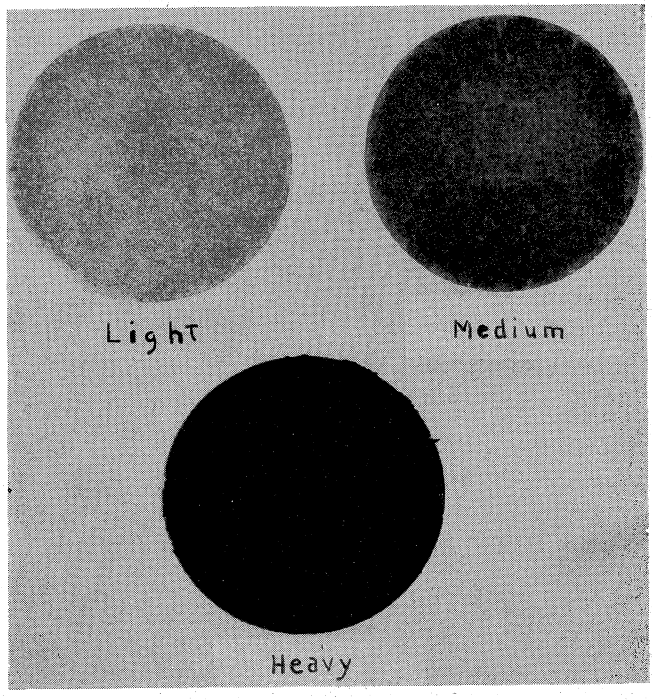

Fig. 9. Smut standards.

rectly related (Fig. 8), a method was developed for determining smut content in wheat using this technique.

Smut spores were washed from 100-g. representative portions of 138 samples of smutty wheat with 120 ml. of Tween-water solution (1:1000) and 5 minutes of agitation. The smut suspensions were decanted through a 70-mesh screen onto a 7.0-cm. no. 5 Whatman filter paper in a Buechner funnel and rinsed with several washings. The dried smut deposits on the filter paper were graded visually by comparison with three standard filter paper deposits which represented light, medium, and heavy smut infestations (Fig. 9). In this manner it was possible to grade the sample into one of the three categories in a matter of seconds. The actual microscopic spore count of 114 or 83.3% of the 138 samples visually tested was within the accepted range of the standard with which it was matched.

To evaluate the unknown sample more precisely, the 138 filter papers containing smut spores were also tested with the Agtron reflectance meter. Two plastic standards were used in the manner described previously. The individual filter papers containing spore deposits were placed in the recessed compartment and readings observed after 10 seconds.

Figure 10 shows the curvilinear relationship which exists between spore counts from the 138 wheat washings and their respective light-reflectance meter readings. The data obtained were statistically analyzed and an equation for the graph was derived. The regression equation for this relation was found to be $Y = 97.2 - 44.35 \log x$. The correlation coefficient for the relation between the meter readings and

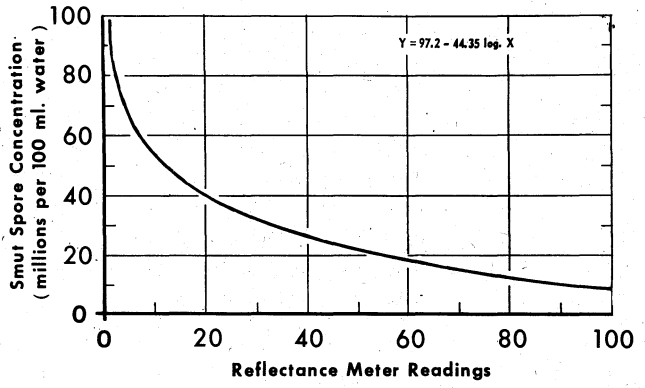

Fig. 10. Relationship between spore counts and reflectance meter readings. Y = predicted value; x = variable which is determined.

microscopic spore counts was −0.80**. The standard error of estimate was 2.3 million spores.

Reflectance meter readings corresponding to smut dockage grades are as follows:

Meter Readings	*Grade*
30.0–100	Light smut
4.0– 29.9	Medium smut
<4.0	Heavy smut

Light Absorption Method

The second general category of methods for determining the amount of smut is its measurement directly on the bulk wheat. Grain inspectors and dealers interested in a quantitative test for smut contamination on samples of wheat prefer a quick, direct test using bulk wheat rather than one in which the smut spores must be separated from the wheat before the analysis is run.

Norris (7) described a method for evaluating the internal characteristics of agricultural commodities. He used a modified Bausch & Lomb Grating Monochromator to measure the spectral transmittance curves for intact samples in the wave length range from 400 to 1000 mμ. This instrument, which was later named "Rephobiospect"[4], has been shown to be applicable for the measurement of internal color of tomatoes (2) and internal characteristics of fruit (1). Measurements made on the Rephobiospect indicated that smut content in wheat could be determined by a transmittance technique, and a simpler instrument (smut meter)[5] operating on the same principle was developed and used in this investigation (Fig. 11).

The Rephobiospect consists essentially of three parts: 1) grating monochromator to illuminate the sample; 2) integrating sphere to collect the light transmitted by the sample; and 3) recording photometer to measure and record the light transmitted by the wheat sample.

Preliminary tests with the Rephobiospect showed that smutty and nonsmutty samples of bulk wheat gave characteristic differences in near infrared transmittance spectral readings. The possibility of using the differences as a basis for an objective method for estimating the degree of smuttiness in wheat was investigated. The spectral transmittance diffierences were observed by comparing smutty and nonsmutty wheat as follows:

An aluminum container with a clear plastic bottom, 1⅜ in. in di-

[4] This instrument is not yet commercially available. It was developed in the Agricultural Marketing Service, Marketing Research Division, Biological Sciences Branch, Quality Evaluation Section Laboratories at Beltsville, Md.

[5] This instrument is available from the American Research & Mfg. Company, Rockville, Md.

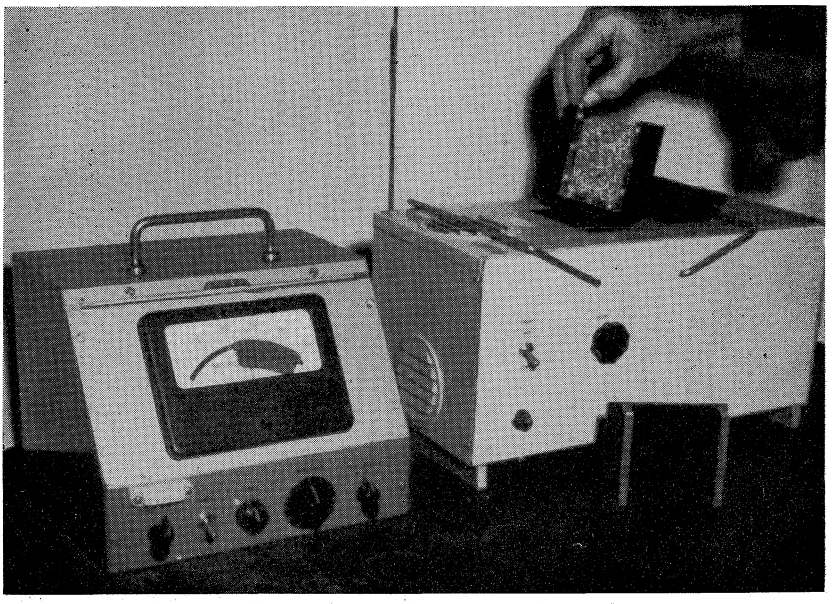

Fig. 11. Smut meter.

ameter and 1 in. in height, was filled with 15 g. of nonsmutty wheat, placed in the sample receptacle of the Rephobiospect, sphere closed, and the spectral transmittance curve recorded. Light, medium, and heavy smut-infected samples were tested in the same manner.

A less expensive portable meter (Fig. 11), which is similar in principle to the Rephobiospect, was designed and constructed for testing samples of bulk wheat for smut content in the field. This smut meter consists of two units: one unit contains the light source, filter assembly, sample holder, and a multiplier phototube; the second unit houses a meter and other electronic components. A piece of amber-colored phenolic laminated cloth (3-3/8 by 3-1/2 by 9/32 in.) was used as the standard.

The principle of operation is as follows: Light energy from a tungsten light source passes through a filter assembly, sample, and into a multiplier phototube. The absorbance difference at two wave lengths (800 and 930 mμ) is read from the meter. Smut spores (dark areas) adhering to the wheat kernels increase the absorption of light at the shorter wave length relative to the longer wave length. Therefore, the greater the amount of smut in a given sample, the greater is the absorbance difference between 800 and 930 mμ.

The group of 138 samples of smutty wheat was tested by the following procedure:

An unweighed sample of wheat (approximately 90 g.) is poured into a 3 by 2 by 1-10/32 in. container. The opposite faces are made of clear plastic, making the light path equal to 1-10/32 in. The container is placed in the sample compartment. The filter knob is pulled out so that the 930-mμ filter is in place, and the zero knob is adjusted so that the meter reads 0; then the filter knob is pushed in so that the 800-mμ filter is in position and the meter reading is taken.

Figure 12 shows that maximum differences between the nonsmutty wheat spectrum (curve I) and the three smutty wheat spectra (curves II, III, IV) are evident at approximately 800- and 930-mμ wave lengths. The system response is indicated by curve V.

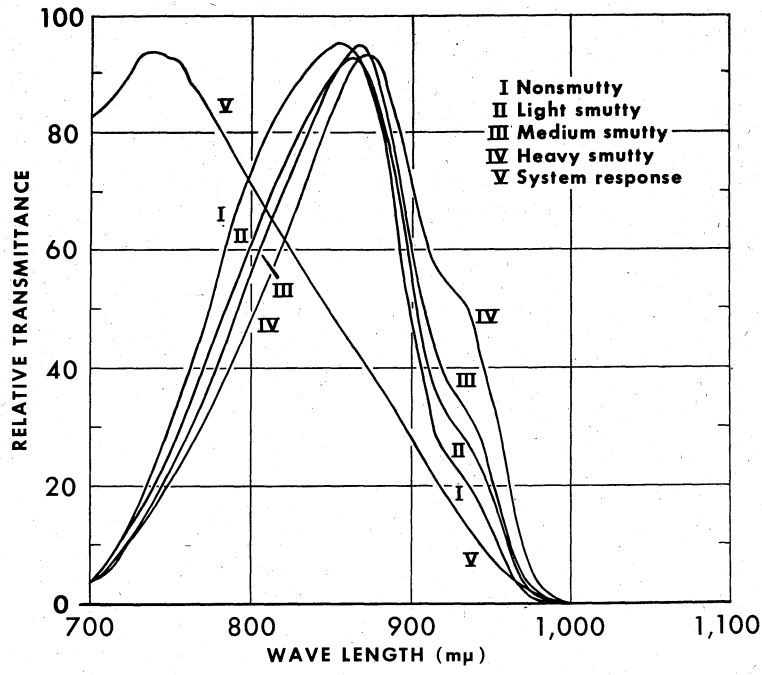

Fig. 12. Relative transmittance curves for four samples of wheat.

The presence of smut in bulk wheat is indicated by a lower relative transmittance value at 800 and a higher value at 930 mμ when compared to nonsmutty wheat samples. By measuring the transmittance at two wave lengths and using the ratio of these values as the measure of smut content, the most meaningful results were obtained. A ratio measurement gives a value which is insensitive to changes in lamp intensity, amplifier gain, size and shape of kernels, and similar factors which affect both wave lengths. A transmittance ratio[6] is equiv-

[6] Transmittance ratio = light energy at 800 mμ/light energy at 930 mμ.

alent to an absorbance difference[7] measurement. The smut meter is designed to measure absorbance differences rather than a transmittance ratio which is measured by the Rephobiospect.

One hundred and thirty-eight samples of smutty wheat were tested for smut using the smut meter. Duplicate analyses showed that the results are reproducible within 5% of the average of ten replicate determinations.

The spore counts from the basic microscopic method for determining smut in wheat were correlated with the meter readings. The curve in Fig. 13 was derived from the data. The regression equation for this relationship was found to be log $Y = 5.388 + 3.815 \log x$. With the use of this equation and plotted logs of the variables, a straight line was obtained. The correlation coefficient for the spore counts and absorption data in the near infrared region was + 0.85**. The standard error of estimate was 0.26 million spores.

The relation between meter readings and smut grades was determined and the results are shown in Table I. The smut meter read-

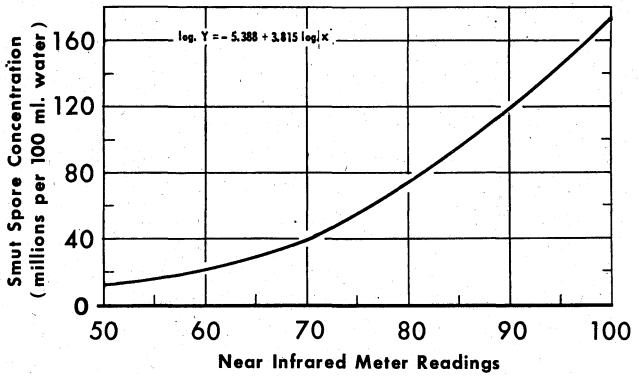

Fig. 13. Relationship between spore counts and near infrared meter readings. Y = predicted value; x = variable which is determined.

TABLE I

RELATION BETWEEN SMUT METER READINGS AND SMUT GRADES

SMUT GRADE	NO. OF SAMPLES	METER READINGS [a]	
		Actual Range	Proposed Range
Light	46	35–67	40–64
Medium	45	56–84	65–85
Heavy	47	85–>100	>85

[a] Meter readings are expressed as the absorbance difference between 800 and 930mμ. A value of less than 40 would indicate no smut.

[7] Absorbance difference = log of energy at 800 mμ — log of energy at 930 mμ.

ings varied from 35 to > 100. A low value denotes little or no smut in the sample, and conversely, a high value indicates a high degree of smut contamination.

A study was also made on 116 samples of nonsmutty wheat to determine what possible influence differences in moisture content and weight per bushel (kernel size) might have on meter readings. Since damaged kernels and foreign material may also affect meter readings, they were removed from the samples by hand separation. Moisture and weight-per-bushel values were obtained from the 116 official grade certificates representing samples of smut-free white wheat obtained from the Pacific Northwest area.

The 116 samples of nonsmutty white wheat were divided into three groups according to their test weight values (Table II). Tests made on samples of like test weight showed no significance at the 5% level for all three groups when meter readings were correlated with moisture contents.

TABLE II

RELATION BETWEEN MOISTURE CONTENT AND METER READINGS OF NONSMUTTY WHEAT

TEST WEIGHT	No. OF SAMPLES	MOISTURE	METER READING	CORRELATION COEFFICIENT
lb		%		
58.0–60.0	44	8.4–15.4	21–46	−0.051
60.1–61.0	36	8.3–13.5	22–44	−0.218
61.1–63.8	36	8.2–12.6	20–49	−0.320

The relation was also determined between the 116 meter readings and respective test weight values. A correlation coefficient of −0.131 indicated no significance at the 5% level.

Therefore, neither moisture content nor test weight of the samples of wheat tested had a significant influence on the meter readings.

Evaluation of Methods for Determining Smut Content in Wheat

For the purpose of selecting the most practical method for determining smut content in wheat, each method was evaluated on the basis of eight important factors and the comparisons are given in Table III.

Discussion

The reference microscopic method served as a basis for calibrating and comparing the five proposed methods for determining smut content in wheat. The reference method is not practical for routine use

TABLE III

EVALUATION OF METHODS FOR DETERMINING SMUT CONTENT IN WHEAT

FACTOR	METHODS						
					Light Reflectance		
	Basic Microscopic [a]	Light Transmittance	Sedimentation	Catalase Activity	Visual Comparison	Instrument	Light Absorption
Simplicity	Complex	Simple	Simple	Complex	Simple	Simple	Simple
Practicability	Poor	Good	Very good	Poor	Good	Good	Excellent
Rapidity [b] (minutes)	30	5	5	20	10	10	0.75
Accuracy	Good	Good	Good	Fair	Fair	Good	Good
Precision	Good	Good	Good	Fair	Fair	Good	Good
Correlation coefficient		+0.81**	+0.82**	+0.75**	[c]	−0.80**	+0.85**
Standard error (million spores)		0.21	0.71	3.5		2.3	0.26
Estimated cost of equipment	$500	$300	$20	$100	$50	$300	$500

[a] "Reference" method against which the five new methods were compared.
[b] Time per determination when run sequentially.
[c] 83.3% of these samples fell within the range of the standard with which it was matched.

because it is slow and tedious, but the other methods which were developed are more rapid and simple.

In the light-transmittance method, agar in the wash water is essential to keep the smut spores in suspension so that reliable meter readings and reproducible results between operators can be obtained. It is believed that the colorimeter responds to the number of heat-resistant thick-walled teliospores of smut present in the suspension, since most other influences have been physically or chemically eliminated. This rapid light-transmittance method for determining smut content in wheat is simple, accurate, and practical. After brief training, nontechnical personnel can make accurate determinations. Fifteen minutes are required for a single determination. When the samples are run sequentially each sample requires only about 5 minutes as compared to about 30 minutes when the basic microscopic method is used. However, the method has a serious limitation, in that the smut spores must be removed from the wheat kernels by the time-consuming washing procedure. It would be more desirable to test bulk wheat directly for smut content, since time is a prime factor in grain inspection work.

The sedimentation test for quantitative determination of smut content in wheat is simple, accurate, inexpensive, and rapid. The most important feature in this quick test is its economy. It is estimated that it would cost an inspection laboratory already equipped with a balance, about $20 for sufficient equipment to determine the smut content on 100 samples of wheat daily. The daily cost for reagents is nominal and each sample requires only about 5 minutes per determination. This test could be used in areas of low smut incidence where an occasional test for smut may be required. This technique was found to be helpful in differentiating between smut and other forms of mold infection in samples of questionable smutty wheat.

The catalase activity method requires inexpensive equipment and reagents and gives fairly reproducible results, but has the disadvantage that it takes approximately 20 minutes per determination when samples are run sequentially. Also, catalase activity decreases during the reaction even at low temperatures because of its partial destruction. In addition, the accuracy of this method may suffer from side reactions between the strong oxidizing action of permanganate and impurities. However, this phenomenon was not observed in filtered samples. Another disadvantage stems from the fact that working at 10°C. is less convenient than working at room temperature. However, with the sacrifice of some degree of accuracy this indirect procedure for determining smut content could be performed at room temperature.

Needless to say, this chemical method would be very impractical in inspection offices where several hundred samples must be graded within a day's time.

The visual comparison test is rapid (10 minutes) and simple to perform; filter paper standards are easily prepared, and the cost per determination is negligible. The Agtron reflectance test, however, could be performed almost as rapidly and with better accuracy than the visual comparison of smut deposits, because it is an objective method.

A number of points should be considered in using the light-absorption method. It has distinct advantages over the other four methods because of its practicability, simplicity, rapidity, accuracy, and good reproducibility. In addition the light-absorption method eliminates the subjective influences usually found in visual grading. The instrument is commercially available at a cost of approximately $500. This procedure should find practical application in grain inspection work where rapid analysis is desirable. With this instrument a single operator can test 500 samples of wheat for smut content in an 8-hour working day.

In all of the proposed methods, dockage was removed from the wheat before the analysis proceeded. Dockage includes such macro contaminants as large stems, weed seeds, and dirt particles, which are removed by passing over a riddle through a series of screens and by means of air. Even so, it is obvious that micro contaminants (non-smut particles) are present in smut-water suspensions, decreasing the accuracy for determining smut concentration in dockage-free wheat. However, in the 138 samples of wheat analyzed for smut content in this study, this apparent factor of contamination did not significantly alter the relation between method results and spore counts, as evidenced by the high correlations obtained in all five proposed methods.

Since the presence of smut within kernels of wheat that were presumedly smut-free has been established by Griffith *et al.* (4) and later by Zscheile (9,10), the light-absorption method, which determines the external as well as the internal infestation of smut, should be more accurate for measuring total smut contamination directly in samples of bulk wheat.

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Literature Cited

1. BIRTH, G. S. Looking inside fruit. Agr. Marketing **2:** 2–3 (1957).
2. BIRTH, G. S., NORRIS, K. H., and YEATMAN, J. N. Nondestructive measurement of internal color of tomatoes by spectral transmission. Food Technol. **11:** 552–557 (1957).
3. CHEREWICK, W. J. An improved method of determining the smut spore load on cereal seed. Can. J. Research **C22:** 120–126 (1944).
4. GRIFFITH, R. B., ZSCHEILE, F. P., and OSWALD, J. W. The influence of certain environmental factors on expression of resistance to bunt in wheat. Phytopathology **45:** 428–434 (1955).
5. HEALD, F. D. The relation of spore load to the percent of stinking smut appearing in the crop. Phytopathology **11:** 269–278 (1921).
6. MORGULIS, S., BEBER, M., and RABKIN, I. Studies on the effect of temperature on the catalase reaction. I. Effect of different hydrogen peroxide concentrations. J. Biol. Chem. **68:** 521–563 (1926).
7. NORRIS, K. H. Measuring light transmittance properties of agricultural commodities. Agr. Eng. **39:** 640–643, 651 (1958).
8. VON EULER, H., and JOSEPHSON, K. Über Katalase. I. J. Justus Liebigs Annalen der Chemie **452** (2): 158–181 (1927).
9. ZSCHEILE, F. P. Search for chlamydospores in wheat seeds grown under conditions favorable to bunt expression. Phytopathology **46:** 182–183 (1956).
10. ZSCHEILE, F. P., and ANKEN, M. Limited development of Tilletia chlamydospores within wheat kernels. Phytopathology **46:** 183–186 (1956).