A photoelectric system for measuring mechanical damage of corn

by

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TABLE OF CONTENTS

INTRODUCTION

American farmers harvest nearly 5.5 billion bushels of corn annually. Of this, nearly 3 billion bushels move in the trade channels. The remainder of the crop is utilized by the producers. Standing in the fields ready for harvest, corn is, sound, undamaged, sweet smelling, shiny and golden, excellent as food for man and beast. The deterioration of corn begins with the harvesting operation. Nearly every operation in the drying, storing and handling processes further decreases the quality of the grain. The rate of deterioration is dependent on the initial injury sustained during the harvest operation. During harvest the corn kernels are subjected to damaging impacts and compressive forces. As a result of mechanical injury encurred the corn may deteriorate in storage to such an extent that it becomes unuseable for food or feed.

Mechanically injured seed is a low quality product and causes direct financial losses from production to consumption. Kaminski (1969) estimated that mechanical damage reduces the market value of corn 3 cents per bushel. This factor represents approximately a 90 million dollar annual loss to corn producers. Small kernel fragments blown out with combine trash causes yield reductions. Mechanical damage may also add to the cost of drying, handling, and processing the corn. Johnson and Lamp (1966) described hidden losses including reduction of weight during storage due to increased respiration of the damaged product and increased biological activity of fungi and molds living in the cracks of damaged kernels and in broken grain parts. It is estimated that mechanically

damaged seed results in a total monetary loss of nearly 0.5 billion dollars annually to the entire corn industry. In addition, an inferior product is being delivered to the consumer.

The level of mechanical damage to corn parallels the increase in field shelling in the corn industry. In 1974, approximately 75 percent of the corn crop was field shelled at high moisture. Ayres and Babcock (1972) reported deliveries of corn having 79.4 percent damaged kernels to country elevators. Mechanically damaged kernels are those that are cracked, broken, scuffed, and/or crushed. Ayres also found that some of the producers were delivering corn with only 16.4 percent damage. Apparently, with proper adjustment of the grain combine, the amount of mechanically damaged seed can be substantially reduced.

One major reason that producers are neither conscientious nor concerned about the adjustment of their machines is that there are no economic penalities applied at the time of sale for delivering a badly damaged product, subject to rapid deterioration. At the present time the USDA grain grading system does not include provisions for accounting for seed that are cracked or scuffed. The only limitation in the present system is on material that will pass through a 12/64 inch round-hole sieve. The penalty on grain containing too many small broken parts is a reduction in grade and possibly a reduction in selling price. This system does not however account for kernels that are only slightly cracked or scuffed and that do not pass through the 12/64 inch round-hole sieve. Such kernels may be equally subject to rapid deterioration in storage.

One of the major reasons that there are no penalties for mechanical damage other than fines is that there exists no fast and reliable method of measuring the total amount of mechanical injury in a sample of corn. The objective of the research reported herein is to develop design parameters for a grain damage meter that can be used in the corn trade. It is imperative that a damage meter be developed so that sources of mechanical damage can be identified and eliminated.

REVIEW OF LITERATURE

Mechanically damaged seed adversely affects all segments of the corn industry. It is estimated that damaged seed cost the industry 0.5 billion dollars annually. The producers lose money directly in lower prices for their product. Kaminski (1969) estimated that the farmer loses 3 cents per bushel for each bushel he sends to market due to mechanical damage. Cleaning the corn cost about 1 cent per bushel which is an indirect cost to the producers. There are many other costs incurred in the drying and handling of the crop that is associated with mechanical damage. Dodds (1972) outlined the costs incurred at grain terminals that could be attributed to mechanical damage. Mechanical damage adversely affects artificial drying which further compounds the damage problem.

The utilization of field shelling at high moisture has necessitated artificial drying of the crop. Saul and Steele (1968) reported that the effect of mechanical damage on the safe storage time for damaged corn can be expressed by the following equation.

$$Md = 2.08\ e^{(-0.0239D)}$$

Where Md = mechanical damage time multiplier

D = percentage of mechanical damage.

This indicates that the time allowed for holding the corn between harvesting and drying is inversely proportional to the degree of mechanical damage.

It is necessary for the producer to maxamize the capacity of his

drying facilities in order to dry his crop as soon as possible after harvest. The amount of heat energy required to dry corn depends upon the initial moisture content and the moisture content of the corn as it leaves the dryer. The heat energy required to dry the corn can be applied to the corn in one of two ways. Low temperatures and long drying time or high temperatures with corresponding shorter drying times. To maximize the capacity of his drying facilities the farmers use high temperatures and short drying times which Foster (1968) has shown increases the brittleness of the grain and causes development of stress cracks and endosperm fissions.

The increased brittleness of the crop results in more of the kernels fracturing with each handling. The increased fines and small particles further aggravate storing and handling. Whenever a granular product is dropped into a bin from a point source the smaller particles tend to remain in the core of material referred to in the corn trade as 'spoutline'. This spoutline decreases the effectiveness of cooling and aeration systems. Dodds (1972) indicated that large amounts of foreign matter may double the cooling time required to cool a bin of corn after drying.

Spoutline formation is of great concern to the export shippers. If there is a large amount of mechanical damage prior to loading into the hold of a ship the corn may show excessive deterioration before it reaches the foreign consumers. Injury to the seed coat provides easy access for storage fungi, insects, and bacteria which find an excellent environment in damaged corn that is stored in the hold of a ship. According to Bailey (1968) most

export corn is discounted from 1 to 4 cents per bushel due to mechanical damage. Mechanical damage is further aggravated by the high volume handling equipment that is used in loading and unloading at export terminals. Bilanski (1966) has shown that high speed impacts such as occurs in high speed handling systems can severely damage seed. The deterioration that occurs due to mechanical damaged seed is further compounded by the marketing system that is used in commercial trade channels.

European importers buy corn by the U. S. grade which sets maximum limits for certain factors as outlined in Table 1. In order to maximize profits export shippers blend the corn to meet the grade ordered by foreign buyers by bringing the corn to the maximum permissible amount of the ingredients mentioned in the grade.

Factors in the Official Grain Standards of the United States for corn as published by the USDA (1966) that determine grades include:

1. Classes or colors, such as yellow corn, white corn or mixed corn,
2. Factors that determine the numerical grade such as moisture content, test weight per bushel, foreign material, damaged kernels, heat damaged kernels and the presence of stones or other substances of similar hardness that do not disintegrate readily in water.

There are six grades for shelled corn. The highest numerical grade is No. 1 and the lowest numerical grade is No. 5, the sixth grade is known as sample grade and is the lowest of all. For all practical purposes, No. 1 grade is not used, even if the grade is No. 1, the purchaser pays the No. 2 price. Buyers seldom if ever order No. 1 grade corn in the hope

that the corn that is actually delivered will be No. 1 for which they have paid the price of No. 2.

If one or more of the grading factors do not meet requirements of the numerical grades, the grain falls into the category of sample grade. Grain that is heating or sour, or has objectionable odor, contains unseparable stones, or unsafe for storage or transportation is, also, graded as sample grade. The numerical grade of corn is determined by the factor on which it grades the lowest in accordance with the U. S. grading specifications shown in Table 1.

Table 1. USDA grades and grade requirements for yellow corn, white corn, and mixed corn.

Grade No.	Minimum test weight per bushel	Moisture Content	Broken Corn and Foreign Material	Damaged Kernels	
				Total	Heat Damaged
	lbs	%	%	%	%
1	56	14.0	2	3	0.1
2	54	15.5	3	5	0.2
3	52	17.5	4	7	0.5
4	49	20.0	5	10	1.0
5	46	23.0	7	15	3.0

Sample grade shall be corn that (a) does not meet the requirements for any of the grades from No. 1 to No. 5, inclusive, (b) contains stones, (c) is musty, or sour, or heating, (d) has any commerically objectionable odor, or (e) which is otherwise of distinctly low quality.

The grain elevator purchasing the corn may run newly purchased high moisture corn through a grain cleaner to remove the fine material so that the driers will function better. After drying the corn, they then blend the fines back into the dried corn to meet a specified grade which is being shipped that day. The corn may be reblended at the export terminal to meet an even lower grade that had been purchased by a foreign buyer. In this way corn, hanging in the stalk, undoubtedly of No. 1 grade, may leave the country elevator as No. 2 corn at 15.5 percent moisture content and leave the export terminal as No. 3 corn of 16.5 percent moisture with 4 percent foreign matter. It is thus little wonder that corn reaches foreign users in the sprout and sour condition.

The wet milling industry uses about 5 percent of the total U. S. domestic production of corn. Freeman (1972) reported corn damaged during harvesting, drying, storage, or handling can reduce production capacity of the wet milling plant and result in reduced yields of primary products and impair the quality of the products. Among the most serious problems caused by grain damage are poor millability, low oil recovery, low starch viscosity, and low pigment content of gluten.

Although the dry processing industry accounts for less than 4 percent of the total U. S. production, Roberts (1972) reported that the dry millers are losing nearly one million dollars annually as a direct result of mechanical damage of the corn kernels. One of the principal products of the dry millers is the flaking grit, used for the production of the very popular breakfast cereal, corn flakes. Fractures and fissions in the corn kernels

result in splits and fractured grits, which result in smaller corn flakes which is a less desirable product. To insure customer satisfaction it is necessary to have large flakes which requires corn that is free from severe mechanical damage.

The largest percentage of the corn crop goes to feeding livestock. Nearly 75 percent of the 1973 corn crop was used for livestock feeds. VanWormer (1972) reported that damaged corn results in nutrient loss which reduces the quality of processed feeds. Generally speaking No. 2 yellow corn is satisfactory for feeding livestock. However, some feeders allow as much as 10 percent damage in the corn prior to processing for livestock feed, which invites severe quality degradation and a severe dust problem.

The most serious problem associated with the corn quality that faces the livestock feeders is the presence of molds capable of producing toxins in the feed. The presence of cracks and breaks in the pericarp of the kernels provide an excellent habitat for the growth of molds. The mold Aspergillis flavus produces a carsinogenic known as aflatoxin. The presence of aflatoxin in a shipment may result in the material being seized and destroyed. VanWormer (1972) reported that in the Fall of 1971 the Food and Drug Administration seized 180,000 pounds of corn meal made from aflatoxin tainted white corn. The levels of the toxin exceeded the FDA guidelines of 20 ppb.

It is likely that regulations regarding toxins in feeds will become more stringent in the future. As methods and techniques are improved for the detection of aflatoxin it is likely that the FDA will decrease the maximum allowable concentration for the toxin. Improved detection techniques

will mean that there will be an increase in the frequency of seizures and destruction of aflatoxin tainted corn and corn products. The seizure and destruction of a shipment of corn could be disastrous to those who produce and handle contaminated corn. It is imperative that an effective method of measuring corn damage be developed and made available to producers and buyers so that they can isolate the sources of mechanical damage and minimize the possibility of their product becoming contaminated with molds that produce aflatoxin or other toxic substances.

Definition of mechanical damage of corn kernels

Ayres and Babcock (1972) defined mechanically damaged seed as any kernels broken, chipped/scuffed, or having any minute cracks in the seed coat. The USDA grain grading system on the other hand uses an official grain standard that classifies any material that will pass a 12/64 inch round-hole sieve as broken corn and foreign matter. This classification includes not only corn chips, but, includes stalk parts, dirt, or other material that passes the sieve.

In general mechanical damage can be classed into two categories, external or internal. There are a variety of methods available for measuring mechanical damage. The methods used depend upon the objective for measuring the damage. Some methods have proven to be satisfactory for their intended purposes. For many applications, however, there is not available at the present time one completely satisfactory method of determining the amount of mechanical damage in corn. Agness (1968) speaking for the farm equipment research industry, stated that none of the available methods were suitable for their needs. He would require that a test

be reliable and take no more than 48 hours, and would prefer one that required less than two minutes!

Methods of measuring mechanical damage

The most reliable method available for measuring external damage of corn is through visual inspection. Hukill (1968) reported rather large variations exist in the estimates of mechanical damage graded by visual inspectors. The precision is affected by size of sample, by operator differences in reading the sample, and by the inability of a man to repeat his readings on the same sample. The standard deviation of a man's reading on a sample was about $\pm$ 5 percent damage when the corn contained 25 percent damaged kernels. There have been many attempts to improve the accuracy obtained through visual inspection. The use of a fast green dye that stains the cracks has been developed and used as an aid to visual inspectors, Koehler (1954). Many attempts have been made to devise a technique that would remove the human judgement in the evaluation of mechanical damage.

The breakage tester has been developed as a method of measuring mechanical damage which does not depend upon human judgement. The breakage tester developed and reported by McGinty (1970) gives a measure of the tendency of the kernels to fracture. However, Chung and Converse (1968) reported that the breakage tester is not a reliable and adequate device to use for wheat samples because of the insignificantly small amounts of breakage obtained and poor reproducibility and uniformity of the results. The inability to distinguish small differences in a sample is the major objection to the breakage tester. There have been many other procedures

tested for evaluating external damage but none have been universally accepted.

There have been many elaborate methods devised for evaluating mechanical damage to grain. X-ray techniques as well as lasers have been applied to check for mechanical damage. These methods, however, have not proven acceptable or practical for measuring mechanical damage in corn.

Table 2 list many of the methods that have been proposed and/or evaluated as a potential means of establishing a uniform test for measur-

Table 2. Methods of measuring mechanical damage in grains.

EXTERNAL	REFERENCES
water absorption	Park (1969)
ion absorption	proposed
sound analysis	Beresford (1974)[1]
color sorting	Boyd et al. (1968)
holographic	proposed
electrostatic separation	Matthes and Boyd (1968)
visual	Anon. (1963)
breakage tester	McGinty (1970)
light reflectance	Anon. (1972)
sieve	USDA (1966)
laser analysis	Yoon (1969)
INTERNAL	
CO_2 production	Steele (1967)
X-ray	Hill (1967)
rheological	Mahmoud (1972)
chemical staining (tetrazolium)	Lakon (1949)
photoelastic and numerical analysis	Arnold and Roberts (1969)
translucence	Beerwinkle and Raymond (1972)
acid treatment and germination	Caldwell and Hampson (1958)
radiographic	Chung and Converse (1968)

[1]Hobart Beresford, Ames, Iowa. Personal communication. 1974.

ing mechanical damage in grains. Some of these test have proven to be extremely valuable in certain applications. However, as a tool to be used by country elevators and possibly even farmers, not one of these techniques have been found satisfactory.

Based on the review of literature, a set of criteria is proposed for evaluating a grain testing system for measuring mechanical damage which should make it universally acceptable. The test must be:

1. reliable and repeatible within less than $\pm$ 5 percent,
2. fast, preferably less than 5 minutes,
3. independent of variety differences and independent of moisture content, and
4. inexpensive and simple enough to be operated by unskilled labor.

A method has been invented which, in my opinion, can be developed to meet these criteria. This method incorporates a fluorescent dye and a photoelectric sensing device and is the subject of this research endeavor.

Photoelectric measuring systems

Photoelectric quality control devices have been used to sort agricultural products for many years. Boyd et al. (1968) reported that it was possible to remove stained damaged seed with available commercial color sorting equipment. The principal disadvantage of the commercial units were high cost, low capacity, and the need for some specialized training for operators.

Beerwinkle and Raymond (1972) utilized a device to facilitate optical sorting of middle rice based on translucence differences. Seventy-five percent of the damaged kernels were removed based on differences of trans-

mittance of individual kernels.

Sallen (1971) discussed the application of a photoelectric system for labeling containers. The containers discussed in his report were railroad cars that had a particular sequence of fluorescent or phosphorescent pigments that were scanned with an optical reader. The use of fluorescent pigmented materials as a code increased the reliability of their equipment to the point that the system was functional under actual conditions as encountered by railroad equipment.

McGinty (1970) found that available color sorters were not suitable in arriving at a satisfactory evaluation of corn damage.

Research workers have for many years utilized fast green dye to facilitate visual inspection for mechanical damage of corn samples. If the same logic applies to photoelectric sensing then there should be a dye that would enhance the discrimination of an electric color sorting device for the detection of mechanical damage in grains.

A commercial sorter developed and marketed through Mandrell Industries has been used to sort aflatoxin tainted materials from food products, A. Rodriguez[1]. This machine uses an ultraviolet light which activates a fluorescent material produced by Aspergillis flavus and a photo detector to distinguish between contaminated material and the product that contained no toxins. In order to adopt the same principle to the detection of damaged corn kernels it is only necessary to make the damaged kernels fluoresce in the same manner as the aflatoxin contaminated material.

The damaged portions of corn kernels can be made to fluoresce by treating with a fluorescent dye that selectively adheres to the damaged

[1]A. Rodriguez, Mandrell Industries, Dallas, Texas. Telephone communication. 1974.

surfaces of the kernels. When the treated kernels are then radiated with an ultraviolet light, the areas of the kernels that have retained the dye will fluoresce.

The light energy generated by the fluorescence can then be sensed with a device equipped with a photomultiplier tube (PMT). The response of the PMT is linear with the amount of incident light energy. It can thus be assumed that the total energy of the fluorescent light is directly related to the area of fluorescing material. This results in a linear response from the PMT relating fluorescent intensity of the area of the exposed damaged surface of the the corn kernels.

The use of fluorescent pigments have found wide acceptance and uses in many areas of research and commercial applications. Fluorescent materials have the property that, when stimulated by an appropriate light source, emit light of a longer wavelength than the incident light. In practice ultraviolet is used as the light source and the emission is in the visible spectrum. Both the relative spectral purity of the emission and the isolation between source and the emitted wavelength are valuable features in the design of optical readers which use fluorescent materials.

The dye

Fluorescent materials have come into extensive use in research and industry. Fluorescence excited by visible light was first discovered in 1602 by V. Cascariola, DeMent (1945). Sir G. G. Stokes described the mechanism of the adsorption and emission process in 1852 according to Guilbault (1973). Stokes also named fluorescence after the mineral fluo-

spar (Latin fluo = to flow + spar = a rock) which exhibits a blue-white fluorescence. Since the time of Cascariola thousands of compounds have been discovered that exhibit some form of fluorescence. DeMent (1945) has tabulated more than three thousand substances that exhibit fluorescence under suitable conditions. Since 1945 many more substances have been shown to fluoresce. Many assay techniques have been developed based on the phenomenon of fluorescence.

Milner et al. (1950) successfully selectively treated insect eggs with a fluorescent dye to aid in the detection of insect damage by visual inspection. With the development of spectrofluorometers many laboratory assay techniques have been developed.

Potter and Shellenberger (1952) reported coupling a substituted quinone with a dihydroxy phenol which occurs in insect cuticles to produce a dye which could then be determined spectrophotometrically.

Cole and Milner (1953) reported fluorescence to be more sensitive than colorimetry in differentiating sound from germ damaged wheat and in revealing the progressive browning in stored germ.

Hansen (1967) used fluorescence as a label for proteins. Udenfriend (1962) reported that dyes could be absorbed onto protein. The labeling of proteins has made possible the use of spectrofluorometers as a laboratory tool in protein research.

Green and Kasarda (1971) discussed binding 2-D-Toluidinylnaphthalene-6-Sulfonate to α-Gliadin of wheat so that the protein could be identified with the aid of a fluorometer. The use of fluorescent probes has been extensively used in analytical chemistry to facilitate the detection and

quantifying of many compounds. Green and Kasarda (1974)[1] suggested that 8-anilino-1-naphthalene sulfonic acid (ANS) would probably be a good stain for indicating mechanical damage in corn and that the ANS was of relative low cost in relation to other possible fluorescent probes for indicating mechanical damage. The ANS is non-fluorescent when in solution but fluoresces strongly when combined with protein.

The corn kernel

The complex structure of the corn kernel has been presented in a detailed analysis by Wolf, et al. (1952). The average composition of whole kernel corn is shown in Table 3 as presented by Inglett (1970).

Table 3. Average composition of whole and hand dissected fractions of corn (moisture free basis).

Fraction	Kernel %	Starch %	Protein %	Lipids %	Sugar %	Ash %
Whole grain	--	71.5	10.3	4.8	2.0	1.4
Endosperm	82.3	86.4	9.4	0.8	0.6	0.3
Germ	11.5	8.2	18.8	34.5	10.8	10.1
Barb(pericarp)	5.3	7.3	3.7	1.0	0.3	0.8
Tip cap	0.8	5.3	9.1	3.8	1.6	1.6

[1]Green and Kasarda, USDA. Telephone communication. 1974.

It is proposed that any external mechanical damage will break through the pericarp and cell wall and expose either the endosperm or the germ. The endosperm consists of two parts, the horny and floury endosperm. The proportions of horny to floury endosperm depend upon the type and variety of corn. However, in either case the endosperm consists of a protein matrix encasing starch granulars, Miller (1938). Any break in the pericarp and cell wall will expose a higher concentration of protein than can be found on the exterior parts of the corn kernel. For a dye to absorb onto the protein of either the endosperm or the embryo it must come into physical contact with the protein. It can be assumed that the only way for the dye to come into contact with the endosperm or embryo is by physical exposure through mechanical damage. Hence, any dye that will selectively absorb onto the protein of the endosperm or germ would be indicative of mechanical damage.

Theory of fluorescence

It is the purpose of this section to describe briefly the phenomenon of fluorescence. A detailed treatise on the subject can be found in the Encyclopedia of Chemical Technology, Voedisch and Ellis (1966).

If a chemical system absorbs energy and then loses energy by emitting electromagnetic radiation, the phenomenon of luminescence has occurred. When both the absorbed and emitted energy is in the form of electromagnetic radiation either fluorescence or phosphorescence has occurred. From a very practical standpoint, it is helpful to distinguish between fluorescence and phosphorescence. The theoretical distinction is based upon the type of transition involved. The more commonly used criterion

is that fluorescence disappears when the excitation source is removed, while phosphorescence has a distinct 'afterglow' or prolonged emission. The second definition is perfectly valid provided one can be certain that there is no delayed fluorescence.

prior to absorption of any type, almost all molecules exist in their lowest energy state, usually called the ground state. The absorption of a quantum of electromagnetic radiation in the ultraviolet and visible regions occurs in a very short period of time (approximately 10^{-15} seconds) and results in the promotion of an electron to a higher energy orbit; thus, the state of the molecule is also changed. The new state in which the molecule finds itself is called the excited state. An individual molecule may have numerous excited states. Each state has superimposed on it a number of vibrational levels, indicative of the various vibrational modes in which the excited molecule can exist.

As implied above, absorption occurs in discrete packets, called quanta. The energy of a quantum of light (photon) is defined by the following expression,

$$E = h\nu$$

Where E = energy

h = Planck's constant

ν = frequency of the absorbed light.

The absorbed energy corresponding to the change in state of the molecule must exactly equal the energy of the quantum of light. For a given molecule, quanta of only certain frequencies will be absorbed, and the structure of the molecule in turn determines these frequencies.

Loss of the absorbed energy can occur by a variety of means. In the ground state, most molecules in solution are in their lowest vibrational state. Absorption usually excites a particular molecule into a higher vibrational level of some excited state. Whether emission will occur from this particular energy level depends on the relative rates of photon emission versus one or more other processes of deactivation. In the process of deactivation to a lower state fluorescence occurs.

Only one photon of fluorescence can be produced per quantum absorbed. The most meaningful physical concept dealing with the efficiency of the fluorescent process is that of the quantum yields or quantum efficiency. Significantly, the quantum yield is usually constant as a function of the frequency of excitation.

$$\Phi = \frac{\text{Number of quanta emitted}}{\text{Number of quanta absorbed}} = \text{quantum yield}$$

The fluorescence quantum yield is affected by temperature. Increases in temperature generally decreases fluorescence and cooling usually increases it.

A physical constant that is characteristic of luminescent molecules is the difference between the wavelength of the excitation and emission maxima. This constant is called the Stokes shift and indicates the energy dissipated during the lifetime of the excited state before return

to the ground state.

$$\text{Stokes shift} = 10^7 \left(\frac{1}{\lambda_{ex}} - \frac{1}{\lambda_{em}} \right)$$

Where λ_{em} = corrected maximum wavelength of emission (nm)

λ_{ex} = corrected maximum wavelength of excitation (nm).

The basic equation defining the relationship of fluorescence to concentration of a fluorescing substance in a liquid as presented by Guilbault (1973) is as follows.

$$f = \Phi I_o (1 - e^{-abc})$$

Where Φ = quantum efficiency

I_o=incident radiant power

a = path length of sensing cell

b = molar absorptivity

c = molar concentration.

When fluorescence is measured at the surface, as is the case for

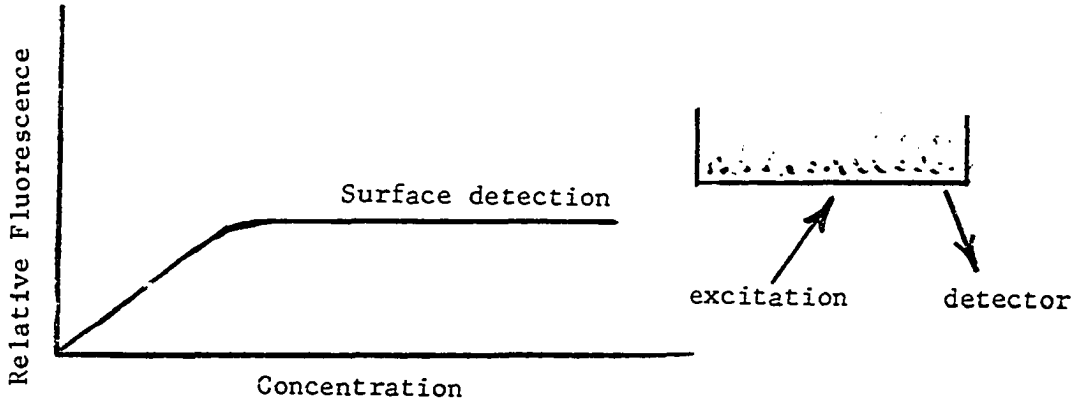


Figure 1. Relationship between fluorescence and concentration.

solids, fluorescence increases linearly, and then levels off as represented in Figure 1.

For solids, the fluorescence (f) increases until the surface is completely covered with the fluorescing material. Hence, the total fluorescence (F) can be found by integrating over the total area,

$$F = \int f \, da$$

Where : F = total fluorescent energy

f = fluorescence per unit area.

Fluorescence is a non-directional phenomenom. That is, the photons are emitted in a random pattern. It can be assumed that a photosensitive surface placed above a uniform fluorescing field would intercept a representative sample of the emitted photons that is proportional to the area of the photosensitive surface. A photomultiplier tube would average over the viewed surface and its response would be a linear function of F. Increasing F would result in a corresponding increase in the output from a photomultiplier tube sensor.

In order to relate the total fluorescence to the area it is desirable to have a photosensing device that responds linearly to the impinging photons. Of the various kinds of photosensitive devices available the photomultiplier tube is the most sensitive in the visible spectrum, Anderson and McMurtry (1966).

Theory of photomultiplier tubes

Photomultipliers convert incident radiation in the visible, infrared, and ultraviolet regions into electrical signals by use of the phenomenon of photoemission and then amplifying the signals by means of secondary emission. Photoemission is a process in which electrons are liberated from the surface of a material by the interaction of photons of radiation energy with the material of the photocathode. The energy of a photon E is given by the following expression,

$$E = h\nu = hc/\lambda$$

Where E = energy

h = Plank's constant

c = velocity of light

λ = wavelength of the incident radiation.

Thus, photons of visible light which are in the wavelength range from 400 to 700 nanometers have energies ranging from 3.1 to 1.8 electron-volts.

An ideal photocathode would have a quantum efficiency of 100 percent; i.e., every incident photon would result in the release of one photoelectron from the material into the vacuum. All practical photoemitters have quantum efficiencies below 100 percent. The more common photoemitters have quantum efficiencies between 0.36 and 28 percent. Amplification of the signal takes place within the photomultiplier by the process of secondary emission. When electrons strike the surface of a material with

sufficient kinetic energy, secondary electrons are emitted. The secondary emission ratio or yield, Q is defined as follows,

$$Q = N_s/N_e$$

Where N_s = average number of secondary electrons emitted

N_e = number of incident primary electrons.

The principal difference between secondary emission and photoemission is that the impact of primary electrons rather than incident photons causes the emission of electrons. With some materials used in the photocathode such as GaP, the secondary emission ratio increases almost linearly up to a very high primary energy; factors up to 130 have been measured. This means, that for relatively low light levels, the response of the photomultiplier will be linear; that is, it is not necessary to have high light levels to accurately measure the quantity of light available.

Based on the phenomenon of fluorescence and the inherent characteristics of photomultiplier tubes a measuring system can be designed that should relate the mechanical damage to the total fluorescence; if, the fluorescence is a function of total mechanical damage.

The light source

The two types of light sources commonly available for fluorescence studies are the mercury vapor and the xenon lamps. The usefulness of a lamp can be evaluated by plotting its relative intensity versus wavelength. Since the total fluorescence observed is proportional to the intensity of the source of excitation, it is necessary to have a significant amount of energy available in the absorption region of the sample to be tested for induced fluorescence.

The spectral characteristics of the 150-W xenon-arc lamp and the Pyrex-jacketed 100 W H-4 mercury lamps are shown in Figure 2.

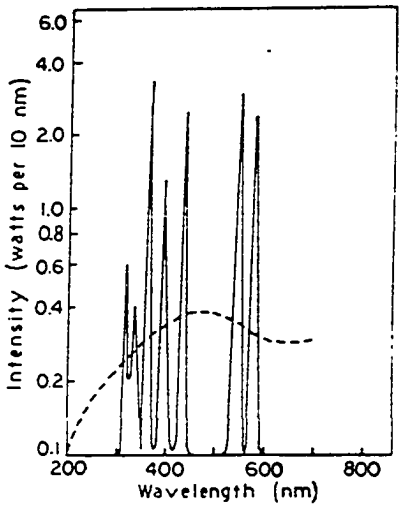


Figure 2. Spectral characteristics of the 150-W xenon-arc lamp (broken curve) and of the Pyrex-jacketed 100-W H-4 mercury lamp (solid curve), Guilbault (1973).

The spectral energy distribution for any mercury lamp or xenon lamp could be expected to be similar to that shown in Figure 2. Individual lamps may vary somewhat from these data, and lamps may change slightly in spectral distribution during life. Principal causes for shifts during life are as outlined in TP-109 published by General Electric

Company, (1971).

1. Changes in transmission of arc tubes and outer bulbs as a result of radiation,
2. Depreciation of phosphor efficiency, and
3. Changes in internal vapor pressure of certain arc materials.

The most commonly used wavelength in the mercury vapor lamp is the resonance lines at 365-366 nm. In this investigation a mercury lamp, model 100, manufactured by Ultraviolet Products, Inc., San Gabriel, California, with appropriate filters to minimize the effects of the light above 400nm was used in the early preliminary testing. Due to the large size of the mercury lamp and the difficulty of incorporating it into the sample compartment a smaller and more convenient light was used in the actual testing of the measuring system. A model UVS-12 manufactured by Ultraviolet Products, Inc. was utilized. The emission curves from this lamp are shown in Figure 3a.

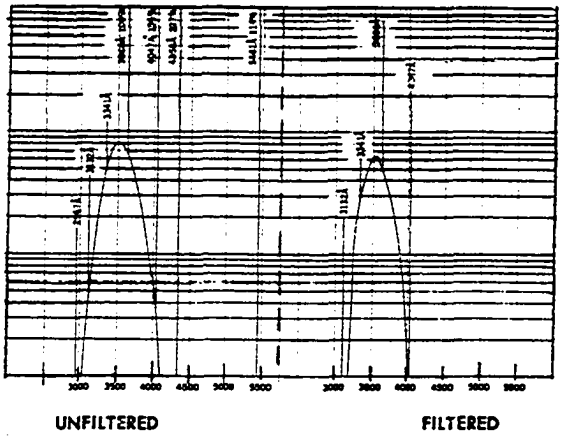


Figure 3a. Emission energy distribution for ultraviolet lamp, model UVS-12.

A significant advantage of a device that uses a fluorescent dye as an indicator for damage would be that it could be used as a screening

device for the detection of aflatoxin contaminated material. There is a material that is associated with aflatoxin that glows when viewed under ultraviolet light, Anon. (1973). Marsh et al. (1969) reported that all Aspergillus flavus isolates caused the greenish yellow fluorescence in living cotton fibers, whereas no other field fungi tested did so. Shotwell et al. (1974) reported that all corn kernels that show the bright greenish-yellow fluorescence under ultraviolet light (365 nm) were contaminated with aflatoxin. Robertson and Dons (1969) reported that aflatoxin B_1, B_2, G_1, and G_2 exhibited a solid state fluorescence emission maxima on silica gel ranging from 427 to 455 nm when the excitation source had a maxima at 368-369 nm. It would be advantageous to have a dual purpose testing machine, one that was able to detect aflatoxin as well as being utilized to evaluate mechanical damage.

By utilizing an excitation source of 368-369 nm, which may excite a fluorescent dye as well as the aflatoxin, it will be possible to have a dual purpose machine. For dual mode operation there are several possible alternatives for measuring either for aflatoxin or for kernel damage. One procedure would be to evaluate the sample prior to treatment with a fluorescing dye for aflatoxin, then make a damage determination on the treated sample. Another technique would be to have the dye that is used to treat the cracks fluoresce at a different wavelength than the aflatoxin. This would make it possible to evaluate for either aflatoxin or damage simply by selectively measuring their respective emission peaks. In a commercial machine it is proposed that the second alternative is the most attractive due to the simplicity in switching filters for the particular wavelength that is of interest.

EXPERIMENTAL EQUIPMENT AND PROCEDURES

Equipment

The system The proposed grain damage detection system is composed of three basic components; light source, fluorescent stained damaged corn samples, and a detection device. The principal involved is to have a fluorescing dye adhere to the damaged parts of the corn kernels, radiate this dye with ultraviolet light and observe the resulting fluorescence with a photo detector. A schematic of the system is shown below.

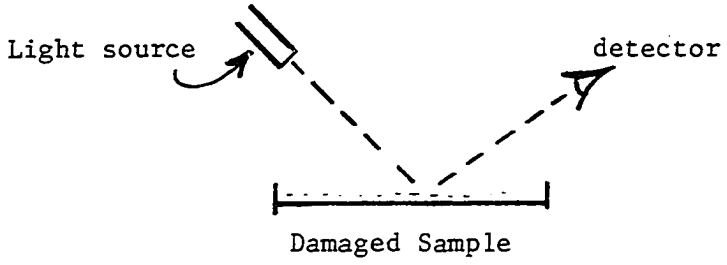


Figure 3b. Schematic of grain damage meter.

The selection of the various components of the system is dependent upon the type of fluorescing dye used to stain the damaged portions of the corn kernels. It is helpful to consider a plot of the adsorption and the emission spectrum of a fluorescing dye in order to gain a better insight into the interdependency of the various components of the damage detection system.

As can be seen from the plot in Figure 4 there is a shift along the wavelength axis in the adsorption peak and the emission peak. This separation allows a system to be designed where the light source can be optically isolated from the detection system. The source should have an emission peak at the adsorption peak of the dye whereas the

detection system should be most sensitive at the emission peak of the dye.

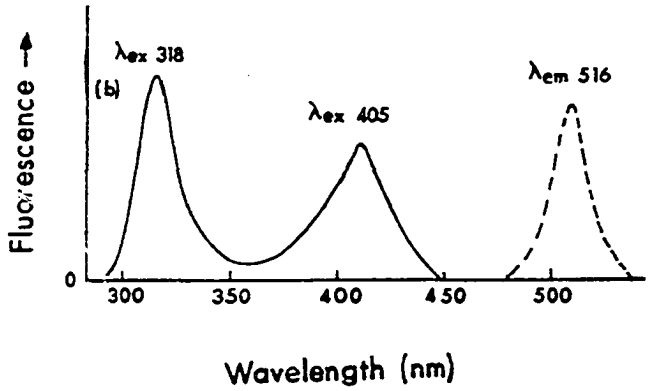


Figure 4. Excitation and emission spectra of naphthol AS-BI, Guilbault (1973).

There are thousands of fluorescent materials available. However, only a few are suitable for a grain damage detection system. The principal criteria used in selecting a suitable fluorescent dye is that the dye stain the damaged parts of the kernels and not the undamaged portions. Two approaches in staining damaged portions of the corn kernels were evaluated. One was to physically trap dye granules in the the cracks and crevices of the damaged kernels and the other was to chemically combine fluorescing compounds to the interior portions of the corn kernels.

The dye Several dyes and dyeing techniques were evaluated in the search for the most satisfactory method of dyeing the damaged portions

of the corn kernels. The following discussion deals with the most promising dyes evaluated in this investigation.

Fluorescent pigmented materials A material known as daylight fluorescent pigments was evaluated as a possible stain for damaged kernels. The theory in using granular materials as an indicator for mechanical damage is that the particles can be physically trapped in the cracks and crevices of the mechanically damaged kernels. There would be no trapping of the dye particles where the pericarp was smooth and undamaged. The available granular fluorescent dyes are pigmented materials that exhibit daylight fluorescence.

Certain substances, especially a number of organic dyes, besides fluorescing under the effect of ultraviolet and shorter wavelengths of light, also have the property of fluorescing when activated by visible light and the blue end of the spectrum—that is, wavelengths in the violet, blue, and blue-green which compose a large portion of daylight. This particular type of fluorescence is called daylight fluorescence.

The fluorescence of organic dyes is associated with the individual molecule of the dyes and in order for then to fluoresce efficiently they must be molecularly dissolved in fairly low concentrations. Since the dyes are organic in nature, it is necessary to have an organic medium or carrier in order to put them into solution, and in order to have a pigment, it is necessary that this medium be solid. The type of material which meets these requirements for a carrier or matrix for the dyes is an organic resin. The daylight fluorescent pigments actually are transparent organic resin particles of glasslike hardness containing dyes in

solid solution which are capable of fluorescing.

The daylight fluorescent pigments used in this investigation was Signal Green in the T-series, Code WT-18 which is distributed by the Day-Glo Color Corp., 4732 St. Clair Avenue, Cleveland, Ohio, 44103. U. S. A. This material radiates predominately at 540-547 mu under the effects of daylight. Signal Green has a luminance factor of 52 percent and is supplied with a purity of 61 percent. The average particle size is on the order of 3 to 5 u. The T-series is provided in an aqueous base which can be diluted with water to the desired concentration for testing and evaluating.

Chemical bond fluorescence Whether an organic compound or dye is fluorescent or not depends upon certain atoms or groups of atoms being present in its molecular structure in a certain way. All fluorescent organic compounds contain an extended series of conjugated double bonds, most of which are present in the form of benzene or heterocyclic rings. They also contain a group of atoms which are electron acceptors and another group ortho, or para to the first, which can act as electron donors, Day-Glo Color Corp. (no date).

There are a number of compounds that can be synthesized that will exhibit fluorescent characteristics that are not found in the parent materials. In the search for a dye that would stain damaged portions of the corn kernels, a material was evaluated that formed double bonds with the interior protein of the corn kernel. As can be seen from the data presented in Table 3, the protein content of the endosperm and the germ are much higher than that of the pericarp, consequently, any material

that chemically combines with protein to form a fluorescent compound would be indicative of kernel fracture, assuming that the endosperm would be exposed upon fracture of the seed coat.

Brand and Gohlke (1972) reported using 1-anilinonaphthalene-8-sulfonate (ANS) as a label for protein in their studies. A similar material was evaluated as a possible indicator of mechanical damage in corn. The ANS used in this study was obtained from the Sigma Chemical Co., St. Louis, Mo.

Other dyes Various other dyes were evaluated and found to be unacceptable as possible indicators for mechanical damage. These have been summarized in Table 4. The carrier used and the major objections are listed. All dyes were evaluated by soaking fractured corn kernels in solution of the various dyes for several minutes and then observing the treated kernels visually under an ultraviolet light.

Table 4. Fluorescent dyes with comments as to their effectiveness for dyeing mechanically damaged corn kernels.

Dye	Carrier	Comment
Auromine O	Ethanol	non-selective
	Benzene	non-selective
Fluorscein	Ethanol	non-selective
	Benzene	non-selective
	Acetone	non-selective
	water	non-selective
Ammonium acetate	none	no visible fluorescence
Ethyl acetate	none	no visible fluorescence
Sodium lactate	none	no visible fluorescence
1% Salicylhydrazide	none	non-selective

The light source In order to properly select a suitable light source it is necessary to consider the adsorption spectrum of the fluorescing dye. It is desirable to have a light source that has an emission spectrum with a peak at the same wavelength as the peak in the adsorption spectrum of the dye. The light source used in this investigation was of the long-wave ultraviolet type that emits predominately at 365 nm. The ultraviolet light source was Model UVS-12 manufactured by Ultraviolet Products of California, U. S. A. The emission spectrum of this light is shown in Figure 3a.

An undesirable characteristic of this light source is that not only does it have a peak emission in the ultraviolet region of the spectrum but also there is considerable emission in the infrared region of the spectrum as well as a peak near 550 nm.

In order to eliminate the undesirable components of the emission a secondary filter that passes only ultraviolet light was placed between the source and the damaged samples. A Kodak 18A glass filter was used as the limiting filter on the light source. The optical characteristics of this filter is shown below in Figure 5.

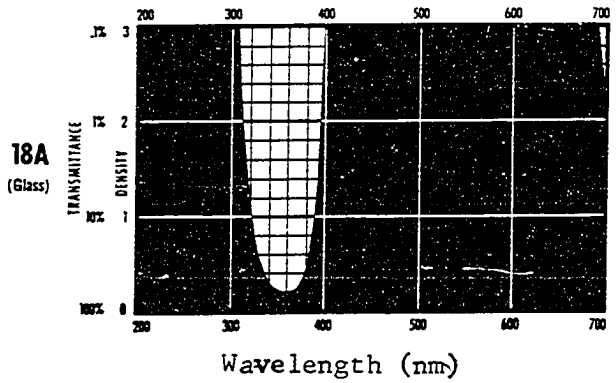


Figure 5. Tramsmittance characteristics of Kodak 18A glass filter.

The photodetector The photosensitive detection device found to be most satisfactory in this investigation was a photomultiplier tube (PMT). An unsuccessful attempt was made in the preliminary investigations to incorporate all solid-state electronics into the measuring system. A photodarlington amplifier, 2N5777, manufactured by Motorola Semiconductor, Inc., Phoenix, Arizona, was evaluated and found to not be sufficiently sensitive to the very low light levels encountered in the fluorescent dye treated corn. No further work beyound the preliminary investigations was done with solid state sensors.

In order to properly select a photodetector it is helpful to consider the emission spectrum of the dye in relation to the sensitivity of the photodetector. The sensitivity curves of the PMT's available from RCA can be found in Appendix A. The emission of all the dyes in this investigation were in the green portion of the spectrum. This means that the PMT should be highly sensitive in the region of the spectrum between 470 and 550 nm.

It is quite obvious that if one has a wide selection of dyes suitable for indicating mechanical damage in grains, then the dye could be selected for an in-house photodetector. In this investigation a Turner Fluorometer, model 110, was available. This fluorometer uses a PMT with a S-4 spectral response. This PMT does not have the most sensitive response curve for fluoresing materials that emit in the green portion of the spectrum. A response curve similar to 129 or 119 would be much more sensitive. However, since the Turner Fluorometer was available and the dye had not been specified prior to the initiation of this investigation, it was decided that the PMT with the S-4 response would be satisfactory for this investigation. It

should be kept in mind that there must be sufficient separation between the light source and the photodetector for optical separation. According to Guilbault (1973) there should be as least 20 to 30 nm separating the two peaks, and preferable 50 or more.

In order to enhance the discrimination of the PMT a second filter was placed between the sample and the photosensitive surface of the PMT. This filter was used to eliminate any scattered light and to permit only the fluorescent light to reach the PMT. The characteristics of the Kodak No. 53 gel filter is shown below. This filter was used to filter the incoming light to the PMT.

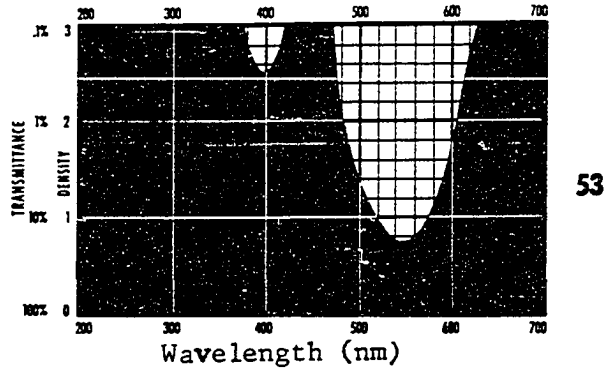


Figure 6. Characteristics of Kodak gel filter No. 53.

It was necessary to modify the Turner Model 110 fluorometer for this investigation. The fluorometer was designed to measure the fluorscence of solutions whereas in this investigation we were interested in measuring the fluorescence from solid state materials not in solution. A schematic of the original equipment is shown in Figure 7.

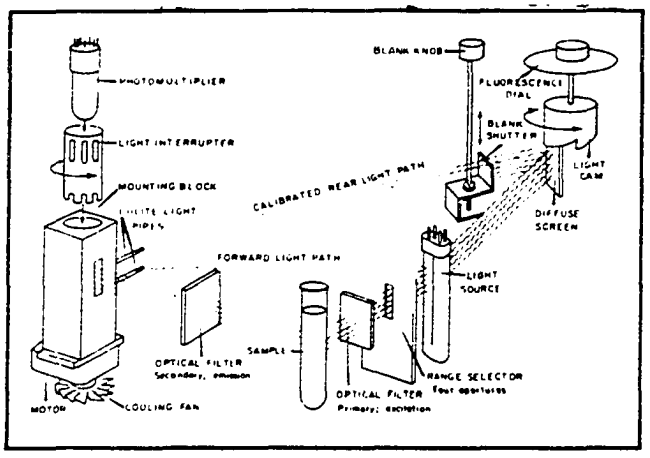


Figure 7. Schematic of Turner model 110 fluorometer.

The modifications included blanking off the internal ultraviolet light source from the sample and building an external sample compartment. An external light source was utilized to excite the damaged samples. A fiber optic was utilized to convey the fluorescent light from the sample to the window of the PMT. Figure 8 shows the fluorometer with external sample compartment removed, voltage regulator, fiber optic, and the external ultraviolet light source.

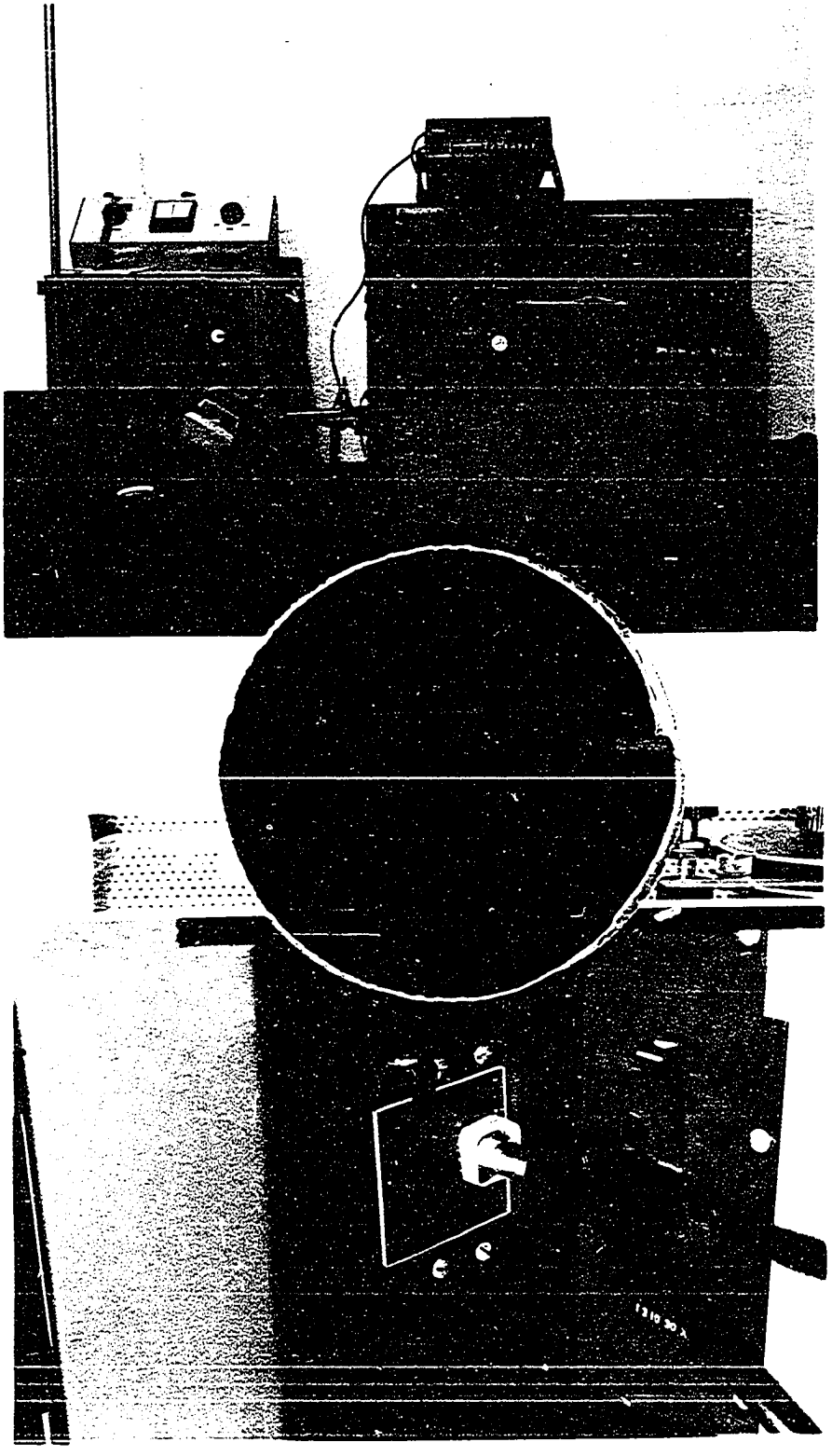
The sample compartment was constructed around the sample holder, fiber optic, and the uv light source to shield the sample from stray light.

Figure 9 shows how the fiber optic was held in place inside the fluorometer. The Kodak No. 53 filter was placed between the end of the fiber optic and photomultiplier tube and was held in place by the spring clip shown in Figure 9. The black plastic tape used to blank off the internal uv light source can also be seen in Figure 9.

The fiber optic used in this investigation was obtained from Edmund Scientific Company. The fiber optic was 12 inches long and ¼ inch in

Figure 8. Research equipment showing fluorometer, voltage regulator, fiber optic, and the external ultraviolet light source.

Figure 9. Modifications made to the interior of the sample compartment of the Turner fluorometer.



diameter with a numerical aperture of 0.55 and an acceptance angle of 60 degrees. About 70 percent of the light striking the end enters the fibers and about 8 percent of this is lost for each foot of fiber. The fiber optic tramsmits wavelengths of 400 to 2000 nm.

The fiber optic was positioned above the sample holder so that the 60 degree cone of light that would be accepted by the fiber optic would encompass the whole of the sample as illustrated in Figure 10.

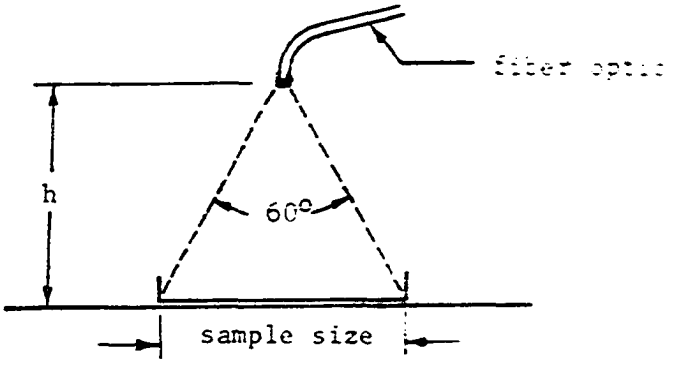
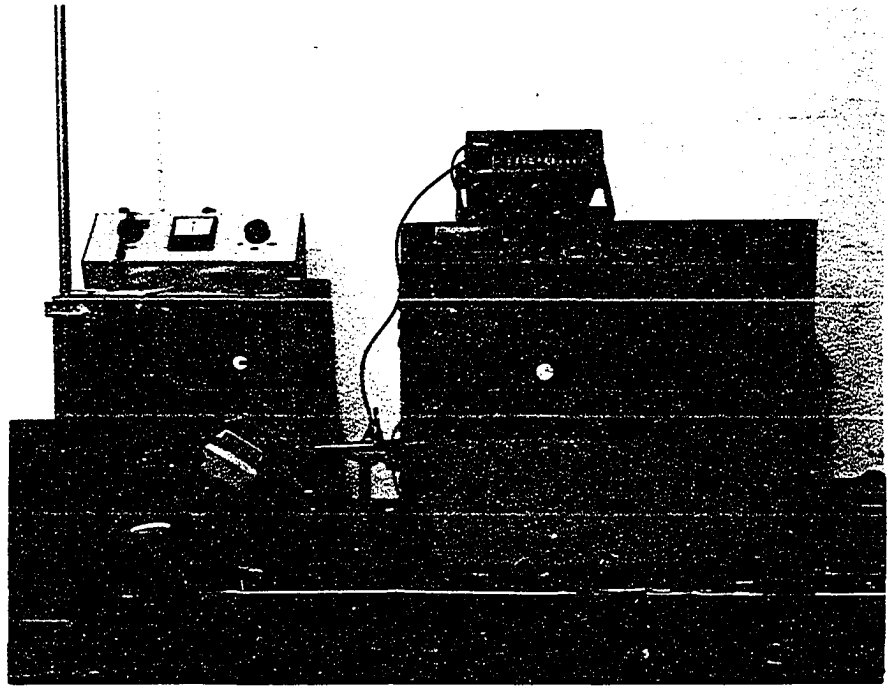
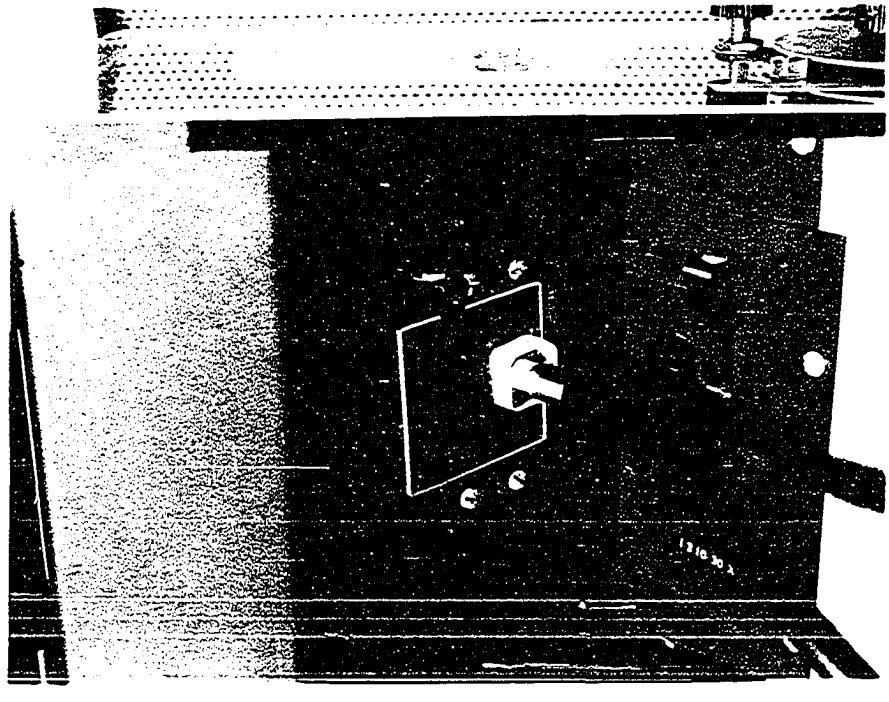


Figure 10. Positioning of the fiber optic in relation to treated sample.

Several sample holders were tested and found to be adequate as long as the sample was sufficiently large to completely cover the bottom of the holder.

The auxiliary equipment utilized in this investigation is shown in Figure 11. The Wiley mill was utilized to grind samples to a uniform particle size. The mill was manufactured by Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, Pa. The grain splitter utilized in separating the samples is manufactured by the Seedburo Equipment Co., Chicago, Ill. The vacuum pump utilized in the preliminary studies was a Model 1400 and manufactured by the Welch Scientific Co., Skokie, Ill.





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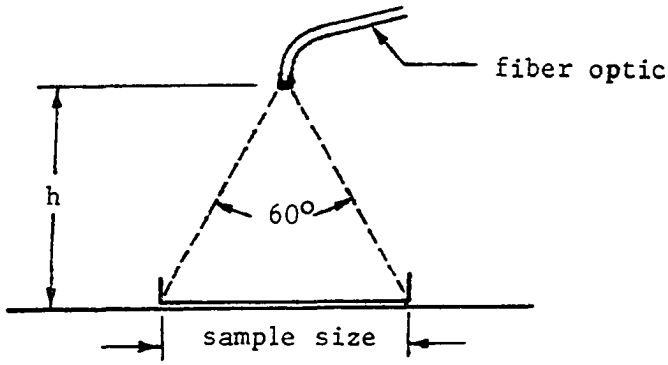

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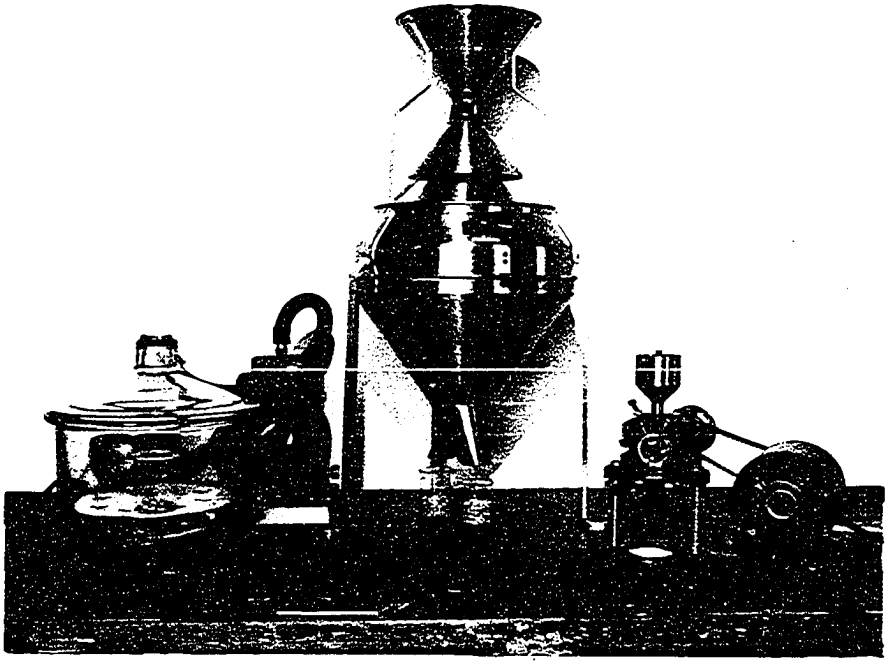
Figure 11. Auxiliary research equipment showing grain splitter, vacuum pump, and the laboratory Wiley mill.



Procedure

The experimental procedure followed in this investigation of methods of evaluating grain damage is outlined in three parts. Part 1 deals with the treatment of damaged grain with granular dyes as an aid to damage evaluation. Part 2 is concerned with the chemical binding of fluorescing materials onto the interior protein of the corn kernel. Part 3 outlines the procedure for evaluating the induced fluorescence in the damaged grain samples.

Granular dyes The procedure of physically trapping the crystal particles of fluorescing dye in the cracks and crevices of damaged corn kernels is similar to a procedure reported by Koehler (1954) for staining damaged corn kernels with fast green dye. Koehler used fast green dye to facilitate the detection of physical damage on corn kernels by visual inspection. He soaked corn kernels for two minutes in a 0.1 percent aqueous solution of fast green dye followed by several washings. In this investigation a standard dye suspension was prepared by mixing 10 grams of pigmented fluorescent dye with 1 litre of distilled water. The dye suspension was vigorously agitated prior to soaking the kernels in the mixture. The damaged corn samples were immersed in the aqueous dye suspension for two minutes and then washed with tap water to remove excess dye. The dyed samples were then dried on paper towels prior to evaluation for kernel damage.

One hundred grams of corn were selected from corn samples that had been harvested in the Fall of 1972. These samples were from an experimental machine harvesting study and had been dried and stored at room

temperature. Extensive damage analysis had been made on these samples by visual inspection and reported by Chowdhury (1973) in an unpublished report to the Garst and Thomas Seed Company of Coon Rapids, Iowa. The results of this analysis are shown in Appendix C for the samples tested in this investigation.

Chowdhury classified the damaged seed into four classes. A kernel was considered damaged it it was broken, cracked, chipped, had a bruised pericarp, or had hairline cracks on the pericarp. Fast green dye was used as an aid to visual inspection in conjunction with a magnifying glass. The kernels were divided into the following five categories:

D_1 = Broken kernels and the fine materials which passed through a 12/64 inch round-hole sieve.

D_2 = Severe Damage - broken, chipped, and crushed kernels (more than 1/3 of the whole kernel missing).

D_3 = Major Damage - kernels with open cracks, chips, and severe pericarp damage.

D_4 = Minor Damage - kernels with hairline cracks and spots of pericarp missing.

D_5 = Whole Kernels - kernels that did not absorb dye on any parts other than the root tip.

Total damage as used in this discussion would include all of the four damage classes, i.e., total damage = $D_1 + D_2 + D_3 + D_4$.

The 100 gram sample of corn was placed in a 150 ml beaker and the standard dye suspension was poured over the corn so that all kernels were completely immersed. The samples were soaked for two minutes, the dye was then poured off and were then rinsed twice with tap water to remove excess dye. The rinse was accomplished by pouring the dye off the kernels and then refilling the beaker with tap water and shaking and pouring off the rinse water. This procedure was repeated for the second rinse. After the final rinse, the samples were placed on absorbent paper towels to dry overnight. The samples were then ready to be tested for retained fluorescence.

Chemical staining A similar procedure to that used by Koehler was utilized in obtaining the fluorescent chemical bonds. A standard dye solution was prepared by mixing 5 grams of 8-anilino-1-naphtalene sulfonic acid (ANS) with 500 ml of distilled water. The ANS was obtained from the Sigma Chemical Company of St. Louis, Missouri. The ANS solution was then poured over the 50 grams of damaged corn kernels in 150 ml beakers. The samples were soaked for two minutes and then the dye solution was poured off and the samples rinsed two times as with the granular pigmented dyes. Following rinsing the samples were placed on absorbent paper towels to dry prior to evaluation for induced fluo-

rescence.

The corn samples for this evaluation were from a harvesting experiment conducted in 1970. These samples had been extensively analyzed for mechanical damage by visual inspection, germination, and with the standard breakage tester. The results from this analysis are shown in Appendix D. These samples had been stored at room temperature.

Fluorescent readings The fluorometer utilized in this investigation is capable of determining relative values of fluorescence. In order to cover the complete range of fluorescence in the damaged grain it was necessary to establish an upper and lower limit of fluorescent intensities that would correspond to the minimum and maximum values indicated on the fluorometer. It is helpful to consider a plot of instrument reading vs relative fluorescence to understand more clearly the utility of the fluorometer as a tool for measuring induced fluo-

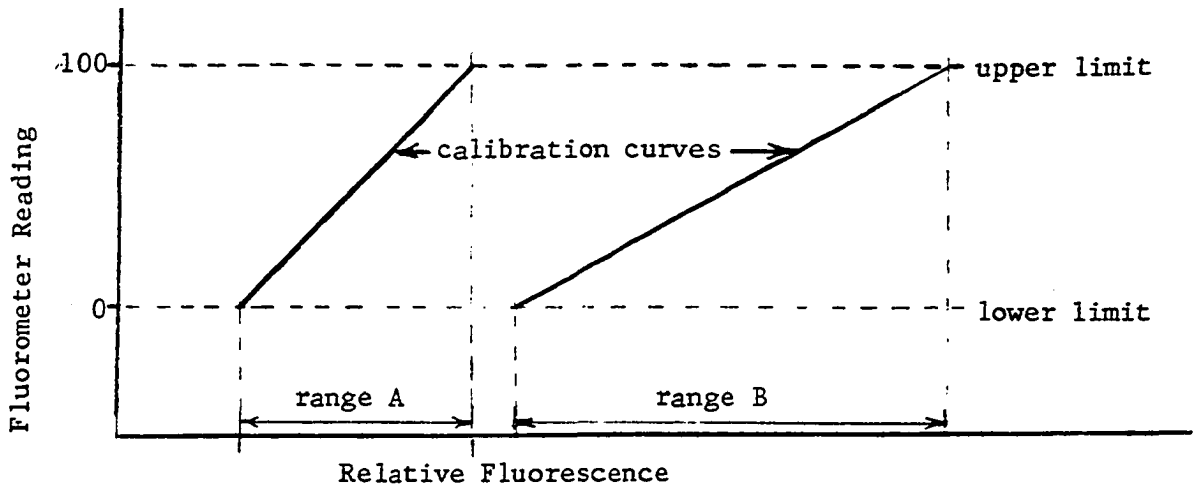
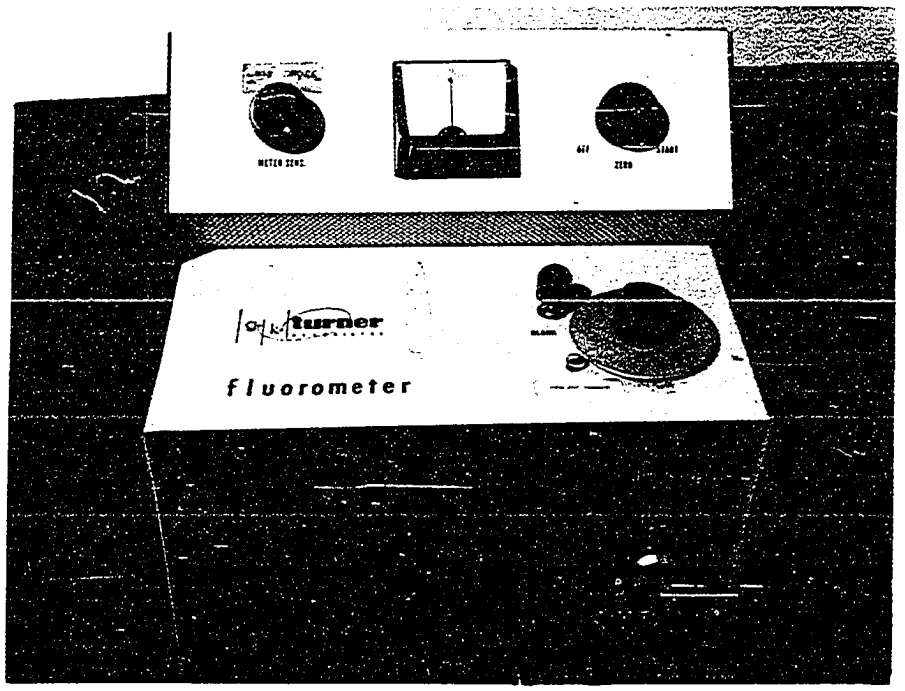


Figure 12. Fluorometer meter reading vs relative fluorescence.

Figure 13. Controls on Turner fluorometer.

Figure 14. Physical arrangemet showing location of sample with respect to the fiber optic and the uv light source.


turner
fluorometer

The fluorometer has a scale that goes from 1 to 100 as shown in Figure 13. Any range of fluorescent intensities can be adjusted to fall within this scale. The lower limit is adjusted by the blank control on the fluorometer. This adjustment sets the level of residual or background fluorescence by balancing the internal optical bridge so that the output meter indicates zero. The upper limit is controlled by the intensity of the fluorescent light that reaches the PMT. An increase in the intensity of the ultraviolet light incident on the damaged stained sample results in increased fluorescence. The intensity of the ultraviolet light affects the background or residual fluorescence; so, there must be some compensation for increased residual fluorescence when adjusting the lower limit. There are two ways of adjusting the intensity of the light that reaches the PMT. One method of controlling the intensity is to control the distance the light probe is from the fluorescing material, see Figure 14. The second control on the fluorescing intensity is to control the intensity of the incident ultraviolet light on the fluorescing sample. Since the quantum efficiency is constant for a particular material, the intensity of fluorescent light is a direct function of incident light energy. Because we are dealing with light intensity which is a square function the incident energy on the sample can be controlled within relative broad limits with only small adjustments in distance between the sample and the light source.

In practice, the lower limit is determined by placing an untreated sample of corn in the sample compartment and adjusting the blank control

and balance control so that the meter indicates zero. This in effect cancels out the affects of the natural fluorescence of the corn. The upper limit is found by placing the sample with the highest degree of fluorescent material in the sample compartment and adjusting the distance that the ultraviolet light source is from the treated sample so that there is an indicated reading of 100 on the fluorometer. The adjustments on the upper limit affect the lower limit. There is some trial and error in finding a suitable upper limit while maintaining the zero output for the lower limit. However, in any case, the upper limit is not a critical adjustment because we can only measure relative values of induced fluorescence which must be related to the quality factor of the grain that is of interest.

Operating procedure The fluorometer, voltage regulator, and the ultraviolet light source were allowed to warm up for thirty minutes prior to taking any fluorescent readings. Once the position of the ultraviolet light and the fiber optic had been determined it was only necessary to adjust the balance control on the fluorometer to bring the instrument to zero for the untreated control prior to taking fluorescent readings on the treated samples.

Several procedures were followed in collecting data for this investigation. The first was to place the whole kernel sample in a five-inch square sample holder and record a reading on the total sample.

The second procedure was used in evaluating single kernels for damage. The kernels were held about 0.5 cm from the end of the fiber

optic and rotated by hand until a maximum value of fluorescence was obtained as indicated by the fluorometer. The maximum value of induced fluorescence was determined by the maximum swing of the needle on the balance indicator of the fluorometer.

A third procedure which seems to have the most promise as a standard method for evaluating damage by induced fluorescence consisted of initially grinding the treated samples on a small laboratory Wiley mill to a uniform particle size. Grinding the samples on the Wiley mill gave a more uniform fluorescing field than whole kernels from which to measure the induced fluorescence. The ground samples were placed in a 4 cm sample holder for evaluation, see Figure 14. The fiber optic was adjusted so that the field of view was 3.5 cm in diameter. Once the initial set-up was made, all testing with the ground samples was made without any further adjustments.

DISCUSSION OF RESULTS

The discussion of results of this investigation is divided into three parts. The first part deals with the instrumentation developed for evaluating induced fluorescence. The second part discusses the results obtained when using the granular dyes as an indicator for mechanical damage. The third part of the results is concerned with the results found when using chemically combined dyes as an indicator for mechanical physical damage in corn.

Calibration of fluorometer

A calibration curve was developed to verify the linearity of the grain damage metering system. The curve is a plot of relative fluorescence vs area where the area consisted of pieces of paper cut from a sheet of uniformly fluorescing paper furnished by the manufacturer of the pigmented dyes. For recording, these fluorescing pieces of paper were placed directly below the end of the fiber optic. The uv light source was adjusted so that the fluorometer read zero with no fluorescing paper in the sample compartment. The height of the fiber optic from the sample was adjusted so that the largest area of paper had an indicated meter reading of 100. The results of this test is shown graphically in Figure 15.

The results of this test substantiate the theory that the response from the photomultiplier tube (PMT) is linear with the amount of fluorescent energy reaching its photosensitive surface.

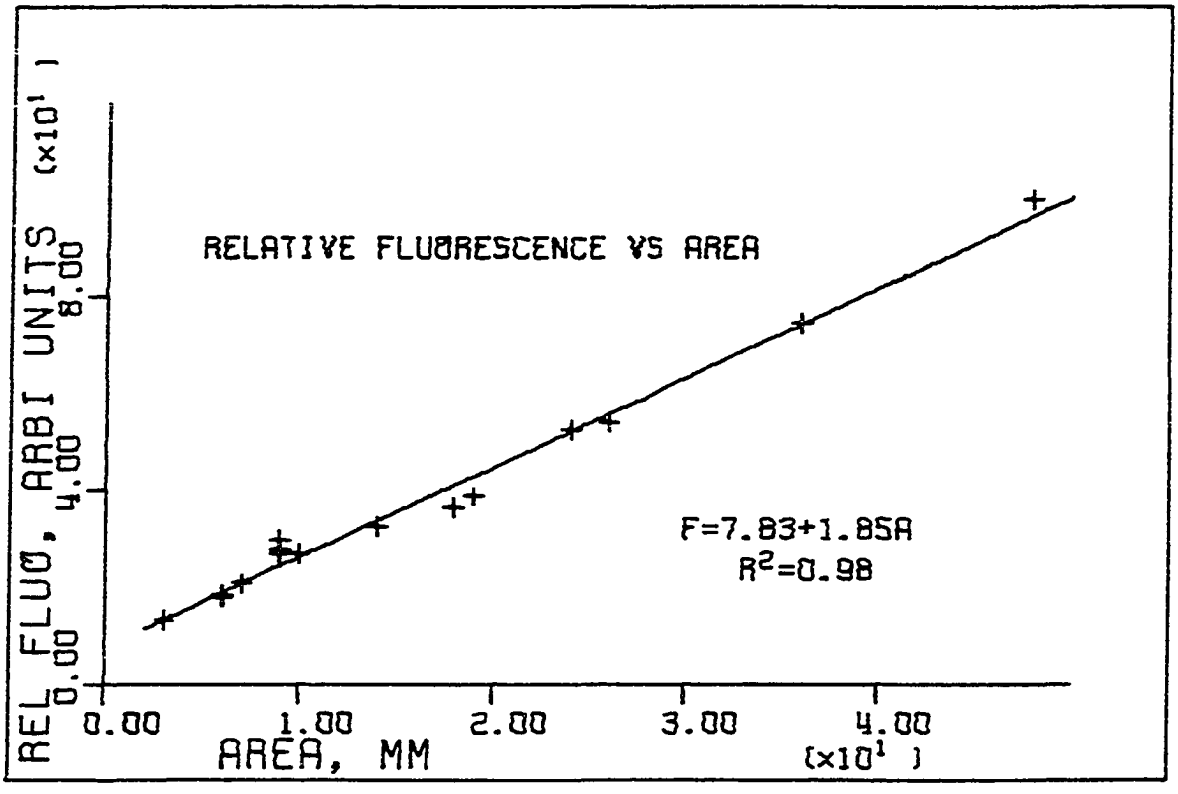


Figure 15. Relative response of fluorometer vs area fluorescing.

Granular dyes

Staining The amount of fluorescent pigmented dyes remaining in the cracks and crevices of the damaged kernels was dependent upon the dyeing technique used.

Several methods were evaluated in an effort to improve the dyeing technique. It had been suggested that dyeing the kernels under vacuum would be beneficial, resulting in better staining of the damaged portions of the kernels. This approach was evaluated by placing the corn kernels in a beaker of dye and then placing the beaker in an evacuation chamber. The pressure was reduced to near zero with a Duo-Seal vacuum pump, model 1400 manufactured by the Welch Scientific Co., Skokie, Ill. The vacuum

was held on the test sample for five minutes and then released.

The only obvious effect of the vacuum procedure was that the attachment end of all the kernels was stained with the dye. This was considered highly undesirable as fluorescence was retained on undamaged portions of the kernels. No further work was conducted beyound the initial test with the use of the vacuum as an aid to staining.

In developing an acceptable staining method several carriers for the dye were evaluated. These carriers have been summarized in Table 5. None of the carriers appeared to increase the retention of the granular dyes on the damaged portions of the corn kernels over that of distilled water.

Table 5. Carriers used for the granular dye.

1. distilled water
2. benzene
3. acetone
4. ethanol

Shaking the kernels in dry pigmented powder was tried. The results from this test was that the pigmented material stuck to all portions of the pericarp regardless of whether there was any physical damage present or not. It did not seem that mixing the kernels with the dry powder warranted further work beyound the preliminary trials. The powder was used directly from a sample container as furnished by the manufacturer of the pigmented dye. No test was conducted to determine the moisture

content of the dry powder.

Following the preliminary trials it was decided that the most practical method of physically trapping the granular dye in the damaged portions of the kernels was to soak the kernels in an aqueous dye suspension for two minutes followed by several washings. The amount of dye remaining in the damaged portions of the kernels was extremely sensitive to the washing operation. Too much washing tended to remove nearly all the dye from the damaged areas whereas too little washing did not remove the dye from the undamaged parts of the corn kernels. After several preliminary trials it was found that two washings gave the best results as far as removing excess dye without removing all the dye from the damaged portions of the corn kernels. The procedure that was selected was to place 100 grams of damaged corn in a 150 ml beaker and then pour the dye suspension over the kernels. The dye suspension was vigorously agitated prior to pouring it over the kernels. The kernels were allowed to soak for two minutes and then rinsed two times with tap water. The stained samples were then placed on paper towels to dry before fluorescent readings were made to determine the relative magnitude of the retained fluorescence.

The two minute soak time was selected as a convenient working time for the treatment as there did not appear to be any significant beneficial effects of longer soaking times. There was no detrimental effects from longer soaking times.

Fluorometer readings The results from this investigation using granular pigmented dyes was non-conclusive. Considerable difficulty was

encountered in getting consistent readings. Several procedures were tested in order to isolate the problems of inconsistency within a given sample. Fluorescent readings were taken from several sample sizes, from one kernel up to sample sizes of 6 inches in diameter. There was considerable inconsistencies in all the samples evaluated when using the granular dyes with whole kernels. As an example of the variability, the standard deviation as calculated from three readings was 11.3 when the mean was only 10.0. As would be expected when there is such wide variability in the data there was no meaningful relationship between the readings taken and the actual damage as reported by Chowdhury(1973). The results of this analysis is shown in Figure 16.

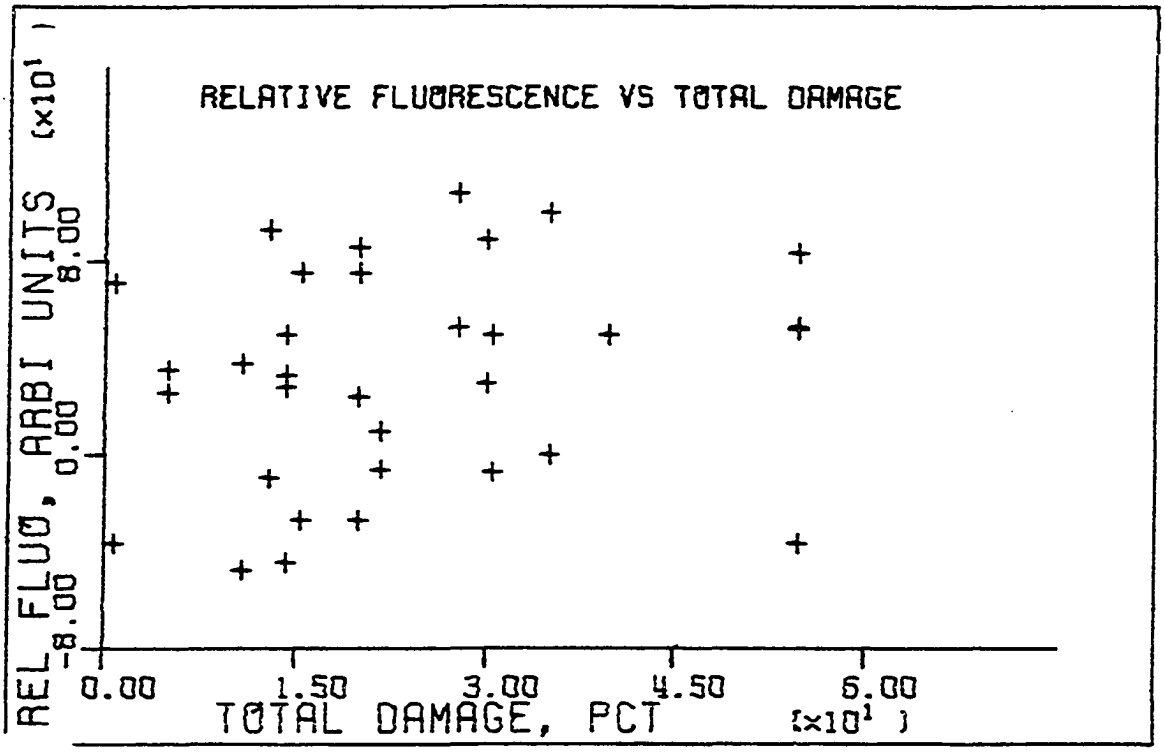


Figure 16. Fluorescence from granular pigmented dye stained whole kernels vs actual damage.

Several reasons are postulated for the inconsistencies found when using the granular dye. The first and probably the most significant is the effects of using whole kernels in the sample. If a damaged kernel happened to be directly beneath the end of the fiber optic with the ultraviolet light impinging directly, a relative large reading would be recorded. It would not be expected to get a badly damaged kernel in the same position for subsequent readings when there were only several damaged kernels in the sample. See Figure 18a, page 59.

Another factor of considerable weight causing the inconsistencies was the non-uniformity in treating the various samples with the dye. Since the quantity of retained dye was particularly sensitive to the washing operation it is unlikely to have several samples treated identically. The inherent differences in the types and kinds of cracks would result in considerable variations in the amount of pigmented dyes retained by different samples. Even when treating samples from the same parent lot, it was difficult to obtain similar readings from the fluorometer.

Another variable which must be considered is the natural fluorescence of the untreated corn. To extract the effect of the natural fluorescence of the samples a comparison of treated vs untreated was conducted. This test was accomplished by first placing the untreated sample in the sample compartment, recording the fluorescence, and then recording the fluorescence from the treated sample. A plot of the differences vs damage is shown below. No correlation could be found between actual damage and

the differences in fluorescence readings. The standard deviation of the samples remained relative high at 26.4 when the mean was 25.

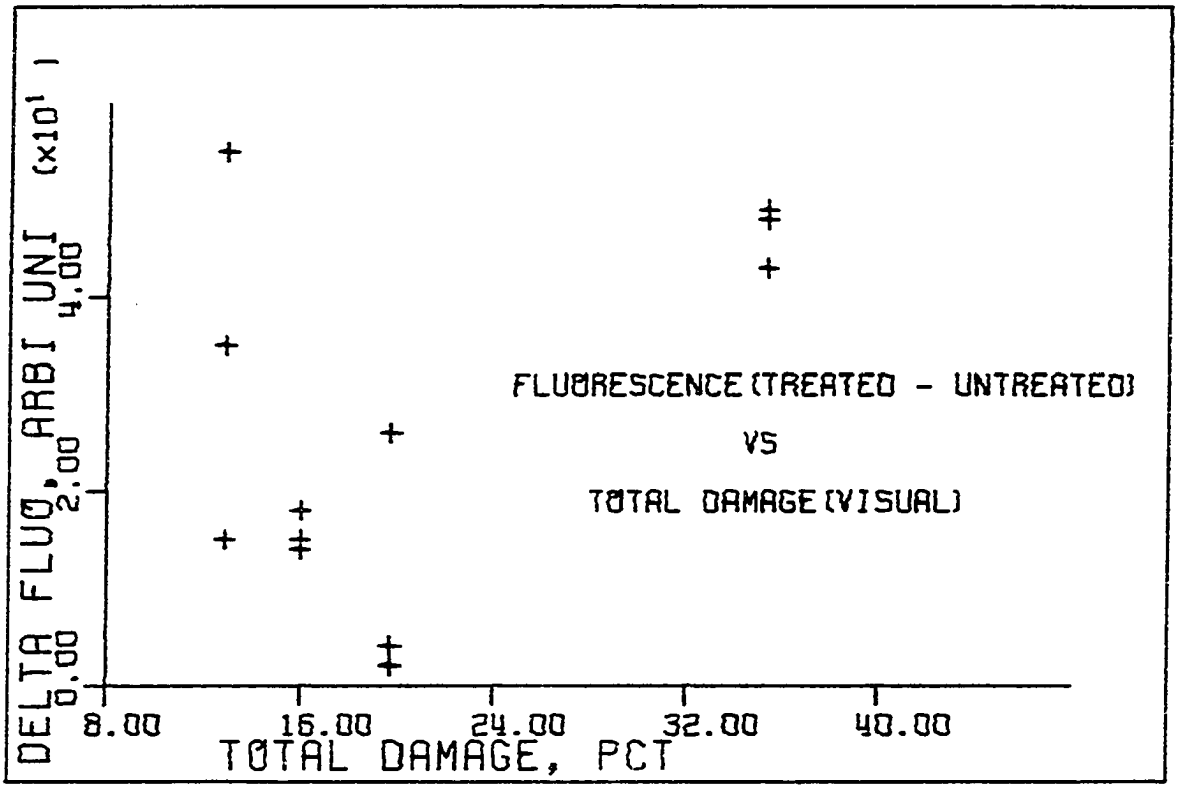


Figure 17. Differences in retained fluorescence between treated and untreated vs actual damage.

To circumvent some of the problems with isolated damaged kernels in a large sample and the natural fluorescence of the kernels single kernels were evaluated. The kernels were held about 0.5 cm from the end of the fiber optic and rotated by hand until a maximum value of indicated fluorescence was obtained. Due to the instability of the hand holding the kernels it was difficult to get an exact reading of the induced fluorescence. Therefore, the maximum swing of the balance meter was used as the indicator of degree of damage. This procedure

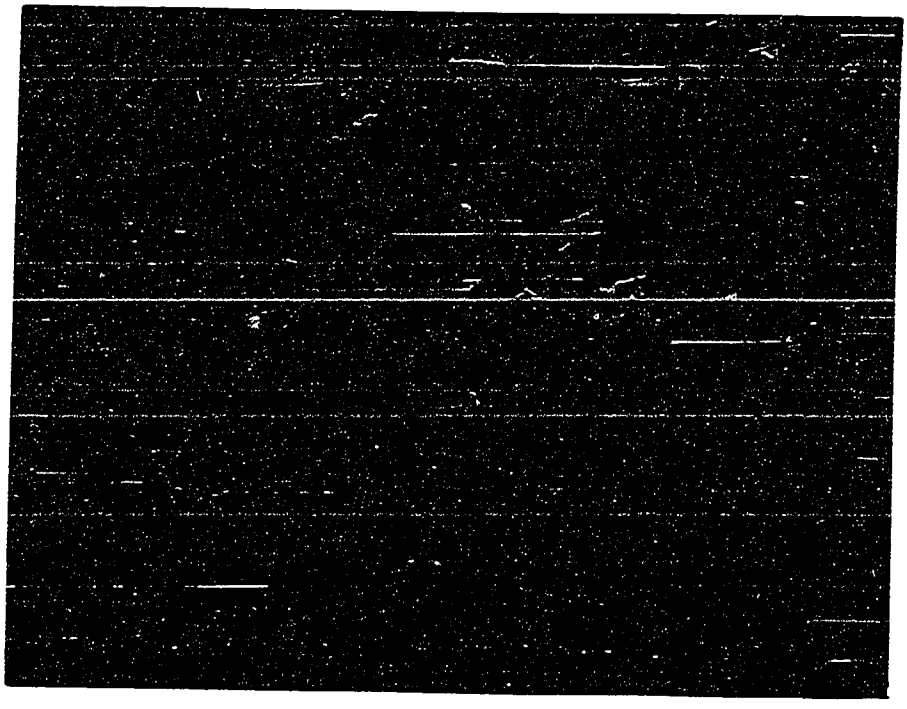
made it possible to separate the kernels into three classes, undamaged, slight damage, and severe damage. The severe damage class consisted mostly of kernel fragments. Figure 18b shows the kind of separation possible using this equipment and technique.

The single kernel evaluation procedure eliminated some of the objections found when using large sample sizes. By careful rotation it was possible to consistently separate the kernels into the three classes. The total magnitude of the natural fluorescence was relatively small in the presence of large amounts of induced fluorescence in the damaged portions of the kernels. This method, however, was found to be unacceptable because it was rather awkward and there was still an element of human judgement that was quite arbitrary.

In conclusion, the test with the granular pigmented dyes was not satisfactory for damage evaluation primarly due to the difficulties encountered in obtaining uniform treatment and the fact that there was no correlation between retained fluorescence and the actual damage as determined by visual inspection. The search for a suitable technique using the granular dyes was abandoned when a more suitable dyeing method was discovered. The use of a material that chemically combines with the interior constituents of the corn kernels made it possible to obtain more reliable staining of the damaged portions of the kernels than with the pigmented granular dyes that were only physically trapped in the cracks of the damaged corn kernels.

Figure 18a. Fluorescing whole kernel sample showing isolated damaged kernels.

Figure 18b. Damage classes obtained by hand rotation of single kernels of corn to find maximum value of fluorescence.



Chemical bonding to form fluorescent compounds

Preliminary investigations The preliminary investigations consisted of testing the ANS solution as an indicator of mechanical damage for contact time, rinsing effects, pH, concentration, and surface tension of the carrier.

An aqueous solution of ANS was prepared for evaluating concentration and contact time for optimum staining of damaged corn kernels. Concentrations of 0.05%, 0.025%, 0.0125%, 0.0062%, 0.0031%, and 0.0016% ANS were prepared for evaluation. The highest concentration was prepared by dissolving 5 grams of ANS into 1 litre of distilled water. Twenty-five ml of the 0.05% solution was mixed with 25 ml of distilled water to form the second highest concentration. Twenty-five ml of the second concentration was extracted and mixed with 25 ml of distilled water to form the third highest concentration. This procedure was repeated until all six dilutions were prepared.

For testing induced fluorescence vs contact time, split corn kernels were immersed in the prepared dilution for their respective treatment times. The times used were 1 minute, 3 minutes, 10 minutes, 1 hour, 22 hours, and 48 hours. The treated kernels were removed after their respective soaking times and dried on paper towels. The treated samples were evaluated by visual inspection under the effects of ultraviolet light. The treated kernels were arranged in an array of increasing concentration

and increasing contact time for visual analysis.

There was no increase in the apparent intensity of the induced fluorescence with increased contact (immersion) time. There was an increase in the intensity of the induced fluorescence due to increased concentration of the ANS solution. The differences in adjacent concentrations in the array were not visually detectible, however, over several concentrations, i.e., between 0.05% and 0.0062%, the differences in the induced fluorescence was quite noticeable. Based on this test it was concluded that the highest concentration would be the most useful for further research. Since there was no detectible differences in contact times used, a soaking time most convenient for laboratory use was used as the standard for all further testing. A soak time of 2 minutes was selected as adequate for all test as this would minimize the processing time for treatments.

Washed and unwashed samples were compared in the preliminary testing. Fifty grams of corn were placed in each of two beakers. The ANS solution was poured over the kernels and allowed to soak for two minutes. The ANS from the first beaker was poured off the corn which was then placed on paper towels to dry. The sample in the second beaker was rinsed twice with tap water prior to placing it on the paper towels to dry. There was a noticeable reduction in surface fluorescence for the sample that was rinsed. There was no visible reduction in the fluorescence on the damaged portions of the corn kernels due to the washing. These conclusions were based on visual inspection of the samples which were visually analyzed under the effects of ultraviolet light. Based on the

reduced surface fluorescence for the washed sample it was concluded that all further testing would include a double rinse after treatment with the standardized ANS solution.

The pH of the dye solution was considered as a variable in the preliminary evaluation of ANS as an indicator of mechanical damage. ANS solutions with a pH of 1, 3, 5, 7, 9, and 11 were prepared by adding either 0.1 N NaOH or 0.1 N HCL. The pH was determined by pH indicating papers manufactured by Micro Essential Laboratory, Brooklyn, 10, N. Y. Split corn kernels were soaked for two minutes and then washed two times prior to being dried on paper towels. Based on visual inspection there was no influence of pH as all samples exhibited similar intensities of induced fluorescence. From this test it was concluded that it would be unnecessary to adjust the pH of the standardized dye solution and dissolving the ANS into distilled water would be satisfactory.

It had been proposed that a reduction in the surface tension of the solvent would enable the dye to better penetrate the cracks and crevices in the pericarp of damaged kernels. A hand soap solution was prepared as a means of reducing the surface tension of the ANS dye. The ANS soap solution was poured over damaged corn kernels and soaked for two minutes. The damaged samples were prepared by making deep cuts with a knife over the endosperm and the embryo. After treatment, the samples were evaluated by visual inspection. There appeared to be no differences in the staining characteristics between the soap solution and the distilled water that was used as the carrier for the dye. Only

the exposed floury endosperm showed signs of induced fluorescence. There was no noticeable staining of the cuts made over the horny endosperm or the embryo.

As a further test in the evaluation of reducing the surface tension of the carrier as a means of enhancing the dyeing, acetone was used as the carrier for the dye instead of the distilled water used in the standardized solution. The surface tension of acetone is near 26.2 dynes/cm whereas water is near 70 dynes/cm at the conditions found in the laboratory. Again there was no obvious increased staining of the damaged portions of the corn kernels when acetone was used as the carrier over that of distilled water. Samples were prepared and analyzed in the same manner as with the hand soap solution testing.

The laboratory procedure developed based upon the preliminary investigations was to soak the damaged samples in a 5% aqueous solution of ANS for two minutes followed by two rinses in tap water. The samples were then dried on paper towels prior to evaluation for induced fluorescence. Drying did not appear to change the fluorescent intensities of the samples. Drying was done as a matter of convenience in handling the samples. Figure 19a shows the equipment and materials for preparing the samples for evaluation for induced fluorescence.

Damage analysis Ten samples of corn were selected from the machine harvest studies referred to earlier in this report. The results of the visual analysis of these samples as reported by Chowdhury are in Appendix C. Fifty grams from each sample of corn were treated with the standardized aqueous ANS dye solution. These samples were soaked for two minutes, then rinsed twice with tap water and dried on paper towels prior to evaluation

for induced fluorescence. Several procedures were followed in evaluating for damage.

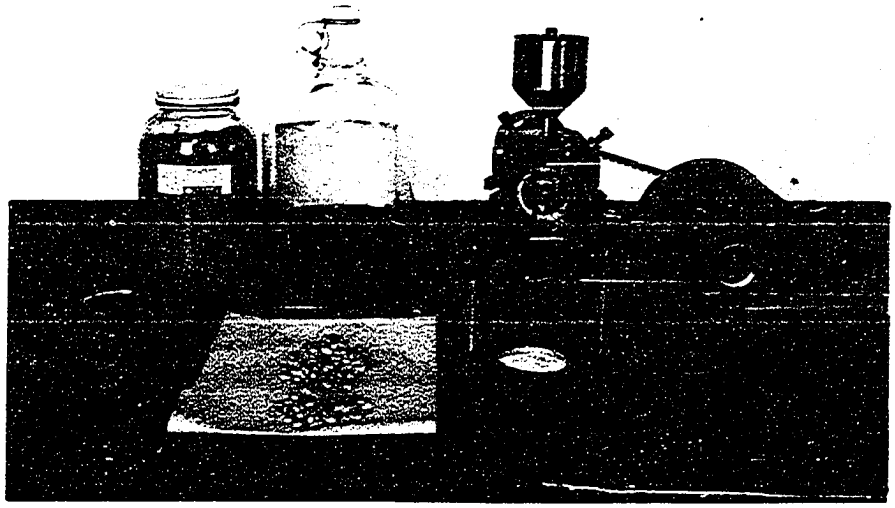
The first procedure followed in evaluating for damage was to place the whole kernels in the damage meter and record the total induced fluorescence. Similar difficulties were encountered with this procedure as with the studies with the granular dyes. It was difficult to obtain consistencies in repeated readings on the same sample. The induced fluorescence was dependent upon the orientation of the kernels in the sample holder. Reorienting the sample within the sample compartment resulted in rather large variations in the recorded fluorescence.

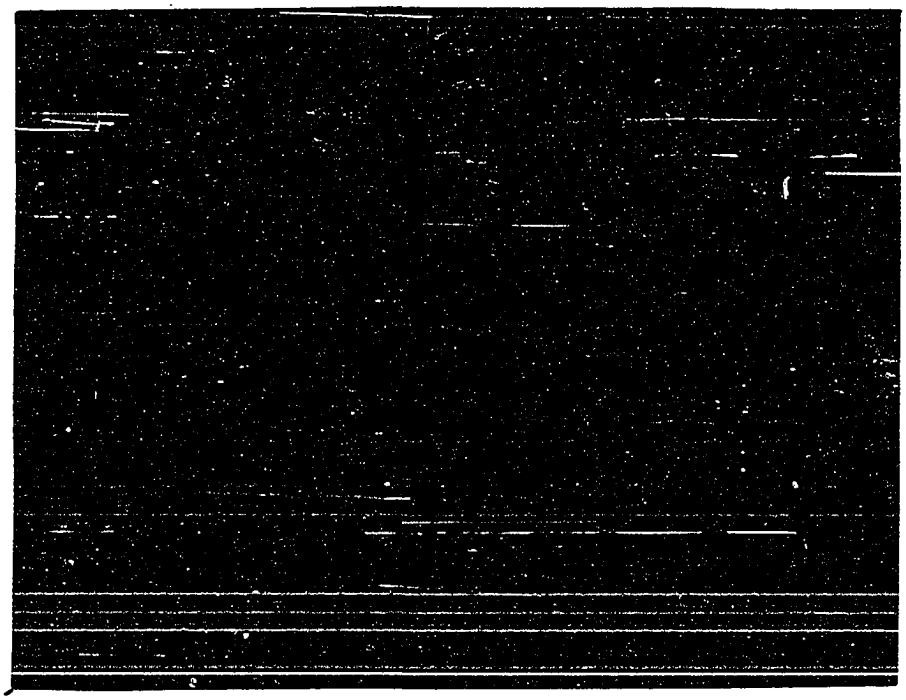
Individual kernels were evaluated by holding them about 0.5 cm from the end of the fiber optic and rotating for maximum fluorescence. Similar results as obtained with the granular dye was found. It was possible to separate the corn according to the amount of induced fluorescence into several classes. However, similar objections to this method as with the granular dye are advanced. A human judgement factor is present in the determination. It would be possible to design the equipment so that it was unnecessary to make the human decision, however orienting the kernels for maximum fluorescence would probably require rather sophisticated and costly equipment. A more practical solution was found to give consistent readings within a given sample.

It was found that more consistent results could be obtained by grinding the samples prior to evaluation. The treated samples were ground on a small laboratory Wiley mill using a No. 20 screen. A ground sample gave repeatable readings of fluorescence intensity regardless of the orientation of the particular sample. The standard deviation of

Figure 19a. Materials and equipment used in preparing damaged corn samples for evaluation of induced fluorescence showing the dry ANS, standardized dye solution, soaking kernels, dried kernels, and the Wiley mill used to grind the treated sampls prior to evaluation.

Figure 19b. Ground corn samples as viewed under the effects of ultraviolet light. From left to right, low damage, medium damage, and high damage. The lower three samples are the corresponding untreated checks. Note calibration strip at top of figure.





The ground samples was on the order of 2.0 when the mean was 10.0. It was concluded from these data that the ground samples were far superior to the whole kernel samples for evaluating induced fluorescence. Materials and equipment used to treat the damaged samples are shown in Figure 19a. Treated ground samples as viewed under ultraviolet light are shown in Figure 19b. There are three levels of damage along with their corresponding untreated checks. Note the calibration strip along the top of Figure 19b. which was used to demonstrate the linearity of the measuring system.

Grinding the samples make it possible to obtain more reasonable quantitative results. Since the procedure had been developed for recording quantitative values of induced fluorescence the contact time was again evaluated as a possible variable to be considered. Samples of corn were taken from one parent lot of corn for evaluation induced fluorescence as a function of contact time. Five 50 gram samples were placed in 150 ml beakers for testing. The first sample was considered the control and did not have any solution poured over it. The second sample was covered with distilled water as a secondary control. The third sample was covered with the ANS solution and soaked for 1 minute. The fourth sample was in the ANS solution for 2 minutes. The fifth sample was soaked for 5 minutes in the ANS solution. All samples were rinsed twice with tap water and then dried on paper towels. The samples were ground on the Wiley mill using the No. 20 screen after they were dry. The results of this test are shown in Figure 20.

This data shows that there is an increase in induced fluorescence with increasing contact time. The increase is explained by an increased penetration of the ANS into the corn kernels with increasing time.

By grinding the samples we are now looking at a value of fluorescence that is a function of the total volume of material that had been in

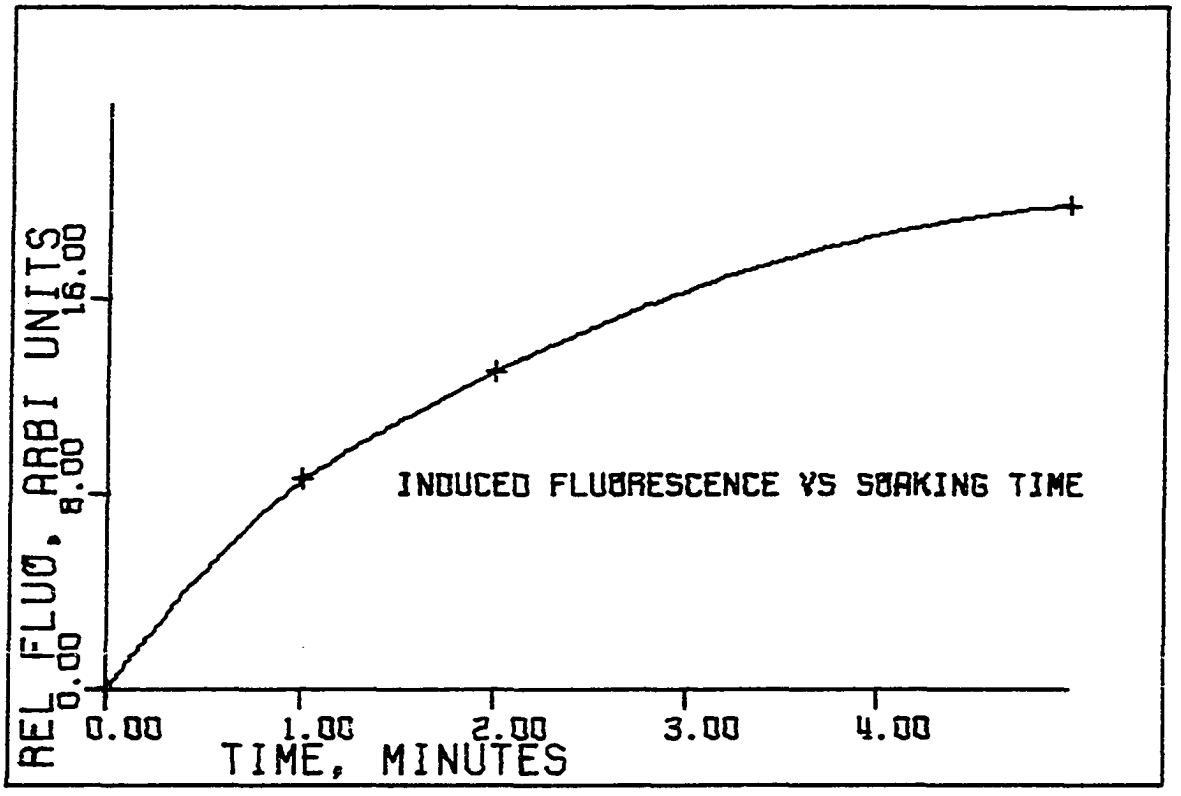


Figure 20. Relative fluorescence as a function of contact time.

contact with the ANS. That is, the total induced fluorescence is a surface times depth phenomena. For a given surface area the deeper the penetration of ANS, the larger the total volume or quantity of fluorescing material in the ground sample. Conceivably if sufficiently long contact time were allowed, all parts of the kernels would fluoresce, regardless of whether it was damaged or not.

To show that the volume of fluorescing material increased with increased soaking time, a test was conducted to measure the depth of

penetration of the ANS into the kernel with time. The surface tension of the carrier was also considered as a possible factor in increasing the depth of penetration. Samples of corn with an initial moisture content of 8 and 17 percent were used in this test to determine whether initial moisture content influenced the depth of penetration.

Split kernels were allowed to soak in the solution for 30 seconds, 1 minute, 2 minutes, 5 minutes, 10 minutes, 30 minutes, and 1 hour. The depth of penetration was measured visually with the aid of a Bausch and Lomb stereozoom binocular dissecting microscope in conjunction with an optical micrometer. The optical micrometer has 0.1 mm as the smallest

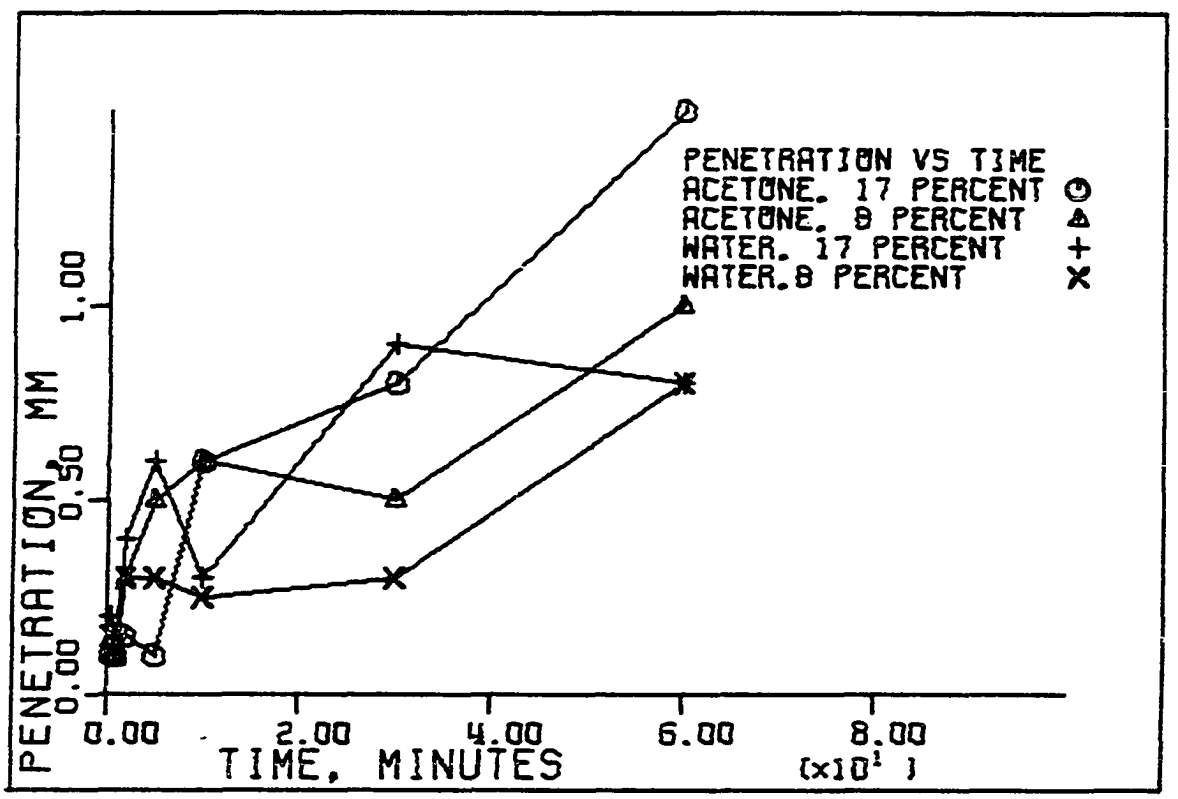


Figure 21. Depth of penetration of ANS with increasing contact time.

scale division. Sections of the split kernels were taken perpendicular to the exposed surface area for evaluation. The sectioning was done with a razor blade. The results of this test are shown in Figure 21.

It was found that the depth of penetration could be expressed by the following equation,

$$\text{Average penetration} = 0.14\ T^{\frac{1}{2}}$$

Where T = contact time.

This equation takes the same form as that for the advance of a wetting front for soil moisture movement in a homogeneous medium as discussed by Baver et al. (1972). Baver states that the advance of a wetting front for time intervals of a few hours and for uniform materials can be expressed by the following equation,

$$S = E\ t^{F}$$

Where F = close to but not always 0.5

E = constant

S = advance of wetting front

t = time.

The depth of penetration when plotted on log-log paper plots as a straight line as shown in Figure 22. Since not enough data was collected to perform any meaningful statistical analysis, the average penetration for each soak time was used in plotting Figure 22.

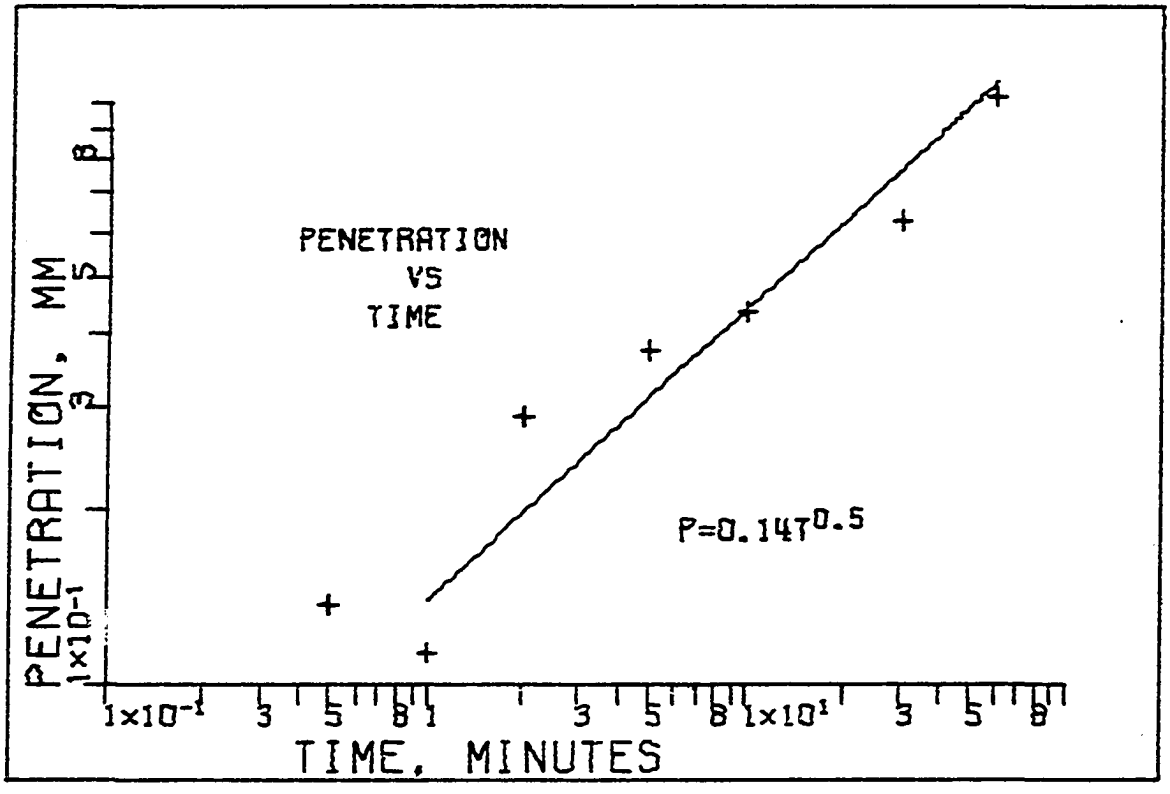


Figure 22. Average penetration of ANS into the floury endosperm vs time.

It was not possible to establish any significant differences in the depth of penetration when either acetone or distilled water was used as the carrier from the data collected. There did not appear to be any large differences in the penetration as a function of the initial moisture content. There is an increase in the depth of penetration as was predicted from the studies with the fluorometer when time of contact between the ANS and the sample was considered. The conclusions from this test is that there is no meaningful difference between water and acetone as the carrier for the ANS. The initial moisture content does not greatly influence penetration for short contact times.

The total volume of induced fluorescence is highly dependent upon the treatment time. For this reason it is important that the treatment time be consistent for any meaningful comparisons between samples.

The results of the depth of penetration studies did not indicate that acetone with a surface tension 1/3 that of water was better as a carrier for ANS than distilled water. A practical method of evaluating the carriers was to treat samples from the same lot with either water or acetone as the carrier for the ANS and record the resulting induced fluorescence on the fluorometer. The results of this test are shown below.

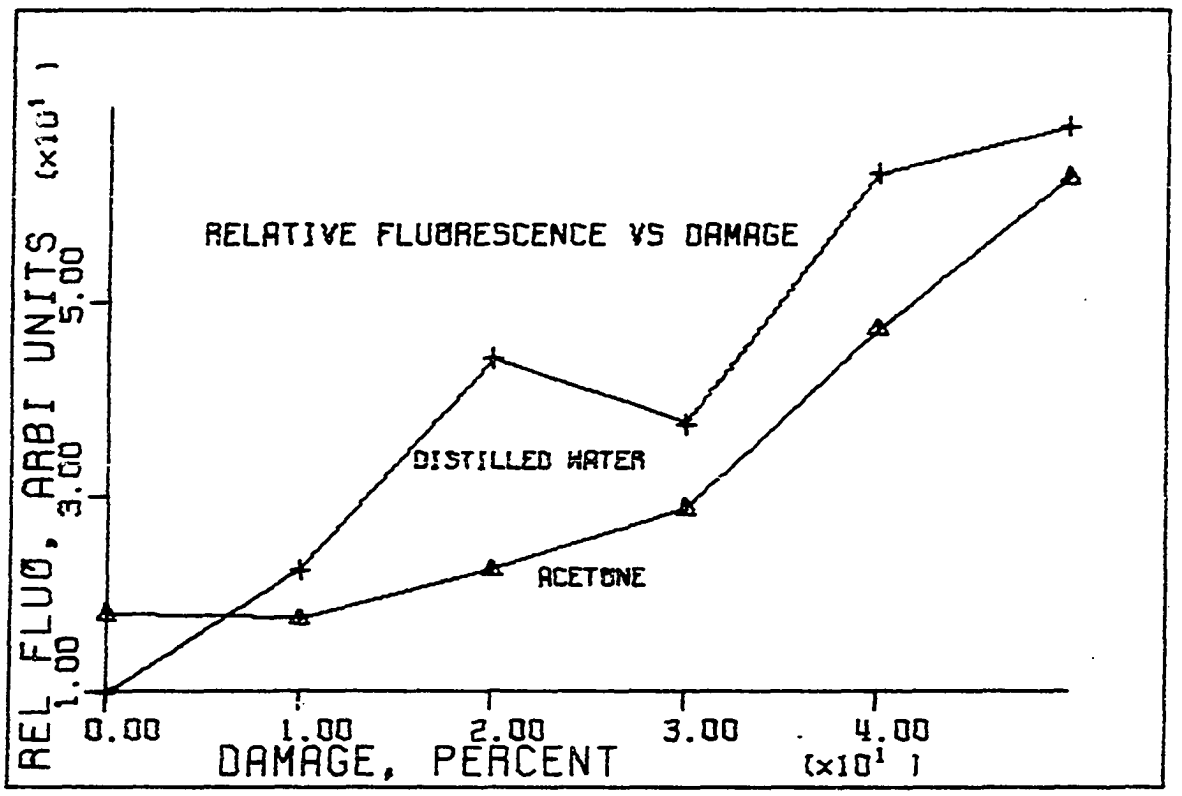


Figure 23. Relative fluorescence vs damage for acetone and water.

For this test samples of corn with a known percentage of cracked corn

were prepared and evaluated. The cracked samples were prepared by crushing kernels of corn between the jaws of a pair of pliers so that they were only slightly cracked and not broken into fragments.

The results from this test are similar to the depth of penetration studies with the optical micrometer indicating that there is no real differences between the ability of the ANS to better stain the kernels when either acetone or distilled water is used as the carrier. Although most tests in the development of the grain damage meter were conducted with an aqueous ANS solution, it is proposed that for some uses acetone or some other solvent with a surface tension different than that of distilled water may be more suitable for indicating the particular quality feature that is of interest.

To test for induced fluorescence as a measure of actual corn damage samples of corn were prepared with a known percentage of damage for evaluation. The damaged samples were prepared by splitting corn kernels through the embryo and then mixing the split kernels with sound undamaged kernels from the same lot. The samples were mixed by weight to give 0%, 20%, 40%, 60%, 80%, and 100% damage. The samples were soaked for 2 minutes in the aqueous ANS solution, rinsed twice, dried, and then ground on the Wiley mill using the No. 20 screen. The results of this test is shown in Figure 24.

The conclusions drawn from this test is that there is a linear increase in induced fluorescence with a linear increase in exposed internal surface area. The percent damage can be expressed by the

following equation as a function of induced fluorescence,

$$D = 1.08\ F - 32.3$$

Where D = equivalent damage percentage of split kernels

F = induced fluorescence.

When viewing the treated samples under the microscope it was found that only the floury endosperm showed any large degree of fluorescence

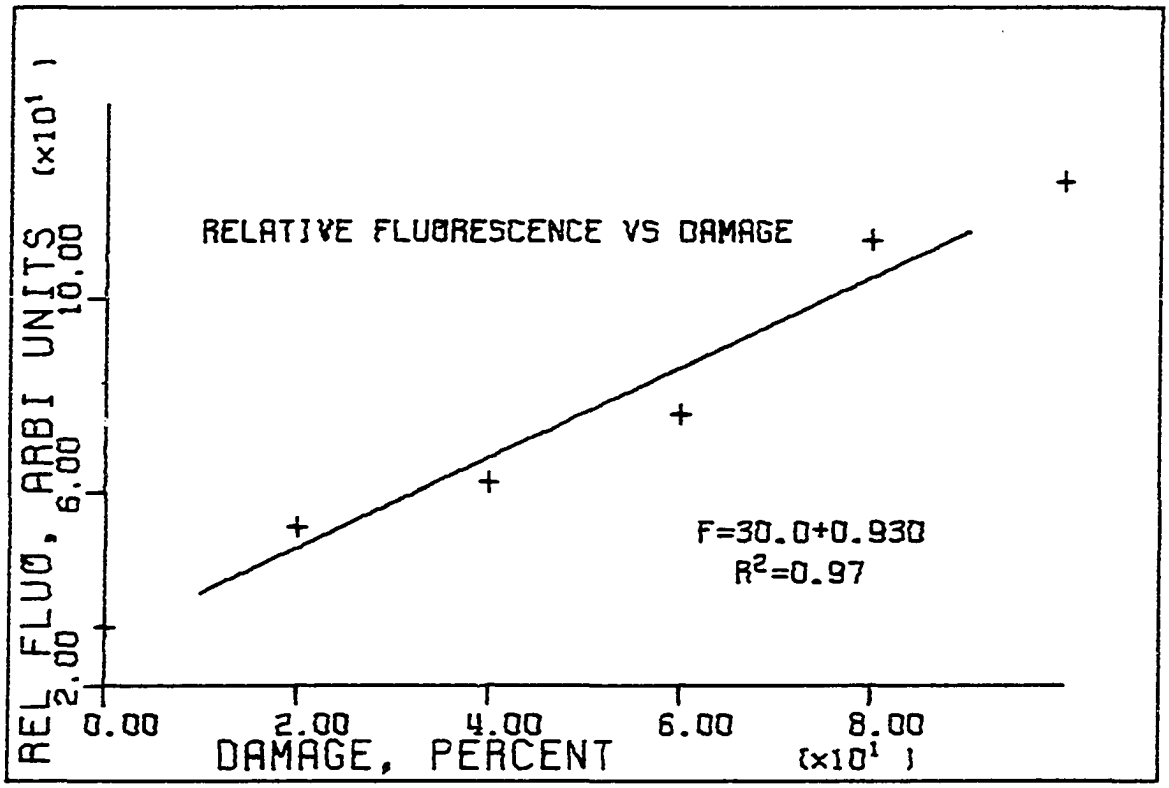


Figure 24. Relative fluorescence vs actual damage.

due to the penetration of the ANS for short contact times. It was possible to measure the penetration of the ANS into the horny endosperm

only for the 1 hour contact time. The horny endosperm and the embryo did not shown any visible ANS penetration when only treated for two minutes. Two minutes had been used as the standard treatment time for all testing. There was some fluorescence on the surface of the horny endosperm and the embryc but it was not possible to measure the depth of penetration with the optical micrometer that was calibrated in 0.1 mm increments. With the contact time of 1 hour a measurable line of fluorescence developed on the horny endosperm not unlike that found on the floury endosperm. Due to the greater penetration of the ANS into the floury endosperm than into either the horny endosperm or the embryo, it is concluded that the floury endosperm contributes the largest percentage to the total volume of material that exhibits the induced fluorescence.

Although there was no distinct line of fluorescence showing in the embryo that would mark the depth of penetration of the ANS there did seem to be an increase in the fluorescence over that of an untreated sample for the longest soak time. For these reasons it is postulated that the indicated response of induced fluorescence is actually related to the amount of the floury endosperm that comes in contact with the ANS.

The induced fluorescence is a measure of a quality factor of corn that has not previously been measured. For some uses it may be desirable to measure the total volume of floury endosperm in a particular variey of corn. If the corn were ground prior to treating with ANS, then all the floury endosperm would fluoresce. This could be used as a quantative measure of the total floury endosperm. No attempt is made here to list the possible uses that may be developed that relate to the area of exposed

floury endosperm. An attempt was made to relate the readings obtained with the induced fluorescence with some quality of the grain that has been used in the industry previously.

Samples of corn were available that had been evaluated with the Stein Breakage Tester, for germination, and visual counts of damage, as well as the USDA official grading techniques. These samples were treated with the ANS and evaluated on the newly developed grain quality meter.

The results of the prior evaluation of these samples is shown in Appendix D. There was no significant correlation between any of the previously measured quality factors and the induced fluorescence. The highest correlation was between the induced fluorescence and the results from the breakage tester. A plot of this data is shown in Figure 25.

Although the F test indicates that there is a highly significant relationship between the results of the breakage tester and the new damage meter the correlation coefficient is only 0.45. This would indicate that there is some correlation, however, the data are spread over a relatively broad range. This spread is not totally unexpected as we are not measuring the tendency of the kernels to fracture with the induced fluorescence, but only the exposed surface area of the floury endosperm.

The linear prediction equation and the corresponding correlation coefficient for the parameters tested are shown in the following table.

Table 6. Prediction equations relating induced fluorescence to various parameters.

Parameter	Prediction equation	Correlation coefficient (R^2)
Breakage tester	F = 8.77 + 7.63BT	0.45
Germination	F = 51.93 — 0.15G	0.01
Broken corn and foreign matter (12/64)	F = 25.39 + 35.59(BC & FM)	0.36
Stained seed and damaged pericarp	F = 4.33 + 8.58(SS & DP)	0.40

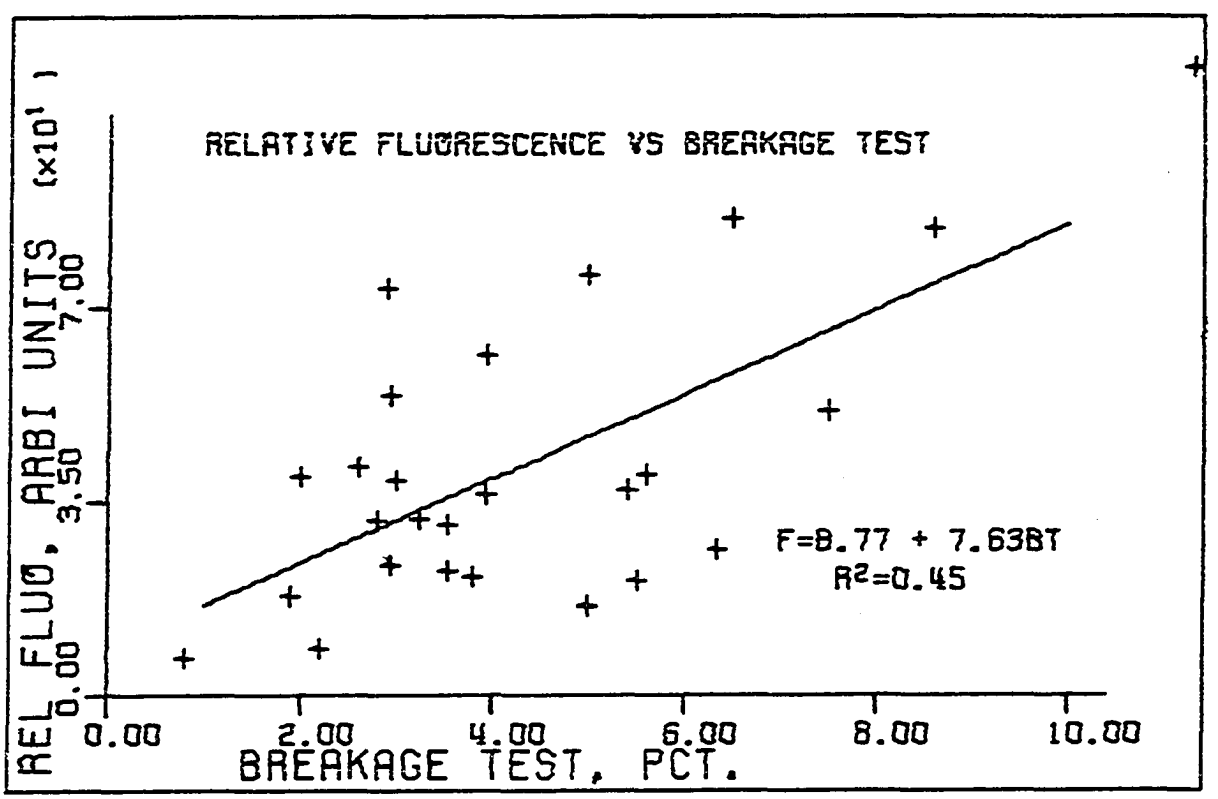


Figure 25. Induced fluorescence vs breakage tester results.

In another series of test evaluating induced fluorescence as a function of a known quality factor, the samples referred to earlier in this report that had been visually inspected and evaluated by Chowdhury were evaluated on the new grain damage meter. Chowdhury classified the damaged kernels into four classes. The material that passes the 12/64 round hole sieve, minor damage, major damage, and severe damage. As with the previous test, there was no significant correlation between any of the damage classes and the results from the fluorescence damage meter. An example of a typical plot of the data is shown below for minor damage vs induced fluorescence.

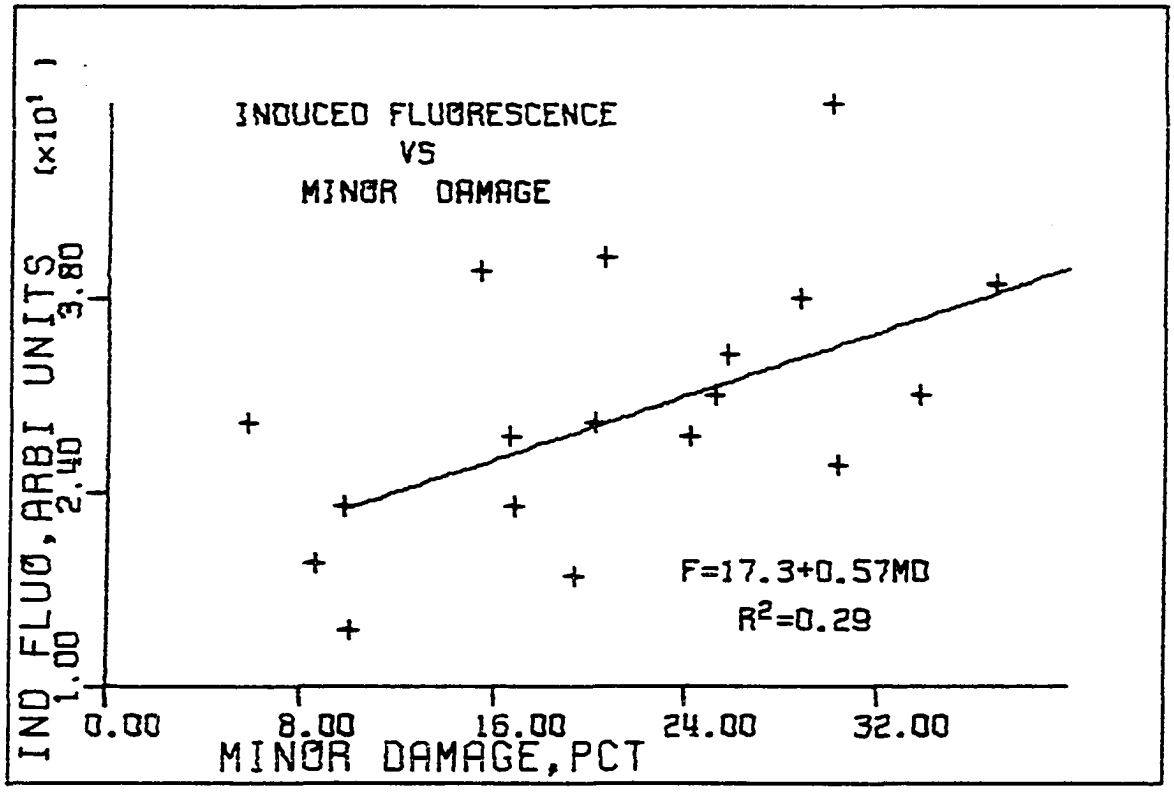


Figure 26. Minor damage vs induced fluorescence.

The linear prediction equations and the corresponding correlation coefficient for the damage classes reported by Chowdhury is given below.

Table 7. Prediction equation relating induced fluorescence to various damage classes.

Damage class	Prediction equation	Correlation coefficient (R^2)
Through 12/64	F = 21.6 + 0.94(12/64	0.33
Severe	F = 21.2 + 6.97(S)	0.41
Major	F = 21.0 + 0.63(M)	0.25
Minor	F = 17.3 + 0.57(m)	0.29
12/64 + severe	F = 19.7 + 4.83(12/64 + S)	0.45
12/64 + severe + major	F = 19.8 + 0.63(12/64 + S + M)	0.31
Total damage	F = 15.6 + 0.38(12/64 +S +M +m)	0.38
Severe + major	F = 20.4 + 0.63(S + M)	0.28
Severe + major + minor	F = 15.8 + 0.38(S + M + m)	0.37
Major + minor	F = 16.0 + 0.31(M + m)	0.35

Machine parameters

Throughout the initial stages of testing it was observed that there was a zero shift from one day to the next in the instrumentation, or even from one hour to the next during the day or night. The output from the ultraviolet light is voltage sensitive. It was proposed that the apparent zero shift was due to voltage fluctuations. The line voltage was monitored for one day and it was found that there was a shift of as much as 4 volts from morning until afternoon. In fact, the line voltage was observed to change as much as 2 volts within a 30 minute time interval. It was concluded that the line voltage shift was the probable cause of the apparent zero shift in the instrumentation from the time one set of data was taken to the time of the next test.

In order to control this zero shift due to fluctuating voltage a line voltage regulator was used to control the voltage to the instrumentation to within $\pm$ 0.2 volts. The voltage regulator was a type 1570-AS15 manufactured by the General Radio Company of Cambridge, Mass. Controlling the line voltage eliminated the apparent zero shift that had been present in the earlier testing.

A plot of relative fluorescence vs line voltage was made as an adjunct to the characteristics of the measuring system. It was possible to adjust the output of the voltage regulator so that a response curve could be developed. A sample of damaged corn was placed in the sample compartment and left there throughout the test. The results of this test are shown in Figure 26.

There was no hysteresis effect in the readings as shown in the

data used to plot the graph in Figure 27 as shown in Appendix B. The

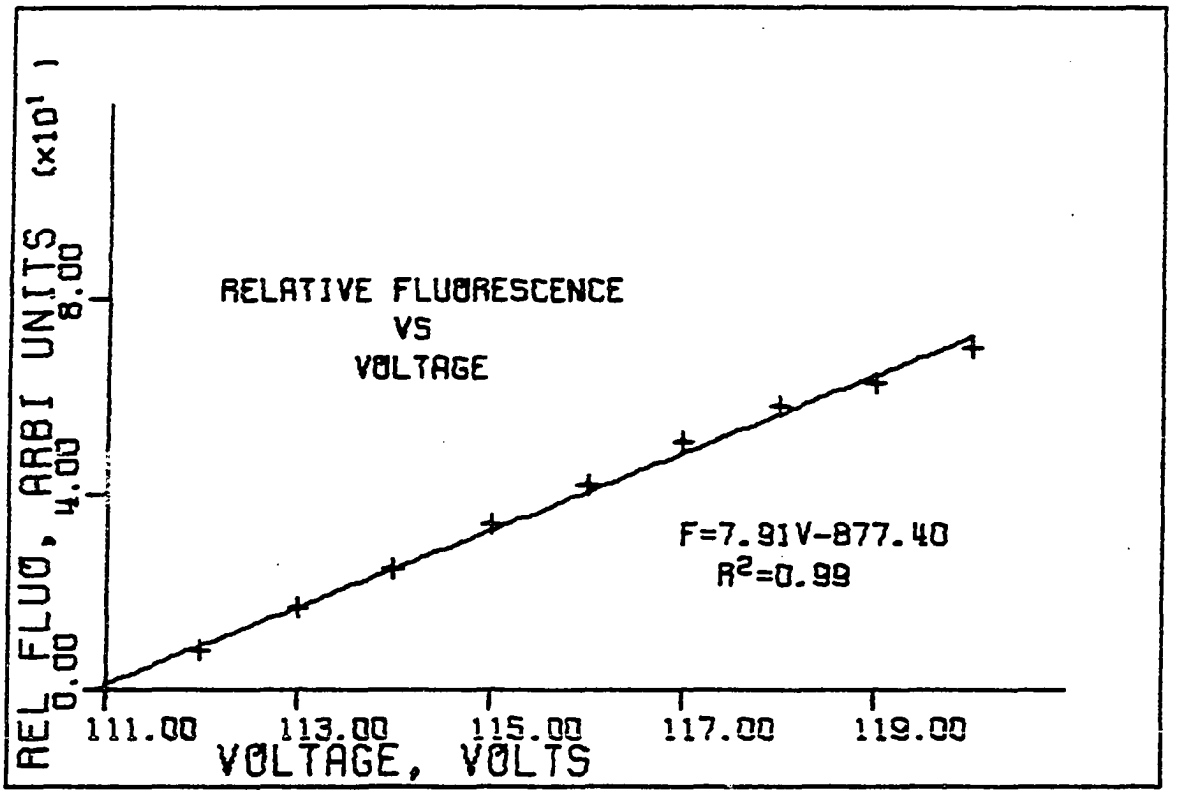


Figure 27. Relative fluorescence vs line voltage.

data were collected starting at 111.0 volts, going by 1.0 volt steps up to 120 volts, and then returning by 1.0 volt steps to 111.0 volts.

The statistical analysis of this data indicates that the relative fluorescence as a function of line voltage can be expressed by the following equation.

$$F = 7.91LV - 877.40$$

Where F = relative fluorescence

LV = line voltage(volts).

Many of the inconsistencies found in the earlier testing can be attributed to the fluctuation in the line voltage.

Once the fluctuation due to line voltage was eliminated it was possible to evaluate the effect of sample preparation on variability of the recorded induced fluorescence. A series of tests was conducted to evaluate sample surface preparation on indicated induced fluorescence. The surface preparation was varied through a range that might be encountered in preparing a large number of samples. All testing was done with one sample, only the surface was changed. The details of this test are outlined in Table B-12, page 106. From this test it was concluded that a standard deviation of 4.4 could be explained by the technique used in preparing the surface of the ground samples prior to being read on the damage meter. It is apparent that a uniform method of preparing the surface of the samples must be used at all times when comparing sample readings.

SUMMARY AND CONCLUSIONS

This study was designed to provide the basic information needed for designing and developing commercial grain damage meters. The conclusions from this investigation have been divided into the following three areas: measuring system, dye, and application of the system to the measurement of mechanical damage in corn.

Measuring system

1. A photomultiplier sensor is capable of detecting and responding to the intensity of the induced fluorescence that is generated by mechanically damaged fluorescent dye treated corn.

2. The system developed in this study responds linearly to a linear increase in the quantity of fluorescent light. For the measuring system used in this study, the following equation can be used to relate fluorescence to the area of fluorescing material.

$$F = 7.83 + 1.85A$$

Where F = induced fluorescence
A = fluorescing area.

3. Fiber optics can be utilized to extend the versatility of the measuring system.

4. Sample surface preparation affects fluorescent output. All sample surfaces must be uniformly prepared so that results may be compared.

5. Whole kernel samples of more than one kernel are not suitable for measuring induced fluorescence. Single kernels can be classified into three categories according to the amount of induced fluorescence

which is proportional to kernel damage.

6. The samples must be ground to a uniform particle size to create a uniform fluorescing field from which to measure the induced fluorescence.

7. The total measuring system is composed of standard components which should make it possible to design, develop, and market a complete package at a relatively low cost.

Dye

The following conclusions were made concerning the dye and the dyeing technique used in this investigation.

1. Granular dyes are not suitable indicators of mechanical damage in grain. This is due primarly to the inability to obtain uniform treatments of samples.

2. Vacuum procedures do not materially increase the staining ability of the granular dyes.

3. Chemically combined substances that form fluorescent compounds with the internal components of the corn kernels offer the most promising method for relating induced fluorescence to physical damage in grain.

4. The total quantity of induced fluorescence is dependent upon the soaking time. The advance of the wetting front into the floury endosperm can be expressed by the following equation.

$$P = 0.14T^{0.5}$$

Where P = penetration (mm)
T = time (minutes).

5. The 8-anilino-1-naphthalene sulfonic acid (ANS) utilized in

this study did not penetrate into the horny endosperm or the embryo for short contact times (soaking times).

6. The soaking time should be held to a practical minimum because long soaking times results in a disproportionate percentage of the undamaged floury endosperm showing fluorescence.

7. There was no detectible increase in the staining characteristics of the dye when a carrier with a surface tension 1/3 that of distilled water was used compared with distilled water. It was concluded that distilled water would be a suitable carrier for the ANS in most applications for measuring mechanical damage in grain.

Application

1. Artificially prepared corn samples with known amounts of split kernels can be correlated ($R^2 = 0.97$) with induced fluorescence as recorded by a fluorometer. The following equation expresses the relationship between kernel damage and induced fluorescence.

$$F = 30.0 + 0.93D$$

Where F = induced fluorescence
D = kernel damage (split kernels by weight).

2. The regression correlation coefficient (R^2) relating induced fluorescence to germination, breakage test, or with visual counts of mechanical damage is less than 0.5.

3. The time required for a complete analysis of a sample, excluding the time used to dry the treated sample, is very short, approximately 5 minutes. Soaking the kernels requires 3 minutes, grinding the samples requires 1 minute, and reading the induced fluorescence requires 1 minute.

SUGGESTIONS FOR FURTHER STUDY

The research carried out in this study should form a basis from which a commercial machine could be designed and evaluated for measuring mechanical damage in grains. It is suggested that a fluorometer be designed specifically for the purpose of measuring the induced fluorescence from ground corn samples. This machine should include a photomultiplier sensor selected for maximum response at the emission spectrum peak of the dye used to stain the damaged grain, an internal ultraviolet light source to radiate the treated grain samples, and it should be designed for the purpose of evaluating samples for aflatoxin as well as damage.

It is suggested, although not required, that the machine be designed so that it would accommodate a sample size of approximately 10 cm in diameter. This would require a larger sample than was used in this investigation and hence should be more representative of the lot of grain being tested. A grinding apparatus needs to be incorporated in the system to grind the samples as fine as possible. It was found that samples of corn could be ground in the laboratory very nicely with the No. 20 screen, but the corn tended to plug the grinder with the No. 40 screen in place.

Provisions need to be made so that the surface of the samples are prepared in the same manner prior to taking a fluorescent reading.

Once a damage meter had been designed to handle the ground samples, it could then be evaluated and appropriate standards developed for specifying the degree of induced fluorescence that is related to a

specific level of damage.

Further study is warranted in the area of dyes and dyeing techniques to be used as indicators of mechanical damage. This research should be conducted by someone with a strong background in chemistry as the chemical bonding of fluorescent indicators offers the most promise as a standard procedure for relating induced fluorescence to a particular damage level. It is recommended that the relation between the induced fluorescence and exposed area of the floury endosperm be studied when ANS is used as the staining agent. It is believed that any mechanical damage that results in exposure of the floury endosperm of corn would increase the quantity of induced fluorescence.

Investigations into other possible indicators of mechanical damage should be conducted. ANS does not penetrate the horny endosperm and the embryo as well as the floury endosperm. It is possible that some other indicator may be more representative of the actual internal exposed area of the grain.

Further study is warranted to ascertain the effects of moisture content of the grain on the staining characteristics of the dye. A commercial grain damage meter must be able to accurately represent the mechanical damage for a wide variety of grain moisture conditions as would be encountered in the grain trade.

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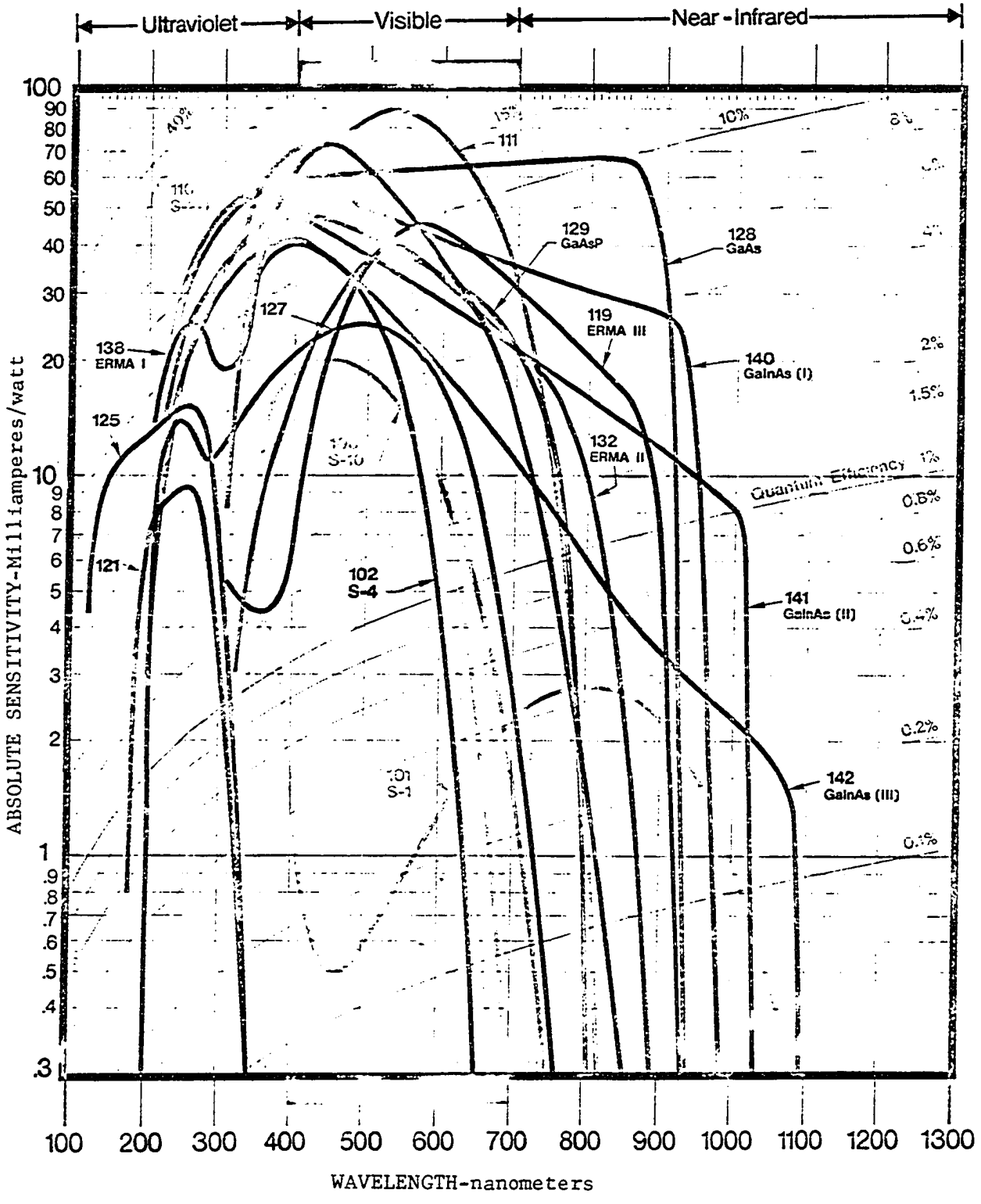
APPENDIX A: SPECTRAL RESPONSE CHARACTERISTICS OF PHOTOMULTIPLIER TUBES

These data were taken directly from Photomultipler Tubes, Photodiodes, and Electron Multipliers; PIT-700B 12/71. RCA/ Electronic Components/ Harrison, N. J. 07029.

Typical Photocathode Spectral Response Characteristics



APPENDIX B: EXPERIMENTAL DATA

Table B-1. Difference in indicated fluorescence (treated -- untreated) for whole kernel granular stained corn kernels vs total mechanical damage as recorded by visual counts.

Sample No.		Replication 1	2	3	Total damage %
	treated	50	-15	45	
3119AH	untreated	-5	-20	30	12.94
	difference	55	35	5	
	treated	28	29	62	
6817B42	untreated	-20	-20	19	35.4
	difference	48	49	43	
	treated	23	3	32	
8318HP	untreated	5	-12	18	16.0
	difference	18	15	14	
	treated	0	-20	48	
1820AH	untreated	44	6	50	19.8
	difference	4	26	2	

Table B-2. Indicated induced fluorescence from whole kernel corn samples treated with granular dye.

Sample No.	Replication No.	
	1	2
9020AM(control)	50	50
3388NIR	-6	10
8516A	33	28
6818A	26	35
7518B3	53	83
1818A	53	108
7121B15	-7	50
0615B26	30	89
3388NIS	-48	38
1820AH	24	86
3390HP	-45	50
9016B49	-37	52
3388HP	-37	71
3117A	-27	75
0615JD	-27	75
3119AH	-10	93
6817B42	0	100

Table B-3. Relative fluorescence for samples harvested in the fall of 1971 in experimental machine harvest studies and treated with the standardized ANS solution and ground on the laboratory Wiley mill prior to evaluation.

Sample No.	Replication No.		
	1	2	3
1816A	23	32	26
6817B36	14	11	10
6817B40	52	54	50
7516B6	31	37	32
7516B9	39	42	38
7516B8	41	37	38
7516A	29	22	25
7519B1	34	30	31
6823B16	18	15	14
6817B37	38	34	37
8520AM	19	17	18
7516AL	40	40	35
9016A	26	25	28
1820B14	28	27	28
9018A	29	29	25
7517B13	31	30	24
8520AH	23	23	22
8520AL	28	26	27

Table B-4. Relative fluorescence for samples evaluated by official grading techniques. Samples treated with standardized ANS solution and ground on Wiley mill prior to testing for induced fluorescence. Voltage regulated.

Sample No.	Replication No.			
	1	2	3	4
Belle P1	23	17	19	13
Belle P2	60	65	60	62
Belle P3	29	23	27	26
Wills 1	73	81	75	75
What C1	54	57	53	41
What C2	57	52	50	58
What C3	43	35	36	34
Oska 1	43	41	38	36
Oska 2	46	43	43	33
Seymour 1	37	32	30	27
Seymout 2	28	25	24	13
Seymour 3	28	28	20	18
Seymour 4	40	30	31	22
Seymour 5	14	6	6	7
Corydon 1	39	40	37	30
N. Hamp. 1	87	85	90	75
N. Hamp. 2	85	85	90	84
N. Hamp. 3	40	43	41	35
N. Hamp. 4	22	16	16	10
Osceola 1	77	70	76	70
Bloomfield A	108	105	120	120
Bloomfield B	22	22	20	19
Bloomfield C	25	22	20	19
Bloomfield D	41	37	40	37
Bloomfield F	17	6	7	-4
Bloomfield G	37	31	32	27

Table B-5. Relative fluorescence for varying line voltage from one prepared sample.

Order of reading	Line voltage	Fluorescent reading
1	111.0	0
2	112.0	7
3	113.0	17
4	114.0	25
5	115.0	34
6	116.0	42
7	117.0	51
8	118.0	58
9	119.0	63
10	120.0	70
11	119.0	64
12	118.0	58
13	117.0	50
14	116.0	42
15	115.0	34
16	114.0	27
17	113.0	17
18	112.0	8
19	111.0	0

Table B-6. Calibration data relating indicated fluorescence to area fluorescing.

Area $(mm)^2$	Replication No.		
	1	2	3
3	12	14	14
6	18	18	19
7	20	20	22
9	29	29	31
9	26	29	28
6	16	20	18
9	26	27	28
14	32	33	32
19	40	37	39
24	52	52	53
10	26	28	27
18	36	37	36
26	54	53	55
36	73	75	76
48	100	100	100

Table B-7. Relative fluorescence for samples with pre-determined damage levels (split kernels mixed with whole kernels by weight) and voltage regulated.

Sample, percent damaged	Replication No.		
	1	2	3
Control (untreated)	30	33	33
0	30	33	33
20	52	52	57
40	62	60	63
60	75	75	79
80	112	112	112
100	124	124	124

Table B-8. Relative fluorescence for crushed samples with acetone or water acting as the carrier for the ANS.

Sample percent crushed	Carrier							
	Acetone				Distilled Water			
	1	2	3	4	1	2	3	4
0	19	19	18	16	11	11	10	7
10	20	14	20	16	27	20	23	20
20	26	20	21	23	50	43	41	43
30	30	30	27	28	38	40	38	34
40	51	49	45	44	58	67	60	68
50	65	67	55	64	66	70	70	66

Table B-9. Relative fluorescence vs contact time for samples treated with the standardized ANS solution.

Time (minutes)	Replication No.	Relative fluorescence			
		Replication Number			
		1	2	3	4
1	1	8	7	7	10
	2	8	11	9	9
2	1	13	10	14	15
	2	10	11	9	10
	3	14	15	17	12
	4	12	16	16	15
5	1	15	23	20	23
	2	19	18	19	23

Table B-10. Penetration of ANS into the floury endosperm with time for acetone and distilled water mixture and distilled water alone as the carrier for two moisture content levels of the corn sample.

Time (minutes)	Penetration of ANS into floury endosperm (mm)			
	Carrier			
	Acetone & water (50/50)		Distilled water	
	moisture content		moisture content	
	17.1%	8%	17.1%	8%
0.5	0.1	0.1	0.2	0.15
1.0	0.1	0.1	0.15	0.1
2.0	0.15	0.3	0.4	0.3
5.0	0.1	0.5	0.6	0.3
10.0	0.6	0.6	0.3	0.25
30.0	0.8	0.5	0.9	0.3
60.0	1.5	1.0	0.8	0.8

Table B-11. Relative fluorescence vs time for evaluating effects of sample preparation and loading into sample compartment.

Elapse time (minutes)	Treatment T_1	T_2	T_3
0	57	57	56
	58	62	57
	59	60	66
	57	56	64
5	56	54	57
	57	55	57
	57	56	60
	56	55	62
	59	57	71
10	58	64	68
	57	64	62
	58	55	60
	59	57	62
15	56	54	60
	57	57	57
	59	52	60
	59	55	64
	57	55	63
20	57	55	51
	56	57	63
22	60	57	63
Statistics			
n = 22			
$\bar{X}$	57.4	56.9	61.05
S_x	1.3	3.09	4.37
$S_{\bar{x}}$	0.28	0.66	0.93

T_1 = Sample no moved during testing, surface undisturbed.

T_2 = Sample repeatedly placed in sample compartment, surface undisturbed

T_3 = Sample surface prepared anew for each reading, surface and position disturbed

Table B-12. Influence of sample surface preparation on induced fluorescence.

Method of preparing sample	Fluorometer reading
Thin layer on bottom of sample holder	38
Strike-off level with top of container, no compression	53
Strike-off level with top of container, compressed surface	55
Compressed sample and then strike off level with top	55
Slightly rounded sample surface	60
Strike off level with the top of container, surface made smooth as possible	50
Strike off compressed rounded	45
Strike off level with top, no compression	52
Rounded, strike off level with top	47

Statistics: $n = 9$

$\bar{X} = 51.5$

$S_x = 4.69$

$S_{\bar{x}} = 1.66$

APPENDIX C: DAMAGE ANALYSIS BY CHOWDHURY

Table C-1. Visual analysis of mechanical damage of corn samples, Chowdhury (1973).

Sample No.	Damage classes				
	Passes 12/64 sieve	Severe	Major	Minor	Total
6816A	0.3	0.82	2.0	1.84	4.96
6817B47	0.2	0.56	6.24	7.44	14.44
3119AM	0.4	1.12	8.24	11.36	21.12
3119AH	0.04	1.8	6.8	4.3	12.94
8520AL	1.0	1.0	7.4	16.6	26.0
7516B6	1.2	0.4	10.2	25.2	37.0
8520AH	0.6	0.8	9.6	16.8	27.8
6823B16	0.6	0.0	1.0	19.4	21.0
7517B13	0.4	1.2	20.6	33.8	56.0
1820B14	0.6	1.8	25.0	24.2	51.6
1821B18	0.8	1.0	19.8	25.2	46.8
9018A	0.4	1.4	8.2	5.8	15.8
9020AM	0.2	1.6	21.6	16.2	39.6
8520AM	0.4	0.6	11.4	8.6	21.0
7516AL	0.2	1.0	14.2	20.2	35.6
7516A	0.6	1.2	11.1	15.8	28.3
0615B26	0.2	0.6	8.0	21.2	30.0
3117A	0.4	0.3	7.1	7.6	15.4
3388LWFHP	0.0	0.0	0.0	0.8	0.8
7516B9	2.6	3.2	25.8	37.0	68.6
6822AM	0.2	0.4	2.8	5.4	8.8
7518B3	1.8	3.8	18.0	31.2	54.8
8516A	0.2	1.2	6.0	6.8	14.2
1816A	0.2	0.2	6.8	9.8	17.0
7516B8	1.6	1.2	19.4	20.6	42.8
6817B37	0.6	0.4	18.4	28.8	48.2
9016B49	0.4	1.4	16.2	36.8	54.8

APPENDIX D: DAMAGE ANALYSIS OF CORN SAMPLES FROM MACHINE HARVEST STUDIES

Table D-1. Damage analysis for corn samples from machine harvest studies.

Sample No.	Broken corn & F. M. %	Stained seed broken, chipped & crushed %	Germination %	Breakage Test (broken corn) %	Official USDA grade No.
Belle P1	0.45	1.76	34	1.9	5
Belle P2	0.32	5.93	67	3.95	4
Belle P3	0.07	2.63	32	6.35	5
Wills 1	1.21	5.65	74	5.0	4
What C1	0.52	5.76	64	7.5	5
What C2	0.04	4.55	78	2.95	3
What C3	0.38	4.5	82	5.6	4
Oska 1	0.10	5.23	83	2.0	4
Oska 2	0.05	3.13	83	2.6	4
Seymour 1	0.47	3.84	82	2.8	4
Seymour 2	0.26	7.55	65	3.55	4
Seymour 3	0.18	1.97	53	2.95	4
Seymour 4	1.10	3.29	61	3.55	4
Seymour 5	0.85	2.54	64	2.2	4
Corydon 1	0.32	4.67	92	3.75	3
N. Hamp. 1	0.43	3.60	17	8.6	SG
N. Hamp. 2	1.30	5.35	39	6.5	4
N. Hamp. 3	0.31	1.80	9	5.6	SG
N. Hamp. 4	0.20	4.22	22	5.0	SG
Osceola 1	0.40	5.16	74	2.9	4
Bloomfield A	1.88	10.96	44	11.3	4
Bloomfield B	0.46	4.71	48	5.5	5
Bloomfield C	0.21	4.42	59	3.8	4
Bloomfield D	0.28	4.37	72	3.8	4
Bloomfield F	0.09	2.45	92	0.8	3
Bloomfield G	0.20	3.83	92	3.25	4