# Reevaluation of Grading Standards and Discounts for Fungus-Damaged Soybean Seeds

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**ABSTRACT:** The proportion of off-colored seed is a primary quality-rating factor that influences the market grade of soybean, *Glycine max* L. Merrill. Off-colors, attributed to biological agents such as fungi and viruses, are visual characteristics that arbitrarily result in lower grade ratings when soybeans show more than ten percent discoloration. These damaged seeds may be further classified under current United States Department of Agriculture, Federal Grain Inspection Service standards as: moldy, weathered, bicolored, mottled, or other colors. Presently, all types of fungal damage and virus symptoms are evaluated equally in the application of discount schedules at grain elevators. However, recent information shows that superficial discoloration caused by some biological agents does not lower grain quality. Indeed, methods are available to distinguish those symptoms that actually reduce seed quality from those that are more cosmetic in nature. This communication provides guidance on fungi that are involved most often in seed damage or discoloration. The following organisms are listed in descending order of importance: *Phomopsis longicolla, Alternaria, Nematospora coryli, Fusarium graminearum, Colletotrichum*, and *Cercospora kikuchii*. Discoloration caused by *Peronospora manchurica* (downy mildew) and the soybean mosaic virus have limited effect on seed quality. Discoloration caused by each of these agents may be identified by recognizable characteristics. Therefore, knowledge of the relative impact of these organisms on seed quality should be practiced in the application of soybean grading standards and discount schedules.
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**KEY WORDS:** *Alternaria, Cercospora*, fungal damage, *Fusarium, Glycine max*, grading standards, *Phomopsis*, seed discoloration, seed quality, soybean.

U.S. grade standards are established and administered by the United States Department of Agriculture–Federal Grain Inspection Service to evaluate the relative quality and market value of soybeans, *Glycine max* L. Merrill, in an objective manner (1). The standards used to evaluate soybean quality are based on a number of physical and biological characteristics. Physical characteristics may include heat; insect and mechanical damage; test weight; cracks or splits; immature seed; and foreign matter. Maximum limits for each category define No. 1 through No. 4 and sample-grade soybeans. Sample-grade refers to soybeans of extremely low seed quality. Although chemical characteristics are not specified in the standard, factors such as protein and oil concentration, and the levels of free fatty acids, tocopherols, chlorophyll, and nonhydratable phosphatides in the oil also impact soybean value. Both chemical and physical attributes are definitive factors that help establish grade and price. However, biological factors that affect grain quality cannot be quantified as easily. Indeed, criteria for these visual judgements have been defined only recently (2–4).

Discoloration of the seed coat caused by biological factors is a principal factor in determining soybean quality. This visual characteristic may be subclassified in six nondefinitive categories: weathered, moldy, bicolored, discolored, mottled, and other colors. Among these categories, seed lots with a cumulative total greater than ten percent discoloration generally are considered sample-grade soybeans. If a market exists for sample-grade soybeans, the contract price is discounted severely.

The tolerance or maximum limits on discoloration caused by biological factors are even more stringent among the best grades for soybean. The threshold for off-colors in a seed lot of No. 1 soybeans is $\geq 1\%$, and $\geq 2\%$ for No. 2 soybeans. Hence, a small degree of variation in estimates of discoloration may have a significant effect on grade and market value of good-quality soybeans. Because this rating is determined visually, off-colors, regardless of cause or source, are presumed to have equivalent impact on seed quality. However, this clinical approach may not be appropriate in all cases. Further examination of this process reveals that various biological factors that cause seed-coat discoloration do not impart the same effect on seed quality. Indeed, some give only superficial discoloration that is not necessarily related to the quality of the seed.

The following discussion considers some of the major fungal (biological) factors that affect gram quality, grading standards, and discount schedules. Emphasis will be placed on the seedborne field fungi and soybean mosaic virus (SMV). Storage fungi such as *Aspergillus, Botrytis*, and *Penicillium* will not be covered. Based on this information, it should be possible to distinguish among signs and symptoms caused by these organisms in regard to their actual impact on seed quality. However, to reduce this information to practice, grading stan-

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dards for soybeans must be amended to allow distinctions among soybeans that have significant or merely superficial damage.

## MATERIALS AND METHODS

Seed samples of unknown mixed cultivars were acquired from farmers' fields in the autumn of 1986. In mid-summer, weather conditions at these field sites were favorable for seed infection by fungi; unusually warm and wet conditions at maturity favored spread of fungi (5). Most seed lots came from the northeastern region of Illinois. A large sample of soybean seeds (cv. Beeson), which showed symptoms of infection by various fungi, also was obtained from the same area. These seeds were sorted manually according to symptoms. Measurements of physical properties were conducted with original samples at 7% (w/w) moisture and with samples that were conditioned in controlled chambers to 13% (w/w) moisture (6). High-moisture seed samples were sealed in plastic bags and stored at 1.7°C for up to two days.

Seed density was determined at constant temperature with a liquid density gradient column (7). This apparatus was filled with a 6:4 (w/w) distilled water/sodium bromide solution. The solution density was 1.42 g $cm^{-3}$. A linear relation was found between floats of known density and the depth at which they reached equilibrium in the column. The resultant equation was used to calculate the density of individual seeds from their recorded depth at equilibrium.

Seed volume was measured with a helium gas pycnometer. Skeletal volumes of individual seeds were determined by gas displacement from the sample chamber. Because helium penetrates pores of irregular surfaces, the volume obtained represented the solid mass of the seed sample. Susceptibility to seed breakage was determined with 100-g samples in a model CK-2M Stein Breakage tester (Fred Stein Laboratories, Atchison, KY) (8). Seed breakage was measured after treatment for 2 min. Seed mass also was based on 100-g samples. Seed surface area was calculated from measured diameters by using Equation 1:

$$\text{surface area} = 2\pi(b^2) + 2\pi[(ab)/e]\sin^{-1}e \qquad [1]$$

where $e$ defines eccentricity and is given by Equation 2:

$$e = [1 - (b/a)^2]^{1/2} \qquad [2]$$

where $a$ and $b$ are major and minor semi-axes of the seed, respectively (9). These data were analyzed by comparing the means of damaged seed with means of sound (asymptomatic) seed in control treatments. Statistical differences among these data were estimated from $t$-tests for small samples at the 5% level ($P = 0.05$) level of significance (10).

## DISCUSSION

Discoloration of soybean seed caused by various microorganisms and viruses has long been used for diagnostic purposes. Now, these signs or symptoms are being used to indicate seed quality. Such changes in seed color may be associated naturally with seed quality because pathogens affect the physical and chemical characteristics of the seed (3,10). Discoloration also may suggest the presence of toxic metabolites or other undesirable seed characteristics. Yet, visual observations may not accurately define the complex interaction of fungal pathogens with soybean seed. Damaged seeds rarely are infected by a single organism. Soybeans may be infected latently by more than one organism or virus (11). Although the signs or symptoms of one pathogen may prevail on a single seed, these indicators often preclude expression of symptoms associated with other pathogens. Thus, the absence of distinguishing symptoms does not necessarily indicate the absence of that pathogen. When symptoms of more than one pathogen are expressed, the mixture of discoloration increases the difficulty in evaluating seed quality. As a convenience, determination of seed quality may be based on total discoloration observed. The problem with this convention is that some pathogens that discolor the seed coat may have little or no effect on the physical properties of the seed. Evidence to support this contention is shown in Table 1, where soybeans were inoculated with pure cultures of *Alternaria, Fusarium graminearum,* and *Phomopsis longicolla* (10). Results show that *Phomopsis* caused significant reduction of seed density, volume, mass, and surface area. Excluding estimates of susceptibility to seed breakage, the impact of the *Alternaria* and *Fusarium* spp. was practically indistinguishable from the control treatment.

Various diagnostic and research methods are available to detect the presence of specific seed pathogens. Unfortunately, only visual evidence is used for grading. As a result, all types of discoloration except for purple stain, regardless of cause, are grouped into a single standard or class. This may lead to overestimation of the effect of fungal damage on seed quality and the unnecessary downgrading of a given lot of soybeans. This situation may be avoided through application of basic knowledge and understanding of how these pathogens affect seed quality and the degree of actual damage they cause.

Approximately 15 of the 40 bacteria, fungi, and viruses that affect soybeans are of major economic importance (12). The following discussion lists the symptoms and causal

**TABLE 1**
**Physical Properties of Beeson Soybean Seeds Infected by Three Fungi[a]**

| | Property | | | | |
|---|---|---|---|---|---|
| Treatment | Density (g $cm^{-3}$) | Volume ($cm^3$) | 100-seed wt (g) | Breakage susceptibility (%) | Surface area ($cm^3$) |
| *Alternaria* | 1.143 A[b] | 0.170 A | 21.2 A | 4.10 A | 1.62 A |
| *Fusarium graminearum* | 1.121 B | 0.164 B | 20.3 A | —[c] | 1.61 A |
| *Phomopsis longicolla* | 1.101 C | 0.140 C | 17.5 B | — | 1.56 B |
| Control | 1.145 A | 0.168 A | 20.8 A | 0.77 B | 1.62 A |

[a]Seeds at 13% moisture content (wet basis).
[b]Means followed by the same letter are not significantly different at $P = 0.05$.
[c]Data not available.

**FIG. 1.** *Phomopsis* seed decay. Soybeans infected with *Phomopsis longicolla.*

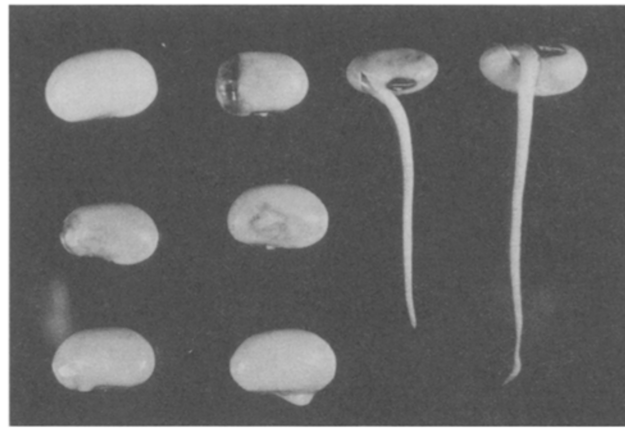

**FIG. 3.** Soybean seeds showing symptoms of yeast spot caused by *Nematospora coryli.* Seed in the upper left corner is uninfected.

agents of eight of these diseases in descending order of importance.

Phomopsis *seed decay.* This disease is cause primarily by *P. longicolla* T.W. Hobbs and is one of several soybean diseases attributed to members of the *Diaporthe/Phomopsis* fungal complex. It is the most destructive disease of soybean seed (4,12). Severely affected seeds are elongated, heavily cracked or fissured, shriveled, and covered with white-to-gray mycelium (Fig. 1). These seeds exhibit less mass, volume, density, and surface area compared to uninfected seed (10,13).

Alternaria *seed decay.* This disease is associated with *Alternaria alternata* (Fr.:Fr.) Keissl and *A. tenuissima* (Kunze:Fr.) Wiltshire (3,12,14). These opportunistic fungi decay pods and seeds after senescence, frost damage, insect injury, or wounding. Transmission of this disease usually is associated with insect injury caused by the beetle *Cerotoma trifurcata* (Forster). Infected seeds have dull gray to deep brown patches and dark, irregular, spreading, sunken areas on the seed coat (Fig. 2). Seeds infected with *A. alternata* exhibit reduced seed mass, volume, density, and increased susceptibility to seed breakage (10,15).

*Yeast spot. Nematospora coryli* Peglion causes this disease and is always associated with stink bug (*Acrosternum hilare* Say) damage (12). At maturity, diseased and injured seeds have sunken light or cream-colored spots, some with dark borders (Fig. 3). Affected areas of the embryo are off-white and cheesy in texture. Severely infected seeds do not mature and are greatly shrunken and wrinkled. However, little is known about the effect of yeast spot on other seed characteristics.

Fusarium *discoloration.* As shown in Figure 4, a salmon-pink-to-red discoloration has been associated with various *Fusarium* spp., particularly *F. graminearum* Schwabe and *F. sporotrichioides* Sherb (12,14,16). These symptoms usually appear after long periods of warm and wet weather at harvest. Although discoloration has been attributed to *Fusarium* spp., a direct association has not precisely been described or proven by Koch's postulates (17). Likewise, shriveled and more ellipsoid seed symptoms, arbitrarily linked to *Fusarium*

**FIG. 2.** Soybean seeds showing severe (left) and mild (right) symptoms caused by *Alternaria* spp.

**FIG. 4.** Soybean seed with discoloration associated with *Fusarium* spp.

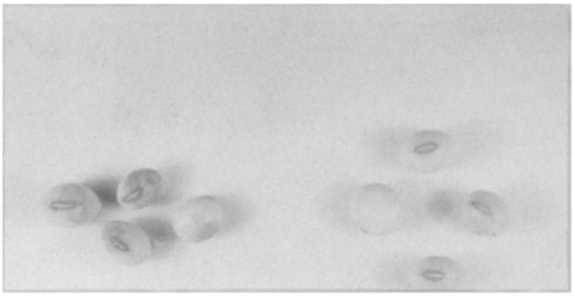

FIG. 5. Soybean seed with symptoms of anthracnose caused by *Colletotrichum truncatum.*

spp., also may be caused by other fungi. In pure culture, infected seed are less dense and have reduced volume compared to control treatments (16). Other *Fusarium* spp. associated with discolored seed are *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. moniliforme* J. Sheld., *F. semitectum* Berk & Ravenele, and *F. solani* (Mart.) Sacc (12). Infection with *F. semitectum* is associated with insect injury caused by the lima bean pod borer, *Etiella zinckenella* Treitschekel (8). In general, *Fusarium*-associated discoloration is superficial (18). However, mycotoxins in the seed meal may be of greater concern to the processing industry.

*Soybean anthracnose.* This disease, caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, primarily affects vegetative tissues. However, the pathogen may colonize and infect seed (12). Infected seeds appear dirty, with irregular brown or small uneven gray areas with black specks (Fig. 5). Infection generally is confined to the seed coat, and the embryo often is not affected. Seeds with superficial infection may be used for processing.

*Purple seed stain.* This discoloration is caused by *Cercospora kikuchii* (Matsumoto & Tomoysau) M.W. Gardner. The symptoms are common and easily recognized. Color may vary from violet to dark purple and is confined to the outer two layers of the seed coat (Fig. 6). Discolored areas range from scattered specks to large irregular blotches over the entire seed surface. The embryo is not discolored, and infected seeds retain normal shape. Unless the strain of *C. kikuchii* is especially aggressive, seeds lose little density or mass (19). Degradation of seed-coat protein by this fungi has been reported (20). A high proportion of purple-stained seed is a criteria that may reduce the grade of a sample.

*Soybean mosaic.* Seeds from plants infected with SMV are mottled with black or various shades of brown bleeding from the hilum (Fig. 7). Seeds of cultivars with colorless hilum may show mottling. Various environmental stresses and other viruses also can cause such bicolor staining or hilar bleeding. SMV does not reduce seed density, volume, or mass; it does not affect breakage susceptibility, surface area, or shape (10).

*Downy mildew.* Downy mildew, caused by *Personospora manshurica* (Naumov) Syd., in Guam, appears as a superficial milky white encrustation of mycelium and oospores on seeds (12). These features may be removed from the seed without damaging the seed coat (Fig. 8). Seeds of most soybean cultivars retain normal characteristics, but highly infected seed may be smaller or lighter in mass.

In conclusion, discoloration of the seed coat caused by fungi and viruses is a principal factor in determining soybean quality. All off-colors, regardless of source, are considered to have an equal effect on seed quality under present grading standards. However, distinction should be made to discount superficial seed-coat discoloration in evaluation of fungal damage. Many economically important fungal pathogens exhibit relatively innocuous symptoms relative to seed-coat color. The following organisms are listed in descending order of importance: *P. longicolla, Alternaria, N. coryli, F. graminearum, Colletotrichum,* and *C. kikuchii.* Discoloration caused by *P. manchurica* (downy mildew) and SMV have limited effects on seed quality. Discoloration caused by each of these agents may be identified by recognizable characteristics. Therefore, knowledge of the relative impact of these organisms on seed quality should be practiced in the application of soybean grading standards and discount schedules.

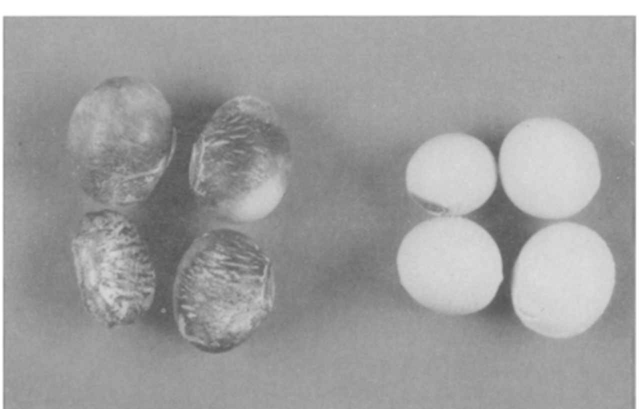

FIG. 6. Soybean seed with purple stain (left) caused by *Cercospora kikuchii.*

FIG. 7. Soybean seed showing bicolor and hilum bleeding from plants infected with soybean mosaic virus.

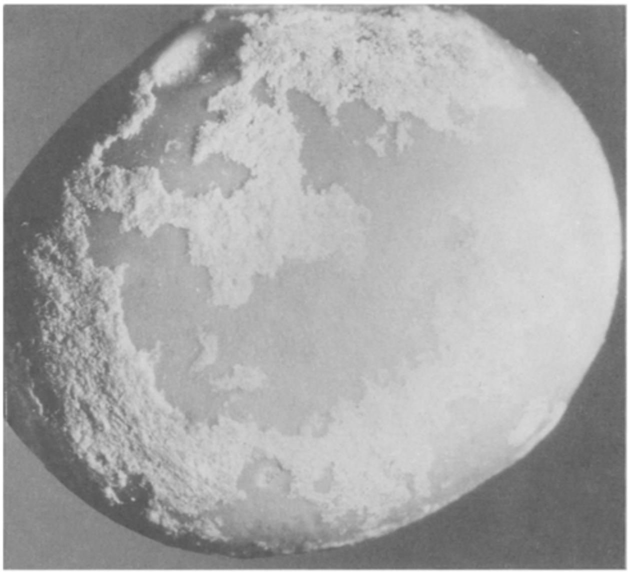

**FIG. 8.** Soybean seed encrusted with oospores of *Peronospora manschurica*, which causes downy mildew.

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