

Discoloration of Soybean Seeds— An Indicator of Quality

The discolorations caused by various microorganisms on soybean (*Glycine max* (L.) Merr.) seeds, long used for diagnostic purposes, are now being used to indicate quality. This is possible because almost all commercially produced soybeans have beige seed coats, which is relatively uncommon among large-seeded legumes. Seed coats of common bean cultivars, for example, have a wide range of colors.

Coats of infected soybean seeds are frequently discolored. Such terms as "weathered" or "off-color" can no longer be used to describe soybean seeds with abnormal coloration. Weather conditions may intensify or otherwise affect discoloration but do not cause the problem. In addition to changing the color, pathogens affect the physical and chemical characteristics of the seeds. Discoloration can indicate undesirable physical qualities, the presence of toxic metabolites, or other unfavorable seed characteristics. Thus, discoloration can be used to determine soybean seed quality and relative market value as well as to diagnose the cause of an infection.

Pathogens Causing Discoloration

Of the more than 40 bacteria, fungi, and viruses that can affect soybean seeds, 15 are of major economic importance (23). Of these, eight are listed here in descending order of importance.

Phomopsis seed decay. Caused primarily by *Phomopsis longicolla* T.W. Hobbs, this is one of several soybean diseases caused by members of the *Diaporthe/Phomopsis* fungal complex (23) and is the most destructive disease of soybean seeds. Severely affected seeds (Fig. 1) are shriveled, covered with whitish mycelium, deeply fissured, and, compared with uninfected seeds, small,

low in volume and density, and elongated, with significantly less surface area (11,17).

Alternaria seed decay. This has been associated with *Alternaria alternata* (Fr.:Fr.) Keissl. and *A. tenuissima* (Kunze:Fr.) Wiltshire (23). These opportunistic fungi decay pods and seeds after senescence, frost damage, insect injury, or wounding. This disease is usually associated with beetle (*Cerotoma trifurcata* (Forster)) feedings, and the incidence increases with the frequency of insect injury. Infected seeds have dull gray to deep brown patches and dark, irregular, spreading, sunken areas occurring anywhere on the seed coat (Fig. 2). Lowered bulk density, volume, and weight; reduced surface area; and increased susceptibility to breakage have been associated with *A. alternata* infections (6,17).

Yeast spot. *Nematospora coryli* Peglion causes this disease, which is always associated with stink bug (*Acrosternum hilare* (Say)) damage (23). Diseased and injured mature 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. Little is known about the effect of yeast spot on other seed characteristics, however.

Fusarium discoloration. A salmon or pink to red discoloration (Fig. 4) has been associated with various *Fusarium* spp., particularly *F. graminearum* Schwabe and *F. sporotrichioides* Sherb. (7,12,23,29). However, discoloration has been neither precisely described nor proved by Koch's postulates to be caused by *Fusarium* spp. (30). Seeds may be shriveled and more ellipsoid than symptomless ones, but other fungi may cause these symptoms. Other *Fusarium* spp. associated with discolored seeds are *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. moniliforme* J. Sheld., *F. semitectum* Berk & Ravenel, and *F. solani* (Mart.) Sacc. (23). Infection with *F. semitectum* has been associated with damage caused by the lima bean pod borer (*Etiella zinckenella* (Treitschke)

(10). *Fusarium*-associated discoloration is generally superficial (27); affected seeds may be used for processing unless the red pigment, mycotoxins, or pod borer injury causes problems.

Soybean anthracnose. This disease, caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, primarily affects vegetative tissues, but the pathogen can colonize and infect seeds (23). Infected seeds appear dirty, with irregular brown areas or small, uneven gray areas with black specks (Fig. 5). Infection is generally confined to the seed coat, and the embryo is often not affected. Seeds with superficial infection can be used for processing.

Purple seed stain. The discoloration caused by *Cercospora kikuchii* (Matsumoto & Tomoyasu) M.W. Gardner is common and easily recognized. The color varies from violet to pale purple to dark purple (Fig. 6) and is confined to the upper two layers of the seed coat. The discolored areas range from scattered specks to large irregular blotches over the entire seed surface. The embryo is not discolored, and infected seeds retain their shape. Unless the strain of *C. kikuchii* is especially aggressive, seeds lose little in density or weight (19). A high proportion of purple-stained seeds or stain amounts exceeding 50% per seed may reduce the grade of a sample.

Soybean mosaic. Seeds from plants infected with soybean mosaic virus (SMV) are mottled with black or various shades of brown "bleeding" from the hilum (Fig. 7). Seeds of cultivars with so-called colorless hila may show mottling. Various environmental stresses and other viruses can also cause such bicolor staining or hilar bleeding. SMV does not reduce seed density, volume, or weight and does not affect breakage susceptibility, surface area, or shape (17).

Downy mildew. Downy mildew, caused by *Peronospora manshurica* (Naumov) Syd. in Gäum., appears as a superficial milky white encrustation of mycelium and oospores on seeds (Fig. 8) that can be removed without damaging the seed coat. Seeds of most cultivars retain their normal characteristics, but those of highly susceptible cultivars may be

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smaller or lighter than normal as a consequence of plant infection.

Effect of Discoloration on Grade Standards

Soybean seed quality is determined by biological, chemical, and physical characteristics that often are poorly defined in commerce and categorized on the basis of visual observation as "soybeans of other color," "weathered," "moldy," "bicolored," "discolored," "mottled," etc. These visual classifications can affect grade, i.e., the greater the proportion of off-color seeds, the lower the grade or acceptance rate. For example, seeds showing more than 10% discoloration are assessed as damaged by the USDA Federal Grain Inspection Service (Table 1) (26). All types of fungal damage are treated equally, regardless of source or type.

Deterioration from cracks in the seed coat, splitting of the seed coat along the sutures, insect injury, heat and mechanical damage, immaturity, and foreign



Fig. 1. Soybean seed infected with *Phomopsis longicolla* is elongated, smaller than normal, deeply fissured, and covered with whitish mycelium.



Fig. 2. Dull gray to deep brown patches and scattered, dark sunken areas on soybean seed infected with *Alternaria* sp.



Fig. 3. Sunken, cream-colored spots with dark borders on soybean seeds with yeast spot, caused by *Nematospora coryli*.

matter also are important factors in determining grade. Cracks or splits lead to further breakage. Economically important physical properties, such as density, shape, size, surface area, volume, and weight, are affected by species of *Alternaria*, *Fusarium*, and *Phomopsis* but not by SMV (17). For example, *Phomopsis* spp. reduced seed density by 4% and volume and weight by 13%, with a resultant potential for seed breakage 20 times greater than that for asymptomatic seeds (17). Effects were similar but less dramatic for seed infected with *Alternaria* or *Fusarium* (17).

Chemical and physical characteristics of soybean seeds are the basis of certain national and international grades and standards that provide information desired by commercial interests. Soybean seeds are a primary source of vegetable oil and protein and of many feed and food products. So, processors are con-



Fig. 4. Salmon or pink to red discoloration anywhere on a soybean seed coat may be associated with infection by *Fusarium* sp.



Fig. 5. Irregular brown or gray areas with black specks develop on soybean seeds infected with *Colletotrichum truncatum*.



Fig. 6. Soybean seed with purple seed stain caused by *Cercospora kikuchii*. Stains vary from violet to pale purple to dark purple and tend to "bleed" from the hilum.

cerned with such definitive chemical properties as level of free fatty acids, yield of oil and protein, and quality of derived oil.

Seeds of most commercially grown soybean cultivars are ellipsoid and have a bright, uniformly beige seed coat with a smooth texture. Unblemished seeds are preferred in the market, and discolored or misshapen seeds have lower market value. Black, brown, gray, green, purple, or red coloration may appear on seed coats that are immature (green), damaged by heat or frost (black or brown), injured by insects, or colonized by plant-pathogenic fungi or viruses. Discoloration caused by plant pathogens varies predictably with the microorganism or virus involved, and symptoms on seeds are useful for diagnosis. However, the absence of symptoms does not necessarily mean that a seed is free of pathogens or other microorganisms (22,23).

Discoloration as Indicator of Toxic Metabolites

Discoloration may be an indicator of mycotoxins in soybean seeds (14), which have not been considered as important as mycotoxins in other crop seeds. Until recently, all soybean seeds used for feed or food had to be heated or cooked to inactivate trypsin (a proteolytic enzyme present in pancreatic secretion) inhibitors; such treatment also destroys most mycotoxins that might be present. However, certain new soybean cultivars with reduced levels of trypsin inhibitors (9,18) can be fed to animals without cooking, and the presence of mycotoxins becomes a concern when these cultivars are used.

Zearalenone, deoxynivalenol (vomitoxin), and T-2/HT-2 toxin have been



Fig. 7. Soybean seeds from plants with symptoms of soybean mosaic are mottled with black or brown, depending on the color of the hilum.



Fig. 8. Seed from soybean plant with downy mildew is encrusted with oospores of *Peronospora manshurica*.

detected in whole soybeans and in soybean seed coats, meal, and oil; zearalenol and diacetoxyscirpenol in seed coats and meal; and aflatoxin B (low levels, up to 5.8 ppb) in seed coats (12). Four isolates of *A. alternata* from soybean seeds produced alternariol monomethyl ether, alternariol, altenuene, and altertoxin I both in culture and on symptomatic seeds (6). Soybean seeds stained pink to red by *Fusarium* spp. were found to contain diacetoxyscirpenol, deoxynivalenol, T-2 toxin, zearalenol, and zearalenone (7,15,28). Fusarin-C was also produced in soybeans infected with *F. moniliforme* (3).

Metabolites from several other fungi growing in soybean seeds have been shown to be toxic to animals. In sheep, isolates of *Phomopsis* sp., *P. phaseoli* (Desmaz.) Sacc., and *Diaporthe* sp. grown on autoclaved wheat grain induced necrosis of the forestomach epithelium (1). In young rats, an intraperitoneal dose of an extract from infected wheat (0.5 ml, or 3.2 g of culture per kilogram liveweight) was lethal within 4 hours; cytochalasins or trichothecenes were suspected as the toxic agents (1).

Identification of factors affecting soybean seed quality is increasingly important in commercial grading of soybean seeds and for the eventual development

of tolerance standards for toxin levels. The production of mycotoxins by fungi associated with soybean seeds requires further investigation.

Effect of Discoloration on Product Quality

Ideally, soybeans used for oil and food production should be high in protein and

oil and low in fatty acids. Seeds infected by *Fusarium* or *Phomopsis* spp. have lower quality oil, higher amounts of free fatty acids, poorer meal color, and other lesser qualities than uninfected seeds (7,29). For example, soybean seeds contaminated with *F. solani* and stored at 80% relative humidity produced rancid, turbid oil high in free fatty acids (2). Similarly, the level of free fatty acids in

Table 1. U.S. Department of Agriculture soybean grades and grade requirements^a

Grade ^b	Minimum test weight/bu (lb)	Maximum limits				
		Damaged seeds		Foreign material (%)	Splits (%)	Soybean of other colors (%)
		Heat (%)	Total (%)			
No. 1	56	0.2	2	1	10	1
No. 2	54	0.5	3	2	20	2
No. 3	52	1.0	5	3	30	5
No. 4	49	3.0	8	5	40	10
Sample

^aFrom *Official United States Standards for Grains* (26).

^bPurple mottled or stained soybeans are graded not higher than No. 3, and materially weathered soybeans are graded not higher than No. 4. Sample grade soybeans 1) do not meet the requirements of No. 1, 2, 3, or 4; 2) contain eight or more stones with an aggregate weight in excess of 0.2% of the sample weight, two or more pieces of glass, three or more seeds of *Crotalaria* spp., two or more castor beans (*Ricinus communis* L.), four or more particles of an unknown foreign substance(s), or a commonly recognized harmful or toxic quantity of other animal filth per 1,000 soybeans; 3) have a musty, sour, or commercially objectionable foreign odor (except garlic odor); or 4) are heating or are otherwise of distinctly low quality.

crude oil derived from seeds infected with *Alternaria* sp. was higher than that from uninfected seeds (6). Another study (11) revealed that *Phomopsis* sp. was more important than *Fusarium* sp. in seed deterioration; flour and oil derived from seeds infected with *Phomopsis* sp. were unmarketable.

Generally, purple seed stain affecting the entire surface tends to lower oil and increase protein content. When seeds were collected from field plots in Illinois in which soybean plants were inoculated with one of several isolates of *C. kikuchii*, those from plants inoculated with a virulent isolate had higher levels of free fatty acids and protein and lower levels of oil than those from plants inoculated with less virulent isolates or not inoculated (19). In another study (13), *C. kikuchii* did not affect protein content of seeds but did increase lipids and lower saccharide content. In a third study (25), *C. kikuchii* infection only slightly affected the hardness of cooked whole beans, the color of soybean curd, and the texture of miso (fermented soybean paste).

Soybean seeds from plants infected with SMV had higher levels of protein and amino acids but lower levels of oil than seeds from uninfected plants (8,24). Similarly, encrusted seeds from soybean plants infected with *Peronospora manshurica* contained more protein and free fatty acids and less oil than seeds from healthy plants (16).

Computer Vision Algorithm

The various factors causing discoloration have been studied in many laboratories, including ours, and the effects of these factors on the quality of soybean seeds are now clearly understood. Some pathogens affect only the color of the seed coat, causing "cosmetic" or "superficial" damage, while other pathogens also damage tissue in the seed coat and embryo. The relationship of seed coat color to infection by fungi and viruses and to chemical and physical properties of seeds provides a potential means for automatic determination of seed quality by color image classification techniques (4,5,20,21).

The distinct types of discoloration caused by different species of fungi and by viruses can be used to indicate seed quality. Computer image analysis, a new technology being studied at the University of Illinois at Urbana-Champaign, shows promise for assisting in fast, accurate determination of pathological factors affecting soybean seed quality and grade. A computer vision algorithm developed to detect and classify soybean seeds damaged by fungi differentiated damaged from undamaged seeds with an accuracy of 98% (4,20). On the basis of color, the algorithm identified the fungal species causing the damage with 66-96% accuracy. Causes are classified as *Phomopsis*

seed decay, *Alternaria* seed decay, pink seed stain, purple seed stain, and unidentified. Affordable units may soon be available to seed dealers and operators of grain elevators.

Results of Collaboration

Discoloration in soybean seeds can no longer be used arbitrarily as an indication that all such seeds are low in quality and unsuitable for seed stock or industrial use. For example, the quality of seeds showing bicolor or hilar bleeding or of seeds covered with the white growth of the downy mildew fungus is comparable to the quality of seeds without such symptoms. Similarly, infection by *C. kikuchii* reduces quality significantly only when seeds are 85-90% purple-stained. Seeds with these types of discoloration can be used commercially.

Infection by some fungi, such as *Phomopsis* spp., can cause sufficient discoloration and damage to justify discounting the affected seed lots. Soybean seeds damaged by insects and colonized by *Alternaria* spp., the yeast spot fungus, or saprophytic microorganisms also should be discounted; whether the insects or the microorganisms are responsible for the greatest damage is not known.

Information is available for assessment of the overall quality of soybean seed lots, and a computer-based technology has been developed to assist in rapid assessment of such quality according to coloration. Plant pathologists and agricultural engineers have collaborated to provide an automatic indicator system that differentiates, on the basis of symptomatology, soybean seeds of potentially high quality from those of potentially low quality. This system provides a more equitable pricing mechanism and a means of identifying seeds useful for feed or processing.

Acknowledgments

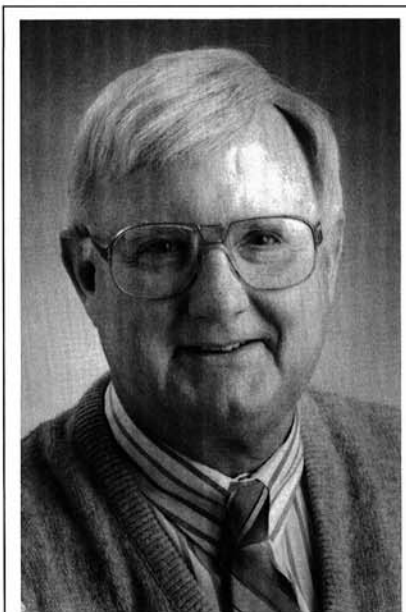
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