An Evaluation of "Heat Damage" and Fungi in Relation to Sunflower Seed Quality

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ABSTRACT

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Thirteen samples of sunflower oilseed graded No. 1, and 21 samples of Sample grade (SG) were analyzed for percent moisture, percent free fatty acids (FFA), fungal colony-forming units per gram of seed (cfu), percent seed yielding fungi, the kinds of fungi present, and total isolates of *Aspergillus*. The relationship of "heat damage" (visible darkening of the meat of sliced seeds that may be caused by the heat applied to dry the seeds or by postharvest fungal invasion during storage), an important grading character, to these other parameters was studied by using correlation and linear regression analyses. Several samples of No. 1 seed had little or no heat damage but unacceptably high FFA values. Almost half of the Sample grade samples produced oil with acceptably low levels of FFA but had high heat damage scores. Apparently, overheating of sunflower seed during drying can cause heat damage without producing high FFA levels. Correlation and linear regression analyses failed to reveal adequate relationships between heat damage and the other quality parameters that were studied. For No. 1 seed, percent FFA showed a positive correlation with percent seed yielding fungi. For SG seed, percent FFA correlated positively with percent seed yielding fungi and percent moisture, percent seed yielding fungi and cfu, and cfu and percent recovery of *Aspergillus*. Heat damage has been incorporated into the new Federal standards for sunflower seed, but the results of this study indicate that heat damage scores in the traditional sense do not always accurately reflect sunflower seed and oil quality as determined by chemical analyses. It is suggested that a more quantitative evaluation, such as percent FFA, be used in conjunction with the more traditional criteria for grading sunflower oilseed.

Additional key words: *Helianthus annuus*.

The maintenance of high quality in stored oilseeds has always been a problem where storage conditions permit the invasion of the seeds by fungi. For sunflower seed, this problem has become acute in recent years with the increased U.S. production and export of sunflower oilseed to Europe, Africa, and Central America and the accompanying expectations of those countries of receiving seed whose quality is accurately reflected by grade. In the past, the Federal Grain Inspection Service had no standards for sunflower seed destined for domestic or export markets (1), but such standards have recently been adopted (5). Heat damage has traditionally been one of the criteria used to evaluate a variety of grains and seeds (3); by definition, it is meant to describe damage (discoloration) caused by heat (1). According to Christensen and Sauer (13), however, discoloration of whole seeds or portions of them including the germ, or embryo, may also be caused by the invasion of both field and storage fungi. Thus, the term heat damage (because the dark color suggests burning) used in grain inspection may also include discoloration due to the activity of fungi and not directly due to heat, although both factors are present when stored grain undergoes heating due to fungal growth.

Schroeder and Sorenson (15) reported that there was no correlation between heat damage and the temperature of rice, and that heat damage was associated with a high level of invasion by storage fungi. Christensen et al (12) found that the embryos of all corn kernels from more than 100 lots of 100% damaged kernels were decayed to some extent by fungi. Christensen (7) also reported that in commercial wheat storage, the invasion of the wheat germ by common species of *Aspergillus* was probably the cause of "sick wheat" (seed with obviously dark germs). The major fungi present in the "sick" seeds were *Aspergillus restrictus* Link, *A. repens* de Bary, *A. candidus* Link, and *A. flavus* Link. Christensen and Kaufmann (11) stated, "Neither in the laboratory of the Department of Plant Pathology of the University of Minnesota . . .nor at the Grain Research Laboratory at Cargill, Inc. . . .have we ever encountered a single case of germ damaged or sick wheat in which storage fungi were not involved." The growth of *Alternaria* spp., *Aspergillus* spp., *Rhizopus* spp., and other fungi on oilseeds has been reported to result in lowered oil quality and increased levels of free fatty acids (FFA) (14,16), but the lack of inoculation studies with living, fungus-free seed as controls has hindered the elucidation of the precise role that these and other fungi play in the lowering of oil quality.

Heat damage is measured by the visual evaluation of seed discoloration and is a component of the grading standards used by state and federal grain inspection services for sunflower seeds, with maximum limits of 0.5% for grade No. 1 and over 1.0% for Sample grade. According to the Minnesota Department of Agriculture, Grain Inspection Division, "Heat damaged sunflower seed shall be seed and pieces of seed, which, when sliced open, show evidence of meats that have been discolored by heat (2)." Although not a part of Federal grain standards, percent FFA is another important parameter of oilseed quality. According to the National Cottonseed Products Association Trading rules, grade No. 1 sunflower seed shall contain. . ."not more than 1.8% free fatty acids in the oil and seed. . .and seed containing over 3.0% free fatty acids shall be Sample grade and subject to rejection." Parameters of seed quality such as percent FFA, percent seed yielding fungi, and percent moisture are more quantitative than heat damage and may more accurately reflect oilseed quality. The relationship of heat damage to these other parameters of seed quality has not been clearly established.

The purpose of this study was to evaluate heat damage as a major criterion of sunflower seed quality by examining its relationship to percent moisture, percent FFA, fungal colony-forming units per gram of seed (cfu), percent seed yielding fungi, and total number of isolates of *Aspergillus* spp.

MATERIALS AND METHODS

The sunflower seeds used in this study were commercial oilseed hybrids obtained from and graded by the North Dakota Grain Inspection Service, Fargo, according to the grade standards prepared for sunflower seeds by the State of Minnesota (2). These samples were representative of commercial samples received by the North Dakota Grain Inspection Service for grading purposes; the origin, lot size, and history of each sample was unknown. Samples received in this laboratory for analysis weighed approximately 450 g. Thirteen samples were graded Number 1 (No. 1), which has maximum limits of 10% moisture, 5% total damage, or 0.5% heat damage. Twenty-one samples were graded Sample grade (SG) which refers to seed with more than 12% moisture, 10% total damage, or 1.0% heat damage.

FFA were determined by AOCS method (4), which involves titration of an ethanolic extract of crude oil with 0.1 N standardized NaOH. The number and kinds of fungi present within the seed were determined by the procedure of Christensen (9). Yeasts that appeared on isolation plates were not considered; the term fungi in this paper refers exclusively to filamentous forms. Approximately 50 seeds were shaken for 1.5 min in 2.6% NaOCl, rinsed twice with sterile distilled water, plated onto tomato juice agar containing 6% NaCl (ten seeds per plate), and incubated for 7 days at 27 C. The fungi growing from these seeds were either identified directly or (when the salt appeared to alter fungal morphology or interfere with sporulation) were subcultured onto other media (usually half-strength V8 juice agar). Total cfu were determined by the method of Christensen (8) by grinding 11.0 g of seed in a sterile Waring blender with 99 ml of sterile distilled water for 1.5 min, making serial dilutions with the resulting suspensions, and plating the dilutions by swirling with molten acidified potato-dextrose agar. Colonies on plates showing from 30 to 300 colonies were counted after 48–72 hr of incubation at room temperature. All analyses were conducted in duplicate, and the data were analyzed by using correlation and linear regression methods from SAS (6).

RESULTS

A total of 50 fungal species belonging to 23 genera were isolated (Table 1). Forty-one species in 16 genera were isolated from grade No. 1 seed; 35 species in 19 genera were isolated from SG seed. In grade No. 1 seed, *Alternaria, Aspergillus*, and *Scopulariopsis* were the genera most frequently isolated. Although *Aspergillus* spp. accounted for 21.4% of the total isolates from grade No. 1 samples, their occurrence was limited primarily to only two (samples 5 and 10) of the 13 samples that were analyzed. The two grade No. 1 samples that yielded the majority of *Aspergillus* isolates (samples 5 and 10) also produced 89% of the *Scopulariopsis* isolates, and had high FFA values (Table 2). Several taxa were recovered more frequently from No. 1 than from SG seed, including *A. alternata* (Fr.) Kiessler, *Alternaria* sp., *Penicillium* spp., *Scopulariopsis* spp., *Phoma* spp., and *Cladosporium* spp.

The characteristics of the sunflower seed graded No. 1 are presented in Table 2. Although heat damage ranged from 0.0 to 0.4%, three of the samples (samples 5, 8, and 10) had unacceptable FFA values for grade No. 1 seed (>1.8% FFA). Neither correlation coefficients nor regression analysis revealed any relationship between heat damage and any of the other parameters studied for No. 1 seed. The FFA values for No. 1 seed, however, were positively correlated ($r = 0.73$) with percentage of seed yielding fungi.

In the SG samples, *Aspergillus, Alternaria,* and *Scopulariopsis* were the genera most frequently isolated. *Eurotium repens* de Bary was the most frequently isolated storage fungus species (13.5%) from SG samples. The species of fungi most often recovered from SG seed were *Alternaria alternata* (16.1%), *Eurotium rubrum* Konig, Speick., and Brem. (9.5%), *E. amstelodami* Mangin (6.8%), *Microascus cirrosus* Curzi (6.5%), and *Aspergillus candidus* (Link) Fr. (4.4%) (Table 1). Taxa recovered more frequently from SG than from No. 1 seed were *Rhizopus* spp., *Eurotium* spp., and *Aspergillus* spp. not producing teleomorphic states. Genera isolated exclusively from SG samples were *Circinella, Nigrospora, Paecilomyces, Stemphyllium, Trichothecium, Sordaria,* and *Thermoascus*.

The characteristics of SG sunflower seed samples are presented in Table 3. Although all SG samples had heat damage scores above the NCPA limits for grade No. 1 seed, 11 of the 21 samples had FFA levels which were within the limits of grade No. 1 seed (less than 1.8%). In particular, samples 12 and 14 had high heat damage scores (10.6 and 14.0%, respectively) and high total damage scores

TABLE 1. Genera of fungi isolated from 13 grade No. 1 and 21 Sample grade sunflower seed samples

Class and genus	Grade No. 1: Number of isolates[c]	Grade No. 1: Number of samples yielding genus	Sample grade: Number of isolates[d]	Sample grade: Number of samples yielding genus
Zygomycetes				
Circinella	–	–	2	1
Mucor	1	1	1	1
Rhizopus	9	6	55	12
Deuteromycetes				
Alternaria	526	13	360	18
Aspergillus[a]	24	4	154	8
Botrytis	14	2	10	6
Cladosporium	22	7	27	7
Fusarium	1	1	2	1
Nigrospora	–	–	1	1
Paecilomyces	–	–	10	4
Penicillium	36	8	41	11
Phoma	34	8	6	6
Scopulariopsis[b]	19	2	5	2
Stemphyllium	–	–	1	1
Trichothecium	–	–	1	1
Ascomycetes				
Ascotricha	1	1	–	–
Chaetomium	1	1	–	–
Emericella[a]	–	–	17	7
Eurotium[a]	203	12	627	15
Microascus[b]	169	9	184	9
Sordaria	–	–	1	1
Thermoascus	–	–	2	1

[a] *Aspergillus* isolates producing teleomorphs are counted with the appropriate isolates of *Eurotium* or *Emericella*.
[b] Isolates of *Scopulariopsis* producing teleomorphs are counted with isolates of *Microascus*.
[c] From 1,300 seeds.
[d] From 2,100 seeds.

TABLE 2. Characteristics of grade No. 1 sunflower seed

Sample number	Heat damaged[a] (%)	Moisture[a] (%)	FFA[b] (%)	cfu[c] (×1,000)	Seeds yielding fungi (%)	Total isolates of *Aspergillus*
1	0.0	8.8	0.51	6	37	10
2	0.4	9.0	0.69	160	36	3
3	0.0	10.0	0.75	31	58	0
4	0.0	9.0	0.59	20	75	12
5	0.0	9.8	2.51	1,800	100	66
6	0.0	7.4	0.46	40	68	0
7	0.0	<8.5	0.52	10	40	14
8	0.0	<8.5	2.65	150	85	3
9	0.0	<8.5	0.93	13	82	0
10	0.0	8.6	2.45	530	100	104
11	0.0	9.3	1.20	2	49	6
12	0.0	<8.5	0.64	8	14	9
13	0.0	9.1	0.63	1	52	0

[a] Determined by North Dakota Grain Inspection Service.
[b] FFA = free fatty acids (as oleic).
[c] Colony-forming units per gram of seed.

TABLE 3. Characteristics of Sample grade sunflower seed

Sample number	Heat damaged[a] (%)	Moisture[a] (%)	FFA[b] (%)	cfu[c] (×1,000)	Seed yielding fungi (%)	Total isolates of *Aspergillus*
1	3.4	13.0	2.24	1,900	91	41
2	2.5	8.6	0.98	6	10	5
3	4.2	12.3	1.26	52	58	4
4	1.6	8.8	0.96	34	5	1
5	2.1	9.4	0.84	15	9	0
6	1.4	9.4	1.24	3	10	0
7	1.7	9.3	0.90	6	8	0
8	2.9	12.6	3.13	1,900	47	19
9	0.7	11.5	1.67	900	48	3
10	4.1	12.8	3.08	1,100	41	7
11	3.0	11.4	1.59	100	24	2
12	10.6	...	1.97	143	18	4
13	1.7	12.5	2.54	17	100	104
14	14.0	...	1.58	3	19	1
15	1.5	12.4	2.17	37	99	120
16	3.0	15.5	3.32	700	100	139
17	1.0	...	1.14	33	92	63
18	1.5	4.9	1.82	865	100	157
19	5.9	5.2	1.99	350	93	67
20	7.8	5.3	3.20	385	95	66
21	3.5	8.5	1.20	61	66	0

[a] Determined by North Dakota Grain Inspection Service.
[b] FFA = free fatty acids (as oleic).
[c] Colony-forming units per gram of seed.

(36.6 and 30.6%, respectively), but had FFA values within or just exceeding limits for No. 1 seed (1.97 and 1.58%, respectively). The recovery of fungi from these two samples was very low as well (18.0 and 19.0%, respectively).

Statistical analysis of SG sample data showed no adequate correlation ($r > 0.7$) of heat damage with any other single component of seed quality. R^2 values from the regression model for heat damage did not reach values high ($R^2 > 0.5$) enough to adequately explain heat damage variation until at least three other variables were introduced ($R^2 = 0.61$) for combined total damage, percent seed yielding fungi, and total number of isolates of *Aspergillus*. Variation in percent FFA, however, could be reasonably explained ($R^2 \geqslant 0.5$) when only two variables were introduced. When percent moisture and percent seed yielding fungi, or cfu and total number of isolates of *Aspergillus* or cfu and percent seed yielding fungi were introduced into the regression model for percent FFA, R^2 values of 0.51, 0.55, and 0.59, respectively, were obtained.

DISCUSSION

The growing number of requests by importers of U.S. sunflower oilseed for federal standards for oilseed quality has necessitated an examination of the criteria currently used to grade sunflower seed lots. Although heat damage is a major component of total damage and therefore has been important in the determination of seed grade, it is based upon a visual, nonquantitative evaluation of seed color and therefore may be subject to variability from inspector to inspector. Another disadvantage of heat damage is that the term by definition implies that the discoloration of the seed was caused by heat when in fact discoloration of seed meats has also been shown to occur after the seeds have been invaded by fungi (7,13,15). Stored seed also may become discolored from the heat caused by the growth of fungi (hot spots) when moisture levels are adequate for fungal growth. In this case, no distinction is needed between discoloration caused by fungal growth and that caused directly by heat; they are concurrent. The practice of artificially drying sunflower seed so that moisture levels meet grade requirements has confused the issue of heat damage and seed quality. The drying process can discolor the seed somewhat, and seed which otherwise would produce oil of high quality and be of low storage risk might be placed in a lower grade than appropriate. Samples SG 12 and SG 14, with 10.6, and 14.0% heat damage, respectively, were apparent victims of such an error because both had low FFA and low percent recovery of fungi. Heat damage as it is presently used, therefore, is an ambiguous term because it does not distinguish between discoloration caused by fungal growth without heating, fungal growth accompanied by heating, and overheating during the drying process.

The lack of heat damage values for grade No. 1 seed samples (0.0–0.4%) precluded any correlation of heat damage with other seed quality variables, but the inclusion of grade No. 1 samples in this study provides evidence of the inability of heat damage scores to consistently reflect seed quality. Three grade No. 1 samples (samples 5, 8, and 10) or about 23% of the samples, had 0.0% heat damage and moisture <10.0% but had FFA values that clearly should have excluded them from grade No. 1. This indicates that a certain degree of error can be incorporated into grading decisions when heat damage is included as a criterion.

Those who have requested that federal quality standards be developed for sunflower oilseed are concerned primarily with the quality of the extracted oil. Although discolored oil will result in some refining losses, the percent FFA in the extracted oil is by far a more serious concern because the removal of FFA from sunflower oil causes the greatest refining losses. The percent FFA of seed lots is, therefore, a more direct and quantitative estimation of seed quality than is heat damage at the time of sale. Percent FFA, used in conjunction with percent recovery of storage fungi and percent moisture as suggested by Christensen (10), would probably give a more accurate evaluation of both quality and storage risk than current evaluation standards. From a practical standpoint, the routine use of percent FFA to evaluate seed quality awaits the development of detection techniques that are less time consuming than the AOCS method currently employed, permitting the rapid estimation of percent FFA for large numbers of samples.

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Cytology and Histology

Structural Comparison of Xylem Occlusions in the Trunks of Citrus Trees with Blight and Other Decline Diseases

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ABSTRACT

Brlansky, R. H., Lee, R. F., and Collins, M. H. 1985. Structural comparison of xylem occlusions in the trunks of citrus trees with blight and other decline diseases. Phytopathology 75:145-150.

Xylem occlusions were compared in citrus trees with blight (cause unknown); tristeza (caused by citrus tristeza virus); psorosis A and concave gum (caused by graft-transmissible agents); stubborn (caused by *Spiroplasma citri*); Phytophthora foot rot; and citrus slump (caused by *Pratylenchus coffeae*). Filamentous plugs observed in trees with citrus blight were also present in the xylem vessels of trees affected with stubborn, citrus slump, tristeza, and foot rot as well as in many healthy trees. Amorphous plugs were more numerous in trees with blight than in healthy trees. A similar type of plug was observed by light microscopy in trees with psorosis and concave gum. However, these amorphous-like plugs in trees with psorosis and concave gum could be differentiated from those present in trees with citrus blight by scanning and transmission electron microscopy. Thus, amorphous plugs are characteristic of blight trees and can be used for diagnosis along with zinc accumulation and reduced water uptake if care is taken to separate them from the amorphous-like occlusions that form in plants affected by other diseases.

Additional key words: *Citrus paradisi*, *C. sinensis*, young tree decline.

Citrus blight is a decline disease of unknown etiology. Some other diseases produce canopy decline symptoms that often resemble those of blight. Therefore, blight cannot be diagnosed from canopy symptoms alone (1). Blight is characterized by reduced water conductivity of the trunk and root xylem (8,11,20), elevated zinc levels of the trunk wood (14,19), and the presence of occlusions in xylem vessels (4,6,8,10,15). Two types of occlusions or plugging materials described as filamentous or fibrous plugs (6,8) and amorphous or gum plugs (8,11) have been observed in the xylem of blight-affected trees. Filamentous plugs consist of a mass of fine fibers (0.2–0.7 μm in diameter) and are usually found at vessel end walls. Amorphous plugs appear to be solid and may completely block the vessel lumen.

Childs and Carlysle (6) reported the presence of filamentous plugs in the xylem of roots of blight-affected trees and concluded they were the mycelia of *Physoderma* sp. Vandermolen (15) found that the ultrastructure of the filamentous plugs in roots from blight-affected trees did not resemble that of fungi, actinomycetes, bacteria, mycoplasmas, or other organisms.

Nemec and Kopp (12) reported that lipid (filamentous) plugs were prevalent in healthy as well as blight-affected trees and that they appeared to be the likely obstruction to water movement. Childs (5) suggested that the blockage in the xylem of blight-affected trees was caused by fibrous plugs. Nemec et al (11) studied the two types of plugging materials in the xylem and found that the number of filamentous plugs did not differ in blight-affected and healthy trees but that a higher percentage of vessels in the roots of blight-affected trees contained amorphous and filamentous plugs. They concluded that amorphous plugs in the trunk wood of blight-affected trees appeared to be the main resistance to water movement in vessels along the cambium. In 1983, Cohen et al (8) studied the two types of occlusions and found that amorphous plugs were associated with a reduction in water conductivity in the trunk. Brlansky et al (3) showed that as the number of amorphous plugs in trunk wood increased, water uptake was reduced and the canopy decline progressed. No such association existed with the filamentous plugs.

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