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Research Article

Sorting of Fungal-damaged White Sorghum

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Abstract

A high-speed, color image-based sorting machine was modified to separate white sorghum with symptoms of fungal damage. Most of the sorghum tested was typically white, but over 27% of the bulk contained grains with fungal damage of various degrees, from severe to very slight. Grains with slight fungal damage were characterized as having several tiny black spots randomly spread across the pericarp surface. To identify small dark spots or blemishes, real-time spot detection algorithms were implemented on a field-programmable gate array (FPGA) directly linked to a color image sensor. Concurrently, grains with large amounts of fungal damage were identified using color histogram algorithms. With the FPGA communicating directly with the camera, image analysis speed was maximized by performing many operations in parallel, including inspection of up to four grains at any given time. Sorting tests indicated that after two passes through the sorter, over 90% of the grains with slight fungal damage and nearly 100% of the grains with large amounts of fungal damage were separated from the original bulk. The germination rates of the grains classified by the sorter as having fungal damage were about half of those that were accepted by the sorter as undamaged. The hardness of the grains accepted by the sorter was also 4% higher after sorting when compared with the original sample and the rejected grains. This sorting system can be used to improve the sorghum quality of food products and seed germination rates and might also be used for other grains or pulse crops for which seeds with localized spots need to be removed.

Keywords: Sorghum; Fungal damage; Sorting; High-speed

1. Introduction

Cultivated sorghum (Sorghum bicolor (L.) Moench) is widely grown in the Great Plains for feed, grain ethanol, and increasingly as a gluten-free food crop. However, because the grain head (panicle) of the sorghum plant is exposed to the environment, the grain (caryopsis) is susceptible to fungal colonization and infection. This can make the grain less desirable for use as a food ingredient and, in the case of non-hybrid lines, reduces germination rate if some of the crop is to be replanted. White-tan sorghum hybrids are more desirable as food-based products than high-tannin or red

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pericarp-colored sorghum varieties but are somewhat more susceptible to fungal infection (Harris and Burns, 1973; Esele et al., 1993; Fredrickson and Odvody, 2000). In previous testing, commercial high-speed sorting machines have been found to be somewhat effective in separating fungal-infected grains from wholesome "clean" grains, but their accuracy is limited (data not published). The most obvious impact of fungal infection on white-tan sorghum hybrids is discoloration; often resulting in grayish-black "weathered" grains, which when milled can lead to off-colored flour. Grain "weathering" occurs when fungi colonize the grain pericarp after physiological seed maturity, as indicated by the deposition of the black abscission layer (Eastin et al., 1973). Fungal infection can cause a breakdown of the grain endosperm, resulting in changes to the chemical and physical properties of the grain and a decrease in processing quality (Waniska et al., 1992). Grains exhibiting greater amounts of fungal damage are also more likely to be contaminated with mycotoxins or to develop off odors, making such grains unsuitable for use in human food applications (Leslie et al., 2005; Sauer et al., 1978; Seitz et al., 1983; Williams et al., 1986).

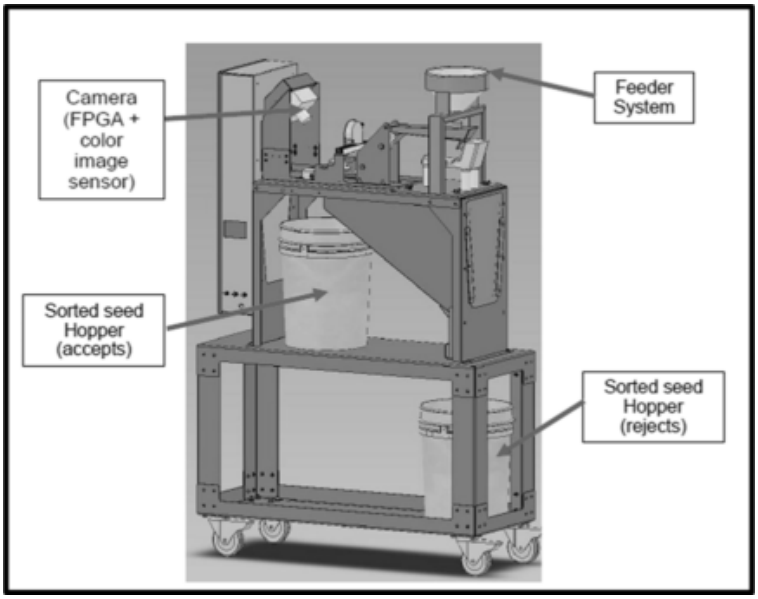

Fig 1. Overview of the sorting system.

A recently developed high-speed sorting machine (Pearson, 2010) performs the separation of grains based on color images of every grain, using color image processing on a field-programmable gate array (FPGA) directly linked to a color image sensor (Figure 1). The FPGA is able to perform many operations in parallel, which permits real-time image processing based inspection of up to four separate sorting streams with one image sensor/FPGA module. Compared with mono- or bi-chromatic high-throughput sorters, this machine has been demonstrated to separate grains more accurately based on color and, with additional programming, can be used to separate grains based on several localized dark spots on the grain surface, a characteristic of many fungal-infected sorghum grains (Figure 2b). The spots on sorghum grains induced by fungal infection do not greatly affect the overall color of the grains and are difficult, if not impossible, to sort on the basis of color by standard commercial color sorters, which have very low spatial resolution. However, by counting the number of times the color substantially changes across a grain, an accurate discrimination can be made between wholesome grains and those with several localized

discolorations and can identify grains with more severe discolorations due to fungal damage, such as those shown in Figure 2c and 2d.

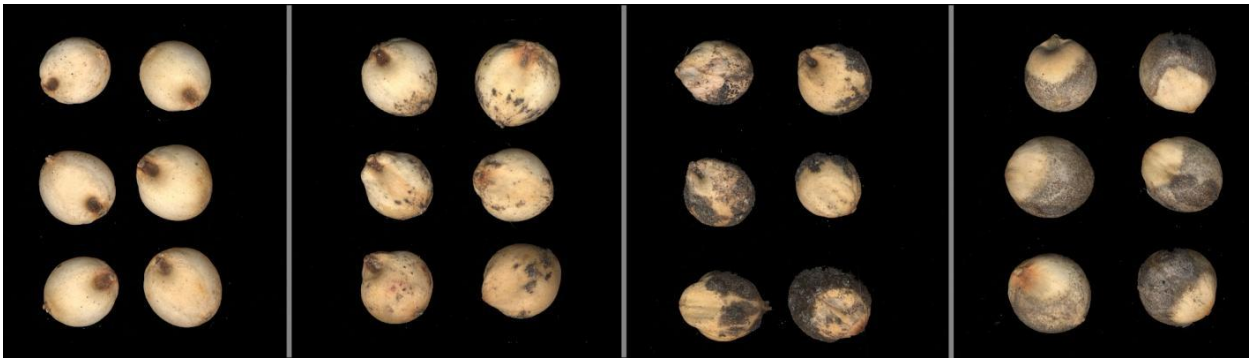

Fig 2. Images of non-infected sorghum and fungal-damaged sorghum grains: (a) non-infected and symptomless, (b) scattered tiny black spots, (c) irregular black blotches, (d) diffuse grayish-black growth (left to right).

The objective of this study was to modify the image processing software of the sorter described by Pearson (2010) so that the number of significant color transitions across a sorghum grain can be counted. This data can be combined with global color features, and all fungal-infected sorghum grains can be separated from non-infected sorghum grains in real time. After sorting, the accepted and rejected fractions were evaluated for physical properties, germination, and fungal species identification to help quantify the differences between the accepted and rejected grains and the benefits of sorting.

2. Materials and Methods

2.1 Samples

White sorghum grain of the Macia variety, grown commercially at three locations (approximately 10 km apart) near Scott City, Kansas and harvested in October 2007 were used in this study. In 2007, late-season rains occurred after the sorghum was mature and delayed harvest. These conditions favored fungal colonization of the sorghum grain heads (Seitz et al., 1983). Samples from each location had similar appearance and approximately 25 to 30% of the grain showed symptoms of fungal-related grain discoloration and deterioration, referred to as "grain weathering." Most of the fungal-damaged grains were characterized as having tiny black spots, while a smaller but significant portion had more obvious symptoms such as irregular black blotches and diffuse grayish-black fungal growth on pericarp surfaces not covered by glumes (Figure 2). From each location, 10-kg samples were collected and blended to form one 30-kg sample. The grain was then transferred to Manhattan, KS in April 2008 and stored in a freezer (at -10°C) until sorting tests were performed in 2011.

2.2 Image software

The image software used in the sorter, described in (Pearson, 2010), segments the grain portion of the image from the background and computes averages and produces histograms of the red, green, and blue pixels. Additionally, for every pixel, the processor subtracts the red from the green value

(R-G) and the green from the blue value (G-B) and produces histograms of these two differences. A total of 83 color features are computed from each grain, as each histogram contains 16 bins plus the averages of the red, green, and blue values. The sorter is calibrated by collecting data from 200 symptomless grains and 200 fungal-infected and discolored grains that should be removed from the product stream. From these data, the camera software uses a discriminant analysis routine to determine the best combination of the three color averages or histogram bin value features to use for sorting. To add spot detection to the camera software, images of 2,000 non-infested sorghum grains and 2,000 fungal-infected and discolored grains were acquired and saved by the sorter camera and transferred to a personal computer for off-line processing.

For spot detection, a simple algorithm was developed to count the number of times that the intensity of the green pixels changed from a fairly high level, representing areas of the non-molded white pericarp surface, to a lower level, representing a spot, possibly caused by fungal infection. This was performed for all horizontal lines. An example of how the algorithm works is shown in Figure 3, in which the pixel intensity values across a fungal-infected and a non-infected grain are shown for one line, located by the yellow line across the grains. For the fungal-infected grain, there are several dips, or valleys, in the profile plot where the line crosses dark spots, indicating fungal infection. Conversely, the profile plot across a non-infested grain is fairly smooth.

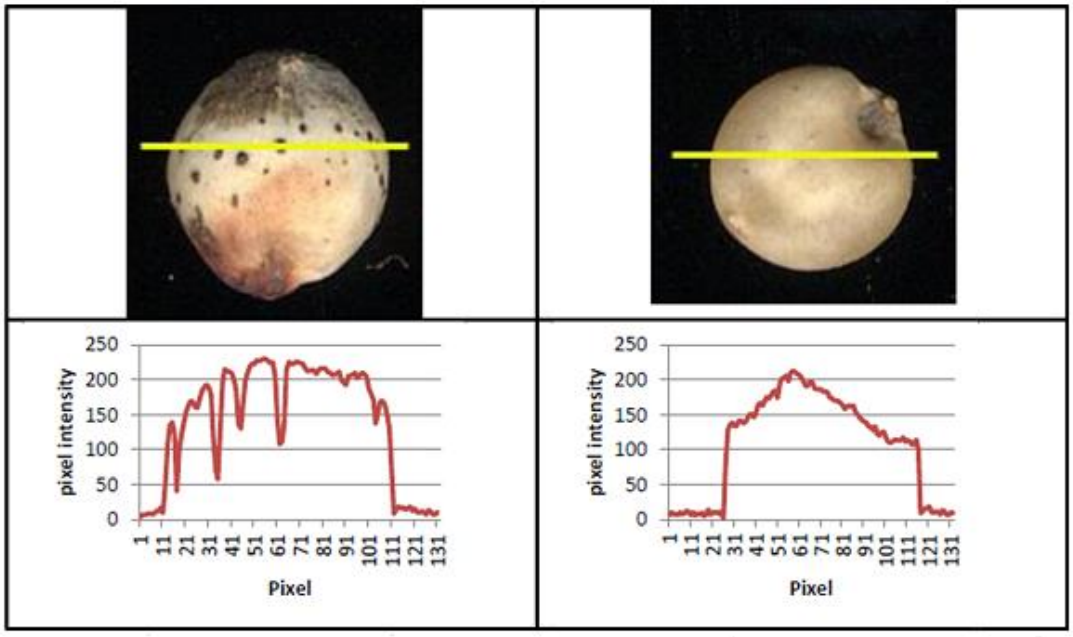

Fig 3. The spot identification algorithms use pixel intensity profile plots. Shown are example images and plots of fungal-infected (left) and non-infected (right) sorghum grains. Note how the dark spots on the infected grain cause the pixel intensity to drop sharply, whereas the non-infested grain profile plot is fairly smooth.

To count the number of valleys or dips in the profile plots, let xp be the green intensity value of a pixel at point p and let xp-n and xp+n be the green intensity of a pixel that are n pixels to the left (-n) or to the right (+n), respectively, of point p. Any pixel for which xp has an intensity value that is

a preset value (L) less than both xp-n and xp+n was considered a point that had enough of an intensity difference that it was counted as a potential spot. In the data set of 4,000 images, five different values for L (10, 25, 50, 75, and 100 intensity levels) and five different values of n (3, 6, 9, 12, 15 pixels) were investigated for all possible combinations of L and n. Thus, 25 different combinations of L and n were computed. The hilum scar at the tip of one end of a sorghum grain is normal but can also be counted as a dark spot. However, these are generally ignored by the algorithm, as one or two sides are often very close to the dark background and therefore not counted as they do not represent a sharp drop on both sides of the scar. The 83 color features that the camera normally extracts from each image were combined with the 25 counts of pixels considered to be part of a spot using different combinations of L and n. The features used for sorting were the combination of three features that yielded the best classification accuracy, based on an exhaustive feature search using linear discriminant analysis. Data means and variances for feature selection were computed for half of the 4,000 original grain images, randomly selected, while classification accuracy was computed for the other half of the data.

2.3 Sorting

Implementation of the spot detection algorithm for real time sorting was accomplished by modifying the camera FPGA software using the Verilog HDL programming language and compiled using software provided by the FPGA manufacturer (Quartus II v9.1 sp1, Altera Corporation, San Jose, CA). The spot detection method was added so that these features were computed in parallel with the color features so that the rate of inspection was not reduced. Sorting was performed on the entire 30 kg of sorghum run through the sorter at a rate of approximately 30 kg/hr, per the recommendation of the sorting machine manufacturer (National Mfg, Lincoln, NE). All grains were sorted twice, producing three fractions: 1) accepted twice, 2) accepted once–rejected once, and 3) rejected twice. After sorting, each fraction was weighed and stored in a freezer at -10°C until fungal evaluations, sprouting tests, and physical property tests were performed, as described below. After sorting, 200-g sub-samples were removed, and each grain was categorized into one of four symptom categories: mostly symptomless, scattered tiny black spots, large black blotches, or diffuse grayish-black growth. The diffuse grayish-black growth category was characterized as a uniform fungal colonization/discoloration of $\geqslant$ 30% of the pericarp surface protruding above the glumes. Examples of each symptom are shown in Figure 2.

2.4 Physical tests

Single-grain hardness, weight, and diameter were measured for 300 single-grain samples using a modified Single Kernel Characterization System (SKCS 4100, Perten Instruments, Springfield, IL). Random samples from the three sort streams were analyzed in the SKCS system. Duplicate runs were performed.

2.5 Fungal evaluations

Samples of accepts and rejects after just one pass through the sorter were evaluated for fungal identification. Two sets of 100 randomly selected grains from each stream were surface-disinfected in a 1% sodium hypochlorite solution for 2 min, washed twice in sterile water, plated on 3% Difco malt extract agar (Becton, Dickinson & Co., Franklin Lakes, NJ) and incubated for seven days at 25°C. Fungi growing from the individual grains were identified, and the frequency (%) of grain colonization was calculated. It was noted if no fungi grew from individual grains. A record was also made of the numbers of grains that had germinated on the agar after 48 h, 72 h, and 96 h. Isolations

of representative fungal cultures were deposited with the ARS Culture Collection (NRRL) of the National Center for Agricultural Utilization Research in Peoria, IL.

2.6 Wet towel germination test

Seed germination (%) was also evaluated for both sorter accepts and rejects following the first sort by distributing 20 randomly selected seeds on each of 5 rolled paper towels (=100 seeds for each sort stream). The towels were moistened with sterile water until thoroughly damp and wrapped in aluminum foil to retain moisture prior to incubation for 4 days (96 h) at 25° C. In this trial, all of the seeds that germinated produced roots and shoots measuring ⩾ 1–2 cm. All germinated seeds were removed from the towels after 4 days (96 h), the towels were rolled up again, and the seeds that had not germinated were re-incubated for an additional 7 days to determine whether any further germination occurred. However, no further germination was recorded after 96 h.

3. Results and Discussion

3.1 Imaging algorithm

The best feature for distinguishing fungal-infected from non-infected grains was the count of pixels belonging to a spot with L=25 and n=9. This feature is used to identify most of the kernels with small spots and these comprised the majority of fungal damaged grains. The second-best feature selected by the feature selection procedure was the count of pixels for which G-B was at least 10. The third-best feature selected was the average of the red pixel intensities. These later two features are global color features which help detect grains with larger discolorations. The more bluish-gray-discolored grains had lower G-B counts and lower average red values. The classification accuracy achieved using these three features on the 2,000-grain validation set was 98% for non-infested grains and 96% for fungal-infected grains.

To compare this classification accuracy to what might be accomplished with a mono- or bi-chromatic commercial color sorter using one or two visible light spectral bands, the feature selection was run again using features that only included histograms of red, green, or blue pixels, as these are the only types of features that commercial color sorters are able to compute. Limiting the feature selection to one such feature, simulating a mono-chromatic sorter, results in a classification accuracy of 80% for non-infected grains and 77% for fungal-infected grains. Using two features, as a bi-chromatic sorter would, the classification accuracy improves to 96% and 83% for non- and fungal-infected grains, respectively. These results indicate the sorting performance that can be achieved using mono-chromatic and bi-chromatic sorting machines and are consistent with the findings of previous studies conducted using commercial machines to sort fungal-damaged sorghum (data not published). Using only one or two color histogram features causes most of the grains with tiny black spots to be incorrectly classified. This is to be expected, as these features are only a measure of the overall color of the grain, and the tiny black spots do not contribute enough to the overall color to be detected by color histograms alone.

3.2 Sorting Results

Figure 4 displays an image of grains from each of the three sorting fractions. Table 1 lists the percentages of grains from the original sample that were sorted into the three sorting streams for each of the four symptom categories. Overall, 89.5% of the symptomless grains remained in the

"accept twice" stream, while no grains with irregular black blotches or diffuse grayish-black discolorations were found in the "accept twice" stream. Most of the grains with irregular black blotches (82.8%) or diffuse grayish-black growth (90.3%) were found in the "reject twice" stream. In two sorts, the sorter removed 90.2% of the grains with small spots from the symptomless grains, with 68.5% in the "accept once–reject once" stream and 21.7% in the "reject twice" stream. Many of the dark spots on grains are only visible from one side, so it is not surprising that removal of many of the grains with spots requires at least two passes through the sorter. These results indicate that the more visibly damaged grains, those with large black blotches or diffuse grayish-black discolorations, could be removed in one pass through the sorter.

Fig 4. Images of each sorting fraction, (left) accept twice; (middle) accept once–reject once; (right) reject twice.

Table 1 Sorting results show the percentage of grains from the original sample in each sorting stream and each symptom category

Symptom category	original		accept twice		reject once–accept once		reject twice	
	weight (kg)	wt% per symptom	weight (kg)	wt% per symptom	weight (kg)	wt% per symptom	weight (kg)	wt% per symptom
1. Symptomless	21.7	72.40%	19.4	89.50%	2.2	10.10%	0.1	0.40%
2. Scattered tiny black spots	6.2	20.50%	0.6	9.80%	4.2	68.50%	1.3	21.70%
3. Irregular black blotches	1.1	3.80%	0	0.00%	0.2	17.20%	0.9	82.80%
4. Diffuse grayish-black growth	0.9	2.90%	0	0.00%	0.1	9.70%	0.8	90.30%
Total weight distribution	29.9	100%	20	67.20%	6.7	22.40%	3.2	10.50%

3.3 Physical properties

Grains from the sorter reject streams were found to have lower hardness and slightly lower weights and diameters. Grains accepted twice had an average hardness index of 80.7, while the grains accepted once and rejected once and the grains rejected twice had average hardness indices of 71.6 and 69.4. These results indicate that the fungal infections affected the endosperm of the grains and reduced their hardness. Grain hardness in sorghum has been related to quality in several food products. Grain hardness has been found to influence the cooking quality of pearled sorghum (Cagampang and Kirleis 1984), sorghum porridge (Rooney et al., 1986), couscous quality (Aboubacar and Hamaker 1999) and sorghum bread quality (Schober et al., 2005). Grain hardness has also been reported to play a role in the milling quality of sorghum (Maxson et al., 1971, Munck et al., 1981, Munck 1995, Rooney and Waniska 2000) and is related to mold susceptibility and resistance in sorghum breeding lines (Jambunathan et al., 1992; Menkir et al., 1996b).

3.4 Fungal and germination evaluations

Alternaria alternata, represented by isolates NRRL 54805 and NRRL 54806, was the most prevalent fungal colonist recorded from both the sorter accepts (45%) and sorter rejects (59%). The European Food Safety Authority (2011) reviewed the safety of Alternaria toxins in food and feed and reported having insufficient information on the toxic effects of Alternaria toxins on farm animals to assess the health risks to different species. For the genotoxic Alternaria toxins, alternariol and alternariol monomethyl ether, the estimated chronic dietary exposure of humans exceeds their threshold of toxicological concern, indicating a need for additional toxicity data. Other fungi were recorded as minor colonists for 5% of grains or less, including the following: Aureobasidium pullulans NRRL 62715; Chaetomium globosum NRRL 54813; Cladosporium cladosporioides NRRL 54811; Cochliobolus sativus NRRL 62716; Epicoccum nigrum NRRL 54809 and NRRL 54810; Fusarium thapsinum NRRL 54812; Fusarium verticillioides NRRL 62720; Phoma glomerata NRRL 54807 and NRRL 62718; Phoma sorghina NRRL 54808; and Spegazzinia tessarthra NRRL 62724 and NRRL 62725. No fungi grew from 54% of the grains from sorter accepts and 38% of the grains from sorter rejects, including kernels exhibiting symptoms of fungal discoloration. It is possible that the surface disinfection with sodium hypochlorite solution may have eliminated some superficial fungal growth on the pericarp surface (Prom, 2004). Furthermore, it is well documented that Alternaria, Bipolaris, Cladosporium, Curvularia and other so-called “field fungi” can rapidly lose their viability in grain storage (Christensen, 1957).

Evidence of seed viability and seedling vigor was also noted while the seeds were incubating on the agar plates. After 48 h of incubation on malt extract agar, the sorter-rejected grains had a germination rate of 25.5%, approximately one third of the rate for the accepted grains, for which the germination rate was 74%. The results of the wet towel germination method also exhibited considerable differences between the accept and reject streams. The germination rate for the rejected seeds (42%) was approximately half of the rate for the accepted seeds (79%). Thus, sorting might be used to increase germination rates if the sorghum are used as seed and can also serve as a means of evaluating mold-susceptible and mold-resistant sorghum genotypes (Bandyopadhyay and Mughogho, 1988; Menkir et al., 1996a; Menkir et al., 1996b). While these results indicate the substantial improvement in the germination rate using this sorting method, analysis of a larger and more diverse sample set of sorghum is needed. A larger study could establish the means and standard deviations of germination rate improvements achieved through

sorting, and decisions based on these statistics could be made to determine whether this method is economically advisable.

4. Conclusion

The sorter appeared to separate nearly all of the symptomless grains from those that were obviously fungal-infected and damaged (those with irregular black blotches or diffuse grayish-black growth). In two passes through the sorter, over 90% of the sorghum grains with tiny black spots were separated from symptomless grains, with an error rate for the symptomless grains of 10 approximately 10%. While commercial sorters could remove most of the grains with obvious fungal damage, they are not likely to be nearly as effective in separating out the grains with tiny black spots as the image-based sorter used in this study. This sorter could be used to remove fungal-infected sorghum grains from food processing lines and might be used for sorting of other commodity grains and seeds with small spots and/or more global discolorations. The results of this study show that this sorter may be able to substantially improve germination rates for non-hybrid seeds; however, more research is needed to fully assess the capability of the sorter for this purpose.

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