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Use of Optical Sorting to Detect Karnal Bunt-Infected Wheat Kernels

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Abstract**.** A high-speed sorter was used to remove kernels infected with Karnal bunt from 1800g wheat samples. When the sorter removed about 8% or more of the sample, the reject portion contained 100% of the bunted kernels. Concentrating the bunted kernels in a smaller sample size will reduce sample inspection time and should reduce inspection errors. One high-speed sorter can process up to 8800 kg/hr, thus bunted kernels can be rapidly removed from samples or large lots. The instrument sorted each sample in less than 1 minute. This technology provides the wheat industry with a tool to rapidly inspect samples to aid in regulating Karnal bunt, and to remove bunted grains from seed wheat and wheat destined for food or feed use.

Keywords. electric eyes, inspection, sampling error

Introduction

Karnal bunt (Kb) in wheat (Triticum aestivum L.) is caused by the smut fungus Tilletia indica Mitra [=Neovossia indica (Mitra) Mundkur]. The fungus survives in the soil, and primary and secondary sporidia from germinating teliospores can infect developing wheat kernels at the time of heading if environmental conditions are suitable. The teliospores produced in infected kernels result in a black sorus that can be confined to a small portion of the germ end of the kernel, or that can progress to attack the entire kernel. The disease can be spread by the wind, contaminated equipment, straw, chaff, soil, seed, or animal feces (7, 8). The fungus generally causes yield losses of 1% or less, but flour with more than 3% bunted kernels can be unfit for human consumption because of the unpleasant, fishy odor associated with the fungus (5). T. indica is subject to international regulation by 78 countries, and U.S. economic costs from market loss could exceed $1 billion if major portions of the U.S. wheat supply were to be affected. In 1996, Kb was found in durum wheat in Arizona and in hard red spring wheat in California. In 1997, Kb was found in hard red winter (HRW) wheat in San Saba County, Texas. Kb was found in HRW wheat in Young, Throckmorton, Baylor, and Archer counties in Texas in 2001. As a result of these findings, the Animal Plant Health Inspection Service (APHIS) implemented quarantines, survey methods, and containment programs to protect wheat producers in bunt-free areas and to protect export markets.

The surveys implemented by APHIS include a national survey for all wheat production areas where Kb has not previously been found, and a survey of fields in regulated areas. The national survey is conducted by collecting 2500g wheat samples from each county at points of first aggregation such as local elevators, and examining a portion of the sample for the presence of teliospores using a size-selective sieving method. If teliospores are found, the sample is further examined for the presence of bunted kernels. Each sample theoretically represents up to 1 million bushels of wheat. For field surveys in regulated production areas, 1800g grain samples are taken from the combine at harvest, from wheat hay, or from seed lots. All kernels are visually inspected for the presence of bunted kernels. If a suspect kernel is found, the teliospores are examined to confirm the presence of Kb. Samples must be inspected before combines can be moved from a suspect field, and before hay or seed can be shipped (V. Malik, Unpublished)

A field survey sample requires up to 1 hour for an inspector to visually examine all kernels. This labor-intensive process can delay harvesting, and some infected kernels may be missed due to inspector fatigue or if the infected portion of the kernel is not oriented towards the inspector.

High-speed sorters have commonly been used in other commodities such as with peanuts to removed discolored seeds (1). Dowell et al. (3) showed that common bunt (Tilletia tritici and T. laevis) could be detected in single kernels with greater than 93%

accuracy using optical sensors, including kernels with low levels of infection. To reduce the sample processing time and the chance of missing bunted kernels, we examined the potential of high-speed optical sorting technology for removing bunted kernels from large samples and concentrating the kernels into a smaller sample for subsequent visual inspection.

Materials and Methods

Sorter Specifications

A ScanMaster II SM100IE (Satake USA Inc, Houston, Texas) sorter was used in all tests. The tests occurred in Olney, Young County, Texas, which is within the Kb quarantine area. The sorter has 10 parallel channels that singulate kernels before each is viewed from two sides by a high-resolution CCD camera filtered at 675 nm. The filter maximizes the color difference between asymptomatic and bunt-infected kernels. The CCD camera can detect a defect as small as 0.3 mm diameter. If a kernel defect is detected, the kernel is sorted into a “reject” bin by an ejector air blast. Other kernels pass through the sorter into an “accept” bin.

Inspection and sorting with this instrument can occur at a rate as high as 10,000 kernels per second, or about 1100 kg/hr (40 bu/hr). The feed rate was set to about 200 kg/hr for these tests. A full-size instrument typically has 80 channels and, therefore, can inspect and sort 80,000 kernels/s (8800 kg/hr). Sorting accuracy and amount of product ejected can be affected by a “dark trip” setting that determines the degree of kernel discoloration that must be exceeded before it is ejected.

Wheat Samples

HRW wheat samples (1800 g) that were known to be Kb-free (El Dorado variety) were spiked with 1, 3, or 10 bunted kernels (Table 1). The bunted kernels were collected from crop year 2001 samples obtained from wheat in storage at Olney, Texas. Bunted kernels were classified as tip infected (the fungus was only present in a small amount on the germ-end), typical bunt (the fungus affected about 30-50% of each kernel), or “canoes” (the fungus affected the majority of the ventral surface and kernel endosperm and most of the spores were gone, leaving a hollowed-out kernel that resembled a canoe). Bunted kernels were treated with an invisible dye (UV 50, Theatre Effects, Hagerstown, Md.) to facilitate rapid evaluation of the sorter efficiency. The invisible dye fluoresces under a black light (380 nm).

Dye Evaluations

To determine if the invisible dye affects sorting, 10 asymptomatic kernels and 10 kernels with Kb obtained from the same sample were treated with the dye and mixed into three samples known to be bunt-free. This was repeated for three samples. Evaluation of rejects and accepts after sorting would reveal if the dye treatment was affecting sorting efficiency. This test also indicated if the samples the bunted kernels originated from had different color characteristics than the bunt-free samples.

To further test whether the bunt samples had a color characteristic different from the bunt-free samples, eight bunted kernels that had not been dyed were spiked into two HRW bunt-free samples. The samples were run through the sorter and inspected for bunted kernels.

Test Procedures

For each test, we cleaned the sorter to insure no kernels were left from previous runs, spiked a bunt-free 1800g sample with a known number of bunted kernels, recorded the initial sample weight, recorded the dark trip level, ran the sample through the sorter, weighed the accepts and rejects, and examined the rejected seeds under a black light for bunted kernels. If the rejected seed did not contain all bunted kernels, the accepts were run through the sorter again and any rejects were examined. If all bunted kernels were not found after the second pass, then the accepts were examined until all bunted kernels were found. The trip rate was varied to eject from about 0.2% to 12% of the original sample weight.

Results

Dye and Spike Evaluations

Evaluation of the rejected seeds from samples containing dyed asymptomatic and bunted kernels showed that 3% of the asymptomatic kernels and 90% of the bunted kernels were rejected when using a setting that rejected about 5% of the sample weight. Thus, the fluorescent dye did not change the appearance of the kernels in the wavelength range used by the sorter and did not increase the likelihood that a kernel would be rejected. In one set of tests using bunted kernels that had not been treated with dye, all bunted kernels were rejected when using a setting that rejected 0.2% of the sample weight.

Sorter Performance

Table 1 and Figure 1 show that the sorter removed 100% of bunted kernels when 8% or greater of the sample was rejected. At a confidence limit of 95%, the minimum likely true proportion of bunted kernels that can be expected to be recovered at this reject rate is about 99.0%. Even when as little as 0.2% of the sample was rejected, greater than 70% of the bunted kernels were contained in the reject portion. When a bunted kernel was missed in the first sorting attempt, which occurred in eight of 99 samples, resorting the accepted portion resulted in recovery of all bunted kernels in six of those samples. Thus, a two-pass sort increases the chance of removing all bunted kernels at low sample rejection rates.

Tip-infected kernels were more likely to be missed than other types of bunted kernels, which was expected since some tip-infected kernels had an area of infection that only slightly exceeded the resolution of the sorter. The sorter functioned with no mechanical failures, and the typical time required for the sorter to remove kernels from an 1800g sample was about 45 s.

Discussion

The sorter was able to rapidly (~ 1 minute) reduce the portion of kernels that must be visually inspected to about 8-12% of the original sample weight without failing to remove any bunted kernels. Since the sorter removes kernels based on their optical properties, kernels exhibiting a discoloration such as black point or common bunt, which is similar in appearance to Kb, are ~~also~~ contained in the reject portion. Also, the air blast used to eject bunted kernels typically removes one or more kernels immediately adjacent to the discolored kernels. Thus, the reject portion will contain bunted kernels, kernels with similar discolorations, and asymptomatic kernels. Although the reject portion contains kernels with Kb in addition to other kernels, reducing the portion that must be visually inspected to as little as 8% of the original sample size should reduce error caused by inspector fatigue and subjectivity. Significantly reducing the portion that must be inspected can allow the original sample size to be increased but still reduce the current inspection time.

The total error associated with detecting Kb is a function of sampling error and measurement error. Previous research on detecting aflatoxin, fumonisin, and damaged kernels in corn, cottonseed, and peanuts shows that sampling error can account for 40-93% of the total error in measuring grain attributes, and sampling error can be 2-90 times greater than measurement error (2, 4, 9, 10, 11, 12). Reducing measurement error can be achieved by replacing the visual detection method used in ~~the~~ field surveys with the size-selective sieving method used in the national survey, or with a chemical means of detecting spores such as with enzyme-linked immuno-sorbent assay (ELISA) tests that are currently under development. However, these two methods can require at least 1 hour to complete, and the spores detected may not have come from bunted grains in that sample.

Increasing sample size may significantly reduce total detection error. For example, doubling the current sample size obtained from the field from 1800g to 3600g can reduce the sampling variance by 50% (2). Thus, with rapid sorting technology, a larger sample can be run through the sorter and inspected for Kb in much less time and with less error than with the non-sorted procedures. This sorter could also be used to remove bunted kernels from large seed wheat lots, or lots destined for mills or export. If bunted kernels are removed from large lots, it may be possible to kill any remaining spores in the wheat with a seed treatment such as propionic acid (6) to allow the wheat to be used for seed, feed, or food purposes. Additional research is needed to optimize sorter settings for other wheat classes and varieties. Also, reductions in total error achieved with this technology need to be quantified.

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Table 1. Results from removing T. indica infected seed from samples using a high-speed sorter. Samples were ca.1800 g.

No. bunted kernels in sample	No. samples	Bunted kernel type[1]	Avg. portion of sample rejected (%)	Std. dev. of rejects	Avg. no. bunted kernels detected
1	10	Tip	12.0	0.28	100%
1	9	Typical	11.2	0.38	100%
1	10	Canoe	10.1	0.52	100%
3	9	Tip	13.2	0.33	100%
3	7	Typical	12.8	0.40	100%
3	9	Canoe	13.1	0.82	100%
10	3	Tip	13.2	0.18	100%
10	3	Typical	13.0	0.28	100%
10	3	Canoe	12.5	0.47	100%
10	3	Tip	8.1	0.04	100%
10	3	Typical	8.0	0.18	100%
10	3	Canoe	8.1	0.19	100%
10	3	Tip	5.3	0.13	96.7%
10	3	Typical	5.2	0.08	100%
10	3	Canoe	5.4	0.04	100%
10	3	Tip	0.60	0.06	96.7%
10	3	Typical	0.57	0.09	100%
10	3	Canoe	0.51	0.03	86.7%
10	3	Tip	0.24	0.02	83.3%
10	3	Typical	0.22	0.01	93.3
10	3	Canoe	0.24	0.01	100%

[1]Tip = The fungus was only present in a small amount on the germ-end; Typical = The fungus affected about 30-50% of each kernel; Canoe = The fungus affected the endosperm and ventral side of the kernel, leaving only the outer portion of the kernel which resembled a canoe.

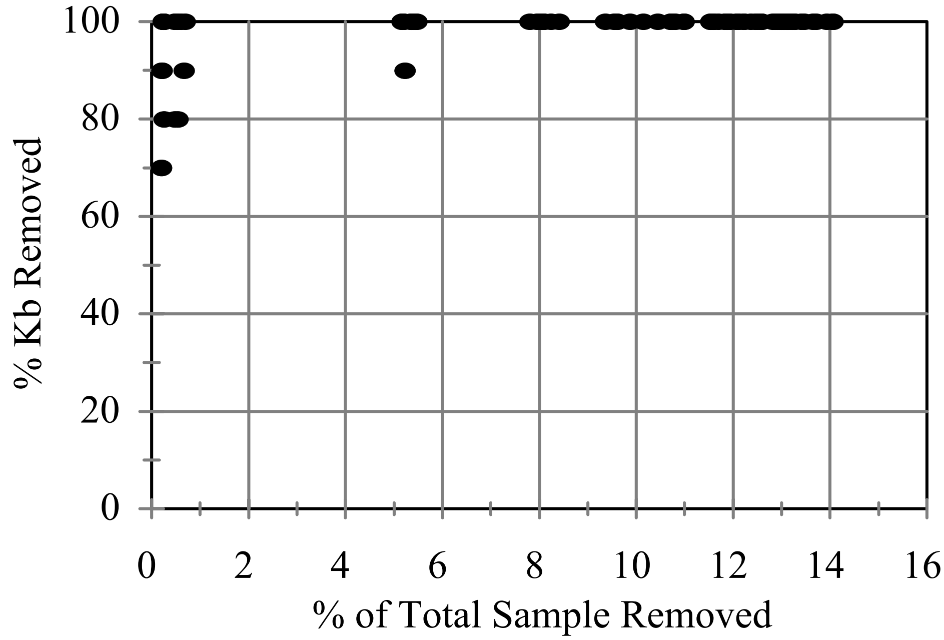

Figure 1. Percentage of kernels infected with Karnal bunt (Kb) removed by a high-speed sorter.