Use of Optical Sorting to Detect Wheat Kernels Infected with *Tilletia indica*

Floyd E. Dowell, Research Leader, United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Grain Marketing and Production Research Center, Manhattan, KS 66503; **Theodore N. Boratynski,** Project Coordinator-Mexico, USDA Animal Plant Health Inspection Service (APHIS) PPQ, Brawley, CA 92227; **Ronald E. Ykema,** Plant Pathologist, Arizona Department of Agriculture, State Agricultural Laboratory, Phoenix 85009; **Alan K. Dowdy,** National Science Program Leader, Agriculture Quarantine Inspection and Port Technology, USDA APHIS PPQ, Raleigh, NC 27606-5202; and **Robert T. Staten,** Laboratory Director, USDA APHIS PPQ, Phoenix, AZ 85040

ABSTRACT

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Wheat infected with *Tilletia indica* is subject to international regulation by 78 countries, and U.S. economic losses could exceed $1 billion if *T. indica* was found throughout major wheat-producing regions and caused wheat exports to be halted. Samples are currently manually inspected for the presence of kernels with Karnal bunt as part of routine survey methods. This visual inspection of all seed in a sample can result in harvest delays due to long inspection times and missed kernels due to inspector fatigue. A high-speed sorter was tested to determine if infected kernels could be rapidly removed from 1,800-g 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 8,800 kg/h; thus, bunted kernels can be rapidly removed from samples or large lots. Each sample was sorted in less than 1 min. 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.

Additional keywords: electric eyes, inspection, sampling error

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 as teliospores, 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. Wind, contaminated equipment, straw, chaff, soil, seed, or animal feces (1,8,10) can spread the disease. The fungus generally causes yield losses of less than 1%, but flour milled from infected grain with more than 3% bunted kernels can be unfit for human consumption because of the unpleasant, fishy odor associated with the fungus (6). There are no toxic affects to either humans or animals associated with Kb. However, *T. indica* is regulated internationally by 78 countries, and U.S. economic losses could exceed $1 billion if *T. indica* was found throughout major wheat-producing regions and caused wheat exports to be halted. 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, TX. 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) in cooperation with state Departments of Agriculture, implemented quarantines, surveys, 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 of all wheat-production areas where Kb has not previously been found, and field surveys in regulated areas where Kb has previously been found. The national survey is conducted by collecting 2,500-g 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. One sample is taken for every million bushels of grain production in a county. For field surveys in regulated production areas, 1,800-g 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, an examination for teliospores is conducted to confirm the presence of Kb. Samples must be inspected before combines can move from a suspect field, and before shipping hay or seed.

A field survey sample requires up to 1 h 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 toward the inspector.

High-speed sorters commonly have been used with peanut to removed discolored seed (2). Dowell et al. (4) showed that common bunt (*T. tritici* and *T. laevis*) could be detected in single kernels, including kernels with low levels of infection, with greater than 93% accuracy using optical sensors. 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 kernels with Kb 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, TX) sorter was used in all tests. The tests were conducted in Olney, Young County, TX, 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 with a 675-nm filter. The filter maximizes the color difference between asymptomatic and kernels with Kb. The CCD camera can detect a defect as small as 0.3 mm in diameter. If a kernel defect is detected, the kernel is sorted into a "reject" bin by an ejector air blast. Other kernels

Corresponding author: F. E. Dowell
E-mail: fdowell@gmprc.ksu.edu

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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/s, or about 1,100 kg/h (40 bu/h). The feed rate was set to about 200 kg/h for these tests. 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. In all, 1, 3, or 10 bunted kernels were added to each of 92 HRW wheat samples (1,800 g) that were known to be Kb-free (cv. El Dorado; Table 1). The bunted kernels were collected from crop year 2001 samples obtained from wheat in storage at Olney, TX. Kernels with Kb 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 to 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). Kernels with Kb 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).

Table 1. Efficiency of a high-speed sorter in rejecting seed infected with *Tilletia indica* from samples (1,800 g) of wheat

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

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

[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to least significant difference.

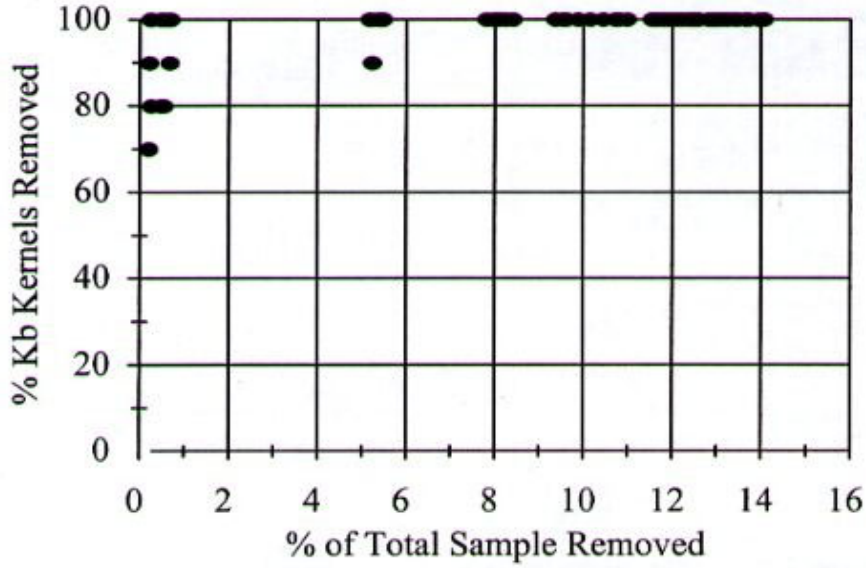

Fig. 1. Percentage of kernels with Karnal bunt (Kb) removed from each of 99 samples by a high-speed sorter.

Dye evaluations. To determine if the invisible dye affects sorting, 10 asymptomatic kernels and 10 kernels with Kb were treated with the dye and mixed into three 1,800-g samples known to be bunt free. Evaluation of rejects and accepts after sorting would reveal if the dye treatment affected the sorting efficiency.

Test procedures. For each test, we cleaned the sorter to insure that no kernels were left from previous runs, added a known number of bunted kernels to a bunt-free 1,800-g sample, 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 seed under a black light for bunted kernels. If the rejected seed did not contain all bunted kernels, accepted kernels were run through the sorter again and any rejects were examined. If all bunted kernels were not found after the second pass, then the accepted kernels were examined until all bunted kernels were found. The trip rate was varied to eject from about 0.2 to 13% of the original sample weight (Table 1). From the original reject rate of about 13%, subsequent reject rates were set to reject 50 to 90% less material than the previous setting. Means of the number of bunted kernels rejected for each trial were separated using least significant difference ($P \leq 0.05$) (9).

RESULTS

Dye evaluations. Evaluation of the rejected seed from individual samples containing dyed asymptomatic and dyed bunted kernels showed that an average of 3% of the asymptomatic dyed kernels and 90% of the bunted kernels were rejected when using a setting that rejected about 5% of the sample weight. We expected about 5% of the dyed asymptomatic kernels to be rejected in the 5% of the sample that is rejected; therefore, we can conclude that 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.

Sorter performance. The sorter removed 100% of bunted kernels when 8% or greater of the sample was rejected (Table 1 and Fig. 1). At a confidence limit of 95% and assuming a binomial distribution, 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 8 of 99 samples and only when the sorter was set to reject less than about 5% of the sample, resorting the accepted portion resulted in recovery of all bunted kernels in 6 of those samples. For the other two samples, a bunted kernel remained in the accept portion, but this was at a rate that rejected less than 1% of the sample. Thus, all bunted kernels are removed when 8% or more of the sample is rejected, and a two-pass sort increases the chance of removing all bunted kernels at lower sample rejection rates. Unpublished APHIS research shows that experienced personnel recovered only 77% of kernels with Kb from 30 samples containing from 3 to 10 bunted kernels.

Tip-infected kernels and canoes were more likely to be missed than other types of bunted kernels, which was expected because some tip-infected kernels had an area of infection that only slightly exceeded the resolution of the sorter, and some canoes were only fragments of whole kernels. The sorter functioned with no mechanical failures, and the typical time required for the sorter to remove kernels from an 1,800-g sample was about 45 s.

DISCUSSION

The sorter was able to rapidly (approximately 1 min) reduce the portion of kernels that must be visually inspected to about 8 to 12% of the original sample weight without failing to remove any bunted kernels. The sorter removes kernels based on their optical properties; therefore, kernels exhibiting a discoloration such as black point or common bunt, which is similar in appearance to Kb, are 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 and 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 peanut shows that sampling error can account for 40 to 93% of the total error in measuring grain attributes, and sampling error can be 2 to 90 times greater than measurement error (3,5,11–14). Reducing measurement error can be achieved by replacing the visual detection method used in 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 immunosorbent assay tests that are currently under development. These two methods can require at least 1 h to complete, and the spores detected may have come from other sources such as contamination during harvesting or handling.

Increasing sample size may significantly reduce total detection error. For example, doubling the current sample size obtained from a lot from 1,800 g to 3,600 g 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 nonsorted procedures. This sorter also could be used to remove bunted kernels from large seed wheat lots or lots destined for mills or export because the full-sized instrument can inspect and sort up to 8,800 kg/h. 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 (7) 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 cultivars; however, rejecting 8% or more of the mass should be sufficient for rejecting kernels with Kb. Also, reductions in total error achieved with this technology need to be quantified.

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