Practice change especially around improvements to hygiene of grain storage was observed in many participants throughout the surveillance program.

In South Australia (SA), the programs significance was also acknowledged through additional industry funding (in the form of a SA grains industry trust grant to state diagnostics) as the extension of the program provided a unique opportunity to investigate the by-catches and the related native species composition in SA. The grant has allowed for further analysis, curation and permanent lodgement of reference material into a nationally recognised collection (Waite insect and Nematode Collection).

Biosecurity strategies emphasis the need for industry and community participation. Clearly this type of biosecurity surveillance program is a lot of work, expensive and time consuming, but has made a beneficial contribution in the collection of proof of absence data and industry awareness and education. Future engagement, cost effective resourcing, collaboration and value adding are required along with evaluating the real value of this type and source of surveillance data.

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Testing Wheat for Internal Infesting Insects with an Electrically Conductive Roller Mill

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Abstract

Although grain is always inspected for adult insects and insect damaged kernels upon shipping and receiving, immature insects living inside the kernels of grain cannot be readily detected. A laboratory roller mill was modified to measure and analyze the electrical conductance of wheat as it was crushed. The electrical conductance of normal wheat kernels is low and fairly constant. In contrast, the electrical conductance of infested wheat kernels produces a sudden change in the electrical signal. The peak height of the electrical spike depends on the size of the larvae and the resulting contact of the crushed larvae between the rolls. This instrument was designed to test wheat with moisture content of 13.5% or less. The laboratory mill can test a kilogram of wheat in less than 2 min. Hard red winter and soft red winter wheat samples were used in experiments. Known numbers of infested kernels were added to the wheat samples. The infested kernels contained larvae of rice weevils and lesser grain borers sorted into large, medium, and small size groups. The instrument detected ~8 of 10 infested kernels per 100 g of wheat with large-larvae (fourth instar or pupae). It detected ~7 of 10 infested kernels with medium-larvae (second or third instar) and ~5 of 10 infested kernels infested with the small-larvae (first or second instar). Under reasonable grain moisture and careful sample handling, there were no non-infested kernels classified as insect infested. The mill can lead to rapid and automated detection of infested wheat.

Keywords: rice weevil, lesser grain borer, x-ray, insect fragment test

Introduction

Grain is commonly inspected for insect contamination using visual indicators such as sieving for adult insects or inspection of a 100-g sub-sample for insect-damaged kernels (GIPSA, 2009). However, internal infestations by insects such as *Rhyzopertha dominica*, lesser grain borer, and *Sitophilus* spp. are not easily detected with visual methods alone. With subsequent storage, these hidden infestations can lead to increased pest populations that require treatment such as fumigation and potentially contaminate resulting flour with insect fragments. Many methods of detecting infested wheat have been developed and are available, but all are relatively expensive and/or time consuming. Some of these methods include staining the wheat to detect weevil egg plugs (Milner et al. 1950), microphones for listening to insects feeding (Hagstrum et al. 1990), single kernel compression testing (Pearson et al. 2003), single kernel NIR measurements (Dowell et al. 1998, Perez-Mendoza et al. 2003, 2005), and x-ray imaging (Karunakaran et al. 2004; Haff and Slaughter 2004, Fornal et al. 2006).

X-ray images provide accurate determinations of infested seeds and larvae stages and number of internally infested kernels. However, x-ray systems are expensive and are only able to test a single layer of wheat and small sample sizes. NIR systems were able to correlate actual and predicted fragment levels over a range of 0 to 300 fragments. However, measurements below 100 fragments contained too much variability to clearly determine whether the flour is above the FDA (1988) defect level of 75 fragments from an average of six 50-g flour samples. Fragments in flour are estimated using a chemical method, AOAC 972.32.

The laboratory mill developed by Pearson and Brabec (2007) monitors electrical conductance through crushed wheat. The conductance mill can detect over 70% of the kernels infested with medium and large larvae and pupae, and is able to test 1 kg of wheat in about two minutes. If infested grain is detected, management could react by rejecting the lot, fumigating, storing the lot separately, or quickly milling the grain before insects have time to multiply. The objective of this study was to investigate the ability of the conductance mill to detect different size larvae in infested kernels (experiment 1) and to determine relationship between insect detections and subsequent insect fragment counts in milled flour (experiment 2).

Materials and Methods

A laboratory roller mill was fabicated and consisted of two, 8 cm diameter by 10 cm wide rolls which were mounted on a 2.5 cm diameter shaft. One mill-roll was electrically grounded through the gear motor. The slave roll was mounted into delrin bearings which made the roll electrically isolated. A 5 Vdc supply was electrically connected via a motor brush and contacted this roll. A schematic diagram of the system is shown in Figure 1.

Hard red winter wheat was obtained from a farm in central Kansas at time of harvest and stored in small barrels in a large refrigerator. This wheat was considered non-infested. The grain was cleaned by passing it through a Carter Dockage tester (Carter-Day, Minneapolis, MN) using the dockage configuration for wheat. The moisture content of the wheat was 12.0%. For experiments, two moisture contents were created: 11% and 13%.

Experiment 1. Approximately 250 *R. dominica* or *Sitophilus oryzae*, rice weevil, adults were added to ~500g of wheat which was tempered to 13% moisture and stored at 27°C for 4-5 weeks. This infested wheat was x-ray imaged (MX20-dc44, Faxitron X-ray Corp., Wheeling, Il.) and infested kernels with large, medium, and small larvae were selected based on the x-ray images (Fig. 2).

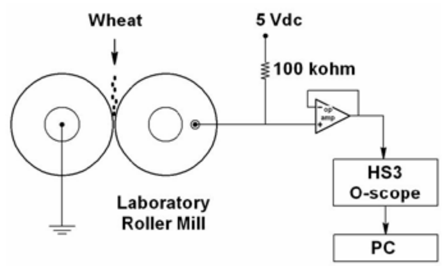

Fig. 1 Schematic diagram of the electrically conductive wheat mill and the associated circuit and basic data acquisition.

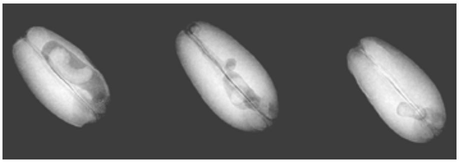

Fig. 2 X-ray image of infested kernels showing the large, medium, and small sized larvae from rice weevil.

Then 10 infested kernels of a given larvae size were added to 100g of sound wheat and crushed in the conductance mill. A micro-controller (Model EL, Tern Inc. Davis, CA) collected and processed the derivative of the conductance signal. The insect counts for a wheat sample were intermittent signal spikes above the baseline of the derivative signal (Fig. 3). The number of detects were recorded. Experimental variables were 11% and 13% moisture content wheat, rice weevil and lesser grain borer infestations, and three larval size categories.

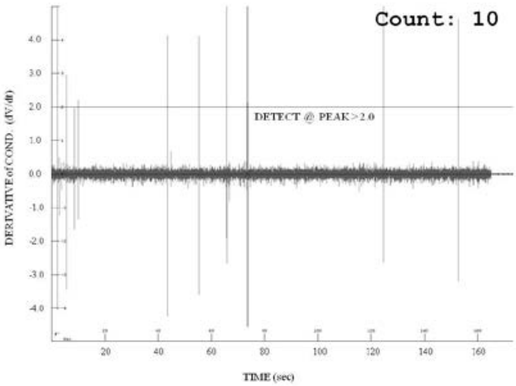

Fig. 3 Example of the software output and the derivative signal collected during the conductance milling of a 1 kg wheat sample. The signal spikes are from infested kernels containg large lesser grain borer larvae as they were compressed between the mill-rolls.

Experiment 2. To study the relationship between conductance detects and insect fragments in flour, infested kernels with lesser grain borers were prepared and added to 1 kg of sound wheat. A spoon of infested grain (~7.0 g) was taken from insect colonies and placed in small plastic bags, and x-rayed. The x-ray images were inventoried for infested kernels. For experiment #2, the initial infested kernels in the plastic bags had size distributions of ~60% large larvae and ~40% medium and small larvae. The original colonies were prepared and adults were removed after 3 weeks causing a bias in the larvae size distribution. The bags of infested kernels were added to the 1 kg of sound grain at three levels of infestation: 11-13 infested kernels (low), 23-25 infested kernels (medium), or 47-49 infested kernels (high) (Brabec et al. 2010). The infested grain samples were evaluated using the conductance mill at two times; day 0 and after six weeks of storage. After six weeks, any emerged adults were removed by sifting and the remaining grain was passed through the conductance mill.

Before each conductance test, a 300 g portion of clean wheat was passed through the conductance mill and the pre-sample was discarded. Then, the 1 kg test sample was passed through the conductance mill. The crushed wheat samples were bagged and stored at 7 °C until they were milled into flour for fragment testing.

Crushed samples were further milled using the Quadramat Jr. milling system (Quad Jr.) and AACC Experimental Milling method 26-50. Flour samples were sent to two U.S. cereal chemistry laboratories for insect fragment analysis. Both laboratories used acid hydrolysis methods. However, laboratory #1 performed the AOAC protocol (1996) using a five minute heating cycle in an autoclave at 121°C and 103 kPa. Laboratory #2 performed the AACC method 28-41b, using a 15 minute heating cycle in the autoclave. A single technician from each laboratory performed the wet chemistry and counted the fragments on filter paper using microscopy techniques.

Results

Experiment 1. The conductance mill is able to detect internal insects, but its ablility to detect varies with the size of the internal larvae. Small larvae (1^{st}-2^{nd} instar) were only detected on average ~50% of the time. The standard error of estimate for the small larvae was 1.5, thus for some samples with small larvae, only 2-3 infested kernels were detected. The large larve and pupae were detected ~80% of the time (Tab. 1).

Tab 1 Detection levels of the 10 infested wheat kernels within 100 g of wheat using the conductance mill for three different size classes of internally infesting larvae of *Rhyzopertha dominica* and *Sitophilus oryzae*.

	Number (+/-SE) of Infested Kernels Detected	
Larvae	***R. dominica***	***S. oryzae***
large	7.9 (1.4)	8.6 (1.1)
medium	7.1 (1.6)	7.7 (1.2)
small	5.5 (1.5)	6.3 (1.5)

Experiment 2. For the 1 kg samples prepared for the insect fragment testing, the infested kernels were a mixed population. At week 0, the lowest density infested samples had detection of ~75% of the infested kernels while the high density infested sample had detection of ~56% of the infested kernels. While accuracy was lower, detection of 28 infested seeds in a kilogram of wheat is already above the level that should raise concerns and therefore the reduced count accurary may be less of an issue. After samples were incubated for 6 weeks, the lowest infested sample went to 67 detects while the highly infested sample went to 120 detect. The insect fragment counts were significantly different between the two commercial laboratories. For laboratory #2, the week 0 samples all had insect fragments below the FDA threshold of 75. For laboratory #1, the fragment counts tended to be higher, even the control samples had fragment counts averaging over 15 counts.

Tab 2 Detection of infested seeds with the conductance mill for mixed infestions in a 1 kg sample of wheat. After the conductance milling, the crushed material was milled for flour and tested for insect fragments.

	Number (+/-SE) Detections	
Infested kernels	**0 wks**	**6 wks**
Control	0 (1)	2 (1)
Low 12	9 (1)	67 (11)
Med 25	16 (2)	88 (16)
High 50	28 (2)	120 (20)

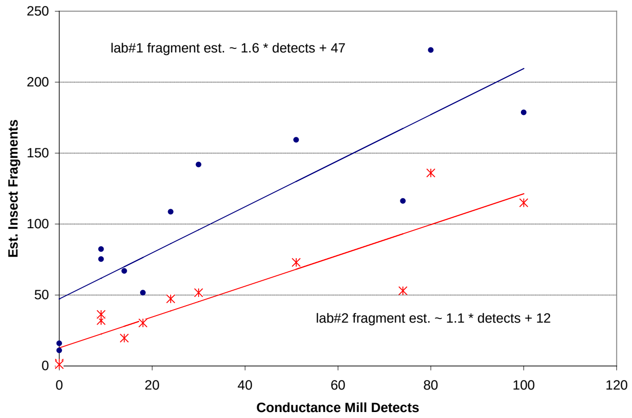

Fig. 4 Estimated insect fragment count verses the conductance mill detects. Two commercial cereal chemistry laboratories analyzed the samples; lab#1 and lab#2.

Discussion

The biggest challenge for sampling wheat for internally infesting insects is that detection is time consuming and that large amounts of wheat need to be sampled to detect low infestation levels. The conductance mill can process 1 kg in a couple minutes thus it is possible to quickly test wheat as grain is received from trucks or rail. Storey et al. (1982) studied over 2,000 wheat samples from many U.S. export grain terminals and found ~8% contained rice weevils and ~6% contained lesser grain borers after incubation, although less than 1% of their samples were graded as "weevilly". Perez-Mendoza et al. (2004) studied grain samples from eight rail cars, or 24 rail car compartments, at a grain processing facility. The study found that 20 of the 24 rail car compartments averaged less than one insect per kg of wheat. However, four compartments averaged 2, 6, 17, and 19 internal insects per 3 kg sample. Probing railcars and inspecting samples and storing samples for later insect emergence requires significant time and effort. Also, the visual sample obtained during inspection often did not match the internal infestation samples in terms of insect density. The conductance mill works well at detecting samples with lower infestation levels that are more realistitic in terms of what the industry needs to be able to detect. And the conductance mill can handle 15-20 kg of samples per hour as might be required while unloading railcars or truck.

There are different factors that can impact the accuracy of detection. False positive counts were caused by small clods of dirt in the wheat, so cleaning the wheat before processing by passing over some sieves is recommended. Also, any external moisture, such as rain or snow, could add signal noise, but usually this is not detected. Additionally, the conductance mill cannot detect internal infestations if the insects have died and are dried up, such as occurs after a fumigation and this will effect estimations of insect fragment levels in flour but will not be a factor in terms of estimating risk of insect population growth in a bin.

The conductance mill has also been test with rice and popcorn (Brabec et al., 2012, 2017). Rice is smaller than wheat and popcorn is larger than wheat, so each grain size needs appropriate mill gaps for the material to grind smoothly. For rice, the mill design included differential gearing during milling. Early test using 1:1 gearing and laboratory mill gaps of 0.018" and 0.028" show that detection decreased as roll gap increased, particularly from the small larvae (Pearson and Brabec 2007). Detection sensitivity was improved with the shearing action from differential rolls.

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Survey of *Trogoderma* species (Coleoptera: Dermestidae) Associated with International Trade of Dried Distiller's Grains and Solubles in the USA

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Abstract

Dried distiller's grains and solubles, DDGS, is a valuable commodity with substantial international trade. Vietnam discovered an infestation of *Trogoderma inclusum*, an actionable quarantine pest, in DDGS from the USA in 2012.