



Detection of internal insects in wheat using a conductive roller mill and estimation of insect fragments in the resulting flour[☆]

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ABSTRACT

A laboratory roller mill that monitors the conductance of kernels that pass through it was tested for its ability to estimate the number of insect fragments in flour after milling. This system can test a kilogram of whole wheat in approximately 1 min and requires little sample preparation. Hard red winter wheat samples were infested with lesser grain borers and stored at 24 °C. Infestations ranged from 12 to over 2000 infested kernels per 1 kg or per 30,000 kernels. After crushing of samples in the conductance instrument, the samples were milled into flour and sub-samples were sent to two laboratories for insect fragment analysis. The insect fragments were proportional to the number of detection incidences obtained using the conductance instrument and X-ray images. Insect fragment counts per 50 g of flour ranged from 0 to over 5000. For insect fragment counts from 0 to 250, correlations between fragment counts and conductance mill detection were 0.75 and 0.80 from two separate cereal chemistry laboratories. Therefore, the conductance mill is potentially a good method for testing incoming grain for live internally infesting insects; it is able to test 1 kg of grain in about 1 min and can detect low levels (as low as three insects) of live internal infestations in a 1- or 2-kg sample.

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1. Introduction

Adequate sample sizes, inspection accuracies, and defect incidence are concerns when inspecting grain lots for hidden internal insects. Storey et al. (1982) studied over 2000 wheat samples from many US export grain terminals. They found that 7.7% contained rice weevils and 5.6% 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 8 railcars, or 24 railcar compartments, at a grain processing facility. They probed each compartment at six locations and three depths per location. The study found that 20 of the 24 railcar compartments averaged less than one insect per kilogram of wheat. However, four compartments averaged 2, 6, 17, and 19 internal insects per 3 kg of sample. Sampling at discharge provided similar indications of insects as did probe sampling, if several sub-samples were obtained during discharge.

Grain is commonly inspected for insect contamination using visual indicators such as sieving grain samples for adult insects or inspection for insect-damaged kernels (GIPSA, 2009). However, internal infestations are not evident with visual methods alone. With subsequent storage, these hidden infestations can develop and contribute to insect fragments that appear in processing. Some of the methods available for estimating internal insect infestations in wheat include crack and flotation test, immunoassay biochemical test, X-ray analysis, and near-infrared reflectance spectroscopy (NIR) analysis which have been overviewed by Brader et al. (2002) and Neethirajan et al. (2007). X-ray images provide clear images of internal insects and are good references (Milner et al., 1950; Haff and Slaughter, 2004; Karunakaran et al., 2004).

The lesser grain borer, *Rhyzopertha dominica* (F.), is one of the main internal infesting insect in US wheat (Flinn and Hagstrum, 1990). The adults lay eggs in the grain. After the eggs hatch, the first instar larva bores into a kernel where it develops to the adult stage (Elek, 1994).

Quantifying insect fragments in flour and correlating it to NIR spectroscopy has been studied by Toews et al. (2007) and Perez-Mendoza et al. (2005). The NIR systems were able to correlate actual and predicted fragment levels over a range of 0–300 fragments per 50 g of flour. However, measurements below 100 fragments contained too much variability to clearly determine whether

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the flour is above the FDA defect level. The FDA (2010) has a defect action level of 75 fragments from an average of a set of six 50-g flour samples. Fragments in flour are measured using a chemical method to digest the flour and then microscopy to identify and count the resulting fragments (AOAC, 1996).

The laboratory mill developed by Pearson and Brabec (2007) monitors the electrical conductance through crushed wheat. The conductance mill can detect over 70% of the kernels infested with medium and large larvae and pupae of *R. dominica*, and is able to test 1 kg of wheat in about 1 min. Although infested grain can be detected with the conductance mill, it is not certain how many of these detections are required before management should be concerned about insect fragments in the resulting milled flour. The objective of this study was to investigate how insect detection with the conductance mill might correlate with subsequent insect fragment counts in milled flour. Thus, this information might help management decide whether to reject the lot, store the lot apart from non-infested grain for immediate or later control, or quickly mill the grain before the insect population can develop further.

2. Materials and methods

2.1. Preparation of infested wheat samples

Hard red winter wheat was obtained from a farm in central Kansas in 2007 and therefore never exposed to stored grain insects. 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% as determined by a Perten SKCS 4100 (Springfield, IL).

Initial small colonies of *R. dominica* were started and incubated. Approximately 250 adult *R. dominica* were added to 400 g of wheat which was tempered to 13% moisture. Sample lots were stored at 27 °C for 4–5 weeks. The adults and insect frass were removed. The infested grain was thoroughly mixed. The grain was separated into 6.5–7.0-g portions, placed into 5 cm × 5 cm plastic bags and then X-rayed (MX20-dc44, Faxitron X-ray Corp., Wheeling, IL). The X-ray images were analyzed for stage of development, such as small larvae (first–second instar), medium larvae (third instar), large larvae (fourth instar), pupae, and internal adults (Kirkpatrick and Wilbur, 1965). Initial infested samples were grouped to contain either 11–13 infested kernels (low), 23–25 infested kernels (medium), or 47–49 infested kernels (high). Their average life stages are shown in Table 1. The initial infested grain weighed 7–20 g. The infested grain samples were added to 1-kg samples of non-infested wheat and thoroughly mixed to start the colonies at low, medium, or high infestation levels, then placed in 1/2-gallon glass jars. Non-infested wheat or control samples were included.

Three sets of conductance mill samples were prepared. Each sample set contained four infestation levels (control, low, medium, high) and four replicates giving a total of 16 × 1-kg samples per set. One set was tested on the conductance mill immediately after preparation. The other two sets were incubated for 6 and 10 weeks to allow the infestation levels to increase. Incubated samples were

stored in an environmental cabinet maintained at 24 ± 1 °C and 60 ± 5% relative humidity. A total of 3 sets × 16 samples per set or 48 samples were prepared for the conductance mill test.

2.2. X-ray of 1-kg wheat samples

X-ray images were taken of sub-samples from each sample prior to crushing with the conductance mill (Faxitron X-ray Corp., Wheeling, IL, MX-20). X-ray data were collected from 120 g of the 1-kg sample. Each 1 kg of sample was sieved to remove emerged adults after the wheat was stored for 6 or 10 weeks. The 120 g of sub-sample was divided into 16 × 7.5 g-portions, placed into 5 cm × 5 cm plastic bags, and X-rayed. The X-ray images were analyzed by a technician who identified and counted infested seeds as either small larvae, medium larvae, large larvae, pupae, or internal adults depending on the size and morphological features. The small larvae occupied an area that was approximately 10% of the kernel's two-dimensional (2D) view. This size of larva corresponds with the first or second instar maturity stage. The medium larvae corresponded to the second or third instar maturity stage and occupied between 10% and 25% of the kernels 2D view. The large larvae, corresponding to the fourth instar or pupal stages (Sharifi and Mills, 1971), occupied over 25% of the 2D view (Fig. 1). The sub-samples were added back to the bulk before crushing.

2.3. Insect detections in wheat with conductance rolls

Before each conductance test, a 300-g portion of non-infested clean wheat was passed through the conductance mill, the mill was brushed, and the pre-sample discarded. Then, the 1-kg sample was passed through the conductance mill. The micro-controller (Model EL, Tern, Inc., Davis, CA) collected and stored the conductance signal from each sample to a memory card. The insect counts for a 1-kg wheat sample were determined by detecting signal peaks using the slope algorithm and thresholds as developed by Pearson and Brabec (2007). Then, the micro-controller displayed the number of infested kernels which were detected. The crushed wheat samples were bagged and stored at 7 °C to prevent deterioration until they were milled.

2.4. Milling the crushed wheat into flour

Crushed samples were milled and sieved using the Quadrumat Jr. milling system (Quad Jr.) and AACC Experimental Milling Method 26–50 (AACC, 2000). The milling procedure was modified in that a Strand shaker (Minneapolis, MN) was used with US Standard sieves #40 and #100 rather than using sifting with a reel sifter. The sifting durations were 3 min for material from the break roll and 4 min for material from the reduction rolls. The AACC experimental milling method called for milling of 100-g portions. The crushed sample was about 1 kg. Thus, 10 × 100-g portions were milled, which produced about 700 g of flour. The resulting break and reduction flours were combined and thoroughly mixed. Flour was milled from 2 replicates at each infestation level and storage time (24 of 48, 1-kg crushed wheat samples).

2.5. Insect fragment testing in flour

Flour samples were sent to two US cereal chemistry laboratories for insect fragment analysis. A single technician from each laboratory was assigned to perform the wet chemistry on all samples and to count the resulting fragments which were collected on filter paper using microscopy techniques. Both laboratories used acid hydrolysis methods. However, laboratory #1 performed the AOAC protocol (1996) using a 5-min heating cycle in an autoclave at

Table 1
Initial (w0) average distributions of infested kernels used to spike the 1-kg samples.

	Internal adult	Pupae	Large larvae	Medium larvae	Small larvae	Total
w0-lo	2	4	4	2	1	13
w0-m	2	8	9	6	2	27
w0-hi	4	14	19	11	4	52

These seeds were selected from X-ray images. The allowed standard deviations for low (lo), medium (m), and high (hi) infestations of pupae and larvae were 1, 2, and 3, respectively. The allowed deviation for internal adults was 1 for all cases.

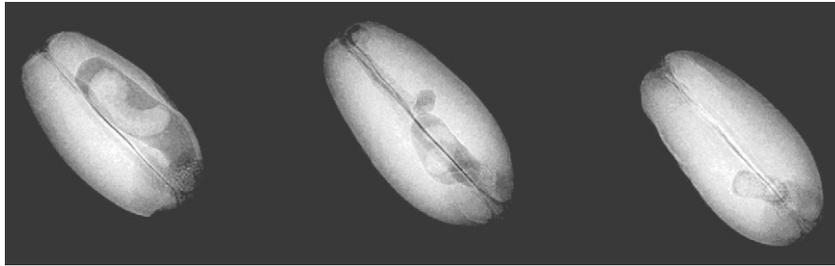


Fig. 1. X-ray image of large, medium, and small larvae in wheat grains (left to right).

121 °C and 103 kPa. Laboratory #2 performed the AACC method 28–41B, using a 15-min heating cycle in the autoclave. Twenty-four of the 48 crushed wheat samples were milled into flour. Each wheat sample yielded about 700 g of flour. Three × 50-g flour samples were drawn from each 700-g flour sample per cereal laboratory. Thus, 24 crushed wheat samples × 3 flours × 2 laboratories or 144 insect fragment analyses were obtained from the crushed wheat that was milled into flour.

Reference samples were prepared from 100-g portions of non-infested and clean wheat. The wheat was crushed through the conductance mill. Then, the crushed wheat was milled with and without infested seeds using the Quadrumat Jr. milling system. Next, 50 g of this flour was sent for fragment testing. The observations included control samples, samples infested with 10 large larvae, or 10 pupae. Also, 10 dead adults *R. dominica* were added to crushed wheat instead of using infested kernels containing pre-emerged adults. Three replicates were prepared per observation and per cereal laboratory and thus 4 infestation types (larvae, pupae, adults, and control) × 3 replicates × 2 laboratories yielded 24 insect fragment analyses from the reference samples.

2.6. Data analysis and statistics

The X-ray, conductance mill detections, and flour insect fragment data were summarized into Excel spreadsheets. The experiment conducted was a randomized block design with 4 replications per treatment combination. The mean, standard error (SE), comparison of means, and R^2 values were determined using SAS (SAS Institute, 2008). Insect counts were transformed using the natural log to stabilize variance for all analyses. When insect counts were observed to be 0, an offset of 0.5 was added to avoid an invalid log function. The log-transformed data were then fitted to a two-way general linear model using the MIXED procedure. The main factors of this model included week of incubation with levels of 0, 6 and 10 weeks. Sub-factors were infestation levels; control, low, moderate and high. Tests of main effects and interactions were conducted using *F*-tests. Significant interactions were followed by separations of infestation level means for each time period using Tukey's Honestly Significant Difference procedure. Mean separations were conducted using the SAS macro PDMIX800 (Saxton, 1998). Means reported in Tables 2–4 are untransformed, on the original scale not on the log scale.

3. Results

3.1. X-ray data and conductance data

The X-ray results per 120 g are listed in Table 2 and show the number of different life stages that were present in the low-, medium- and high-density samples. The total number of infested seeds per 120 g were 2–7 (week 0), 18–50 (week 6), and 98–258 (week 10). The week-6 samples had few large larvae, adults and

pupae, but contained many small and medium larvae. This gap in life stages occurred because no adults were available to lay eggs during the early days of storage. By week 10, all life stages were abundant.

Table 3 shows the conductance mill insect detections and X-ray results per 1 kg. The mean X-ray values from Table 2 were multiplied by 8.3 to adjust the counts from 120-g to 1-kg sample size. The ratio of conductance insect detections versus X-ray (Cond/X-ray) shows that the conductance mill detected about 50% of the infested seeds at week 0. Detection efficiency dropped during week 6. There are a couple factors which could cause this drop. The week-6 samples contained a high percentage of small larvae. Small larvae are less detectable than larger larvae with the conductance mill (Pearson and Brabec, 2007). Secondly, the detection efficiency of the mill dropped as the level of infestation increased. This may have been due to multiple infested seeds being crushed at the same time. Week-10 samples demonstrated the current conductance mill design's upper detection limit of around 350 detections per kilogram of grain.

3.2. Insect fragment data

Table 4 gives the insect fragment data for the samples crushed in the conductance mill. The insect fragment counts ranged from 34 to over 5000. The current FDA insect fragment defect action level is an average of 75 fragments per unit lot of flour. This threshold was passed after 30–60 detections with the conductance mill. Laboratory #1 exceeded this threshold with the first treatment level (week 0, low density). However, the second treatment (week 0, medium density) measured fewer fragments. This is difficult to

Table 2

Mean (\pm SE) number of infested seeds found from X-ray images of 120-g sub-samples of each 1 kg grain sample.

	Internal adults	Pupae	Large larvae	Medium larvae	Small larvae	Total/120 g
w0-c	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
w0-lo	0 ± 0	0 ± 0	1 ± 1	1 ± 0	0 ± 0	2 ± 1
w0-m	0 ± 0	1 ± 0	2 ± 0	1 ± 0	0 ± 0	4 ± 1
w0-hi	1 ± 0	1 ± 0	5 ± 2	1 ± 1	0 ± 0	7 ± 2
w6-c	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
w6-lo	0 ± 0	1 ± 0	0 ± 0	6 ± 2	11 ± 3	18 ± 2
w6-m	1 ± 0	0 ± 0	0 ± 0	6 ± 2	18 ± 3	24 ± 5
w6-hi	1 ± 1	1 ± 0	2 ± 1	16 ± 3	31 ± 3	50 ± 6
w10-c	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
w10-lo	8 ± 3	12 ± 2	21 ± 5	30 ± 6	27 ± 6	98 ± 11
w10-m	20 ± 4	26 ± 3	43 ± 7	54 ± 6	40 ± 6	183 ± 17
w10-hi	26 ± 5	40 ± 3	63 ± 8	76 ± 3	53 ± 8	258 ± 25

Samples are designated by incubation week (w0, w6, w10) and infestation level (control (c), low (lo), medium (m), high (hi)). The infestation life stages were identified and counted.

Table 3
Mean (±SE, TSD) number of conductance mill and X-ray detections for infested wheat samples.

	Cond-mill	X-ray	Small larvae	Cond/X-ray
w0-c	0 ± 0 d	0 ± 0 c		
w0-lo	9 ± 1 c	17 ± 3 b	0%	53%
w0-m	16 ± 2 b	31 ± 6 ab	0%	52%
w0-hi	28 ± 2 a	58 ± 10 a	0%	48%
w6-c	0 ± 0 c	0 ± 0 c		
w6-lo	67 ± 6 b	147 ± 24 b	63%	46%
w6-m	88 ± 8 ab	197 ± 32 ab	74%	45%
w6-hi	120 ± 10 a	415 ± 67 a	62%	29%
w10-c	0 ± 0 c	0 ± 0 c		
w10-lo	255 ± 10 b	813 ± 130 b	28%	31%
w10-m	322 ± 8 a	1515 ± 241 ab	22%	21%
w10-hi	356 ± 2 a	2139 ± 341 a	21%	17%

X-ray data were adjusted to reflect a 1-kg sample versus the 120-g sub-sample. Samples are designated by incubation week (w0, w6, w10) and infestation level (control, low, medium, high). Significant interactions were followed by mean separations of infestation level means for each time period and within each column using Tukey's Honestly Significant Difference (TSD) procedure. Also, included are columns containing the percentage of small larvae and the relative efficiency of the conductance mill (Cond/X-ray).

explain because the medium infested samples were given about 25 infested seeds per 1 kg while the low infested samples contained about 12 infested seeds per 1 kg. The mean fragments for week-0 treatments were not statistically significantly different. Laboratory #1 reported an average value of 16 insect fragments in the control samples. The control samples should have contained 0 insect fragments, so laboratory #1 may be misreading plant material as insect parts.

Table 4
Mean (±SE, TSD) insect fragments determined by two US cereal chemistry laboratories from flour produced from the crushed grain.

	Insect fragments (lab #1)	Insect fragments (lab #2)
w0-c	19 ± 3 b	2 ± 1 b
w0-lo	79 ± 5 a	34 ± 4 a
w0-m	59 ± 6 a	25 ± 3 a
w0-hi	125 ± 17 a	50 ± 5 a
w6-c	15 ± 2 c	1 ± 1 c
w6-lo	138 ± 26 b	63 ± 6 b
w6-m	192 ± 37 ab	140 ± 13 ab
w6-hi	323 ± 53 a	318 ± 27 a
w10-c	14 ± 3 d	2 ± 1 c
w10-lo	1168 ± 120 c	582 ± 48 b
w10-m	2560 ± 212 b	1167 ± 96 ab
w10-hi	5724 ± 246 a	2004 ± 163 a

Samples are designated by incubation week (w0, w6, w10) and infestation level (control, low, medium, high). Significant interactions were followed by mean separations of infestation level means for each time period and within each column using Tukey's Honestly Significant Difference (TSD) procedure. The TSD was determined on log-transformed value, although the table values are not transformed.

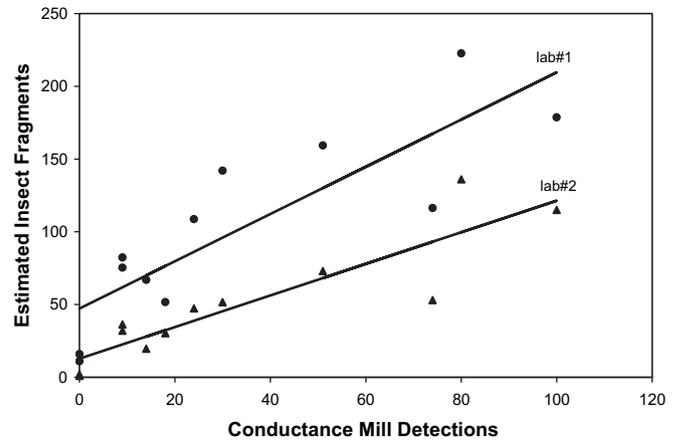


Fig. 2. Estimations of potential insect fragments in flour versus conductance mill detections of whole grain from two US cereal chemistry laboratories. The analytical procedures followed by each laboratory are mentioned in Section 2. Laboratory #1: $y = 47 + 1.6 \times x$ ($R^2 = 0.73$, $P = 0.0003$, $n = 12$). Laboratory #2: $y = 12 + 1.1 \times x$ ($R^2 = 0.80$, $P = 0.0001$, $n = 12$).

Fig. 2 displays a plot of the number of conductance mill detections versus insect fragments for samples containing less than 250 fragments. This insect density range is near the FDA insect fragment threshold. Also, the conductance mill detection efficiency drops significantly at the higher insect density. For this data range, the estimate of insect fragments for laboratory #1 was 1.6 times the number of conductance detections and the R^2 was 0.75 ($n = 12$) and standard error of prediction was 36. Laboratory #2 fragment counts were 1.1 times the number of detections and the R^2 was 0.80 ($n = 12$) and a standard error of prediction of 19. Data from these two laboratories show how variable the fragment counts can be. Much of this variability is reduced by averaging 3×50 -g flour analysis per test. However, differences between technicians or laboratories are clearly evident and must be considered in the conductance mill versus insect fragment models.

3.3. Insect fragments per life stage

Table 5 shows the average fragments per insect found from the reference samples. In this study, the number of fragments from the pupae and larval stages were approximately 3–7 fragments per insect. The internal adults produced significantly more fragments than earlier life stages. Fragments from internal adults averaged 34 from laboratory #2. This agrees with a study by Perez-Mendoza et al. (2005) who found that lesser grain borer larvae and pupae yielded 1–2 fragments, while the internal adults produced 22–31 fragments. Also, Toews et al. (2007) found that, for the rice weevil (*Sitophilus oryzae* (L.)), larvae accounted for 1 fragment, pupae had 2 fragments, and adults produced around 27 fragments.

4. Discussion

It is presumed that live infestations contain a range of insect life stages. If a circumstance occurred where only dead insects were present then the number of insect fragments in milled flour would

Table 5
Mean (±SE) insect fragments produced at three life stages from reference wheat samples as determined by two US cereal chemistry laboratories.

	Lab #1	Lab #2
Larvae	4 ± 1	3 ± 1
Pupae	7 ± 1	5 ± 1
Adult	160 ± 27	25 ± 4

be underestimated. Also, if only insect eggs or first-instar larvae are present, such as may be the case shortly after fumigation, then the storability of the grain may be underestimated again. However, most circumstances would have very low numbers of insects or a diverse population of all maturity levels.

Under many grain storage scenarios, the internal adult stage is a small fraction of the total distribution of the live insects (Hagstrum et al., 1995). The internal adult stage for the lesser grain borer includes roughly the last 5 days of development (Stemley, 1962). For week-10 test, the internal adults represented about 10–15% of the samples in the X-ray data in Table 2. Thus, a high percentage of detections with the conductance mill comes from larvae or pupae, which produce fewer fragments.

From results of Perez-Mendoza et al. (2005), Toews et al. (2007), and laboratory #2 results from this study, 1 adult produces roughly 30 insect fragments, a pupae produces 6 fragments, and a larva produces 3 fragments. While the adults produce more insect fragments on a per insect basis, they may only contribute about half the fragments from a mature population due to more pupae and larvae being present. Assuming a population distribution of 70% larvae, 20% pupae, and 10% internal adults, the larvae and pupae combine to contribute nearly identical numbers of fragments to the flour as the adults do.

If it is assumed, for simplicity, that the insect-infested kernels are uniformly distributed in a railroad car compartment, then the number of infested kernels in a small sample from the population can be approximated by the Poisson distribution given by Eq. (1) (Gotelli and Ellison, 2004).

$$P(x) = \frac{\lambda^x e^{-\lambda}}{x!} \quad (1)$$

where $P(x)$ is the probability of a sample containing an insect count, x , when the given sample contains an average number of insects, λ .

For the following example, consider a grain truck that actually contains 6 insects per kg. The sample averages, λ , would be 0.6 insects per 100 g, 3 insects per 500 g, 6 insects per 1000 g, and 12 insects per 2000 g. Given 100-, 500-, 1000-, and 2000-g sample sizes and 95% confidence intervals, the range of insects randomly found within a given sample size are 0–20, 0–12, 2–10, and 3–9, respectively. With a 100-g size, there is a 54% probability that the sample would contain 0 insects, indicating the inadequacy of this sample size. The probability of zero detection is lowered to 5% for a 500-g sample and 2.5% for a 1-kg sample. For the 1-kg sample size, 95% of the samples would contain 2–10 insects. For the 2-kg sample size, 95% of the samples would contain 3–9 insects. This example illustrates the need for larger sample sizes when estimating insect populations. Insect-infested kernels may not be distributed absolutely uniformly in railcars. However, mixing during bin unloading and loading of the car will cause considerable scattering of infested kernels. The ranges listed above serve as a guide for selecting sample sizes when inspecting for insect-infested kernels. A 100-g sample is obviously too small, while a 2000-g sample is superior, but this requires more time involved with sampling and handling of samples so, in practice, a 1000-g sample may be the optimal sample to use until more data are available to suggest otherwise.

The conductance mill is a potentially good method for testing incoming grain for live internally infesting insects. The conductance roller can process a 2-kg sample in under 2 min, and would have a 95% chance of detecting 6 ± 3 insects per kg of grain from a railcar with uniformly distributed infested kernels. The testing in this study was performed with lesser grain borer but results should be very comparable if grains were infested by other species, such as the rice weevil (Pearson and Brabec, 2007). The number of

detections by a conductance mill can be used to give some estimation of potential fragments resulting in the flour. Future research is planned to study the use of the conductance mill to estimate initial insect density, and using this estimate combined with a population growth model to predict insect density in stored grain over several months of storage.

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