Detection of Lesser Grain Borer Larvae in Internally Infested Kernels of Brown Rice and Wheat Using an Electrically Conductive Roller Mill

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ABSTRACT

Modifications were made to a small laboratory mill to enable the detection of rice kernels internally infested by immature grain insects. The mill, which was originally designed for wheat, monitors electrical conductance through the grain and detects kernels that are infested with live insects based on abrupt changes in electrical conductance as the insects inside the kernels are crushed between the mill rolls. The mill was adapted to detect rice infested by immature lesser grain borers (LGB) by altering the gearing and reducing the gap between the two mill rolls to produce shear between the rolls. Samples of LGB infested long, medium, and short grain (dehulled) brown rice and hard red winter wheat were tested in both the modified and original mills. The detection rates for long grain brown rice kernels infested with large, medium, and small LGB larvae were 97, 83, and 42%, respectively, with the modified mill and 61, 22, and 4%, respectively, with the original mill. Similar detection rates were observed for medium and short grain brown rice with the modified mill. The detection rates for hard red winter wheat kernels infested by large, medium, and small LGB larvae were 98, 94, and 78%, respectively, with the modified mill and 78, 67, and 38%, respectively, with the original mill. More time was required to process a sample through the modified mill than through the original mill. For rice, a 500 g sample could be processed in ~150 sec, making the instrument useful for quality control checks of incoming and outgoing product and for monitoring grain during storage to determine whether fumigation is necessary. However, for drier wheat kernels, the flattened teeth in the modified mill allowed kernel slippage; as a result, the benefit of increased accuracy might not outweigh potential feeding issues.

Rice and wheat are both vulnerable during storage to insect pests that degrade quality and value. Rice is especially vulnerable because it is generally grown and stored in warm, humid climates. Stored-grain insects can cause significant economic losses for grain handlers and processors. Traditional methods for detecting internally infesting, stored-grain insects involve time-consuming X-rays (4,7) or chemical tests to extract insect fragments from milled grains (AACCI Approved Method 28-41B [1]). Because these methods are expensive, can only analyze very small amounts of grain, and are time-consuming, they are not usually performed during grain storage or shipment. This leads managers of grain storage facilities to fumigate at regular intervals regardless of whether the grain is actually infested. In addition, the lack of quick methods for detecting infested grain makes quality control checks of incoming and outgoing shipments of grain nearly impossible.

Rice and wheat are considered infested when two or more live insects are found during inspection (13). Live adult insects found in the grain represent an obvious sign of potential infestation. However, grain can be internally infested without any external evidence. Perez-Mendoza et al. (12) showed that ~90% of 2.75 kg wheat samples from rail cars that were infested with stored-grain insects had no adults in the samples. Thus, checking only for adult insects in a grain sample can lead to missed infestations, which can then cause significant damage over the course of a few months if the temperature and humidity are favorable for insect growth.

In previous studies by Brabec et al. (2) and Pearson et al. (11), instruments were developed that monitor electrical conductance through grain as it is milled. When an insect-infested kernel is crushed between two mill rolls, the moisture from the insect causes a spike in conductance that is detected by a computer connected to the mill rolls. This conductance mill can crush 1 kg of wheat in less than 2 min and count kernels infested with live larvae, pupae, and pre-emergent adult stored-grain insects. Two major internally infesting insect species were tested: the rice weevil, Sitophilus oryzae (L.), and the lesser grain borer (LGB), Rhizopertha dominica (F.). The processing speed of the conductance mill makes it a useful tool for grain receiving stations. Insect-infested kernel counts can be used by grain storage managers to make informed decisions regarding the storability of the grain and whether a bin should be fumigated.

In 2008–2010 the United States produced around 150 million cwt of long grain rice in several southern states, including Arkansas, Louisiana, Mississippi, and Texas. In 2009 California produced 45 million cwt of medium grain rice and more than 3 million cwt of short grain rice. The total U.S. rice crop was valued at more than US$3 billion in 2008, 2009, and 2010 (14). Rice is a major food source throughout the world, particularly in Asia and South America.

Preliminary studies with the conductance mill found much lower detection rates for internally infested brown rice than for wheat. Although brown rice is a cereal grain that is similar in shape to wheat, brown rice has a significantly smaller diameter than wheat. Also, larvae tend to be smaller in rice, as observed in X-ray images, possibly because of the harder composition of rice. These factors combine to make the detection of internally infested rice more difficult than that of internally infested wheat.

The objectives of this research were threefold: 1) to modify the conductance mill previously developed for detection of insect-infested wheat to improve its ability to detect insect infestations in brown rice; 2) to quantify the detection accuracy of
the modified mill for long, medium, and short grain brown rice kernels infested with various sizes of larvae; and 3) to compare the modified mill to the original mill for the detection of infested wheat and rice.

MATERIALS AND METHODS

Conductance Mills

Two laboratory mills constructed by National Manufacturing were tested. Both mills share a similar exterior appearance (Fig. 1); the only differences involve the internal parts (Fig. 2). The first mill, henceforth referred to as Mill(1), has design features similar to those of the original instrument developed by Pearson and Brabec (10). This mill crushes the grain using a 1:1 gear ratio between the two rolls, which results in no shear being applied to the grain as it is crushed. The tooth profile of Mill(1) is sharp, with a tooth depth of 0.64 mm (0.025 in.). The sharp tooth profile facilitates uniform feeding of wheat through the tight gap, because the sharp teeth strongly grip the kernels. The gap between the two rolls from peak to peak is 0.45–0.51 mm (0.018–0.020 in.). More importantly, the gap between the tooth valleys of the two rolls is \( \approx 1.73 \) mm (0.068 in.). This 1.73 mm gap corresponds to the diagonal of a 1.2 mm \( \times \) 1.2 mm square void that allows plenty of room for smaller larvae to pass through without being detected.

Detection of infested kernels only occurs if larval fluid (hemolymph) is released during the milling of the kernels and larvae, causing an electrical short circuit between both rolls. The rolls and teeth have some spaces between them, and a larva could be oriented such that it might not be crushed or detected. Larger spaces allow more chances for infested kernels to pass undetected through the mill. Large LGB larvae reared in long grain brown rice are \( \approx 1.6 \) mm long and 0.8 mm in diameter. Medium LGB larvae in long grain brown rice are \( \approx 1.1 \) mm long and 0.55 mm in diameter, which is smaller than the 1.2 mm \( \times \) 1.2 mm void space between the rolls shown in Figure 3. In a worst case scenario, if a medium larva is positioned perpendicular to the mill teeth, there is a chance that no contact will be made between the larva and both rolls, allowing the infested kernel to go undetected.

Several modifications were made to Mill(1) to facilitate the detection of internally infested rice kernels, resulting in Mill(2). Mill(1) is driven by a pair of gears that rotate at the same speed and basically crush the kernels. Mill(2) uses drive gears that do not rotate at the same speed and cause shearing of the kernels. The differential pair of drive gears used in Mill(2) have a gear ratio of 1:1.4. This modification reduces the space between the rolls, because the teeth always pass over each other. The shape of the teeth also was modified. Shearing between the rolls increases the wear on teeth, and sharp teeth would eventually wear down. Thus, the teeth used in Mill(2) have flattened tops. Also, a smaller tooth depth was used, 0.46 mm (0.018 in.), as shown in Figure 3. The changes to mill roll shearing and smaller tooth profiles reduce the maximum void space between the rolls and enhance the detection of smaller larvae.

The gear box for Mill(1) (F832-18K-B5, Boston Gear) operates at 96 rpm and a maximum torque of 78 N·m (690 lbf·in).
Additional torque was required for Mill(2) because of the shearing and 1:1.4 gear ratio. The Mill(2) gear box has a maximum torque of 165 N-m (1,457 lb-in) (F842B-36K-B5, Boston Gear) but operates more slowly at 48 rpm. Thus, grain sample throughput is slowed, and sample processing time increases.

During the process of milling grain, heat is created by the friction between the grain and the mill rolls, especially when shear is produced between the rolls. These laboratory mills are intended to operate intermittently and not continuously, and a short pause between sample runs allows the rolls to cool. However, even with intermittent operation, heat in the mills increases. Temperature sensors were mounted on the side of each mill, and the temperature of the mill side plate was measured between samples. A cooling fan was mounted on the back of Mill(2) to circulate air around the sides for cooling. Mill operation was limited to temperatures lower than 40°C because temperatures higher than 50°C resulted in noninfested kernels being counted as infested.

Grain Samples

For the tests described below, 22 kg (50 lb) bags of long, medium, and short grain (dehulled) brown rice (LBR, MBR, and SBR, respectively) were obtained from commercial sources in the United States. The hard red winter wheat (HRW) was obtained from the Kansas State University Foundation seed facility. All bags of grain were inspected, and the grain appeared sound, with a normal odor and moisture content <12%. The bags were stored in a large refrigerator at ≈5°C until use to prevent any potential insect activity. Control samples were crushed in a conductance mill, and no insects were detected. Before the control or infested samples were tested, the rice samples were conditioned to 14.2% moisture by tempering and drying 1–2 kg portions. This moisture level was selected because it represents the maximum moisture level at which grain can be safely stored (3,6). Also, brown rice containing more than 14.5% moisture would be less likely to be found in commercial markets because it is considered sample grade (9). Other moisture content levels of noninfested grain samples were tested using the conductance mill as described below.

Insect Colonies for Internally Infested Kernels

To acquire a sufficient density of infested kernels for easier x-ray imaging and sorting, 0.8 g of adult LGB (≈600 insects) were added to the 250 g samples of HRW and LBR. Only 0.4 g of adults was needed to acquire densely infested colonies in MBR and SBR samples, because these colonies could reproduce more easily. All grain samples were initially tempered to 13% moisture before adding the insects. The colonies were stored at 27°C and 60% RH for 8 weeks. New jars of colonies were started each week of the 8 week incubation period. Adults were maintained within each jar over the incubation period. Sufficient quantities of small, medium, and large larvae were available from several of the jars.

X-ray Imaging of Rice and Wheat Kernels

X-ray images were used to manually separate the infested rice and wheat kernels and to determine the size of the infesting larvae (usually there is only one larva per kernel). Approximately 6 g of kernels was placed on a plastic dish and x-rayed using a digital imaging system (MX20-dc44, Faxitron X-ray Corp.). The infested kernels were sorted into three size categories based on the size of the infesting larva (small, medium, or large), according to the following guidelines. Kernels containing larvae and tunnels that occupied more than half the length of the kernel were considered to be infested by large larvae. Kernels that contained larvae and tunnels occupying between one-quarter and one-half of the kernel length were considered to be infested by medium larvae. Kernels that contained larvae and tunnels occupying less than one-quarter of the kernel length were considered to be infested by small larvae. X-ray photos of the larval stages in LBR, MBR, and SBR are provided in Figure 4. According to Kirkpatrick and Wilbur (8), this size classification roughly corresponds to large larvae as pupae and fourth-instar larvae (∼1 mm × 3 mm), medium larvae as third-instar larvae (∼0.6 mm × 1.8 mm), and small larvae as second-instar larvae (∼0.3 mm × 1 mm).

Experimental Test and Design

Infested and noninfested rice and wheat grains were milled using both Mill(1) and Mill(2) to establish the detection rates for rice infested by LGB larvae of various sizes and to compare the detection rates between the two mills. Mill(1) was tested using two grain types: LBR and HRW. Mill(2) was tested using four grain types: LBR, MBR, SBR, and HRW. The experiment contained six grain × mill combinations: LBR × Mill(1), HRW × Mill(1), LBR × Mill(2), MBR × Mill(2), SBR × Mill(2), and HRW × Mill(2).

A randomized block design was used, with each grain × mill combination repeated three times. Within each block, four 500 g samples were tested for each of the three larval sizes and the control. Four samples and three replications yielded twelve samples for each larval size and grain type. Each 500 g sample of noninfested grain was spiked with 12 infested kernels just prior to milling. Thus, a total of 144 large, 144 medium, and 144 small larvae were used with each grain type. There were also 12 noninfested control samples tested for each grain × mill combination.

Fig. 4. An X-ray image shows infested kernels of long grain (top row), medium grain (middle row), and short grain (bottom row) brown rice. Each kernel contains a lesser grain borer larva of a different size: small (left kernels), medium (middle kernels), or large (right kernels). The length of the long grain rice measured ∼7–8 mm.
RESULTS AND DISCUSSION

Detection of Internally Infested Brown Rice

For LBR infested by large, medium, and small larvae, 61, 22, and 4%, respectively, of the infested kernels were detected using Mill(1) (Table I). The addition of shear to and the tighter teeth profiles of Mill(2) resulted in significantly higher ($P < 0.001$) detection rates. Using Mill(2), detection of kernels infested by large, medium, and small larvae averaged more than 97, 83, and 42%, respectively, for LBR. Similar detection rates were observed for MBR and SBR using Mill(2). The disadvantage of Mill(2) was the feed rate. It took $\approx 150$ sec to mill a 500 g sample of brown rice, which was approximately twice the time required for Mill(1).

After the conductance signal was collected, it was processed by computing the derivative of the signal, smoothing the derivative signal, and counting the peaks above the threshold. All of the peaks in the derivative signal that were higher than 2% of the full scale were counted as infested kernels. A computer screen image of the conductance signal for Mill(2) for a 500 g sample of LBR kernels with 14% moisture mixed with 12 kernels infested with medium larvae is shown in Figure 5, and its derivative is shown in Figure 6. In this example, 10 of 12 in-

**Table I. Infested kernel detection rates for the original conductance mill (Mill(1)) and the modified mill (Mill(2)) for long (LBR), medium (MBR), and short (SBR) grain brown rice**

<table>
<thead>
<tr>
<th>Larval Size</th>
<th>LBR × Mill(1)</th>
<th>LBR × Mill(2)</th>
<th>MBR × Mill(2)</th>
<th>SBR × Mill(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Large</td>
<td>61  11</td>
<td>57  11</td>
<td>54  11</td>
<td>50  11</td>
</tr>
<tr>
<td>Medium</td>
<td>22  14</td>
<td>20  14</td>
<td>18  14</td>
<td>16  14</td>
</tr>
<tr>
<td>Small</td>
<td>4  4</td>
<td>4  4</td>
<td>4  4</td>
<td>4  4</td>
</tr>
</tbody>
</table>

* Mean = average detection rate, and SD = standard deviation of detection of infested kernels. Each size category contained 12 samples, and each sample contained 12 lesser grain borer-infested kernels, for a total of 144 infested kernels per larval size.
fested kernels were detected. It was apparent that two peaks slightly below the 2% threshold were not counted; these were presumably from the two infested kernels that were not detected. It is possible to lower the slope threshold so lower peaks can be detected. However, lowering the slope threshold level increases the risk of false detections. As discussed below, the grain moisture tests indicated that the 2% slope threshold was a reasonable choice.

Peak height in the derivative signal (dV/dt) is not a clear indicator of the size of the infesting larva. However, large larvae tend to produce higher peaks relative to smaller larvae. Table II shows the detected peak heights versus larval size for LBR for Mill(2); 55% of the peaks from the large larvae were above the threshold level of 5.0, and 83% of the peaks from the small larvae were below the 5.0 threshold. The contact of the larvae with the rolls was variable; thus, no conclusions about larval size can be drawn from specific peaks.

Detection of Internally Infested Wheat

The detection rates for infested HRW are provided in Table III. Large, medium, and small larvae (Fig. 3) were detected at 78, 67, and 38%, respectively, using Mill(1) and 98, 94, and 78%, respectively, using Mill(2). Although mean detection significantly (P < 0.002) improved with use of Mill(2), there were problems with feeding the wheat through the mill as a result of the flat tooth design. On several occasions, the wheat kernels stopped feeding, and the motor needed to be reversed and restarted to allow the wheat to pass through, thus requiring 190–240 sec to feed 500 g of wheat through the mill. In contrast, Mill(1) operated without any feeding interruptions, and 500 g was crushed within 40 sec. This might be attributable to the sharper tooth design and faster roll rotational speed. It is possible that for wheat other tooth designs could be used with shear and a roll gap similar to Mill(2) that would facilitate more efficient feeding. However, these experiments appear to indicate that the increased detection rate for Mill(2) for wheat may not provide enough of an advantage to outweigh the feeding issues and the faster sample run time of Mill(1).

More experimentation with different roll designs is planned to improve the feeding of wheat through a mill with shear and a gap similar to that of Mill(2). A limited number of experiments with the sharp tooth rolls used in Mill(1) combined with the 1:1.4 gear ratio used in Mill(2) were performed and indicated that wheat would feed reliably with this configuration. However, a long-term study needs to be performed to determine the wear on sharp teeth used with shear before a specific combination can be recommended.

False Detections in Noninfested Rice and Wheat

False detections occur when noninfested grain is erroneously counted as infested. Because a 500-g sample of wheat or brown rice may contain 15,000 kernels, the false detection rate of any Table II. Frequency distribution of detected peaks for large, medium, and small larvae in long grain brown rice in the modified conductance mill

<table>
<thead>
<tr>
<th>Larval Size</th>
<th>Detected Count</th>
<th>No. of Infested Kernels Detected at Different Threshold Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Large</td>
<td>Accumulated</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Incremental</td>
<td>6</td>
</tr>
<tr>
<td>Medium</td>
<td>Accumulated</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Incremental</td>
<td>23</td>
</tr>
<tr>
<td>Small</td>
<td>Accumulated</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Incremental</td>
<td>23</td>
</tr>
</tbody>
</table>

*For each larval size, 144 kernels were prepared and tested.

Table III. Infested kernel detection rate for the original conductance mill (Mill(1)) versus the modified mill (Mill(2)) for hard red winter wheat (HRW)

<table>
<thead>
<tr>
<th>Larval Size</th>
<th>HRW × Mill(1)</th>
<th>HRW × Mill(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Large</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td>Medium</td>
<td>67</td>
<td>13</td>
</tr>
<tr>
<td>Small</td>
<td>38</td>
<td>14</td>
</tr>
</tbody>
</table>

*Mean = average detection rate, and SD = standard deviation of detection of infested kernels. Each size category contained 12 samples, and each sample contained 12 lesser grain borer-infested kernels, for a total of 144 infested kernels per larval size.
Table IV. Average conductance signal, maximum derivative signal \((dV/dt)\), and feed rates for the modified conductance mill for noninfested long (LBR), medium (MBR), and short (SBR) grain brown rice kernels at various moisture levels

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>Signal (mean)</th>
<th>(dV/dt) (max)</th>
<th>Feed Rate (sec/500 g)</th>
<th>Signal (mean)</th>
<th>(dV/dt) (max)</th>
<th>Feed Rate (sec/500 g)</th>
<th>Signal (mean)</th>
<th>(dV/dt) (max)</th>
<th>Feed Rate (sec/500 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.2</td>
<td>1.3</td>
<td>0.2</td>
<td>206</td>
<td>1.7</td>
<td>0.2</td>
<td>180</td>
<td>1.4</td>
<td>0.1</td>
<td>190</td>
</tr>
<tr>
<td>13.2</td>
<td>3.8</td>
<td>0.2</td>
<td>176</td>
<td>4.4</td>
<td>0.3</td>
<td>170</td>
<td>4.1</td>
<td>0.2</td>
<td>170</td>
</tr>
<tr>
<td>14.2</td>
<td>9.0</td>
<td>0.6</td>
<td>162</td>
<td>18.3</td>
<td>0.8</td>
<td>138</td>
<td>12.5</td>
<td>0.8</td>
<td>144</td>
</tr>
<tr>
<td>15.2</td>
<td>23.1</td>
<td>0.8</td>
<td>156</td>
<td>39.4</td>
<td>1.1</td>
<td>130</td>
<td>30.3</td>
<td>1.2</td>
<td>130</td>
</tr>
</tbody>
</table>

* Conductance signal mean was 0.2% while empty (no grain). Signal mean and \(dV/dt\) values are percentages of the full scale.

Considering that cereal grains such as brown rice and wheat should be stored at <14% moisture to avoid mold growth (6), the performance of the conductance mill can be considered acceptable when the grain remains at a safe moisture level. Additionally, the instrument indicates when the grain has a high moisture level that will increase the risk of mold growth during storage. It should be noted that it does not take as long to mill a high-moisture sample because the kernels become softer as moisture increases.

**CONCLUSIONS**

The modified conductance mill has the potential to detect brown rice and wheat that is internally infested with LGB; in tests Mill(2) detected 97, 83, and 42% of LBR kernels infested by large, medium, and small LGB larvae, respectively. Additionally, a 500 g sample of rice could be processed in ≈150 sec.

Brown rice is thinner and harder than wheat, requiring a narrower gap and shear between mill rolls to effectively detect infestations in rice. The modified mill is best suited for detection of internal infestation in rice. While the detection rate for wheat was higher in the modified mill, there were grain feeding problems that should be considered before using the modified mill for wheat.

The system is vulnerable to false detections resulting from the presence of soil clumps or mishandling. However, positive detections by this instrument should make the user wary of the presence of soil clumps or mishandling. Additionally, a 500 g sample of rice could be processed in ≈150 sec.

Considering that cereal grains such as brown rice and wheat should be stored at <14% moisture to avoid mold growth (6), the performance of the conductance mill can be considered acceptable when the grain remains at a safe moisture level. Additionally, the instrument indicates when the grain has a high moisture level that will increase the risk of mold growth during storage. It should be noted that it does not take as long to mill a high-moisture sample because the kernels become softer as moisture increases.

**Acknowledgments**

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**References**