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 slipping; as a result, the benefit of increased accuracy might not outweigh potential feeding issues.

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http://dx.doi.org/10.1094/CPLEX-2012-0316-01R

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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 × 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. Larger 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 × 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).

![Fig. 1. Photograph of a conductance mill. The modified mill is shown, but the original mill is nearly identical.](image1)

![Fig. 2. Schematic of a conductance mill. The basic electronic circuit created between the rolls and the grain is used to detect infested kernels of rice.](image2)

![Fig. 3. Roll teeth and gap geometry for the original conductance mill (left) and the modified mill (right). The shearing action of the modified mill reduces the space between the rolls, enabling more larvae to be detected.](image3)
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 lbf·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-raying 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.
Computer and Software for Monitoring Conductance Signals

Data collection and analysis were accomplished using a laptop computer and software provided by the manufacturer of the conductance mills (National Mfg.). The software allows the user to specify certain parameters for data smoothing and counting infested kernels. In this study, the slope generator parameter was set at 10, and the slope threshold constant was set at 2%. The slope generator parameter is used to smooth the signal to make the signal produced by infested kernels more distinguishable. The first derivative of the crushed grain signal is used to determine the potential infested kernels, as described by Pearson and Brabec (10). The slope threshold constant is the threshold value for classifying a peak in the derivative signal as an infested kernel and is given as a percentage of the full scale. The 2% level was used with the rice data to maximize true detections and minimize false detections. The software also contains features for motor control and automatic shut off. The mill was allowed to run empty for 10 sec after the material finished passing through the mill to allow the mill and motor to cool and to help remove any residual grain that might be stuck to the mill rolls.

Testing the Effects of Grain Moisture

Electrical conductance through the grain, as measured by the mill, is somewhat logarithmically proportional to the moisture content of the grain. This characteristic was the basis of the concept of the Tag Heppenstall moisture meter (5). At lower moisture levels, the conductance of the grain is low. As seen in the current study, the conductance signal of the grain became more variable as the grain moisture content increased. At 15% moisture, the ability to distinguish between infested kernels and high-moisture grain diminishes because the added moisture from the crushed larvae can be relatively small compared with the relatively high conductance signal. The bandwidth of the conductance signal increases as moisture content increases, and in addition, the bandwidth of its derivative signal \( (dV/dt) \) increases. This effect requires higher values for the \( dV/dt \) threshold level if the grain sample moisture could be 14% or higher to ensure that noninfested, higher moisture kernels are not counted as infested kernels.

To measure the effect of grain moisture on the conductance signal and its derivative signal, LBR, MBR, SBR, and HRW samples were conditioned to 12, 13, 14, and 15% moisture. The moisture levels were determined using the 1 hr ground-grain and oven-air method (AACCI Approved Method 44-15A [1]). Each grain moisture level was tested six times using a 250 g sample per test. The conductance signal averages were calculated. Additionally, the maximum peak height of the conductance signal derivative was recorded so a safe threshold level could be selected for classifying derivative signal peaks as infested kernels.

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>Detection Rate (%)</th>
<th>LBR × Mill(1)</th>
<th>Mean</th>
<th>SD</th>
<th>LBR × Mill(2)</th>
<th>Mean</th>
<th>SD</th>
<th>MBR × Mill(2)</th>
<th>Mean</th>
<th>SD</th>
<th>SBR × Mill(2)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>61</td>
<td>11</td>
<td>97</td>
<td>5</td>
<td>97</td>
<td>4</td>
<td>98</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>22</td>
<td>14</td>
<td>83</td>
<td>1</td>
<td>10</td>
<td>89</td>
<td>11</td>
<td>11</td>
<td>89</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>4</td>
<td>7</td>
<td>42</td>
<td>11</td>
<td>40</td>
<td>11</td>
<td>57</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></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.

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 infested kernels were detected by computer software.
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 de-
tected. 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 in-
dicator 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 lar-
va 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 signifi-
cantly (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 re-
started 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 pos-
sible 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 indi-
cate 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 experi-
ments with the sharp tooth rolls used in Mill(1) combined with
the 1:1.4 gear ratio used in Mill(2) were performed and indi-
cated that wheat would feed reliably with this configuration.
However, a long-term study needs to be performed to deter-
mine 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
insect-infested kernel detection system must be extremely low.
Typical infestation rates are fewer than 20 infested kernels per
2.75 kg sample (12). Thus, more than one or two false detec-
tions per sample can render a method ineffective. When the
slope threshold was set at 2%, 2 of 36 control samples each had
one false detection (one in SBR and one in MBR). When the
slope threshold was lowered to 1.5%, 5 of 36 control samples
had one false detection. In this case, false detections were ob-
served in the two MBR samples and the three SBR samples,
with no false detections observed in the LBR control samples.

One source of false detection is contamination with a small
piece of soil. Thus, all samples should be sieved with a coarse
sieve to remove any soil. This step can be accomplished by pass-
ing the sample through a dockage tester or using a separate
sieve (e.g., Tyler no. 12 sieve).

Another potential source of error is moisture added to the
outside of kernels, such as happens when collecting a sample
under rainy or snowy conditions or handling grain with wet
hands. Thus, grain samples should be handled in a manner that
will not increase moisture prior to testing. Plant materials and
foreign kernels that had a chance to equilibrate to the same
moisture content as the sample were not found to be a source
of false detections.

A third type of false detection was noted when samples with
large larvae were tested. On several occasions, a large larva
would create multiple peaks. For example, sometimes 13 peaks
were detected when only 12 infested kernels were present. Ad-
ditional software code was added to avoid counting peaks
within 150 msec of a positive detection. However, this change
did not completely solve the problem, and other possible solu-
tions are being evaluated.

Performance of Conductance Mill(2) at Varying
Rice Moisture Contents

The average electrical conductance and range of derivative
signals for rice at various moisture levels are shown in Table
IV. As the moisture content increased, the average conductance
signal and noise on the derivative signal also increased. At
14.2% moisture, the maximum derivative signal (dV/dt) for all
three types of rice was 0.8% of the full scale. This value was
slightly less than half the threshold of 2% used for classifying
peaks as infested kernels. Thus, the 2% threshold was a safe
level for all grain moisture contents <14.2%. Although the de-
ivative signal did not exceed 1.2% of full scale at 15.2% mois-
ture, the ability of the instrument to detect infested kernels was
greatly diminished because the average conductance signal be-
came highly elevated (23.1–39.4%), such that any peak created
by the moisture of an insect was obscured by the high grain
moisture. Therefore, the detection rate of the conductance mill
would be decreased for rice at a moisture level of 15.2%. How-
ever, using a 2% slope threshold should avoid false detections
with 15.2% moisture rice as well.

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>No. of Infested Kernels Detected at Different Threshold Levels</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Large Accumulated</td>
<td>136</td>
<td>130</td>
<td>109</td>
</tr>
<tr>
<td>Large Incremental</td>
<td>6</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>Medium Accumulated</td>
<td>117</td>
<td>94</td>
<td>72</td>
</tr>
<tr>
<td>Medium Incremental</td>
<td>23</td>
<td>22</td>
<td>36</td>
</tr>
<tr>
<td>Small Accumulated</td>
<td>59</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Small Incremental</td>
<td>23</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

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>Detection Rate (%)</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>

* For each larval size, 144 kernels were prepared and tested.
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 sample and encourage further analysis of the grain for insect infestation before long-term storage or shipping to consumers. The modified mill provides storage managers with a new tool to detect internally feeding insects in stored rice, thereby reducing losses resulting from insect feeding and unnecessary fumigation. In addition, the instrument has the potential to allow processors to deliver a safer and higher quality product to the consumer.

Acknowledgments

We thank Ken Freisen and Ann Redmon for their support in caring for and managing the insect colonies. In addition, we thank Mark West (USDA-ARS Northern Plains Offices) for statistical support.

References