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Research

Stored-Product

Performance of a Low-Cost Acoustic Insect Detector System with *Sitophilus oryzae* (Coleoptera: Curculionidae) in Stored Grain and *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Flour

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Abstract

Reduction of postharvest losses is gaining increased priority in warm regions where insect infestation may cause rapid deterioration of staple commodities. Acoustic detection can be used to assess the likelihood of insect infestations in bags of grain, flour, and other commodities that are stored in small holdings in developing countries, enabling rapid targeting of treatments. A portable postharvest insect detection system was developed with the goal to provide low-cost capability to acoustically assess infestations in small-scale storage facilities. Electret microphones input pest insect sounds to a 32-bit microcontroller platform that digitized and stored the signals on a digital memory card transferable to a portable laptop computer. The insect sounds then were analyzed by custom-written software that matched their spectra to those of known pests. Infestations of *Sitophilus oryzae* (L) in 2.6-kg bags could be detected down to densities of 1.9 adults/kg in grain and *Tribolium castaneum* (Herbst) down to 3.8 adults/kg in flour in laboratory settings. Also, differences in the rates of sounds per insect in treatments with different numbers ranging from 5 to 50 insects suggested that the sound rates of adults of different species at different population densities may be noticeably affected by aggregation pheromones or other behaviorally active semiochemicals. Further testing is needed but previous experience with acoustic detection systems suggests that the prototype has potential for use in small storage facilities where early detection of infestations is difficult to provide.

Key words: food security, postharvest, infestation likelihood

Stored product insect pests cause 20–30% or more economic loss to staple commodities yearly in developing countries (Mwololo et al. 2010, George et al. 2011, Wijayaratne et al. 2018). Harvest handling processes, transportation, and proper storage methods often are inadequate, and are among the most important biotic factors causing postharvest loss (Kumar and Kalita 2017, Abass et al. 2018, Quellhorst et al. 2020). The long-term prevalence of such losses led the United Nations (FAO 1983) to urge that greater priority should be given to postharvest loss reduction. The Covid-19 pandemic is now exacerbating continued prevalence of losses by increasing the occurrence of transportation delays, allowing pests to propagate within delayed shipments (FAO 2020).

Local and global efforts towards reduction of pest-insect postharvest losses in developing countries currently include general treatments such as hermetic storage, controlled atmospheres, and phosphine fumigation (Murdock and Baoua 2014, Kumar and Kalita 2017, Njoroge et al. 2019a, b) as well as targeted postharvest treatments, such as heat exposure against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Yan et al. 2014) and essential oils against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Mondal and Khalequzzaman 2006), and preharvest treatments such as entomopathogenic fungi and botanicals against aphids (Abebe et al. 2020). Monitoring is performed, when feasible, to reduce treatment costs by quickly identifying and targeting infestations

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when and where they begin. Effective monitoring methods include visual inspection, random sampling, use of pheromone traps, and acoustic monitoring (Njoroge et al. 2019a, b).

Acoustic methods of detecting insects in stored products have been available since the 1920s (Schwab and Degoul 2005, Mankin et al. 2011, Njoroge et al. 2019a) but have not become widespread, due partly to the high costs of most commercially available equipment. The AED-2000L and 2010L, two portable acoustic systems used frequently to detect stored product insects (Potamitis et al. 2009; Eliopoulos et al. 2015, 2016; Kiobia et al. 2015; Inyang et al. 2019; Njoroge et al. 2019a, b), ceased production in 2019 and had cost over 2,000 USD apiece. Alternatives have been developed, e.g., Mankin et al. (2010), Eliopoulos et al. (2018), Potamitis et al. (2019), Njoroge et al. (2019a), but remain most readily available in developed countries.

Recently, a prototype was developed for a portable Postharvest insect Detection System (PDS, Custom Engineered Solutions, West Hempstead, NY) (Fig. 1) with the goal to determine whether low-cost contact microphones and microcontroller systems can be incorporated into robust devices for small-scale use in regions where postharvest insect infestations develop rapidly and cause severe economic loss. The PDS collects acoustic signals from a microphone embedded in a Plexiglas base and stores them for digital signal analyses. Bags of postharvest commodities can be placed on the base to assess insect infestation. To conduct an initial assessment of the PDS performance as a low-cost postharvest insect detection system, bags containing insect-infested grain or flour were acoustically monitored and analyzed by previously developed digital signal processing methods as a means of estimating the likelihood of infestation. The bags contained adult *S. oryzae*, an important pest in stored grain (Lee et al. 2001, Yan et al. 2014) and the flour contained adult *T. castaneum*, an important pest in flour mills (Campbell and Arbogast 2004).

Materials and Methods

Insect Rearing and Treatment Combinations

Adult *S. oryzae* and *T. castaneum* were obtained from colonies reared at CMAVE (Mankin et al. 1999) in incubators at 25.1 ± 1°C, 50 ± 5% RH. The *S. oryzae* were reared on organic soft wheat (*Triticum aestivum* L.) grain kernels (Purcell Mountain Farms, Moyie Springs, ID). The *T. castaneum* were reared on wheat flour (Fresh Harvest, Clarkston, GA), organic medium white corn meal (Palmetto Farms, Ferry, SC), and Brewer's Yeast (Puritan's Pride, Oakdale, NY) mixed in ratios of 2:2:1 by weight. For exploratory studies, storage bags with different amounts of grain or flour and different numbers from 0 to 100 insects were tested. This led to tests of several treatments of different numbers of *S. oryzae* on wheat grain and *T. castaneum* on flour for this study, primarily using polyethylene, 26.8 by 27.3 cm zipper-locking storage bags (International Plastics, Greenville, SC) filled with 2.6 kg of wheat grains or flour. A combination of an insect-free bag containing 2.3 kg of wheat grains was included to provide additional grain control replications. Combinations of 0, 5, 10, 25, or 50 *S. oryzae* were added to grain treatment bags and likewise, *T. castaneum* to flour. In all cases, the wheat and flour had been purchased and stored in a freezer at −17.8°C for at least 6 mo to ensure no other live insects were present when testing began (Flinn et al. 2015). To reduce interference from background noise, the bags were placed on the Plexiglas base connected to the PDS inside a sound- and vibration-shielded room described previously by Vick et al. (1988a). The temperature and humidity conditions in the shielded room were approximately 26 ± 1°C, 50 ± 5% RH. There were three replicates of each treatment combination.

Signal Collection and Processing

The PDS (Fig. 1) included a 32-bit microcontroller with a 10-kHz, 16-bit analog to digital converter, and an amplifier with 40–80 dB user-adjustable gain (ATSAMG54, Microchip Technology, Inc., Chandler, AZ). The microcontroller received input from an electret microphone (Model WM-63GNT, Panasonic Corp., Newark, NJ) embedded at the center of a 30 × 30 × 0.635 cm Plexiglas base. The signal was band-pass filtered between 20 and 5,000 Hz to reduce noise and avoid aliasing. The PDS stored the collected signals on a removable, commercially available Secure Digital (SD) memory card from which the .wav files were transferred to a laptop or desktop computer for further processing.

Sounds were collected from each treatment bag replicate for a 20-min period after the bag was placed on the Plexiglas base, first allowing 20 min or more resting time for grain settling sounds to decrease to negligible levels (Mankin et al. 1999). Bags of flour were not observed to exhibit significant rates of settling sounds, but the same waiting period was observed to allow for mitigation of any effects of disturbance on *T. castaneum* behavior. The recording was prescreened with Raven Pro Software (Charif et al. 2008) to ensure that minimal background noise was present, preventing interference with analysis of insect-produced sounds.

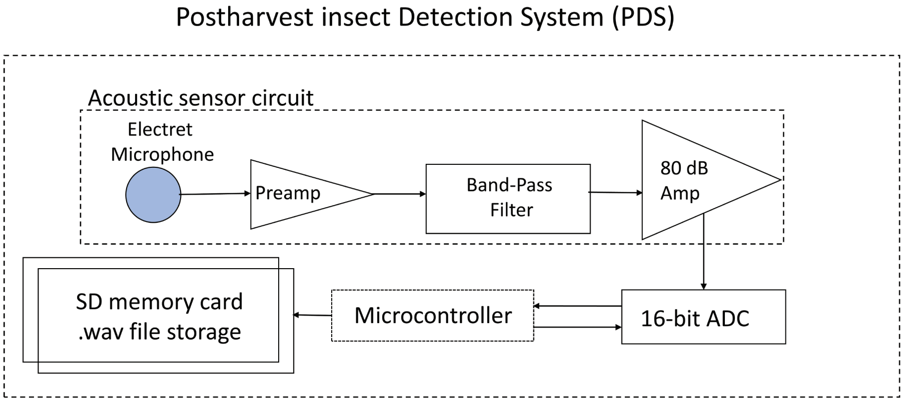

Fig. 1. Diagram of Postharvest insect Detection System (PDS) showing microphone, preamplifier (Preamp), 20–5,000 Hz band-pass filter, 40–80 dB user settable amplifier (Amp), 16-bit Analog to Digital converter (ADC), Microcontroller, and removable, Secure Digital (SD) memory card system for sound file storage.

During exploratory studies with bags of each species, 1-min intervals were identified which contained insect sounds without significant background noise. A 1-min interval recorded from each species was used to generate a mean spectral profile that was applied to discriminate the sounds of that species from background sounds using DAVIS, a custom-written insect sound analysis program (Mankin et al. 2011, Dosunmu et al. 2014). In subsequent tests with treatment bags, sounds were deleted from further analysis if the summed least-square differences of their spectral profiles from the mean spectral profile over frequencies between 500 and 2,500 Hz were greater than a predetermined threshold based on user assessment of the Raven prescreening (Mankin et al. 2011). The times of sounds that met the threshold criterion were saved in a spreadsheet for analysis of the numbers of insect sounds per second, r_s, as in Mankin et al. (2011).

Additional analyses considered the rates of insect sounds in relation to the numbers of insects placed into a bag for testing. One of the goals of the study was to use the magnitude of r_s measured from a bag as an indicator of the likelihood that the test bag was infested (Mankin et al. 2007, 2008). Previously it was determined that acoustic indicators for infestation likelihood could be set as *low* if $r_s < 0.02$ s^{-1}, *medium* if 0.02 s$^{-1} \le r_s < 0.06$ s^{-1}, and *high* if $r_s \ge 0.06$ s^{-1} for wood-feeding insects (Mankin et al. 2008). The magnitude of r_s is highly variable due to effects of physiological state, temperature, and other factors (Mankin et al. 2007, 2011). The mean rates of sounds produced by insects in stored products is proportional to the number of insects present although the relationship has a large standard deviation, e.g., Njoroge et al. (2017). Consequently, the use of indicators for the likelihood of infestation often provides a more easily interpretable assessment of infestation presence or absence than direct use of sound rate alone (Mankin et al. 2007).

Statistical Analyses

The data from all treatments were combined for comparisons of actual infestation with the infestation likelihoods predicted from rates of insect sounds. The actual and predicted likelihood of infestation in each treatment were compared using a Wilcoxon test in Proc Univariate (SAS Institute 2013). The normality of the distribution of sound rates per insect across treatments was tested also using Proc Univariate (SAS Institute 2013).

Results

Signals Recorded from *S. oryzae* in Grain and *T. castaneum* in Flour

Two 13.2-s periods of signals recorded from 100 *S. oryzae* adults (Fig. 2) and 50 *T. castaneum* adults (Fig. 3) are representative of oscillograms and spectrograms recorded from the two species during the study. The primary differences among tests with different numbers of each species were in the rates of sounds. Figure 3 is an example that contains both background noise and insect sounds. Two high-amplitude impulses marked by a dashed box in Fig. 3 were classified as background noise by the DAVIS analysis, and the other impulses were classified as *T. castaneum* sounds. Individual sound impulses produced by movement and feeding activity of both species in their respective substrates were 2–3 ms in duration and had peak energy between 0.5 and 2.5 kHz (Figs. 2 and 3B).

The mean spectral profiles calculated to distinguish *T. castaneum* and *S. oryzae* sounds from background noise are shown in Fig. 4. As in the spectrograms of Figs. 2 and 3B, the greatest signal energy was between 0.5 and 2.5 kHz.

Insect Sound Rates in Relation to Infestation Likelihood

The means of sound rates measured from bags with treatments of different numbers of *S. oryzae* on grain and *T. castaneum* on flour were assessed in Table 1 relative to previously determined sound rate ranges that have been used as indicators of infestation likelihood (see section on Signal Collection and Processing). A Wilcoxon test confirmed that the use of such indicators provided statistically significant assessments of infestation for *S. oryzae* and *T. castaneum*. The results suggested that *S. oryzae* at a density of 1.9 insects/kg or *T. castaneum* at a density of 3.8 insects/kg can be easily detected by the PDS system (i.e., the mean rate exceeded 0.06 sounds/s, indicating a *high* likelihood of infestation), and the threshold for detection of *S. oryzae* is lower than 1.9 insects/kg. In this study, only one failure to detect an infestation occurred, that of the combination

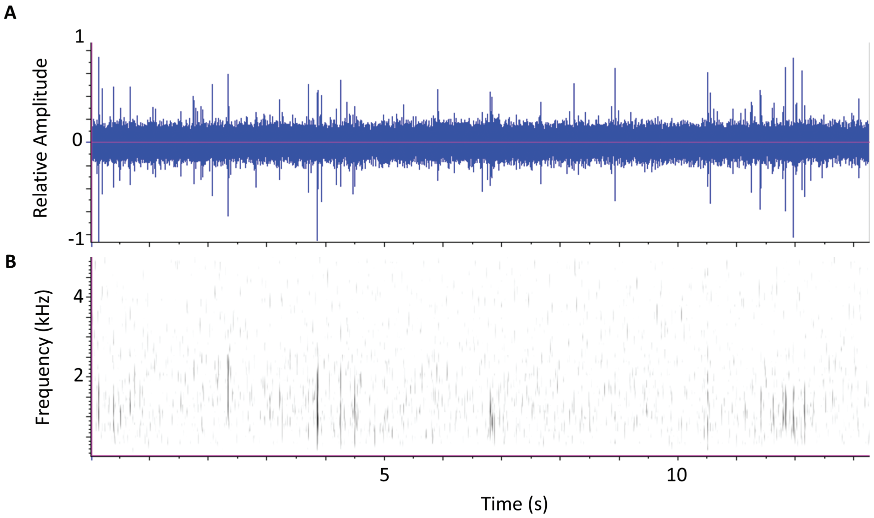

Fig. 2 (A) Oscillogram and (B) spectrogram of signals recorded from 100 *Sitophilus oryzae* adults in a bag of grain. Darker areas in the spectrogram (analyzed at 128 points/spectrum) indicate greater energy at specific frequencies and times.

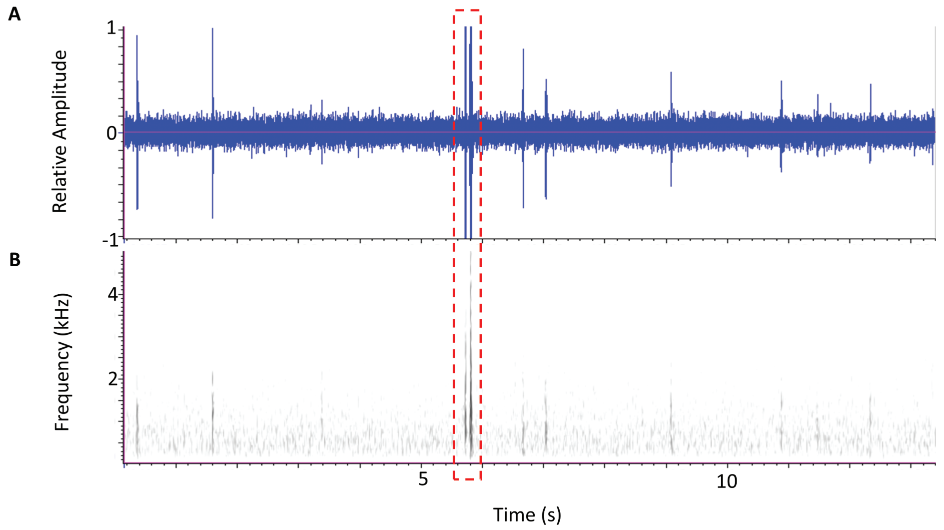

Fig. 3 (A) Oscillogram and (B) spectrogram of signals recorded from 50 *Tribolium castaneum* adults in a bag of flour. Two sounds marked by the dashed box were classified by the DAVIS automated signal analysis program as background noise. Darker areas in the spectrogram (analyzed at 128 points/spectrum) indicate greater energy at specific frequencies and times.

Table 1. Mean rates of sounds produced in treatment combinations containing 0, 5, 10, 25, or 50 *Sitophilus oryzae* (SO) in grain or *Tribolium castaneum* (TC) in flour, and sound-rate-based assessments of infestation likelihood

Combination (insect sp: grain/flour)	No. insects	Density (insects/kg)	Mean no. sounds/s	Infestation Likelihood[a]
SO: Grain	50	19.2	14.19	high
SO: Grain	25	9.6	2.24	high
TC: Flour	50	19.2	1.88	high
SO: Grain	5	1.9	1.66	high
TC: Flour	10	3.8	1.39	high
SO: Grain	10	3.8	1.26	high
TC: Flour	25	9.6	0.26	high
TC: Flour	5	1.9	0.004	low
Grain	0	0	0.002	low
Grain[b]	0	0	0.002	low
Flour	0	0	0.002	low

[a] A Wilcoxon test was performed to compare the distributions of infestation likelihood values for infested ($n = 8$) and uninfested ($n = 3$) treatment combinations ($n = 3$ replications per combination). The infestation likelihood distributions were found to be significantly different: statistic S = 7.5, $Z = -2.4398$, $P > |Z| = 0.0147$.

[b] Combination contained no insects in 2.3 kg of grain.

of 5 *T. castaneum* adults in a 2.6 kg bag of flour. No combinations tested in this study had a likelihood of infestation assessed as *medium*, between *low* and *high* infestation likelihoods.

Distributions of *S. oryzae* and *T. castaneum* Sound Rates per Insect

The sound rates in the different treatment combinations had been expected to be species-dependent and proportional to the number of insects in each bag, as noted in the Signal Collection and Processing section. Figure 5 shows the ranges of distributions of insect sounds/insect/s for the treatment combinations of 5, 10, 25, and 50 *S. oryzae* in grain and the combinations of 10, 25, and 50 *T. castaneum* in flour that had been assessed at high likelihood of infestation in the previous section. The vertical axis is on a Log scale to highlight the relative difference in the range of magnitudes of sounds/insect/s of the two species at the low end of the range. The range for *T. castaneum* is considerably larger than for *S. oryzae*, due primarily to several low magnitudes of No. sounds/insect/s for *T. castaneum* at intermediate densities, and is considered further in the discussion. The magnitudes range from 0.45 to 0.07 sounds/insect/s for *S. oryzae*, a factor of 6.4 between lowest and highest measurement, and the range was 0.26–0.02 sounds/insect/s for *T. castaneum*, a factor of 13 between lowest and highest. The magnitudes were not normally distributed (Shapiro–Wilk W = 0.905, with $P < W = 0.0433$).

Discussion

A primary goal of the study was to explore portable, robust, inexpensive alternatives to existing insect acoustic detection systems that could be used by regulatory agencies, agricultural extension personnel, grain traders, or warehouse managers in developing countries to identify insect infestations during storage. Capacitive microphones, sometimes used with acoustic couplers, and associated amplifiers have been the gold-standard of detection systems for airborne since the 1960s due to their high fidelity and sensitivity (Mankin et al. 2011). Less expensive electret microphones like those in this study (Lujo et al. 2016, Mankin et al. 2016) and laser vibrometers (Gordon et al. 2019) have been used to detect substrate-borne vibrations (Hill et al. 2019) including those produced by insects in grain and flour underlain by a solid Plexiglas plate. Piezoelectric sensors, some of which are inexpensive, also work well to detect sounds in solid substrates along with accelerometers, geophone systems, and low-cost magnetic cartridges (Shuman et al. 1993, Mankin et al 2011, Lima et al. 2020). Previous studies have found that the rates of insect sounds recorded by microphone-based systems are proportional to those recorded by piezoelectric-sensor-based systems when the recordings are collected simultaneously from the same bags of grain (Njoroge et al. 2019a). The prototype system has been demonstrated in this study to detect important stored product insect pests over an informative range of densities (1.9–19.2 *S. oryzae* per kg in grain and 3.8–19.2 *T. castaneum* per kg in flour) under quiet conditions (Table 1). The full range of detectability remains to be determined in

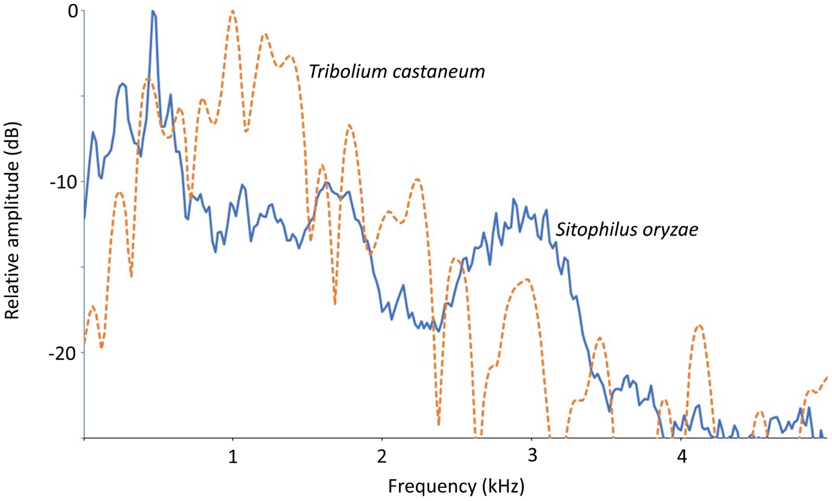

Fig. 4. Mean spectral profiles: 10 *Sitophilus oryzae* adults moving and feeding on wheat during a 60 s interval (solid line), and 25 *Tribolium castaneum* adults moving and feeding in flour during a 60 s interval (dashed line).

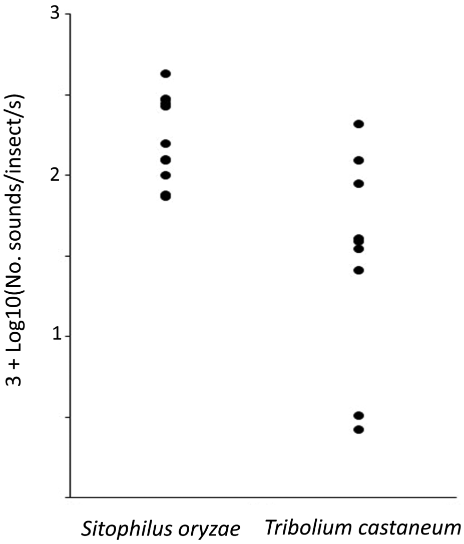

Fig. 5. Distribution of *Sitophilus oryzae* and *Tribolium castaneum* sound rates per insect from treatment combinations with different numbers of insects.

different storage contexts. It should be noted with respect to insect density that in the United States, Federal Grain Inspection Standards indicate that 0 or 1 insect/kg of grain is acceptable for human consumption (Shuman et al. 1993). If 1.9, i.e., 2 insects/kg can be detected, this implies that the sensitivity of the detection system may suffice to detect *S. oryzae* at the minimum densities considered to be an infestation.

The mean spectral profile for *S. oryzae* in Fig. 4 shares similarities with the different mean profiles that Kiobia et al. (2015) reported for different types of *S. oryzae* mean spectra below 5 kHz in maize. In addition, it shares similarities with the spectrum determined in this report from *T. castaneum* sounds. The Kiobia et al. (2015) spectra extended to higher frequencies because they had been digitized at 44.1 kHz, enabling spectra to be calculated up to 22.05 kHz before the necessity of filtering to avoid artifacts from aliasing (Inyang et al. 2019). The system used by Kiobia et al. (2015) is highly sensitive to insect sounds over a large range from 1 to 22.05 kHz. Also, the *S. oryzae* and *T. castaneum* signals in Figs. 3 and 4 were similar to those displayed by these species in Geng and Shang (2006). The similarities among the spectra of sounds produced by these and other insects documented in the literature suggest that inexpensive detection systems like the PDS have sufficient sensitivity to be useful for detection of a variety of different stored product insect pests like the more expensive microphone- and piezoelectric-based systems that have been used for two decades.

Observations of the behaviors of the *S. oryzae* and *T. castaneum* as they were being monitored suggested that the two species may have somewhat different behavioral reactions to conspecifics at different population densities. Thus, it was of interest that the magnitudes of sound rates per insect varied across a larger range for *T. castaneum* than for *S. oryzae*, 13 versus 6.4, as noted in the results. Both species are known to have aggregation pheromones that affect their movements and orientation towards conspecifics (Phillips and Burkholder 1981, Suzuki et al. 1984, Athanassiou et al. 2006, Dissanayaka et al. 2020), but *S. oryzae* females may orient more strongly to food odor than pheromone (Likhayo and Hodges 2000, Togola et al. 2014). *Tribolium castaneum* may emigrate when overcrowding causes food deterioration (Faustini and Burkholder 1987). Increased levels of searching behavior or emigration behaviors that adults of different species may or may not perform in different contexts can affect the rate of sounds produced and thus the detectability of different insect species in storage bags. The two lowest values in the *T. castaneum* treatment measurements in Fig. 5 were from replicates with 25 insects, where there was opportunity for reduced levels of movement in the presence of aggregation pheromone. Behavioral interactions like these would play a lesser role in the rates of sounds produced by *S. oryzae* larvae that feed individually inside grain kernels (Shuman et al. 1993) and have only minor interactions with conspecifics.

It should be noted also that differences in the structure of the storage media, e.g., grain kernels and flour, also play a role in sound transmission and thus in the acoustic detectability of different insect species (Mankin et al. 2011). Improved knowledge of variability of behaviors such as those above and others that may be of importance for a pest species of special interest can assist development of appropriate acoustic indicators for infestation likelihood in different storage contexts.

The results in Fig. 5 and Table 1 serve as examples where use of acoustic indicators can reduce the need for high numbers of replications in insect detection field studies where it may be difficult to collect large numbers of samples. In Fig. 5, the rate of sounds per insect varied over a considerable range of magnitudes, 6.4 to 13, depending on the *S. oryzae* and the *T. castaneum* behavioral variation noted earlier. To visualize how such variation might affect predictions about infestation likelihood such as those in Table 1, consider that, if the relationship between sound rate and insect number were fixed precisely, an observed magnitude of 14 sounds/s for 50 *S. oryzae* in grain 'predicts' 7/s for 25, 2.8/s for 10, and 1.4/s for 5 *S. oryzae*. In Table 1, the measured mean sound rate was lower than 'predicted' for 25 and 10, but higher for 5 *S. oryzae*. More replications likely would smooth out the relationship between insect numbers and sound rates, but in this case all the measurements were correctly assessed at a *high* likelihood of infestation. Similarly, in the case of a precise relationship, the observed magnitude of 1.9 sounds/s for 50 *T. castaneum* in flour 'predicts' a rate of 0.95/s for 25 and 0.38/s for 10 *T. castaneum*. In Table 1, the mean rate was lower than 'predicted' for 25 but higher for 10 *T. castaneum* although additional replications might have smoothed out the observed results. Nevertheless, as in the case with *S. oryzae*, all the *T. castaneum* sound rate measurements resulted in correct assessments of *high* infestation likelihood.

Numerous questions remain whether systems like the PDS will operate well enough for successful use in rugged environments with higher background noise. Indeed, background noise was initially a problem in the Kenyan warehouses during the Njoroge et al. (2019a) study, but temporary reductions in human and mechanical activity approved by the warehouse managers enabled assessment of storage bag infestations. Each warehouse facility has noise of different magnitudes from different sources and there will be times when background noise levels will overwhelm the insect-produced signals although incorporation of improved algorithms based on temporal-envelope or energy-based analysis can bring the capability of insect sound signal processing software closer to the capability of the human ear to detect signals of interest in background noise (Jennings and Chen 2020). If high noise levels are constantly present, a noise-shielded chamber could be constructed to reduce the background noise sufficiently for insect detection (Vick et al. 1988a).

Since the initial testing of the insect detection device, there have been several suggestions for improvements, including extension of testing to consider the detectability of internally feeding larvae in grain, increasing the numbers of sensors up to the maximum (eight) available to the microcontroller, removing or otherwise customizing the Plexiglas base to meet the needs of a specific environment, and transferring more signal processing from a laptop computer to the microcontroller, especially if such systems ultimately would be operated from cell phones (Mukundarajan et al. 2017, Lima et al. 2020). The duration of sampling could be reduced to periods as small as 3–5 min, but movement and feeding activities of stored product insects are not continuous and recording periods less than 3–5 min would result in reduced accuracy of assessment (Vick et al. 1988b). Implementation of further improvements would depend on availability of robust components, cost, and the needs of regulatory agencies, extension agents, and other users (Kumar and Kalita 2017). As noted in the introduction, there is longstanding need for reduction of pest-insect postharvest losses, but acoustic and other monitoring of pest insects is not likely to be performed if the instrumentation is not readily available, field-effective, and low-cost.

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Author Contributions

B.R., E.J., M.Y., and R.W.M. conceived and designed the experiments; E.J. and M.Y. performed the experiments; E.J., M.Y., B.R., and R.W.M. analyzed the data; R.W.M. and B.R. contributed acoustic measurement equipment; B.R., E.J., M.Y., and R.W.M. wrote the paper; R.W.M. secured the funding.

Conflicts of Interest

The authors declare no conflict of interest.

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