



# Frequency and time pattern differences in acoustic signals produced by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in stored maize



A.W. Njoroge<sup>a,b,\*</sup>, H. Affognon<sup>c</sup>, C. Mutungi<sup>a</sup>, B. Rohde<sup>d</sup>, U. Richter<sup>b</sup>, O. Hensel<sup>b</sup>, R.W. Mankin<sup>e</sup>

<sup>a</sup> International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya

<sup>b</sup> University of Kassel, Faculty of Agricultural Engineering, Nordbahnhofstraße 1a, 37213, Witzenhausen, Germany

<sup>c</sup> International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), BP 320, Bamako, Mali

<sup>d</sup> Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL 32611, USA

<sup>e</sup> U S Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608, USA

## ARTICLE INFO

### Article history:

Received 30 December 2015

Received in revised form

6 June 2016

Accepted 10 June 2016

### Keywords:

Detection

Sensor

Pest management

Postharvest

## ABSTRACT

Frequency spectra and timing patterns of brief, 1–10 ms broadband sound impulses produced by movement and feeding activities of *Prostephanus truncatus* and *Sitophilus zeamais* last instars and adults in maize were investigated to find spectral and temporal pattern information useful for distinguishing among these species and stages. The impulse spectra were categorized into five different types of frequency patterns (profiles), designated Broadband, HighF, MidF1, MidF2 and LowF to indicate differences in their peak energies and broadness of frequency range. Groups (trains) of three or more closely spaced impulses, termed bursts, were observed to occur frequently in all recordings, as has been reported for sounds produced by other insects. Mean rates of bursts, mean counts of impulses per burst, and mean rates of impulses in bursts were calculated and compared among the two species and stages. The counts of broadband and MidF2 impulses per burst and the rates of broadband and MidF2 impulses in bursts were significantly different for adult than for 4th instar *S. zeamais* and either stage of *P. truncatus*. These findings can be useful in developing an acoustic sensor system for automated detection of hidden insects including *P. truncatus* and *S. zeamais* in bulk storage warehouses. The findings are discussed in relation to different movement and feeding behavior patterns that have been identified in these important pests.

© 2016 Elsevier Ltd. All rights reserved.

## 1. Introduction

In sub-Saharan Africa, maize is a key source of nutrition and incomes for individual farmers, grain traders and feed manufacturers. Consequently, the harvested crop is often stored seasonally to smooth out inter-seasonal supply fluctuations, for marketing timing, or for other strategic reasons (Stephens and Barrett, 2010). However, insect pests are a major constraint to grain storage in many parts of the world (Abebe et al., 2009). Under the current global grain trade standards, the grain industry maintains a “nil

tolerance” for live insects as a means of ensuring all grain is compliant with feed and food safety requirements (Neethirajan et al., 2007). Often, insect absence is checked visually on a representative sample withdrawn from the lot. A disadvantage of visual examination is the inability to detect hidden infestations by pre-emergent stages of the pests, whose population may be many times higher than free-living adults (Fleurat-Lessard, 1988).

The larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) are the main damaging storage insect pest for maize (García-Lara and Bergvinson, 2013) regionally. The *P. truncatus* is more injurious, and in endemic situations, extensive grain damage results in over 30% dry weight loss losses (Farrell and Schulten, 2002; Borgemeister et al., 2003).

\* Corresponding author. P.O. Box 30772-00100, Nairobi, Kenya.

E-mail addresses: [anjoroge@icipe.org](mailto:anjoroge@icipe.org), [annwanjiru608@gmail.com](mailto:annwanjiru608@gmail.com) (A.W. Njoroge).

Because *P. truncatus* and *S. zeamais* larvae remain hidden inside the maize kernels, early detection of these species is an important concern in stored maize. Detection methods that determine presence, absence and magnitudes of these hidden infestations are useful for enabling mitigatory actions before economically significant damage become evident (Fleurat-Lessard et al., 2006; Leblanc et al., 2011; Mankin and Hagstrum, 2011). Early detection can inform when to apply pesticides, fumigate or dispose of the grain.

Acoustic devices enable non-destructive, automated detection, and monitoring of insect infestations in grain (Fleurat-Lessard, 1988; Weinard, 1998; Gobernado et al., 2005; Schwab and Degoul, 2005; Eliopoulos et al., 2015), including pre-emergent stages of postharvest pests (Mankin et al., 2011; Kiobia et al., 2015). These devices can be incorporated in continuous insect pest surveillance during storage and at ports of exit/entry during grain trade. To the best of our knowledge, no past research has been conducted on the acoustic detection and monitoring of *P. truncatus*, probably because the pest is a fairly recent invasive species that was for long regarded as an occasional pest for maize, and whose distribution is not widespread (Boxall, 2002). However, considerable research has been conducted on its close cousin *Rhyzopertha dominica* (lesser grain borer) on wheat (Hagstrum et al., 1988, 1990; Hagstrum and Flinn, 1993). Similarly, little research work has been conducted on the acoustic detection of *Sitophilus zeamais* although considerable research exists on the rice weevil *Sitophilus oryzae* (L.) and the granary weevil *Sitophilus granarius* (L.) (Mankin et al., 1996, 1999; Schwab and Degoul, 2005; Pittendrigh et al., 1997; Potamitis et al., 2009). Such studies have determined that internally feeding larvae in grain produce movement and feeding sounds of relatively low intensity, 15–35 dB/ref: 20  $\mu$ Pa Sound Pressure Level (SPL) at a distance of 3 cm between a sensor and a larva inside a grain sample, with greatest energy primarily at frequencies of 2–6 kHz (Mankin et al., 1996). Typically the sounds consist of trains of short (1–10-ms) broadband impulses, while background noises often occur as continuous signals with harmonic peaks that can be discriminated from insect sounds either by automated computer analysis or experienced listeners (Mankin et al., 2011). Nevertheless, as a precaution, a majority of insect acoustic detection studies conducted in laboratory settings use acoustic shielding to reduce interference from unwanted background noise.

Understanding the characteristics of sounds produced by *P. truncatus* and *S. zeamais* would be helpful in developing tools that could be used for timely determination of the presence or absence of hidden stages of these pests, as well as their level of infestation in stored maize. It is of potential interest also to explore software tools developed recently whereby differences in spectral and temporal patterns of insect signals have been correlated with differences in physiological states or differences in behavioral activities. For example, energetic scrapes, snaps, and feeding movements of insects are expected to generate impulses with a broader, higher-frequency spectrum than low-energy movements (Mankin et al., 2010, 2011). In addition, groups (trains) of consecutive sound impulses separated by 200 ms or less, termed bursts, have been found to be more reliable indicators of insect presence than individual impulses alone, which may be more difficult to distinguish from spurious background noise (Mankin et al., 2008a,b). Differences in the mean counts of impulses per burst and the rates of impulses in bursts (i. e., the numbers of impulses occurring within bursts, divided by the total duration of bursts) have been found to be different for movement and feeding behaviors of insects in different physiological states (Jalinas et al., 2015).

In the present study, the objectives of these experiments were to characterize the spectral and temporal patterns, i. e., the frequencies and timing of sound impulses produced by *P. truncatus* and *S. zeamais* in stored maize. They were conducted under

acoustically shielded conditions. Sounds recorded from separate groups of the last instars and adults were analyzed to consider frequency and temporal pattern differences that could be used to distinguish among the species and stages.

## 2. Materials and methods

### 2.1. Insect colonies

*Prostephanus truncatus* and *S. zeamais* were obtained from infested maize purchased from a local grain marketer in Nairobi, Kenya. Clean maize (13% moisture content) for rearing was obtained from the same marketer and was disinfested by storing at  $-18^{\circ}\text{C}$  for 14 days. Approximately 100 unsexed adults of each species were isolated from the infested grain and reared in multiple 1.45 L glass jars containing about 1 kg of clean (uninfested) maize each to give rise to the F1 generation. The rearing was carried out in an environmental chamber whose conditions were: temperature  $28 \pm 1^{\circ}\text{C}$ ; relative humidity  $65 \pm 5\%$  RH and photoperiod 12:12 (L: D). Acoustic measurements were made on a set of 12 jars comprising samples of 50 third instars or emerging adults of *P. truncatus* as well as the 4th instars or emerging adults of *S. zeamais* extracted from the jars. Third instars (final larval stage) of *P. truncatus* were identified by their C-shaped body and head retracted into the prothorax (Farrell and Haines, 2002), and were isolated 27 days after oviposition. Fourth instars (final larval stage) of *S. zeamais* were identified by their white colour and length of about 4 mm (Hill, 1983), and were isolated 30 days after oviposition. To ascertain the insect stages being observed, prior to acoustic measurements, preliminary assessment of colony development for the two insects was carried out. For both insects the presence of larvae within the kernels was determined by dissecting maize kernel samples of the correct day after oviposition.

### 2.2. Experimental design

The experiments included 4 different treatments: 3rd instar and adult *P. truncatus*, 4th instar and adult *S. zeamais* each replicated three times with separate insects. Each experimental unit (sample) was a glass jar holding 200 g of maize infested with 50 individuals of a specific treatment. For an exploratory investigation of the types and rates of signals produced by the different species and stages, 2 h of signals were recorded separately from each of the twelve samples in a shielded chamber (see 2.3 below). To reduce signal processing to manageable proportions, ten-minute sections from the recording of each sample then were prescreened to survey the different types of frequency spectra and rates of impulses that had been recorded and to establish general characteristics of representative signals (Fig. 1). Intervals of 180 s or longer were observed to contain approximately the same rates and types of sound impulses as the 10-min sections. Therefore, to approximate the durations of recordings typically collected in field environments (Jalinas et al., 2015), a 180-s interval was selected at random from a 10-min section of each sample to characterize representative impulse frequency spectra and timing patterns.

### 2.3. Acoustic shielding chamber

A portable noise shielding chamber was constructed (Fig. 2) to reduce vibrational and impact noises of below 20 kHz in the vicinity of the acoustic measurement area. The chamber was a rectangular cuboid box comprising of three wooden boxes (18 mm thick). The outermost box dimensions (length by width by height) were 61 by 66 cm by 117 cm; the middle box dimensions were 50 by 35 by 70 cm while the inner box was 40 by 20 by 50 cm. The two smaller

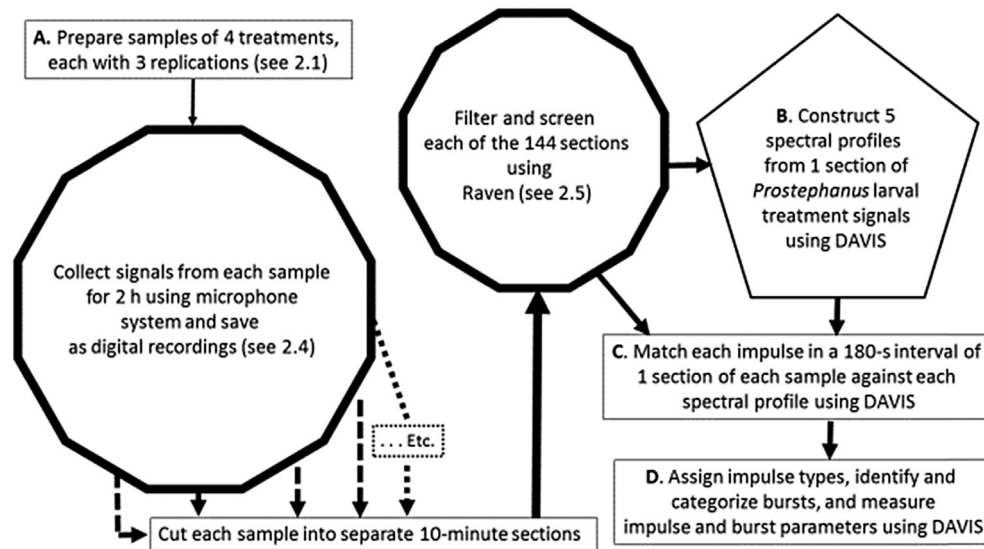


Fig. 1. Process flow of experimental data recording and signal analyses.

inner boxes were assembled together and suspended in the larger outer box with metal springs, so that they hung inside the outer box with a 5 cm layer of air around them. The construction was based on review of published sound transmission loss characteristics of composite materials (Lord et al., 1980). All boxes were made of Marine plywood (Timber Corner Limited, Nairobi, Kenya). The outer box was lined on the inside with 50 mm thick, polyethylene acoustic foam (Jumbolene®, Jumbo Chem Ltd., Nairobi, Kenya). Jumbolene foam reduces airborne noise in medium and high frequency ranges. The 50 mm foam sheets are rated at a Sound Transmission Coefficient (STC) of 63 and a Noise Reduction Coefficient (NRC) value of 0.85. The foam was glued to the interior of the box using Henkel Conta® contact adhesive. The inner boxes were lined on the inside with removable foamposite comprising three layers of open-cell/reticulated polyethylene foam namely: high, medium and low density polyethylene, each 50 mm thick. The three foams ensemble was tied together with duct tape to reduce their overall thickness to 100 mm. The doors to the boxes were lined with foam, and the outermost was fastened closed during measurement using strips of hard synthetic rubber. A 0.5 cm diameter hole was drilled at the top traversing all the boxes through which the microphone cables were inserted into the inner box and the hole sealed completely with foam.

#### 2.4. Acoustic measurements

Insect sound recordings were performed inside the shielded acoustic chamber in an isolated quiet room at ambient temperature (22–25 °C), with fluorescent lighting supplemented by ambient sunlight from nearby windows. Maize grains (200 g) to which 50 *P. truncatus* or *S. zeamais* larvae or adults had been added were placed in a 13-cm diam., 4.5 cm deep stainless steel container and covered with a fitting lid having a slit opening at the center through which a 0.5" microphone attached to a preamplifier system (Model 378B02, PCB Piezotronics Inc., NY), was positioned to make contact with the maize surface. The 20-dB-preamplified signals from the microphone were amplified an additional 10× using a 4-analog-, 8-digital-input measurement device (imc C-SERIES, CS-3008-N, imc Meßsysteme GmbH, Frankfurt, Germany) and recorded at 20 kHz sampling rate with 16-bit resolution in .ccv (curve configuration

file) format. These amplification levels were standardized throughout the experiment; consequently the relative amplitudes are the same for all the oscillograms shown in this report.

#### 2.5. Signal processing

First, the recorded signals were converted from .ccv (curve configuration files) to .wav (wave audio files) format using a custom program written in MATLAB Release 2012b (The MathWorks Inc., Massachusetts, United States). The signals from each .wav file were band-pass filtered between 0.2 and 10 kHz. Ten-minute sections were prescreened and independently verified to contain insect sounds by playback, oscillogram, and spectrogram analysis with Raven Pro 1.5 Beta Version software (Cornell Lab of Ornithology, New York, United States; Charif et al., 2008). To recognize potential differences among acoustic behaviors of insects in the different adult and larval treatments, the most commonly detected types of impulses in the recordings were characterized using a spectral profiling approach described by Mankin et al. (2011). A custom-written insect signal analysis software program DAVIS (Digitize, Analyze, View, Insect Sounds) (Mankin, 1994; Mankin et al., 2002; Herrick et al., 2013) was used to conduct automated analyses to distinguish insect sounds from unshielded background noise and consider whether there were differences in the sounds produced by the different species and stages tested in the study.

To characterize spectral patterns, mean spectra (profiles) were calculated by DAVIS fast Fourier transform and other algorithms from one of the 10-min prescreened records obtained from the *P. truncatus* 3rd-instar treatment that contained several series of distinctive sound impulses relatively uncontaminated by background noise. In performing these calculations, a spectrum was constructed from 512-point time slices centered on the peak of each impulse, and the profile was calculated as the average of the individual spectra in the series. Five spectral profiles were constructed from different distinctive series of impulses, as described in the Results. Then the DAVIS program least-squares matched all the impulses in 180-s samples randomly selected from one of the 10-min sections recorded from *P. truncatus* and *S. zeamais* instars and adults to these 5 insect profiles (Fig. 1). Each impulse detected in the recording then was assigned to the type from which it had

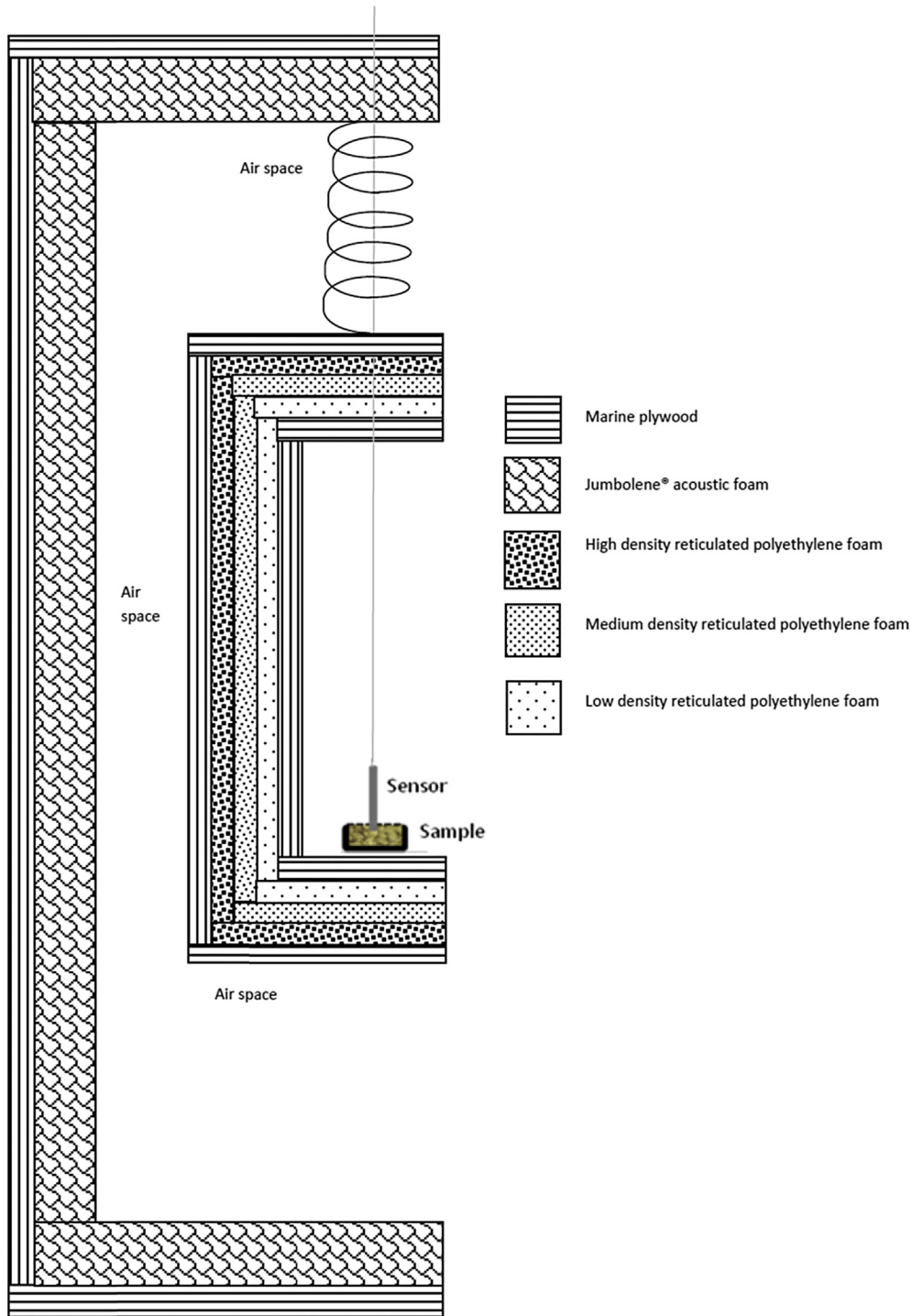


Fig. 2. Schematic diagram of the acoustic shielding chamber showing position of the sample and sensor.

the smallest total mean-square difference (Dosunmu et al., 2014). Impulses whose spectra failed to match any profile within a preset least-squares threshold were classified as noise, typically only 1–2% of the signals. For each sample, the DAVIS program identified and timed groups (trains) of insect sound impulses separated by intervals <200 ms, storing the beginning and end time of each train in a spreadsheet along with the number of impulses in the train. Trains that contained at least three impulses whose spectra

matched one of the 5 insect profile types were categorized as insect sound bursts and classified as one of five burst types, based on the type of impulses most frequently occurring in the burst. For each recording, the types and rates of bursts (the number of bursts divided by the recording duration), the numbers of impulses per burst, and the rates of impulses in bursts (the number of impulses occurring within bursts divided by the total duration of bursts) were calculated as in Jalinas et al. (2015).



## 2.6. Statistical analyses

All data were analyzed using Stata SE Version 12 (StataCorp, 2011). Analysis of variance and Tukey's Studentized range tests were performed to compare mean rates of impulses in bursts, rates of bursts and number of impulses per burst among larval and adult treatments. Depending on the question under analysis, the bursts of different types were either considered separately or combined together into an overall total (e. g., when considering detection thresholds).

## 3. Results

### 3.1. Acoustic characteristics of *P. truncatus* and *S. zeamais* sound impulses

Larvae and adults of *P. truncatus* and *S. zeamais* all produced sound impulses with a broad range of amplitudes, spectral features, and temporal patterns. Fig. 3a shows an example of several impulses and an impulse train in a 4 s record of *S. zeamais* larvae. Fig. 3b shows an inset expansion of the larval impulse train at a higher temporal resolution. The amplitudes and spectra of the signals were well within the range of those observed previously for stored product insects (Mankin et al., 2011); consequently, the detectability of larvae of these two species is expected to be similar to the detectability of *S. oryzae* reported in Kiobia et al. (2015), who found that an individual *S. oryzae* larva can be detected by state-of-art sensors and amplifiers in maize over a range of about 30 cm.

Five audibly distinct types of spectra (Fig. 4) were identified in the prescreening of a single, high quality acoustic recording of the 3rd instars of *P. truncatus*. For designative purposes, the five signal profiles are labeled: Broadband, HighF, MidF1, MidF2 and LowF, based on their peak energies and breadths of spectral range. Spectral profiles of each type were generated by calculating the mean spectrum of a series of consecutive impulses of the same type observed in this single recording. For the Broadband profile, spectra from 303 impulses in 40 trains were averaged. For the HighF profile, spectra from 139 impulses in 13 trains were averaged. For the MidF1 profile, spectra of 100 impulses in 10 trains were averaged. For the MidF2 profile, spectra of 36 impulses in 8 trains were averaged, and for the LowF profile, spectra of 17 impulses in 3 trains

were averaged.

Using DAVIS, the impulses in each of the twelve recordings under analysis were least-square matched against each of the five profiles (see Signal Processing above). Playback of the records by experienced listeners suggested that trains with >3 impulses were recognizable as insect sound bursts. For this reason, we set a minimum count of 3 impulses per train of a given insect sound type (e.g., inset in Fig. 3a) to classify the train as an insect sound burst in this study.

The numbers of bursts detected of each type in the recordings from the different treatments are listed in Table 1. The MidF2 bursts had the highest frequency of occurrence for both species with higher numbers recorded for the larvae as compared to the adults. It was notable also that none of the adult *P. truncatus* bursts matched the HighF profile. The MidF2 and LowF bursts had impulses with relatively low signal levels at high frequencies, which suggests they were produced by less energetic movements compared to those producing Broadband, High F and MidF1 impulses. Statistical analyses for bursts combined over all types are presented below in Table 2, and separately for bursts of each type in Table 4.

### 3.2. Temporal patterns of larval and adult sound impulses

Examples of oscillograms and spectrograms of sounds produced by adults and last instars of *S. zeamais* and *P. truncatus* are shown in Figs. 5a, b - 6a, b, respectively. Each example contains numerous impulses and bursts. Fig. 7 displays a burst from a *P. truncatus* 3rd instar (Fig. 5a), classified as MidF2, the most frequently occurring profile type (Table 1). Throughout the recordings, consecutive impulses often differed in amplitude, duration, and time interval, as in the examples of Figs. 3b and 7. Such variation has been observed also with many other insect species (e.g., Mankin et al., 2008a,b). In all of the spectrograms, dark lines spanning broad frequency ranges mark impulses associated with higher energy levels.

### 3.3. Comparison of overall bursts rates across treatments

An important aim of acoustic detection of insects in stored products is to discriminate whether or not infestations exist, and where there are multiple infestations such as *P. truncatus* and

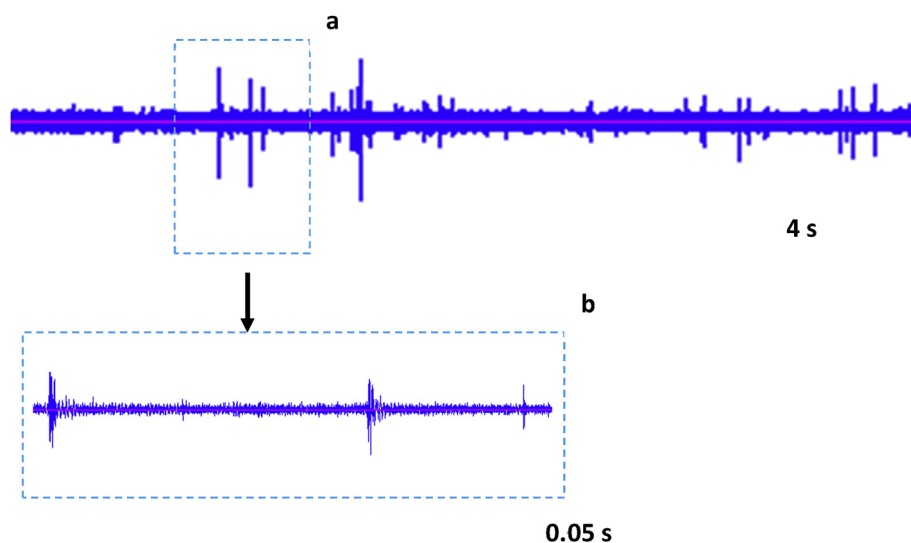
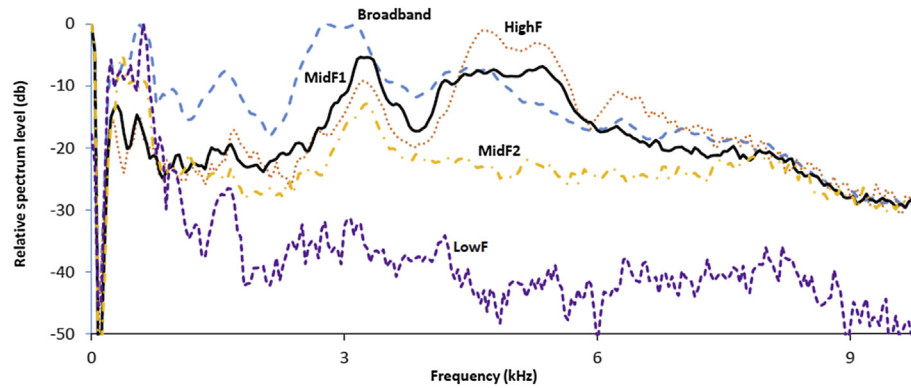


Fig. 3. Examples of (a) typical impulses recorded from *S. zeamais* 4th instars, and (b), higher resolution inset displaying a 0.05 s interval of impulses.



**Fig. 4.** Frequency spectral profiles of five distinct types of impulses identified from an individual acoustic recording of the third instars of *P. truncatus*. Broadband, long dashed line; LowF, dashed line; MidF2, dash-dot-dotted line; MidF1 solid line, and HighF, dotted line. Horizontal axis indicates frequency in kHz and vertical axis indicates relative spectrum amplitude in dB.

*S. zeamais* in maize, to identify the kind of insects present and their relative abundance. Such accomplishments have been demonstrated in previous studies based on quantitative and/or qualitative characteristics of detected sounds (Mankin et al., 2007, 2008a,b). In this study, the number of impulses per recording varied between 5 and 1301 for *P. truncatus* adults, 4–15,760 for *S. zeamais* adults, 20–1854 for late instar *P. truncatus* and 5–399 for late instar *S. zeamais*. The overall number of impulses per burst ranged from 3 to 213. Table 2 shows the burst rates, impulse rates and impulses per burst of the last instars and adults of *P. truncatus* and *S. zeamais*, combining bursts of all types. There was a significant differences in the counts of impulses in bursts for *S. zeamais* adults compared to other treatments.

It was of interest to consider what would have been the minimum number of individuals per sample that would be needed for reliable detection of infestation by the microphone system used in this study. Using the value of 0.02 bursts/s, estimated in Mankin et al. (2008a) as a threshold below which the likelihood of detection is low, and assuming that each of the 50 larvae produced bursts at approximately the same mean rate, a minimum of 4 *P. truncatus* 3rd instars would have been needed per treatment for reliable estimation that the sample was infested, and a minimum of 6

*S. oryzae* 4th instars would have been needed using the mean values listed for *P. truncatus* and *S. oryzae* larval burst rates in Table 2.

#### 3.4. Comparisons of burst rates across treatments with different profile types considered separately

Analysis of variance of the effects of the four treatments on the rates of bursts, numbers of impulses per burst, and rates of impulses in bursts (Table 3) revealed that the counts of impulses per burst and the rates of impulses in bursts were significantly different among treatments for bursts of Broadband and MidF2 impulses. Table 4 displays the results of a Tukey's Studentized range test on the measurements where significant differences were found. The adult *S. zeamais* mean values for numbers of broadband and MidF2 impulses per burst, and for rates of broadband and midF2 impulses in bursts were significantly greater than for other treatments.

## 4. Discussion

The characteristics of the larval signals detected in this study suggests that their detectability is similar to that found previously for the close relatives, *S. oryzae*, *R. dominica*, and other stored product insect pests (Mankin et al., 2011; Kiobia et al., 2015; Eliopoulos et al., 2015). Consequently, acoustic devices already in use for detection of stored product insects can be readily adapted to the particular environmental conditions and storage structures in sub-Saharan Africa where *P. truncatus* and *S. zeamais* are prominent. Improved automation of the insect detection and noise discrimination process would be especially beneficial in these regions due to the minimal technical training levels of many farmers in the region. For this reason, the measurements of spectral and temporal patterns of larvae and adult *P. truncatus* and *S. zeamais* in this study

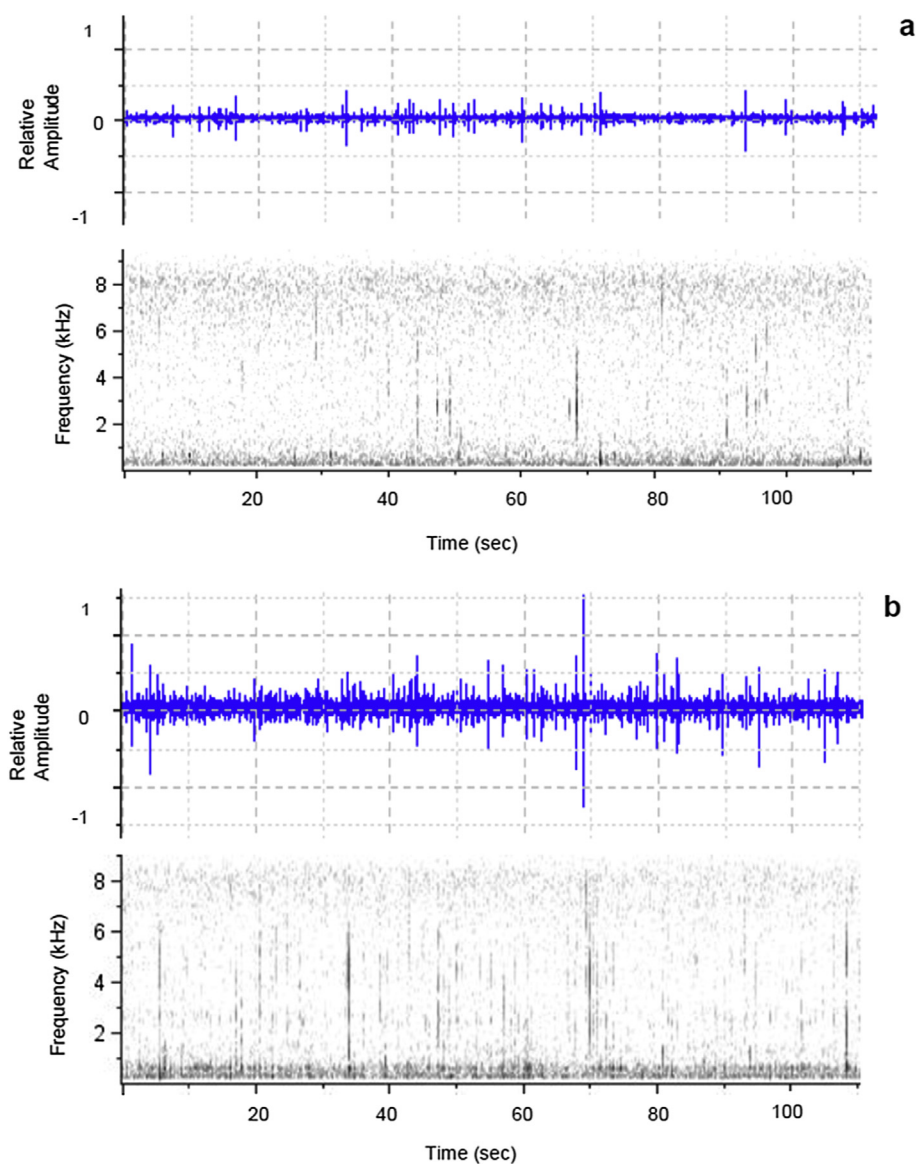
**Table 1**  
Numbers of bursts of each profile detected in 180-s recordings from last instars and adults of *P. truncatus* and *S. zeamais*.

Species	Stage	No. bursts detected of each profile type				
		Broadband	HighF	MidF1	MidF2	LowF
<i>P. truncatus</i>	Larvae	27	12	57	88	32
	Adults	13	0	11	358	47
<i>S. zeamais</i>	Larvae	12	10	12	195	40
	Adults	24	7	84	212	6

**Table 2**  
Analysis of variance of burst rates, rates of impulses in bursts and counts of impulses per burst, combined over profile types, for last instars and adults of *P. truncatus* and *S. zeamais*.

Species	Stage	Burst rate (No./s)	Impulse rate (No./s)	Impulses per burst
<i>P. truncatus</i>	Larvae	0.30 ± 0.49a	1.73 ± 2.97a	5.85 ± 2.26a
	Adult	0.33 ± 0.42a	1.80 ± 2.41a	5.64 ± 2.55a
<i>S. zeamais</i>	Larvae	0.19 ± 0.26a	0.78 ± 0.97a	4.48 ± 1.86a
	Adult	0.17 ± 0.23 a	14.99 ± 28.51a	57.51 ± 64.45b

Means in the same column, followed by same letters are not significantly different ( $P < 0.05$ ).



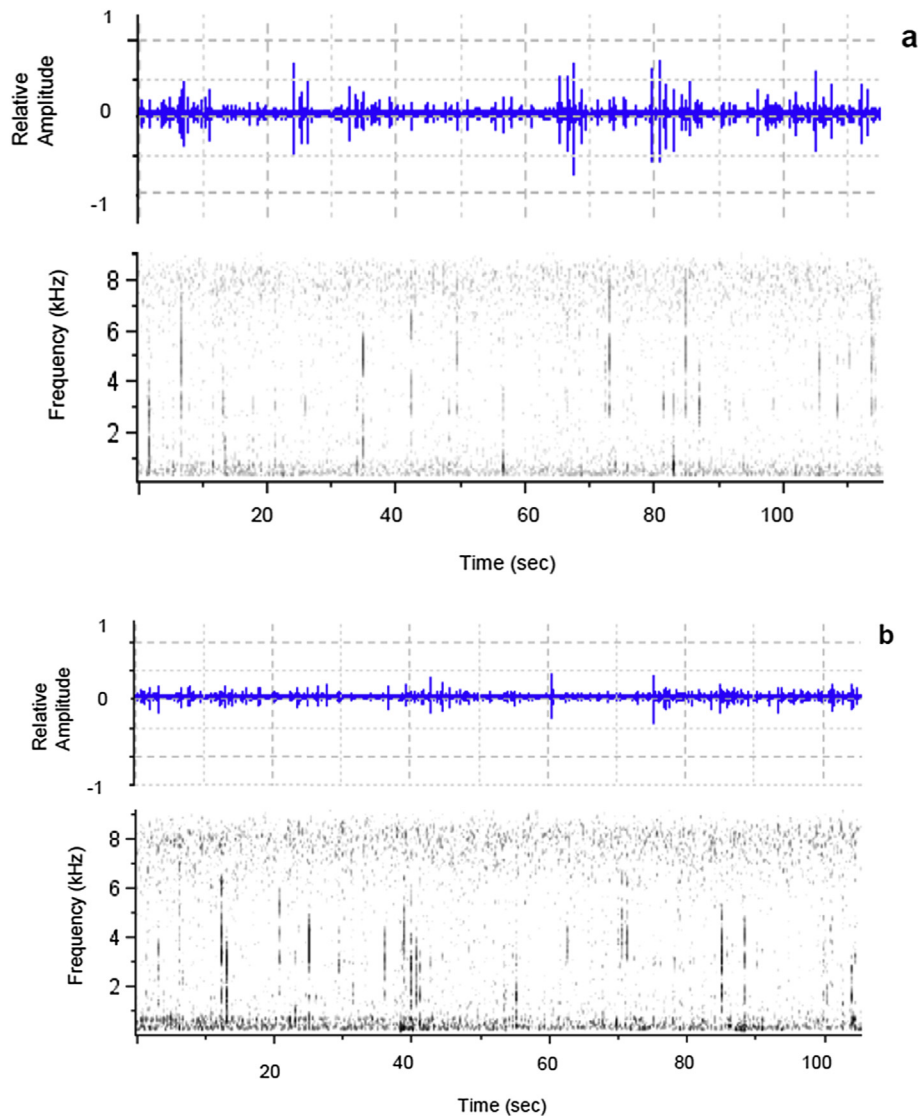
**Fig. 5.** Oscillogram and spectrogram, of a 100 s period of signals recorded from maize infested with (a) 4th instar of *S. zeamais* larvae, and (b) *S. zeamais* adults. Darker shading in spectrogram (256 points per spectrum, 50% overlap) indicates greater energy at that frequency.

can be of assistance in future development of tools that enable both automated detection and distinguishing among species. In addition, some of the differences found in the spectral and temporal patterns produced by insects in the different larval and adult treatments may have relevance to understanding of differences among their behaviors, as is considered in the next sections. A better understanding of how specific behaviors produce sound impulses of different types may have relevance not only for *P. truncatus* and *S. zeamais*, but for other stored product insect pests as well.

#### 4.1. Differences in *S. zeamais* larval and adult behaviors

The sounds of *S. zeamais* adults, see Table 1 and Fig. 5b, were characterized by higher amplitude impulses than those of the larvae. This could be explained by the higher activity level of the adults as compared to the larvae of *S. zeamais*. In addition to feeding, adults exhibit locomotory activity. Fleurat-Lessard et al., 2006 also reported lower larval activity for *S. oryzae* with a lower

range of acoustic peak energy, spanning from 1.3 to 2.0 kHz, while for the adult stage the frequency range was higher, spanning from 1.8 to 3.0 kHz. During oviposition, adult *S. zeamais* pierce through the grains, particularly into the endosperm, to create holes into which eggs are deposited and covered with waxy secretion (Dobie, 1974; Urrelo and Wright, 1989). This activity creates considerable movement in and out of the grains by the adults whereas the larvae are predominantly confined inside the maize kernel. Larval activity is mainly feeding (Fleurat-Lessard et al., 2006). In addition, *S. zeamais* females may move more actively in search of oviposition sites than *P. truncatus* because they oviposit only one egg per kernel (Kossou et al., 1992). Such differences in behaviors may have contributed to the result that the mean count of impulses per burst was significantly greater for *S. zeamais* adults than for larvae (Table 2), as well as the result that the mean counts of broadband and MidF2 impulses per burst and the mean rates of broadband and midF2 impulses in bursts were significantly greater for *S. zeamais* adults than larvae (Table 4).



**Fig. 6.** Oscillogram and spectrogram of (a) a 100 s period of signals recorded from maize infested with 3rd instar of *P. truncatus* larvae, and (b) *P. truncatus* adults. Darker shading in spectrogram (256 points per spectrum, 50% overlap) indicates greater energy at that frequency.

#### 4.2. Differences in *S. zeamais* and *P. truncatus* adult behaviors

The oscillogram of *P. truncatus* adults (Fig. 6b) was uniquely characterized by lower amplitude impulses than those of the *S. zeamais* adults. This could be attributed to the fact that *P. truncatus* females may be less active during oviposition because they do not necessarily deposit their eggs inside the grains but instead in the created flour, frass, in tunnels or at the bottom of the maize container (Rugumamu, 2009). Another notable distinction relates to the mouth parts of the two pests in that adult *S. zeamais* possesses a characteristic rostrum for piercing into grains. *P. truncatus* adults have exposed mandibles on the head that is firmly retracted in the thorax for effective tunneling from grain to grain. Such differences in behaviors may have contributed to the result that the count of impulses per burst was significantly greater for *S. zeamais* adults than for *P. truncatus* (Table 2), and the numbers of broadband and MidF2 impulses per burst and the rates of broadband and midF2 impulses in bursts were significantly greater for *S. zeamais* adults than *P. truncatus* (Table 4).

#### 4.3. Differences in *P. truncatus* and *S. zeamais* larval behaviors

It has been reported that *Prostephanus* causes more severe damage compared to *S. zeamais*. (Rugumamu, 2009; Makundi et al., 2010). Some evidence for behavioral differences among the larvae that might cause differences in infestation damage is suggested in Table 1, where *P. truncatus* larvae had greater numbers of HighF and MidF1 bursts with profiles containing high energy at frequencies >4 kHz than *S. zeamais* larvae, while *S. zeamais* had greater numbers of MidF2 bursts with profiles containing low energy at frequencies >4 kHz. A comparison of the spectrogram of the larvae in Fig. 5 in relation to the *P. truncatus* larval spectrogram in Fig. 6, suggested also that signals produced by the *S. zeamais* larvae had less energy at frequencies >4 kHz than *P. truncatus* larvae on infested maize. However, there was sufficient variation among measurements in this study that the differences did not reach the level of statistical significance (Table 3).



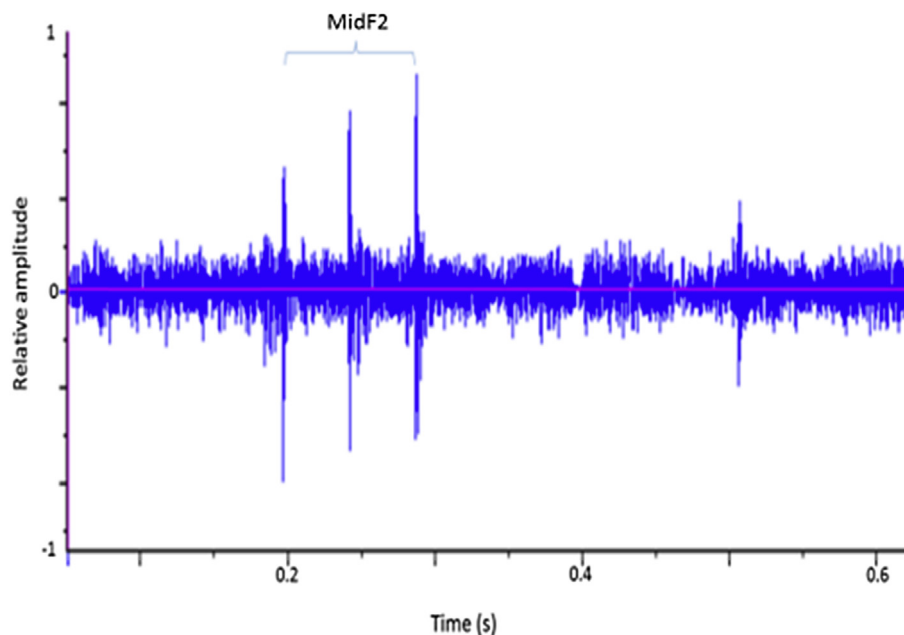


Fig. 7. Example of an impulse train consisting of 3 MidF2 impulses that was classified as an insect burst.

**Table 3**

Analysis of variance of effects of insect species (*Prostephanus truncatus* and *Sitophilus zeamais*) and stage (last instar and adult) treatments on the rates of bursts, counts of impulses per burst, and counts of impulses in bursts of different spectral profile types.

Parameter	Error mean square	F	P
Rate of Bursts ( $s^{-1}$ )			
HighF	0.0672	1.21	0.366
Broadband	0.0016	1.26	0.352
MidF1	0.0168	0.83	0.513
MidF2	0.076	1.44	0.301
LowF	0.002	0.69	0.581
No. impulses per burst			
HighF	72.39	1.05	0.423
Broadband	54.45	7.76	0.001*
MidF1	308.37	3.43	0.073
MidF2	2153.14	4.49	0.040*
LowF	1763.34	1.94	0.202
Rate of impulses in bursts (No. impulses/s)			
HighF	0.115	0.72	0.567
Broadband	0.122	9.08	0.006*
MidF1	37.102	1.42	0.307
MidF2	391.5	5.59	0.023*
LowF	5.013	1.12	0.396

\*indicates values of  $P < 0.05$ .

**Table 4**

Means of insect sound parameters with significant differences among insect species and stages (Table 3).

Parameter	<i>P. truncatus</i>		<i>S. zeamais</i>	
	3rd instar	Adult	4th instar	Adult
No. broadband impulses/burst	3.83a	3.0a	4.08a	27.36b
No. MidF2 impulses/burst	6.28a	6.47a	4.73a	119.38b
Rate of broadband impulses in bursts ( $s^{-1}$ )	0.396a	0.084a	0.104a	1.379b
Rate of midF2 impulses in bursts ( $s^{-1}$ )	5.56a	4.60a	1.89a	57.94b

Means in a row followed by the same letter are not significantly different using the Tukey Studentized range test ( $df = 8$ ).

#### 4.4. Use of acoustic detection methods for management of stored product infestations in sub-Saharan Africa

*Prostephanus truncatus* and *S. zeamais* cause severe damage and weight losses in stored maize in sub-Saharan Africa, and are difficult to control because the larvae are not easily detected. It was thus of interest in this study that the frequency spectra of sound impulses produced by adults and larvae of both species have similar characteristics, and that significant differences were found in temporal patterns of sound impulses produced by *S. zeamais* adults. If the impulse spectra of both insects are similar, a common acoustic sensor can be developed to detect both species in field environments. Further study may reveal additional impulse temporal pattern differences that could be used to distinguish among insect stages or species. There is need to develop algorithms capable of identifying insect sounds of varying frequency, amplitude, and duration from audio recordings in farmers' stores.

This study contributes to knowledge of acoustic detection technology which is much needed for improving timely detection for farmers who store their grain as well as for the inspection of grain at points of entry and for 'at-origin' inspections. With increasing smart phone use and adoption in sub-Saharan Africa, acoustic detection apps can be developed using existing data on postharvest pests for specific species and installed on mobile

phones for farmer use.

## Acknowledgement

Authors are thankful to the German Ministry for Economic Cooperation and development (BMZ) under the Reduction of Postharvest Losses and Value Addition in East African Food value chains (RELOAD) project and the International Centre for Insect Physiology and Ecology (*icipe*) for facilitating the study.

## References

- Abebe, F., Tefera, T., Mugo, S., Beyene, Y., Vidal, S., 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). *Afr. J. Biotechnol.* 8, 5937–5943.
- Borgemeister, C., Holst, N., Hodges, R.J., 2003. Biological control and other pest management options for larger grain borer *Prostephanus truncatus*. In: Neuenschwander, P., Borgemeister, C., Langewald, J. (Eds.), *Biological Control in IPM Systems in Africa*. CAB International, Wallingford, pp. 311–328.
- Boxall, R.A., 2002. Damage and loss caused by the larger grain borer *Prostephanus truncatus*. *Integr. Pest Manag.* 7, 105–121.
- Charif, R.A., Waack, A.M., Strickman, L.M., 2008. Raven Pro 1.3 User's Manual. Cornell Laboratory of Ornithology, Ithaca, New York.
- Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). *J. Stored Prod. Res.* 10, 183–197.
- Dosunmu, O.G., Herrick, N.J., Haseeb, M., Hix, R.L., Mankin, R.W., 2014. Acoustic detectability of *Rhynchophorus cruentatus* (Coleoptera: Dryophthoridae). *Fla. Entomol.* 97, 431–438.
- Eliopoulos, P.A., Potamitis, I., Kontodimas, D.Ch., Givropoulou, E.G., 2015. Detection of adult beetles inside the stored wheat mass based on their acoustic emissions. *J. Econ. Entomol.* 108, 2808–2814.
- Farrell, G., Haines, C.P., 2002. The taxonomy, systematics and identification of *Prostephanus truncatus* (Horn). *Integr. Pest Manag. Rev.* 7, 85–90.
- Farrell, G., Schulten, G.G.M., 2002. Larger grain borer in Africa; a history of efforts to limit its impact. *Integr. Pest Manag. Rev.* 7, 67–84.
- Fleurat-Lessard, F., 1988. Determination of insect infestation. In: Multon, J.L., Reimbert, A.M., Marsh, D., Eydt, A.J. (Eds.), *Preservation and Storage of Grains, Seeds and Their Products: Cereals, Oilseeds, Pulses and Animal Feed*. Lavoisier Publishing Inc., New York, pp. 495–515.
- Fleurat-Lessard, F., Tomasini, B., Kostine, L., Fuzeau, B., 2006. Acoustic detection and automatic identification of insect stages activity in grain bulks by noise spectra processing through classification algorithms. In: Lorini, I., Bacaltchuk, B., Beckel, H., Deckers, D., Sundfeld, E., Dos Santos, J.P., Biagi, J.D., Celaro, J.C., D'Alfonso, L.R., de, L., Bortolini, O.F. (Eds.), *Proceedings of the 9th International Working Conference on Stored Product Protection*, 15–18 October 2006, Sao Paulo Brazil, pp. 476–486.
- García-Lara, S., Bergvinson, D.J., 2013. Identification of maize landraces with high level of resistance to storage pests *Sitophilus zeamais* Motschulsky and *Prostephanus truncatus* Horn in Latin America. *Rev. Fitotec. Mex.* 36 (3), 347–356.
- Gobernado, P.R., Fleurat-Lessard, F., Villamayor, J., Sautereau, B., Brioni, G., Gondolo, J., Tomasini, B., 2005. Method and device for detection, identification, and densimetric quantification of grain-eating insects in stocks of cereals. US Patent No. 6,923,064 B2, August 2, 2005.
- Hagstrum, D.W., Flinn, P., 1993. Comparison of acoustical detection of several species of stored-grain beetles (Coleoptera: Curculionidae, Tenebrionidae, Bostrichidae, Cucujidae) over a range of temperatures. *J. Econ. Entomol.* 86, 1271–1278.
- Hagstrum, D.W., Webb, J.C., Vick, K.W., 1988. Acoustical detection and estimation of *Rhyzopertha dominica* (F.) larval populations in stored wheat. *Fla. Entomol.* 71, 441–447.
- Hagstrum, D.W., Vick, K.W., Webb, J.C., 1990. Acoustical monitoring of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) populations in stored wheat. *J. Econ. Entomol.* 83 (2), 625–628 (4).
- Herrick, N.J., Mankin, R.W., Dosunmu, O.G., Kairo, M.T.K., 2013. Ecology and detection of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), and related weevils for the protection of palm tree species in the United States. In: *Palm Pest Mediterranean Conference*, Nice France, pp. 269–283.
- Hill, D.S., 1983. *Agricultural Insect Pests of Tropics and Their Control*, second ed. Cambridge University Press, Cambridge, p. 746.
- Jalinas, J., Güerri-Agulló, B., Mankin, R.W., Lopez-Follana, R., Lopez-Llorca, L.V., 2015. Acoustic assessment of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) effects on *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) larval activity and mortality. *J. Econ. Entomol.* 108 (2), 444–453.
- Kiobia, D.O., Tumbo, S.D., Cantillo, J., Rohde, B.B., Mallikarjunan, P.K., Mankin, R.W., 2015. Characterization of sounds in maize produced by internally feeding insects: investigations to develop inexpensive devices for detection of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in small-scale storage facilities in sub-Saharan Africa. *Fla. Entomol.* 98, 405–409.
- Kossou, D.K., Bosque-Perez, N.A., Marek, J.H., 1992. Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* Motschulsky. *J. Stored Prod. Res.* 28, 187–192.
- Leblanc, M.P., Gaunt, D., Fleurat-Lessard, F., 2011. Experimental study of acoustic equipment for real-time insect detection in grain bins – Assessment of their potential for infestation risk prediction during long term storage periods. *IOBC/wprs Bull.* 69, 79–88.
- Lord, G., Gatley, W.S., Evensen, H.A., 1980. *Noise Control for Engineers*. McGraw-Hill, New York.
- Makundi, R.H., Swila, N.N., Misangu, R.N., Reuben, S.W.M., Mwatawala, M., Sikira, A., Kilonzo, B.S., Lyimo, H., Massawe, A.W., Ishengoma, C., 2010. Dynamics of infestation and losses of stored maize due to the larger grain borer (*Prostephanus truncatus* Horn) and maize weevils (*Sitophilus zeamais* Motschulsky). *Arch. Phytopathol. Pflanzenschutz* 43 (14), 1346–1355.
- Mankin, R.W., 1994. Acoustical detection of *Aedes taeniorhynchus* swarms and emergence exoduses in remote salt marshes. *J. Am. Mosq. Control Assoc.* 10, 302–308.
- Mankin, R.W., Hagstrum, D., 2011. Acoustic monitoring of insects. In: Hagstrum, D.W., Phillips, T.W., Cuperus, G. (Eds.), *Stored Product Protection*. Publication S156-22. Kansas State University Press, Manhattan, KS, pp. 263–269.
- Mankin, R.W., Hagstrum, D.W., Smith, M.T., Roda, A.L., Kairo, M.T.K., 2011. Perspective and Promise: a century of insect acoustic detection and monitoring. *Am. Entomol.* 57 (1), 30–44.
- Mankin, R.W., Hodges, R.D., Nagle, H.T., Schal, C., Pereira, R.M., Koehler, P.G., 2010. Acoustic indicators for targeted detection of stored product and urban insect pests by inexpensive infrared, acoustic, and vibrational detection of movement. *J. Econ. Entomol.* 103 (5), 1636–1646.
- Mankin, R.W., Hubbard, J.L., Flanders, K.L., 2007. Acoustic indicators for mapping infestation probabilities of soil invertebrates. *J. Econ. Entomol.* 100 (3), 790–800.
- Mankin, R.W., Mizrach, A., Hetzroni, A., Levsky, S., Nakache, Y., Soroker, V., 2008a. Temporal and spectral features of sounds of wood-boring beetle larvae: identifiable patterns of activity enable improved discrimination from background noise. *Fla. Entomol.* 91, 241–248.
- Mankin, R.W., Osbrink, W.L., Oi, F.M., Anderson, J.B., 2002. Acoustic detection of termite infestations in urban trees. *J. Econ. Entomol.* 95, 981–988.
- Mankin, R.W., Smith, M.T., Tropp, J.M., Atkinson, E.B., Jong, D.Y., 2008b. Detection of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) larvae in different host trees and tissues by automated analyses of sound-impulse frequency and temporal patterns. *J. Econ. Entomol.* 101, 838–849.
- Mankin, R.W., Shuman, D., Weaver, D.K., 1999. Thermal treatments to increase acoustic detectability of *Sitophilus oryzae* (Coleoptera: Curculionidae) larvae. *J. Econ. Entomol.* 92, 453–462.
- Mankin, R.W., Sun, J.S., Shuman, D., Weaver, D.K., 1996. A sound-insulated enclosure to shield acoustical insect detectors from grain elevator background noise. In: *Proc. Second Symposium on Agroacoustics*, pp. 89–104.
- Neethirajan, S., Karunakaran, C., Jayas, D.S., White, N.D.G., 2007. Detection techniques for stored-product insects in grain. *Food Control* 18, 157–162.
- Pittendrigh, B.R., Huesing, J.E., Shade, R.E., Murdock, L.L., 1997. Monitoring of rice weevil, *Sitophilus oryzae*, feeding behavior in maize seeds and the occurrence of supernumerary molts in low humidity conditions. *Entomol. Exp. Appl.* 83, 225–231.
- Potamitis, I., Ganchev, T., Kontodimas, D., 2009. On automatic bioacoustic detection of pests: the cases of *Rhynchophorus ferrugineus* and *Sitophilus oryzae*. *J. Econ. Entomol.* 102, 1681–1690.
- Rugumamu, C.P., 2009. Influence of simultaneous infestations of *prostephanus truncatus* and *sitophilus zeamais* on the reproductive performance and maize damage. *Tanz. J. Sci.* 31 (1), 66–72.
- Schwab, I., Degoul, P., 2005. Automatic acoustical surveillance system of grains in silos. In: Fleurat-Lessard, F., Ndiaye, A., Knight, J.D. (Eds.), *Stored Malting Barley: Management of Quality Using an Expert System*. INRA-Editions, Paris, France, pp. 203–218.
- StataCorp., 2011. *Stata Statistical Software: Release 12*. StataCorp LP, College Station, TX.
- Stephens, E.C., Barrett, C.B., 2010. Incomplete credit markets and commodity marketing behaviour. *J. Agric. Econ.* 62 (1), 1–24.
- Urrelo, R., Wright, V.F., 1989. Oviposition performance of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) on resistant and susceptible maize accessions. *J. Kans. Entomol. Soc.* 62, 23–31.
- Weinard, W., 1998. Larvae eavesdropper- or where the beetles suddenly appear from. *Muhle + Mischfuttertechnik* 135, 654.