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Acoustic detection of insect pests of stored grains in Kenya

Dissertation **Witzenhausen 2017**

Universität Kassel
Fachbereich Ökologische Agrarwissenschaften
Fachgebiet Agrartechnik
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Acoustic detection of insect pests of stored grains in Kenya

Dissertation zur Erlangung des akademischen Grades Doktor der

Agrarwissenschaften (Dr. agr.)

Fachbereich Ökologische Agrarwissenschaften

von
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aus Kenia
2017

Die vorliegende Arbeit wurde vom Fachbereich für Ökologische Agrarwissenschaften, Fachgebiet Agrartechnik der Universität Kassel als Dissertation zur Erlangung des akademischen "Grades Doktor der Agrarwissenschaften" angenommen.

Tag der mündlichen Prüfung: 26.09.2017

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DEDICATION

To my dear parents Nellius and James who held my hand and took parental care for my daughter Nelly as I pursued this PhD. They stood by me throughout this journey and have been my greatest source of inspiration and blessings

Acknowledgement

My utmost gratitude is to the Almighty God for His abundant blessings upon me and my family.

“*Joshua 21:45 Not one of all the Lord’s good promises to Israel failed; every one was fulfilled.”*

I would like to thank Prof. Dr. Oliver Hensel for supervision and allowing me to carry out my research as an external student. He always had his door open whenever I wished to visit the department. I would also like to thank Prof. Dr. Maria Finckh, Department of Ecological Plant Protection, University of Kassel, Germany for agreeing to assess this thesis, and Prof. Dr. Rainer Jörgensen and Dr. Christian Hülsebusch for their participation as oral examiners.

My most profound gratitude goes to Dr. Hippolyte Affognon for giving me a great opportunity to work within the RELOAD-GIZ project in *icipe* and for his professional guidance throughout this PhD work.

I am grateful to Dr. Uwe Richter, Dr. Sascha Kirchner, Hans Hermann Kaufmann, and Dr. Hubertus Siebald for their training on acoustic data collection and analysis and support towards my research work. Dr. Richter’s technical advice, support with the set-up of the acoustic lab at *icipe* and review of the work are gratefully acknowledged.

Special thanks to Michael Hesse the RELOAD Project coordinator who facilitated my thesis defense in terms of my travel and stay in Witzenhausen. I cannot forget the friendly atmosphere at the Department of Agricultural Engineering whenever I came to visit from Kenya. I am very thankful to Christian Schellert, Stefanie Retz, Carolina Bilibio, Franz Roman and Barbara Sturm.

I cannot forget my friends who made my settling in Germany easy whenever I visited; Jackline Ogolla, Michael Mgalula, Ivan Adolwa, Lunah Shrestha, Guyo Roba, Aditya Parmar, Joseph Korese, Chemeda Abdeta, Sirawdink Forsido and many others who I may forget to mention.

In a special way I would like to acknowledge Dr. Richard Mankin from USDA-ARS CMAVE for his great input towards acoustic signal analysis and manuscript development. He welcomed me to his lab in Gainesville, Florida in 2014 marking the beginning of a great collaboration. He taught me many virtues of a true scientist among them patience and resilience. I am greatly indebted to him. Special thanks to Barukh Rohde his PhD student from the University of Florida and Everett Foreman in his lab for their inputs and training on data conversion and analysis.

I am sincerely grateful to the students and staff of *icipe*, Duduville campus for their direct or indirect help towards the successful completion of my study as well as for their friendly atmosphere throughout my stay. I am very thankful to my fellow scholars for their encouragement and friendly atmosphere; Edoh Ognakossan, Zoul-Kifouly Midingoyi, Peter Denis Muiruri, Juliet Wanjiku, Allan Mueke, Vincent Nyasembe, Beatrice Nganso, Frank Thomas Ndjomatchoua, Rosaline Wanjiru, Crispus Njeru, Chia Shaphan, Marwa Shumo, Akua Konadu Antwi and many others. My sincere gratitude goes to Lilian Igweta-Tonnang and Dr. Robert Skilton of the Capacity Building and Institutional Development for their support towards the completion of my PhD.

My heartfelt appreciation goes to Prof. Dieudonne Baributsa of Purdue University for moral support and consistent encouragement throughout the course of this work. As my mentor in postharvest research I am greatly indebted to the values and principles I have learnt from him.

With great benevolence, I cannot forget the late Prof. Corrine Alexander of Purdue University who stood by me and motivated me to grow my career from the beginning of my Doctoral Studies.

I am greatly indebted to the University of Kassel, Department of Agricultural Engineering, for offering me a chance to pursue my Doctoral degree. I gratefully acknowledge the financial support for this research by the Deutsche Gesellschaft für Internationale Zusammenarbeit, GmbH (GIZ) under the Reduction of Postharvest Losses and Value Addition in East African Food value chains (RELOAD) project (*project number 12.1433.7-001.00 contract number 81161243)*. I was supported by a RELOAD Project scholarship awarded by the International Centre for Insect Physiology and Ecology (*icipe*) through the Dissertation Research Internship Program (DRIP).

My deepest appreciation goes to all my family members for their constant support and inspiration.

List of original publications

This thesis is based on original manuscripts published/ submitted for publication as follows:

Chapter 3 Njoroge AW, Affognon H, Mutungi C, Rohde B, Richter U, Hensel O, Mankin RW. 2016. Frequency and time pattern differences in acoustic signals produced by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in stored Maize. Journal of Stored Products Research 69: 31-40.

Chapter 4 Njoroge AW, Affognon H, Mutungi C, Rohde B, Richter U, Hensel O, Mankin RW. 2016. Bioacoustics of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) on *Phaseolus vulgaris* L. (Fabaceae). Florida Entomologist 100(1):109-115.

Chapter 5 Njoroge AW, Affognon H, Mutungi C, Rohde B, Richter U, Hensel O, Mankin RW. 2017. Acoustic survey in Kenya's grain storage facilities and the promise of acoustic detection of pests. (Accepted at the 1st All Africa Postharvest Conference for publication in a special edition book by the CRC Press (Taylor and Francis); Status: Accepted).

Parts of this thesis have also been presented at the following conferences

AW Njoroge, H Affognon, C Mutungi, U Richter, O Hensel, and RW Mankin (2016). XXV International Congress of Entomology 2016, September 25-30, 2016, Orlando, Florida, USA

AW Njoroge, H Affognon, C Mutungi, U Richter, O Hensel, and RW Mankin (2015). The XXV International Bioacoustics Congress (IBAC), September 7-12, 2015, Murnau, Bavaria, Germany

AW Njoroge, H Affognon, C Mutungi, U Richter, O Hensel, and RW Mankin (2015). TROPENTAG, September 16-18, 2015, Humboldt Universitat, Berlin, Germany

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Acronyms and Abbreviations

AEC	Acoustic Emmissions Consulting
AGRA	Alliance for Green Revolution in Africa
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APHLIS	African Postharvest Losses Information System
ARS	Agricultural Research Services
CABI	Centre for Agriculture and Biosciences International
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
CMAVE	Center for Medical Agricultural and Veterinary Entomology
DAVIS	Digitize Analyze View Insect Sounds
DRIP	Dissertation Research Internship Programme
EAC	East African Community
EAGC	East African Grain Council
EPZ	Export Processing Zones
ERA	Economic Review of Agriculture
ESA	Eastern and Southern Africa
FAOSTAT	Food and Agriculture Organization Statistical Databases
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit, GmbH
icipe	International Center for Insect Physiology and Ecology
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics

IPM	Integrated Pest Management
ISO	International Standards Organization
KARLO	Kenya Agricultural and Livestock Research Organization
kHz	Kilohertz
MATLAB	Matrix Laboratory
MoA	Ministry of Agriculture
MSE	Mean Squared Error
MT	Metric tones
NCPB	National Cereals and Produce Board of Kenya
NRC	Noise Reduction Coefficient
PHLs	Post-Harvest Losses
RELOAD	Reducing Losses Adding Value along East African Food Value Chains
RH	Relative humidity
SEM	Standard Error of the Mean
SPL	Sound Pressure Level
SS	Sum of Squares
STC	Sound Transmission Coefficient
USAID	United States Agency for International Development
USD	United States Dollar
USDA	United States Department for Agriculture

1. General introduction

1.1. Background information

More than 75% of the Kenyan population earns its livelihood from agriculture; the pillar for Kenya's economic development (Nukenine et al. 2010). The bulk of agricultural produce in Kenya is grain which is seasonally produced but consumed throughout the year. Therefore; grain storage becomes a particularly important activity. The storage is either done on-farm or in community stores and large warehouses. Grain storage is plagued with a myriad of problems the major one being quantitative and qualitative loss. The greatest grain loss agents are postharvest insect pests.

Annual average grain losses due to insect attack are in the range of 20-30% (Boeke, 2002). Stored product insects are endemic throughout the storage and handling systems of Kenya's grain industry. The rate of insect proliferation in storage warehouses could be alarmingly high, especially with the warm climate in Kenya (Nukenine et al. 2010). Existing grain procurement and sampling procedures at bulk grain storage warehouses increase the risk of accidentally allowing infested grain to pass. Some invasive species are introduced in stores where they did not exist before. The presence of live insects affects the value of food grains, is unacceptable in grain trade and threatens the food security (Darby, 2007). Measures are therefore put in place to monitor infestation in stores.

The sampling and sieving method is commonly used due to its simplicity. However, it is not suitable for early detection of hidden infestation in form of eggs and larvae. This method cannot be automated, is not continuous and involves destructive sampling (Mankin et al. 2011, Yigezu et al. 2010).

Based on these shortcomings, research efforts have been directed towards detection of internal insect infestation of grains. Scientists have tested possibilities in flotation, radiographic techniques, acoustic techniques, uric acid measurement, ninhydrin-impregnated paper, nuclear magnetic resonance, and immunoassays (Pedersen, 1992). Among these methods it is only acoustical methods that have the potential for automation of insect monitoring hence gives it a major advantage over other methods.

Acoustic methods rely on detecting the sound generated by the movement and feeding of insects on grain. Recent developments in acoustic technology have enabled detection of larval stages, estimate population density, identify insect species and map distributions of some postharvest insect pests (Fleurat-Lessard et al., 2006, Hagstrum et al. 1988, Mankin et al. 2010).

Within bulk storage facilities in Africa, the acoustic technique is potentially valuable for detection and monitoring of insect activity. The efficacy of acoustic devices in detecting cryptic insects and pre-emergent life stages of insects can be enhanced by using suitable sensors for the frequency range of noises caused by the insect of concern. To achieve this, acoustic profiles of insects of important food grains of Africa need to be studied and process these acoustic signatures into numeric data suitable for an automatic recognition of insect sound spectrum.

Based on this, this research work was conceived with the overall objective to undertake acoustic fingerprinting of postharvest insect pests' sound spectra for long term monitoring of storage pests of grains in bulk storage warehouses in Kenya.

The first activity was to conduct a review on the postharvest loss situation in Kenya, in terms of grain production, consumption, storage and postharvest losses associated with insect pest attack. This review shed light on the magnitude of the problem in grain storage, advised the next set of steps and justified the need to carry out this work. The second activity was to set up an acoustic laboratory in Kenya for the investigation of acoustic signals of several postharvest insect pests. The laboratory was equipped with state-of-the-art equipment described in this thesis for acoustic profiling of sounds produced by adult and immature stages of 3 postharvest pests namely *Prostephanus truncatus, Sitophilus zeamais,* and *Acanthoscelides obtectus*. The data obtained in this work helped in the selection of specific unique frequency identifiers for these pests. The third activity of this research work aimed at surveying selected maize storage warehouses in Kenya located in different climatic zones in order to establish the possibility of acoustic detection and trap capture of postharvest insect pests. The study created a basis for sensor development and identified the need to discriminate the insect signals from non-target background noise which was present in all stores surveyed.

The following dissertation structure and road map are based on the sequence of the above activities and research publications emanating from this work.

1.2.Dissertation structure

This dissertation comprises of 8 discrete chapters:

- Chapter 1 covers the introduction, dissertation structure and the dissertation road map
- Chapter 2 the state of the art identifying gaps in knowledge and articulation of the research objectives and questions
- Chapter 3 the acoustic characteristics of postharvest insect pests of maize *Prostephanus truncatus* and *Sitophilus zeamais*
- Chapter 4 the bioacoustics of a postharvest insect pest of common beans *Acanthoscelides obtectus*
- Chapter 5 the acoustic survey of selected grain storage warehouses in Kenya
- Chapter 6 the overall discussion and synthesis of results obtained from this research and an outlook with recommendations for further research.
- Chapter 7 constitutes the summary and provides conclusions on the work undertaken within this dissertation.
- Chapter 8 the appendix which provides captions of acoustic shielding chamber constructed and acoustic recording equipment employed in this research.

Chapters 3, 4 and 5 are stand- alone topics each with its own abstract, introduction, materials and methods, results and discussion. The results of the overall research are given within these 3 chapters.

1.3.Dissertation road map

The chapters in this thesis are arranged to follow the stages of automated acoustic detection which begins with identifying the problem pests followed by lab-based and field-based studies to characterize their acoustic emissions. Overall this dissertation deals with experimental studies on the efficiency of acoustic methods in the detection of larvae and adult insects. The acoustic emissions of the insects during feeding and locomotion are the main agenda for this research.

The laboratory activites begin with rearing the colonies of selected major postharvest insect pests that devour and attack major cereal and legume crops of food security importance in Kenya particularly *Prostephanus truncatus, Sitophilus zeamais,* and *Acanthoscelides obtectus*. Each pest is monitored throughout its developmental stages from egg to adult using acoustic detection equipment in a sound proof chamber. These studies help to realize the first 2 objectives of this thesis elaborated in chapter 2 and the lab- based studies are presented in chapters 3 and 4.

The success of this stage leads to the field- based studies of detecting the pests in their natural habitat in grain storage warehouses whereby noise shielding is not done.

The field- based approach is embraced to validate the magnitude of losses in grain storage facilities as identified in chapter 2. This approach is implemented through an acoustic survey in selected grain storage warehouses in various geographical zones of Kenya and with varying presence of insect pests. This study helps realize the third objective of this thesis and the results emanating from this work are presented in chapter 5.

The research conducted in this dissertation was a part of the Reducing Losses and Adding Value along East African Food Value Chains (RELOAD)-project funded by the Deutsche Gesellschaft für Internationale Zusammenarbeit, GmbH (GIZ) under the Staple Foods Value Chain, Subproject 5 (SP5) Work Package 1 (WP1) whose overall objective was to develop an acoustic early warning system for insects and rodents control in storage.

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2. State of the art

2.1. Grain production in Kenya

Grains are the most important food staple in Sub-Saharan Africa. Kenya's economy largely depends on the agricultural sector with about 75% of Kenyans owing their livelihood to agriculture (Nukenine, 2010). Grain storage at farm level and in strategic reserves of developing countries plays a critical role to food security since it mitigates the impact of poor and non-consistent harvests. Grain production does not tally with the national consumption patterns and the deficit has to be met by importation. The most widely produced and consumed cereal and legume in Kenya are maize and beans. Other important grains that are less produced and consumed are wheat, rice sorghum, millet, green grams, cowpeas, and pigeon peas.

Maize (*Zea mays* L.) is Kenya‘s main staple food and the most widely cultivated and has the highest production as shown in Table 1. It contributes to household and national food security accounting for 65% of total staple food caloric intake and 36% of total food caloric intake (Food and Agriculture Organization Statistical Databases (FAOSTAT), 2009). The average person consumes 98 kg of maize products per year in green, milled or dry grain form (Ariga et al., 2010) and the low income earners spend about 28 % of their revenue on maize. The bulk of maize production (50%) in Kenya takes place in the North Rift Valley (Trans Nzoia, Uasin Gishu and Nakuru), 14% takes place in the Nyanza and Western regions and the rest is from small scale production in the rest of the country (United States Agency for International Development (USAID), 2010).

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in Kenya. Kenya is ranked as the seventh largest world producer of dry beans which are the third most important staple food nationally (USAID, 2010). Beans constitute a significant portion of total caloriesapproximately 5-9% of the Kenyan diet (Kirimi et al., 2010) hence having a critical relevance to national food security. Because they can be consumed as dry or green seed as well as provide a vegetable source in form of leaves, they are an important factor in improving food security and combating hunger and malnutrition (Korir et al., 2003).

National production of beans in the years from 2006 to 2010 as demonstrated by Table 1 below has not been stable however between 2011 and 2012 production increased at an estimated compound growth rate of 14 % (Ministry of Agriculture (MoA), 2015). Common bean production takes place mainly in high and mid altitude areas. Over 75% of annual production occurs in Rift valley, Nyanza, and Western Provinces. The Rift Valley contributes about 33% while Nyanza and Western provinces account for 22% each. Output from eastern parts of the country and the coast is constrained by adverse climatic conditions.

2.2.Fluctuation of grain production

Overall, grain production has fluctuated widely over the seven years presented in Table 1 below depending on changing weather patterns, availability of seed and fertilizers and ready market for the grain. Despite Kenya being a major consumer of dry beans, the demand for the commodity outweighs production (USAID, 2010). The country consumes approximately 500 Metric Tonnes (MT) against a local production of 125MT of dry beans harvested from 500,000 hectares (MoA, 2015). With increasing population and urbanization, there is persistent supply shortage in the face of rising demand for the dry beans especially in urban areas.

Table 1 Grain production in Kenya

Grain type	Yearly Production in MT								
	2006	2007	2008	2009	2010	2011	2012	2013	2014
Maize	3,247,777	2,928,793	2,369,569	2,442,823	3,464,541	3,376,862	3,603,338	3,592,688	3,513,171
Wheat	358,061	354,241	336,688	219,301	511,994	268,481	441,756	449,641	328,637
Rice	64,840	47,256	21,881	42,202	80,042	111,465	122,323	125,256	112,263
Beans	531,800	383,900	261,137	465,363	390,598	577,673	657,740	714,492	615,992

Source: MoA Economic Review on agriculture; 2015

2.3.Grain Stocks and Bulk Grain Storage Practices in Kenya

2.3.1. Importance of storage

The importance of enhancing post-harvest storage and handling of maize, wheat and rice in Kenya cannot be overemphasized (Nduku et al. 2013). The national food security in Kenya is often pegged on availability and adequate supplies of maize to meet domestic demand. Storage evens out the seasonal supply, stabilizes prices and reduces food insecurity (Komen et al. 2006). Storage is also aids in seed preservation, quality improvement and quantity equalization (Adetunji, 2007). Incentives to store are mainly dependent on profits accrued from sale of stored grain (Komen et al. 2006).

2.3.2. Grain stocks in Kenya

In Kenya, the storage of grains is undertaken by farmers, farmer groups, traders, millers and private or government warehouses or silos in various capacities. Large scale farmers in the North Rift have the resources to construct their own godowns for storage while most small scale farmers sell out most of their produce and are left with just enough for consumption. Therefore, both on farm and off farm storage are of key importance. The total grain stocks of maize, wheat, rice and beans in Kenya in the year 2012 are shown in Table 2.

Table 2 Grain stocks in Kenya

Grain type	Quantity in MT
Maize	2,379,190
Beans	230,760
Wheat	49,008
Rice	64,539

Source: MoA Economic Review on agriculture; 2015

2.3.3. Strategic maize grain reserves in Kenya

Strategic grain reserves are the Kenyan government's strategy to deal with the seasonal nature of grain production. The National Cereals and Produce Board of Kenya (NCPB) established in 1985 is mandated by the Government to regulate and control the marketing and processing of grains in Kenya in all aspects of growing, procurement, research, distribution, storage, trade and promotion in both the local and the international markets. It does this through licensing and regulating the key players in the sector, who include traders, farmers and millers among others. However, after liberalization of the sector, the NCPB has faced competition from independent players especially in procurement, distribution, storage, and grain processing. Most of the harvested grain is held by the farmers and stockists as shown in Table 3 for maize stocks in 2012.

Table 3 Maize grains holding in Kenya in 2012

Stakeholders	Quantity in MT
Farmers	1,891,119
Traders	241,422
Millers	42,004
NCPB	204,463
Total	2,379,190

Source: MoA Economic Review on agriculture; 2015

2.3.4. Storage by farmers, farmer groups and traders

The bulk of grain storage in Kenya is undertaken by farmers, farmer groups and traders who have invested in large warehouses where they stack bags of grain. Farmers do this to avoid middlemen who pay them rock-bottom prices for their harvests. They are given incentives via the warehouse receipting system whereby they can use the receipts to access credit. There are innovative schemes, like the East African Grain Council (EAGC), which enable small farmers to collectively store their produce in certified warehouses and use it to obtain credit from banks and enabling them to buy good seeds and fertilizer. The warehouses are collectively or individually owned and they store grains in bags stacks. Both small- and large-scale farmers bring their grain in trucks, donkey carts or on bicycles.

2.3.5. Storage by millers

Millers also play an integral part in Kenya's grain storage. There are a total of 22 millers, of which 18 are large capacity (150 tons/24 hours) and 4 are medium capacity (50-150 tons/24 hours). The country's installed milling capacity is about 3,500 tons per day. However, some millers operate below capacity with the majority of mills operating at capacities of between 100-300 tons per day.

2.4.Grain Postharvest Losses in Kenya

2.4.1. Grain loss estimates

Food valued at over United States Dollar (USD) 4 billion dollars is lost every year in Africa as a result of post-harvest inefficiencies across the staples agricultural value chain (Alliance for Green Revolution in Africa (AGRA), 2013). Kenya experiences an estimated 20-30% loss of staple grains, which poses great challenges to the country's food security and economic development (George, 2011). Post-harvest losses significantly endanger the livelihoods of stakeholders across the value chain by reducing valuable incomes and profitability. Reduction of one percent of postharvest losses can lead to saving of USD 40 million annually. Poor storage facilities and substandard storage pesticides aggravate insect attack and losses. Losses during storage are largely overlooked because the damaged grain is often fumigated to kill the existing storage pests then mixed with freshly harvested grains to make the damaged grain palatable. Rembold et al. 2011 designed loss estimation tables for various grains in Eastern and Southern Africa for The Africa PostHarvest Losses Information System (APHLIS). The figures quoted in the APHLIS tables are estimates of cumulative weight loss from production incurred during harvesting, drying, handling operations, farm storage, transport and market storage from the year 2006 to 2014 (Rembold et al. 2011). Some of the loss estimates are shown in Table 4.

Table 4 Grain loss estimates for major cereal grains in Kenya

Grain type			**Percentage loss (%)**						
Year	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Maize	17.9	18.9	19.9	17.8	18.8	17.8	18	17.8	18.6
Wheat	9.9	12.8	12.6	15.1	14	13.1	12.9	15.2	-
Rice	11.8	11.8	12.1	12	12.6	12	13.9	12.1	-

Source: APHLIS Storage loss estimates (retrieved January 2017)

These percentage losses can be used to derive generalized loss estimates for the major food grains produced in Kenya from 2006 to 2014 in Kenya as shown in Table 5.

Table 5 Generalized loss estimates for major cereal grains in Kenya

Grain type	Yearly Grain losses in MT								
	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Maize	581,352	553,541	471,544	432,379	627,081	601,081	652,204	639,498	653,449
Wheat	35,448	45,342	42,759	32,895	67,071	35,171	56,544	68,345	-
Rice	7,651	5,576	2,647	5,064	10,085	13,487	17,736	13,583	-

Source: Author estimates based on APHLIS Storage loss estimates (retrieved January 2017)

2.4.2. Postharvest handling and reducing food losses

Efficient post-harvest handling, storage and marketing can tremendously contribute to social economic empowerment of rural communities (George 2011). Thus, reducing food losses increases food availability without requiring additional production resources and in least developed countries, and it also contributes to rural development and poverty reduction (Hodges et al. 2011). Storage of cereals plays an important role in evening out fluctuations in production from one season or year to the other (Kimenju and De Groote 2010).

2.5.Grain Storage Management and Insect Pest Control in Kenya

2.5.1. Measures to combat insect attack on stored grain at farm level

During grain storage pest control measures have to be put in place to combat imminent insect pest attacks. These measures include but are not limited to: synthetic pesticides, plant botanicals and physical control methods. Other measures put in place to reduce postharvest losses (PHLs) include proper drying of grain to 13-14% moisture content, use of suitable storage methods like hermetic storage. In hermetic environments, low oxygen conditions are created by metabolism of insects, fungi and grain itself leading to the death of same insects by asphyxiation (Murdock et al. 2012). Additional coping strategies include the use of herbs like the Mexican marigold and hot pepper in storage, selling grain soon after harvest and fumigation (Bett and Nguyo 2007). Chemical pest control is common when grain is to be stored for 6 months or more. The effectiveness is highly dependent on proper use of the chemicals. Poorly treated grain with hidden infestation finds its way to storage warehouses thus spreading the problem.

2.5.2. Measures to combat insect attack on stored grain in bulk storage facilities

Once the grain gets to the bulk storage facilities and strategic grain reserves, fumigation with Phosphine gas (hydrogen phosphide, PH_3) is done. During fumigation, the grain is held under gas tight conditions for approximately 7 days. However, some insects like *Rhyzopertha dominica* (F.) and *Tribolium* castaneum (Herbst) have developed resistance to phosphine (Opit et al. 2012). In most cases gas tightness cannot be ensured and hence the gas escapes with the subsequent result of insect resistance to the fumigant (Song et al. 2011) and migration of resistant populations through grain trade (Opit et al. 2012).

2.5.3. The concept of integrated pest management

Overall, Integrated Pest Management (IPM), a pest risk management tool that combines biological, cultural, physical and chemical tools, is used to combat PHLs effectively especially in bulk storage facilities. It includes understanding interactions between stored product environments, insects associated with stored products , and replacing chemical control with non-chemical alternatives such as good sanitation, continuous monitoring, and physical control (Subramanyam, 1995). Improved store sanitation reduces the frequency of fumigation because it takes a longer time before re-infestation occurs. Monitoring entails timely inspection of grains to reduce the probability of infestation and to identify the existing and potential pest problem in the storage facility (Koul et al. 2004). Records of the infested area and the insect density over time are kept. These records are used to verify the effectiveness of a control measure and for pest management decisions to avoid unnecessary or late implementation of control measures. Visual inspections in and around storage facility, examination of grain samples, monitoring changes in temperature and insect trapping are among the methods used (Fleurat-Lessard ,2011).

2.6.State of the art of bioacoustics insect monitoring

2.6.1. Economically important postharvest insect pests in Kenya and their acoustic detection

Two major groups of insects harbour the mostly economically important post-harvest insect pests of food grain in Kenya: Coleoptera (beetles) and Lepidoptera (moths and butterflies) (Sallam, 2008). Several Coleopteran and Lepidopteran species attack crops both in the field and in storage. The order Coleoptera is the largest order of insects, constitute the primary pests and are therefore of the greatest importance (Salunke et al. 2009). They inhabit a wide variety of habitats and can be found almost everywhere. They constitute the most important pests of maize, wheat, rice and beans (Salunke et al. 2009). They include *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) *and Acanthoscelides obtectus* Say (Coleoptera: Bruchidae).

Acoustic detection is a very promising method for early detection of insects inside the grain mass.The acoustics of infestation during insect feeding, moving, moulting, and pupating including the activity noises produced during the development of various insect pests has been studied. Adult and immature stages of stored product insect pests vary considerably in size and in the amplitudes and rates of sounds they produce (Mankin et al. 1997). The first studies on acoustical detection of pests were based on detection inside kernels using low frequency detectors like microphones, phonograph cartridges, and earphones or speakers coupled with mechanical counters or strip chart recorders (Adams et al. 1953, Bailey & McCabe 1965, Street 1971, Vick et al. 1988). Next followed a series of studies on the use of high frequency detectors (40 kHz) like piezoelectric sensors for *Sitotroga cereallela* Olivier (Lepidoptera: Gelichiidae), *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) and *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae) *(*Hagstrum et al. 1988, Webb et al. 1985, Shade et al. 1990, Litzkow et al. 1990). Advances in attempts to automate acoustical monitoring of postharvest insect pests have also been made (Vick et al. 1990). More recently, the efficacy of bioacoustics in detecting the presence of adult beetles in wheat was studied (Eliopoulos et al. 2015). Furthermore, a comparison of the performance of a laboratory acoustic device and an acoustic probe in the detection of infestation within grain bulks was tested in a field study in the cereal production area of Western France, (Leblanc et al. 2011). Review articles have also demonstrated acoustic detection as the future of pest management in storage facilities (Mankin et al. 2011, Mankin and Hagstrum 2011).

***Prostephanus truncatus* Horn (Coleoptera: Bostrichidae)**

Prostephanus truncatus Horn (Coleoptera: Bostrichidae) is the most important storage pest for maize and dried cassava (Meikle et al. 2002). The most recent published data on the geographical distribution of *P. truncatus* indicate that the pest currently occurs in at least 16 African countries (Nansen and Meikle 2002). *Prostephanus truncatus* is more injurious, and in endemic situations, extensive grain damage results in over 30% dry weight loss (Cugala et al. 2007; Mutambuki and Ngatia 2012). Little research has gone into the acoustic detection and monitoring of *P. truncatus*. However, a lot has been done on its close cousin *Rhyzopertha dominica* also known as the lesser grain borer. *Rhyzopertha dominica* larvae damage intact wheat kernels more than any other stored product pest (Edde 2012). Acoustical monitoring of *R. dominica* immature stages and different populations in wheat kernels has been done (Hagstrum et al. 1988, 1990). The acoustical detection of *R. dominica* among other insects over a range of temperatures has also been studied (Hagstrum and Flinn 1993). More recently, a digital X-ray image was demonstrated as an application to detect storage pests, including *R. dominica*, in wheat kernels (Karunakaran et al. 2003).

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae)

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), also known as the maize weevil, is a pest of stored maize and of cob maize prior to harvest (Adedire 2001). It is one of the major maize storage pests among smallholder farmers in Eastern and Southern Africa (ESA). It starts to infest the ripening maize crop in the field when the grain moisture content is still 50-55% (Adedire 2001).

It is closely related to the rice weevil (*Sitophilus oryzae* and the granary weevil (*Sitophilus granarius*). Heavy infestation may cause weight losses of as much as 30-40% of produce (Commonwealth Agricultural Bureaux International (CABI), 2005). Adult weevils and larvae feed on undamaged grains and reduce them to powdery form (Adedire 2001). Several detection techniques have been researched on for the various *Sitophilus spp*. For instance, acoustic detection of *S. oryzae* has been greatly emphasized in several research works for example, the possibility of thermal treatment to increase acoustic detectability of *Sitophilus oryzae* in stored grain (Mankin et al. (1999). Fleurat-Lessard et al. (2006) also developed classification algorithm for the automatic recognition of recorded insect noise signals of *S. oryzae*. More recently, Mankin et al. (2010) studied the crawling and scraping activity of *S. oryzae* among other insects to by developing indicators for their targeted/ focused detection. Potamitis et al. (2009) also dedicated research efforts towards the development and evaluation of a unified framework for automatic bioacoustic recognition of *S. oryzae by* capturing and automatically recognizing the acoustic emission resulting from typical behaviors like locomotion and feeding.

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae)

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae), is an externally feeding secondary pest of stored products, mainly flour and stored grain (Grünwald et al. 2013). It is an opportunist pest that attacks grain that has already been compromised by other internally feeding primary pests. The insect reduces food quality and its economic significance cannot be underestimated. It causes major economic losses, quantitative and qualitative losses. It has been estimated that economic losses caused by stored-product pests can range from 1.25 to 2.5 billion dollars annually in the United States (Flinn et al. 2007). In the last decade, little research has been dedicated towards acoustical detection of *T. castaneum*. Hagstrum et al. 1991 evaluated automated an acoustical detection system for monitoring *T. castaneum* populations in stored wheat. Hagstrum and Flinn, 1993 studied the acoustical detection of *T. castaneum* among other insects, over a range of temperatures.

Acanthoscelides obtectus **(Coleoptera: Chrysomelidae: Bruchinae)**

Acanthoscelides obtectus (Say) (Coleoptera: Chrysomelidae: Bruchinae) the bean beetle of Mesoamerican origin (Oliveira et al. 2013) is a serious post-harvest and field bruchid pest species of wild and cultivated common beans (*Phaseolus vulgaris* (L.) in the tropics (Paul et al. 2009). Beans among other edible legumes are a key source of dietary protein throughout much of the world. In Kenya, common bean is the most important food legume and second to maize as a staple (Wagara et al. 2011). Both *A. obtectus* and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae) bruchid species overlap in both niche and range, frequently co-occurring in bean stores. *A. obtectus* is reportedly more widely distributed in Eastern and Southern Africa (Ngamo and Hance 2007) and with a high predominance in bean stores of Uganda, Zimbabwe, and the Eastern highlands Tanzania (Msolla and Misangu 2002).

In Africa the economic importance of *A. obtectus* cannot be underestimated with many small-scale farmers in Africa relying on the production and sale of beans as an important source of household income. Farmers respond to the bruchid problem by selling their commodity at harvest, when market prices are at their lowest. It causes dry weight losses of between 10-40% in less than six months, and up to 70% grain damage rates have been recorded in the same time period (Paul et al. 2009). Grain damage, manifested by insect emergence holes in beans, cause significant price discounts, resulting to about 2.3% decrease in price for every hole per 100 beans (Mishili et al. 2011). All the larval instars are voracious feeders and develop at the cost of legume proteins so that heavily infested beans are often reduced to empty shells

Since *A. obtectus* has a short life cycle, just under 3-4 weeks, and high reproductive potential it can give rise to several generations per year under favorable conditions (Soares et al. 2014). To the best of our knowledge, little research on sound production by *A. obtectus* has been done in Africa. However, Andrieu and Fleurat-Lessard (1990), studied the type of sensor that can be used to identify *A. obtectus*.

2.7.Gap in acoustic research and the need for further research

Despite advances in acoustic detection techniques little effort has been directed towards real sensor development for application in storage facilities in Africa. Detection of insect eggs and larvae in internal infestation in bulk grain storage facilities in Kenya is a major challenge. The sampling and sieving method is commonly used due to its simplicity, however, it is not sensitive to pre-emergent forms of insects and when the infestation level is low (less than 5 insects/kg).

Improved postharvest pests' detection systems with increased sensitivity and affordable maintenance costs are needed in Africa. There is a need for tools for remote sensing of insects and detection of internal infestations for bulk stored grain. Automated detection methods for bulk grain storage are also needed to reduce the risks associated with entry into grain silos and grain stacks.

Acoustic methods have been applied successfully for grain inspection, estimations of population density and mappings of various stored product insect pest distributions namely: *R. dominica* larvae (Hagstrum et al. 1988); *T. castaneum* adults (Hagstrum et al. 1991); *S. oryzae*, *T. castaneum* and *Stegobium paniceum* L. (Coleoptera: Anobiidae) (Mankin et al. 2010); *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionida), *Sitophilus granaries* L. (Coleoptera: Curculionidae), and *Oryzaephilus surinamensis L.(Coleoptera: Silvanidae)* (Schwab and Degoul 2005); and *C. maculatus* on cowpea (Shade et al. 1990). Acoustic profiles of insects of important food grains of Africa need to be studied and process these acoustic signatures into numeric data suitable for an automatic recognition of insect sound spectrum and an easy discrimination from the noises in the storage environment or from the grain handling machinery.

All this is important in designing an early warning system for insect pest monitoring to be applied in the African setting to help prevent PHLs in bulk storage warehouses in Kenya.

2.8.Research questions and objectives

Based on the identified gaps in knowledge and need for research, the aim of this dissertation is to provide insight towards acoustic sensor development for postharvest insect pests.

In particular, the dissertation focuses on characterizing insect sounds of different species and different developmental stages as well as discrimination of background noise from target insect sounds.

The following is an overview of the objectives and corresponding research questions of this dissertation.

The overall objective is to undertake acoustic fingerprinting of postharvest insect pests' sound spectra for long term monitoring of storage pests of grains in bulk storage warehouses in Kenya. To achieve this, a set of sub-objectives were formulated:

1. The first sub-objective was to characterize the acoustic profiles of late instar larvae and adult stages of two major maize pests *Prostephanus truncatus* and *Sitophilus zeamais*

   Research question 1.1: Is there a difference in the acoustic characteristics of the developmental stages i.e the larvae and adults of these insects?

   Research question 1.2: Is there a difference in the acoustic characteristics of the two species?

2. The second sub-objective was to determine the bioacoustics of late instar larvae and adult stages of one major common bean pest *Acanthoscelides obtectus*

   Research question 2.1: Is there a difference in the acoustic characteristics of the larvae and adults of *A. obtectus*?

   Research question 2.2: Which stage of development is easily detected using the acoustic device?

3. The third sub-objective was to determine insect presence in selected grain storage warehouses in Kenya located in different geographical zones by means of acoustic survey and trap capture techniques

   Research question 3.1: Is there a relationship between the number of insects captured in the traps and acoustic emissions recorded during the acoustic survey?

   Research question 3.2: Is it possible to discriminate background noise from target insect sounds in the busy grain storage facilities?

   Research question 3.3: Are data collected using two different acoustic recording equipment comparable?

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

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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.

3.1. 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 (Garcia-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). 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 1988, 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; Hagstrum and Flinn, 1993) Similarly, little research work has been conducted on the acoustic detection of *S. zeamais* although considerable research exists on the rice weevil *Sitophilus oryzae* (L.) and the granary weevil *S. granarius* (L.) (Mankin et al. 1996, Schwab and Degoul 2005; Mankin et al. 1999; 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: 20μPa 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.

3.2. Materials and Methods

3.2.1. 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°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 ± 1°C; relative humidity 65 ± 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 & 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.

3.2.2. 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. 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 (Figure 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 (Mankin et al. 2015, 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.

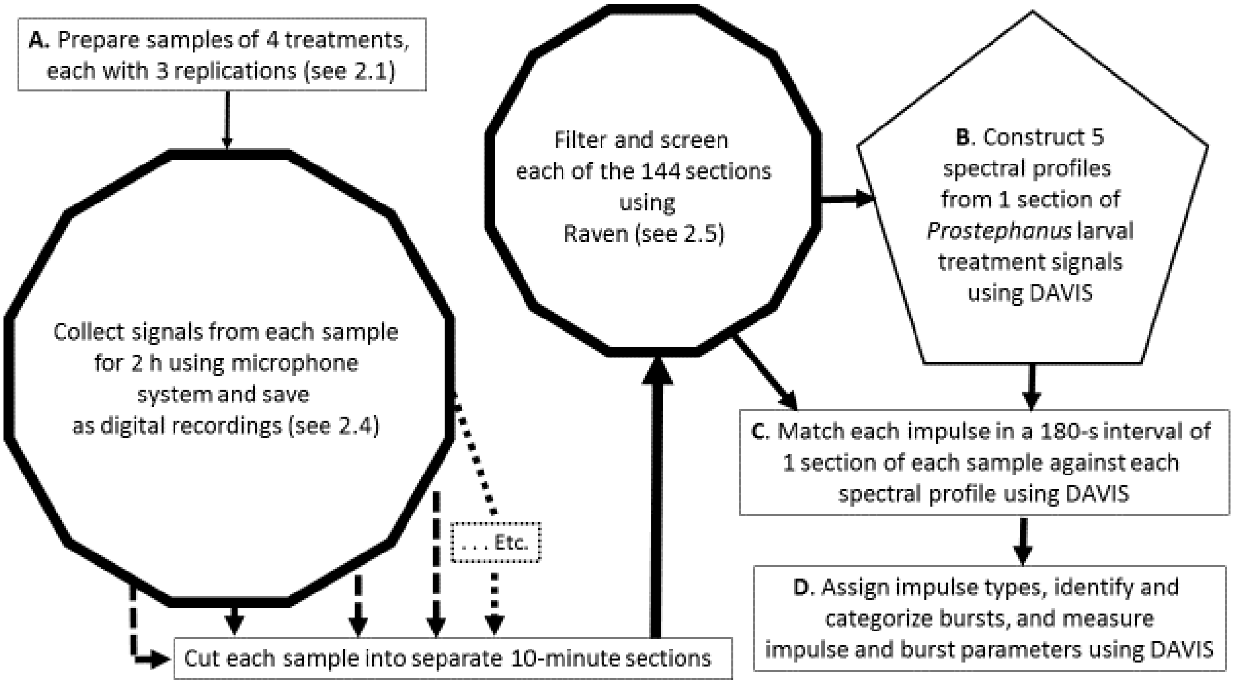

Figure 1 Process flow of experimental data recording and signal analyses

3.2.3. Acoustic shielding chamber

A portable noise shielding chamber was constructed (Figure 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 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.

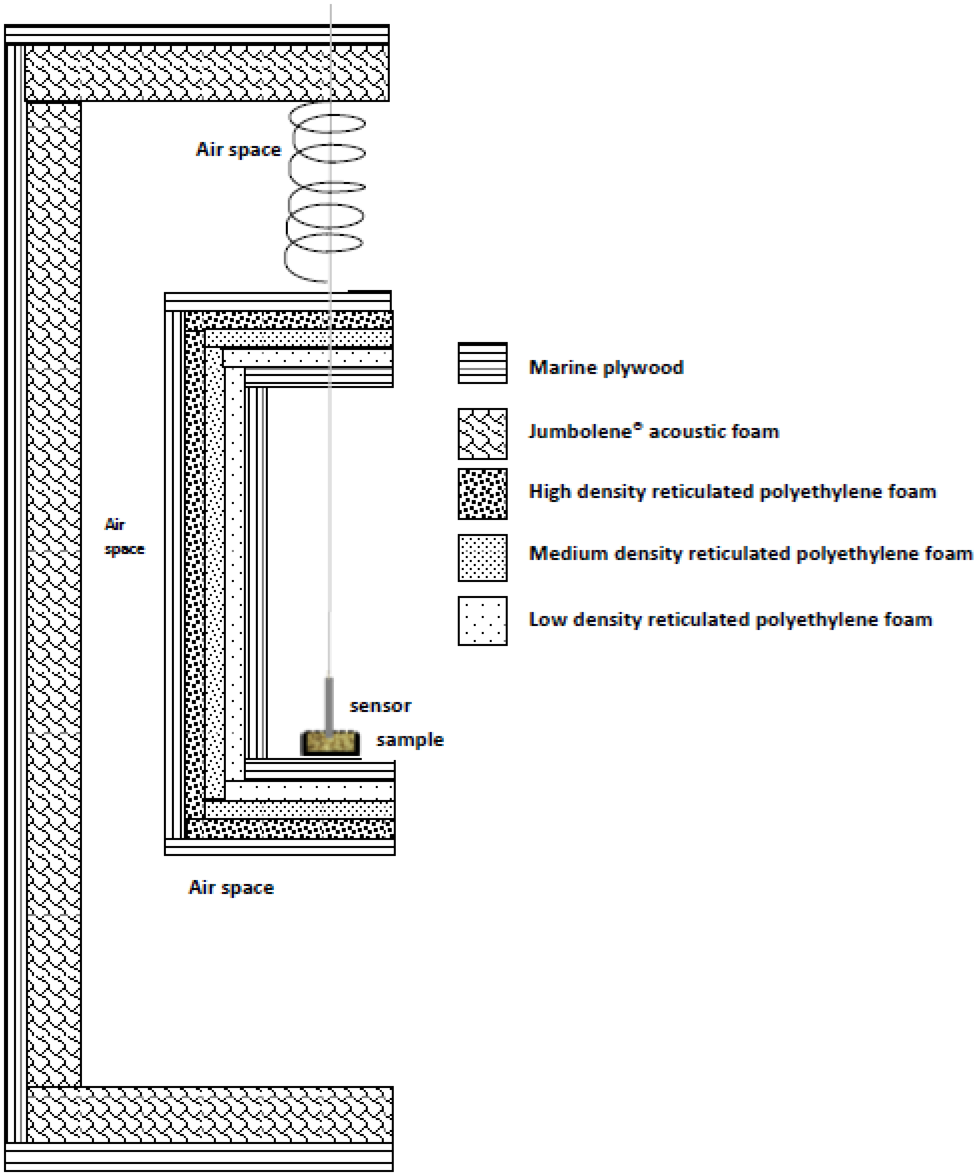

Figure 2 Schematic diagram of the acoustic shielding chamber showing position of the sample and sensor

3.2.4. 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 10X 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.

3.2.5. 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. 2000, 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 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).

3.2.6. Statistical Analyses

All data were analyzed using Stata SE Version 12 (Stata Corp 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).

3.3. Results

3.3.1. 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. Figure 3a shows an example of several impulses and an impulse train in a 4 s record of *S. zeamais* larvae. Figure 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.

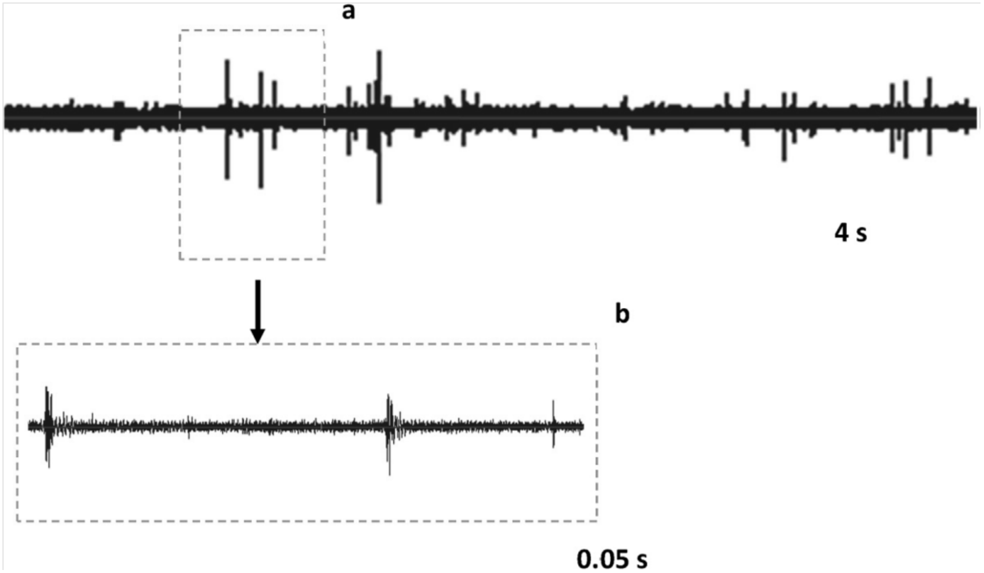

Figure 3 Examples of (a) typical impulses recorded from S. zeamais 4th instars, and (b), higher resolution inset displaying a 0.05 s interval of impulses.

Five audibly distinct types of spectra (Figure 4) were identified in the prescreening of a single, high quality acoustic recording of the 3^{rd} 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.

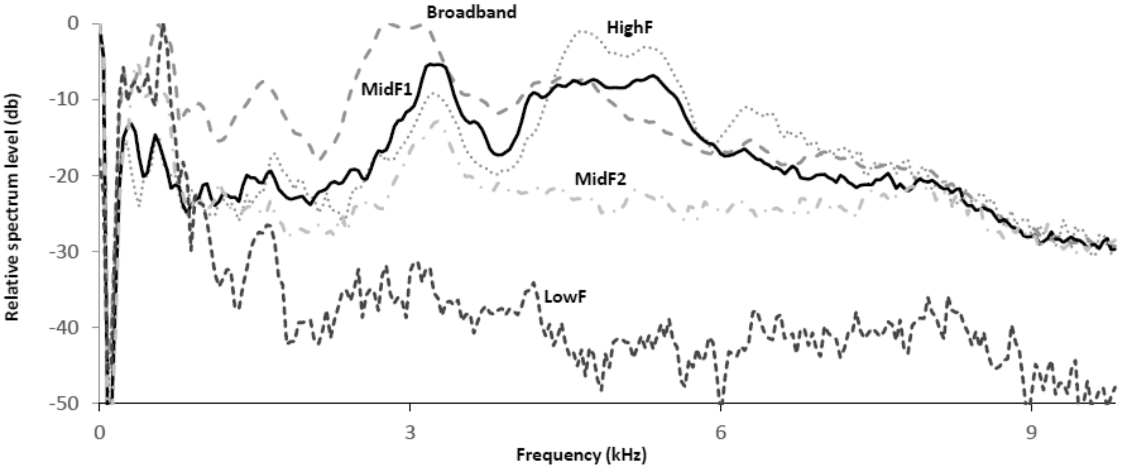

Figure 4 Frequency spectral profiles of five distinct types of impulses identified from an individual acoustic recording of the third instars of *P. truncatus*. Broadband, long dashed line; Low F, dashed line; MidF2, dash-dot-dotted line; MidF1, solid line, and HighF, dotted line. Horizontal axis indicates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

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 Figure 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 6. 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 in Table 7, and separately for bursts of each type in Table 9.

Table 6 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
P. truncatus	Larvae	27	12	57	88	32
	Adults	13	0	11	358	47
S. zeamais	Larvae	12	10	12	195	40
	Adults	24	7	84	212	6

3.3.2. 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 Figure 5a, b - Figure 6a, b, respectively.

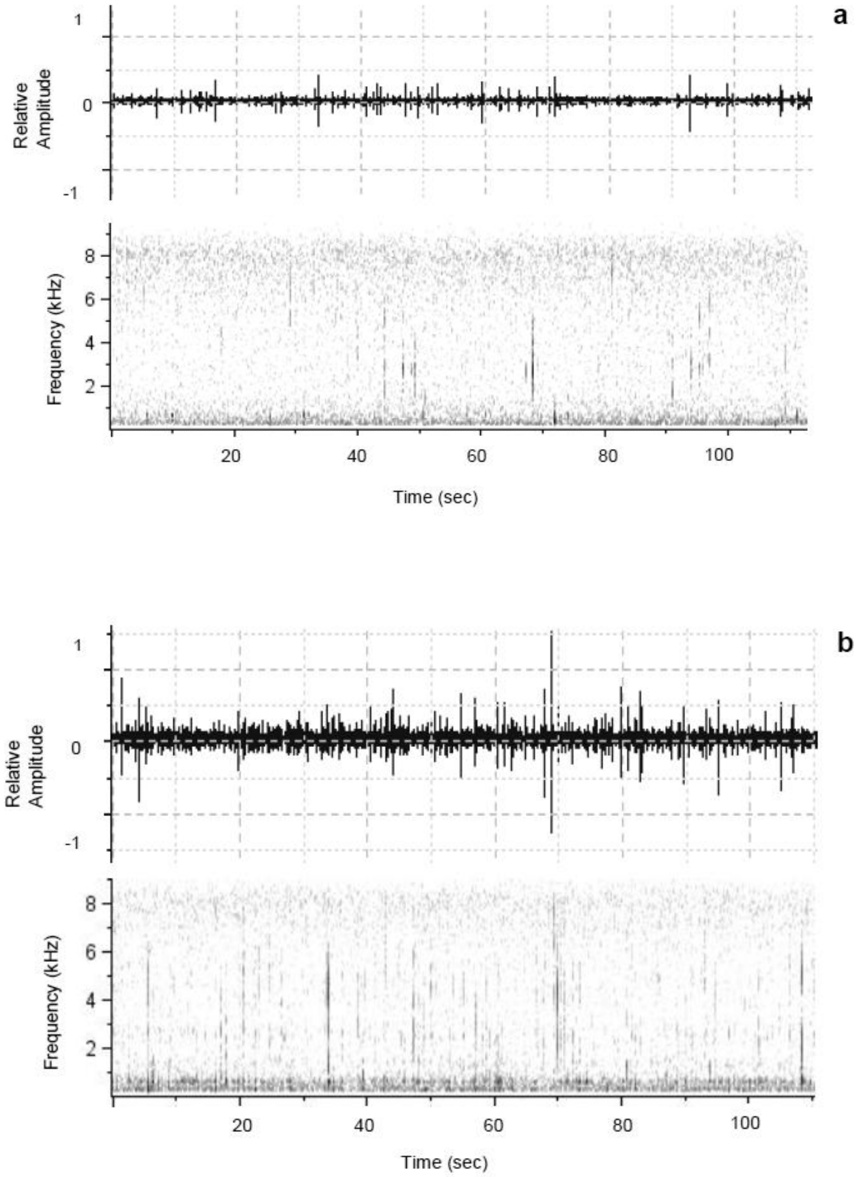

Figure 5 Oscillogram and spectrogram, of a 100-s period of signals recorded from maize infested wih (a) 4th instar of *S. zeamais* larvae, and (b) *S. zeamais* adults. Darker shading in spectrogram (256 points per spectrum, 50% overlap) indicates greater energy at that frequency

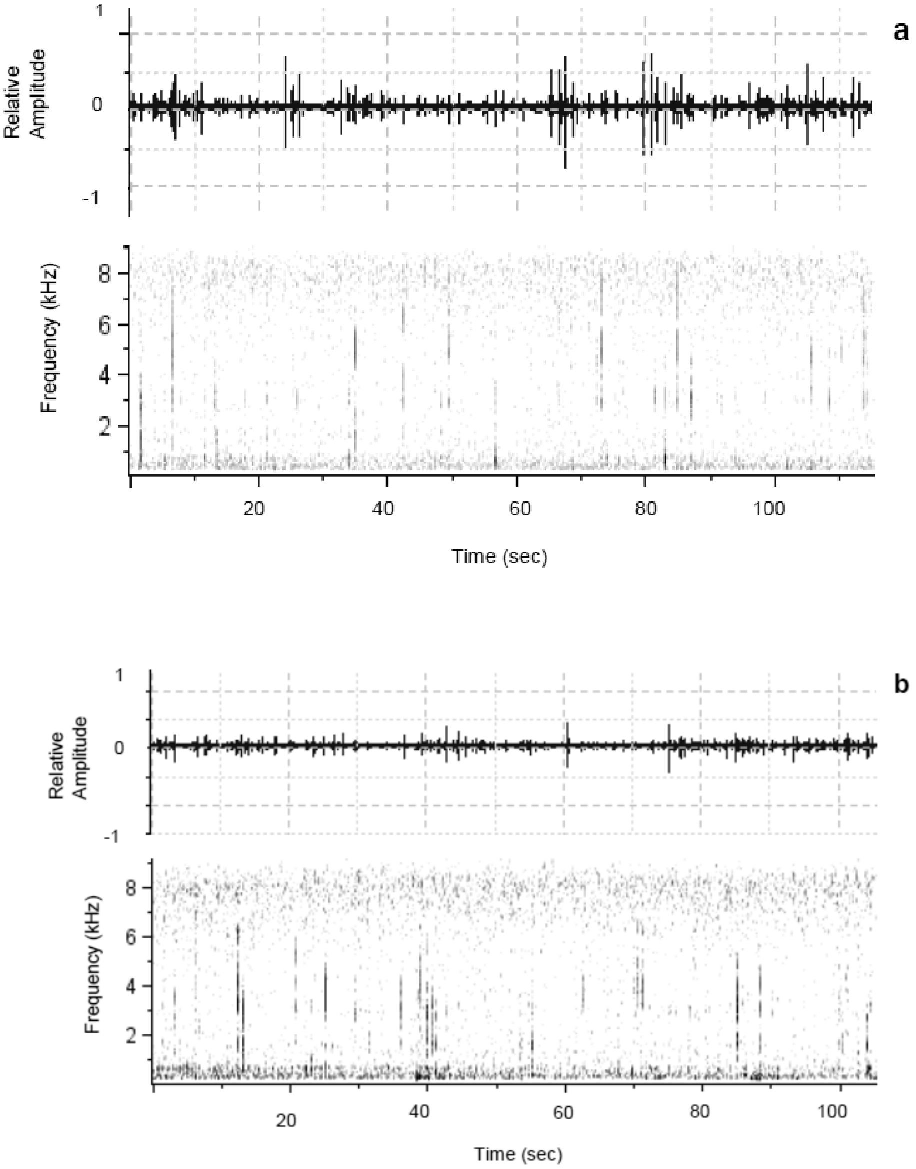

Figure 6 Oscillogram and spectrogram of (a) a 100-s period of signals recorded from maize infested with 3rd instar of *P. truncatus* larvae, and (b) *P.truncatus* adults. Darker shading in spectrogram (256 points per spectrum, 50% overlap) indicates greater energy at that frequency

Each example contains numerous impulses and bursts. Figure 7 displays a burst from a *P. truncatus* 3rd instar (Figure 5a), classified as MidF2, the most frequently occurring profile type.

Throughout the recordings, consecutive impulses often differed in amplitude, duration, and time interval, as in the examples of Figure 5b and Figure 6. 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.

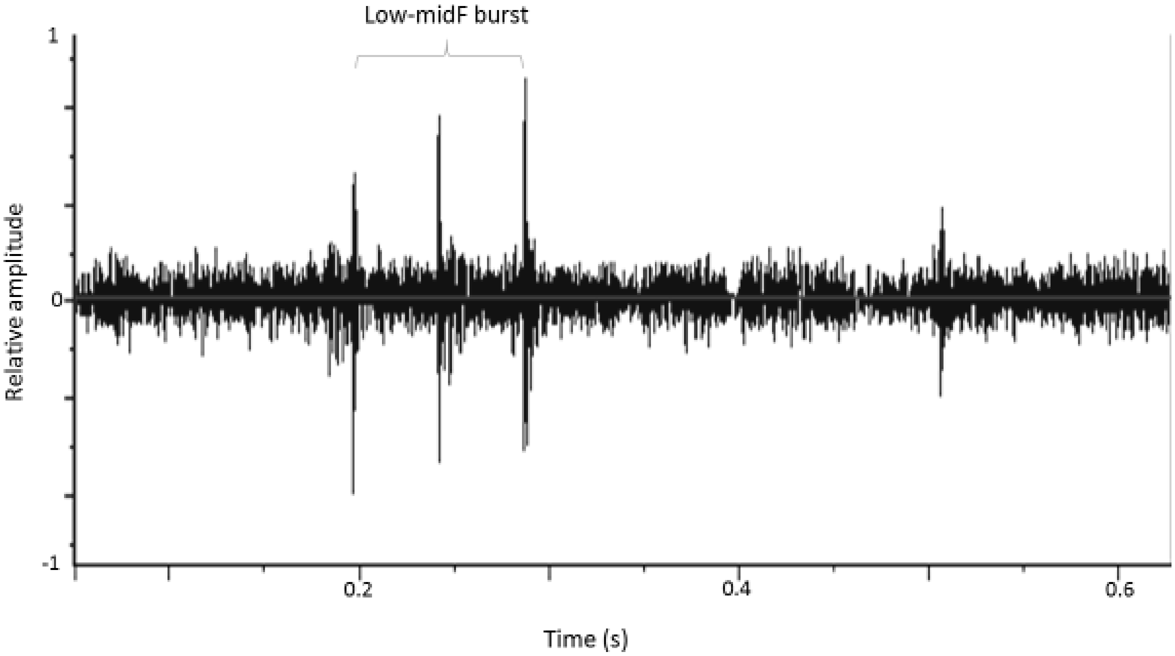

Figure 7 Example of an imulse train consisting of 3 MidF2 impulses that was classified as an insect burst

3.3.3. 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 *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 - 1301 for *P. truncatus* adults, 4 – 15,760 for *S. zeamais* adults, 20 – 1,854 for late instar *P. truncatus* and 5-399 for late instar *S. zeamais*. The overall number of impulses per burst ranged from 3- 213. Table 7 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 difference 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 7.

Table 7 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
P. truncatus	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
S. zeamais	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 ($P < 0.05$).

3.3.4. 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 8) 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 9 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.

Table 8 The effect 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 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 9 Means of insect sound parameters with significant differences among insect species and stages

Parameter	*P. truncatus*		*S. zeamais*	
	3^{rd} instar	Adult	4^{th} 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)

3.4. 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 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.

3.4.1. Differences in *S. zeamais* larval and adult behaviors

The sounds of *S. zeamais* adults, 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, 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.

3.4.2. Differences in *S. zeamais* and *P. truncatus* adult behaviors

The oscillogram of *P. truncatus* adults 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 no 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*, 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*.

3.4.3. 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, 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 relation to the *P. truncatus* larval spectrogram, 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.

3.4.4. 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.

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4. Bioacoustics of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) on common beans *Phaseolus vulgaris* L. (Fabaceae)

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Abstract

Acanthoscelides obtectus (Say) (Coleoptera: Chrysomelidae), is a serious pest of common bean *Phaseolus vulgaris* L. (Fabaceae) in the tropics and subtropics. It is difficult to detect the presence of *A. obtectus* because the larvae are cryptic and spend most of their developmental time inside the bean seeds. Their presence is almost imperceptible except for circular emergence holes created by the last instar larvae as they exit as adults. We believe that inexpensive acoustic means can be used to detect these larvae. To that end, laboratory experiments were conducted to estimate the acoustic characteristics of *A. obtectus* larvae and adults on stored common beans. Spectral and temporal features of sound signals recorded in an anechoic chamber were analyzed.

The larvae displayed continuous low-amplitude insect sound impulses frequently occurring in trains (bursts) of two or more impulses. In contrast, the adults displayed lower-amplitude impulses with less distinct bursts. The rates of bursts and the impulses per bursts for the larvae and the adults were significantly ($P \leq 0.05$) different. Overall, the larvae and adults of *A.*

obtectus produced varied acoustic signals that could be harnessed to acoustic sensor development. The use of acoustic sensors for real-time detection of *A. obtectus* infestation in stored common beans in sub-Saharan Africa may contribute to efforts to alleviate hunger and poverty in the region.

Key words: Acoustic detection, grain storage, Sub-Saharan Africa

4.1. Introduction

Acanthoscelides obtectus (Say) (Coleoptera: Chrysomelidae: Bruchinae), of Mesoamerican origin (Oliveira et al. 2013), is a serious post-harvest and field pest species of wild and cultivated common beans (*Phaseolus vulgaris* (L.) (Fabaceae) in the tropics (Alvarez et al. 2005; Keals et al. 2000; Paul et al. 2010; Thakur 2012). Beans and other edible legumes are a key source of dietary protein throughout much of the world. In Kenya common bean is the most important food legume and second to maize as a staple (Wagara et al. 2011). Both *A. obtectus* and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae) overlap in both niche and range, frequently co-occurring in bean stores. The *A. obtectus* is reportedly more widely distributed in Eastern and Southern Africa (Masolwa and Nchimbi 1991; Ngamo & Hance 2007; Mutungi et al. 2015) and with a high predominance in bean stores of Uganda, Zimbabwe, and the eastern highlands of Tanzania (Giga et al. 1992; Msolla & Misangu, 2002).

In Africa the economic importance of *A. obtectus* cannot be underestimated, with many small-scale farmers in Africa relying on the production and sale of beans as an important source of household income. Farmers respond to infestations by selling their commodity at harvest, when market prices are at their lowest. Infestations cause dry weight losses of between 10-40% in less than six months, and up to 70% damage rates have been recorded in the same time period (Paul et al. 2009). Postharvest bean damage causes significant financial loss to African small-scale farmers; Mishili et al (2011) estimated a 2.3% decrease in price per insect emergence hole in 100 beans. All the larval instars are voracious feeders and develop at the cost of legume proteins. Heavily infested beans are often reduced to empty shells.

Acanthoscelides obtectus has a short life cycle, just under 3-4 weeks, and has high reproductive potential. It can therefore give rise to several generations per year under favorable conditions (Soares et al. 2014). The females deposit eggs in clusters inside the pods in the field or on the shelled stored bean seeds (Godrey & Long 2008). One larva generally infests each seed; however, multiple infestations sometimes occur. In such cases, later instar larvae enter the seed through the same burrow initially excavated by the first. The final instars excavate a chamber just below the seed testa for pupation to take place. The presence of final-instar larva or pupa can be visibly detected by a small window composed almost entirely of testa, for easy emergence of the adult. After eclosion the adult chews a hole in the seed coat and pulls itself out of the seed, ready to mate.

As with many other postharvest pests of stored grain, *A. obtectus* infestation begins in the field, where adults lay eggs in dried bean pods. By late harvest, the damage to the beans can be so extensive that there is sometimes is no harvest at all (Schmale et al. 2001; Schmale et al. 2003; Velten et al. 2007). The best preventative measure is to plant seeds free from weevils, but careful visual inspection of every single seed is not practicable. And even if the planting seed is clean, the nearby bean fields must also be clean in order to ensure that the harvested crop will be free of weevils. This is a difficult task for bean farmers; harvested beans are therefore often infested. The internal mode of life of *A. obtectus* protects them from temperature and humidity variation, and enables them to be carried unnoticed during trade. Beans with undetected early-instar larvae move across geographical boundaries in import/export consignments, and pose a great phytosanitary threat in new ecological niches due to the absence of natural enemies.

Acoustic detection is a promising method for detecting insect larvae inside stored product grain kernels. The first studies on acoustical detection of pests were based on detection inside kernels using low-frequency microphones and phonograph cartridges which transmitted signals to earphones or speakers, which were coupled with mechanical counters or strip chart recorders (Adams et al. 1953; Bailey and McCabe 1965; Street 1971; Vick et al. 1988). Next followed a series of studies on the use of high-frequency (up to 40 kHz) piezoelectric sensors combined with powerful amplifiers, used to detect a variety of stored product insect pests, including *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *A. obtectus* and *Z. subfasciatus* (Webb et al. 1985; Hagstrum et al. 1988; Shade et al. 1990). Adult and immature stages of stored product insect pests vary considerably in size and in the amplitudes and rates of sounds they produce (Arnett 1968; Mankin et al. 1997). However, most of the movement and feeding sounds produced by these insects are in the form of groups (trains) of short, 3-10 ms broadband sound impulses that can be processed to enable their classification as insect sounds and distinguish them from background noise (Mankin et al. 2011).

Attempts to automate acoustical monitoring of postharvest insect pests began in the last century (Vick et al. 1990) and continue unto this day (Eliopoulos et al. 2015; Kiobia et al. 2015). The performance of a laboratory acoustic device and an acoustic probe in the detection of infestation within grain bulks was tested in a field study in the cereal production area of Western France (Leblanc et al. 2011). Review articles have also documented the applicability of acoustic detection for pest management in storage facilities (Mankin et al. 2011; Mankin & Hagstrum 2011). Frequency and time patterns of signals emitted by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera*:* Curculionidae) have been elucidated also (Njoroge et al. 2016). Though Andrieu and Fleurat-Lessard (1990) studied the type of sensor that can be used to identify *A. obtectus*, little research has been done on their automated detection. In the present study, experiments were carried out to characterize the spectral and temporal patterns of sound impulses produced by *A. obtectus* in dry common beans under laboratory conditions. Sound signals of separate groups of the larvae and adults of this pest were recorded in an acoustically shielded chamber.

4.2. Materials and Methods

4.2.1. Sample preparation

The initial stock of *A. obtectus* was obtained from infested pesticide-free dry common beans bought 6 months prior to the experiments. The common beans were a Rosecoco variety, cultivar of *Phaseolus vulgaris* L., cultivated in Kenya and procured from farmers through traders at Nyamakima Market, Nairobi. This first population of *A. obtectus* were raised and kept in a dark chamber under a 12:12 L:D photoperiod at 27-28 ºC and 70±/5% relative humidity (RH). The colony was maintained in multiple glass jars fed on 1 kg Rosecoco bean (15% m. c.). No other food or water was provided.

For acoustic measurements, 100 *A. obtectus* adults were randomly selected from the second generation and introduced into 3 sets of 1.45 L glass jars. Two hundred (200) g cleaned previously-frozen common beans were put in each glass jar and the adults were allowed to oviposit. Common beans with single eggs on the seed coat were selected using a microscope. The oviposited beans were divided into 100-g jars, and held in an environmental chamber at 30 ± 1ºC and 70 ± 5% RH. Each day the jars were checked for eggs that had hatched and larvae that had penetrated into the beans. The majority of the eggs hatched on the 7th day, and neonate larvae penetrated into the beans on the 8th day. After 15 days, the sample beans were dissected, and final-instar larval presence was ascertained by their morphological characteristics and by the presence of exuviae in the galleries, as described by Pfaffenberger (1985). Fifty (50) infested bean seeds were isolated from this stock and used for acoustic recordings of larval signals. After 26 days, 50 more bean seeds bearing adults were used for acoustic recordings of the adult signals. For uninfested control samples, 100 g of undamaged beans were randomly selected from the initial sample lot.

4.2.2. Recording setup and procedure

All insect sound recordings were carried out in a portable noise shielding chamber constructed as described in Njoroge et al. 2016 to reduce the possibility of false positives due to vibrational and impact noises in the vicinity of the acoustic measurement area. This chamber was kept in an isolated quiet room at ambient temperature (22-25 °C), with fluorescent lighting supplemented by ambient sunlight from nearby windows. Common bean seeds (100 g) infested with 50 *A. obtectus* larvae or adults were observed in a 13-cm diam., 4.5 cm deep stainless steel container using the method described in Njoroge et al. (2016). A 0.5" microphone of a preamplifier system (Model 378B02, PCB Piezotronics Inc., NY), positioned to make contact with the bean's surface, collected 20-dB-preamplified signals which were later amplified an additional 10X using a 4-analog-, 8-digital-input measurement device (imc C-SERIES (CS-3008-N) imc Meßsysteme GmbH, Frankfurt, Germany). All signals were saved at a 44.1 kHz sampling rate with 16-bit resolution. The measurement device was configured and operated using an integrated software package (imc STUDIO, imc DataWorks, LLC, Novi, MI). Six two-hour recordings were taken of each *A. obtectus* adult and larva treatment in our study, and saved in a .ccv (curve configuration file) format.

4.2.3. Signal processing

Signal processing was done as described in Njoroge et al. 2016, starting with conversion of the recorded signals from .ccv (curve configuration files) to .wav (wave audio files) format using a custom program written in MATLAB (Release 2012b, The MathWorks Inc., Newton, MA). After file conversion, the signals were band-pass filtered between 0.2 and 10 kHz, and several sections were taken from each two-hour recording with Raven Pro 1.5 Beta Version software (Cornell Lab of Ornithology, New York, United States; Charif et al. 2008).

A three-minute filtered recording from each jar was automatically analyzed using an insect signal analysis software program DAVIS (Digitize, Analyze, View, Insect Sounds) (Herrick et al. 2013; Jalinas et al. 2015). The analysis identified insect sound impulses of different spectral types and distinguished them from occasionally occurring background noise. In addition, we considered whether there were differences in the sounds produced by the adults and larvae tested in the study. To make spectral comparisons, we compared each impulse detected in each test recording against four insect sound profiles that had been employed in a previous study (Njoroge et al. 2016) to distinguish stored product insect sounds from background noise. Based on their peak energies and breadths of spectral range, the profiles had been labeled Broadband, HighF, MidF1, and MidF2. A fifth profile, LowF, was considered initially but was dropped from this study because none of the impulses detected from *A. obtectus* matched this profile. To perform each comparison, a spectrum first was constructed from a 512-point time slice centered on the peak of each impulse. The impulse then was classified as one of the four insect sound profile types, based on the profile from which it had the smallest least-squares difference (Mankin et al. 2011). Impulses whose spectra failed to match any profile within a preset least-squares threshold were classified as noise impulses—typically less than1-2% of the signals in this study because it was conducted in a sound-shielded chamber. For each recording, the DAVIS program identified and timed groups (trains) of two or more insect sound impulses separated by intervals < 200 ms as bursts. The beginning and end times, types of each burst and the count of impulses in each burst (burst impulses) were saved in a spreadsheet for subsequent analysis as in Njoroge et al. (2016) and Jalinas et al. (2015). The type of burst was classified as the predominant type of impulse in the burst, or in case of a tie, it was classified as the type of the first impulse that occurred in that burst.

4.2.4. Statistical analyses

All data were analyzed using Stata SE Version 12 (Stata Corp 2012). Analysis of variance (ANOVA) was performed to compare mean rates of impulses in bursts, rates of bursts and number of impulses per burst among larval and adult treatments.

4.3. Results

4.3.1. Spectral profiles

In comparisons of spectra of each detected impulse in each recording against the four insect sound profiles described above, adults produced impulses that matched the Broadband, HighF, MidF1 and MidF2 profiles, while larvae produced impulses that matched the Broadband, MidF1 and MidF2 profiles. The counts of impulses of each type detected in the study are shown in Figure 8 and the rates of impulses in bursts are shown in Figure 9.

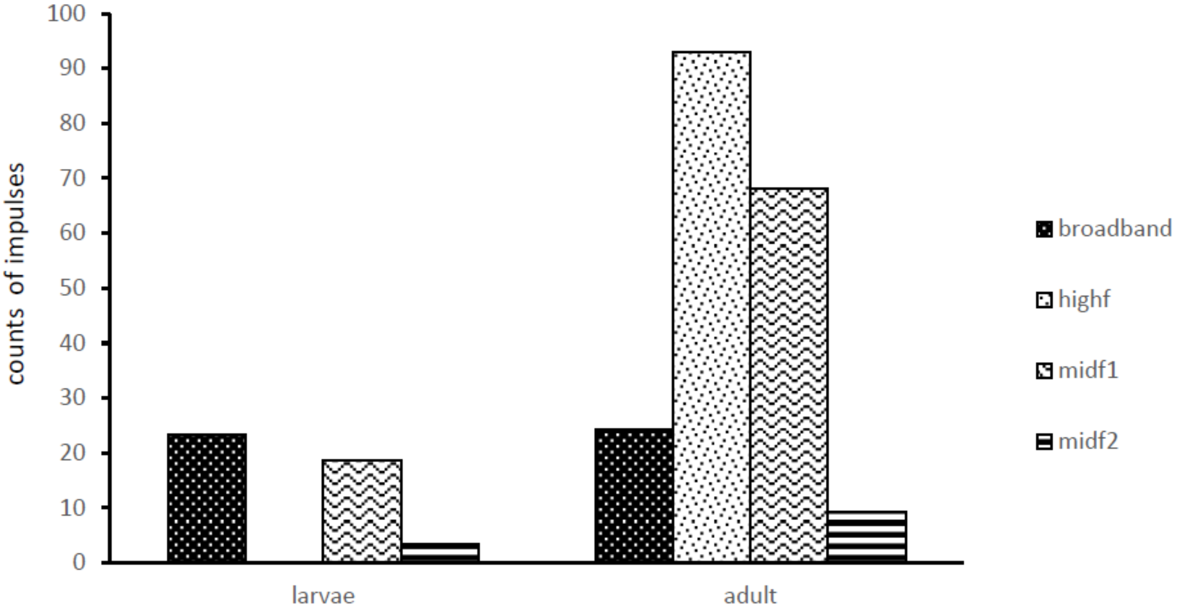

Figure 8 Counts of impulses of each type detected from the larvae and adults of *A. obtectus*

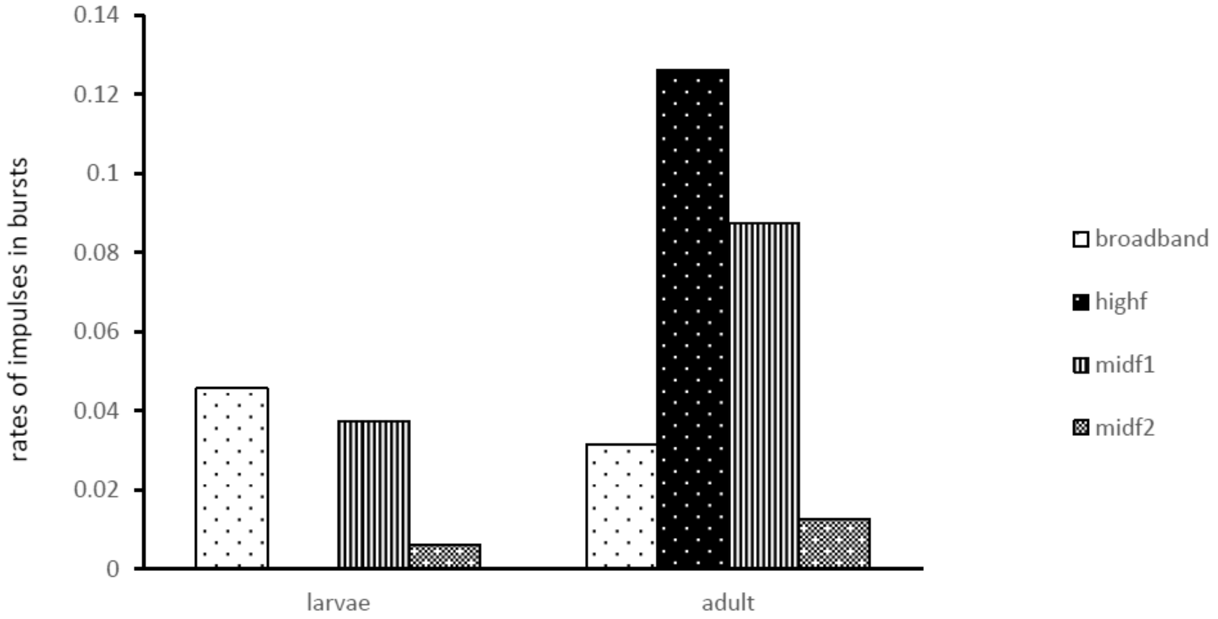

Figure 9 Rates of impulses in bursts of each profile type detected from the larvae and adults of *A. obtectus*

4.3.2. Larval and adult impulse characteristics

Examples of sounds produced by moving and feeding larvae and adults, respectively, are shown in Figure 10, Figure 11and Figure 12. The recorded signals display some of the similarities and differences frequently observed with *A. obtectus* signals recorded in the acoustic shielding chamber. The characteristic larval signals consisted primarily of Broadband or MidF1 impulses frequently occurring in bursts of two or more impulses (Table 10). The adult signal comprised primarily HighF and MidF1 impulses (Figure 8) with fewer impulses per burst than occurred with larvae (Table 10).

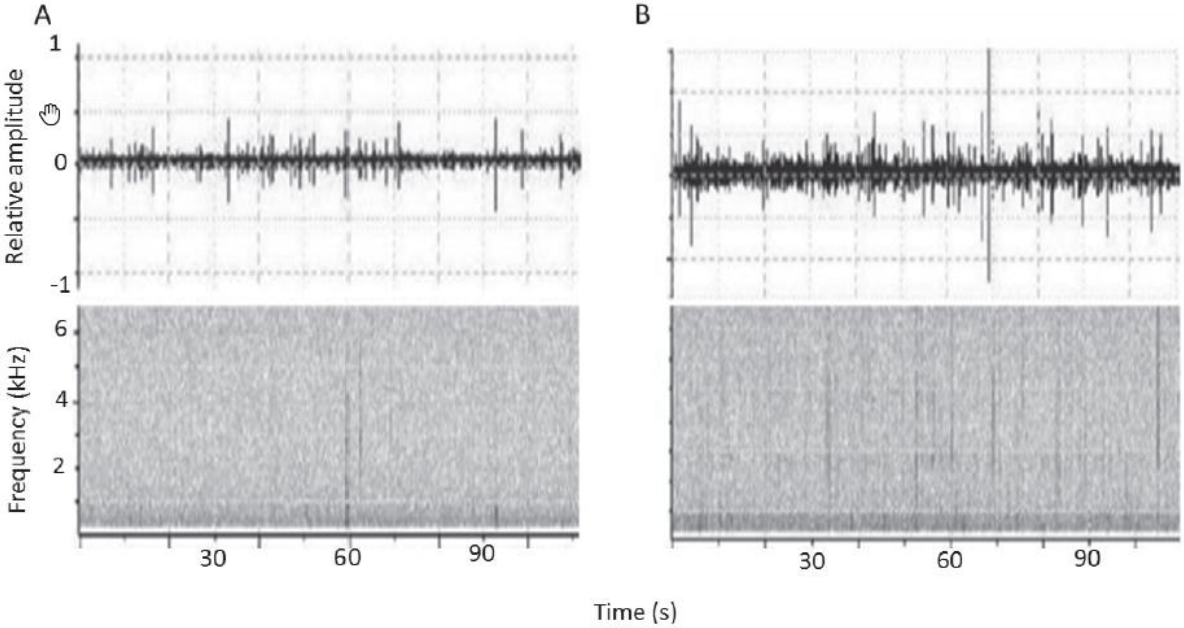

Figure 10 Oscillograms and spectrograms of a 100-s period of signals recorded from beans infested with *A. obtectus* adults A and larvae B

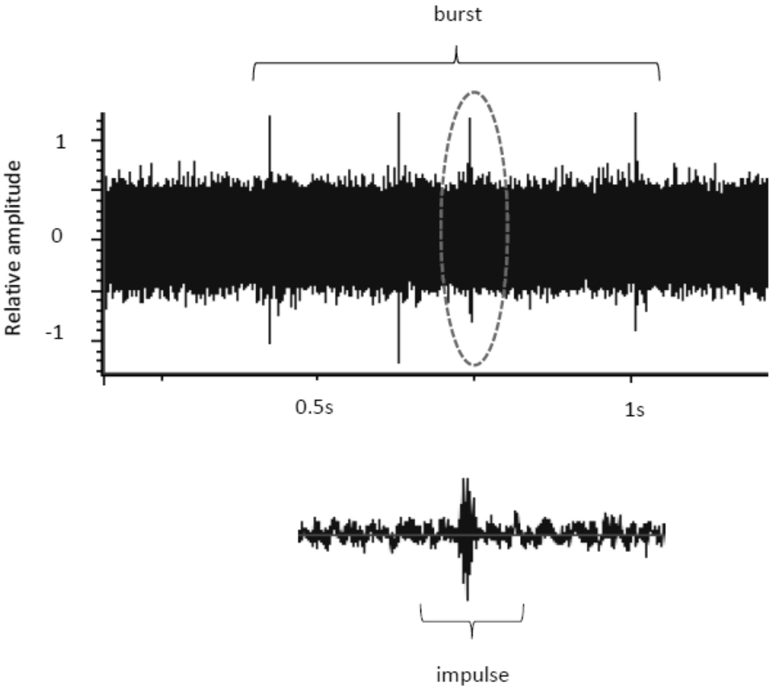

Figure 11 Oscillogram showing a single burst emitted by *A. obtectus* larvae and an example of a single impulse from the burst at a higher temporal resolution

It was observed that single bursts of the larvae consisted of distinct impulses combined with more or less regular intervals as shown in Fig 5.

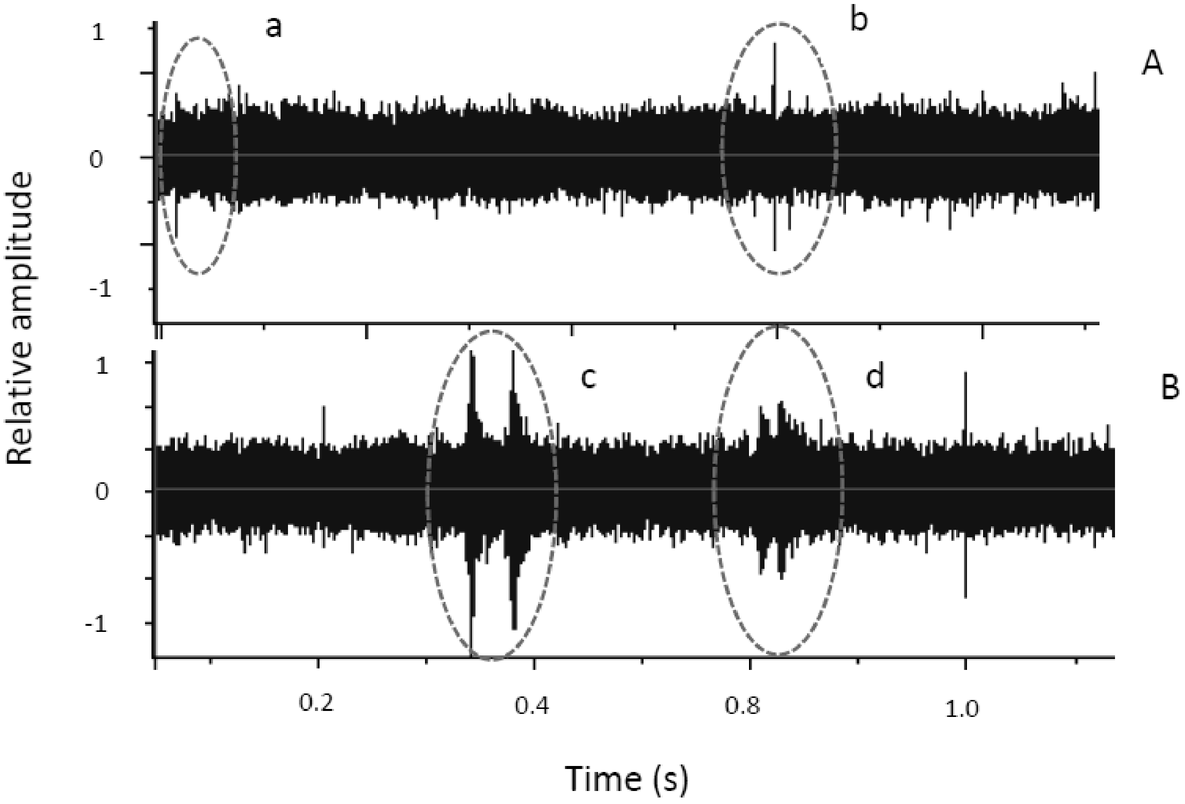

Figure 12 Oscillograms of a 1-min period of signals recorded from beans infested with *A. obtectus* adults A and larvae B. Signals enclosed by a dashed oval indicate bursts of the adults (a and b) and larvae (c and d)

4.3.3. Impulse-train and burst analyses

The number of impulses per burst varied significantly between the larvae and adult of *A. obtectus* (Table 10). The number of impulses per recording varied between 2 - 151 for the adults, and 1 - 40 for the larvae. The overall number of impulses per burst ranged from 2 to 20 with a majority being below 4.5.

Table 10 shows the burst rates, impulse rates and impulses per burst of the last instar larvae and adults of *A. obtectus*. There was no significant difference between the larvae and the adults for rates of burst impulses. However there was a significant difference for the rates of bursts ($P \leq 0.05$) and the impulses per burst ($P \leq 0.05$) of the larvae and adults.

Table 10 Mean (± SE) rates of bursts, rates of impulses in bursts and impulses per burst for adults and larvae of *A. obtectus*

Stage	Rates of bursts (No. bursts s^{-1})	Rates of impulses in bursts (No. impulses s^{-1})	No. impulses per burst
Adult	0.023 ± 0.007a	0.054 ± 0.031a	2.24 ± 1.00a
Larvae	0.014 ± 0.011b	0.061 ±0.045a	3.62 ± 1.42b

4.3.4. Mean rates of larval and adult impulses in bursts

The mean rate of impulses from the larvae was subtracted from the mean of the adults, the mean difference expressed as mean ± SEM, was 0.04 ± 0.03 impulses / s and indicated that there was no significant difference between the impulses of the larvae and the adults (df = 11, $t = 1.61$, $P = 0.135$). On the contrary, the difference between the mean rates of bursts of the adults and the larvae was 0.09 ± 0.03 bursts / s and differed significantly for the adult and larval stages of *A. obtectus* (df = 11, $t = 2.11$, $P = 0.05$).

The analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was done to test the effect of developmental stage on the counts of impulses per burst, and counts of burst impulses of Broadband, HighF, MidF1 and MidF2 spectral profiles. There was no significant effect of developmental stage on any of the above mentioned parameters (Table 11).

Table 11 Analysis of the effect of developmental stage on the rates of bursts, counts of impulses per burst, and rates of impulses in bursts of HighF, Broadband, MidF1 and MidF2 spectral profiles

Parameter	Error Mean Square	*F*	*P*
Rate of Bursts (s^{-1})			
HighF	0.0207	4.79	0.123
Broadband	0.0012	0.33	0.594
MidF1	0.0090	3.42	0.138
MidF2	0.0008	1.04	0.365
Number of impulses per burst			
HighF	0.809	4.00	0.116
Broadband	0.0527	0.03	0.865
MidF1	58.62	1.07	0.360
MidF2	2.597	0.74	0.438
Rate of impulses in bursts (s^{-1})			
HighF	0.026	3.81	0.123
Broadband	0.003	0.36	0.583
MidF1	0.0034	1.09	0.355
MidF2	0.0008	0.81	0.420

4.4. Discussion

The results suggest that differences in sound-producing locomotory and feeding behaviors of *A. obtectus* larvae and adults can be assessed by measurements of differences in the rates of bursts, the counts of impulses per burst. In addition, it was observed that only adults produced signals of the HighF profile type; consequently, it was possible to distinguish larvae and adults by spectral as well as temporal pattern differences.

4.4.1. Spectral and temporal patterns of larval and adult signals

The differences in the spectral and temporal patterns of sounds produced by larvae and adults of *A. obtectus* correlate well with aspects of previous studies conducted on other postharvest insect pests that reported effects of insect size and stage on acoustic signal production (Rajendran, 2005; Mankin et al. 2011; Njoroge et al. 2016). However, the results differ from numerous previous studies which found adult stages of insects to be producers of greater rates and louder sounds than the larvae. For example, Hagstrum et al. (1990) reported that *R. dominica* moving on the outside of the grain kernels produced 37-fold more sounds than larvae feeding inside the grain. In another study with *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), adults produced 80 times more sounds than larvae (Hagstrum et al. 1991). Other work by Pittendrigh et al. (1997) and Hickling et al. (2000) considering rates of sounds produced by *S. oryzae* in grain, as well as work by Shade et al. (1990) with *C. maculatus* larvae in cowpeas *Vigna unguiculata* (L) Walp (Fabaceae), found that sound rates increased with instar. Studies on adult *S. oryzae* and *T. castaneum* showed that both species were more readily detectable than smaller *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) or *Oryzaephilus surinamensis* (*L.*) (Coleoptera, Silvanidae), while *R. dominica* was intermediate to them (Hagstrum and Flinn, 1993; Mankin et al. 2011).

Feeding events from the early instar through to the last instar have been detected previously in cowpea weevil, and the rate of feeding events has been found to be directly proportional to the population of larvae present per seed (Shade et al., 1990). Other research efforts have proven that a considerable amount of larval time is spent just feeding only. For instance Vick et al. (1988) showed that, in grain samples, *R. dominica*, *S. oryzae* and *S. cerealella* larvae spent 61 to 90% of their time feeding and thus producing sounds.

The unusual result in the acoustic signals of *A. obtectus* possibly can be explained by observations of its behavior. It has been shown previously, for example, that some insect females react to host deprivation by retarding egg maturation (Sadehi and Gilbert, 2000) or by delaying oviposition (Asman and Ekborn, 2006). During our experiments we transferred the insects from their mother culture to a new set of beans before acoustic measurements. The females may have perceived this transfer as disturbance or host deprivation and their adaptive response could have been to postpone egg laying.

Another possible explanation for the relatively silent behaviour of *A. obtectus* adults is the fact that the beetle is aphagous and females at emergence contain sufficient energy to develop and lay eggs without feeding. The females diapause for more or less extended periods after landing on their host before the second mating for oogenesis to take place. This lower level of feeding activities could be explained by the larger interpulse intervals observed with the *A. obtectus* adult signals in this work. The feeding of adults has attracted little research attention in the past but acoustic recordings can help to better understand their feeding behaviour. With the exception of *Bruchus pisorum* (L.) (Coleoptera: Chrysomelidae) whose females are known to be able to reproduce only after feeding on pollen of *Pisum sativum* (L.) (Fabaceae) their host plant, little is known about the feeding of other adult Bruchinae. Most of these species are able to oviposit without feeding due to the important reserves accumulated in their body fat (Godrey and Long 2008). Feeding of *A. obtectus* has almost never been observed. Like other Bruchinae, adult *A. obtectus* are also weak flyers.

The low acoustic signal rates recorded from the *A. obtectus* adults could also be due to their ability to feign death when disturbed. Some insects become quiet when they are disturbed, and the time needed for them to return to normal activity after a disturbance must be taken into account when they are monitored (Arnett 1968; Mankin et al. 2011).

4.4.2. Application of acoustic detection methods in the control of *A. obtectus* infestations

There is considerable need to study the potential of eavesdropping on this very quiescent postharvest pest because, from the moment when the first instar larva bores into the seed, it feeds, grows and molts into successive instars and there is no visible sign of insect presence on the infested seed. The only sign of infestation is manifested when the prepupa gnaws a neat circular hole on the already damaged bean seed to facilitate adult emergence. Such hidden infestations move across geographical boundaries as import/export consignments and pose great phytosanitary threats in new ecological niches. Acoustic technology can tap into this system and create a means for detection at ports of entry/exit whereby larval infested consignments can be separated from clean consignments with accuracy and precision. The ultrasonic signals produced when the larvae strike the seed tissue during feeding and as they tunnel and turn within the bean seed also can be harnessed for acoustic sensor development.

Previous research has documented the magnitude of postharvest losses and the importance of controlling various postharvest pests in Sub-Saharan Africa. (Njoroge et al. 2014; Affognon et al. 2015; Mutungi et al. 2015).This study of adult and larval sounds recorded on stored beans provides insights on timely detection of postharvest insect pests. Understanding the behaviour of bean beetles and the characteristics of the signals they emit during feeding and locomotion can be useful for pest surveillance in storage warehouses using acoustic technology. It is of interest that we were able to distinguish between the larvae and adults of *A. obtectus* based on both spectral and temporal patterns so as to improve the automation of the detection of these stored product insects. This will help make it possible for engineers to adapt existing acoustic sensors for the detection of this prevalent pest in African stores. This result possibly can also be extrapolated to detection and management of other bean beetles like *Callosobruchus spp* that cause postharvest loss of other pulses and legumes.

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5. Acoustic survey in Kenya's grain storage facilities and the promise of acoustic detection of pests

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Abstract

Grain production by Kenyan farmers, an important resource for their food security, faces constant challenges from pre- and post-harvest conditions favorable to rapid growth of insect populations. Currently, Kenya must import grain to meet consumption needs; however, if losses due to insects in storage facilities could be reduced, significant reductions in grain imports could be achieved. A review of current grain resources available in Kenya indicated that its grain production has increased over the last decade, but storage capacity has remained constant, with continued losses of up to 20-30% due to inadequate control of postharvest insect pests. Early warning of pest infestations can help managers reduce postharvest losses by enabling them to target and eliminate infestations before they increase to economically damaging levels. Because acoustic methods have been successfully used previously for early detection of infestations, an acoustic survey was done in selected maize grain stores in storage facilities in five Kenyan counties, Nairobi, Nakuru, Nyeri, Kirinyaga and Kiambu. Stores visited during the survey exhibited significant presence of *Sitophilus zeamais* Motschulsky, *Prostephanus truncatus* Horn and *Tribolium castaneum* Herbst. It was demonstrated that the use of acoustic technology can help managers identify and target infestations within their warehouses, enabling them to reduce postharvest losses.

Key words: postharvest losses, Kenya grain storage, postharvest insect pests, pitfall traps, acoustic detection

5.1.Introduction

Food valued at over USD 4 billion dollars is lost every year in Africa as a result of post-harvest inefficiencies across the staples agricultural value chain (Zorya et al., 2011). Kenya has developed a Strategic Grain Reserve to store sufficient grain for release into markets if supplies fall below typical levels of consumption (Murphy, 2009). The government dedicates funds every year to ensure there is backup maize that can be released in an emergency. There is wide recognition that strategic grain reserves play a vital role in ensuring Kenyan food security.

Kenya experiences an estimated 20-30% postharvest loss of staple grains, which poses great challenges to the country’s food security and economic development (George, 2011). *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) are the major maize pests in Sub Saharan Africa (Vowotor et al. 2005). Postharvest losses significantly endanger the livelihoods of stakeholders across the value chain by reducing valuable incomes and profitability.

Managers of bulk storage facilities frequently fumigate with phosphine gas; however, *Rhyzopertha dominica* (F.), *T. castaneum* and potentially other postharvest pests have been developing resistance to phosphine (Opit et al. 2012). In many facilities, gas tightness is not complete and fumigation needs to be augmented with additional tools. Routine monitoring and timely inspection of grains to identify problems enables removal infestations before they cause economic damage. Common monitoring methods make use of visual inspections in and around storage facility, examination of grain samples, measurements of temperature changes in bulk grain, and widespread placement/inspection of insect traps (Toews et al. 2012). More often than not, this monitoring is not completely effective because of hidden infestations of larvae. However, acoustic detection (Mankin et al. 2011) is a promising technology which can detect hidden larval infestations and advise store managers on timing and targeting of grain preservation efforts. Acoustic methods have been applied successfully for grain inspection, estimations of population density and mappings of various stored product insect pest distributions. Recent efforts have been directed towards integrating acoustic technology in grain storage in Africa (Kiobia et al., 2015, Njoroge et al., 2016, 2017).

Prior to this acoustic study review was done to provide an insight on the current food-grain resource structure of Kenya. The postharvest loss situation in Kenya was assessed based on available literature on production consumption, storage and loss estimates. Reference was made to the most recent economic review on agriculture to get production data for maize, wheat and rice from 2006 to 2014 (Ministry of Agriculture (MoA), 2015). Then loss estimates for Sub Saharan Africa were derived from the Africa Postharvest Loss Information System (APHLIS) loss tables (retrieved January 2017). The losses tables designed by Rembold et al., 2011, represented cumulative weight loss of cereal from harvesting, drying, handling operations, farm storage, transport and market storage for various grains in eastern and southern Africa. The loss estimate figures for this review were from the year 2006 to 2014 and ranged from 17. 8 -19.9%, 9.9-15.2% and 11.8-13.9% for maize, wheat and rice, respectively.

These loss figures were then used to derive generalized loss estimates for the major food grains produced in Kenya from 2006 to 2014 in Kenya. This gave a representation of the magnitude of postharvest losses for the three major cereals in Kenya; maize, wheat and rice in that order. A general increase in production of maize, wheat and rice was observed over the years under review. However there was a marked decrease in maize production in 2008 and 2009 due to the effects of the post-election violence in 2007/2008. However, of interest was the fact that percentage loss remained almost constant over the years under review and therefore loss estimates increased with increasing production. About 500,000-600,000MT, 30000-60000MT and 5000-17000MT of maize, wheat and rice, respectively are lost annually between harvest and consumption. Most of this grain is lost during storage.

The aim of this acoustic survey was to detect the abundance and presence of otherwise not visible stored product insect pests hence add to existing knowledge that acoustic detectors can be applied in monitoring target species in stored grain.

5.2.Materials and Methods

5.2.1. Recording sites

Recordings were collected from maize grain bags in June 2016 from five grain storage facilities in Kenya. A preliminary visit was conducted in May 2016 at the sites of grain storage to gain consent to participate in the acoustic survey for the detection of hidden stages and adult insects within their premises.The stores were located in 5 separate counties: Nairobi, Kiambu, Kirinyaga, Nyeri and Nakuru as shown in Figure 13. The actual store locations were Nakuru, Thika, Sagana, Kiganjo and Nakuru. These sites had similar climatic conditions of the subtropical highland type with slight variations in altitude namely Nairobi 1795m, Thika 1631m, Sagana 1762m, Kiganjo 2161m and Nakuru 1850m.

5.2.2. Sampling in the stores

Each grain store had several grain stacks most of which exhibited various levels and types of infestation. All store managers fumigated the grain routinely. Visual inspection for infestation was carried out to identify the stacks to work with. The stacks that exhibited more likely places where insects may be found were selected. The best sites were along the stack edges, at the top of the bulk and areas with spillages. This was according to the recommendation of the ISO Standard 6322 for the search of an infestation on a static grain bulk.

After identifying a stack likely to contain infested bags, 12 50-kg bags were drawn randomly from the surface of the stack and brought to the ground for the acoustic recordings to be done. For high stacks, the recording equipment was brought to the top of the stack for signal collection.

5.2.3. Insect trapping

For each of the 12 selected bags non-pheromone Storgard WB Probe II traps (pitfall traps) were set up prior to acoustic recording to collect samples of free-moving insects in the grain. The grain probe trap works on the principle that excludes grain kernels but permit insect entry through its perforated walls after which the insects fall into the pit of the collecting vial where they cannot escape. Insects moving through the grain walk into the holes of the probe shaft, drop through the void and right through the funnel into a collection vial whose wall angle and constrcted opening deter the escape of the trapped insects. The trap is placed below the grain surface.

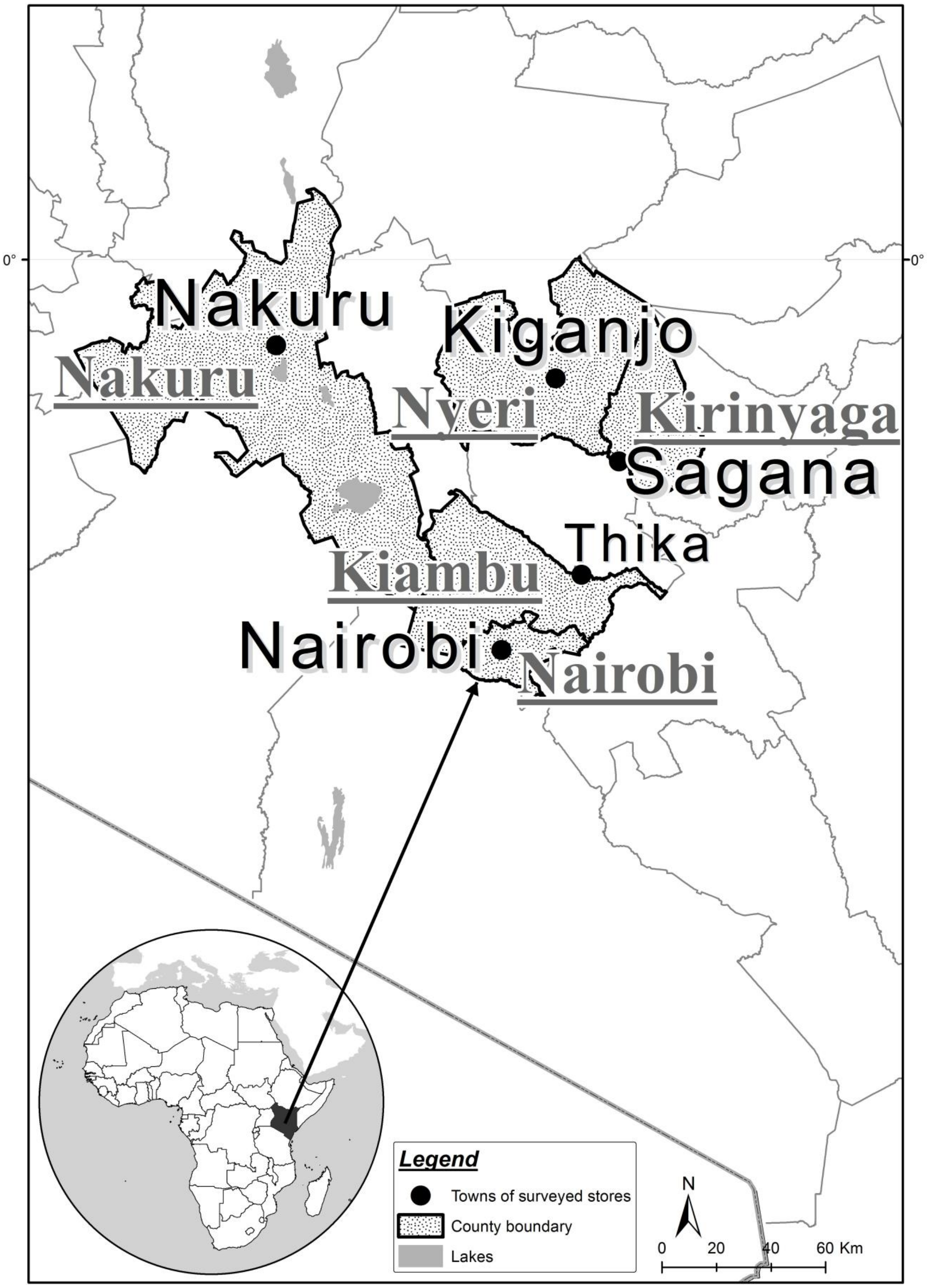

Figure 13 Locations of maize stores acoustically surveyed in Kenya.

The bags were placed vertically, opened at the top, and the traps were pushed into the grain. The traps were placed near the surface of the grain in the sampled bags for ease of retrieval and also because more insects congregate at the upper layer of the grain (Flinn et al. 2010). All traps were labelled, as well as the plastic containers in which the trap contents were emptied. The traps were retrieved 2-3 hrs later, at the end of the acoustic recordings, and the contents were taken to the laboratory for identification and counting.

5.2.4. Insect identification

At the end of each trapping, traps were retrieved and the contents of probe traps were poured into vials and sealed. The insects were identified to species level, and counted. When large numbers (more than several hundred) of insects were caught, samples were subdivided as needed and total counts were estimated by multiplying the number of insects in 1 subsample by the number of subsamples.

The insects were distinguished based on their morphological features (Greig and Reeves 1985). *Sitophilus zeamais* was identified by its long beak (or rostrum) about 2.5 to 4 mm long, dark brown colour, sometimes with four lighter soots on the wing cases. *Prostephanus truncatus* was recognized by the appearance of its head "tucked" under the thorax so that it is invisible from above and the prominent pattern of tubercles on the thorax. *Tribolium castaneum* were identified as elongated reddish-brown beetles. Other insects that did not match these three were counted as other species. Larvae were not identified to species level and were reported as mixed larvae.

5.2.5. Recording set-up and equipment

The recordings were taken using two different sets of equipment. One was the imc microphone sourced from *icipe* Nairobi Kenya and the other an AEC probe from USDA-ARS CMAVE Gainesville, Florida USA. The purpose of using two sets of equipment was to compare the detection ranges and background noise discrimination capabilities of each system in the warehouse environment.

The imc microphone equipment included a 0.5” microphone (Model 378B02, PCB Piezotronics Inc., NY) attached to a preamplifier system (imc C-SERIES, CS-3008-N, imc Meßsysteme GmbH, Frankfurt, Germany), as described in Njoroge et al. (2016, 2017). The AEC probe equipment included a 16-cm-length x 6-mm diam stainless steel probe attached to a sensor–preamplifier module (model SP-1L, Acoustic Emission Consulting [AEC] Inc., Sacramento, CA) connected to an amplifier (AED-2010, AEC Inc. Sacramento, CA), leading to a digital audio recorder (model HD-P2, Tascam, Montebello, CA) that stored signals at a 44.1-kHz digitization rate. The recording procedures were similar to those described by Mankin (2011) and Kiobia et al. (2015). Records of 3 min each were collected over a 5-day period from a total of 60 different bags. Testing began at approximately, 10:00 A. M. and continued for 3 or more h. For each bag, recordings were made simultaneously with both systems except at the Thika stores, where imc microphone data was not collected due to power failure.

Weather conditions were dry with no rain or wind present throughout the survey periods. Each store location was unique, with some stores located in very busy environments characterised by background noise. Sources of noise included but were not limited to birds singing, vehicle movement and beeping, on-site machine noises, and human activity. For these reasons, recordings were made when noise was reduced and monitoring with headphones was done to help identify times when recordings needed to be repeated. Nevertheless, recordings were not free of noise and signal processing was conducted in a manner that discriminated the targeted insect signals from the untargeted background noise.

5.2.6. Automated Classification of insect sounds and Background Noise Signals

Signals recorded with imc microphone 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). Signals recorded with the AEC probe were already in the .wav format. The recordings were band-pass filtered between 1 and 10 kHz and pre-screened using Raven Pro 1.5 Beta Version software (Cornell Lab of Ornithology, New York, United States; Charif et al., 2008). Prescreening entailed playback, oscillogram, and spectrogram analysis of each file to locate periods of insect sound impulses and discarding periods of loud background noises.

To test for potential differences among spectral and temporal patterns of sound impulses from insects in the bags in the different stores, we applied a methods developed previously in Mankin et al. (2008). Mean spectral profiles of impulses recorded under each condition then were calculated using DAVIS, and designated as separate profiles for “AEC” and “imc”data. They were designated profile 0 and profile 1 for data recorded by the “AEC” and “imc”, respectively. In addition, the prescreening identified bird noise that occurred frequently in all the stores visited and therefore a bird profile was also calculated to facilitate discrimination between insect sound impulses and background noise, as described in Mankin et al. (2008) and below.

The profiles were constructed using the custom-written insect signal analysis program: “Digitize, Analyze, View, Insect Sounds” (DAVIS) (Mankin et al. 2011). The sound impulses in each “imc” or “AEC” recording were least-squares matched by DAVIS against each of the two spectral profile types and the bird noise profile and were assigned to the profile type of best fit as in Mankin et al. 2011. Impulses classified as background noise were discarded. DAVIS classified impulse trains containing >6 and <200 impulses that matched insect sound profiles, as insect bursts in each recording, based on the high likelihood that they were produced by insects and not by background sounds (Mankin et al. 2008). The discrimination was based on the fact that insect movement and feeding activity generates trains (groups) of 3 - to 30-ms impulses (Potamitis et al. 2009, Mankin et al. 2011).

Times and types of each burst and the count of impulses in each burst (burst impulses) were saved in a spreadsheet for statistical analyses. On the spreadsheets, rates of bursts, impulses per burst, and rates of impulses in bursts were calculated for each profile, and totals for rates of bursts, impulses per burst, and rates of impulses in bursts were calculated as the sums the of separate values for each profile, as in Njoroge et al. (2016, 2017).

5.3.Results

5.3.1. Insect traps analysis

Acoustic survey as a research method can detect the abundance, monitor activity patterns and determine behavior of target species. It has the advantage of being conducted over greater spatial extents in many habitats with little cost, but drawbacks are difficulty in species identification and quantification of species present. Despite the drawbacks, acoustic survey remains as an important tool to address postharvest pest monitoring because determining their presence/absence is of key importance. Different detectors can be used to determine the presence of insects in stored grain. To achieve this, it is important to discern the difference between typical insect sounds and other interfering noise such as bird calls, traffic noise (vehicles and machines), and human activity. Before deploying this acoustic survey, separate insect species (*P. truncatus, S. zeamais* and *A. obtectus*) had been studied under laboratory conditions in a sound-shielded chamber (Njoroge et al. 2016, 2017). Therefore, their acoustic emissions at different stages of development were clearly understood and facilitated the discrimination of insect sounds recorded in new environments.

Though there were considerable amounts of grain dust, all recorders and amplifiers performed at their optimum conditions. As evidenced by insects captured by the pitfall traps, adults and/or larvae of at least 2 species of postharvest insect pests were present in all stores visited thus making it possible to collect recordings with different numbers of each or either species present (Table 12) under varying conditions of wind, bird noise, and machinery noise.

Although the pitfall traps are originally designed for use in concrete silos, steel bins, and flat storages, we modified their use to fit in 50kg bags in this study. By keeping the sampled bags in a vertical position during the acoustic recordings, present insects were able to fall through the perforated walls of the traps and collect in the vial attached on the bottom.

As shown in Table 12, *P. truncatus* and *S. zeamais* were present in 4 out of 5 store locations, while *T. castaneum* was present in all 5 store locations surveyed. Other species and mixed larvae were observed in 3 and 4 locations respectively. It was worth noting that *T. castaneum* recorded the highest numbers in all 5 stores. The highest numbers recorded per store were *P.truncatus* in Kiganjo at 39.08, *T. castaneum* in Nairobi, Nakuru, Sagana and Thika at 39.42, 27.11, 5.86 and 12.50, respectively. Of interest was the presence of large numbers *Sitotroga cereallela* larvae in

Thika stores. They were not captured in the traps since they were mostly on walls, floors, on top of bags with some also dropping from the roof top.

Table 12 Analysis of variance of counts of insects captured and identified in each county (Mean ± SEM)

	Number of insects				
Species	Kiganjo	Nairobi	Nakuru	Sagana	Thika
P. truncatus	37.69±13.80a	0.25±0.25a	0.89±0.54a	0.57±0.42a	0.00±0.00a
S. zeamais	6.23±5.82b	20.42±6.71b	24.56±8.70b	0.00±0.00a	0.25±0.25a
T. castaneum	39.08±7.37a	39.42±4.75c	27.11±7.64b	5.86±1.74b	12.50±1.84b
Other species	4.07±0.78b	0.08±0.08a	0.56±0.44a	0.00±0.00a	0.00±0.00a
Mixed larvae	7.54±1.75b	0.00±0.00a	3.67±1.09a	0.14±0.14a	0.42±0.42a

Means followed by the same letter in the same column are not significantly different (*P*>0.05)

5.3.2. Infestation assessment

Sound impulses matching the insect spectral profiles were detected in recordings from all stores. All bags tested were identified as infested based on the total rates of insect sound bursts exceeding a detection threshold of 0.02 burst/s previously established in Mankin et al. (2008).

It was of interest to sum the trap counts of the two most important pests, *P. truncatus* and *S. zeamais*, as a single total, T_c. Previous studies (Mankin et al. 2011) suggested that the insect sound burst rates from both the AEC and the imc sensors would be approximately proportional to T_c, i. e., the statistical model would be:

$$r_b = T_c \qquad \text{(Eq. 1)}$$

In addition, the AEC sensor and the imc microphone were expected to detect sound bursts at different rates in different bags due to differences in the positions of the insects relative to the detectors as well as differences in the range of detection. The microphone detected insects over approximately 25-cm distances from the top of the bag, while the AEC sensor was attached to a probe, enabling detection of insects up to 25-cm distances along its 16-cm length (Kiobia et al.

2015). However, the insect sound burst rate detected by imc, imcr_b, was expected to be proportional to the rate detected by the AEC, aecr_b, and the statistical model would be:

$$\text{imc}r_b = \text{aec}r_b \qquad \text{(Eq. 2)}$$

The models were tested for insect sound burst rates from 21 bags at the Kiganjo, Nairobi, Nakuru, and Sagana warehouses in which recordings were obtained from both the imc microphone and the AEC probe. The slopes of the regressions were statistically significant for each of the models (Table 13).

Table 13 Intercepts and slopes (±SEM) for regression equations fitting the models in Eqs. 1-2

Model	Intercept ±SEM	*t*	*P* > *t*	Slope ±SEM	*T*	*P* > *t*
aec$r_b = T_c$	0.326 ± 0.210	1.55	0.137	0.0132 ± 0.0039*	3.34	0.004
imc$r_b = T_c$	0.078 ± 0.039	2.0	0.063	0.0016 ± 0.0007*	2.13	0.047
imcr_b = aecr_b	0.070 ± 0.041	1.72	0.102	0.073 ± 0.033*	2.16	0.044

Statistically significant values of *t* ($P < 0.05$) for regression parameters are marked by asterisk.

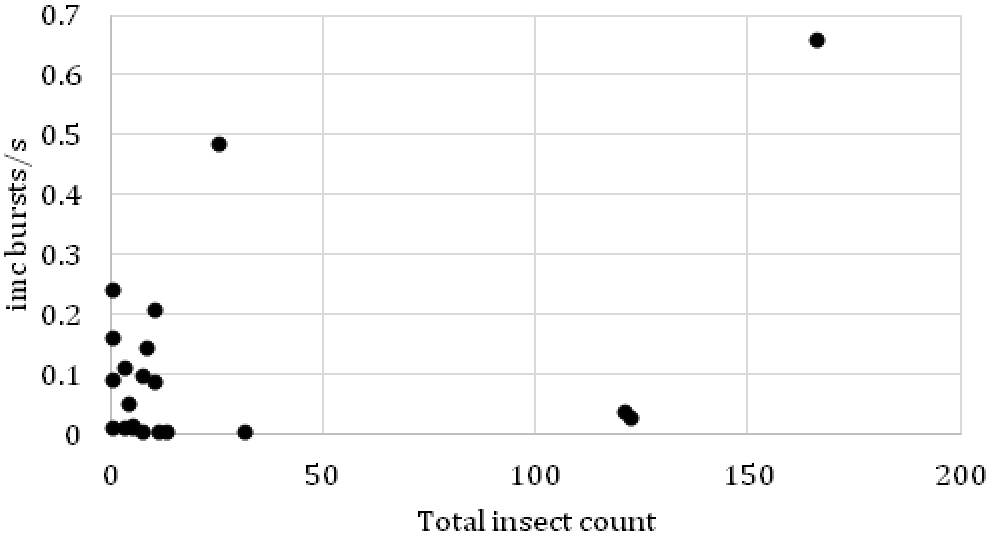

Figure 14 Relationship between insect sound burst rates and total counts of *P. truncatus* and *S. zeamais* in using the imc probe in recordings from 21 bags at Kiganjo, Nairobi, Nakuru, and Sagana warehouses.

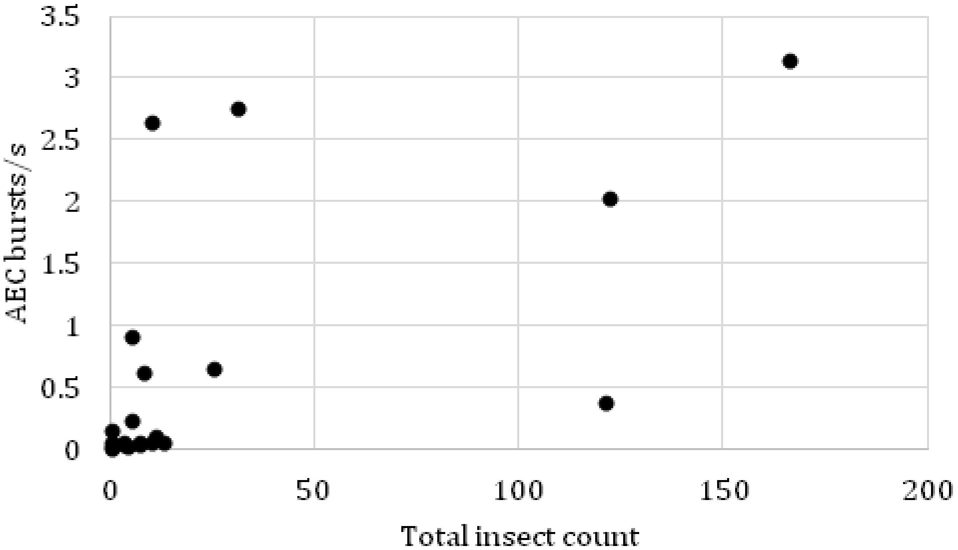

Figure 15 Relationship between insect sound burst rates and total counts of *P. truncatus* and *S. zeamais* using the AEC probe in recordings from 21 bags at Kiganjo, Nairobi, Nakuru, and Sagana warehouses.

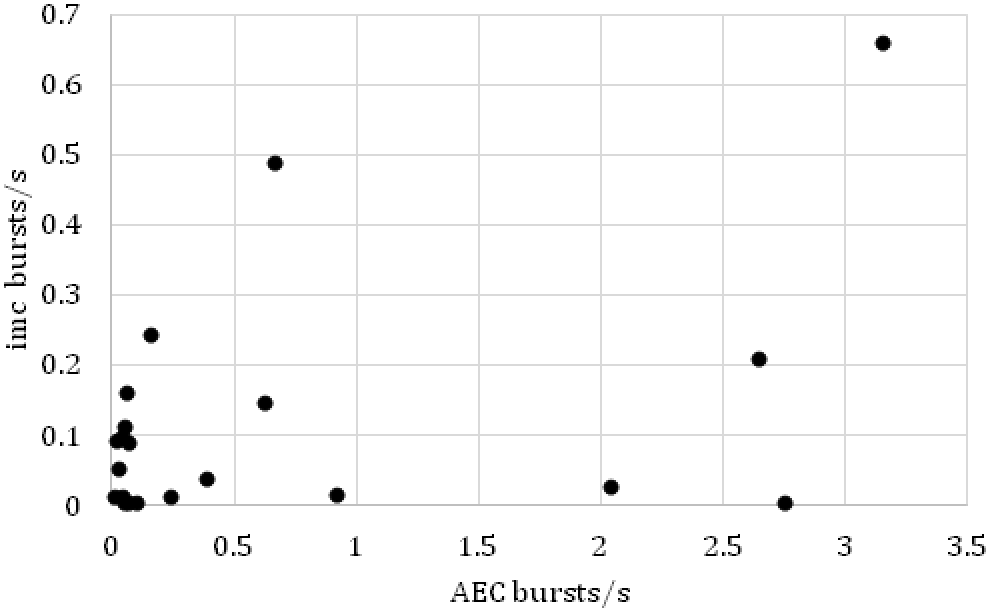

Figure 16 Comparison of insect sound burst rates from 21 bags at the Kiganjo, Nairobi, Nakuru, and Sagana warehouses where recordings were obtained from both the imc microphone and the AEC probe.

An overall comparison between the stores was done using the AEC data (Figure 16). There was a significant difference in the burst rates and impulse rates recorded in all stores ($P < 0.05$). This was expected given the different types and amounts of infestation observed with the pitfall traps. Kiganjo stores exhibited the highest burst and impulse rates while Sagana had the lowest. This correlated well with the trap catch data whereby Kiganjo had significantly higher counts insects ($P < 0.05$) compared to Sagana.

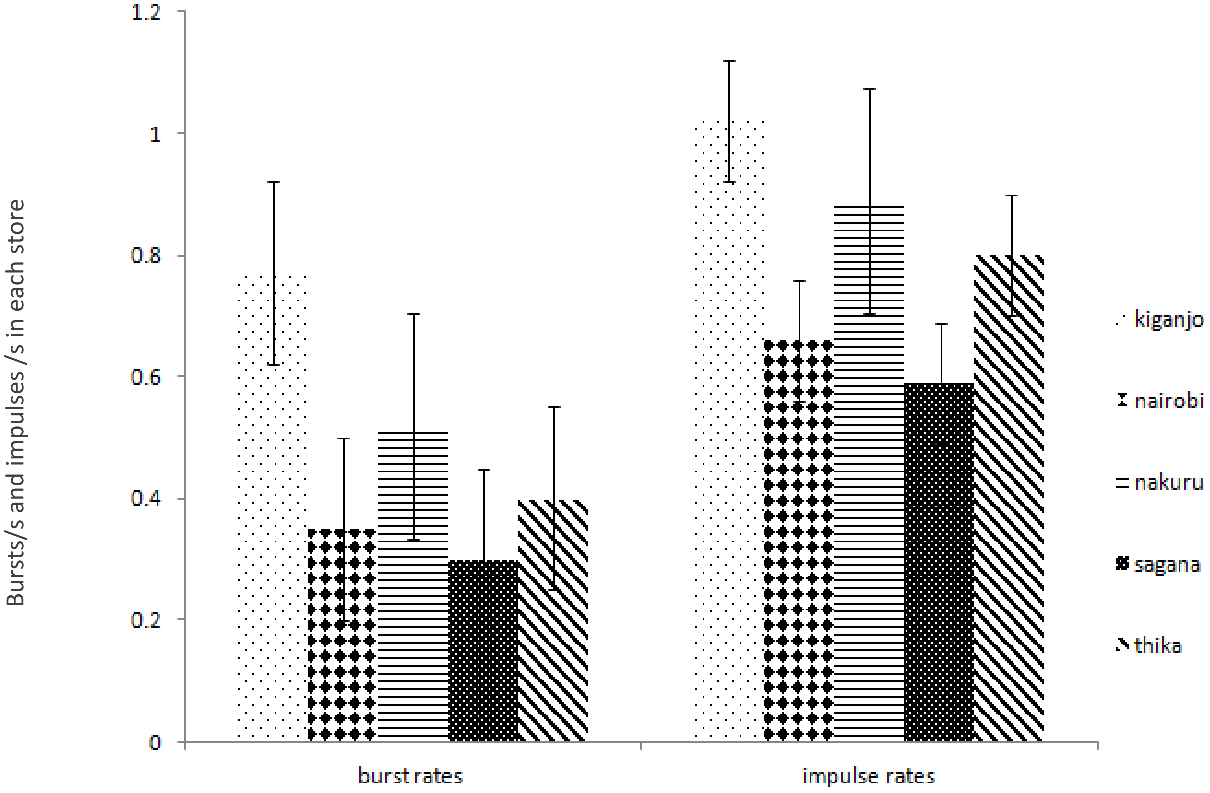

Figure 17 Comparison of burst rates and impulse rates recorded in the surveyed stores

5.4.Discussion

The results of the review indicate that there is cause for alarm in the magnitude of losses associated with storage of cereals in Kenya. The survey results echo the same as evidenced by insect presence of at least 2 species of postharvest insect pests in all stores visited. While visual inspection of the bags in the stores did not give us the true indication of the infestation in the stored visited, pitfall traps helped in estimating the number of insects present at the time of survey. Trap use and interpretation of insect captures provide the foundation for integrated pest management programs and may be considered a method of sampling the insect population (Toews et al., 2012). The insects captured, *P.truncatus, S. zeamais* and *S.cereallela* are of great economic significance in maize storage in Sub Saharan Africa. Their presence in bulk grain storage poses a threat to the food security of the populations depending on that grain for survival. Therefore, there was an economic incentive to fumigate all the stores we visited in response to the presence of these pests mainly because of they are internal infesting species. Other examples of serious internal feeding pests of stored grain are *Sitophilus oryzae, Sitophilus granarius, Rhyzopertha dominica* and all bruchidae species (Toews et al. 2012). Though the presence of T. castaneum was high in all stores visited, it was tolerated because it is an external feeding species amongst others e.g. *Plodia interpunctella* (Hubner), *Oryzaephilus surinamensis* (L.), etc.

In the assessment of the infestation it was evident that both the “AEC” and “imc” equipment gave related results under relative unfavorable conditions and that the most important factor for precise detection was the proximity of the insects to the sensor. In acoustic sensor development the range of detection is an important factor to consider especially because the more sensitive the sensor, the larger the detection range, and thus the fewer the number of sensors needed per volume of stacked bags of grain. The use of waveguides can improve the detection range by increasing the surface area of grain that is in contact with the sensor thus improving the accuracy of detection. Previous research has shown that among the many microphones in the market, piezoelectric sensors are the best option forinsect detection because they reduce attenuation losses as signals traverse across different media (Mankin and Hagstrum, 2011). To improve the accuracy of detection, efforts have been directed towards construction sound attenuating boxes lined with foam and fitted with piezo sensors as shown by Flynn et al. 2016. These innovations are very applicable to Africa since they can be fabricated from locally available material. Such a box coupled with developed prototype sensors as the one by Kiobia et al. 2015 can be used for insect detection in store here in Africa. This will complement prototypes and sensors developed in the past for instance the Early Warning Detector (EWD) (Leblanc et al. 2011)

The convergence of the acoustic sinal analysis and the captured insects demonstrated that indeed acoustic detection is a useful tool in explaining infestations in grain stores in Africa. Acoustic methods should compliment efforts being put in place to reduce economic and qualitative losses in large grain stores in Africa. These results were in line with the study conducted by Leblanc at al 2011 where he compared the convergence of acoustic data collected with a field probe EWD P3™ which was shown to give comparable results to the lab probe EWD LAB™. There are several sound detection apps that have been developed and these can be modified for insect detection by setting the detection threshold at the lowest density detection threshold that picks insect sounds and not background noise. Microphone systems can be coupled with the smart phones and the app creating a useful tool for scouting for insects in grain stores. Sensors can also be routed to wifi and instead of scouting they can be located at strategic locations in the store and programmed to send a text to store managers when insects are detected.

From this survey it is evident that the challenge in designing a sensor would be associated with background noise-cancelling. Spectral and temporal pattern features of the target insects would have to be incorporated in the sensor design. Detection can also be done at night when the insects are more active and the humans are less active.

Overall, from this study, the acoustic assessments of insect infestation in Kenyan warehouses correlated well with pitfall trap assessments and have the advantage of providing early detection of adult and larval infestations. Knowledge of early infestation can assist warehouse managers in maintaining strategic grain reserves with scarce resources. Improved monitoring combined with innovations such as hermetic storage bags may enable reduced reliance on grain imports.

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6. General Discussion

This chapter comprises reflections of the approaches used in this dissertation work for each objective. Results from laboratory and field based approaches have been synthesized and correlated with other scholarly contributions towards acoustic detection of stored-products pests. This chapter culminates to an outlook upon further research research work that may be pursued.

6.1. Reflection on laboratory studies

The laboratory studies in this dissertation aimed at exploring the acoustic characteristics of adult and larval stages of the most economically damaging pests of maize and common beans. Analysis methods used enabled the detection of larval activity as well as adult insect sounds which are low amplitude events. The first objective focused on finding out if it was possible to distinguish between acoustic emissions of two different maize pests; *P. truncatus* and *S. zeamais*. Their signals were characterized by amplitudes and spectra observed previously with other stored product insects (Kiobia et al. 2015, Eliopoulos et al. 2015 and Mankin et al. 2011). The frequency profiles of the larvae of both species mostly occurred within the range of 3 – 8 kHz during periods of high activity and 0.5 kHz during periods of less activity. This was similar to Kiobia et al. 2015 who found that the activity of *S. oryzae* at different times was not uniform. His study showed that insect activity was more during the mornings and evenings than afternoons. He attributed this difference to sensitivity of the insects to changes in ambient temperature. He found that the activity of insects ranged between 1 and 15 kHz and our findings fall within that range. *S. oryzae* is closely related to *S. zeamais* studied in this dissertation. Previous studies on *Rhyzopertha dominica* the close cousin of *P. truncatus* showed that acoustical sensors were able to distinguish between the larval and adult sounds as well as provide estimates of the populations at different temperatures (Hagstrum et al. 1990). He found that adults produced >37 times the number of sounds produced by larvae at 27°C which agreed with our findings. In general, the findings of the first objective complimented previous findings on acoustic detection of related pests. The findings on *P. truncatus* and *S. zeamais* added to existing knowledge and also created new information for *P. truncatus* whose acoustic profiles had not been documented before.

The second objective aimed at exploring the difference in the bioacoustic signals emitted by larval and adult stages of a serious common bean pest; *A. obtectus*. It was possible to distinguish clearly between the adults and larvae based on the temporal and spectral patterns of their acoustic signals. Larval signals mainly exhibited peaks between 4 and 8 kHz and were easily detected than the adults which were more quiescent. Previous research on *A. obtectus* provided scanty information and dated far back in time. The only work in literature was that by Andrieu and Fleurat-Lessard 1990, who studied insect noise on food stuffs. Research on other bean beetles like *Callosobruchus maculatus* is also scanty. Shade et al. 1990 showed that the activity of *C. maculatus* increased with increase in larval instar and decreased with increase in temperatures above 38 ºC. Our findings made a break through in identifying the frequency range for the detection of *A. obtectus* which may be extrapolated for the detection of other bean weevils as well.

The analysis methods used for both objectives were keen to screen out unwanted background noise and only identify insect sounds of interest. Similar studies have shown that it is possible to distinguish between stored-product larvae and adults feeding and locomotory activities based on their spectral profiles provided that the substrate is the same. For instance differentiation of the acoustic profiles of the larvae of *S. granarius, T. confusum* and *R. dominica* has been done successfully (Schwab and Degoul 2005).

6.2. Reflection on the field studies

The field based studies in this dissertation were based on the idea of developing acoustic sensors for insect pest detection in storage warehouses in Kenya and Africa in general. The methods employed were based on comparing two different acoustic detection techniques andtrap-capture counts of insects in the reliability of predicting insect presence in stored maize.

The objective of the study was to determine insect presence in sampled 50 kg grain bags in selected grain storage warehouses in Kenya through an acoustic survey. We used imc (imc C-SERIES, CS-3008-N, imc Meßsysteme GmbH, Frankfurt, Germany) and AEC (AED-2010, AEC Inc. Sacramento, CA) measurement systems and state of the art acoustic sensors. We also used non-pheromone pitfall traps (Storgard WB Probe II traps) to capture live insects that may have been present in the sampled bags.

While during the laboratory studies we worked with known populations, known species and at known environmental conditions; the field did not come with all these conveniences. We experienced mixed species in all sites that we visited with each store having at least 2 species present. We also had different environmental conditions with varied temperatures and relative humidity which had an effect on the pest abundance, presence and their activity if present. Of great significance was the presence of background noise. Each store had various activities going on ranging from vehicular noise, human activity to natural sounds due to bird singing or wind blowing. One particular store in Nairobi was located next to a railway line and road construction was going on. All these factors may have an influence on the acoustic sensor performance and therefore future implications on the design of the acoustic sensors.

The trap capture data correlated with the acoustic data such that where we had high insect counts we also had higher impulse and burst rates. In all stores visited *T. castaneum* was present.

The two acoustic equipment used gave related results for all the stores visited meaning their range of detection was comparable. The AEC sensor had a known detection range spanning 30 cm away from the waveguide probe; however, it was the first time to collect data outside the lab set up with the imc equipment.

There is little effort directed towards acoustic surveys of postharvest insect pests, however, one study was conducted to compare the difference between detection of insects in stores and in the lab on samples carried collected from grain stores in France (Leblanc et al. 2011). A detection probe for detection in grain bulks (EWD P3™) and a laboratory device (EWD LAB™) were compared for accuracy in detecting insect presence or absence and the equipment predicted insect presence with a confidence level of 90% and 79% respectively. Another study conducted in grain bins found that *S. oryzae* and *R. dominica* could be detected effectively at a distance of nore than 20 cm from the probe (Fleurat-Lessard et al. 2006). They also found that the insects could be detected at temperatures between 5 to 30 ºC.

As far as the insect traps were concerned, we were able to capture live adults and larvae during the 2hrs we spent at each location. The main species captured were *P. truncatus, S. zeamais, T. castaneum* and other mixed species. In one store we also encountered *S. cereallela* a major internally feeding maize pest but due to the nature of the traps we could not capture them. The best option for their capture would be light traps since they are mostly in the air around the grain and their larvae crawl on the walls and floors. These were the major pest species in grain storage warehouses and retail stores in Kenya.

In the stores visited during this study, *T. castaneum* was tolerated because it is an externally feeding species and its economic threshold level is higher than that of all other stored-product pests standing at 40 insects/food bait trap (Hodges et al. 1997). Stored-product insect populations are divided into two major groups i.e. insects of immediate economic importance infesting commodities, and insects that live in food residues in equipment and facilities. *T. castaneum* falls in the latter category. The insect infestation economic threshold is not fixed and depends upon the cost of the pest management method used and the market value of the infested commodity. Therefore in developing countries attention is paid more on economically damaging internal-feeders such as *P. truncatus* rather than less significant external feeders like *T. castaneum*.

The findings of this study added to existing knowledge on acoustic surveys aimed at understanding the abundance and distribution of postharvest insect pests in storage warehouses. Insect detection in stores that seemed visibly clean gave a positive sign that acoustic sensors would serve a great purpose in identifying hidden infestation in those stores and advice the managers on when to fumigate.

6.3. Outlook and further research

Early detection and acoustic technology in insect pest detection has been a topic of research for many decades. Stored- product entomologists in Africa now have more tools than did our pioneers in yesteryears towards the detection and mitigation of postharvest losses. Our laboratories are better equipped with state of the art sensors and amplifiers to understand insect behaviour and activity.

We have data on the studies of acoustic detection of a myriad of species that wreak havoc in our grain stores. We also have the knowledge and capacity to develop these sensors. We have attempted to make prototypes (Kiobia et al. 2015). Most importantly store managers are willing to pay for the device.

Despite all this, early detection of insects during storage is still a major challenge because we still rely on primitive methods to check for insect presence within the stores and for incoming consignments i.e. sampling and sieving method. It cannot give an indication of immature stages hidden within the grain kernels. Other parts of the world use better methods like pheromone traps and bait traps but we wait to see or hear an insect sounds in order to take action.

Our economy is mainly driven by agriculture. Most of our energies are directed towards production of food that we knowingly or unknowingly don’t know how to preserve. Cereals and legumes can be stored for more than 1 year in some instances and during this period they become very susceptible to insect pest attack. As a consequence, a significant portion of this food is never directed to its intended use.

Acoustic technology can be incorporated in pest surveillance programs in our major warehouses. Sensors can be developed with accurate precision with the consideration of background noise, infestation size and development stages of the target pests. Sensors with detection ranges of 3-8 kHz can be developed for discriminate identification of larval and adult insect presence. Affordability and ease of application should be considered too since the best adopted technologies are the ones which are easiest to use.

Based on this several recommendations can be obtained from this dissertation work:

1) There is need for research efforts to be directed towards acoustic sensor development ranging from apps that can work with secondary microphone attachments to mobile phones for easy detection to more sophisticated arduino-based sensors for use in stores. The arduino sensors can have wifi and text (SMS) capabilities with the possibility to communicate remotely with the mobile phone of the store managers.
2) There is need to document the soundscape of several grain storage areas in order to come up with a database of background noises to be considered in the acoustic sensor development.

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7. Summary

Kenya experiences persistent food and nutritional security just like other countries in Sub-Saharan Africa (SSA). This is aggravated by low productivity, erratic weather patterns and high postharvest losses ranging from 20-30%. Insect pests are a key constraint to effective preservation of grain crops in sub-Saharan Africa (SSA), with severe damage caused by these pests during bulk storage in warehouses. Although a number of approaches have been advanced for control of storage pests, their elimination is still yet to be realized.

Maize and common beans are Kenya‘s main staple food crops with cereal crops playing a major role in smallholder farmers' livelihoods. To achieve food security and food safety several pest management approaches including the use of pesticides are employed have been advanced for management of post-harvest pests.

The major postharvest pests in Kenya are *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), *Sitophilus zeamais* Motschulsy (Coleoptera: Curculionidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) for maize and *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) for common beans. Their presence often goes unnoticed in freshly harvested grain or during grain procurement in storage warehouses. Their early detection is a challenge because conventional sampling methods cannot detect infestations of less than 5 insects/kg. Based on this, a study was conceived to study the acoustic characteristics of these postharvest pests in the laboratory as well as in bulk storage warehouses. State-of-the-art acoustic measurement equipment and non-pheromone grain probe traps were used for the study. Laboratory studies focussed on the possibility to distinguish between different species as well as between larval and adult acoustic emissions. In the storage warehouses an acoustic survey was done to distinguish the detection range of two acoustic devices as well as compare acoustic data with counts of insects captured using traps.

Lab results showed that the three insect species could be distinguished based on their spectral profiles which ranged from Broadband frequency with multiple peaks from 1-8 kHz, High-Frequency with a band of high energy between 4 and 5.5 kHz, Mid-Frequency1 with a peak near 3 kHz and a smaller peak between 190 3.5 and 5.2 kHz, Mid-Frequency 2 with a band at 3 kHz, and Low-Frequency with a peak between 500-700 Hz. Majority of the insect sounds were between 3 - 8kHz. The differences in their acoustic signals can be harnessed in designing sensors within their frequency range. Their frequency range of detection was identified to be between 3.5 and 5.5 kHz.

Field results from the acoustic survey showed that in their natural habitat postharvest insects existed as mixed species and there were at least two species present in each store that was sampled. There was an economic incentive to fumigate all the stores we visited in response to the presence of these pests mainly because of they are internal infesting species. Background noise was a major challenge in the detection of the infestation especially because most stores were located in urban and peri-urban areas with high human activity. The convergence of the acoustic signal analysis and the captured insects demonstrated that indeed acoustic detection is a useful tool in explaining infestations in grain stores in Africa.

In conclusion, protection of strategic grain reserves can help reduce future food shortages. Since acoustic technology can be incorporated in pest surveillance programs in our major warehouses, further research into affordable acoustic sensor development is recommended.

8. Appendix

Appendix 1: Captions of acoustic equipment used and lab set up