Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author. Acoustic communication and behaviour of the golden haired pine bark beetle, *Hylurgus ligniperda* (Coleoptera: Curculionidae)

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science in Zoology

at Massey University, Palmerston North, New Zealand

> Sunil Sapkota 2017

Abstract

The golden-haired bark beetle, *Hylurgus ligniperda* (Coleoptera: Curculionidae: Scolytinae) imposes significant threats to New Zealand pine log exports. To date, control strategies against this invasive insect have relied heavily upon fumigation treatments. However, novel environmentally friendly and cost-effective strategies that decrease reliance on fumigants and can be used as part of an integrated package of disinfestation methods are urgently needed.

The adults of *H. ligniperda* produce characteristic and species-specific sounds when disturbed or aggregated. Males produce distinct audible simple and interrupted chirps using an elytral abdominal stridulatory mechanism whereby the pars stridens, usually present on the left elytrum, are scrapped by the sclerotized pegs present on the seventh segment of the abdominal tergite, whereas the females (despite having a similar stridulatory mechanism) just produce a click-like sound. Although the ability to produce sounds by *H. ligniperda* has been acknowledged for decades, nothing is yet known as to the relevance of acoustics on the behaviour of this species.

Thus, the main objective of this thesis was to study the sound-related behaviours of *H. ligniperda* under various scenarios (i.e. distress, mating, competition territoriality and colony) and to investigate the functions and characteristics (temporal and spectral) of the acoustic signals produced by this insect and their role in intraspecific communication.

Our results indicate that the role of sound in communication in the case of *H. ligniperda* is oriented more towards communication between the sexes rather than within individuals of the same sex. Depending upon the scenarios studied, the males of *H. ligniperda* can produce different frequencies of acoustic signals, ranging from 232 Hz to 21890 Hz. The minimum and maximum amplitudes of male acoustic signals (chirps) were highest in a colony context (-661270 and 764270), and lowest during competition (-12633 and 190383). The males did not produce any sounds (chirp) during mating. Similarly, the spectral analysis indicated that the females can produce acoustic signals of different frequencies in the range from 256 Hz to 23875 Hz. The minimum and maximum amplitudes of the female acoustic signals (clicks) were highest during competition (-189034 and 1041600) and lowest when they were distressed (-275112 and 191270). Toothstrike duration for male chirps (0.047 sec) and click duration for female clicks (0.012 sec) were longest when the beetles were distressed.

When distressed, the males produced a significantly higher number of simple chirps with a longer chirp duration and higher toothstrike rate. Similar patterns were observed for distressed females, that produced significantly higher number of clicks with a longer click duration. The role of interrupted chirps for distressed males was minor. However, in a mating context, the interrupted chirps seemed to play a more significant role in communication than the simple chirps.

Courtship displays were carried out by the males when the female was a virgin and never occurred when the female was already mated by a different male. The duration of the courtship displays was affected by competition between males. Mating time was also affected by the presence of competing males. When there were no males competing for a female in a mating trial, the duration of the courtship and of the mating was found to be comparatively longer than in the presence of competing males. Although *H. ligniperda* was previously reported as a monogamous species, the observations of this thesis indicate that this insect is a polygamous species with the ability to mate multiple times with multiple partners.

This study provides a good example of acoustics research in insects and a proofof-concept for future research on acoustics as a deterrent or behaviour-modifying tool for *H. ligniperda* control.

Acknowledgement

This thesis would not have been possible without the countenance and input from numerous people. I still remember the days when I was so disquieted about not getting a suitable project for my thesis. It was one of the happiest days in my life when Dr Masha Minor (my supervisor at Massey University) took me to Plant & Food Research and introduced me to Dr Adriana Najar-Rodriguez (Plant & Food Research Ltd, Palmerston North), and I knew about the project. First and foremost, I would like to express gratitude to my supervisor, Dr Masha Minor who conceded my research interests and showed me the direction ultimately linking to Dr Adriana Najar-Rodriguez. I am greatly indebted to Dr Najar-Rodriguez for all encouragements and support and for functioning as my guardian. Thank you, both of you for proofreading, editing and giving feedbacks on every single mistake that I made while writing my thesis. Your enthusiasm to figure out my problems, elucidate concepts and furnish insightful advice on the variety of topics were really helpful for me. Your office door was always open for me whenever I ran into a trouble or had a query about my research or writing.

I have so many other people to whom I'm indebted a lot for their varied assistance over the course of this project. Steven Burgress (Lab technician, Plant & Food Research Ltd, Palmerston North), thank you so much for climbing up the high hills of pine forest to get the cambium for my experiments. Thank you, Fang Tsang (Lab technician, Plant & Food Research Ltd, Palmerston North) for your effort in taking care of my beetles every week by providing them with the modified huhu grub diet. This research would not have been possible without the assistance from Mr Duncan Hedderley (Statistician, Plant & Food Research Ltd, Palmerston North). Thank you, Duncan, for all your help regarding the statistical data analysis. To Jess Sailor (former lab manager of the entomological department at Plant and Food Research Ltd, Palmerston North), thank you for assisting me in buying all different tools and gear needed for my experiment. On top of everything, thank you for checking grammatical errors of my thesis writing. Thank you Nirosha Priyadarshani, for your regular guidance and making me literate on sound analysis using different sound analysing software. You are my guru on this aspect. A huge salute to you.

I am grateful to Mr Liz D. Rowland from Cornell University for regular support and counselling on different aspects and providing me technical support in terms of sound analysis using Raven software. Thank you, Dr Germano Henrique Rosado Neto (Federal University of Parana, Brazil) for giving me some suggestions and feedbacks in terms of sound analysis.

I must express my very profound gratitude to my mother and brother for providing me with unfailing support and continuous inspiration throughout my years of study. My dear dad, although you are not physically with us, your blessings and memories are always with me in every step of my life providing me with the encouragement to climb up the ladder of success. I owe a huge appreciation and thank you to my wife for her daily love, care and support in my day to day activities. Thank you so much for taking over all of my responsibilities and providing me with the favourable environment for the successful accomplishment of this thesis. mating.

I would also like to acknowledge Massey University and Plant & Food Research for providing me with a good platform for the accomplishment of my Master's degree. I would like to remember all other people who are directly or indirectly involved in my two years' journey of my masters' study at Massey University. I am gratefully indebted to your support and best wishes for making my dream come true.

Last but not the least, thank you all the beetles (*Hylurgus ligniperda*) for producing sounds for me. I am really sorry for the pain that I have given to you in the distress experiment. You taught me some portion of your language and now I really can mimic you sound (especially male). Although you are considered as a pest for New Zealand pine forest industry, I consider you as one of my best friends because you are the one who built my career.

It's certainly been a journey of ups and downs but on balance, it's been a great experience and I have learnt so much. Thank you once again, everyone.

Regards,

Sunil Sapkota

Table of Contents

Abstracti
Acknowledgementiii
Table of Contents
List of Figures
List of Tablesix
Chapter 1: General Introduction and Ltterature Review
Acoustic Communication in Coleoptera
Bark Beetles Communication
Sound Production Mechanisms in Bark Beetles
Function of Sound Communication in Bark Beetles7
Behavioural Contexts of Sound Production in Bark Beetles
Study Species: Golden Haired Bark Beetle (Hylurgus ligniperda) (Fabricius, 1787)
Distribution
Host Plants 11
Physiology and Phenology12
Morphology13
Sex Differentiation
Life Cycle 15
Associations with Fungal Pathogens
Sound Production in <i>H. ligniperda</i>
Morphology of the Stridulatory Apparatus in H. ligniperda 19
Research Objectives
Common Terms and Definitions Used in This Study 21
For Males 21
For Females
Chapter 2: Methods and Methodology23
Bark Beetles (Hylurgus ligniperda) Rearing
Experimental Trials
Experimental Set-up
Sound and Video Recording
Sound Analysis in Raven
Temporal Analysis
Spectral Analysis
Sound Recording and Behavioural Observation Specific to Each Trial
Distress
Mating
Competition

Male Territoriality	42
Female Territoriality	42
Colony	42
Statistical Analysis	42
Temporal Analysis	43
Spectral Analysis	44
Behavioural Analysis	44
Chapter 3: Results	
Distress	47
Mating	50
Male and Female Behaviour and Male Sound Production Before and During Matin	g 50
Male and Female Behaviours and Male Sound Production after Mating	55
Female Sound Production Before, During and After Mating	57
Competition	58
Territoriality	62
Male Territoriality	62
Female Territoriality	64
Colony	65
Comparative Analysis	68
Temporal parameters	68
Spectral Comparisons	
Principal Component Analysis (PCA) of the Spectral Data	
Behavioural Comparisons	79
Chapter 4: Discussion	
Sound Production	83
Behaviour	88
Chapter 5: Conclusions and Recommendation	
Conclusions	95
Recommendations for Future Work	
Literature Cited	
Appendix	107
Temporal Data (Male)	107
Temporal Data (Female)	109
Spectral Data (Male)	111
Spectral Data (Female)	114

List of Figures

Figure 1.1: The stridulatory apparatus in pig-bean pods weevil (Bondarius tubercula	atus)
(Curculionidae).	4
Figure 1.2: Waveform and spectrogram of a chirp recorded from coconut rhinoceros	5
beetle (Oryctes rhinoceros, Scarabaeidae) stridulation	4
Figure 1.3: Oscillograms of stridulation of the western pine beetle (<i>Dendroctonus</i>	
brevicomis) showing the male's sub-chirp in between the chirps	9
Figure 1.4: Different body parts of <i>H. ligniperda</i> adult	13
Figure 1.5: Three different bark beetle species which may be mistaken for each other	er. 14
Figure 1.6: The dorsal caudal part of the abdomen of H. ligniperda	15
Figure 1.7: Hylurgus ligniperda, stages of the life cycle	17
Figure 1.8: The stridulatory apparatus of male and female H. ligniperda	19
Figure 2.1: Feeding the larvae of <i>Hylurgus ligniperda</i> with an artificial diet	26
Figure 2.2: Sexing adults of Hylurgus ligniperda	28
Figure 2.3: The location of Gordon Kear Forest (Google Earth)	29
Figure 2.4: Extraction of cambium from a pine tree	30
Figure 2.5: Preparation of cambium for the experiment	31
Figure 2.6: Cambium chamber set up	32
Figure 2.7: Recording instruments used for the experiment.	33
Figure 2.8: Marking the beetles for easy identification.	33
Figure 2.9: Recording setup for distress trial.	
Figure 2.10: The screenshot of the sound recorder TASCAM HD-P2 showing samp	ling
frequency and sample width	
Figure 2.11: Matching the timeline of audio and video recordings in order to pinpoin	nt
the behavioural activities during specific period of sound production.	
Figure 2.12: Screenshot of Raven Pro 1.4 showing a representative waveform and	
spectral pattern of sounds produced by a distressed Hylurgus ligniperda virgin male	
Figure 2.13: Example of the temporal sound analysis	
Figure 2.14: Sample of some of the predefined measurement options (used in this st	udy)
available in Raven.	
Figure 2.15: Spectral measurements of different parameters using Raven software	39
Figure 2.16: Example showing how the 1-minute sample was extracted from three	
different phases under mating trial observed	41
Figure 3.1: Sounds of distressed <i>H. ligniperda</i> male	48
Figure 3.2: Sound of distressed <i>H. ligniperda</i> female; a waveform showing clicks	
produced.	49
Figure 3.3: Waveforms showing variation in the nature of sounds produced by a <i>H</i> .	
<i>ligniperda</i> male, before and after the introduction of a female into the cambium	
chamber.	51
Figure 3.4: Behaviours observed after the introduction of <i>H. ligniperda</i> female into	the
cambium chamber.	51
Figure 3.5: Waveform and spectrogram showing the toothstrikes produced by a virg	in
H. ligniperda male in mating trial, with female present, one minute before mating	
Figure 3.6: Waveforms of sounds produced by a H. ligniperda pair at various stages	sof
mating	52
Figure 3.7: Behaviours observed during interaction of virgin H. ligniperda male and	1
female	54
Figure 3.8: Waveform and spectrogram showing the toothstrikes of a virgin <i>H</i> .	
ligniperda male after mating.	56

Figure 3.9: Sounds produced by <i>H. ligniperda</i> during competition (two males and a	
female)	60
Figure 3.10: Two males and female of <i>H. ligniperda</i> during competition ("red" male	
was introduced together with the female, "green" male introduced 30 min later)	61
Figure 3.11: Sounds of territorial males	.63
Figure 3.12: Different stages in male territoriality	.64
Figure 3.13: Waveforms and spectrogram showing clicks of territorial <i>H. ligniperda</i>	
females.	.65
Figure 3.14: Different stages during female territoriality	.65
Figure 3.15: Sounds of male and female <i>H. ligniperda</i> in a colony	.67
Figure 3.16: Behaviours of <i>H. ligniperda</i> males and females observed in colony	
context	67
Figure 3.17: Means and standard errors (bars) of temporal parameters for H. lignipera	la
female click sound in different context	69
Figure 3.18: Mean values and standard errors (bars) for temporal parameters of	
H.ligniperda male sound (simple chirps) in different contexts	.71
Figure 3.19: Mean values and standard errors (bars) for temporal parameters of <i>H</i> .	
ligniperda male sounds (interrupted chirps) in different contexts	72
Figure 3.20: Mean values and standard errors (bars) of spectral parameters	
(toothstrike/click duration, maximum amplitude, minimum amplitude, high frequency	/,
low frequency and peak frequency) for the sounds produced by H. ligniperda male an	ıd
female in five different behavioural contexts	.76
Figure 3.21: Scatterplots for standardised spectral parameters of sounds produced by <i>I</i>	Н.
<i>ligniperda</i> males and females in different behavioural contexts	.78
Figure 3.22: Mean values and standard errors (bars) for the first courtship duration, fin	rst
mating duration and number of mating observed in different contexts	.79

List of Tables

Table 2.1: Artificial diet used for <i>Hylurgus ligniperda</i> larvae (modified huhu grub
(Prionoplus reticularis) diet
Table 3.1: Means and standard errors (\pm SE) of temporal parameters measured from
sounds produced by distressed <i>H. ligninerda</i> males, and a naired t-test comparison
of simple and interrupted chirps ($\alpha=0.05$) 48
Table 3.2: Means and standard errors (+SE) of different spectral parameters
massured from sounds produced by distressed <i>H</i> ligningrdg males and a paired
t test comparison of simple and interpreted chima($u=0.05$)
t-test comparison of simple and interrupted chirps ($\alpha = 0.05$)
Table 3.3: Means and standard errors (\pm SE) for different temporal parameters
measured from sounds (clicks) produced by distressed <i>H. ligniperda</i>
females (N=10)49
Table 3.4: Means and standard errors (±SE) of different spectral parameters
measured from sounds (clicks) produced by distressed <i>H. ligniperda</i> females
(N=10)
Table 3.5: Means and standard errors (\pm SE) of different temporal parameters
measured from the sounds produced by a H , <i>ligninerda</i> male before mating and a
nonserved from the sounds produced of a fit n_{g}
(N=10)
(N=10)
Table 5.0. Means and standard effors $(\pm 5L)$ of spectral parameters of sounds
produced by virgin <i>H. ligniperaa</i> male before mating and a paired t-test
comparison of simple and interrupted chirps (α =0.05) (N=10)53
Table 3.7: Behaviours recorded during mating of <i>H. ligniperda</i> (single pair of virgin)
adults)55
Table 3.8: Means and standard errors (±SE) of temporal parameters of sounds
made by <i>H. ligniperda</i> males after mating and a paired t-test comparison of simple
and interrupted chirps (α =0.05)
Table 3.9: Means and standard errors (\pm SE) of spectral parameters of sounds made by
<i>H. ligniperda</i> males after mating and a paired t-test comparison of simple and
interrupted chirps (α =0.05)
Table 3 10: Means and standard errors (+SE) of temporal parameters for sounds
produced by H ligningrada female at three stages of mating
(N=10)
(19–10)
Table 3.11: Means and standard errors $(\pm SE)$ of spectral parameters of sounds
produced by <i>H. ligniperda</i> female at three stages of mating $(N=10)$
Table 3.12: Means and standard errors (\pm SE) of spectral parameters of sounds
produced by <i>H. ligniperda</i> males during competition (N=10)60
Table 3.13: Means and standard errors (±SE) of spectral parameters of sounds
produced by <i>H. ligniperda</i> females in the presence of two competing males (N=10)60
Table 3.14: Different behaviours observed during competition between two
<i>H. ligniperda</i> males for a conspecific female
Table 3.15. Means and standard errors (+SE) of spectral parameters of sound
produced by $H_{ligningrdg}$ male (marked red) in the presence of other two males
(N-10)
(1N-10)
Table 5.10. We and standard errors $(\pm SE)$ of spectral parameters of sounds
produced by <i>H. ligniperda</i> temale (marked red) in the presence of other two females
(N=10)
Table 3.17: Means and standard errors (±SE) of spectral parameters of sounds

produced by <i>H. ligniperda</i> male (marked red) and female (marked red) in a colony	
(N=10)	68
Table 3.18: Behaviours observed in a colony of H. ligniperda (three males and thre	e
females)	68
Table 3.19: Analysis of variance (ANOVA) for temporal parameters of sounds	
produced by <i>H. ligniperda</i> female under distress and during mating	70
Table 3.20: Analysis of variance (ANOVA) for temporal parameters of sounds	
(simple chirps) produced by <i>H. ligniperda</i> male under distress and during phases	
of mating	70
Table 3.21: Analysis of variance (ANOVA) for different temporal parameters	
of sounds (interrupted chirps) of <i>H. ligniperda</i> male under distress and phases of	
mating	70
Table 3.22: Means and standard errors (+SE) for spectral parameters of sounds	
produced by <i>H. ligninerda</i> male in five different behavioural contexts.	
Table 3.23. Means and standard errors $(+SE)$ for spectral parameters of sounds	, .
produced by <i>H</i> ligningrdg female in five different behavioural contexts	74
Table 3.24: Analysis of variance (ANOVA) for spectral parameters of sounds	, .
produced by <i>H</i> ligningerda male and female in five different behavioural	
contexts	75
Table 3.25: The correlation matrices (Dearson correlations) of different spectral	
name s.25. The conclusion matrices (rearson correlations) of different spectral	77
Table 2.26. A relation of variance (ANOVA) for first courtship duration	//
Table 5.20. Analysis of variance (ANOVA) for first courtship duration,	
first mating duration and number of mating observed in <i>H. ligniperda</i> in different	0.0
contexts	80