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 proof-of-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

List of Figures

List of Tables

Chapter 1: General Introduction and Literature Review

Acoustic Communication in Coleoptera

Many insects use sound for mate identification and attraction, courtship, expression of aggression, alerting and defence. The signals that are produced in this context must be effectively and efficiently transmitted, processed and received by the targeted receiver and should elicit a behavioural response. Such types of sender-receiver acoustic and vibratory signal interactions have been widely studied for hymenopteran, orthopteran, hemipteran and lepidopteran species (Drosopoulos & Claridge, 2006). However, investigations in regards to acoustic communication in coleopterans have generally been given less consideration (Alexander, Moore, & Woodruff, 1963; Arrow, 1942; Drosopoulos & Claridge, 2006). Sound production in coleopterans has been reported from around 30 families and it seems that this trait has evolved a number of times (Drosopoulos & Claridge, 2006). Indeed, for long-horned beetles, (Family: Cerambycidae), it has been suggested that the synchronization of different rhythmic movements during courtship behaviour could have led to the evolution of diverse stridulatory organs independently (Michelsen, 1966).

Generally, in coleopterans, the sounds are delivered through a mechanism known as stridulation which involves the use of a well-defined structure such as the ‘plectrum’ or the ‘scraper’ (lip or nodule) that is moved across a finely ridged surface commonly known as the ‘file’ or ‘pars stridens’ (Figure 1.1) (Rosado-Neto & dos Santos, 2010). The plectrum or the scraper can be a single sclerotized peg, a series of pegs, single or multiple ridges, tapered edge of an appendage or tubercles, depending upon the species. Similarly, the file or ‘pars stridens’ is made up of a series of transverse ridges (Drosopoulos & Claridge, 2006). Due to strongly sclerotized exoskeletons in many beetles, even the smooth movement can cause body parts to strike and evoke vibrations (Drosopoulos & Claridge, 2006).

There are 14 different types of stridulatory mechanism of sound production that have been discovered in coleopterans. Among these, most are considered to have evolved from the movements produced during biting, walking, wing folding and struggling behaviours (Claridge, 1968; Hyder & Oseto, 1989; Lyal & King, 1996; Masters, 1979; Michelsen, 1966). Not many studies have empirically discussed the elements of acoustic signalling describing stridulatory structures and their mechanisms in coleopterans (Buchler, Wright, & Brown, 1981; Eisner et al., 1974; Masters, 1980). Only few have provided information regarding the production of acoustic signals

(Figure 1.2) (Hirschberger, 2001; Mankin, Moore, Samson, & Chandler, 2009; Schmitt & Traue, 1990).

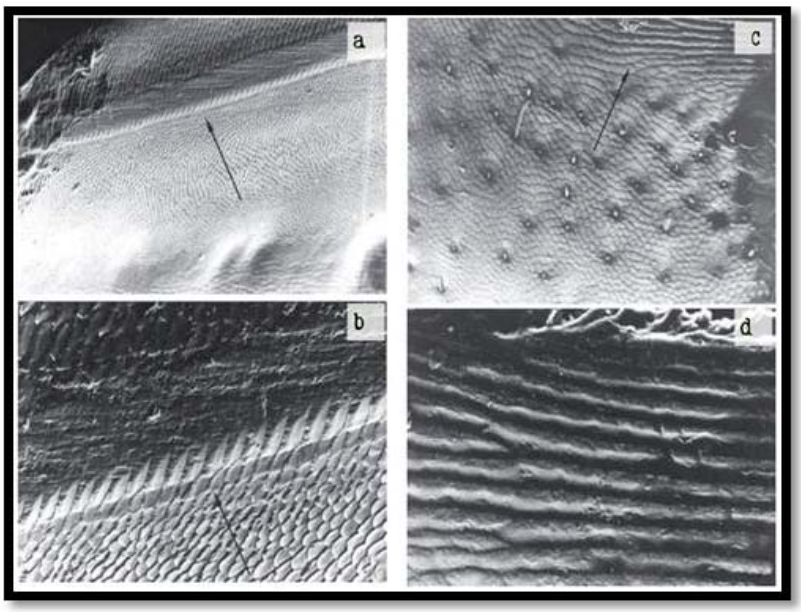


Figure 1.1: The stridulatory apparatus in pig-bean pods weevil (*Bondarius tuberculatus*) (Curculionidae); a) and b) file located internally on the apical third of the left elytron near suture, c) and d) plectrum located transversely at the dorso-apical margin of the seventh abdominal tergite. These structures are present both in a male and a female. Reproduced with permission. Source: (Rosado-Neto & dos Santos, 2010).

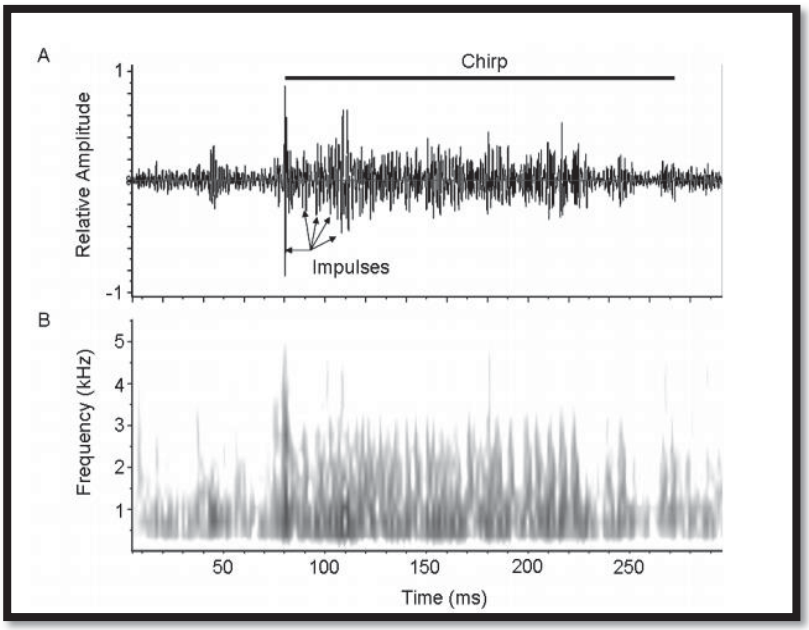


Figure 1.2: Waveform and spectrogram of a chirp recorded from coconut rhinoceros beetle (*Oryctes rhinoceros*, Scarabaeidae) stridulation. The arrow shows the impulses (toothstrikes) of the chirp. Reproduced with permission. Source: (Mankin et al., 2009).

The functions of receptor organs have only been described in few species, for example the tympanal hearing organs in tiger beetles (Cicindelidae) and scarab beetles (Dynastinae). In these beetles, the hearing organs act mainly as sense organs for recognising the ultrasonic sounds from predatory bats (Forrest, Read, Farris, & Hoy, 1997). There is a clear need for more advance research into the functions, characteristics and receptor mechanisms of the acoustic communication in coleopterans.

Bark Beetles Communication

The sub-family Scolytinae (Family: Curculionidae) includes all bark beetles, comprising about 220 genera (Dunn, 1996). Despite the diversity of beetles in this sub-family, and the fact that many species within it seem to use acoustics for communication, bark beetles as a whole have received little attention in relation to the sound communication, compared to other coleopterans.

Bark beetles are ecologically considered to be 'parasites' of coniferous trees of the family Pinaceae (Raffa et al., 2008). They infest and kill young and recently damaged trees. The adult bark beetles bore into the phloem through the bark, mate, build galleries and lay eggs. The matured larvae feed on the host tree, digging tunnels and thereby disrupting the vascular system on the host tree (Sauvard, 2004). Their life cycle involves complex conspecific and heterospecific interactions that help them to overcome the resinous defences of their host trees (Raffa & Berryman, 1983). Generally, bark beetles display conspecific mass aggression (Bentz et al., 2010). During the conspecific mass aggression for the host colonization, bark beetles use short and long range communication cues. Long range cues generally involve the use of pheromones and plant-related chemicals (Symonds & Elgar, 2004), whereas at short range bark beetles use sounds (acoustic) together with pheromones as a means of communication (Ryker, 1988). Long-range host selection, localization and colonisation are governed by the recognition of terpenes of the host plant. The pheromones are then used by the bark beetles to attract their conspecifics. Such pheromones are synthesized through the oxidation of the terpenes of the host plant (Raffa, et al., 2008). The pheromones produced can have multiple functions such as aggregation, mate attraction and inter- and intra-species competition (Symonds & Elgar, 2004). In the case of the mountain pine beetle *Dendroctonus ponderosae*, a species of bark beetle which causes considerable damage to lodge pole pine plantations in North America, the female finds

the host tree and starts its colonisation. As the female starts invading the phloem through the bark of the host tree, it produces aggregation pheromones which attracts males. Once the males arrive to the host tree, they start emitting aggregation pheromones which ultimately attracts additional females. When the group of males and females are in close proximity, the acoustic signalling becomes the predominant form of communication (Ryker, 1988).

Chemical communication in bark beetles has been widely studied (Andersson, Larsson, & Schlyter, 2009; Byers, 2007; Raffa, 2001; Rudinsky, 1962; Wallin & Raffa, 2000). However, the study of sound communication has received relatively less attention (Wyatt, 2003). Understanding the functions of acoustic communication mechanisms in bark beetles could provide valuable information on their evolutionary and behavioural ecology, and could lead to the development of efficient control methods.

Sound Production Mechanisms in Bark Beetles

With the documentation of the presence of stridulatory structures in various species of beetles from the sub-family Scolytinae (bark beetles), it is believed that sound communication forms a major means of communication in many bark beetles (Barr, 1969). Most of the studies on sound communication associated with bark beetles have focused on the mechanism of sound production, including the temporal characteristics of signals and the way signals are involved with various behavioural contexts. Four main kinds of stridulatory mechanisms have been described so far, including: i) gular prosternum, where the pars stridens present beneath the head is scraped against a single or multi-ridged plectrum present at the anterior end of the prosternum. ii) Elytral abdominal; the pars stridens usually present on the left elytrum are scrapped by the sclerotized pegs present on the seventh segment of the abdominal tergite. It has been demonstrated that the elytral-abdominal mechanism of sound production is common across the sub-family Scolytinae, which includes the bark beetles. Such mechanisms are believed to have evolved from the abdominal movements during the folding of the fine transparent wings present under the coleopteran elytra (Lyal & King, 1996). iii) Vertex-protonum; the pars stridens present on the vertex of the head is scrapped against the multiple ridged plectrums present at the ventral anterior side of the protonum (Barr, 1969). iv) In some females of the genus *Dendroctonus*, the sclerotized peg present on

the eighth abdominal segment is scraped against a pars stridens on the posterior region of the last sternite (Rudinsky & Michael, 1973).

The stridulatory mechanism in bark beetles varies depending upon the genus, gender and mating system. For instance, the vertex-protonum mechanism is generally found in non-host selecting females of polygamous species (e.g. *Gnathotrichus sp.*) (Swaby & Rudinsky, 1976; Wilkinson, McClelland, Murillo, & Ostmark, 1967). The elytral-abdominal type of stridulatory mechanism is observed in non-host selecting males of monogamous species (e.g. *Hylesinus sp.*) (Vernoff & Rudinsky, 1980). The gular-prosternum mechanism can be observed in both male and females of both monogamous (e.g. *Scolytus sp*.) and polygamous (e.g. *Ips sp*.) species (Oester & Rudinsky, 1979). However, *Dryocoetes autographus* females (host-selecting) have the elytral-abdominal as well as the vertex-protonum types of stridulatory mechanism, while their males do not produce any type of sound at all (Sasakawa & Yoshiyasu, 1983). Interestingly, *Cryphalus fulvus* males use the elytral-abdominal mechanism during aggression with other males, but use the vertex-protonum mechanism during male-female interactions (Sasakawa & Sasakawa, 1981).

Function of Sound Communication in Bark Beetles

In beetles from the family Scolytidae (now Curculionidae), one sex (either male or female) is responsible for the selection of the host tree and breeding site, and for gallery construction and production of pheromones. This particular sex seems to be normally 'silent' compared to the other one, and thus the stridulating organs are not well developed or in some cases absent (Byrne, Swigar, Silverst, Borden, & Stokkink, 1974). The individuals of the opposite sex, on the contrary, are found to make sounds audible to the human ear during handling or at the entrance of the gallery of the host-selecting sex (Barr, 1969). However, there is not yet a convincing explanation as to why the two sexes have not evolved similar patterns of stridulation.

For bark beetles, some explanations regarding this phenomenon have been put forward. For example, wood has good sound-transmitting properties, while chemical signals such as pheromones might not disperse as effectively as sound through the galleries made by the beetles (Campbell, 2004). Similarly, tree bark has uneven surface, and the air might not efficiently move into the galleries, thus impeding an efficient chemical communication between the beetles present outside of the bark and those in

the galleries (Dumortier, 1963; Rudinsky & Michael, 1972). In many cases, when there is sexual dimorphism in the stridulatory organs (for example, in *Scolytus scolytus* the female has a double and the male has a single-ridged plectrum), the species can produce a wider range of calls. However, the stridulatory apparatus of both male and female *Scolytus multistriatus* has similar morphological features. Therefore, the sounds produced by different genders are differentiated by parameters such as frequency, amplitude, and duration of the pulses, which all together make up the gender-specific acoustic signals (Dumortier, 1963).

Sounds are also produced to express specific behaviours. Some insects stridulate when they are disturbed or attacked (Wood, 1961). The sound emitted in such cases may represent alarm or warning. Among various types of stridulation, rivalry sounds are the most commonly studied phenomena in insect acoustic behaviour and can be classified under 'aggressive' signals (Alexande, 1967). If more than one male is attracted towards a *Dendroctonus* female, the male beetles fight with one another, and stridulate loudly at the entrance of the female gallery. The males of *Dendroctonus ponderosae* have been found to fight with each other with loud stridulation when put together in the gallery. This behavioural activity has been termed as territorial behaviour (McGhehey, 1968).

Behavioural Contexts of Sound Production in Bark Beetles

Generally, bark beetles are found to produce sounds in three situations: i) distress; ii) intra-sexual rivalry interactions; iii) pre-copulatory interactions between male and female (Ryker, 1988). Based on the temporal structure of the sounds produced, the sound signals have been classified into six types: i) single chirps, ii) double chirps, iii) interrupted chirps, iv) multi-component chirps, v) clicks, and vi) trills (Oester, Ryker, & Rudinsky, 1978; Rudinsky, Oester, & Ryker, 1978; Rudinsky & Vallo, 1979; Swaby & Rudinsky, 1976). However, such classifications have created confusion in the acoustic nomenclature, as previous researchers only provided theoretical descriptions of such different types of signals and did not explain how these signals were categorized. For instance, in the case of *Dendroctonus sp.,* males produce 'multi-component' chirps which are composed of fairly consistent multiple 'sub-chirps' in between the chirps (Figure 1.3) (Ryker, 1988). Such chirps are also described as 'interrupted chirps' by Michael & Rudinsky (1972) and Ryker & Rudinsky (1976a). However, for females of

some species of the genus *Ips,* 'interrupted chirps' do not have multiple 'sub-chirps'. Instead, they have 'single' chirps' with gaps of missing pulses which are structurally different than those of the 'multi-component' chirps produced by *Dendroctonus* males (Oester & Rudinsky, 1975; Swaby & Rudinsky, 1976).

Oester and Rudinsky (1975) described 'clicks' produced by the pine engraver beetle *Ips pini* during male-male interaction (aggression) as a function of defending the territory. However, the mechanism of production of the click-like sounds remains unknown. Similarly, it is also not clear how 'clicks' can be distinguished from other sounds produced during digging and walking.

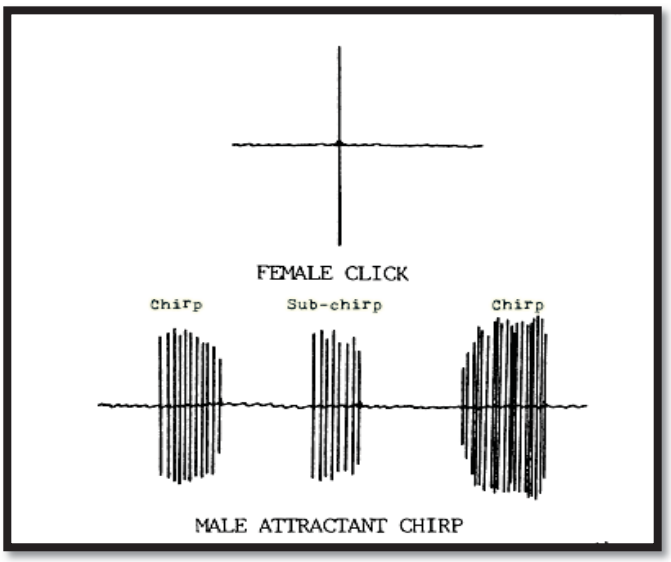


Figure 1.3: Oscillograms of stridulation of the western pine beetle (*Dendroctonus brevicomis*) showing the male's sub-chirp in between the chirps (Ryker, 1988) which was also called 'interrupted chirp' by Michael & Rudinsky (1972) and Ryker & Rudinsky (1976a).

Swaby and Rudinsky (1976) described the sound production mechanism in *Ips pini* females. The females produce their acoustic signals by scrapping the 'pars stridens' present on the vertex of the head against the plectrum present on the ventral anterior side of the pronotum. Swaby and Rudinsky (1976) recorded sounds produced by *Ips pini* females during various behavioural activities such as disturbance ('stress chirps'), pre-mating interactions ('attraction chirps'), female-female aggressive interaction ('rivalry chirps'). In addition, females trying to enter the male nuptial chamber stridulate once they come in physical contact with the male (Swaby and Rudinsky, 1976). In this species, it was observed that stridulation was required to get permission from the male to enter into the nuptial chambers. Swaby and Rudinsky (1976) also analysed the

temporal characteristics of the acoustic signals in terms of chirp durations, number of pulses (toothstrike) and pulse rates (toothstrike rate) and showed that these characteristics differed significantly across different behavioural activities such as ‘stress’ and ‘attraction’.

In addition to their temporal structure, bark beetle acoustic signals have been classified based on the specific purposes for which they are produced (Ryker, 1988). For instance, the signals produced during pre-mating are commonly known as ‘attractant’, ‘greeting’, ‘agreement’ or ‘courtship’ chirps based on the time when these signals are produced during male-female interactions (Ryker, 1988; Ryker & Rudinsky, 1976a). For example, in the red turpentine beetle *Dendroctonus valens*, males produce ‘simple’ chirps in response to female pheromones, described as ‘attractant’ chirps (Ryker & Rudinsky, 1976a). In response to these sounds females produce ‘simple’ chirps which were called ‘agreement’ chirps. Once both male and female come in contact, the females produce comparatively shorter ‘simple’ chirps which were named ‘greeting’ chirps (Ryker & Rudinsky, 1976a). Similarly, in the Douglas-fir beetle *Dendroctonus pseudotsugae*, females produce aggregation pheromones to attract males. Attracted males produce ‘simple’ ‘attractant’ chirps and enter into the female galleries. The males then switch to ‘multi-component’ chirps to express aggression to other males around them. When accepted by the female, the male again produces ‘simple’ ‘courtship’ chirps prior to mating (Rudinsky & Ryker, 1976). However, in both species described above, the quantitative temporal characteristics (e.g., interchirp interval, toothstrike per chirp, chirp duration, toothstrike rate) of different types of simple chirps have not been provided (Ryker, 1988).

There is a lack of specific definitions and criteria for classifying and differentiating different acoustic signal types across bark beetles. Only some of the studies have explained the temporal characteristics of the signals produced by different bark beetles and have demonstrated variation in terms of chirp types under different behavioural contexts (Ryker & Rudinsky, 1976b; Yandell, 1984). For example, signals produced by *D. ponderosae* have been categorised broadly as ‘simple’ and ‘interrupted’ chirps, and temporal parameters such as chirp duration, number of toothstrike per chirp and toothstrike rates have been defined and analysed (Ryker & Rudinsky, 1976b; Yandell, 1984). Apart from few cases, detailed studies on the spectral characteristics of the acoustic signals such as intensity and frequency have been done (Fleming, Lindeman, Carroll, & Yack, 2013; Yturralde & Hofstetter, 2015). When *D. ponderosae*

adults were observed under different behavioural contexts such as distress and male-female interactions, the acoustic signals were produced at a frequency range between 15 to 26 kHz. During distress, the duration of the simple chirp was significantly shorter than that of the interrupted chirp. However, both types of chirps had similar toothstrike rates. During male-female interactions, the males produced shorter simple chirps with smaller number of toothstrikes compared to interrupted chirps (Fleming, et al., 2013). In the Colorado pine beetle *D. approximates* males and females produced simple chirps when distressed (Yturralde & Hofstetter, 2015). The mean duration of male simple chirps under such condition was 108.6 ms, with a mean peak frequency of 5293 Hz. The females produced simple chirps during the same situation with mean duration of 81.4 ms and mean peak frequency of 11348 Hz. Similarly, during male-female interactions, male simple chirps had a mean duration of 124.5 ms with a mean frequency of 5686 Hz. The responding females produced simple chirps with a mean duration of 88.2 ms (Yturralde & Hofstetter, 2015).

Study Species: Golden Haired Bark Beetle (*Hylurgus ligniperda*) (Fabricius, 1787)

Distribution

Hylurgus ligniperda is an invasive species that has spread throughout the world. It is native to the Mediterranean region, southern and central Europe, Russia, Ukraine, Algeria, and Asia Minor (Wood, 1992); It has been introduced and established in various parts of the world such as Brazil, Argentina, Chile, Uruguay, Paraguay, South Africa, USA, Sri Lanka, Japan, Australia and New Zealand (Bright, 2014). In New Zealand, the species was first discovered in April 1974 in Whitford south of Auckland (*New Zealand pest and beneficial insects*, 1984). It was thought to have been introduced in sawn timber from South Australia (New Zealand Forest Service, 1974). However, the exact route(s) of invasion of this beetle into New Zealand is unknown.

Host Plants

Species within the family Pinaceae such as *Pinus armena, Pinus brutia, Pinus canariensis, Pinus elliottii, Pinus halepensis, Pinus nigra, Pinus patula, Pinus pinaster, Pinus pinea, Pinus strobes, Pinus sylvestris* and *Pinus radiata* are considered to be the major hosts for *H. ligniperda* (Eglitis, 2001). Besides pine tree species, *H. ligniperda* has been shown to have an ability to spread to other coniferous species such as spruce

(*Picea* spp.), Douglas fir (*Pseudotsuga* spp.), true fir (*Abies* spp.) and larch (*Larix* spp.) (Eglitis, 2001).

Hylurgus ligniperda breeds in unhealthy pine stumps, in pine logs, usually near the base of the trunk, or in large exposed roots (Fabre & Carle, 1975). In New Zealand, the adults have been found breeding mainly in recently felled stumps and logs of *Pinus radiata,* especially those in contact with the soil (Bain, 1977). The beetles are often extremely abundant in pine plantation regions, especially in the years following tree harvesting. The adults can cause direct damage through brood galleries and feeding tunnels formed under the bark of wind thrown or harvested logs, introducing decay fungi and sapstain which can reduce the quality and value of logs if not promptly processed (Kerr, 2010). In Spain, communal gatherings of 30-40 adults of *H. ligniperda* during winter season can cause ring barking up to 15 cm in diameter, which could eventually kill the pine trees (Stephen, Reay & Patrick, Walsh, 2001). Such evidence of damage has not been reported from other parts of the world. In South Africa, *H. ligniperda* is predominantly a root-dwelling species which is active throughout the year in subterranean habitats that maintain enough moisture and stable environmental conditions all year round; beetles can directly bore and infest the host through the soil (Tribe, 1992). In Spain and Chile, the overwintering adults were found to girdle and kill 1-2 years old young seedlings of *Pinus radiata*. The tree mortality was more severe when the trees had either malformed roots caused by poor planting or were affected by other type of injuries caused by other insects and/or diseases (Ciesla, 1988). However, in other countries where the beetle has established itself, direct mortality of live trees has not been reported (DATA, 1994).

Physiology and Phenology

Hylurgus ligniperda has more than one generation per year. In Chile, three generations per year have been observed (Eglitis, 2001). In southern Europe, the adults fly from March to April and have the ability to disperse over a wide range of areas in response to their host-plant volatiles (Grüne, 1979). In south eastern France, these beetles have two generations per year with the first generation having two successive periods of oviposition and the second generation having two oviposition periods only in favourable conditions (Fabre & Carle, 1975). The peak flight activity occurs in spring followed by a shorter peak in autumn. The peak flight during autumn overlaps with that of the second generation and the adults go into hibernation (Grune, 1979). In South Africa *H. ligniperda* has its peak flight activity during April and May (Tribe, 1991b). In New

Zealand, the adults disperse with two peak flights during spring and autumn, corresponding to the two generations per year (Kerr, 2010). In New York, USA, the species was found to have two generations per year, with the first generation developing from May to June, and the second generation from July to September; the highest flight activity was observed from September to November, corresponding with the emergence of the second generation (Phytosanitary Alert System, 2002).

Morphology

The adult beetles are 6 mm long and 2 mm wide with black-brown cylindrical body (Bain, 1977). The antennae and the terminal segments of the legs are reddish to brown. Most of their body parts are covered with red to yellowish dense hairs which are more obvious on the posterior slope of the elytra and in the frontal region of the head. The dense hairs appear notched when observed under the microscope. The apex of the elytra is convex with a minor indentation (Figure 1.4 a-d) (Cavey & Passoa, 1994).

Figure 1.4: Different body parts of *H. ligniperda* adult; a) close up view of the head, b) lateral view of the head, c) dorsal view of pronotum, d) the elytral apex. Source: www.ipmimages.org, with permission.

Hylurgus ligniperda adults sometimes can be confused with the black pine bark beetle *Hylastes ater*, as the two species are superficially similar in appearance. The two

species can be differentiated by their body size – *H. ligniperda* adults (6 mm in length and 2 mm in width) (Bain, 1977) are bigger than *H. ater* (4-5 mm in length and 1.4 mm in width) (Milligan, 1978).

Both of these species can also be confused with *Pachycotes peregrinus* (4.7 mm in length and 2.3 mm in width) which has similar body length as *H. ater*. However, *Pachycotes peregrinus* has a humped body and a dull pronotum compared to *H. ater*, while *H. ligniperda* has a hairier body than the other two species (Figure 1.5 a-c). Furthermore, the *P. peregrinus* excretes granular white frass out of the entry tunnel whereas the frass of *H. ligniperda* and *H. ater* is normally reddish-brown. It is because *P. peregrinus* tunnels in the wood whereas *H. ater* and *H. ligniperda* tunnels are found in the bark or cambium (Bain, 1977).

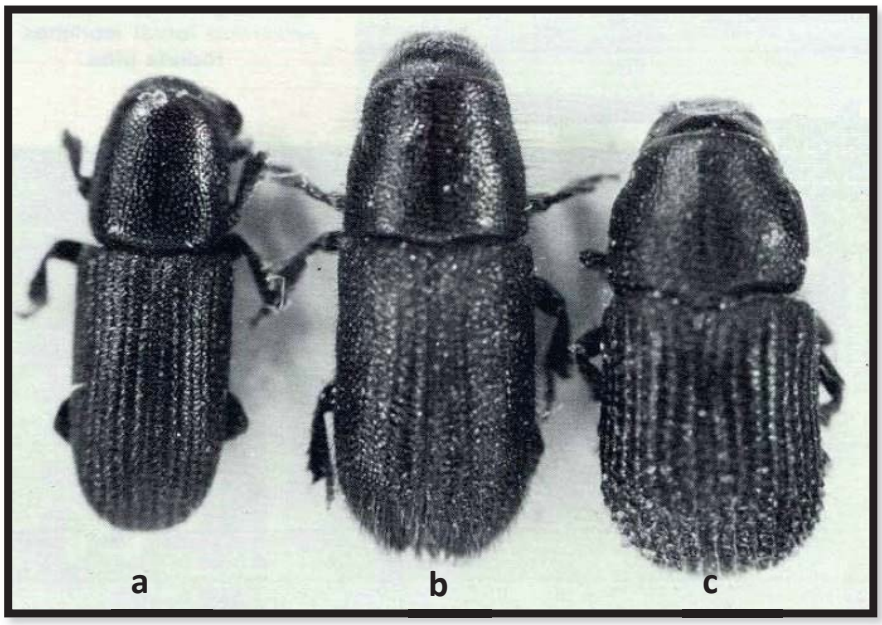


Figure 1.5: Three different bark beetle species which may be mistaken for each other; a) *Hylastes ater*, b) *Hylurgus ligniperda*, c) *Pachycotes peregrinus*. Source: www.nzffa.org.nz, with permission.

Sex Differentiation

The males and females of *H. ligniperda* can be distinguished by acoustic signals produced by the males when disturbed. However, this technique is not always reliable, as the beetles sometimes remain silent even when disturbed or handled (Mausel et al., 2007).

The males have specific granules along the second interstrial space and the females have a slightly impressed second interstrial space in the elytral declivity

(Grune, 1979). However, these morphological characters are difficult to recognise when observed under high magnification (Liu, Flint, & Seybold, 2008).

The other way to differentiate male and female of *H. ligniperda* is by observing morphological characteristics of the genitalia. The posterior margin of the 6th tergite of the male is sclerotized and has more bends and turns than that of the female (Figure 1.6 a & b). The length of the 6th (last) abdominal tergite of the female is equal to the combined length of the 6th and 7th abdominal tergites of the male. The 7th tergite is sealed by the 6th tergite in female (Figure 1.6 c & d). Furthermore, the 7th tergite of the male is highly pubescent whereas the concealed 7th tergite of the female is not pubescent (Figure 1.6 c & d). It is suspected that the distinct sclerotization on the second last abdominal tergite of male helps in the stridulation, thereby producing a unique sound different to that of the female (Liu, et al., 2008).

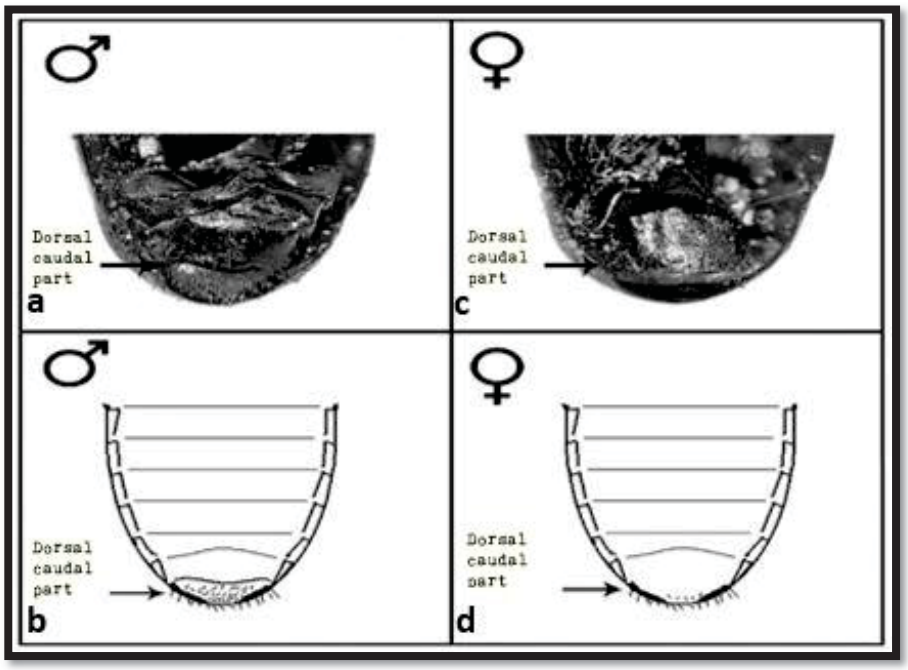


Figure 1.6: The dorsal caudal part of the abdomen of *H. ligniperda*; a) and b) adult male, c) and d) adult female. Source: (Liu, et al., 2008), with permission.

Life Cycle

Hylurgus ligniperda is a monogamous species with a 1:1 male to female sex ratio (Lanfranco, Ide, Ruiz, Peredo, & Vives, 2001). The female enters the host tree through the bark and makes a short entrance tunnel including a slanted or inclined nuptial chamber in the innermost bark (phloem), usually near the base of the stem or in large exposed roots/stumps of recently cut trees buried or touching the soil (Ciesla, 1988). The female selects a particular male out of many males competing outside to enter the

tunnel. The chosen male will then mate with the female in the nuptial chamber. From the nuptial chamber, the female normally starts building long single egg galleries which usually are parallel with the wood grain. The egg galleries can be as long as 1 m long, and might be sinuous, spiral or circular in shape and might even double-back on themselves (Dumouchel & Palisek, 2002). Most of the time, the female is found digging at the end of the tunnel whereas the male assists the female in gallery construction by clearing the frass out of the tunnel (Browne, 1968). Sometimes, the male can be found closer to the nuptial chamber making short feeding tunnels to the egg galleries (Browne, 1968).

A female can lay up to 500 eggs (Eglitis, 2001). The eggs are ovoid and pearly white (Figure 1.7 a) (Clare & George, 2016). The eggs are laid in individual notches made on the walls of the egg gallery and are covered with frass (Figure 1.7 b). Once the first batch of eggs is laid on the notches of the egg gallery, the female starts digging for about 100-200 mm in order to extend the gallery and lay more eggs. This process continues until the egg laying period of the female is over, which may last for up to six weeks (Liu et al., 2007). The females feed between egg laying. During summer, it takes about two weeks for the eggs to hatch (Kimoto & Duthie-Holt, 2004). The larval gallery is normally at a right angle to the egg gallery. However, this arrangement turns into a random pattern and does not follow a distinctive pattern as the larvae mature (Figure 1.7 c). The mature larvae are found close to nuptial chamber followed by medium to smaller-sized larvae at successive intervals along the tunnel (Dumouchel & Palisek, 2002).

The fully-grown larvae are white, legless and with a C-shaped body. The head of the larva is yellowish to brown in colour, with two dark circular protuberances on the front portion of the head just above the jaws (Figure 1.7 d). The smaller larvae have transparent body wall and the reddish content of the guts is visible (Bain, 1977). There are four larval instars. After the completion of the larval development period, the fully-grown larva pupates at the end of their tunnels. The pupae is yellowish in color (Figure 1.7 e). The pupal stage may last from one to two weeks. A complete colony is made up of single, longitudinal or normally slanted egg galleries including long individual larval feeding tunnels turning into pupal cells (Figure 1.7 f) (Brown & Laurie, 1968). It takes about 2-3 weeks for the pupae to turn into a matured adult. Sometimes, the males and females together make galleries extended vertically downwards in order to clear the debris. In such situations, the females may bore extra holes, although the members of

other species such as *Hylastes ater* may alter the gallery patterns. The modification of the shape of the egg galleries is also to protect the brood from predators or specific tree defence mechanisms (Rudnev & Kozak, 1974). In trees with thin bark, the galleries are located close to the sapwood and the larvae pupate in the wood. However, in the trees with thick bark, the pupation occurs in between the bark and the wood (Rudnev & Kozak, 1974).

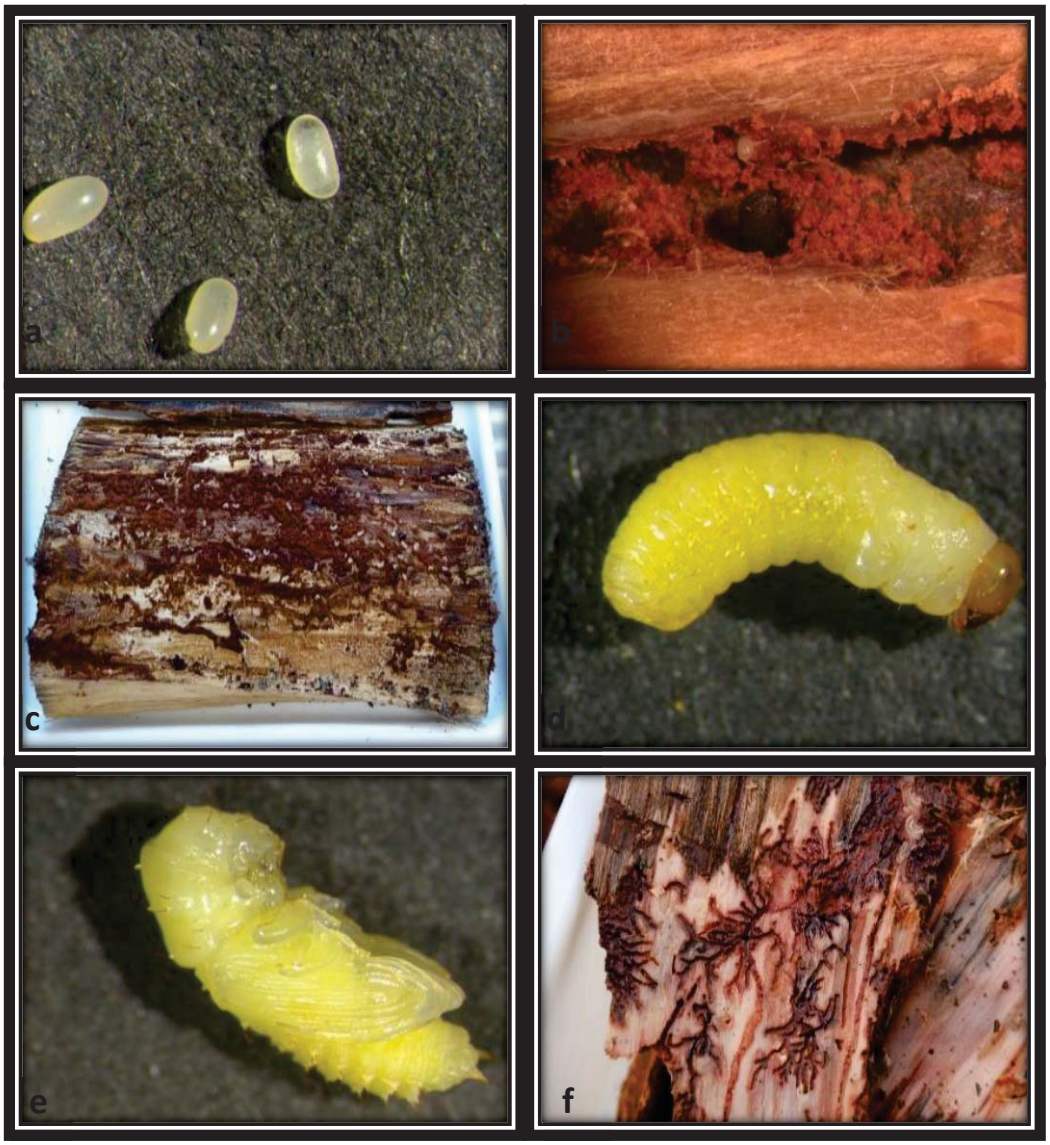


Figure 1.7: *Hylurgus ligniperda*, stages of the life cycle; a) eggs, b) freshly laid eggs covered with frass, c) larval galleries d) grown up larvae, e) lateral view of the pupae, f) gallery made by *H. ligniperda* males and females.

The adults frequently overwinter in aggregation. In New Zealand, it has been reported that the development from the initiation of brood galleries to the appearance of

new adults takes about 10-11 weeks (Bain, 1977). In southern France, the life cycle from egg to adult is completed in 45 days at 25^{o}C (Tribe, 1991a).

Associations with Fungal Pathogens

Fungal species like *Leptographium truncatum*, *Leptographium procerum*, *Ceratocystiopsis minuta*, and *Ophiostoma galeiformis* have been found in association with *H. ligniperda* (Zhou, Beer, Ahumada, Wingfield, & Wingfield, 2004). *L. procerum* has been involved in white pine (*Pinus strobus*) root decline disease in United States (Matusick III, 2010) whereas *L. truncatum* has been reported from Canada (Harrington, 1988). These two fungal pathogens are not extremely infectious (Hausner et al., 2005; S. Reay, Thwaites, & Farrell, 2005; S.D. Reay, Walsh, Ram, & Farrell, 2002). However, in association with bark beetles the fungi can cause substantial pine tree decline (Romon, Zhou, Iturrondobeitia, Wingfield, & Goldarazena, 2007). The spread of *H. ligniperda* in western USA has been a serious concern, as this pest can vector *L. procerum* and increase its distribution to new conifer plantation sites (Viiri, 2004). Both *L. truncatum* and *L. procerum* have been isolated from New Zealand populations of *H. ligniperda* (Ray, Thwaites, & Farrell, 2006). In addition, adults of *H. ligniperda* overwintering gregariously in the bark of larger roots or root collars can cause cross-contamination with other fungal spores. In South Africa, fungi like *Leptographium serpens, Leptographium procerum, Ophiostoma ips, Ophiostoma galeiformis, leptographium lundbergii,* and *Ophiostoma piceae* are found to have an association with *H. ligniperda* in pine plantations (Zhou, Beer, Wingfield, & Wingfield, 2001). There is a major concern that if *H. ligniperda* could reach the conifer forests of western USA, it can become a vector of *Ophiostoma wageneri,* a virulent fungal pathogen that causes black stain root disease in western USA (Romon, et al., 2007). If this happens, the forest dynamics would change dramatically and the beetles would become a serious pest in western USA.

Sound Production in *H. ligniperda*

Hylurgus ligniperda males have been observed to produce distinct audible chirps (Mausel, et al., 2007). However, there has not been any study describing the physical characteristics and nature of the acoustic signals produced by this species or the behavioural function of such signals in intraspecific communication.

Morphology of the Stridulatory Apparatus in *H. ligniperda*

The pars stridens or file of *H. ligniperda* males has sharp distinct serrated transverse ridges or teeth along the sutural margin and tip of the left elytron (Figure 1.8 a). In females, these structures are reduced and are blunt (Figure 1.8 b).

Males have more teeth on pars stridens, which are also longer than those of the female. As a result, the males produce an audible sound when these structures are scrapped against the sclerotized pegs (plectrum or scraper) (Figure 1.8 c & d) present on the 7th segment of the abdominal tergite, while the females just seem to produce clicks.

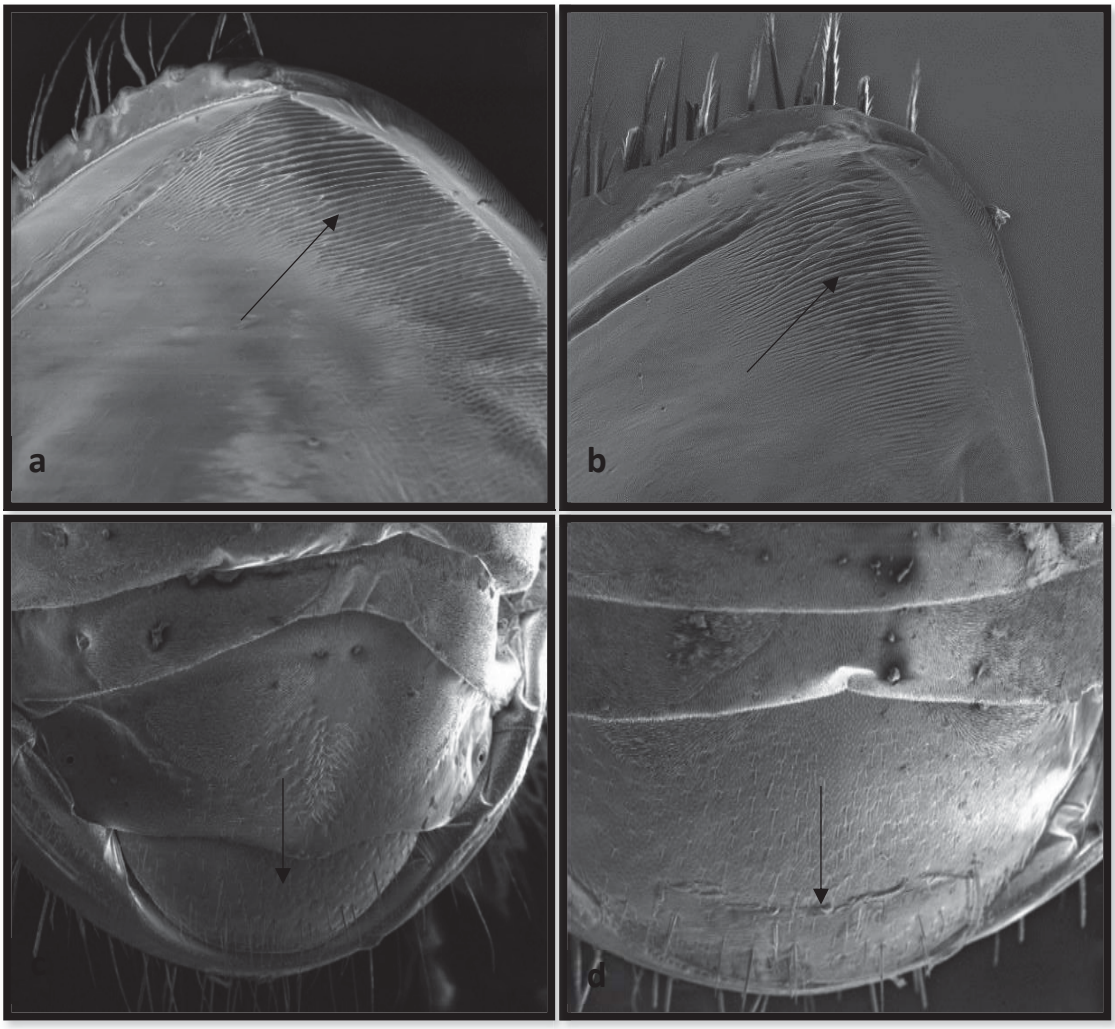


Figure 1.8: The stridulatory apparatus of male and female *H. ligniperda*; a) serrated transverse ridges or teeth along the sutural margin and tip of the male's left elytron, b) serrated transverse ridges or teeth along the sutural margin and tip of the female's left elytron, c) plectrum or scrapper on 7th segment of the male abdominal tergite, d) plectrum or scraper on 7th segment of the female abdominal tergite. Photo by: Ria Rebstock and Adriana Najar-Rodriguez (Plant & Food Research Ltd).

Research Objectives

The golden haired bark beetle *Hylurgus ligniperda* is one of the major species infesting New Zealand pine logs (Kerr, 2010). Introduced bark beetle species such as *H. ligniperda* can establish very well into a new area because of the lack of specific natural enemies or host defences (Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004). Therefore, many countries have increasingly strict quarantine regulations on the import and export of logs and timber, which require the use of various treatments such as fumigation to check the risk of further biological invasions (Zhang, Epenhuijsen, Brash, & Hosking, 2004).

The New Zealand log trade exports in 2014 were valued at $ 2.6 billion, which accounted for 6.9 percent of New Zealand's primary sector earnings. Of the total log exports, about 71.8 percent of logs (*Pinus radiata*) were exported to China (Ministry of Primary Industries, 2015). To meet the requirements of the importing countries, all the logs exported from New Zealand must undergo fumigation in order to confirm that they are free of phytosanitary insects (Somerfield et al., 2013).

Fumigation with methyl bromide and other fumigants is the only effective treatments to provide the quarantine security at the ports. Methyl bromide is a colourless and odourless gas which has the ability to deplete ozone layer and has many toxic impacts on human and animal health (Fields & White, 2002). The methyl bromide use in New Zealand increased from 270 tonnes in 2009 to 406 tonnes in 2010 (New Zealand Ministry for the Environment, 2011). The public and the New Zealand Government have been concerned regarding the use of methyl bromide to fumigate the exporting pines. For the safety reasons, the rules on the use of methyl bromide are becoming increasingly strict, leading to the creation of buffer zones around fumigation sites (New Zealand Environmental Protection Authority, 2011). Furthermore, by 2020 the requirement of destruction or recapture of methyl bromide will be implemented in order to prevent the escape of this gas into the atmosphere. Overall, because of the hazardous impact on human and animal health, the use of such treatments is becoming socially unacceptable (Lanfranco, Ide, & Peredo, 2004). Furthermore, quarantine measures on their own do not solve the issues with this pest. If populations of *H. ligniperda* could be reduced at the source, such as in the forests, then the use of fumigation could be decreased (Mausel et al., 2006).

Such quarantine problems associated with *H. ligniperda* have led to this opportunity to study its communication and behavioural characteristics. The use of acoustics has already been found to effectively deter some bark beetle species (*Dendroctonus frontalis* and *D. brevicomis*) from entering pine logs. In the future, this study could initiate the development of robust monitoring and prevention techniques in order to check and control bark beetle infestation affecting pine forest industry. In the past, most of the studies done on the bark beetles were focused on chemical-based control measures (Pranamornkith et al., 2014; Somerfield, et al., 2013) and none of the studies investigated sound communication or its relationship with different behavioural contexts. Therefore, this study aims to open a pathway towards finding possible alternative measures, which can replace the use of harmful chemicals like methyl bromide in future.

The thesis addresses the following objectives:

1. To characterize the temporal and spectral features of the airborne sounds produced by *H. ligniperda* under different ecological scenarios.
2. To relate sound production to the behaviour of *H. ligniperda* under different ecological scenarios, and to see if sound plays a communication role in *H. ligniperda*

Common Terms and Definitions Used in This Study

For Males

The individual component of sounds produced by the impact of the scraper (plectrum) being rubbed against one ridge or tooth of the file (pars stridens) is called a toothstrike, or pulse (Masters, 1980) A single pass of the scraper over all or part of the file is called a chirp, or burst (Fleming, et al., 2013; Masters, 1980). Chirp has been previously defined as a train of stridulatory pulses or toothstrikes (Barr, 1969). Between each chirp there is a pause, as the movement of the scraper over the file is disengaged causing a period of silence. This pause before the start of another chirp is called the interchirp interval. The chirps are classified as simple and interrupted. Simple chirps have one series of regularly spaced toothstrikes, whereas interrupted chirps have two or more components of interruption with short periods of silence (Ryker & Rudinsky, 1976b). Although there is a clear distinction between the two types of chirps, the duration of silence is not standardized, especially in terms of interrupted chirps. The interrupted

chirps have been recently re-defined as having chirp components with a toothstrike interval of at least 3 ms and containing two or more toothstrikes (Fleming, et al., 2013).

In this study, since the nature of sound production of *H. ligniperda* differed from that of the previously studied bark beetle species, I decided to modify the definition of simple and interrupted chirps based on the temporal analysis (using Raven Pro 1.4) of more than fifty sound recordings from about 20 *H. ligniperda* males. During analysis, two distinct types of chirps were clearly observed: chirps with many toothstikes (normally more than 20) and chirps with fewer toothstrikes (normally less than 10). Therefore, to standardize them, I defined *simple chirps* as those having more than ten toothstrikes and *interrupted chirps* as those having 2 to 10 toothstrikes. This classification was not made based on the time duration of each chirp, as the pace of toothstrike production varied depending upon the behavioural situation of sound production.

Other terminology I have used in this thesis includes: *chirp duration*, i.e. the time taken to produce a single chirp (either simple or interrupted); *toothstrike rate*, calculated by dividing the number of toothstrikes produced in a chirp by the duration of the entire chirp; and *toothstrike duration*, calculated as the time taken to produce a single toothstrike within a chirp.

For Females

As expected, given the variation in the sound producing organs between *H. ligniperda* males and females, the nature of sound production in the females is quite different from that of the males. The females only produce click-like sound (refered to as '*click*' throughout this thesis). So, the term 'chirp' is not used in the case of females. The 'click' is similar to an individual toothstrike of a male. However, the click duration is much shorter than the toothstrike of a male. The *interclick interval* is the time gap between two successive clicks produced. *Click duration* is the time taken to produce an individual click.

Chapter 2: Methods and Methodology

Bark Beetles (*Hylurgus ligniperda*) Rearing

All the experiments described below were conducted in the Entomology laboratory at Plant & Food Research, Palmerston North, New Zealand. In order to have enough insects for the experiments, we first isolated individual larvae of *H. ligniperda* which originated from insect colonies held in Plant & Food Mt Albert Research Centre (Auckland).

The individual larvae were put into single compartments of 24 well-Tissue Culture Plates (Biofil) with about 1gm of artificial diet each (Figure 2.1 a-d). The larvae were transferred into new tissue culture plates every six days until adulthood to avoid the possibility of mould growing on the diet. By keeping larvae in separate wells until adulthood, we secured provision of virgin adults for our experiments. Table 2.1 shows the list of ingredients used for making the artificial diet for the larvae.

Larvae were reared in a temperature-controlled room at 24.3ºC and 50-60% relative humidity. The room was kept in complete darkness.

Once the virgin adults reached full maturity, which was evident by their change in colour from brown to black, they were sexed by gently squeezing their thorax with the help of the forceps tips for about 10-15 seconds and noting the absence or presence of unique gender-audible chirps (Figure 2.2 a).

The sound production by beetles was tested with the help of a microphone (electrate condenser, analog omni-53DB having omnidirection feature with frequency range 100 Hz to 100 kHz) (Figure 2.2 b) connected to a sound recording device (TASCAM DR-05, Linear PCM Recorder, Sampling frequency 96.0 kHz, 24-bit WAV format) (Figure 2.2 c). Males produced longer and distinct chirps whereas females just produced “click-like” sounds.

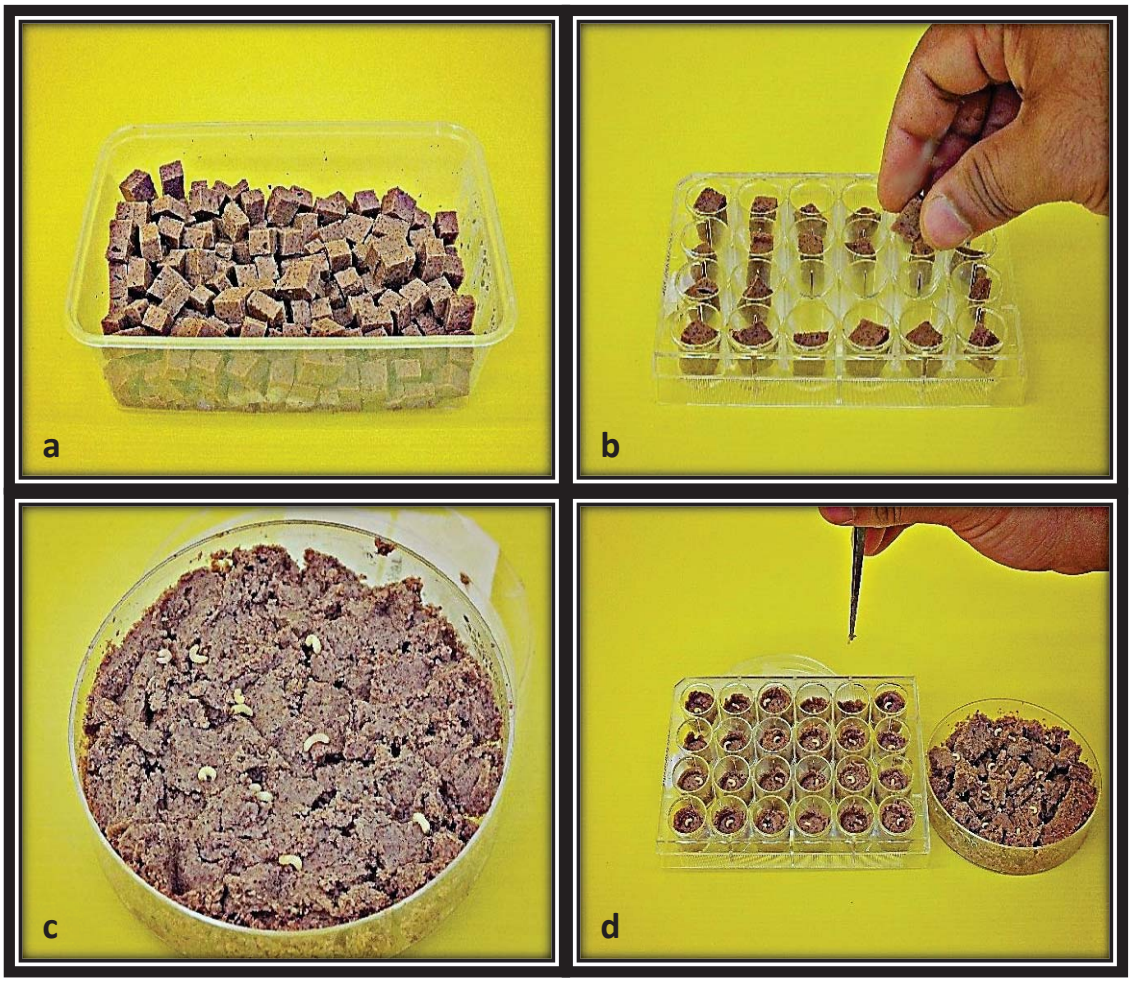


Figure 2.1: Feeding the larvae of *Hylurgus ligniperda* with an artificial diet; a) artificial diet cut into square shapes b) putting artificial diet into Tissue Culture Plates, c) larvae obtained from Plant & Food lab, Mt Albert Research Centre (Auckland), d) transferring larvae into the Tissue Culture Plates with artificial diet.

Experimental Trials

Different behavioural scenarios were tested, including: I) Mating: a virgin male plus a virgin female; II) Competition: a virgin male plus a virgin female in the presence of a second virgin male introduced into the system after the first male; III) Male Territoriality: a virgin male in the presence of two other virgin males; IV) Female Territoriality: a virgin female in the presence of two other virgin females; V) Colony: three virgin males in the presence of three virgin females. As beetles do not produce sounds if alone or undisturbed, our positive control was a 'distress' treatment, whereby a single female or male were forced to produce sounds by physically disturbing them or placing them in an 'out of context' situation. Each treatment was replicated 10 times, with new insects used each time.

Table 2.1: Artificial diet used for *Hylurgus ligniperda* larvae (modified huhu grub (*Prionoplus reticularis*) diet, from Barrington, Logan, & Connolly, (2015).

Ingredients	**Weight (gm) (per 3kg of diet)**	**Percentage (100%)**
Wheat germ	72.9	2.37
Wesson's Salt	24.3	0.8
Agar	97.5	3.18
Cellulose Powder	108	3.5
Sawdust	146.1	4.76
Yeast	72.9	2.37
Casein	72.9	2.37
Sugar	60.9	1.98
Water	2310	75.3
Mix and autoclave all ingredients at 121^{o}C for 25 minutes		
Mould inhibitor	43.8	1.4
Start mixing autoclaved diet and add mould inhibitor		
Ascorbic Acid	10.5	0.3
Vitamin Solution	48.6	1.6
Add ascorbic acid and vitamin solution once diet has cooled to 60^{o}C.Then wrap, seal and store at 4^{o}C for later use.		

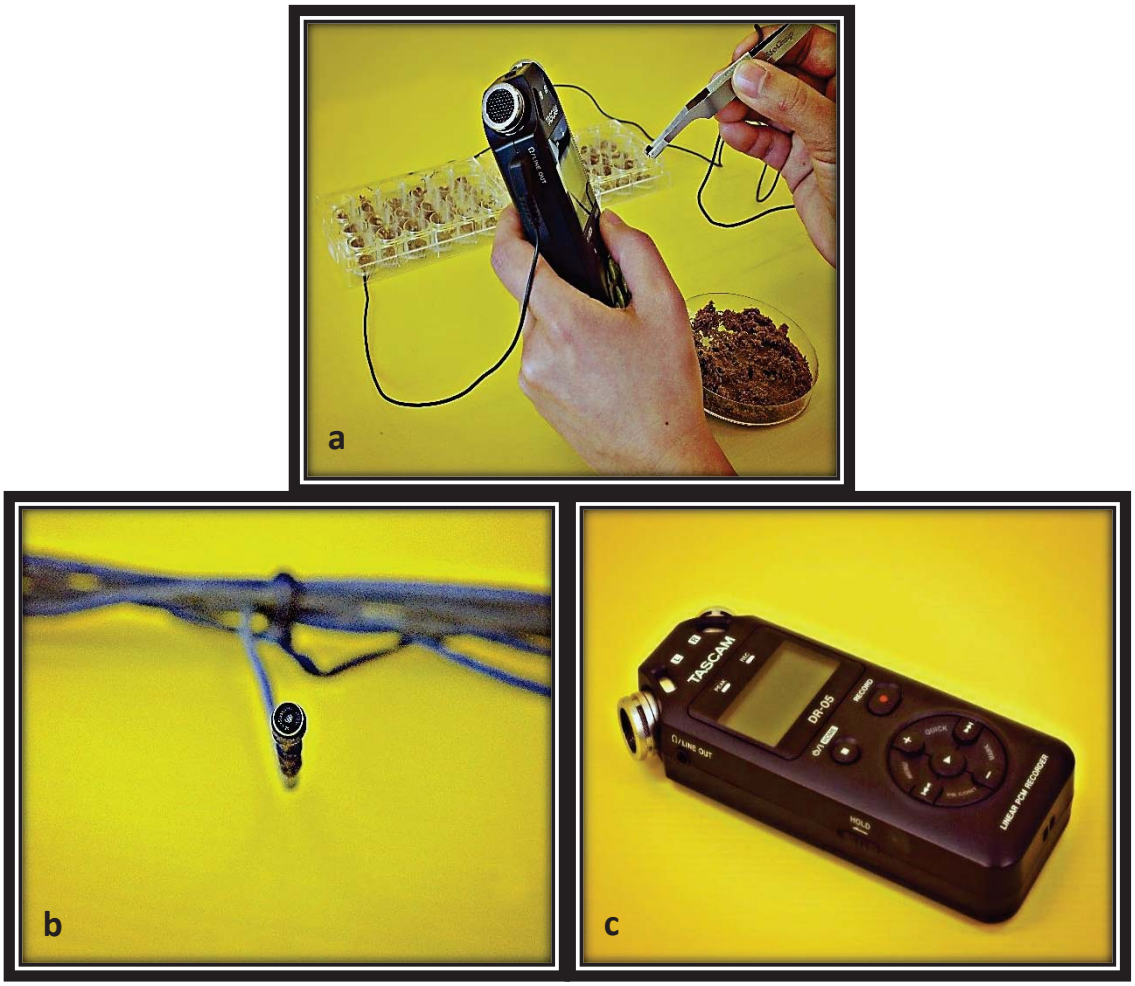


Figure 2.2: Sexing adults of *Hylurgus ligniperda*; a) noting the presence or absence of unique gender audible chirps. The microphone is attached at the tip of the forceps used for holding the beetles by the thorax, b) a microphone (electrate condenser, analog omni-53DB having omnidirection feature with frequency range 100Hz to 100 kHz, c) sound recording device (TASCAM DR-05, Linear PCM Recorder).

Experimental Set-up

For sound recording, virgin adults were placed into a 'cambium sandwich' made from a piece of freshly cut tree cambium placed in between two plexiglass sheets.

The cambium was obtained from pine trees (*Pinus radiata*) located in the Gordon Kear Forest (40^{o} 30' 18'' S, 175^{o} 35' 6'' E) (Figure 2.3). The trees were cut down and the cambium with bark required for our experiments was peeled off using tools such as a spade, bark scrappers and bark peelers (Figure 2.4). The cambium thus extracted (still with bark) was immediately brought back into the lab and cut into small rectangular pieces 10 cm × 8 cm in size (Figure 2.5 a). These pieces were wrapped with a plastic film and placed into a -20^{o}C freezer for future use. The cambium was kept in

the freezer to avoid the mould growth and to suppress the presence of other insects or pathogens.

For making an experimental chamber, the prepared piece of cambium with bark was defrosted and the bark peeled off (Figure 2.5 a-e). A small rectangular section 3 cm × 1.5 cm was cut out of the cambium piece for experimental setup (Figure 2.5 f). Prepared cambium was placed in between two plexiglass plates (21 cm × 12 cm) like a 'sandwich'. Another small rectangular plexiglass plate (10 cm × 8 cm) with interior cut 4.5 cm × 3 cm was placed over the cambium piece and in between the two bigger plexiglass plates to create enough moving space for the beetles around the cambium. The whole set up was clipped together with foldback clips from three sides (Figure 2.6 a). Two microphones (electrate condenser, analog omni-53DB having omnidirection features with a frequency range between 100 Hz to 100 kHz) were then placed 0.5 cm apart right where the small rectangular cut was made, by drilling two small holes in one of the plexiglass plates of the cambium sandwich (Figure 2.6 b).

Figure 2.3: The location of Gordon Kear Forest (Google Earth).

Figure 2.4: Extraction of cambium from a pine tree; a) pine tree (*Pinus radiata*) chopped down, b) scrapping the bark using a bark scrapper to remove unwanted dirt, c) making the edges on a log of pine tree for easy extraction of cambium, d) extraction of cambium with bark using a spade.

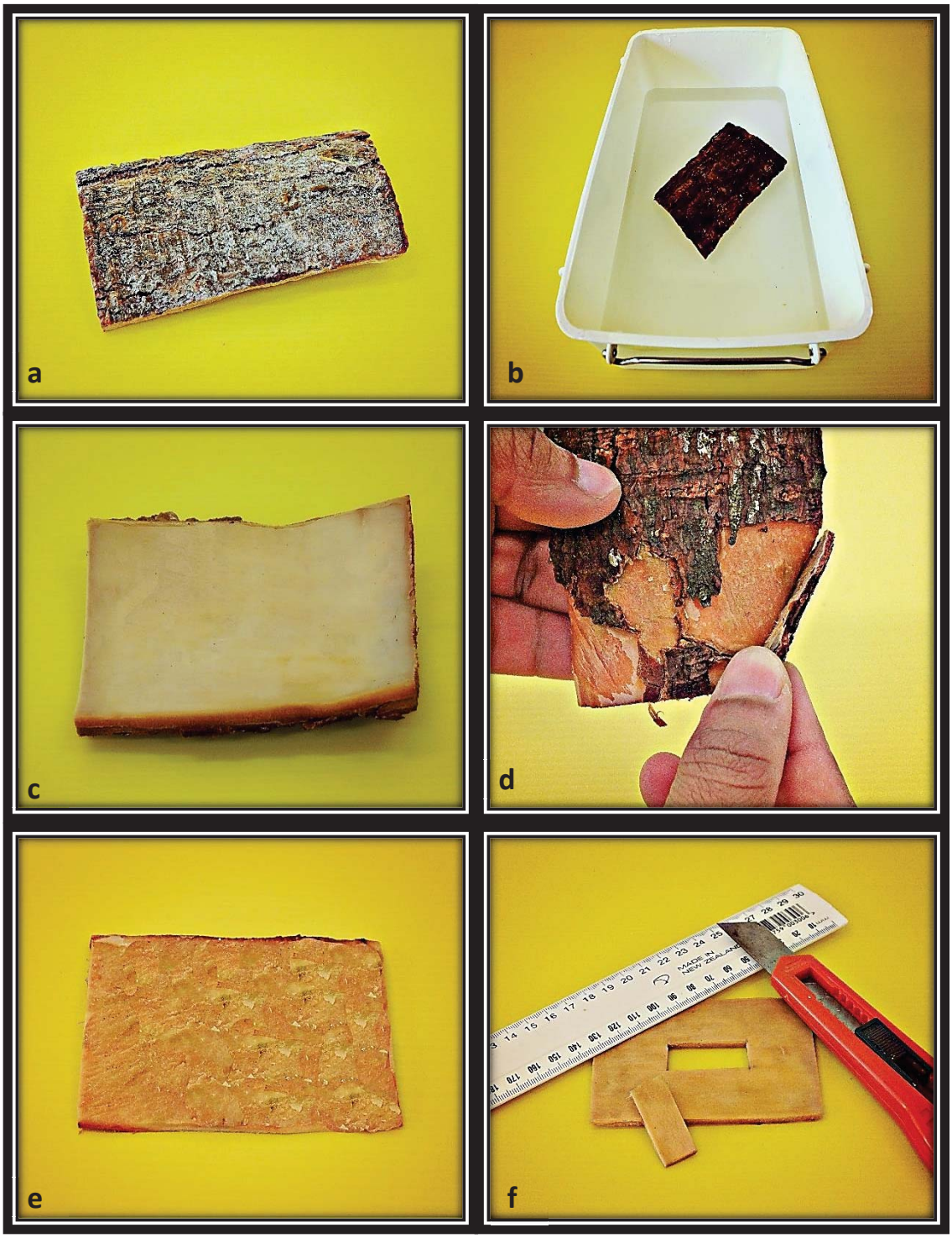


Figure 2.5: Preparation of cambium for the experiment; a) frozen cambium with bark (size 10 cm × 8 cm), b) defrosting the frozen cambium with bark in water, c) defrosted cambium with bark, d) peeling the bark from the cambium, e) prepared cambium piece 10 cm × 8 cm, f) small rectangular section 3 cm × 1.5 cm cut out of the cambium piece for experimental setup.

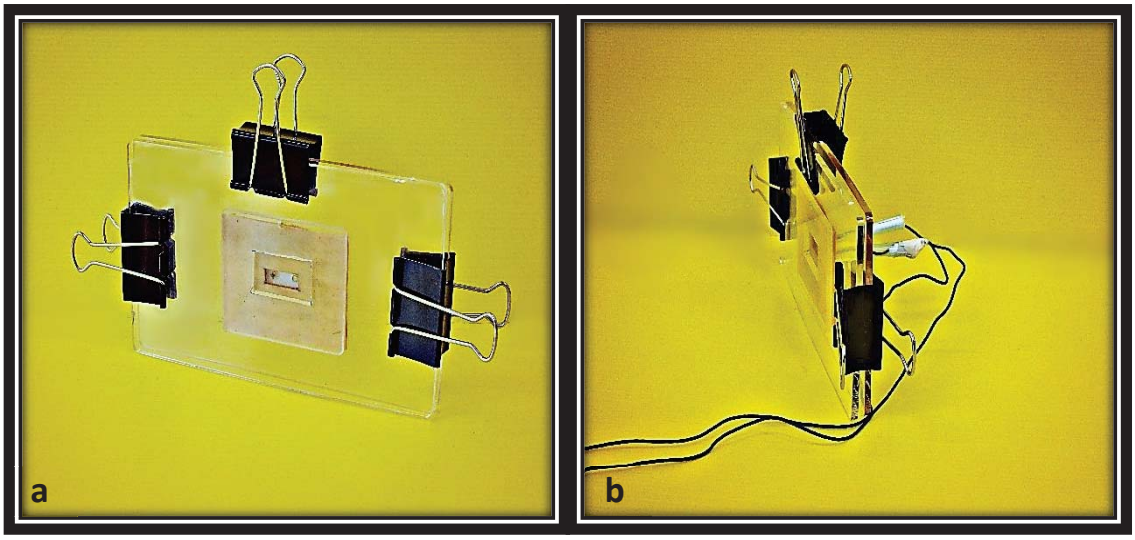


Figure 2.6: Cambium chamber set up; a) cambium piece (10 cm × 8 cm, with 3 cm × 1.5 cm window) and small plexiglass plate (10 cm × 8 cm, with 4.5 cm × 3 cm window) placed between two larger plexiglass plates (21cm × 12 cm) and clipped with foldback clips from three sides, b) two microphones (electrate condenser, analog omni-53DB having omnidirection feature with frequency range 100Hz to 100 kHz) connected by drilling two small holes (0.5 cm apart) in one of the plexiglass plates.

One of the microphones was then connected to a sound recorder (TASCAM DR-05, Linear PCM Recorder) (Figure 2.7 a) whereas the other one was connected to a video camera (Panasonic High Definition-4K Video Camera, Model No: HC-VX870M) (Figure 2.7 b). The camera was set on a mini tripod stand in front of the prepared cambium at a distance of about 20 cm (Figure 2.7 c).

The beetles were placed inside the cambium sandwich through one of the holes where the microphone was connected. To allow identification of individual beetles, each beetle was marked using a Liquid Paper correction fluid and a ‘Sharpie’ permanent marker pen of different colours (Red/Green/Purple) (Figure 2.8).

Figure 2.7: Recording instruments used for the experiment; a) sound recorder (TASCAM DR-05, Linear PCM Recorder), b) video camera (Panasonic High Definition-4K Video Camera, Model No: HC-VX870M), c) camera set-up in front of the cambium sandwich.

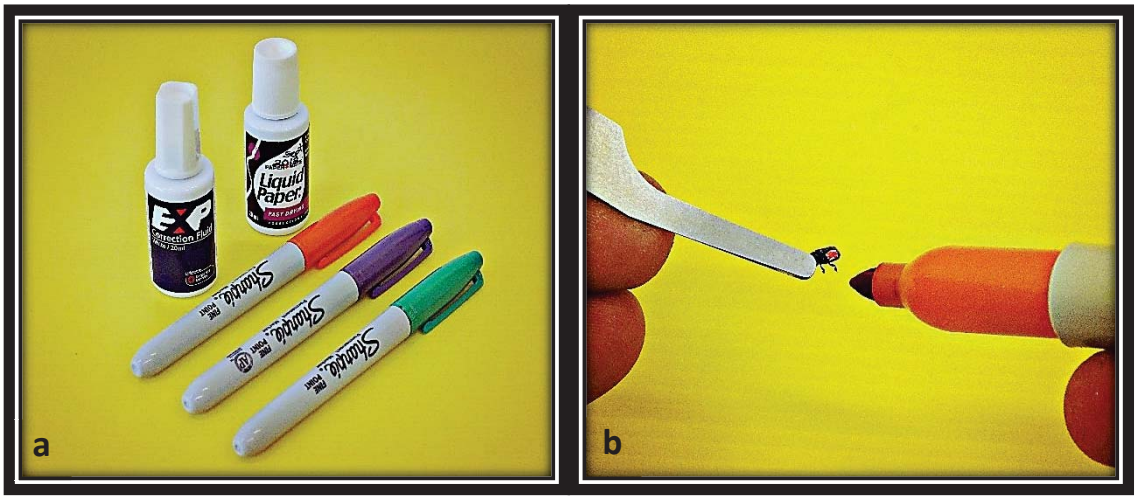


Figure 2.8: Marking the beetles for easy identification; a) Liquid Paper correction fluid and 'Sharpie' permanent marker pens used for marking, b) marking process.

To record the sounds of distressed males and females a different set up to that described above was made. The microphone (electrate condenser, analog omni-53DB having omnidirection feature with frequency range 100 Hz to 100 kHz) was modified (just the physical structure) by placing it inside a teflon tube (5.5 cm long, 0.3 cm internal diameter) ending with a pipette tip. This set up was designed to stop the beetles from scratching the microphones and thus avoid recording unwanted/unnecessary secondary sounds. The Teflon tube was connected to the front part of the microphone and individual beetles were then placed inside the tube and their sounds were recorded (Figure 2.9).

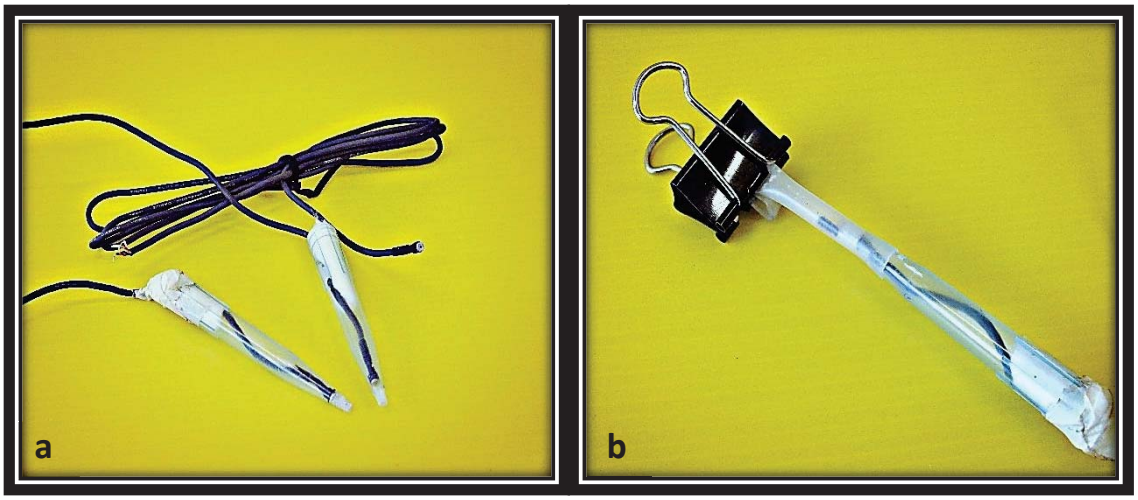


Figure 2.9: Recording setup for distress trial; a) microphone (electrate condenser, analog omni-53DB having omnidirection feature with frequency range 100 Hz to 100 kHz) modified by putting it inside the pipette tip tube, b) modified microphone connected with a rubber tube and clipped with foldback clip. The virgin *Hylurgus ligniperda* adult is put inside the tube for recording sound.

Video recordings were not performed for the distress trials as the beetles were outside their normal habitat and did not show normal behaviour. Regardless of the set-up, all sounds were recorded inside a sound-proof chamber (60 cm × 48 cm) in order to minimise the background noise. Complete darkness was maintained inside the chamber. However, the camera internal low light (lamp) setting with slow shutter speed mode was turned on for maintaining the visibility of video recordings. The recording room was maintained at 25^{o}C.

Sound and Video Recording

For sound recordings, the sampling frequency of the sound recorder TASCAM HD-P2 was set to 96 kHz and a sample width of 24 bits per sample (Figure 2.10). Every recording was saved as '.wav' file. The settings of the camera (Panasonic High

Definition- 4K Video Camera, Model No: HC-VX870M) were set to manual mode with the manual focus system on. The video was recorded in the AVCHD format whereas the recording mode was set to 1080/50p. The microphone amplification setting was set on automatic mode.

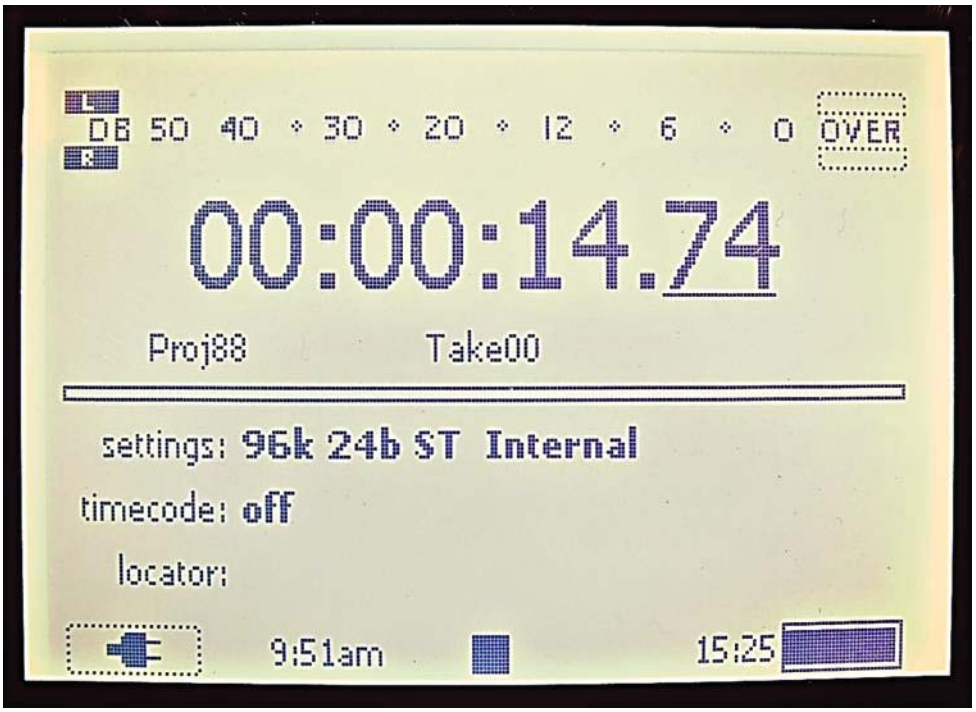


Figure 2.10: The screenshot of the sound recorder TASCAM HD-P2 showing sampling frequency and sample width.

At the beginning of the recording process, sound and video recorders were turned on at the same time. A loud human voice saying 'START' was recorded after pressing the 'RECORD' bottom in both recorders. This was done so that the behavioural activities of beetles recorded in the video camera could be exactly matched in time with sounds recorded with the sound recorder. Sound and video were recorded for 10 hours. After completion of the recording time, the sound and video files were transferred onto a computer. Except for the distress treatment (as there was no video recordings), the sound and video files were opened together using a video editing software NCH VideoPad software (Version 4.8), and brought into the timeline of the software. The timing of the audio recordings were matched exactly (using recorded "START" sound and by observing the sound waves) to the video timeline as shown in the Figure 2.11.

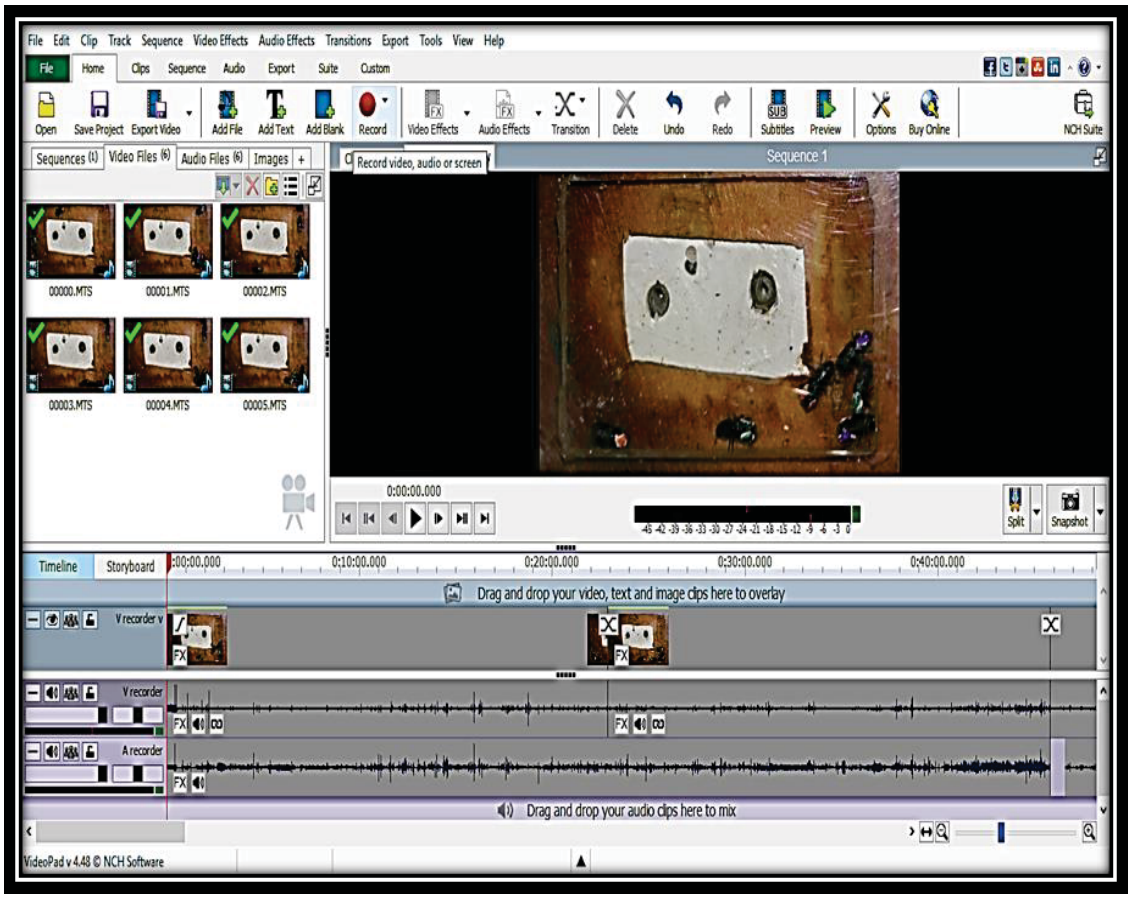


Figure 2.11: Matching the timeline of audio and video recordings in order to pinpoint the behavioural activities during specific period of sound production.

To allocate the recorded sounds to specific behaviours, the video timeline was observed carefully and the duration was marked in [Hours (HH): Minutes (MM): Seconds (SS): Milliseconds (MS)] format. Marked durations were matched with the original sound files obtained from the sound recording device, TASCAM HD-P2 and those specific files were brought into RAVEN PRO 1.4 (Cornell laboratory of Ornithology 2011, Ithaca, NY, USA), a sound-analysis software that allows both temporal and spectral analysis of the sounds (Figure 2.12).

Sound Analysis in Raven

For all replicates, a sampling duration of 1 minute was randomly chosen.

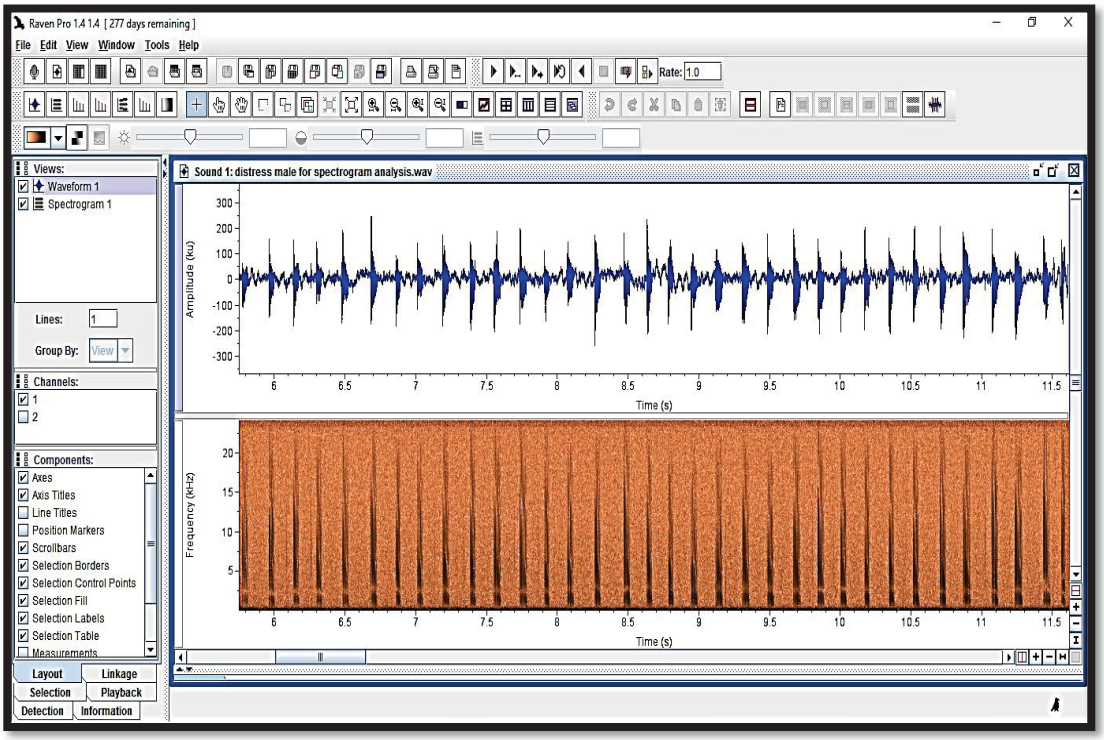


Figure 2.12: Screenshot of Raven Pro 1.4 showing a representative waveform and spectral pattern of sounds produced by a distressed *Hylurgus ligniperda* virgin male.

Temporal Analysis

In the case of male sounds, the parameters obtained for each one minute recording included: the number of chirps, interchirp interval, toothstrike per chirp, chirp duration (in seconds), and toothstrike rate (number of toothstrikes/second). The chirps were classified as: a) simple – chirps having more than 10 toothstrikes or b) interrupted – chirps having 10 or less toothstrikes (Figure 2.13 a).

For females, as they do not produce distinct audible chirps like males, the parameters analysed were slightly different. The sounds produced by females are click-like sounds ('click(s)'), and the parameters measured were: the total number of clicks per minute, the duration of an individual click, inter-click interval and click strike rate (Figure 2.13 b).

In order to measure the correct number of toothstrikes/clicks, manual counting (by visual observation of the waveforms) along with careful listening to the recordings (using 'Loop Playing' tool) were performed. All parameters measured from the sound files were recorded in a Microsoft Excel spreadsheet for later statistical analysis.

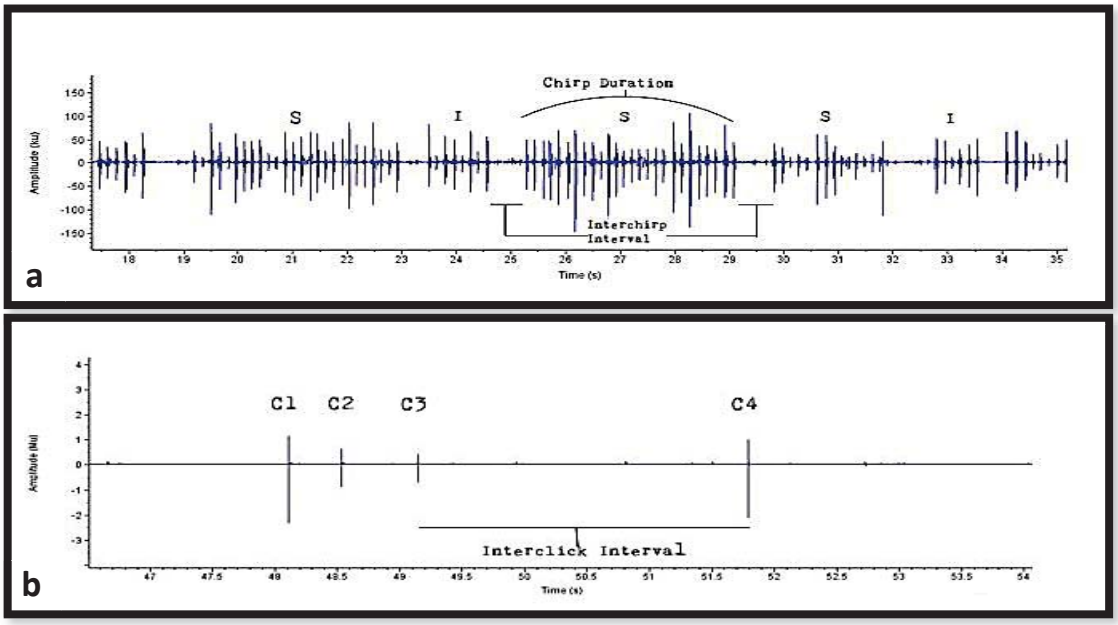


Figure 2.13: Example of the temporal sound analysis; a) waveform of a *Hylurgus ligniperda* male sound, 'S' represents simple chirp and 'I' represents interrupted chirp; b) waveform of a *Hylurgus ligniperda* female sound, C1, C2, C3 and C4 represent different clicks produced by the female.

Spectral Analysis

Toothstrike duration (for males), click duration (for females), maximum/minimum amplitude, high/low frequency and peak frequency were measured from the sound recordings of both males and females. In order to obtain these values, predefined measurements were selected from the Raven's 'Choose Measurements' dialog box (Figure 2.14). The manual selection tool was used to draw a rectangular boarder around the randomly selected toothstrikes/clicks, delimiting time (seconds) on the horizontal edges and frequency (kHz) in the vertical edges of the selection rectangle. On committing each selection, the values for each selected measurements were automatically calculated and added to a 'Selection Table' (Figure 2.15 a-c). All these parameters were recorded in a Microsoft Excel spreadsheet for future statistical analysis.

The temporal analysis is difficult to perform in situations when more than one individual of the same sex were present (particularly for *H. ligniperda* males), because in many cases, sound waves originated from different individuals would overlap. This could lead to substantial errors when counting the number of toothstrikes/clicks produced by a particular individual. Similarly, the measurements of parameters such as chirp duration and inter-chirp interval could be affected. Therefore, the temporal

analysis was only performed for the distress and the mating experiments, while spectral analysis was done for all experiments.

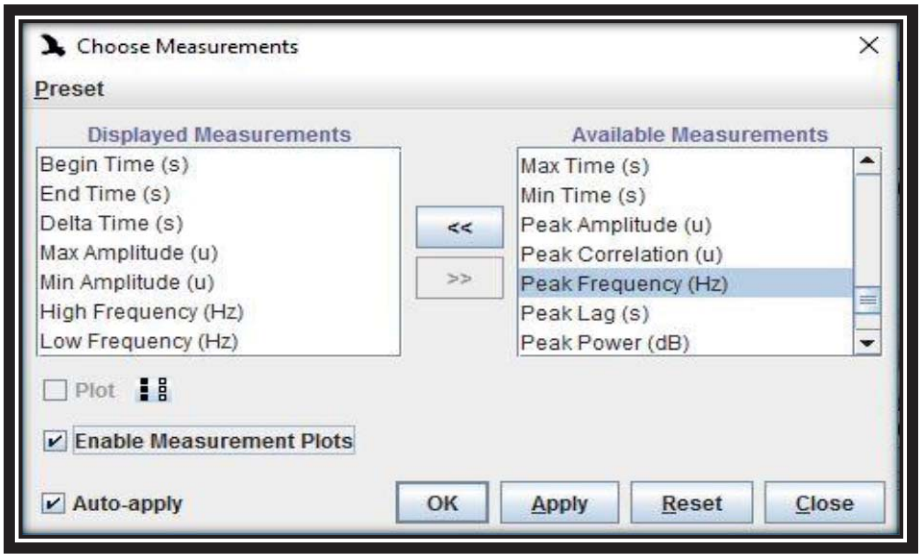


Figure 2.14: Sample of some of the predefined measurement options (used in this study) available in Raven.

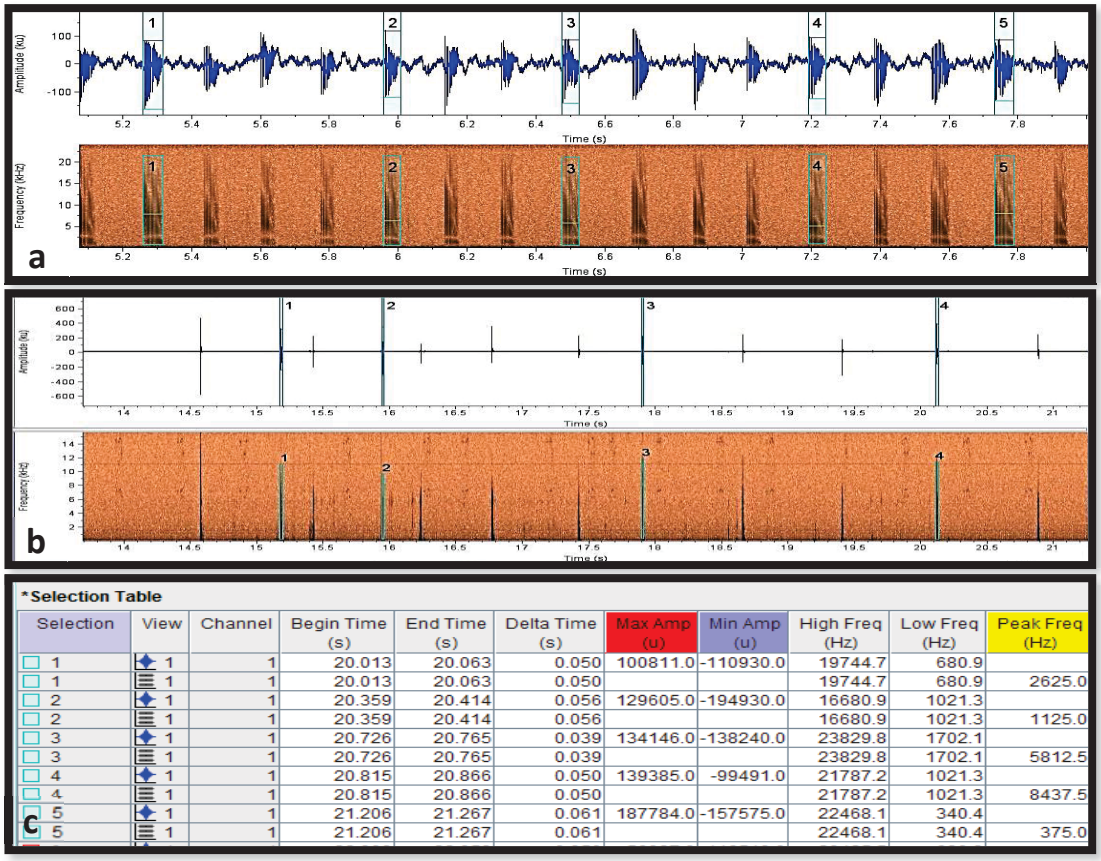


Figure 2.15: Spectral measurements of different parameters using Raven software; a) example of toothstrike measurements of *Hylurgus ligniperda* male sound, b) example of click measurements of *Hylurgus ligniperda* female sound c) values obtained for each parameter in the selection table in Raven.

Sound Recording and Behavioural Observation Specific to Each Trial

Some of the methodology procedures described above were specific to the treatments being tested. Thus, a description of how sounds were analysed and how the associated behaviours were observed in each particular trial is presented below.

Distress

An individual virgin male or female were introduced into the teflon tube and the sound recorder was turned on 5 minutes after the beetle was introduced into the tube and had walked along the tube, to avoid any physical pressure on the microphones by the beetle. The sound was recorded (no video recordings) for the duration of thirty minutes for each replicate. There were 10 replicates for each gender. For temporal and spectral sound analysis, one minute of recording was selected randomly. For spectral analysis of male sounds one toothstrike per chirp (simple and interrupted) was randomly selected. For spectral analysis of female sounds, due to lower number of clicks produced, all available clicks within one minute recording were used. In this experiment, the behaviour of the tested beetles was not observed as they were exposed to an 'out of context' situation.

Mating

Both sound and video recorders were turned on 5 minutes after introducing a virgin male (unmarked) into the cambium sandwich. After 30 minutes, a virgin female (marked white) was introduced. The sounds and videos were recorded for 10 hours for each replicate. The sounds produced by the two beetles were then divided into: before mating, during mating and after mating. The starting and finishing time of the first mating was noted. The sounds recorded during the time prior to the start of mating were considered as 'before mating sounds' and the sounds recorded soon after mating were considered as 'after mating sounds'. A one-minute recording was then selected from each of the three sound groups, i.e. before, during and after mating, for both temporal and spectral analysis (Figure 2.16). The behaviours displayed by both the male and the female prior, during and after mating were also observed and described, and when possible matched to the sounds produced during these three distinctive phases.

For spectral analysis of male sounds, one toothstrike per chirp (simple and interrupted) was randomly selected from one minute sample duration. For female

sounds, due to lower number of clicks produced, all the available clicks within one minute recording were used. Ten replicates were used for this experiment.

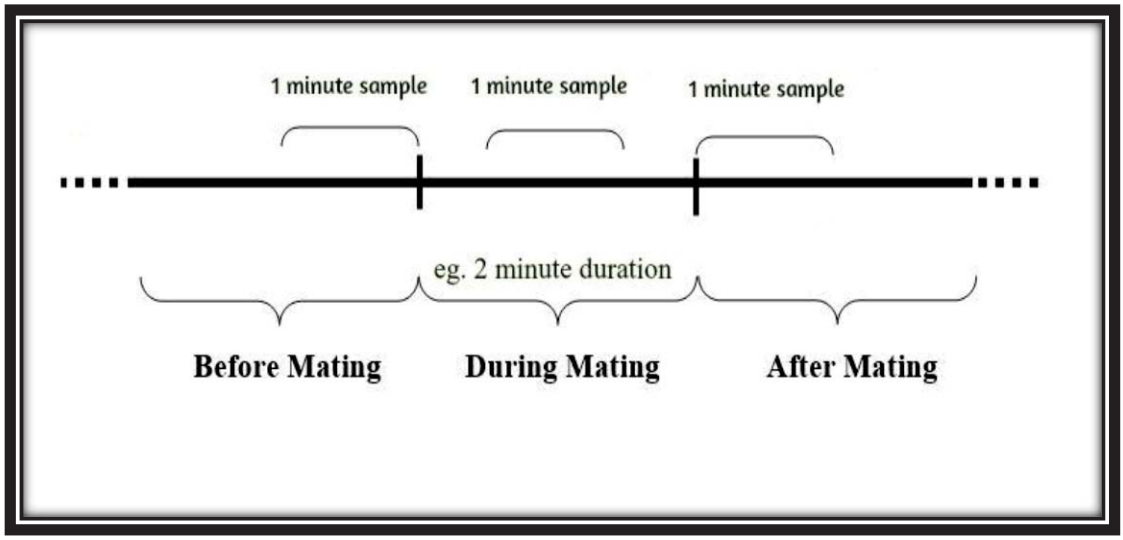


Figure 2.16: Example showing how the 1-minute sample was extracted from three different phases under mating trial observed.

Competition

Both sound and video recorders were turned on 5 minutes after introducing a virgin female (unmarked) and a virgin male (marked red) into the cambium sandwich. After 30 minutes, another virgin male (marked green) was introduced. Observing the video recordings, it was ensured that no mating had occurred between the female and the 'red' male until the 'green' male was introduced.

The reason for introducing the males at different times was to differentiate the toothstrikes of the two males during spectral analysis. It turned out that each male produces sounds slightly different from other males, so the males could be differentiated by listening to the recordings and observing the shape of the toothstrikes. The competition between the two males to mate with the female and the response of the female to the two males were also observed from the video recordings. The sounds and videos were recorded for 10 hours. The sound recordings (one-minute random sample) of the 'red' male and the female were obtained during the time period from the moment when the two males started competing and before the first mating occurred, and then used for analysis. For the spectral analysis of male sounds (from the 'red' male) ten toothstrikes from each one minute recording sample, were randomly selected; for the female, all the available clicks produced within the same period of time were analysed. A total of ten replicates were used for this experiment.

Male Territoriality

Both sound and video recorders were turned on 5 minutes after introducing a virgin male (marked red) into the cambium chamber. Two other virgin males (marked green and purple) were introduced 30 minutes after the introduction of a first virgin male (marked red). The sounds and videos were recorded for 10 hours. The behaviours displayed by the first male ('red') in the presence of the two other virgin males were observed and ten toothstrikes, from each of one minute recording sample per replicate, were randomly selected for spectral analysis. A total of ten replicates were used for this experiment.

Female Territoriality

Both sound and video recorders were turned on 5 minutes after introducing a virgin female (marked red) into the cambium chamber. Two other virgin females (marked green and purple) were introduced 30 minutes after introducing the first virgin female (marked red). The sounds and videos were recorded for 10 hours for each replicate. The behaviours displayed by the first female ('red') in the presence of the other two virgin females were observed and all click sounds produced by the first female ('red') within randomly selected one minute recording sample per replicate were used for the spectral analysis. A total of ten replicates were used for this experiment.

Colony

Three virgin males (marked red, green and purple) and three virgin females (marked red, green and purple) were used in each replicate for this trial. The males were marked on the two sides of their elytra whereas the females were marked on the dorsal side of their thorax. The males and the females marked with green and purple were introduced into the cambium chamber 30 minutes after introducing the male and female marked with red. The behaviours of the 'red' male and the 'red' female upon the introduction of the other individuals were observed. The sound recordings, each of one minute duration, were randomly selected for the spectral analysis. A total of ten replicates were used for this experiment.

Statistical Analysis

The data obtained from the Raven's selection table were transferred into Microsoft Excel spreadsheet. The means and standard errors (SE) were calculated for each of the

temporal and spectral parameters for both sexes for further statistical analysis. The temporal analysis was performed only for distress and mating trials. However, spectral analysis was carried out for all the trials. As the measures for temporal data were different in males and females, they were analysed separately. All statistical analysis was performed using GenStat (version 17, 2014, VSNi Ltd. Hemel, Hempstead, UK).

Temporal Analysis

For male distress and mating trials, the total and average values of each temporal parameter (number of chirps, interchirp interval, toothstrike per chirp, chirp duration and toothstrike rate) for simple and interrupted chirps were calculated from ten replicates. Paired t-tests were carried out to see if the simple chirps tended to have higher values of the temporal parameters than the interrupted chirps.

The temporal parameters (number of chirps, interchirp interval, toothstrike per chirp, chirp duration and toothstrike rate) of simple and interrupted chirps between the two trials (distress and mating) were compared using analysis of variance (ANOVA). As the temporal data measurements from the two stages of the mating trials (before and after mating) were from the same insect, the comparison was split into two parts. The first compared temporal parameters of distress with the mean values of temporal parameters of mating trial (obtained by combining all stages of mating). The second compared the mean values of the different temporal parameters of mating trial from two stages of mating (before and after). Two stages of mating (before and after) were used for males because males did not produce any sounds during the actual mating. The data was log transformed.

For female clicks, the temporal parameters (number of clicks, click duration, interclick interval and click strike rate) were calculated from ten replicates. The measurements of temporal parameters from the distress and mating trials were compared using analysis of variance (ANOVA). As the temporal data measurements from the three phases of the mating trials were from the same insect, the analysis was split into two parts. Firstly, the means of temporal parameters of distress trial were compared with the mean values of the temporal parameters of mating trial (obtained by combining all stages of mating). Secondly, the three stages of mating (before, during and after mating) were compared. The data was log transformed.

Spectral Analysis

The spectral parameters (toothstrike/click duration, maximum amplitude, minimum amplitude, high frequency, low frequency and peak frequency) were the same for both males and females. For male distress trial and mating (before mating and after mating) trial, the average values of each spectral parameter for simple and interrupted chirps were calculated from ten replicates. Paired two tailed t-tests were carried out to see if the means of spectral parameters of simple and interrupted chirps differed As the spectral parameters did not show any significant difference between simple and interrupted chirps, the simple and interrupted chirps were averegaed giving one set of measurements for the distress and mating trial.

For female spectral data analysis, the average values of spectral parameters of clicks were calculated for each trial. The phases of the mating trial (average of before, during and after mating) were averaged to get one set of measurements for the mating trial. Then, the spectral data were compared among all trials using two-factor analysis of variance, with sex and trial as the main factors. As the data obtained from the females tended to be more variable than from the males, a mixed effects model was fitted with insects as the random factor, which allowed different variability for male and female subsets of the data.

Principal Component Analysis (PCA) was also performed to explore the relationships between different spectral parameters. The correlation matrices (Pearson correlations) of different spectral parameters for male and female *H. ligniperda* were calculated.

Behavioural Analysis

For statistical analysis of behaviours, only mating, competition and colony trials were considered, as there were no interesting behaviours in other trials that could be numerically expressed. Therefore, the three behavioural parameters quantified were: first courtship duration, first mating duration and number of matings observed throughout the observation period. A one-way analysis of variance was done to compare the average values of these parameters among mating, competition and colony trials.

Chapter 3: Results

Distress

Upon introduction of *H. ligniperda* males into the rubber tube (experimental setup), they started making rapid continuous intense chirps, with more simple chirps than interrupted chirps being produced (within randomly selected one minute sample), and with the recurrence of interrupted chirps slowly declining with time (Figure 3.1 a). For all males, no more than one interrupted chirp was observed in between two simple chirps, but there were two or more simple chirps produced in succession (Figure 3.1 a). The number of toothstrikes per chirp increased gradually overtime in simple chirps, whereas the number of toothstrikes per interrupted chirp did not (Figure 3.1 a). Having more toothstrikes per chirp, the simple chirp duration was significantly longer than that of interrupted chirps. However, no significant difference was observed in terms of toothstrike rate between simple and interrupted chirps (Figure 3.1 b & c) (Table 3.1). The interchirp interval for simple chirps was significantly higher than that of interrupted chirps (Table 3.1). The exception to the above was replication No. 5, when no interrupted chirps were observed within the one minute sample.

Even though there were significant differences between simple and interrupted chirps in terms of different temporal parameters, there was no significant difference when the physical properties of randomly selected toothstrikes of simple chirps versus interrupted chirps were spectrally analysed. The average toothstrike duration for both simple and interrupted chirps was found to be 0.047 ± 0.001 seconds (Table 3.2).

Similarly, in distressed females the production of sounds (in the form of clicks) increased over time. However, observing the nature of the waveforms and spectrograms, the specific rhythm and pattern of click production could not be identified as easily as for the males (Figure 3.2). The interclick interval, when observed within each replication, did not give a clear indication about the uniformity of production of clicks, either. However, through temporal measurements, it was found that the distressed females on average produced 11.6 ± 1 clicks per minute with an average interclick duration of 5.2 ± 0.4 seconds (Table 3.3).

Based on the spectral measurements of all the available clicks produced per female and per minute of sampling time, I found that females took on average 0.012 ± 0.00016 seconds to produce a single click when distressed (Table 3.4). Upon observing the individual clicks produced within a one-minute sampling duration, there was a

drastic variation in the maximum or minimum amplitude of each successive click within the same replication; the rest of the parameters did not differ much.

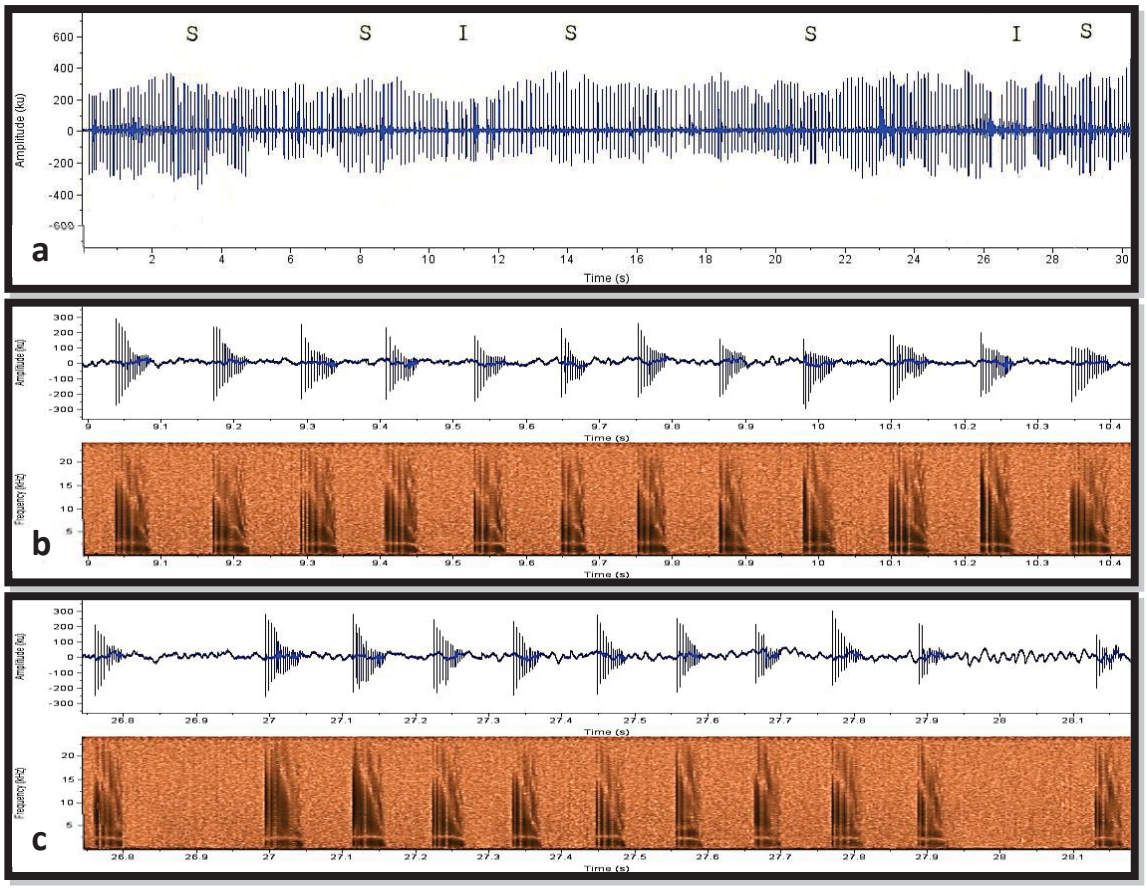


Figure 3.1: Sounds of distressed *H. ligniperda* male; a) waveform showing simple (S) and interrupted (I) chirps, b) waveform and spectral view of toothstrikes of a simple chirp, c) waveform and spectral view of toothstrikes of an interrupted chirp.

Table 3.1: Means and standard errors (±SE) of temporal parameters measured from sounds produced by distressed *H. ligniperda* males, and a paired t-test comparison of simple and interrupted chirps (α=0.05).

Distressed male chirps	No. of chirps	Interchirp interval (sec)	Toothstrikes per chirp	Chirp duration (sec)	Toothstrike rate (No./sec)
Interrupted [1]	3.8 ± 0.6	0.39 ± 0.02	7.6 ± 0.3	0.78 ± 0.05	10.0 ± 0.3
Simple[2]	6.0 ± 0.3	0.58 ± 0.03	80.0 ± 6.3	8.76 ± 0.69	9.2 ± 0.2
P (paired t-test)	0.002	0.001	<.001	<.001	0.025

[1] N= 10 for number of chirps and 9 for all other measurements.

[2] N= 10 for all measurements.

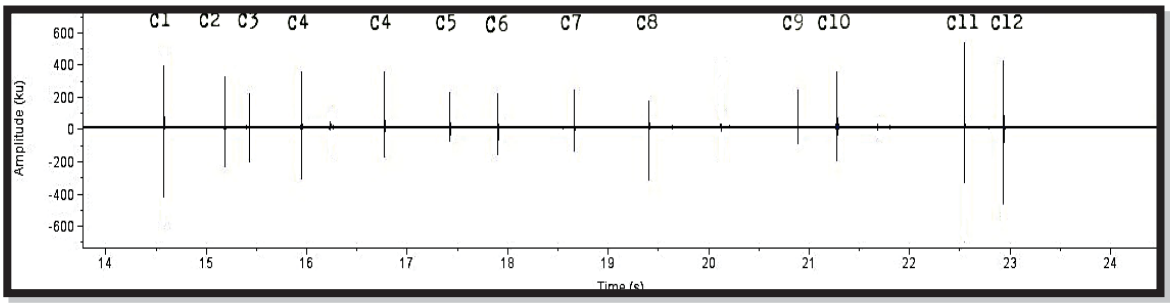


Figure 3.2: Sound of distressed *H. ligniperda* female; a waveform showing clicks produced.

Table 3.2: Means and standard errors (±SE) of different spectral parameters measured from sounds produced by distressed *H. ligniperda* males and a paired t-test comparison of simple and interrupted chirps (α=0.05).

Distressed male chirps	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Interrupted[1]	0.047 ± 0.001	248322 ± 21572	-295827 ± 18076	21967 ± 306	1118 ± 73	4788 ± 372
Simple[2]	0.047 ± 0.001	266798 ± 18098	-300835 ± 16674	21640 ± 455	1047±33	4583 ± 419
P (t-test)	0.719	0.268	0.618	0.490	0.270	0.736

[1] N= 9 for all measurements.

[2] N=10 for all measurements.

Table 3.3: Means and standard errors (±SE) for different temporal parameters measured from sounds (clicks) produced by distressed *H. ligniperda* females (N=10).

No. of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No/sec)
11.6 ± 1.0	0.0122 ± 0.0003	5.2 ± 0.4	84.1 ± 1.8

Table 3.4: Means and standard errors (±SE) of different spectral parameters measured from sounds (clicks) produced by distressed *H. ligniperda* females (N=10).

Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
0.012 ± 0.00016	191270 ± 40321	-275112 ± 68276	10023 ± 685	256 ± 14	1235 ± 117

As video recordings of males and females in distress were not performed, there were not behaviours measured in this context. However, upon taking the beetles out of the experimental tube, it was observed that both males and females moved faster than normal, and males produced chirps (not measured). Clicks by the females could not be heard, although they may have also been produced.

Mating

In order to analyse sound production in a mating context, I separated this complex behaviour into three phases: before, during and after mating. The sounds recorded and associated behaviours observed during these three phases are described below.

Male and Female Behaviour and Male Sound Production Before and During Mating

Before the introduction of the female into the cambium chamber, there was no sound production by the first male (Figure 3.3 a). The male was observed moving around the cambium sandwich and scraping the cambium (in a particular site). Upon the introduction of the female, the male started producing sounds in a form of frequent simple chirps (Figure 3.3 b). The female, upon entering the cambium chamber, moved around the cambium and made frequent physical contacts with the male (Figure 3.4 a & b). Over time, the number of physical contacts between the male and the female increased. The female seemed to move around the cambium chamber trying to find a suitable place to start tunnelling. Although clicks were produced by the female during all this time, the variation in their waveform according to specific behaviours (such as physical contacts with male and tunnelling site selection) could not be identified.

The nature of the sounds produced by the male changed again as it approached the female and were about to initiate mating. By then, the number of simple chirps produced by the male gradually declined with a gradual (and statistically significant) increase in the number of interrupted chirps (Table 3.5). Although there was a significant difference in the number of toothstrikes per chirp and the chirp duration between simple and interrupted chirps, there was no difference in the toothstrike rate between the two types of chirps before mating (Figure 3.5 & Table 3.5). Just before mating, the interchirp interval of interrupted chirps were found to increase gradually, while the number of toothstrikes within those interrupted chirps were gradually decreasing (Figure 3.6 a).

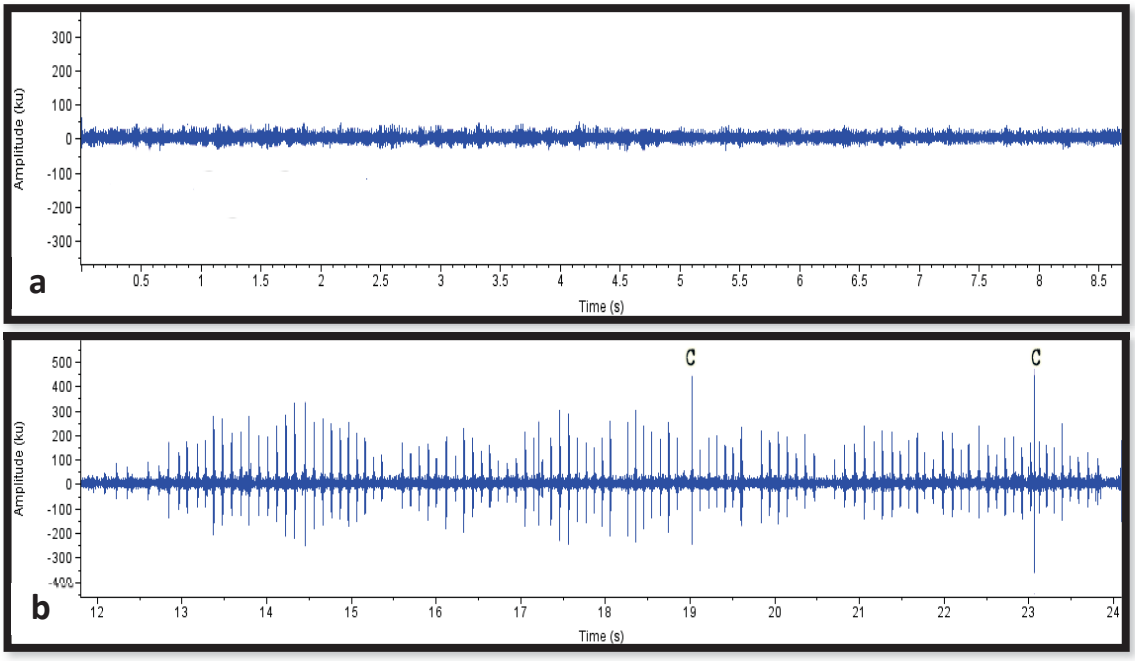


Figure 3.3: Waveforms showing variation in the nature of sounds produced by a *H. ligniperda* male a) before and b) after the introduction of a female into the cambium chamber.

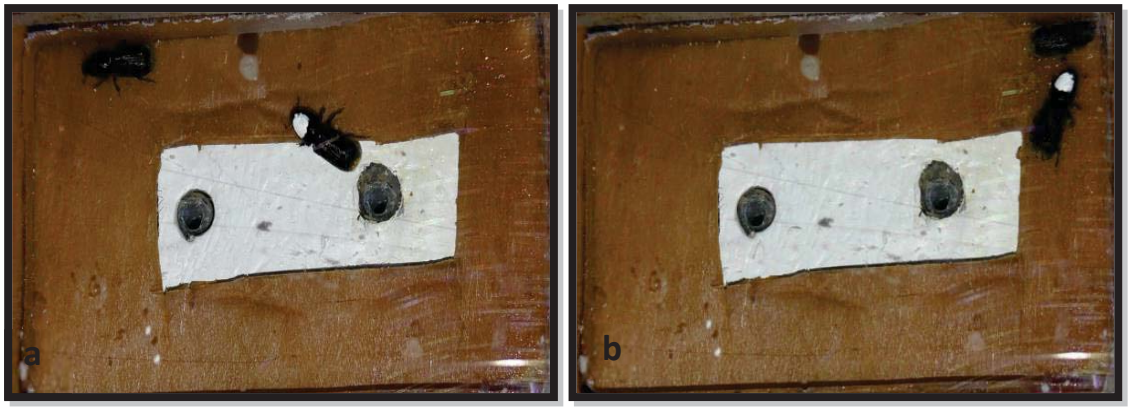


Figure 3.4: Behaviours observed after the introduction of *H. ligniperda* female into the cambium chamber a) male and female moving around the cambium b) female making contact with the male.

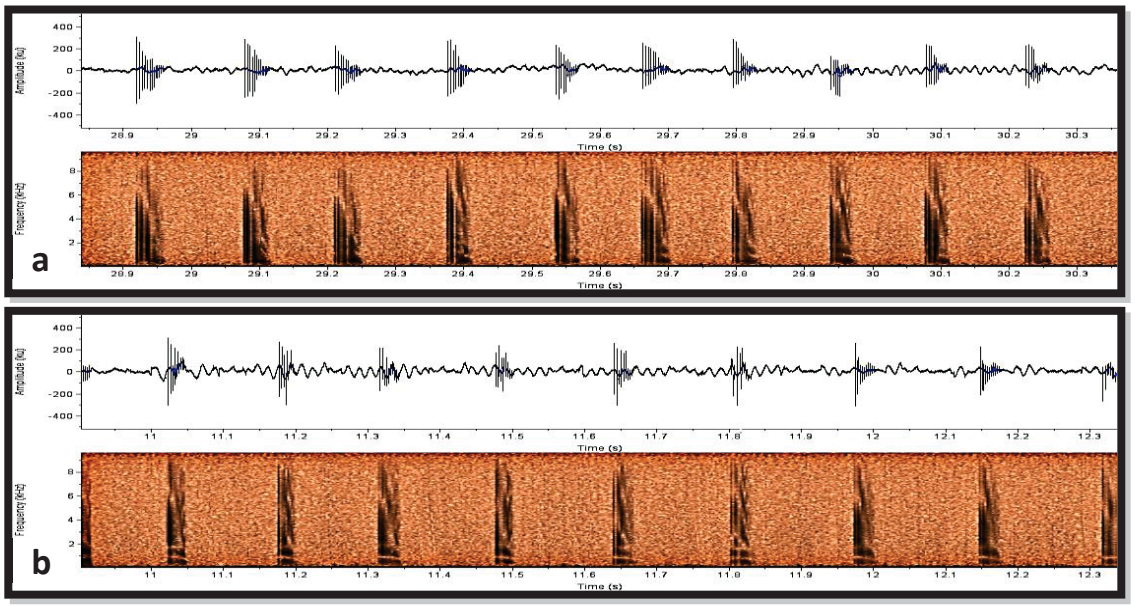


Figure 3.5: Waveform and spectrogram showing the toothstrikes produced by a virgin *H. ligniperda* male in mating trial, with female present, one minute before mating; a) a simple chirp, b) an interrupted chirp.

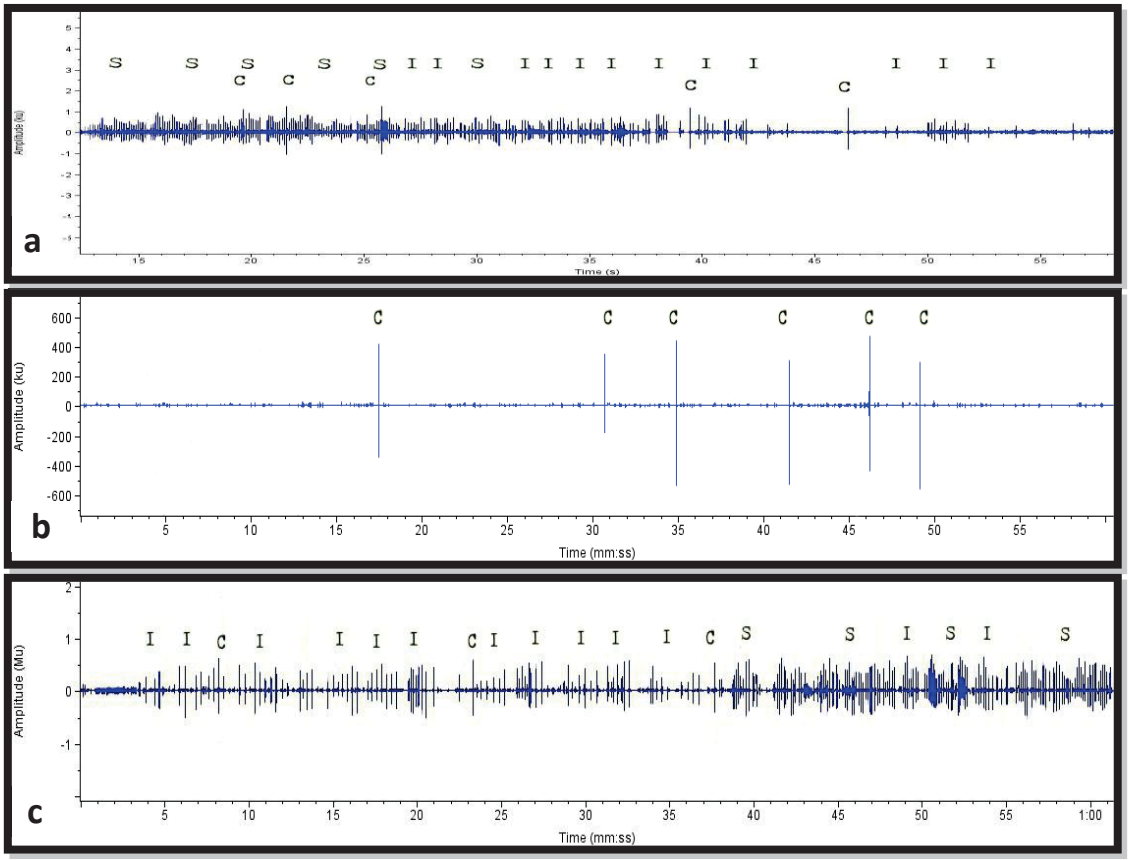


Figure 3.6: Waveforms of sounds produced by a *H. ligniperda* pair at various stages of mating: a) before mating; gradual decline in the simple chirps and increase in the interrupted chirps produced by the male, b) during mating: only female clicks, male chirps are absent, c) after mating: gradual decrease in the interrupted chirps and increase in simple chirps produced by the male. C, S and I represent Click, Simple chirp, and Interrupted chirp, respectively.

Table 3.5: Means and standard errors (±SE) of different temporal parameters measured from the sounds produced by a *H. ligniperda* male before mating (a single virgin male and a single virgin female together) and a paired t-test comparison of simple and interrupted chirps (α=0.05) (N=10).

Male before mating	No. of chirps	Interchirp interval (sec)	Toothstrikes per chirp	Chirp duration (sec)	Toothstrike rate (No./sec)
Interrupted	9.3 ± 0.7	1.52 ± 0.07	6.3 ± 0.3	0.92 ± 0.06	7.1 ± 0.3
Simple	6.0 ± 0.4	1.95 ± 0.18	18.8 ± 0.8	2.60 ± 0.17	7.5 ± 0.3
P (paired t-test)	0.004	0.031	<.001	<.001	0.079

Table 3.6: Means and standard errors (±SE) of spectral parameters of sounds produced by virgin *H. ligniperda* male before mating (a single virgin male and a single virgin female together) and a paired t-test comparison of simple and interrupted chirps (α=0.05) (N=10).

Male (before mating)	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Interrupted	0.015 ± 0.000	295298 ± 8276	-266229 ± 12247	12657 ± 94	425 ± 29	6599 ± 275
Simple	0.015 ± 0.000	295342 ± 7848	-265682 ± 8612	12562 ± 98	399 ± 26	6638 ± 276
P (t-test)	0.739	0.996	0.969	0.440	0.526	0.814

The physical properties of the toothstrikes of simple and interrupted chirps produced by the males before mating were not different (Table 3.6).

In all replications, the males were found to perform courtship activities before mating. These courtship activities included male beetle's head touching around the genital area of the female, and male grooming (using all legs) all around the female dorsal body parts (Figure 3.7 a & b). As soon as the male started mating, there was no male sounds (simple or interrupted chirps) observed (Figure 3.6 b). The females were immobile during courtship and mating.

During mating, the males used the front two legs for grooming the female, mostly around the posterior part of the female body. The other legs were used for grasping and holding the female (Figure 3.7 c). Multiple matings were observed in all replications. Overall, males were found to perform a long courtship activity for the first

mating and no courtship activities (except for three replications) in subsequent mating attempts within the same replication (i.e., same beetle pair) (Table 3.7). Although there were some cases of courtship activities for subsequent matings in some of the replications, the duration of the courtship was shorter compared to the courtship before the first mating.

Figure 3.7: Behaviours observed during interaction of virgin *H. ligniperda* male and female; a) male touching female genital area, b) male grooming the female, c) mating, d) male mating with the female while the female has already started tunnelling, e) male waiting to assist the female in clearing frass out of the tunnel, f) male assisting the female inside the tunnel.

Table 3.7: Behaviours recorded during mating of *H. ligniperda* (single pair of virgin adults).

Mating	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10	Mean
Courtship duration before 1st mating (sec)	135	187	98	109	80	141	122	134	166	113	128.5
Courtship (grooming) observed in subsequent mating	Yes	No	Yes	No	Yes	No	No	No	No	No	-
Mating Duration (1st mating only) (sec)	207	419	188	446	235	309	351	288	254	247	294.4
No. of matings throughout the observation period	2	2	3	2	4	3	3	4	3	2	2.8
Male assisting in tunnelling	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-

Male and Female Behaviours and Male Sound Production after Mating

Once mating was over, the males immediately began to produce sounds again, starting with interrupted chirps. The number of toothstrikes within the interrupted chirps gradually increased, while the interchirp interval decreased. Over time, the number of interrupted chirps gradually decreased as the number of simple chirps increased (Figure 3.6 c).

Analysing the temporal data, I found that there was a remarkable difference in per minute number of simple and interrupted chirps produced by the males after mating. Significantly more interrupted chirps than simple chirps were produced during this phase. Although there were significant differences in the number of toothstrikes per chirp and chirp duration between simple and interrupted chirps, the difference in the toothstrike rate between these two chirp types was negligible (Figure 3.8 and Table 3.8). The males in replications 6, 9 and 10 did not produce any simple chirps after mating (within the one minute observation time period).

Comparing different physical properties of toothstrikes of simple and interrupted chirps produced by males after mating, no significant differences were found across any of the parameters studied, except for the maximum amplitude, which was significantly higher for interrupted compared to simple chirps (Table 3.9).

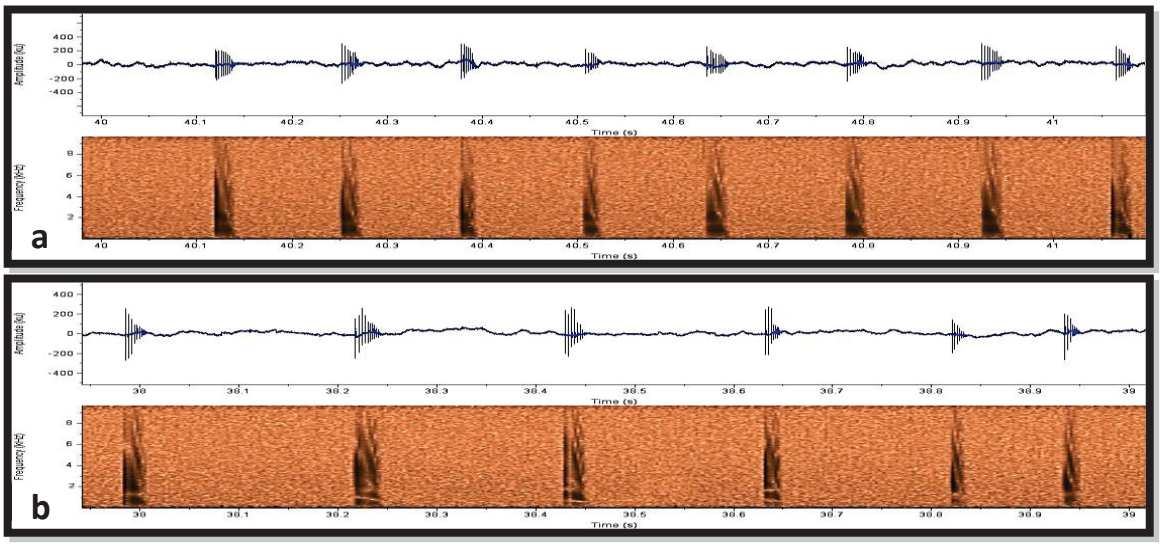


Figure 3.8: Waveform and spectrogram showing the toothstrikes of a virgin *H. ligniperda* male after mating; a) a simple chirp, b) an interrupted chirp.

Table 3.8: Means and standard errors (±SE) of temporal parameters of sounds made by *H. ligniperda* males after mating and a paired t-test comparison of simple and interrupted chirps (α=0.05).

Male (after mating)	No. of chirps	Interchirp interval (sec)	Toothstrikes per chirp	Chirp duration (sec)	Toothstrike rate (No/sec)
Interrupted[1]	8.5 ± 1.0	2.0 ± 0.1	5.3 ± 0.2	0.6 ± 0.0	9.1 ± 0.2
Simple[2]	1.8 ± 0.5	0.7 ± 0.1	32.0 ± 4.8	3.7 ± 0.5	8.5 ± 0.4
P (t-test)	<0.001	<0.001	0.001	0.001	0.319

[1]N=10 for all measures.

[2]N= 10 for number of chirps and 7 for all other measures.

Table 3.9: Means and standard errors (±SE) of spectral parameters of sounds made by *H. ligniperda* males after mating and a paired t-test comparison of simple and interrupted chirps (α=0.05).

Male (after mating)	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Interrupted[1]	0.011 ± 0.000	313632 ± 6913	-295749 ± 7609	12343 ± 66	456 ± 24	6139 ± 148
Simple[2]	0.012 ± 0.000	286167 ± 10044	-275608 ± 16427	12216 ± 153	437 ± 51	6469 ± 496
P (t-test)	0.207	0.005	0.065	0.596	0.638	0.559

[1]N=10 for all measures.

[2]N= 10 for number of chirps and 7 for all other measures.

After mating, the females were found to initiate their tunnelling behaviour in all the replications observed. Males stayed around the female, while the female started tunnelling (Figure 3.7 d). In some of the cases (in three replications), while the females were busy in tunnelling, the males would only scrape themselves. However, scraping by males did not last long and males would finally join the female after a certain time period. During these periods (even when in different sites), males and females were found to produce sounds quite often. The male chirps during this stage were composed of both simple and interrupted chirps, whereas females produced clicks.

The first mating (in all replications) occurred before the start of the tunnelling process. However, in four of the replications, the males were found to be mating (subsequent matings) with females even after the females have already started tunnelling (Figure 3.7 e). Once there was enough room within the tunnel, the male would assist the female by clearing frass out of the tunnel (Figure 3.7 f). There was negligible (almost nil) number of chirps/clicks produced by the male and female once the male started assisting the female in tunnelling.

Female Sound Production Before, During and After Mating

Females were found to produce clicks during all stages of mating (Figure 3.6). However, observing the nature of the waveforms and spectrograms did not allow to identify specific rhythms and patterns of click production. Females were found to produce more clicks with longer click duration before mating than during mating, and the lowest number of clicks after mating. However, the click strike rate was higher after mating than during mating, followed by before mating. On average, the interclick interval was shorter during mating as compared to before or after mating (Table 3.10).

The analysis of the spectral properties of the clicks produced by females at various stages of mating indicated that the clicks with the highest frequency were produced during mating, followed by after mating, and then before mating. The pattern was similar in the case of low frequency as well. However, the average peak frequency was higher for clicks after mating (Table 3.11).

Table 3.10: Means and standard errors (±SE) of temporal parameters for sounds produced by *H. ligniperda* female at three stages of mating (N=10).

Female	No. of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No/sec)
Before mating	4.7 ± 0.4	0.0066 ± 0.0001	9.4 ± 0.5	156.7 ± 4.0
During mating	4.4 ± 0.5	0.0050 ± 0.0002	6.4 ± 0.5	209.2 ± 9.6
After mating	3.8 ± 0.3	0.0043 ± 0.0001	9.5 ± 0.5	240.8 ± 7.5

Table 3.11: Means and standard errors (±SE) of spectral parameters of sounds produced by *H. ligniperda* female at three stages of mating (N=10).

Female	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Before mating	0.007 ± 0.00015	1306701 ± 220340	-1076267 ± 154953	16912 ± 524	528 ± 34	4709 ± 683
During mating	0.005 ± 0.00020	360783 ± 42687	-422257 ± 54930	24811 ± 2383	1702 ± 179	6458 ± 766
After mating	0.004 ± 0.00013	277043 ± 22481	-266701 ± 20569	24390 ± 2762	1612 ± 268	7944 ± 710

Competition

The competition context was recreated by introducing a virgin female into the cambium chamber along with a virgin male (marked red, referred to as 'first' male hereafter), and a second male (marked green, referred to as 'rival' male hereafter) 30 minutes later. The first male, as soon as in contact with the female, started producing a mixture of simple and interrupted chirps. There were some clicks produced by the female in response. As soon as the rival male was introduced into the cambium chamber, the nature of sound production by the first male changed. Both males started producing chirps, with the pace of chirp production by the first male increasing (Figure 3.9). As it was difficult to analyse the temporal parameters of the sounds produced by individual males, only spectral measurements were performed in this context.

During competition for the female, the males had an average toothstrike duration of 0.012 ± 0.00017 seconds (Table 3.12), whereas the females produced a click of 0.005 ± 0.0003 seconds (Table 3.13).

Upon entering the cambium chamber, the rival male made frequent physical contacts with the female and the first male. The first male was also found to move around and make physical contact with the female and with the rival male during

different periods of time (Figure 3.10 a). No signs of aggression from one male to the other were observed up to this point. Sounds were produced by all three individuals upon physical contact with each other. The production of chirps by both males slowly declined over time. However, they were found to produce chirps during specific behavioural activities, such as expression of aggression. Females were found to move around the cambium chamber in order to find a suitable place to start tunnelling. During this period, the female would contact both males (scraping cambium on different sites) (Figure 3.10 b & c) and both males would respond with the production of chirps. As soon as the female found a suitable place to start tunnelling, it stopped moving around and stayed within that specific area. At this point in time, and even though the three individuals were in different sites, there were frequent chirps produced by both males along with the clicks from the female. In three replications, the female would join a male at a particular site, mate, and make that the final tunnelling site. The male would groom and mate with the female (Figure 3.10 d & e). The rival male at a different site could detect the bonding between the first male and the female and was found to produce loud chirps and finally join the pair after a certain interval of time and compete for the female (during or after mating) (Figure 3.10 f-i). The mated male would either defend the female or leave the female and start scraping into another site. As previously described, no sounds were produced by males during the actual mating. The females were found to produce clicks during all matings (first and subsequent mating). Observing the waveforms, no differences in the nature of these clicks could be identified. In cases where the rival male was not trying to physically disrupt mating (during the first mating), the courtship duration was comparatively longer (Table 3.14). In cases where both males were competing to mate with the female, there were frequent disruptions in mating. The mating male was found to restrict the other male from gaining physical contact with the female by blocking it with its head (Figure 3.10 g & h). In some of the cases, loud chirps were produced by the mating male when the other male was approaching the mating pair and showed an aggressive behaviour such as pushing the other male with its head. As mentioned before, courtship activities were not observed in subsequent matings (Table 3.14).

Multiple mating was observed in all replications (Table 3.14). There was no mating observed when the female started tunnelling. In cases where both males stayed together behind the female, none of the males were found to help the female in clearing frass out of the tunnel. In six replications, it was observed that there was only one male

behind the female assisting in frass clearing (Table 3.14), while the second male was scraping cambium by itself in a different location in a close vicinity. Interestingly, the males that were found helping the female were those that had mated with the female.

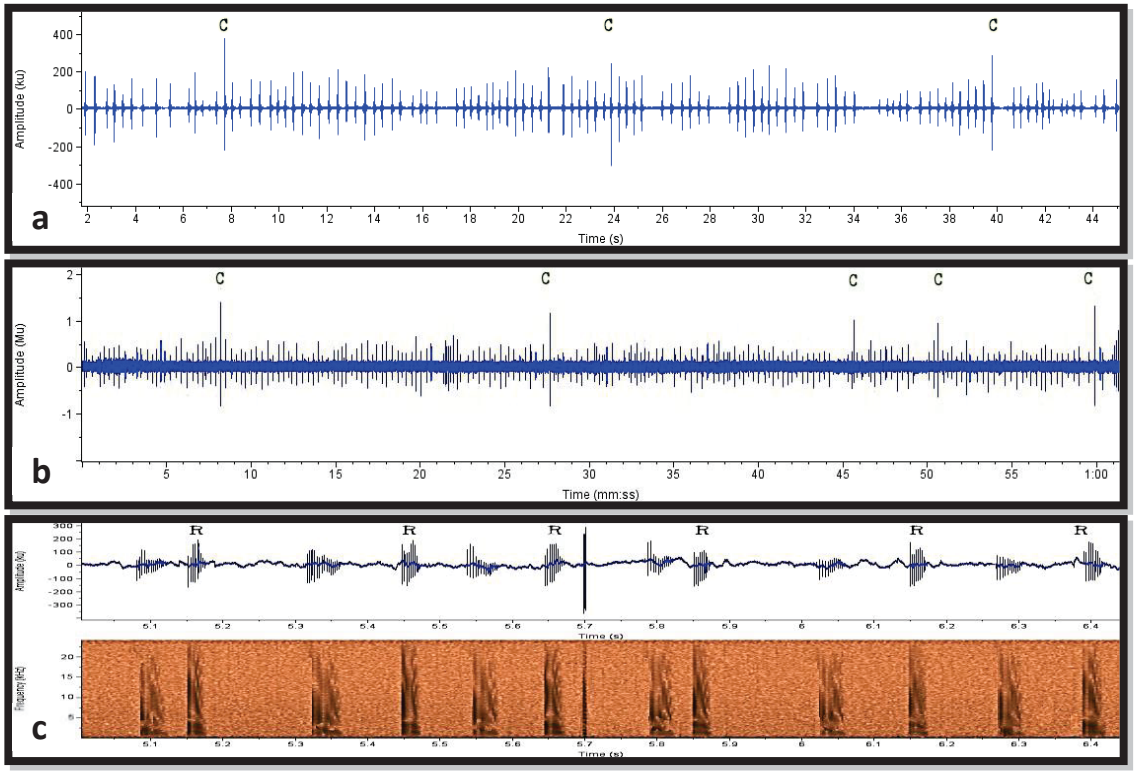


Figure 3.9: Sounds produced by *H. ligniperda* during competition (two males and a female); a) the chirps of the first male (red) with the clicks of a female before the introduction of a rival male (green), b) chirps of both males together along with the clicks of the female, c) waveform and spectrogram showing the toothstrikes of two competing males and a female click. R represents the toorthstrike of a male marked red.

Table 3.12: Means and standard errors (±SE) of spectral parameters of sounds produced by *H. ligniperda* males during competition (N=10).

Parameter	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Competing males	0.012 ± 0.00017	190383 ± 12633	-170817 ± 7209	15740 ± 144	294 ±10	6927 ± 218

Table 3.13: Means and standard errors (±SE) of spectral parameters of sounds produced by *H. ligniperda* females in the presence of two competing males (N=10).

Parameter	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Females	0.005 ± 0.0003	1041600 ± 189034	-767881 ± 152396	17998 ± 788	1631 ± 863	4843 ± 261

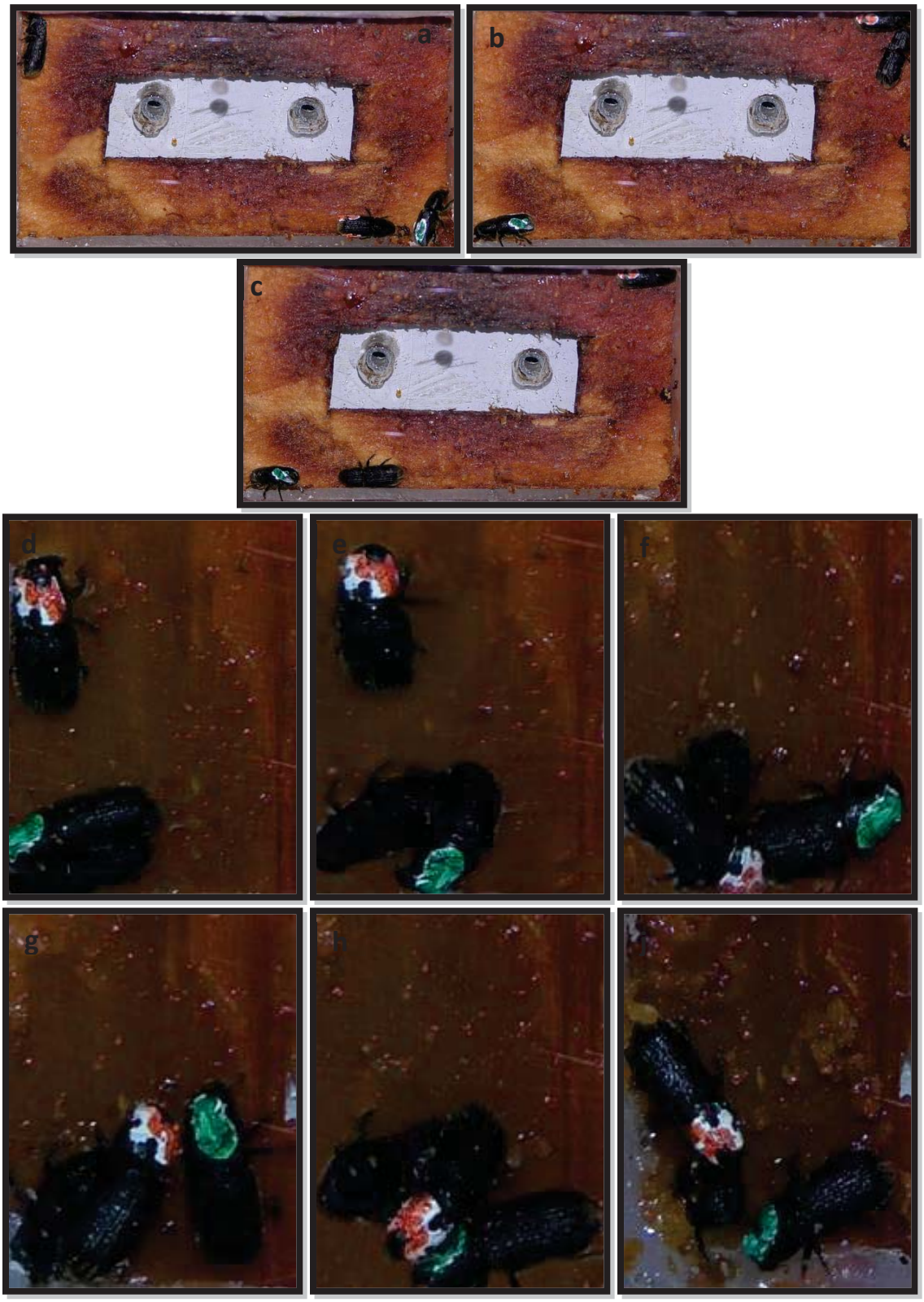


Figure 3.10: Two males and female of *H. ligniperda* during competition ("red" male was introduced together with the female, "green" male introduced 30 min later); a)) red male and green male meeting with each other, b) and c) female approaching two males to make contacts with two males, d) green male grooming female, e) green male mating female while red male is scrapping close by, f) red male competing with green male for mating, g) red male defending (restricting) green male from mating, h) red male pushing green male with its head during mating, i) both males waiting outside the tunnel while female is tunnelling.

Table 3.14: Different behaviours observed during competition between two *H. ligniperda* males for a conspecific female ("1st male" was introduced together with the female, "2nd male" introduced 30 minutes later).

	Rep 1	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Rep 6**	**Rep 7**	**Rep 8**	**Rep 9**	**Rep10**	**Mean**
First male to achieve mating	1st male	1st male	1st male	2nd male	1st male	2nd male	2nd male	1st male	1st male	2nd male	-
First courtship duration (Sec)	76	55	82	167**	77	68	151**	140**	88	41	94.5
Courtship observed in subsequent mating	No	No	No	No	No	No	No	No	No	No	-
First Mating duration (Sec)	166	203	170	251	273	142	224	296	178	254	215.7
Number of matings (both males)	5	4	7	4	4	6	4	4	6	4*	4.8
Male/s assisting in tunnelling	Both males	2nd male	1st male	1st male	Both males	Both males	1st male	Both males	2nd male	2nd male	-

*Only mated by one male. ** When the rival male was not competing, or showing physical disruption.

Territoriality

Male Territoriality

The male territoriality context was tested by introducing two virgin males (marked green and purple) 30 minutes after a virgin male (marked red) was initially introduced into the cambium sandwich. The male marked with red did not produce any sound when by itself. However, as soon as the other males (marked green and purple) were introduced, all three males started making loud chirps (Figure 3.11). The sound production from all three males slowly declined over time and finally there was silence again, except for when physical encounters happened between any of the males. In most

of the cases, head to head contacts were observed. However, no signs of aggression were observed when these encounters took place.

Although two of the males (any colour) met together and stayed close by (scraping the cambium), all three males were never found to be together at any time (Figure 3.12). When a particular male broke bonding with another male and joined the third male in another site, sound was produced by the third male and the joining male. The bonding activity was found to be frequently changing throughout the observation period. There were no signs of tunnelling activities observed, but some minor scraping was made in the cambium (but not a proper tunnel like the one made by a female).

As our set-up did not allow us to study the temporal characteristics of the sounds produced by more than one individual of the same sex at a time, only spectral parameters could be measured in this case. The average toothstrike duration was found to be 0.017 ± 0.00017 sec (Table 3.15).

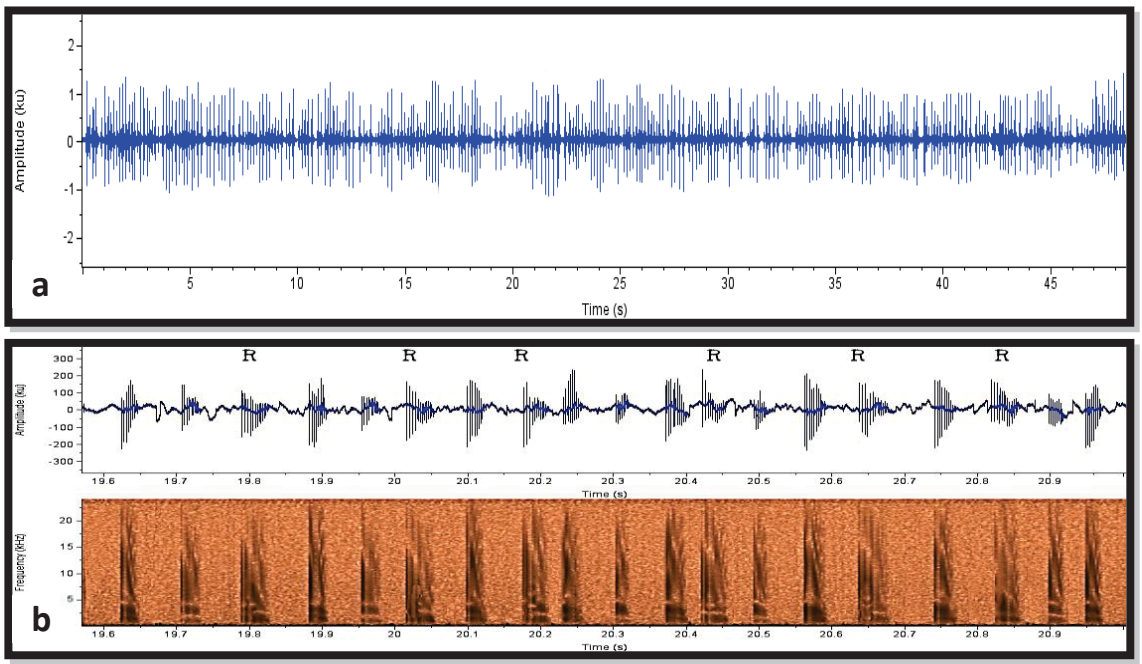


Figure 3.11: Sounds of territorial males; a) waveform showing the sounds of three territorial males, b) waveforms and spectrogram showing toothstrikes of three different territorial *H. ligniperda* males. 'R' represents the toothstrikes of male marked red.

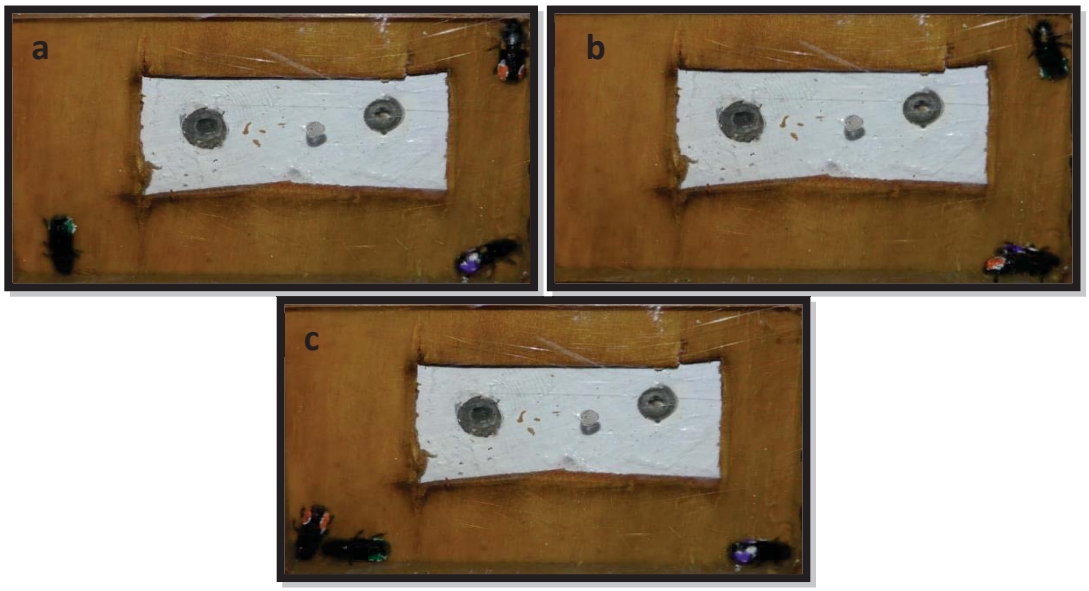


Figure 3.12: Different stages in male territoriality; a) three males scraping in three different sites, b) red male and purple male together, c) red male and green male together.

Table 3.15: Means and standard errors (±SE) of spectral parameters of sound produced by *H. ligniperda* male (marked red) in the presence of other two males (N=10).

Parameter	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Male	0.017 ± 0.00017	649965 ± 62686	-514507 ± 46930	15507 ± 538	515 ± 36	6978 ± 562

Female Territoriality

Two virgin females (marked green and purple) were introduced after 30 minutes of introducing a first virgin female (marked red) into the cambium chamber. The females marked with red were not found to produce any clicks when alone. However, as soon as the females marked green and purple were introduced, all three females started producing clicks (Figure 3.13). Females were observed to stay independently (Figure 3.14) and meet each other momentarily. All three females seemed to move around the cambium chamber in order to find a right place for tunnelling. Although clicks were produced during different behavioural activities (such as physical contacts and site selection), the exact purpose and meaning of the clicks were not identified.

Female aggression was observed in most of the replications. The females were found to push the other females with their heads in order to express aggression. This behaviour was mostly observed when one female made a physical contact with another female that was trying to initiate the tunnelling process. The tunnelling activity seems to

work well until a certain point, but slowed down over time as frass accumulated at the entrance of the tunnel. Spectral properties of randomly selected clicks from ten replications are shown in Table 3.16.

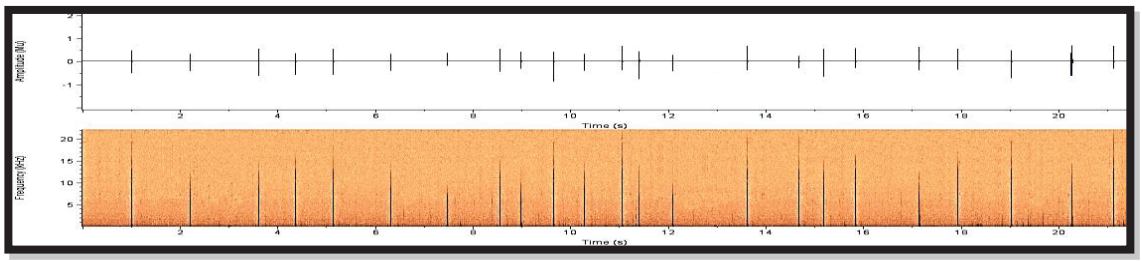


Figure 3.13: Waveforms and spectrogram showing clicks of territorial *H. ligniperda* females.

Figure 3.14: Different stages during female territoriality; a) female moving around to find specific locations for tunnelling, b) three different females tunnelling in different sites.

Table 3.16: Means and standard errors (±SE) of spectral parameters of sounds produced by *H. ligniperda* female (marked red) in the presence of other two females (N=10).

Parameter	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Female	0.005 ± 0.00015	321596 ± 54987	-302851 ± 64984	21503 ± 3205	1435 ± 150	4646 ± 707

Colony

The colony context was tested by introducing a total of three virgin males (marked red, green and purple) and three virgin females (marked with red, green and purple) into the cambium chamber. The males were marked on the two sides of their elytra, whereas the females were marked on the dorsal side of their thorax. The males and the females marked with green and purple were introduced 30 minutes after introducing the male

and female marked with red. The nature of the sounds produced by both the males and the females marked with red changed after the introduction of other males and females (Figure 3.15). All three males produced chirps together, which gradually declined over time. The nature of the sounds changed as the males progressed towards specific behavioural activities such as competition and/or courtship. All individuals were observed to make physical contacts with each other and started displaying behaviours such as courtship, competition, aggression, mating and tunnelling (Figure 3.16). As it was not possible to study the temporal characteristics of the sounds produced by more than one individual of the same sex at once, only spectral measurements were performed for this context (Table 3.17).

Although the females marked green and purple mated with different males at different times, the duration of courtship and mating was only noted for the females marked red. The first courtship duration was comparatively longer in cases where there were no other males competing to mate with that female (Table 3.18). This coincides with the previous observations in competition context. In such cases, the other males were either mating with other females or individually scraping the cambium at a different site. The females marked red were also found to mate with other males in subsequent matings. The males that first mated the red females were also found to subsequently mate with other females.

As previously observed, no courtship behaviours were observed in subsequent matings. However, in replication 2 and 9, courtship behaviours were observed in the second mating with the same male (Table 3.18). As previously observed, no sounds were produced by the males during mating. However, the rival males trying to mate with the same female or other females were found to produce chirps. The females were found to produce clicks during all matings (the first or other subsequent matings). However, as previously noted, the difference in the nature of clicks could not be detected.

Although competition was observed among males in order to mate with a particular female, it did not seem to last for a long time. There were cases where a particular male did not mate with any female or the female was not mated by any male throughout the observation period. In replication 5 and 8, for instance, the females marked red were not mated by any males throughout the observation period. No aggressive behaviour was observed among females to mate with a particular male. Males behind the tunnels (i.e. those helping females) could not be observed as most of

the individuals (either male or female) aggregated close to each other, and frass blocked the vision preventing observation (Figure 3.16 b).

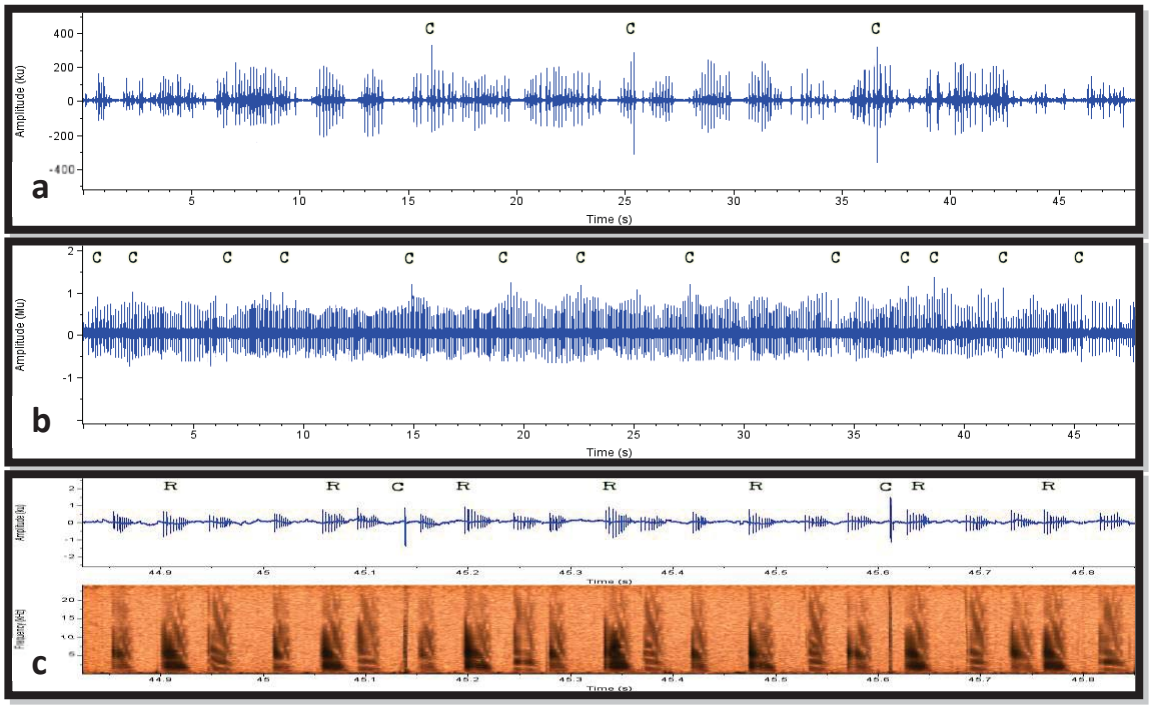


Figure 3.15: Sounds of male and female *H. ligniperda* in a colony; a) waveform showing chirps of a virgin male (marked red) along with clicks (C) of female (marked red) before the introduction of other two virgin males and females, b) waveform showing chirps of three males and clicks (C) of three females, c) waveform and spectrogram showing toothstrikes of three different males (R are chirps of the "red" male) and two female clicks (C).

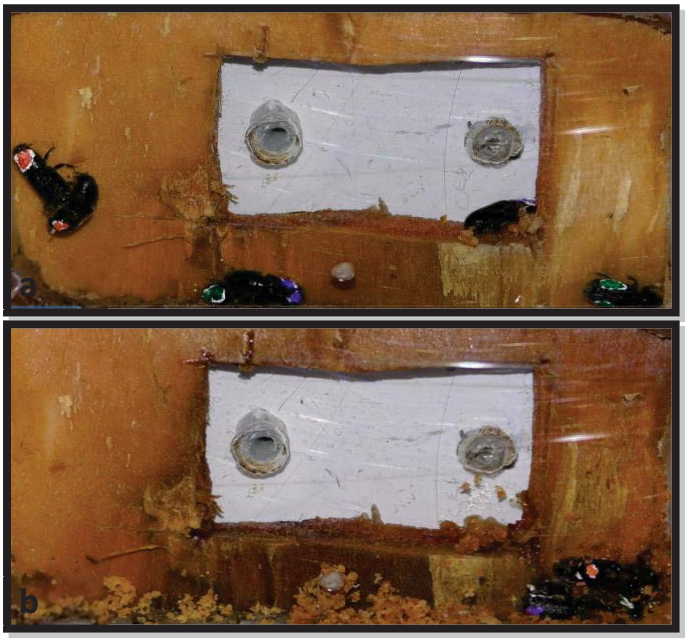


Figure 3.16: Behaviours of *H. ligniperda* males and females observed in colony context; a) female marked red mated by a male marked red, female marked green groomed by a male marked purple, female marked purple tunnelling on its own, the male marked green scraping on its own, b) all individuals gathered together and tunnelling in close vicinity.

Table 3.17: Means and standard errors (±SE) of spectral parameters of sounds produced by *H. ligniperda* male (marked red) and female (marked red) in a colony (N=10).

Parameter	Toothstrike/click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
Male	0.020 ± 0.00041	64270 ± 67039	-661270 ± 25040	15285 ± 254	232 ± 17	5678 ± 216
Female	0.005 ± 0.00028	401590 ± 51705	-416972 ± 64488	23875 ± 1408	999 ± 113	5115 ± 544

Table 3.18: Behaviours observed in a colony of *H. ligniperda* (three males and three females). The "red" male and female were introduced into the cambium chamber first; "purple" and "green" males and females were introduced 30 minutes later.

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep10	Mean
Male to first mate red female	Purple	Green	Red	Green	-*	Purple	Red	-*	Green	Green	-*
First courtship duration (sec)	57	101**	49	68	-	114**	117**	-	97	36	79.9
Courtship observed in subsequent matings	No	Yes***then No	No	No	-	No	No	-	Yes*** then No	No	-
First Mating duration (sec)	137	92	107	126	-	189	241	-	232	133	157.1
No. of matings	6	4	5	4	-	6	5	-	5	5	4.0

* Female marked red was not mated by any males throughout the observation period. ** There were no other males competing. ***Courtship observed again only in second mating with the same (first mated) male.

Comparative Analysis

Temporal parameters

The analysis was split into two components – first, distress mean versus mean of three stages of mating were compared. Second, the stages of mating were compared. Females were found to produce significantly more clicks under distress than in any phase of mating (Figure 3.17). Click duration was longest under distress than in mating whereas it dropped significantly. The inter-click interval was longer before and after mating than during mating or in distress. The click strike rate was higher in mating than in

distress, and increased over the stages of mating (Figure 3.17 and Table 3.19). Other contexts such as competition, territoriality and colony were not used for temporal analysis as most of the sounds from different individuals from the same sex overlapped during recording making difficulty in the temporal analysis.

The number of simple chirps was higher in distress and before mating than after mating. Toothstrike interval was higher before mating than after mating or in distress. Toothstrikes per chirp and chirp duration were higher in distress than after mating, followed by before mating. Toothstrike rate was higher in distress than before and after mating (Figure 3.18 and Table 3.20). The number of interrupted chirps was higher and the interchirp interval was longer in mating context than in distress. On the contrary, there were more toothstrikes per chirp and higher toothstrike rate in distress (Figure 3.19 and Table 3.21). Interchirp interval was longer after mating than before mating. Chirp duration was longest before mating and shortest after mating. Toothstrike rate was higher after mating than before mating, but not as high as when in distress (Figure 3.19 and Table 3.21).

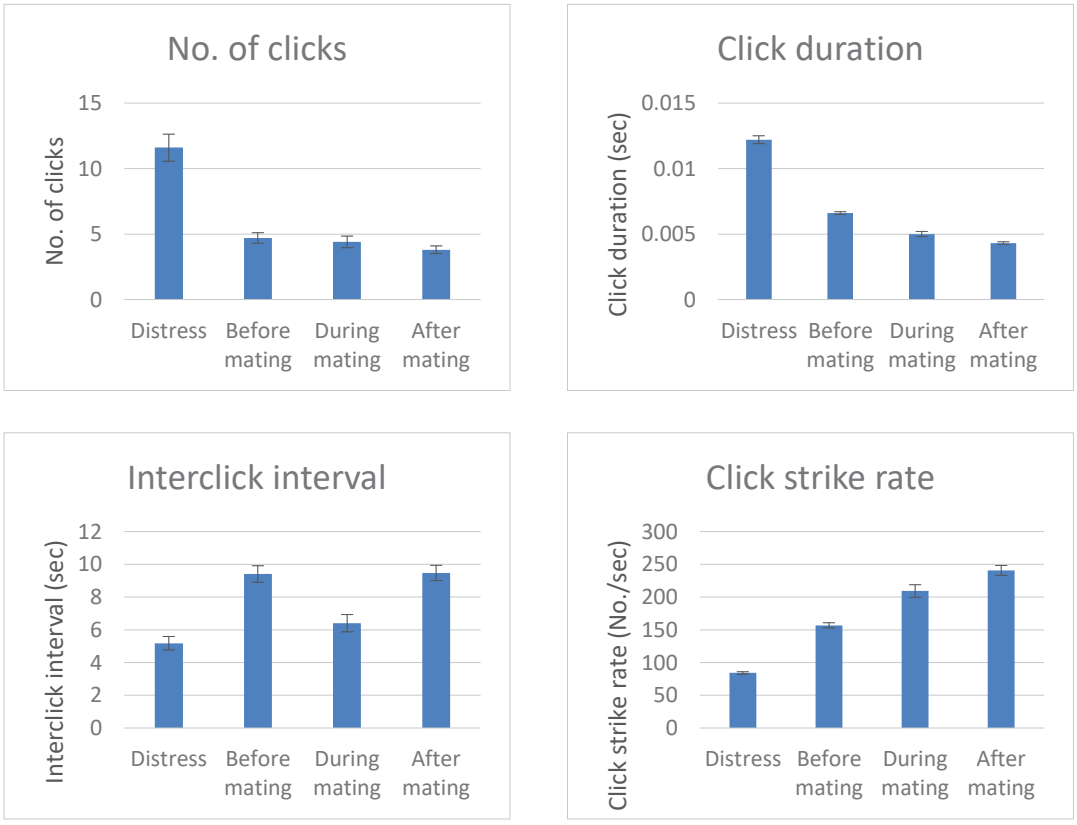


Figure 3.17: Means and standard errors (bars) of temporal parameters for *H. ligniperda* female click sounds in different contexts.

Table 3.19: Analysis of variance (ANOVA) for temporal parameters of sounds produced by *H. ligniperda* female under distress and during mating.

	No. of clicks*		Click duration (sec)		Interclick interval (sec)		Click strike rate * (No./sec)	
	F	P	F	P	F	P	F	P
Distress vs. mating (df 1, 18)	104.9	<.001	760.8	<.001	38.1	<.001	673.0	<.001
Mating phases (df 2, 18)	1.1	0.359	61.3	<.001	12.3	<.001	41.2	<.001

* Log transformed for analysis.

Table 3.20: Analysis of variance (ANOVA) for temporal parameters of sounds (simple chirps) produced by *H. ligniperda* male under distress and during phases of mating.

ANOVA	No. of chirps		Interchirp interval (sec)		Toothstrikes per chirp*		Chirp duration* (sec)		Toothstrike rate (No/sec)	
	F	P	F	P	F	P	F	P	F	P
Distress vs. mating (df 1, 18)	16.6	<.001	17.8	<.001	122.5	<.001	88.8	<.001	14.9	0.001
Before vs. after mating (df 1, 9)**	44.6	<.001	98.8	<.001	40.2	<.001	16.4	0.007	4.4	0.081

* Log transformed for analysis.

** No sounds are produced by males during mating.

Table 3.21: Analysis of variance (ANOVA) for different temporal parameters of sounds (interrupted chirps) of *H. ligniperda* male under distress and phases of mating.

ANOVA	No. of chirps		Interchirp interval (sec)		Toothstrike per chirp*		Chirp duration* (sec)		Toothstrike rate (No./sec)	
	F	P	F	P	F	P	F	P	F	P
Distress v mating (df 1, 18)	39.3	<.001	719.4	<.001	44.8	<.001	0.2	0.670	32.6	<.001
Before vs. after mating(df 1, 9)**	0.3	0.587	9.8	0.012	4.3	0.068	31.0	<.001	35.0	<.001

* Log transformed for analysis.

** No sounds are produced by males during mating.

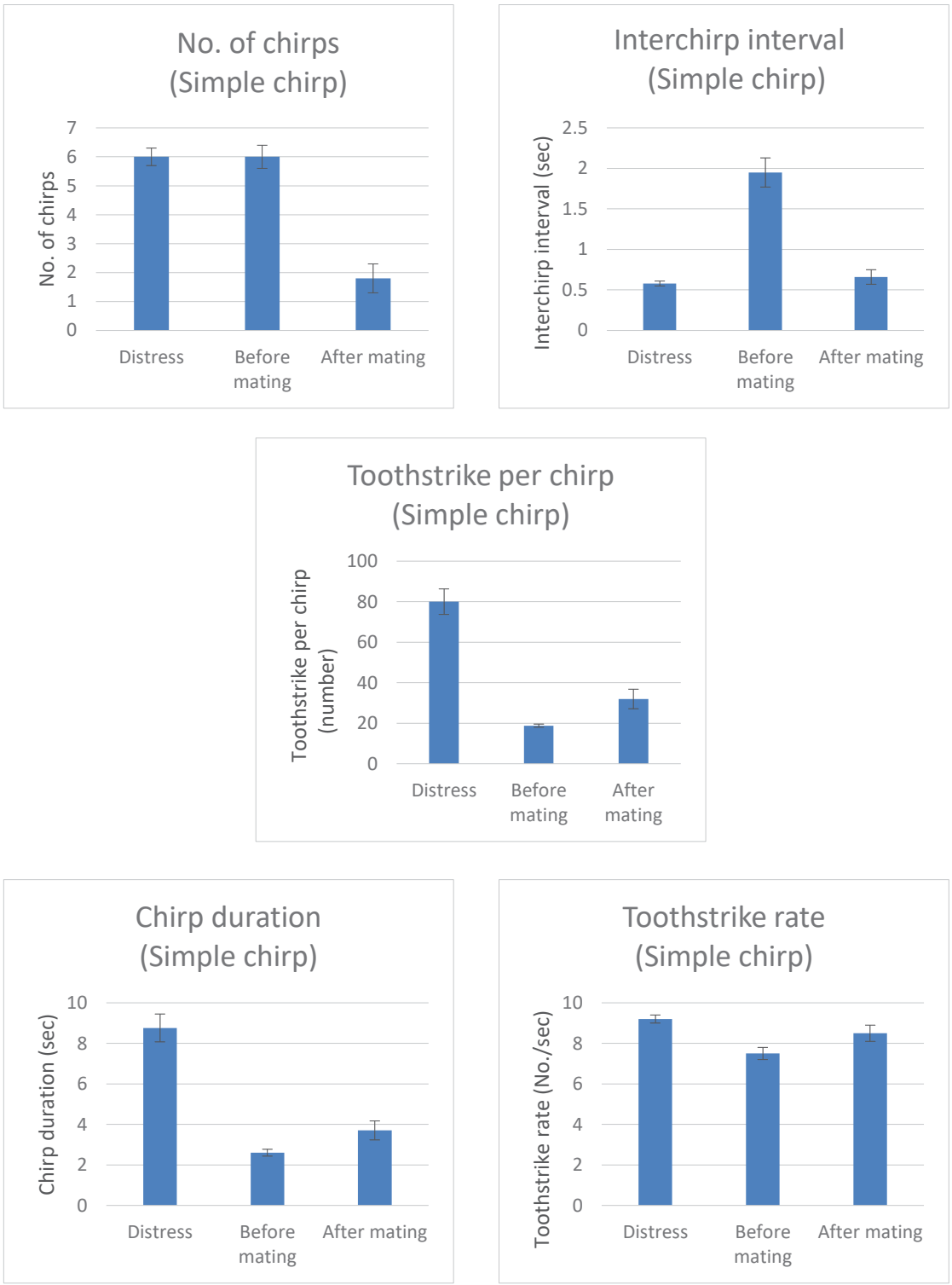


Figure 3.18: Mean values and standard errors (bars) for temporal parameters of *H. ligniperda* male sounds (simple chirps) in different contexts. Note: no sounds are produced during mating.

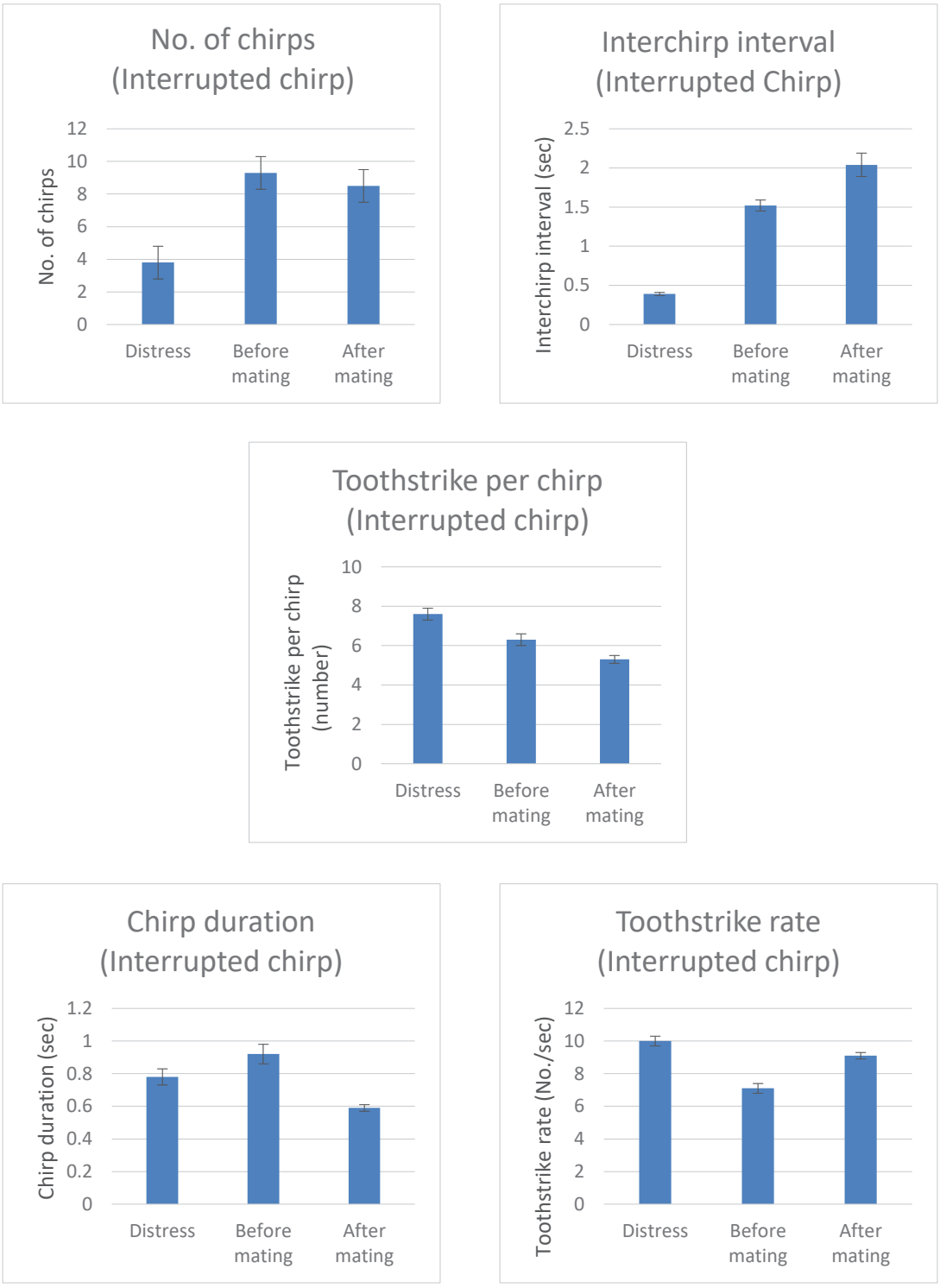


Figure 3.19: Mean values and standard errors (bars) for temporal parameters of *H. ligniperda* male sounds (interrupted chirps) in different contexts. Note: no sounds are produced during mating.

Spectral Comparisons

As the spectral parameters of simple and interrupted chirps did not differ significantly, the data for males were averaged, giving one set of measurements for the male distress trial. The simple and interrupted chirps for both mating phases were also averaged. Furthermore, the data from two mating phases (i.e., before and after mating) were also averaged to get one set of measurements for the mating trial. Then, the spectral data were compared among all trials (Table 3.22). Similarly, in the case of female, the data for different spectral parameters from three different phases of mating (before, during and after mating) were averaged to get one set of measurements for the mating trial. Then the spectral data were compared among all the trial (Table 3.23)

For most parameters the SEs for females were much higher than for males.

There were significant effects of behavioural context for all spectral parameters, and significant gender × context interactions for all parameters, suggesting that the patterns are different between males and females. For some of the parameters there were also significant gender effects, suggesting that one sex had a higher mean than the other. But in most cases the F-values of gender effects were smaller than those for the gender × trial interaction, suggesting the average difference may be lost in the variations between contexts (Table 3.24). Peak frequency was one exception, with values for males being generally higher than those for females (Figure 3.20).

As expected, the toothstrike duration of males was longer than the click duration of females. For both males and females, the toothstrike/click duration was higher in distress than in any other context. Maximum amplitude for males was highest in the colony and territoriality context, and lowest in competition. For females, maximum amplitude was highest in the competition context. The minimum amplitude pattern was similar to the maximum amplitude for both sexes (Figure 3.20).

Table 3.22: Means and standard errors (±SE) for spectral parameters of sounds produced by *H. ligniperda* male in five different behavioural contexts. Letters indicate which means are not significantly different, based on least significant difference test; two means which have a letter in common are not significantly different (α=0.05).

	Toothstrike duration (sec)	**Maximum amplitude**	**Minimum amplitude**	**High frequency (Hz)**	**Low frequency (Hz)**	**Peak frequency (Hz)**
Distress	0.047 ± 0.0066 f	262138 ± 19401 b	-304766±16268 c	21890±295 e	1076±43 ef	4663±239 bc
Mating	0.013 ± 0.0003 c	298227 ± 5343 b	-275479±7268 bc	12465±51 b	430±19 c	6459±164 fg
Competition	0.012 ± 0.00017 b	190383 ± 12633 a	-170817±7209 a	15740±144 c	294±10 b	6927±218 g
Territoriality	0.017 ± 0.00017 d	649965 ± 62686 d	-514507±46930 d	15507±538 c	515±36 d	6978±562 g
Colony	0.020 ± 0.00041 e	764270±67039 d	-661270±25040 e	15285±254 c	232±17 a	5678±216 bdef

Table 3.23: Means and standard errors (±SE) for spectral parameters of sounds produced by *H. ligniperda* female in five different behavioural contexts. Letters indicate which means are not significantly different, based on least significant difference test; two means which have a letter in common are not significantly different (α=0.05).

	Click duration (sec)	**Maximum amplitude**	**Minimum amplitude**	**High frequency (Hz)**	**Low frequency (Hz)**	**Peak frequency (Hz)**
Distress	0.012±0.00016 b	191270±40321 a	-275112±68276 ab	10023±685 a	256±14 ab	1235±117 a
Mating	0.005±0.00015 a	648175±73270 cd	-588408±53509 de	22038±1510 de	1281±99 ef	6370±476 efg
Competition	0.005±0.00030 a	1041600±189034 d	-767881±152396 de	17998±788 cd	1631±863 e	4843±261 bcd
Territoriality	0.005±0.00015 a	321596±54987 b	-302851±64984 bc	21503±3205 de	1435±150 f	4646±707 b
Colony	0.005±0.00028 a	401590±51705 bc	-416972±64488 cd	23875±1408 e	999±113 ef	5115±544 bcde

Table 3.24: Analysis of variance (ANOVA) for spectral parameters of sounds produced by *H. ligniperda* male and female in five different behavioural contexts.

	Toothstrike/ click duration (sec)		**Maximum amplitude***		**Minimum amplitude***		**High frequency (Hz)***		**Low frequency (Hz)***		**Peak frequency (Hz)**	
	F	P	F	P	F	P	F	P	F	P	F	P
Gender (df 1, **)	110.8	<.001	0.1	0.734	0.3	0.583	2.2	0.141	13.3	<.001	36.3	<.001
Context (df 4, **)	250.9	<.001	51.9	<.001	68.7	<.001	112.4	<.001	119.3	<.001	16.8	<.001
Gender*Context (df 4, **)	621.3	<.001	26.9	<.001	17.9	<.001	46.9	<.001	39.3	<.001	5.8	<.001

* Log transformed to stabilise variance.

**Residual degrees of freedom vary between the parameters, depending on the relative size of the male and female variability; values were 68 (toothstrike/click duration), 60 (maximum amplitude), 53 (minimum amplitude), 51 (high frequency), 53 (low frequency) and 79 (peak frequency).

For males, both the high frequency and low frequency were higher in distress than in any other contexts; the peak frequency in distress was lower than in the other contexts. The high frequency in mating was lower than in the colony, competition or territoriality contexts. The low frequency in mating or territoriality was higher than in the colony or competition contexts. The peak frequency in competition or territoriality trials were higher than in the colony context (Figure 3.20).

For females, the high frequency, the low frequency and the peak frequency were lower in distress than in any other context. The high frequencies in the mating and colony contexts were higher than in the competition context. The peak frequency in mating was higher than in competition and territoriality contexts (Figure 3.20).

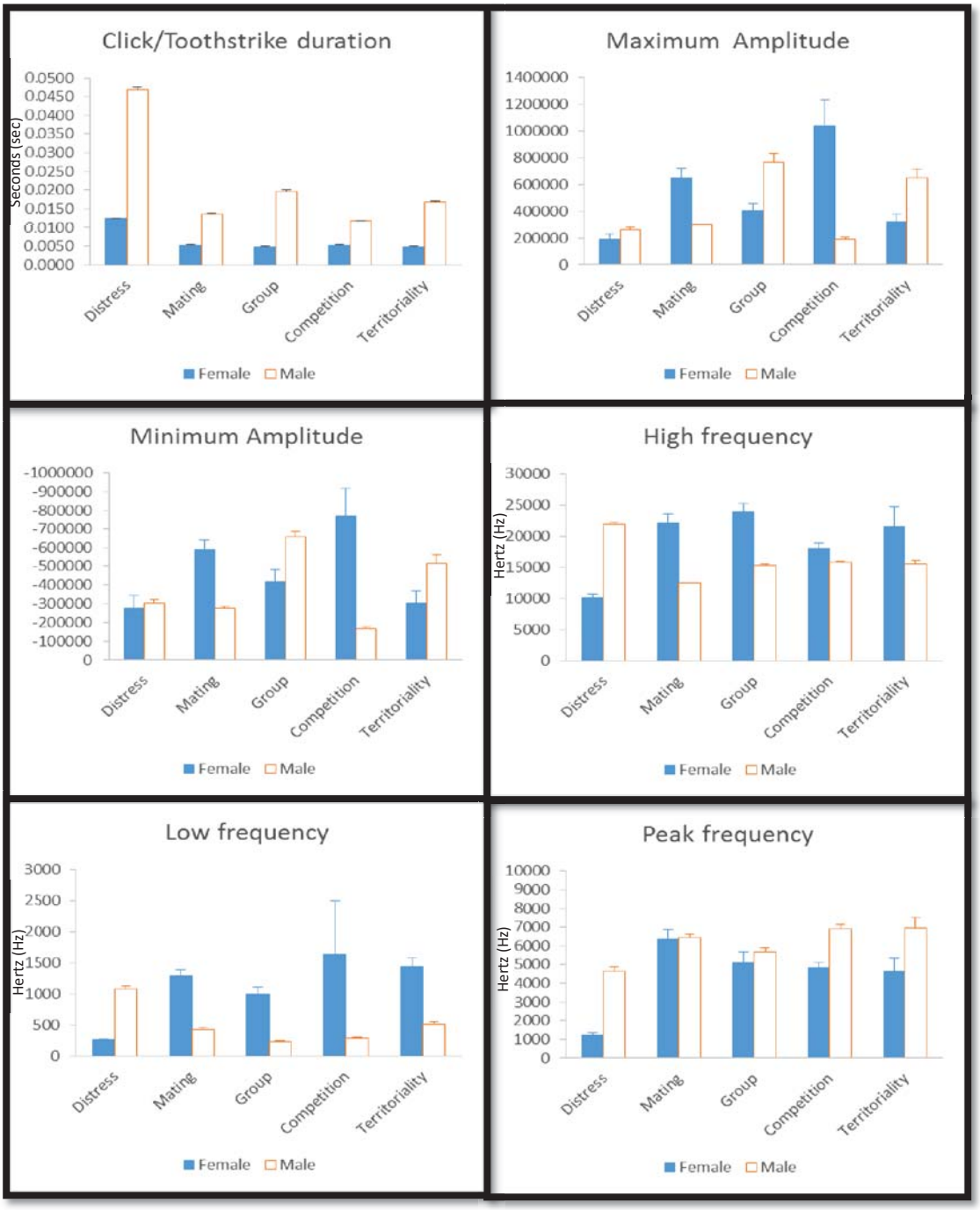


Figure 3.20: Mean values and standard errors (bars) of spectral parameters (toothstrike/click duration, maximum amplitude, minimum amplitude, high frequency, low frequency and peak frequency) for the sounds produced by *H. ligniperda* male and female in five different behavioural contexts.

Principal Component Analysis (PCA) of the Spectral Data

The spectral data were further analysed to look at the correlations between the spectral parameters (Table 3.25). For instance, for both males and females, the maximum and minimum amplitudes are strongly and positively correlated. Similarly, the high and low frequencies are positively correlated. However, in the case of females, the click duration and both the high and the low frequencies were negatively correlated. Similarly, for

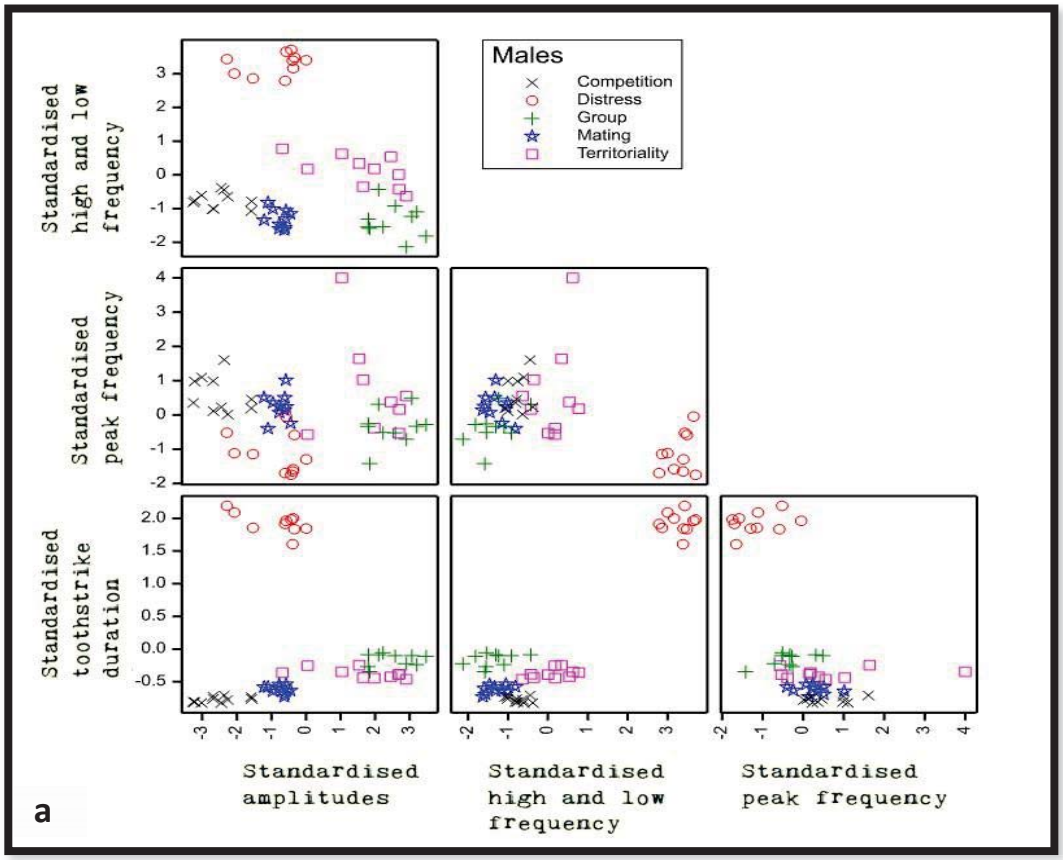
females the peak frequency was positively correlated with high and low frequency whereas in the case of males, the correlation was weak but negative (Table 3.25).

Table 3.25: The correlation matrices (Pearson correlations) of different spectral parameters for male and female *H. ligniperda*. Correlation values >|0.28| are significant at p=0.05; correlations >|0.36| are significant at p=0.01.

Male	**Toothstrike duration (sec)**	**High frequency (Hz) (log 10)**	**Low frequency (Hz) (log 10)**	**Peak frequency (Hz)**	**Maximum amplitude (log 10)**
High frequency* (Hz)	0.85	-			
Low frequency* (Hz)	0.76	0.58	-		
Peak frequency (Hz)	-0.60	-0.35	-0.38	-	
Maximum amplitude*	-0.14	-0.23	-0.26	-0.04	-
Minimum amplitude*	0.06	-0.08	-0.14	-0.14	0.95
Female	**Click duration (Hz)**	**High frequency (Hz) (log 10)**	**Low frequency (Hz) (log 10)**	**Peak frequency (Hz)**	**Maximum amplitude (log 10)**
High frequency* (Hz)	-0.76	-			
Low frequency* (Hz)	-0.75	0.74	-		
Peak frequency (Hz)	-0.72	0.66	0.67	-	
Maximum amplitude*	-0.50	0.55	0.27	0.42	-
Minimum amplitude*	-0.34	0.53	0.20	0.32	0.93

* Log transformed

Principal components analysis (PCA) on male and female spectral data (separately) suggests that three groups of variables account for 92% of the correlation in both male and female data. For males, these groups of variables were (high and low frequency plus toothstrike duration), (maximum and minimum amplitude), and (peak frequency). For females, these groups of variables were (high frequency and low frequency plus click duration), (maximum and minimum amplitude), and (peak frequency). Plotting the pairs of spectral parameters shows some clear trends. First, the sounds produced in distress are clearly separated for both males and females. Second, the sounds produced by males are much more different between different non-distress behavioural contexts, and clusters related to different contexts can be easily identified. On the contrary, for females the distress cluster is clearly separate, but sounds produced in all other behavioural contexts do not group into identifiable clusters (Figure 3.21).



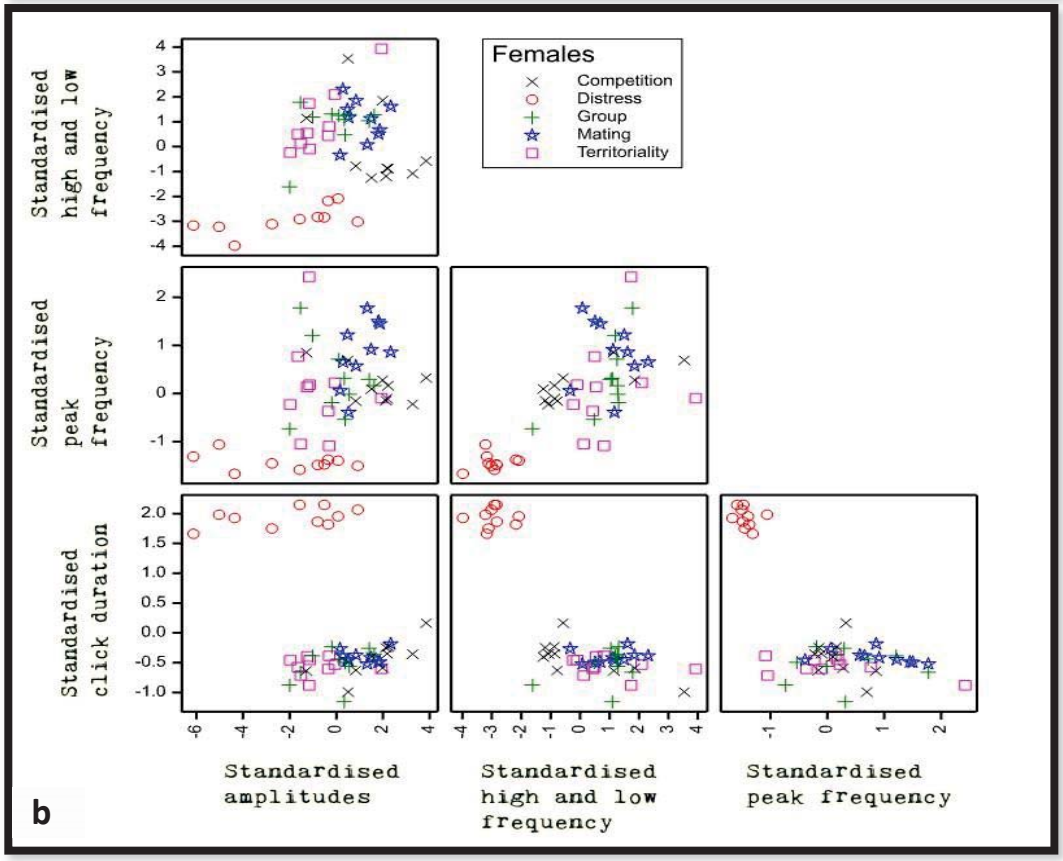


Figure 3.21: Scatterplots for standardised spectral parameters of sounds produced by a) *H. ligniperda* males, b) *H. ligniperda* females in different behavioural contexts.

Behavioural Comparisons

The one-way ANOVA analysis indicated that the first courtship duration, the number of matings, and mating duration differ significantly among the three contexts under which mating occurred (single-pair mating, competition, and colony) (Figure 3.22 and Table 3.26). Both courtship duration and mating duration were significantly higher in single-pair context, and lowest in the colony context. However, the total number of matings was lowest in single pairs, and significantly higher in competition and colony contexts (Figure 3.22).

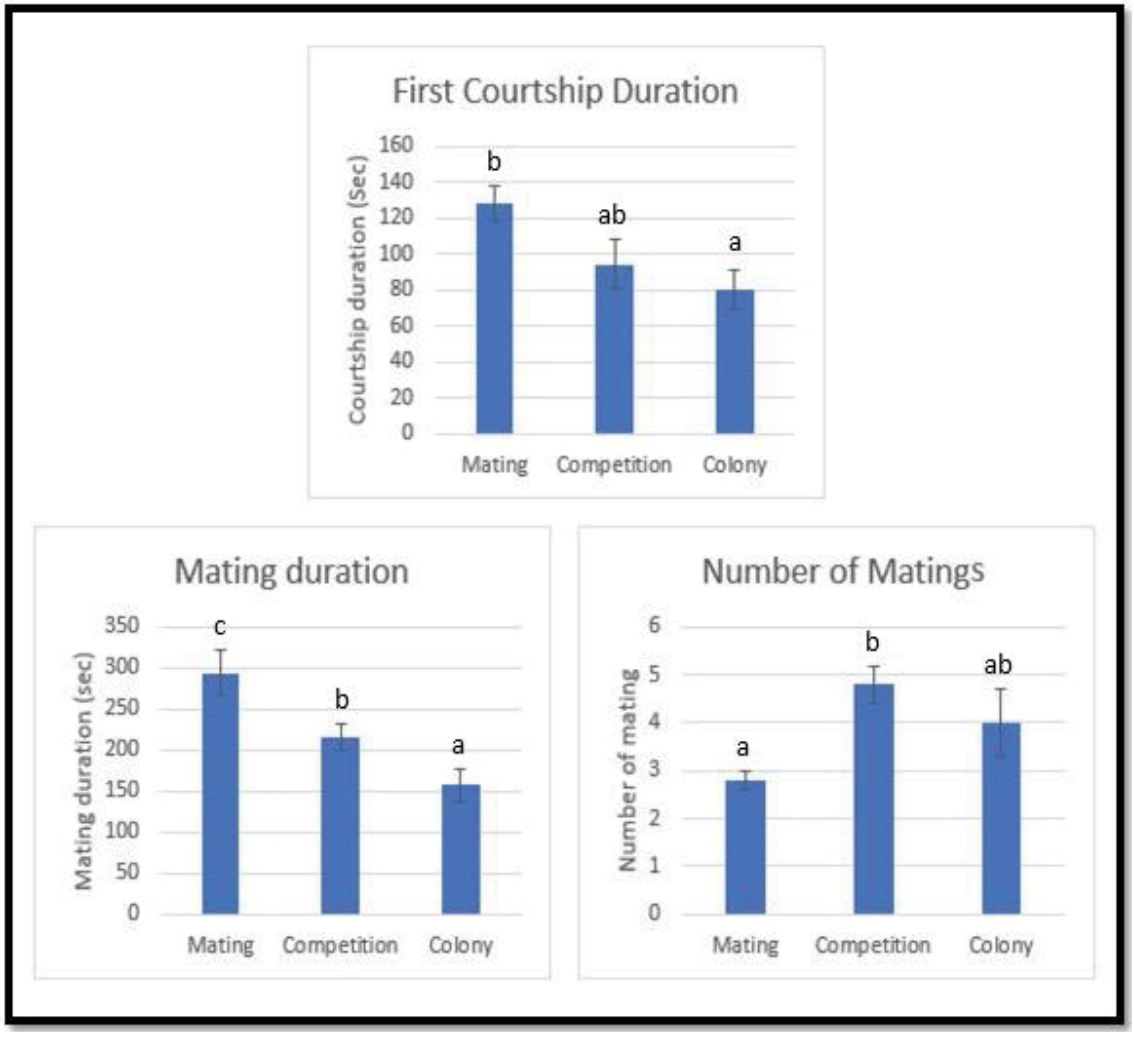


Figure 3.22: Mean values and standard errors (bars) for the first courtship duration, first mating duration and number of mating observed in different contexts: single-pair mating ("mating"), competition and colony. Means with the same letter are not significantly different, LSD, $\alpha = 0.05$.

Table 3.26: Analysis of variance (ANOVA) for first courtship duration, first mating duration and number of mating observed in *H. ligniperda* in different contexts: single-pair mating ("mating"), competition and colony.

	Degrees of freedom	**F-value**	**P-value**
First courtship duration (sec)	2, 25	4.5	0.022
First mating duration (sec)	2, 25	9.3	<.001
Number of matings	2, 27	4.5	0.021

Chapter 4: Discussion

Sound Production

In spite of almost two decades (in the 1970s and 1980s) of bark beetle bioacoustics investigations, this mode of communication still remains one of the least comprehended and to a great extent one of under-appreciated forms of communication in this group of beetles. Compared to the numerous empirical studies in bark beetle sensory physiology and chemical/pheromone ecology, acoustic communication has received relatively little consideration (Ryker, 1988). Given that previous literature in bark beetle acoustics provides limited information on the details of the signal characteristics, transmission and functional significance of signals, and on the receptor mechanisms, this study, which focuses on the acoustic signals produced by *Hylurgus ligniperda* adults in different ecological contexts, aims at understanding the role of acoustics in insect communication. This study is the first study on the acoustic communication of golden haired bark beetles, *Hylurgus ligniperda*, which provide a basis for future investigations. There were three major aspects of this study: the temporal and the spectral comparison of the acoustic signals including the behavioural analysis.

There is a lack of specific definitions and criteria for classifying and differentiating different acoustic signal types across bark beetles. Only some of the studies have explained the temporal characteristics of the signals produced by different bark beetles and have demonstrated variation in terms of chirp types under different behavioural contexts (Ryker & Rudinsky, 1976b; Yandell, 1984). For example, signals produced by *D. ponderosae* have been categorised broadly as ‘simple’ and ‘interrupted’ chirps, and temporal parameters such as chirp duration, number of toothstrike per chirp and toothstrike rates have been defined and analysed (Ryker & Rudinsky, 1976b; Yandell, 1984). The early study done in *Ips pini* by Swaby and Rudinsky (1976) described some temporal characteristics of the signals such as chirp duration, number of toothstrikes, and toothstrike rate. However, those signals were not very broadly analysed. My temporal comparisons gave an idea about the nature of male chirps (simple and interrupted) and the clicks produced by the female. It further explained the role of both simple chirps and interrupted chirps in different behavioural scenarios. However, I could not differentiate the significance and role of the female click sounds although I obtained quantitative measurements of different temporal parameters.

Previous literature on bark beetle acoustics has largely ignored spectral characteristics of the signals, or only provides vague and general statements about the

frequency range (Oester, Ryker, & Rudinsky, 1978; Rudinsky & Michael, 1972; Wilkinson, 1962). Detailed studies on the spectral characteristics of the acoustic signals, such as intensity and frequency, have hardly been done (Fleming, et al., 2013; Yturralde & Hofstetter, 2015). The spectral comparisons in my study gave an indication of the variation in the physical properties of the sounds produced under different behavioural scenarios. The temporal and spectral analyses together suggested that the signals produced are context-specific, indicating that the signals have different specific roles. Similar observations have been found in other bark beetle species such as *Ips pini*, showing the significance of potential hearing organs in order to discriminate and identify such varying signals (Swaby & Rudinsky, 1976). The temporal measurements of the acoustic signals are more related to the size and weight of the individuals, and other experimental factors (Sivalinghem, 2011). The spectral parameters, such as frequency, provide information about signalling being specific to behavioural context (Sivalinghem, 2011). In my experiments, female *H. ligniperda* receiving acoustic signals (chirps) of specific frequency from the males seemed to be potentially extracting messages from the males. The data also indicate that individual males can be identified by the characteristics of the sound produced by them. The possible reasons behind this individual difference could be the variation in the shape and size of the serrated ridges or the teeth of the pars stridens present along the suturnal margin and the tip of the left elytron. This variation could be also due to the age or size of the adult male. However, I could not differentiate two adult females just by listening to their click sounds. To get to the specific conclusions related to these aspects, further studies describing the temporal and spectral characteristics of other species across genera are needed. Given that *H. ligniperda* live sympatrically with *Hylastes ater* (Reay & Walsh, 2001), it would be interesting to compare the sound frequencies of these two species in order to determine the degree of overlap between them. A similar frequency range may indicate that these signals are probably shaped by the signalling environment, whereas the signal variation may indicate species-specific signals (Endler, 1992).

In all contexts tested, except distress, the individuals were found to produce sounds upon detecting the presence of another individual of the same species in the cambium chamber, regardless of the sex. This was similar to the results obtained by Fleming et al. (2013), where the male of *Dendroctonus ponderosae* upon encountering females started producing interrupted chirps, ultimately leading to the mixture of simple and interrupted chirps. Similar behaviour was observed when two of the males were

introduced to each other (Fleming et al., 2013). During male territoriality trials in my experiment, chirps (either simple of interrupted) were produced when one male was making physical contacts with other male(s). The sound was not produced when the males were in different locations scrapping the cambium and without any contact. However, males were found to produce more chirps in other trials (mating, competition and colony) where females were present (even if they were physically apart). Neither males nor females were observed to produce sounds when by themselves. This verifies that sound plays a role in communication of *H. ligniperda* and seems to play a role in sexual communication.

Although the females were also found to produce sounds, the characteristics of the clicks could not be differentiated among different behavioural activities. Furthermore, when observing the waveforms and spectrograms of clicks produced within a specific context (such as distress or mating), and comparing successively produced clicks, I could not find any visible pattern. Therefore, as compared to females, the male-produced sounds seem to play more roles in the communication system of this species, whereas for females, olfaction might have a more relevant role in their communication through pheromone-recognition. As mentioned in the literature, the sex responsible for selecting host tree and breeding site, gallery construction and production of pheromones is normally silent as compared to the other one (Byrne et al., 1974). It has already been found that the females of *H. ligniperda* are the ones responsible for such activities (Ciesla, 1988). Our behavioural study also shows the leading role of female in the tunnelling (gallery construction). Meanwhile, the males of *H. ligniperda* have the ability to produce an audible sound, which may be important in communication with the females.

Furthermore, acoustic studies in *Ips pini* (Sivalinghem, 2011) and *Dendroctonus ponderosae* (Fleming et al., 2013) have shown that acoustic signals are transmitted through both the air and the solid phloem substrate. Most of the communication between males and females occurs inside galleries, where the sound vibrations can theoretically be transmitted through the legs, elytra and pronotum (Sivalinghem, 2011). Understanding the transmission properties of the phloem substrate, and how effectively the vibrated signals are transmitted, is important for determining possible functions of vibratory signals (Stölting, Moore, & Lakes-Harlan, 2002).

In order to have the functional role of sound communication, transmitted signals should be perceived by the receiver. Furthermore, the functional role of the receptor

organ have hardly been studied in some species such as scarab and tiger beetles (Forrest, Read, Farris, & Hoy, 1997; Spangler, 1988). It is surprising that to date, no detail study has examined the receptor mechanisms involved in perceiving these acoustic signals in any bark beetle species. Future studies of such receptor organs in *H. ligniperda* would help to solve the queries about their sound communication system.

Observing the sounds produced by distressed males, I noted that sounds produced under distress are very different in nature compared to the other contexts, indicating either pain or stress. These sounds do not seem to have a communication role because the characteristics of the sounds produced throughout the observation period were similar in every single individual tested. However, whether this distress sounds could be used as an alarm system to warn other conspecifics of the presence of a predator or any other threat, remains to be tested.

In a study of acoustic signals of *Dendroctonous ponderosae* male during distress, both simple and interrupted chirps had similar toothstrike rates (Fleming, et al., 2013). In the Colorado pine beetle *D. approximates* males and females were found to produce more simple chirps than interrupted chirps when distressed (Yturralde & Hofstetter, 2015). This is similar to the results obtained in my experiment. There were always more simple chirps than interrupted chirps produced under distress, whereas there was no significant difference in the toothstrike rate between these two types of chirps. This indicates that the pace of production was similar, probably because the file and the plectrum were continuously scrapping each other in order to express pain or stress. The production of an interrupted chirp in between two simple chirps seems logical. Interrupted chirps in many bark beetle species are considered to be the result of plectrum temporarily being dislodged from the pars stridens (Michael & Rudinsky, 1972; Oester, et al., 1978; Rudinsky & Ryker, 1976). The file and plectrum are disengaged for a certain period of time after producing a certain number of toothstrikes (below ten), in order to gain enough energy to produce another simple chirp (which has a longer toothstrike duration with an average of 80 toothstrikes per chirp) immediately after. When different physical properties (spectral measurements) of simple and interrupted chirps produced by males under distress were compared, there was no significant difference in each of the parameters compared, which further indicates that the physical properties of the distressed sounds are similar in simple and interrupted chirps. Interrupted chirps in this context could be functioning for the production of successive simple chirps with longer chirp duration. Similarly, although the nature of

production of sounds in between males and females is different, the significant increase in the number of clicks produced per minute by distressed females, when compared to mating, also indicated that the females express their stress/pain by producing more clicks with longer durations.

Observing the three phases of mating demonstrated that male-produced sounds play a role in the communication between the sexes, particularly before and after mating. As the males approached the females, the number of interrupted chirps increased while the simple chirps slowly declined. The sound production by males showed an overall decline as mating approached, when more emphasis was given to stimulating the female for mating. As soon as the male started mating, sound production by the male declined to nil. Such behaviour has not been reported in any of the bark beetles species yet. As soon as mating finished, males reinitiated sound production by emitting interrupted chirps. As time elapsed, the number of interrupted chirps slowly declined while the number of simple chirps slowly increased. Compared to the sound production during distress, the simple chirps produced before and after mating were significantly lower in terms of toothstrikes per chirp. On the contrary, the number of interrupted chirps was significantly higher (with longer interchirp interval) before and after mating as compared to the distress context. This indicates that interrupted rather than simple chirps might play specific roles in communication between the sexes before and after mating. There were not many significant differences in the spectral parameters of sounds produced by males when simple and interrupted chirps produced before and after mating were compared. However, significant differences in temporal parameters (except toothstrike rate) between simple and interrupted chirps were observed. This indicates that (a) the requirement for sound production is less during this stage (during mating and a period just before and after mating) and (b) interrupted chirps play a more important functional role than simple chirps. It has been reported that the interrupted chirps do not have any specific communication function and are to be caused by some obstruction during stridulation (Oester & Rudinsky, 1978). However, my data suggest that in *H. ligniperda* interrupted chirps have the role in communication, especially during the event of mating. Similar results were obtained in the acoustic study of *Dendroctonous ponderosae* male (Fleming et al., 2013). Males signalled consistently throughout in the presence of female, and females signalled intermittently. During male-female interactions, the males were found to produce shorter simple chirps than in distress, with smaller number of toothstrikes. There was higher number of interrupted

chirps as compared to simple chirps. Interrupted chirps had significantly lower toothstrikes and longer interchirp interval than simple chirps (Fleming, et al., 2013).

Similarly, although *H. ligniperda* females were observed to produce click-like sounds during all stages of mating, there was significantly lower number of clicks produced during mating as compared to when females were under distress. The interclick interval, on the contrary, increased significantly during mating compared to when under distress. This indicates that the role of female-produced sounds decreases during mating. Furthermore, when the male and the female start tunnelling, negligible production of chirps or clicks is observed. This also indicates that the role of female-produced sounds is minor after mating, when the focus of the females turns to gallery construction and egg-laying.

When *Dendroctonous. ponderosae* adults were observed under different behavioural contexts such as distress and male-female interactions, it was shown that the acoustic signals were produced at a frequency range between 15600 to 26400 Hz (Fleming, et al., 2013). I determined that the males of *H. ligniperda* can produce the high frequency of acoustic signals in the range from 12465 Hz to 21890 Hz, with the low frequency ranging from 232 Hz to 1076 Hz. The Colorado pine beetle, *D. approximates* males and female produced an acoustic signal of peak frequency 5293 Hz and 11348 Hz under distress condition. The males, upon the interaction with a female, were observed to produce acoustic signals with the mean frequency of 5686 Hz, while the female signal was not reported (Yturralde & Hofstetter, 2015). In my experiments, the peak frequency was within the level of 4663 Hz to 6978 Hz for males and 1235 Hz to 6370 Hz for females. Observing the frequencies reported in other bark beetle species, it seems that bark beetles have similar range of sound frequency, which probably indicates that this range is ideal for the transmission of sound waves through the airborne or vibratory substrate.

Behaviour

It has been shown that male mate choice and associated behaviours in some bark beetles, such as *I. pini,* depends on different factors such as host-tree quality, population density on a given host tree, quality of the male, and female quality (Sivalinghem, 2011). Addressing the importance of acoustic signals during male mate choice is fundamental to understanding the behaviours of different bark beetle species. Furthermore, the properties of different ecological behaviours and associated acoustic

signals vary depending upon the local environment, for example temperature and humidity, and other factors such as presence of local predators (Nevo & Capranica, 1985).

Within my experimental set up, males of *H. ligniperda* were found to perform long courtship displays mostly prior to the first mating. It seems that the courtship is always displayed by the males for the first mating when the female is a virgin. Courtship during subsequent matings (after the first mating) was only observed in three replications out of ten in mating trial. In competition trial, none of the males engaged in courtship prior to subsequent matings. In a colony, only two males (each from different replications) displayed courtship in subsequent matings; in both cases the female was mated by the same male previously, and no other males had mated the female in between. Courtship displays never occurred when the female was already mated by a different male. In such cases, the male was only found to use their front two legs for grooming. It also appears that competition between males affects the duration of courtship displays. When there were no other males competing for a female (such as in the single-pair mating trial), the duration of courtship was comparatively longer than when males were exposed to competition or when in a colony. In other species (pine engraver beetle, *Ips pini*), it has been demonstrated that female signal parameters were correlated with female size and weight, and it is suggested that males may use these signals to determine female size during mate choice (Robertson, 1998; Robertson & Roitberg, 1998). Therefore, parameters such as courtship displays duration and mating duration may be related to female quality, especially during competition or where the males have more choice. Before determining whether males use acoustic signals to assess female size (and choose the partner), it is important for future studies to first determine whether males reject signalling females, and whether female size is related to reproductive success in *H. ligniperda*.

The male *Dendroctonous frontalis* beetles have been found to fight and stridulated loudly when other males are attracted towards the female at the entrance of the gallery (Rudinsky & Michael, 1973). Similarly, McGhehey (1968) observed two males of *D. ponderosae* fighting with loud chirps when males were put into the female gallery. The territory of the beetle pair is considered to be established by the female as soon as the female starts constructing gallery and starts attracting males for mating. The female acoustic signals apparently have spacing effect in this site selection (Rudinsky & Michael, 1973). Male physical aggression in my experiments was apparent when in

competitive situation or when in a colony. Aggression was also found to be expressed with the production of loud distinct chirps. However, aggression was not observed in male territoriality trials in the absence of females. On the other hand, some forms of physical aggression were observed among *H. ligniperda* females in the absence of males (i.e. in female territoriality trial), when a particular female was trying to stop another female from tunnelling around the same area. Interestingly, competition among females was not observed when in a colony. This clearly shows that the role of competition is different for the two sexes. While males seem to compete more for females while trying to mate, females seem to compete more among themselves when engaged in tunnelling.

Although the literature seems to indicate that *H. ligniperda* adults are monogamous (Lanfranco, et al., 2001), my behavioural observations showed that both males and females are able to mate with multiple partners multiple times, making them a polygamous species. However, this discrepancy may be due to my experimental setup. In the natural environment, the female enters through the bark and makes a short entrance tunnel, including a slanted or inclined nuptial chamber in the innermost bark (phloem) of the pine trees, near the base of the stem or large exposed roots/stumps of recently cut trees buried or touching the soil (Ciesla, 1988). The pheromones are then used by the female to attract many males. The female then chooses one potential male. The chosen male then enters through the entrance tunnel and mates with the female. However, in my experimental set up, the females were not required to make an entrance tunnel and were exposed to more than one male at a time. Thus, either the courtship process was simplified under these conditions, which allowed more males to have access to females, or perhaps the females did not have enough sperm for egg fertilization during the first mating and thus were required to mate a second time. However, why another male instead of the same one would be chosen after the first mating remains unclear. Mating with more males would occur if the frequency and duration of copulation is short (Gromko, Gilbert, & Richmond, 1984; Ridley, 1988), if the sperm has not been transferred well into the spermathecae, or if there is a deficit in the amount of sperm (not enough for the fertilization of the eggs) (Pitnick, Wolfner, & Suarez, 2009; Warner, Shapiro, Marcanato, & Petersen, 1995). Furthermore, mating with multiple partners could also happen from the attraction of other potential mating partners (Jones, McNamara, Colvin, Featherston, & Elgar, 2006). Reid and Stamps (1997) reported that the males of *Ips pini* indiscriminately accept and can mate with two

to three females, but become choosier later on. The mate choice later becomes judgemental, depending upon the factors such as population density, female quality, and host tree quality (Robins & Reid, 1997). The female *I. pini* have an adjustable mate selection threshold (Stamps, 1997). Further research is needed to see whether *H. ligniperda* males/females discriminate between females/males, and whether this is correlated with the type of the signal produced or with any physical parameters such as body size or age. Mate selection criteria should also be studied for females, to understand potential mate selection, and explain the reason behind multiple matings with multiple partners.

It seems that *H. ligniperda* females have well-developed mechanisms to bore tunnels compared to males. Although the males were observed to make some tunnels when in a territoriality context (in the absence of females), their tunnelling was slower and less productive than that of females. Similar behaviour has been reported in Mountain Pine beetle *Dendroctonous monticolae* (Ridley, 1988) The female *D. monticolae* has been observed to initiate the tunnelling behaviour and pass the tunnelled frass to the male behind her. When the bulk has been collected, the male has been found to push frass out of the entry tunnel. The female then moved forward, continuing tunnelling (Ridley, 1988). Similar behaviour was observed in the case of *Ips pini* (Schmitz, 1972). Immediately after mating, the female was observed to construct an egg gallery in the artificial experimental chamber. The female bit off pieces of the phloem and pushed those particles beneath and behind her, using all legs, and passed it to the male in order to throw it out of the entrance hole. During the final phase of gallery construction, some of the frass were kept in order to cover the eggs (Schmitz, 1972). Interestingly, in my experiments, although females were very capable of making their own tunnels when in a territoriality context (in the absence of males), clearing frass out of the tunnel took females very long, which reduced the efficiency of tunnel-making. In contrast, tunnelling process was efficient in mating and competition contexts, where a clear division of labour was observed – the female was always leading and digging the tunnel while the male was assisting the female by clearing out frass. Interestingly, in the absence of females males were observed to work together, i.e. scrapping the cambium together. However, this bonding did not last long as compared to when a male and a female were working together. This signifies that the tunnelling process seems to be a task requiring both sexes.

Chapter 5: Conclusions and Recommendations

Conclusions

I was successful in fulfilling the objectives of my experiments. It is clear that sound plays an important role in communication for *H. ligniperda*. Although females also produce click-like sounds, the sounds made by males seem to have a leading and dominant role in the communication system in this species. Both males and females were observed to produce sounds upon feeling the presence of another individual in the close vicinity. Observing the sound production under different behavioural contexts, it seems that the role of sound in communication to be oriented more towards the opposite sex than towards individuals of the same sex. When distressed, the males produce significantly higher number of simple chirps with a longer chirp duration and higher toothstrike rate. I also observed similar pattern in the case of distressed female. The role of interrupted chirps seems to focus on helping in the production of successive simple chirps. However, in a mating context, I observed that interrupted chirps seemed to play a more significant role. I also determined that the males of *H. ligniperda* can produce the high frequency of acoustic signals in the range from 12465 Hz to 21890 Hz, with the low frequency ranging from 232 Hz to 1076 Hz. The peak frequency was within the level of 4663 Hz to 6978 Hz. The minimum and maximum amplitudes for male acoustic signals were highest when in a colony context (-661270 and 764270) and lowest during competition (-12633 and 190383). The males did not produce any sounds during mating.

Females can produce high frequency of acoustic signals in the range from 10023 Hz to 23875 Hz with the low frequency ranging from 256 Hz to 1631 Hz. The peak frequency was within the level of 1235 Hz to 6370 Hz. The minimum and maximum amplitudes for the female acoustic signals were highest during competition with other females (-189034 and 1041600). The minimum and maximum amplitudes were lowest when they were distressed (-275112 and 191270). Toothstrike and click duration were longest for both males (0.047 sec) and females (0.012) when distressed.

Behavioural observation indicated that the courtship is always displayed by the males for the first mating when the female is a virgin. Courtship displays never occurred when the female was already mated by a different male, and the duration of the courtship displays was affected by the competition between the males to mate with a female. The duration of courtship was found to be comparatively longer when there were no other males competing for a female. Although the *H. ligniperda* were reported

as a monogamous species, my experimental observation indicate that they may be polygamous in some situations. The tunnelling activity was always more meaningful in the presence of both sexes together.

Recommendations for Future Work

During the experiments, I really struggled with the background noise which hampered the accuracy of data collection. A temperature-controlled and sound-proof room specifically designed for recording sound should be used for future experiments. Similarly, as I was doing sound and behaviour analysis (video recordings) together, I had to synchronise the time code manually using a video editing software. The matching up the timeline of both audio and video recordings for the behaviour observation process was very time-consuming. Having a sound recorder that can perform the synchronisation process automatically will be desirable for such kind of combined acoustic and behavioural study, both in terms of time saving and accuracy. Furthermore, I modified the natural habitat of the beetles for my experiments. I extracted the cambium and made a cambium chamber ("sandwich") where the beetles were artificially introduced. I only observed behavioural activities up to tunnelling activities because of time constraints. However, it could have been more meaningful if mating, egg laying and gallery construction process were observed in a real pine log. It could also help us to know more about the role of acoustics in different life stages. Likewise, from my experiments, I observed that sound seems to be have less functional role in *H. ligniperda* female communication. The females might rely more on communication through pheromones. In future experiments, looking at pheromones along with acoustics could further help to unravel the functioning of beetle communication. In addition, as I could not identify the reason for multiple matings, microscopic examinations of female spermathecae could have helped in solving this issue.

Timeframe of my project did not allow playing back different sounds produced by both males and females to determine if they induce any behavioural changes in adults of either sex. These experiments should be included in future work as part of the main aim of determining the role of acoustics in beetle communication.

Literature Cited

Alexande.Rd. (1967). Acoustical communication in arthropods. *Annual Review of Entomology, 12*, 495-+. doi: 10.1146/annurev.en.12.010167.002431

Alexander, R. D., Moore, T. E., & Woodruff, R. E. (1963). The evolutionary differentiation of stridulatory signals in beetles (insecta: coleoptera). *Animal Behaviour, 11*((1)), 111-115. doi: 10.1016/0003-3472(63)90018-6

Andersson, M. N., Larsson, M. C., & Schlyter, F. (2009). Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: Single-cell responses to ecologically relevant odors. *Journal of Insect Physiology, 55*(6), 556-567. doi: 10.1016/j.jinsphys.2009.01.018

Arrow, G. J. (1942). The origin of stridulation in beetles. *Proc Roy Ent Soc London Ser A, 17*((7/9)), 83-86.

Bain, J. (1977). *Hylurgus ligniperda (Fabricius),(Coleoptera: Scolytidae)*: Forest Research Institute, New Zealand Forest Service.

Barr, B. A. (1969). Sound production in Scolytidae (Soleoptera) with emphasis on the genus *Ips*. *The Canadian Entomologist, 101*(06), 636-672.

Barrington, A. M., Logan, D. P., & Connolly, P. G. (2015). A method for rearing *Arhopalus ferus* (Mulsant) (Coleoptera: Cerambycidae) larvae on a modified artificial diet. *New Zealand Plant Protection, 68*, 353-359.

Bentz, B. J., Regniere, J., Fettig, C. J., Hansen, E. M., Hayes, J. L., Hicke, J. A., . . . Seybold, S. J. (2010). Climate change and bark beetles of the western United States and Canada: direct and indirect effects. *Bioscience, 60*(8), 602-613. doi: 10.1525/bio.2010.60.8.6

Bright, D. E. (2014). A catalogue of Scolytidae and Platypodidae (Coleoptera), Supplement 3 (2000-2010), with notes on subfamily and tribal reclassifications. *Insecta Mundi*(0356), 1-336.

Browne, F. G. (1968). *Pests and diseases of forest plantation trees. An annotated list of the principal species occurring in the British Commonwealth*: Oxford, Clarendon Press.

Buchler, E. R., Wright, T. B., & Brown, E. D. (1981). On the functions of stridulation by the passlid beetle *Odontotaenius disjunctus* (Coleoptera, Pasalidae). *Animal Behaviour, 29*(MAY), 483-486. doi: 10.1016/s0003-3472(81)80108-x

Byers, J. A. (2007). Simulation of mating disruption and mass trapping with competitive attraction and camouflage. *Environmental entomology, 36*(6), 1328-1338.

Byrne, K., & Swigar, A. Silverst. Rm, Borden JH, Stokkink E (1974) Sulcatol—population aggregation pheromone in Scolytid beetle, *Gnathotrichus sulcatus*. *J Insect Physiol, 20*, 1895-1900.

Campbell, S. A. (2004). *Integration of visual and olfactory cues by host-seeking forest coleoptera (Scolytidae, Cerambycidae)*. Simon Fraser University.

Cavey, J., & Passoa, S. (1994). *Screening aids for exotic bark beetles in the Northeastern United States*: United States Department of Agriculture, Forest Service, Northeastern Area.

Clare, G., & George, E. (2016). Life cycle and mass-rearing of *Hylurgus ligniperda* using a novel egg-collection method. *New Zealand Plant Protection, 69*, 143-152.

Claridge, L. C. (1968). Sound production in species of *Rhynchaenus* (Orchestes) (Coleoptera-Curculionidae). *Transactions of the Royal Entomological Society of London, 120*, 287

Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters, 7*(8), 721-733.

DATA, U. D. (1994). Npga data: *Hylurgus ligniperda* red haired bark beetle. December, 1994.

Drosopoulos, S., & Claridge, M. F. (2006). *Insect sounds and communication: physiology, behaviour, ecology and evolution*. Boca Raton,: CRC Press LLC.

Dumortier, B. (1963). *The physical characteristics of sound emissions in arthropoda Insecta, Crustacea, Arachnida*: American Elsevier Publication Co.

Dumouchel, L., & Palisek, S. (2002). Red-haired Pine Bark Beetle, *Hylurgus ligniperda* (F.). Pest Risk Assessment. *Plant Health Risk Assessment Unit. Science Division. Canadian Food Inspection Agency*.

Dunn, G. A. (1996). *Insects of the Great Lakes region*: University of Michigan Press.

Eisner, T., Aneshansley, D., Eisner, M., Rutowski, R., Chong, B., & Meinwald, J. (1974). Chemical defense and sound production in Australian Tenebrionid beetles *Adelium* spp. *Psyche (Cambridge), 81*(1), 189-208. doi: 10.1155/1974/63815

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *The American Naturalist, 139*, S125-S153.

Fabre, J. P., & Carle, P. (1975). Contribution to the biological study of *Hylurgus ligniperda* Coleoptera Scolytidae in the southeastern part of France. *Annales des Sciences Forestieres (Paris), 32*(1), 55-71.

Fields, P. G., & White, N. D. (2002). Alternatives to methyl bromide treatments for stored product and quarantine insects 1. *Annual review of entomology, 47*(1), 331-359.

Fleming, A. J., Lindeman, A. A., Carroll, A. L., & Yack, J. E. (2013). Acoustics of the mountain pine beetle (*Dendroctonus ponderosae*) (Curculionidae, Scolytinae): sonic, ultrasonic, and vibration characteristics. *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 91*(4), 235-244. doi: 10.1139/cjz-2012-0239

Forrest, T., Read, M., Farris, H., & Hoy, R. (1997). A tympanal hearing organ in Scarab beetles. *Journal of Experimental Biology, 200*(3), 601-606.

Gromko, M. H., Gilbert, D. G., & Richmond, R. C. (1984). Sperm transfer and use in the multiple mating system of Drosophila. *Sperm competition and the evolution of animal mating systems*, 371-426.

Grune, S. (1979). *Brief illustrated key to European bark beetles*. Hannover, German Federal Republic: Verlag M. & H. Schaper.

Grüne, S. (1979). *Handbuch zur Bestimmung der europäischen Borkenkäfer: Brief illustrated key to European bark beetles*: M. & H. Schaper.

Harrington, T. (1988). Leptographium species, their distributions, hosts and insect vectors.

Hausner, G., Iranpour, M., Kim, J.-J., Breuil, C., Davis, C., Gibb, E., . . . Hopkin, A. (2005). Fungi vectored by the introduced bark beetle Tomicus piniperda in Ontario, Canada, and comments on the taxonomy of Leptographium lundbergii, Leptographium terebrantis, Leptographium truncatum, and Leptographium wingfieldii. *Botany, 83*(10), 1222-1237.

Hirschberger, P. (2001). Stridulation in Aphodius dung beetles: Behavioral context and intraspecific variability of song patterns in *Aphodius ater* (Scarabaeidae). *Journal of Insect Behavior, 14*(1), 69-88. doi: 10.1023/a:1007801713479

Hyder, D. E., & Oseto, C. Y. (1989). Structure of the stridulatory apparatus and analysis of the sound produced by *Smicronyx fulvus* and *Smicronyx sordidus* (Coleoptera, Curculionidae, Erirrhininae, Smicronychini). *Journal of Morphology, 201*(1), 69-84. doi: 10.1002/jmor.1052010107

Jones, T. M., McNamara, K. B., Colvin, P. G., Featherston, R., & Elgar, M. A. (2006). Mating frequency, fecundity and fertilization success in the hide beetle, *Dermestes maculatus. Journal of insect behavior, 19*(3), 357-371.

Kerr, J. L. (2010). Olfactory and visual cues in host finding by bark beetles.

Kimoto, T., & Duthie-Holt, M. (2004). Exotic forest insect guidebook. *Plant Pest Survey Unit, Canadian Food Inspection Agency*.

Lanfranco, D., Ide, S., & Peredo, H. (2004). An analysis of health risk reduction in Chilean primary forest products for export. *Forestry, 77*(3), 193-203.

Lanfranco, D., Ide, S., Ruiz, C., Peredo, H., & Vives, I. (2001). Sex ratio of *Hylurgus ligniperda* (F.), *Hylastes ater* (Paykull) and *Gnathotrupes* spp. (Coleoptera: Scolytidae)

Liu, D., Bohne, M. J., Lee, J. C., Flint, M. L., Penrose, R. L., & Seybold, S. J. (2007). New introduction in California: The redhaired pine bark beetle, *Hylurgus ligniperda* Fabricius.

Liu, D. G., Flint, M. L., & Seybold, S. J. (2008). A secondary sexual character in the redhaired pine bark beetle, *Hylurgus ligniperda* Fabricius (Coleoptera : Scolytidae). *Pan-Pacific Entomologist, 84*(1), 26-28. doi: 10.3956/2007-21.1

Lyal, C. H. C., & King, T. (1996). Elytro-tergal stridulation in weevils (Insecta: Coleoptera: Curculionoidea). *Journal of Natural History, 30*(5), 703-773. doi: 10.1080/00222939600770391

Mankin, R. W., Moore, A., Samson, P. R., & Chandler, K. J. (2009). Acoustic characteristics of Dunastid beetle stridulations. *Florida Entomologist, 92*(1), 123-133. doi: 10.1653/024.092.0119

Masters, W. M. (1979). Insect disturbance stridulation - its defensive role . *Behavioral Ecology and Sociobiology, 5*(2), 187-200.

Masters, W. M. (1980). Insect disturbancestridulation-characterization of airborne and vibrational components of the sound. *Journal of Comparative Physiology, 135*(3), 259-268.

Matusick III, G. (2010). *Pathogenicity and virulence of root-inhabiting ophiostomatoid fungi on Pinus species of the southeastern United States.* Auburn University.

Mausel, D. L., Gara, R. I., Lanfranco, D., Ruiz, C., Ide, S., & Azat, R. (2006). The introduced bark beetles *Hylurgus ligniperda* and *Hylastes ater* (Coleoptera: Scolytidae) in Chile: seasonal flight and effect of *Pinus radiata* log placement on colonization. *Canadian Journal of Forest Research, 37*(1), 156-169.

McGhehey, J. H. (1968). Territoral behaviour of bark - beetle males. *Canadian Entomologist, 100*(11), 1153-&.

Michael, R., & Rudinsky, J. (1972). Sound production in Scolytidae: specificity in male Dendroctonus beetles. *Journal of Insect Physiology, 18*(11), 2189-2201.

Michelsen, A. (1966). On the evolution of tactile stimulatory actions in Longhorned beetles (Cerambycidae, Coleoptera). *Zeitschrift fur Tierpsychologie, 23*(3), 257-266.

Milligan, R. H. (1978). *Hylastes Ater (Paykull),(Coleoptera: Scolytidae): Black pine bark beetle*: Forest Research Institute, New Zealand Forest Service.

New Zealand Forest Service. (1974). *New bark beetle Hylurgus ligniperda (Family: Scolytidae) discovered in New Zealand.* Wellington, New Zealand. [4]pp.

New Zealand pest and beneficial insects. (1984). Canterbury, New Zealand: Lincoln University College of Agriculture.

Oester, P., Ryker, L., & Rudinsky, J. (1978). Complex male premating stridulation of the bark beetle *Hylurgops rugipennis* (Mann.). *The Coleopterists' Bulletin*, 93-98.

Oester, P. T., & Rudinsky, J. A. (1975). Sound production in scolytidae stridulation by silent Ips bark beetles. *Zeitschrift fuer Angewandte Entomologie, 79*(4), 421-427.

Oester, P. T., & Rudinsky, J. A. (1979). Acoustic behaviour of three sympatric species of *ips* (Coleoptera - Scolytidae) co-inhabiting *Sitka spruce*. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 87*(4), 398-412.

Oester, P. T., Ryker, L. C., & Rudinsky, J. A. (1978). Complex male premating stridulation of the bark beetle *Hylurgops rugipennis* (Mann.). *Coleopterists Bulletin, 32*(2), 93-98.

Pitnick, S., Wolfner, M. F., & Suarez, S. S. (2009). Ejaculate-female and sperm-female interactions. *Sperm biology: an evolutionary perspective*, 247-304.

Pranamornkith, T., Hall, M., Adlam, A., Page, B., Connolly, P., Somerfield, K., & Brash, D. (2014). Relative methyl bromide tolerances of *Arhopalus ferus* (Mulsant), *Hylurgus ligniperda* (F.) and *Hylastes ater* (Paykull) adults. *New Zealand Plant Protection, 67*, 80-85.

Phytosanitary Alert System. (2002). *Hylurgus ligniperda* Fabricius. Retrieved from www.pestalert.org

Raffa, K. F. (2001). Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology, 11*(2), 49-65. doi: 10.1007/pl00001833

Raffa, K. F., Aukema, B. H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G., & Romme, W. H. (2008). Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. *Bioscience, 58*(6), 501-517. doi: 10.1641/b580607

Raffa, K. F., & Berryman, A. A. (1983). The role of host plant resistance in the colonization behaviour and ecology of bark beetles (Coleoptera, Scolytidae). *Ecological Monographs, 53*(1), 27-49. doi: 10.2307/1942586

Ray, S., Thwaites, J., & Farrell, R. (2006). Survey of Ophiostomataceae associated with *Hylurgus ligniperda* (Curculionidae: Scolytinae) in New Zealand. *New Zealand Entomologist, 29*(1), 21-26.

Reay, S., Thwaites, J., & Farrell, R. (2005). A survey of Ophiostoma species vectored by *Hylastes ater* to pine seedlings in New Zealand. *Forest Pathology, 35*(2), 105-113.

Reay, S. D., & Walsh, P. J. (2001). Observations of the flight activity of *Hylastes ater* and *Hylurgus ligniperda* (Curculionidae: Scolytinae) in *Pinus radiata* forests in the central North Island, New Zealand. *New Zealand Entomologist, 24*, 79-85.

Reay, S. D., Walsh, P. J., Ram, A., & Farrell, R. L. (2002). The invasion of *Pinus radiata* seedlings by sapstain fungi, following attack by the Black Pine Bark Beetle, *Hylastes ater* (Coleoptera: Scolytidae). *Forest Ecology and Management, 165*(1), 47-56.

Ridley, M. (1988). Mating frequency and fecundity in insects. *Biological Reviews, 63*(4), 509-549.

Robertson, I. C. (1998). Paternal care enhances male reproductive success in pine Engraver beetles. *Animal behaviour, 56*(3), 595-602.

Robertson, I. C., & Roitberg, B. D. (1998). Duration of paternal care in pine Engraver beetles: why do larger males care less? *Behavioral Ecology and Sociobiology, 43*(6), 379-386.

Romon, P., Zhou, X., Iturrondobeitia, J. C., Wingfield, M. J., & Goldarazena, A. (2007). Ophiostoma species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. *Canadian Journal of Microbiology, 53*(6), 756-767.

Rosado-Neto, G. H., & dos Santos, G. B. (2010). The elytro-tergal stridulatory apparatus of the genus *Bondarius Rosado-Neto* (Coleoptera, Curculionidae). *Revista Brasileira De Entomologia, 54*(2), 337-338. doi: 10.1590/s0085-56262010000200020

Rudinsky, J. (1962). Ecology of scolytidae. *Annual review of entomology, 7*(1), 327-348.

Rudinsky, J., & Michael, R. (1972). Sound production in Scolytidae: Chemostimulus of sonic signal by the Douglas-fir beetle. *Science, 175*(4028), 1386-1390.

Rudinsky, J., & Ryker, L. C. (1976). Sound production in Scolytidae: Rivalry and premating stridulation of male Douglas-fir beetle. *Journal of Insect Physiology, 22*(7), 9971001-9991003.

Rudinsky, J. A., & Michael, R. R. (1972). Sound production in Scolytidae - chemostimulus of sonic signal douglas-fir beetle. *Science, 175*(4028), 1386-&. doi: 10.1126/science.175.4028.1386

Rudinsky, J. A., & Michael, R. R. (1973). Sound production in Scolytidae-stridulation by female Dendroctonus beetles. *Journal of Insect Physiology, 19*(3), 689-705. doi: 10.1016/0022-1910(73)90078-4

Rudinsky, J. A., Oester, P. T., & Ryker, L. C. (1978). Gallery initiation and male stridulation of polygamous Spruce bark beetle *Polygraphus rufipennis*. *Annals of the Entomological Society of America, 71*(3), 317-321.

Rudinsky, J. A., & Ryker, L. C. (1976). Sound production in Scolytidae: rivalry and premating stridulation of male Douglas-fir beetle. *Journal of Insect Physiology, 22*(7), 997-1003.

Rudinsky, J. A., & Vallo, V. (1979). Ash bark beetles *Leperisinus-Fraxini* and *Hylesinus-oleiperda* stridulatory organs, acoustic signals and pheromone production. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 87*(4), 417-429.

Rudnev, D. F., & Kozak, V. T. (1974). Factors associated with the variability of galleries made by bark-beetles (Coleoptera, Ipidae). *Zoologicheskii Zhurnal, 53*(9), 1420-1423.

Ryker, L. C. (1988). Acoustic studies of Dendroctonus bark beetles. *Florida Entomologist, 71*(4), 447-461. doi: 10.2307/3495004

Ryker, L. C., & Rudinsky, J. A. (1976a). Sound production in scolytidae - acoustic signals of male and female *Dendroctonus valens leconte*. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 80*(2), 113-118.

Ryker, L. C., & Rudinsky, J. A. (1976b). Sound production in Scolytidae - aggressive and mating behaviour of mountain pine beetle. *Annals of the Entomological Society of America, 69*(4), 677-680.

Sasakawa, M., & Sasakawa, T. (1981). Stridulatory organs of the minute pine bark beetle *Cryphalus fulvus* Coleoptera Scolytidea and role of male sound in aggression behaviour. *Kontyu, 49*(3), 461-469.

Sasakawa, M., & Yoshiyasu, Y. (1983). Stridulatory organs of japanese pine bark beetles coleoptera scolytidae. *Kontyu, 51*(4), 493-501.

Sauvard, D. (2004). General biology of bark beetles. *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, 63-88.

Schmitt, M., & Traue, D. (1990). Morphological and bioacoustic aspects of stridulation in Criocerinae (Coleoptera, Chrysomelidae). *Zoologischer Anzeiger, 225*(5-6), 225-240.

Schmitz, R. F. (1972). Behavior of *Ips pini* during mating, oviposition, and larval development (Coleoptera: Scolytidae). *The Canadian Entomologist, 104*(11), 1723-1728.

Sivalinghem, S. (2011). *Acoustic communication in the pine engraver bark beetle, Ips pini (Coleoptera: Scolytinae).* Carleton University Ottawa.

Somerfield, K., Bycroft, B., Brash, D., Clark, J., Hedderley, D., & Armstrong, J. (2013). Efficacy of methyl bromide used at reduced rates to control larvae and adults of goldenhaired bark beetle, *Hylurgus ligniperda*. *New Zealand Plant Protection, 66*, 82-88.

Spangler, H. G. (1988). Hearing in tiger beetles (Cicindelidae). *Physiological entomology, 13*(4), 447-452.

Stölting, H., Moore, T. E., & Lakes-Harlan, R. (2002). Substrate vibrations during acoustic signalling in the cicada *Okanagana rimosa*. *Journal of Insect Science, 2*(1), 2.

Swaby, J. A., & Rudinsky, J. A. (1976). Acoustic and olfactory behaviour of *Ips pini* (say) (Coleoptera-Scolytidae) during host invasion and colonization. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 81*(4), 421-432.

Symonds, M. R. E., & Elgar, M. A. (2004). Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. *Ecology Letters, 7*(3), 202-212. doi: 10.1111/j.1461-0248.2004.00571.x

Tribe, G. D. (1991a). Phenology of *Pinus radiata* log colonization by the red-haired pine bark beetle *Hylurgus ligniperda* (fabricius) Coleoptera, Scolytidae) in the south-western cape province. *Journal of the Entomological Society of Southern Africa, 54*(1), 1-7.

Tribe, G. D. (1991b). Phenology of three exotic pine bark beetle species Coleoptera Scolytidae colonising *Pinus radiata* logs in the south-western cape province. *South African Forestry Journal*(157), 27-31.

Tribe, G. D. (1992). Colonization sites on *pinus-radiata* logs of the bark beetles, *Orthotomicus erosus, Hylastes angustatus* and *Hylurgus ligniperda* (Coleoptera, Scolytidae). *Journal of the Entomological Society of Southern Africa, 55*(1), 77-84.

Vernoff, S., & Rudinsky, J. A. (1980). Sound production and pairing behaviour of *Leperinus-californicus swaine* and *Leperisinus oregonus blackman* (Coleoptera, Scolytidae) attacking *oregon ash*. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 90*(1), 58-74.

Viiri, H. (2004). Fungi associated with *Hylobius abietis* and other weevils. *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, 381-393.

Wallin, K. F., & Raffa, K. F. (2000). Influences of host chemicals and internal physiology on the multiple steps of postlanding host acceptance behavior of *Ips pini* (coleoptera: scolytidae). *Environmental Entomology, 29*(3), 442-453.

Warner, R. R., Shapiro, D. Y., Marcanato, A., & Petersen, C. W. (1995). Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females. *Proceedings of the Royal Society of London B: Biological Sciences, 262*(1364), 135-139.

Wilkinson, R. (1962). Stridulating organs in three southeastern Ips bark beetles. *Florida Entomologist, 45*(1), 43-44.

Wilkinson, R. C., McClelland, W. T., Murillo, R., & Ostmark, E. O. (1967). Stridulation and behavior in two southeastern Ips bark beetles (Coleoptera: Scolytidae). *Fla Entomol, 50*((3)), 185-195. doi: 10.2307/3493300

Wood, D. L. (1961). Stridulation in the genus *Ips* De Geer. *Pan-Pacif. Ent, 37*, 187-188.

Wood, S. L. (1992). Nomenclatural changes in Scolytidae and Platypodidae. *Great Basin Naturalist, 52*(1), 89-92.

Wyatt, T. D. (2003). *Pheromones and animal behaviour: communication by smell and taste*: Cambridge University Press.

Yandell, K. L. (1984). Sound production of *Dendroctonus-ponderosae* hopkins (Coleoptera, Scolytidae) - a comparision of populations from three host pines in Oregon. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology, 97*(2), 180-187.

Yturralde, K. M., & Hofstetter, R. W. (2015). Characterization of stridulatory structures and sounds of the larger Mexican pine beetle, *Dendroctonus approximatus* (Coleoptera: Curculionidae: Scolytinae). *Florida Entomologist, 98*(2), 516-527.

Zhang, Z., Van Epenhuijsen, C., Brash, D., & Hosking, G. (2004). Phosphine as a fumigant to control *Hylastes ater* and *Arhopalus ferus*, pests of export logs. *New Zealand Plant Protection, 57*, 257.

Zhou, X. D., de Beer, Z. W., Ahumada, R., Wingfield, B. D., & Wingfield, M. J. (2004). Ophiostoma and Ceratocystiopsis spp. associated with two pine-infesting bark beetles in Chile. *Fungal Diversity, 15*, 261-274.

Zhou, X. D., de Beer, Z. W., Wingfield, B. D., & Wingfield, M. J. (2001). Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. *Sydowia, 53*(2), 290-300.

Appendix

Temporal Data (Male)

Distress (Simple)	Replication	Number of chirps	Interchirp interval (sec)	Toothstrike per chirp	Chirp duration (sec)	Toothstrike rate (No./sec)
	1.000	7.000	0.556	60.571	6.386	9.691
	2.000	7.000	0.678	71.000	6.980	10.348
	3.000	4.000	0.574	118.250	13.114	8.912
	4.000	5.000	0.495	86.800	10.226	8.384
	5.000	5.000	0.669	102.200	11.060	9.187
	6.000	7.000	0.688	56.857	6.770	8.610
	7.000	6.000	0.590	75.333	8.394	8.930
	8.000	6.000	0.495	79.833	8.561	9.088
	9.000	6.000	0.429	90.500	9.027	9.940
	10.000	7.000	0.595	58.857	7.063	8.417
Distress (Interrupted)	1.000	7.000	0.344	8.143	0.863	9.498
	2.000	6.000	0.381	7.200	0.660	11.127
	3.000	3.000	0.371	8.000	0.791	10.125
	4.000	3.000	0.403	5.667	0.538	10.695
	5.000	0.000	0.000	0.000	0.000	0.000
	6.000	5.000	0.399	7.600	0.744	10.256
	7.000	4.000	0.398	8.000	0.806	10.005
	8.000	5.000	0.321	8.000	0.758	10.550
	9.000	2.000	0.499	7.500	0.745	10.074
	10.000	3.000	0.396	8.000	1.126	7.782
Before mating (Simple)	Replication	Number of chirps	Interchirp interval (sec)	Toothstrike per chirp	Chirp duration (sec)	Toothstrike rate (No./sec)
	1.000	4.000	2.483	21.250	2.934	7.077
	2.000	6.000	1.898	21.000	3.002	7.000
	3.000	7.000	2.162	18.000	2.712	6.684
	4.000	6.000	1.366	21.500	3.168	6.856
	5.000	4.000	2.285	19.250	3.067	6.216
	6.000	7.000	2.298	19.429	2.712	7.316
	7.000	7.000	2.809	15.000	1.602	9.198
	8.000	7.000	1.111	14.286	1.792	8.257
	9.000	6.000	1.266	19.167	2.421	7.994
	10.000	6.000	1.849	19.500	2.542	7.984
Before mating (Interrupted)	1.000	7.000	1.677	6.714	1.146	6.277
	2.000	8.000	1.358	6.500	1.045	6.312
	3.000	10.000	1.312	7.800	1.108	7.211

	4.000	11.000	1.855	6.455	0.986	6.887
	5.000	13.000	1.606	5.231	0.789	6.592
	6.000	11.000	1.498	4.364	0.652	6.476
	7.000	9.000	1.642	6.000	0.654	9.402
	8.000	7.000	1.350	6.000	0.884	6.826
	9.000	11.000	1.217	6.364	0.865	7.360
	10.000	6.000	1.694	7.833	1.107	7.218
After mating (Simple)	Replication	Number of chirps	Interchirp interval (sec)	Toothstrike per chirp	Chirp Duration (sec)	Toothstrike rate (No./sec)
	1.000	2.000	0.688	49.000	5.155	9.417
	2.000	3.000	0.471	28.667	3.766	7.402
	3.000	5.000	0.724	25.800	2.890	8.753
	4.000	1.000	0.478	51.000	5.564	9.166
	5.000	2.000	0.688	28.500	3.326	8.123
	6.000	0.000	0.000	0.000	0.000	0.000
	7.000	1.000	1.121	21.000	3.121	6.729
	8.000	4.000	0.476	20.200	2.076	9.732
	9.000	0.000	0.000	0.000	0.000	0.000
	10.000	0.000	0.000	0.000	0.000	0.000
After mating (Interrupted)	1.000	12.000	2.449	5.250	0.598	8.776
	2.000	9.000	1.736	5.667	0.640	8.618
	3.000	9.000	2.961	3.889	0.482	8.283
	4.000	12.000	1.728	5.333	0.568	9.237
	5.000	4.000	2.248	5.750	0.640	9.623
	6.000	7.000	1.585	6.000	0.567	10.518
	7.000	10.000	1.748	5.200	0.561	9.361
	8.000	12.000	1.537	5.667	0.625	9.092
	9.000	5.000	2.408	5.167	0.575	9.218
	10.000	5.000	1.983	5.500	0.637	8.615

Temporal Data (Female)

Distress	Replication	Number of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No./sec)
	1.000	12.000	0.012	4.793	81.665
	2.000	14.000	0.012	4.003	84.209
	3.000	10.000	0.012	5.778	84.099
	4.000	8.000	0.013	7.046	80.570
	5.000	15.000	0.013	3.742	78.742
	6.000	9.000	0.012	6.324	84.929
	7.000	10.000	0.012	5.475	85.498
	8.000	12.000	0.013	4.715	83.806
	9.000	8.000	0.010	6.645	98.485
	10.000	18.000	0.013	3.274	79.398
Before Mating	Replication	Number of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No./sec)
	1.000	5.000	0.006	11.661	168.571
	2.000	5.000	0.006	10.744	177.778
	3.000	4.000	0.006	11.244	160.714
	4.000	3.000	0.007	9.083	150.794
	5.000	6.000	0.007	8.786	161.706
	6.000	4.000	0.007	9.719	154.762
	7.000	5.000	0.008	9.992	134.127
	8.000	5.000	0.007	8.431	141.270
	9.000	3.000	0.006	6.640	158.730
	10.000	7.000	0.007	7.806	158.900
During Mating	Replication	Number of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No./sec)
	1.000	4.000	0.004	4.479	279.167
	2.000	5.000	0.005	5.632	203.333
	3.000	4.000	0.005	7.043	208.333
	4.000	6.000	0.005	5.467	225.000
	5.000	3.000	0.005	10.061	205.556
	6.000	5.000	0.005	5.658	198.571
	7.000	7.000	0.006	5.440	177.551
	8.000	2.000	0.005	5.471	225.000
	9.000	4.000	0.005	6.349	200.000
	10.000	4.000	0.006	8.427	169.048

After mating	Replication	Number of clicks	Click duration (sec)	Interclick interval (sec)	Click strike rate (No./sec)
	1.000	4.000	0.005	10.487	200.000
	2.000	3.000	0.004	8.850	277.778
	3.000	4.000	0.004	11.363	270.833
	4.000	4.000	0.004	11.300	237.500
	5.000	2.000	0.005	10.679	225.000
	6.000	5.000	0.004	9.125	240.000
	7.000	4.000	0.005	9.129	225.000
	8.000	5.000	0.004	8.019	256.667
	9.000	3.000	0.004	9.047	250.000
	10.000	4.000	0.005	6.703	225.000

Spectral Data (Male)

Distress (Simple)	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.046	278430.714	-317168.000	19424.486	1116.329	3962.814
	2.000	0.049	297156.429	-345235.714	19728.943	1111.771	3769.500
	3.000	0.043	285926.750	-329259.250	22397.975	1134.375	3431.125
	4.000	0.048	314658.400	-338420.800	22706.920	1100.600	3344.480
	5.000	0.046	339874.400	-362679.200	22668.280	1023.380	4458.140
	6.000	0.047	291699.000	-325787.714	23515.129	1075.771	5225.329
	7.000	0.047	283974.000	-327520.500	22237.083	1061.983	7457.850
	8.000	0.044	234465.500	-287585.000	21062.483	965.433	4766.017
	9.000	0.046	176915.167	-232672.667	22253.183	820.600	4560.200
	10.000	0.051	164884.143	-203867.857	21432.571	1034.386	4729.186
Distress (Interrupted)	1.000	0.048	276461.571	-317388.286	20255.543	1033.757	3913.743
	2.000	0.047	285128.500	-340507.667	21961.767	1179.650	4403.367
	3.000	0.042	305027.000	-342897.333	21979.633	1029.800	4566.300
	4.000	0.047	298847.333	-297960.667	22912.900	1287.233	4405.100
	5.000	0.000	0.000	0.000	0.000	0.000	0.000
	6.000	0.047	284169.600	-298738.800	20795.380	1448.140	6930.560
	7.000	0.044	296880.000	-372643.750	22422.125	1182.650	3300.350
	8.000	0.048	162270.000	-242399.400	22031.120	791.640	4547.820
	9.000	0.052	162109.000	-229178.500	23073.800	820.600	4823.400
	10.000	0.050	164008.000	-220730.667	22269.267	1287.233	6543.133

Before mating (Simple)	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.015	266657.250	-241818.500	12771.925	387.050	7058.275
	2.000	0.016	300448.333	-276263.667	13269.533	387.033	5598.933
	3.000	0.015	278514.857	-237607.143	12316.657	471.557	6420.057
	4.000	0.015	336336.000	-308097.667	12728.183	328.000	6511.217
	5.000	0.016	276089.750	-270589.250	12545.275	321.675	6755.475
	6.000	0.014	320274.429	-294933.286	12477.657	454.129	8174.514
	7.000	0.016	325193.714	-296611.857	12629.071	281.157	5955.257
	8.000	0.013	298011.286	-245651.000	12261.429	367.629	7854.271
	9.000	0.016	282546.571	-249898.143	12347.929	551.457	5540.214
	10.000	0.017	269343.500	-235348.500	12274.033	441.533	6515.600

Before mating (Interrupted)	1.000	0.016	269074.286	-209229.429	12764.029	458.114	6710.486
	2.000	0.017	333856.625	-308887.375	13076.025	387.025	6022.263
	3.000	0.015	295453.000	-240487.300	12503.620	370.870	5927.100
	4.000	0.013	329346.909	-287958.818	12357.764	357.800	7530.282
	5.000	0.016	294560.231	-288413.385	12412.800	419.208	6747.431
	6.000	0.015	303711.909	-252139.091	12516.018	330.309	7841.700
	7.000	0.014	288866.222	-255975.778	12471.689	588.689	6067.000
	8.000	0.014	281844.143	-263141.714	12758.814	356.814	7831.471
	9.000	0.016	246927.364	-222293.182	13231.636	584.873	5646.610
	10.000	0.016	309337.500	-333762.000	12475.867	391.050	5662.417
After mating (Simple)	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.014	285730.500	-199046.000	12072.250	454.100	7544.250
	2.000	0.013	298432.333	-269364.333	12210.967	428.867	7608.933
	3.000	0.012	270632.600	-278692.800	12639.880	681.200	7818.900
	4.000	0.010	253280.000	-252126.000	11428.900	302.800	4896.300
	5.000	0.012	259902.500	-282191.000	12564.150	340.600	6543.500
	6.000	0.000	0.000	0.000	0.000	0.000	0.000
	7.000	0.013	310597.000	-314950.000	12185.700	529.800	4585.200
	8.000	0.013	324592.750	-332886.500	12412.800	321.675	6287.775
	9.000	0.000	0.000	0.000	0.000	0.000	0.000
	10.000	0.000	0.000	0.000	0.000	0.000	0.000

After mating (Interrupted)	1.000	0.012	303775.417	-269231.333	12299.267	359.542	5894.458
	2.000	0.013	322267.000	-300883.111	12715.556	597.122	6587.067
	3.000	0.012	292733.556	-281162.000	12068.011	512.989	6249.056
	4.000	0.011	316921.000	-293110.667	12374.967	504.592	6350.475
	5.000	0.012	295325.750	-307916.000	12091.150	378.475	5837.400
	6.000	0.011	327843.429	-309148.429	12607.429	464.943	6357.143
	7.000	0.011	355402.400	-344797.300	12458.220	461.690	6693.160
	8.000	0.011	332366.833	-308829.917	12305.583	428.908	5213.517
	9.000	0.011	307938.200	-262492.400	12200.880	484.380	5681.460
	10.000	0.011	281742.800	-279916.200	12306.820	363.300	6528.220

Competition	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.011	145047.900	-144458.700	15505.030	294.910	6603.950
	2.000	0.011	158967.000	-150599.100	16502.160	275.620	7568.370
	3.000	0.013	192585.300	-177538.700	16594.050	296.830	8231.290
	4.000	0.012	170436.800	-167790.800	15434.990	268.560	7427.760
	5.000	0.011	149879.500	-143043.600	15491.540	303.890	7416.370
	6.000	0.011	182491.400	-178616.000	15724.770	360.420	6442.650
	7.000	0.012	184086.700	-157716.800	15279.520	275.610	6287.530
	8.000	0.012	205921.100	-176270.200	15385.520	332.160	6163.320
	9.000	0.012	258673.000	-205505.800	15816.630	282.680	6730.430
	10.000	0.012	255737.400	-206634.800	15668.220	247.350	6394.350
Territoriality	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.016	844195.200	-717135.600	14160.230	424.920	6854.920
	2.000	0.017	352832.100	-247168.500	17926.950	468.210	6373.840
	3.000	0.017	807560.900	-667772.900	14574.140	558.800	5451.190
	4.000	0.017	449592.700	-480602.000	17821.240	437.990	11335.070
	5.000	0.016	761831.500	-489956.600	13879.410	709.840	5643.570
	6.000	0.016	862355.100	-560715.300	14861.110	709.830	6633.730
	7.000	0.017	840008.100	-650418.100	14408.020	453.080	6348.560
	8.000	0.016	571559.100	-533441.500	13864.320	528.600	7474.980
	9.000	0.018	403215.100	-319037.700	15812.570	483.310	5397.780
	10.000	0.019	606498.200	-478826.700	17760.820	377.590	8268.050
Colony	Replication	Toothstrike duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.017	594080.700	-569776.000	14013.490	257.440	4298.020
	2.000	0.019	1073965.30	-677631.900	15614.080	246.230	5710.480
	3.000	0.019	833485.100	-727825.400	14282.130	179.090	5223.680
	4.000	0.018	618343.100	-537468.300	15972.280	179.100	5801.970
	5.000	0.021	587416.900	-564680.100	14550.740	268.660	5708.000
	6.000	0.021	573257.500	-668927.600	15770.790	346.980	6546.470
	7.000	0.020	998762.700	-671545.900	15983.450	212.680	6772.810
	8.000	0.020	698461.000	-719323.900	16408.780	235.060	5459.830
	9.000	0.020	1074471.20	-779403.100	15177.560	179.080	5779.420
	10.000	0.021	590459.600	-696114.500	15076.830	212.670	5476.540

Spectral Data (Female)

Distress	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.012	295331.917	-496498.667	13612.667	254.017	1313.533
	2.000	0.012	233266.357	-313929.714	10666.614	231.086	1115.614
	3.000	0.012	278671.800	-378179.500	13362.050	244.290	1365.320
	4.000	0.013	419953.625	-693026.375	10892.963	189.825	1071.125
	5.000	0.013	248183.067	-370580.867	10118.367	255.280	1136.167
	6.000	0.012	59566.444	-69612.333	7467.367	190.722	704.467
	7.000	0.012	108292.400	-138704.400	8661.550	283.900	1188.340
	8.000	0.013	45979.167	-52631.167	8070.692	302.600	2062.433
	9.000	0.012	32491.698	-30826.573	7902.556	328.388	1500.790
	10.000	0.013	190964.111	-207127.444	9480.150	276.544	887.772
Before Mating	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.006	1573731.60	-1361160.20	17270.840	359.820	10444.740
	2.000	0.006	2339832.60	-1475993.20	18750.020	559.720	4384.660
	3.000	0.006	2001857.50	-1256591.50	16591.175	699.650	4356.625
	4.000	0.007	849617.000	-478411.667	17057.600	533.067	4914.700
	5.000	0.007	702987.000	-475732.333	17190.833	566.383	3821.550
	6.000	0.007	864171.000	-900320.000	18540.150	649.675	5332.300
	7.000	0.008	2352920.60	-2078346.20	18310.260	599.700	4295.840
	8.000	0.007	1016658.20	-1084606.20	15951.520	439.780	3272.100
	9.000	0.006	784255.667	-891736.000	16457.933	466.433	3612.267
	10.000	0.007	580974.571	-759776.714	13004.029	408.714	2651.071
During Mating	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.004	267846.500	-200511.750	15441.925	1278.375	6976.750
	2.000	0.005	336328.200	-458156.200	17861.880	1802.820	7086.400
	3.000	0.005	419588.000	-505161.000	19935.125	1941.525	8286.875
	4.000	0.005	404220.667	-450597.667	34068.850	2033.983	6852.350
	5.000	0.005	361705.667	-424391.333	34300.000	1201.867	5654.400
	6.000	0.005	317488.600	-359706.000	31397.000	1386.800	4625.100
	7.000	0.006	339242.714	-366571.000	20625.329	1984.243	7483.171
	8.000	0.005	691692.000	-844504.000	29591.800	2466.500	11100.400
	9.000	0.005	289006.500	-332002.000	28780.600	2296.000	2379.425
	10.000	0.006	180707.000	-280970.000	16107.400	625.500	4134.400

After Mating	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.005	261126.250	-216802.250	16772.075	1329.250	7838.075
	2.000	0.004	144090.000	-120737.333	16055.300	1876.600	11607.533
	3.000	0.004	316088.000	-259805.000	16381.125	1016.475	10766.625
	4.000	0.004	289562.250	-293095.500	20290.700	1798.400	9742.975
	5.000	0.005	327868.500	-256563.000	36124.450	3753.200	8237.200
	6.000	0.004	384400.000	-340845.800	36793.260	1825.720	7212.020
	7.000	0.005	307575.000	-347173.750	36984.950	1631.700	7314.975
	8.000	0.004	226639.400	-250025.600	20320.840	908.640	5084.420
	9.000	0.004	195497.333	-292640.667	21408.467	1101.433	4699.033
	10.000	0.005	317579.250	-289318.750	22768.025	877.725	6940.025
Competition	Replication	Click duration (sec)	Maximum ampliude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.004	613110.833	-353415.667	22024.267	9031.733	5999.200
	2.000	0.005	756236.000	-922697.143	19326.386	3023.571	5065.286
	3.000	0.005	840098.571	-575480.143	15705.929	371.243	4657.671
	4.000	0.005	690313.400	-408652.600	16071.440	519.740	4096.540
	5.000	0.005	2229704.42	-923541.714	16791.071	371.243	3942.229
	6.000	0.006	1511702.75	-544139.000	15541.725	399.800	4114.275
	7.000	0.007	1672858.00	-1888845.80	18829.960	439.780	5169.620
	8.000	0.005	1115267.22	-744998.667	16746.656	444.222	4212.300
	9.000	0.006	766608.000	-1090437.20	16751.080	439.780	4811.120
	10.000	0.005	220096.286	-226600.857	22194.443	1266.700	6358.214
Territoriality	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.004	251670.167	-219752.667	24506.817	1659.017	9876.550
	2.000	0.005	262885.125	-215894.625	15006.288	1046.288	4853.213
	3.000	0.004	241525.000	-173333.333	17673.733	888.117	2089.017
	4.000	0.005	209240.125	-179531.500	17154.900	1278.663	6162.088
	5.000	0.005	370573.667	-362984.778	25572.289	2034.200	4944.422
	6.000	0.005	337537.833	-318168.500	15905.950	1426.233	3627.533
	7.000	0.005	194149.333	-149022.444	14648.956	983.044	3933.589
	8.000	0.005	320004.167	-344163.500	19011.350	1329.533	2006.850
	9.000	0.005	238800.900	-217700.800	17211.740	1319.860	4749.540
	10.000	0.005	789571.000	-847959.000	48341.700	2387.200	4221.800

Colony	Replication	Click duration (sec)	Maximum amplitude	Minimum amplitude	High frequency (Hz)	Low frequency (Hz)	Peak frequency (Hz)
	1.000	0.005	460769.400	-480055.800	24264.260	1182.520	4410.000
	2.000	0.006	332841.800	-363730.000	21988.460	1476.860	4016.940
	3.000	0.005	385025.000	-399707.667	26460.000	957.217	6039.917
	4.000	0.005	261198.667	-239578.667	25844.633	957.233	7150.533
	5.000	0.003	429473.600	-432340.600	25598.540	902.540	5155.880
	6.000	0.005	473238.200	-406788.800	19833.420	929.680	3244.640
	7.000	0.005	658977.600	-794016.000	27599.580	890.960	4801.960
	8.000	0.006	629968.143	-697014.143	28414.286	691.729	5101.586
	9.000	0.004	178237.000	-158356.286	13464.414	380.929	2801.700
	10.000	0.005	206170.333	-198127.333	25280.950	1617.950	8427.883