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Acoustic signal applications in detection and management of *Rhynchophorus* spp. in fruit-crops and ornamental palms

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Abstract

Rhynchophorus ferrugineus (Olivier) (Coleoptera: Dryophthoridae) is an economically important, internally feeding pest of ornamental and fruit-producing palms in many subtropical regions. A related weevil, *Rhynchophorus cruentatus* (Fabricius) (Coleoptera: Dryophthoridae), is an internally feeding palm pest in the southeastern USA. Acoustic methods for detection of early instars hidden in palms in field environments have been investigated for both species. Acoustic methods also have been used to examine the effectiveness of physical, biological, and other control treatments. This report addresses several physical, physiological, and behavioral factors that influence the spectral and temporal patterns of sounds produced by palm weevil larvae under different laboratory and field experiment conditions, which must be considered carefully in interpreting larval sound production. Such factors include the leakage of fluid from the palm tree tissues into tunnels scraped out by larvae as they move and feed within the tree trunk, as well as occurrences of molting between periods of feeding activity, and frequency-dependent damping that distorts signals as the distance between insects and sensors increases. Methods are discussed that combine effects of environmental, physiological, and behavioral variability to facilitate reliable interpretations of *Rhynchophorus* and other insect larval acoustic activity in hidden environments.

Key Words: red palm weevil; palmetto weevil; biological control

Resumen

Rhynchophorus ferrugineus (Olivier) (Coleoptera: Dryophthoridae) es una plaga económicamente importante que se alimenta internamente de palmeras ornamentales y frutícolas en muchas regiones subtropicales. Un gorgojo relacionado, *Rhynchophorus cruentatus* (Fabricius) (Coleoptera: Dryophthoridae), es una plaga de la palma que se alimenta internamente en el sureste de los EE. UU. Métodos acústicos para la detección de estadios tempranos escondidos en palmas en ambientes de campo han sido investigados para ambas especies. Los métodos acústicos también se han utilizado para examinar la efectividad de los tratamientos físicos, biológicos y otros tratamientos de control. Este informe aborda varios factores físicos, fisiológicos y de comportamiento que influyen en los patrones espectrales y temporales de los sonidos producidos por las larvas del gorgojo de la palma en diferentes condiciones de laboratorio y experimentos de campo, que deben considerarse cuidadosamente al interpretar la producción del sonido larval. Tales factores incluyen la fuga de líquido de los tejidos de la palmera hacia túneles raspados por las larvas a medida que se mueven y se alimentan dentro del tronco del árbol, así como las ocurrencias de las mudas entre los períodos de actividad de alimentación y la amortiguación dependiente de la frecuencia que distorsiona las señales al aumentar la distancia entre los insectos y los sensores aumenta. Se discuten los métodos que combinan los efectos de la variabilidad ambiental, fisiológica y de comportamiento para facilitar interpretaciones confiables de la actividad acústica de larvas de *Rhynchophorus* y otros insectos en ambientes ocultos.

Palabras Clave: picudo rojo de la palmera; gorgojo del palmetto; control biológico

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae), and palmetto weevil, *Rhynchophorus cruentatus* (Fabricius) (Coleoptera: Dryophthoridae), are cryptic stem borers that cause damage to cultivated and ornamental palms (Hunsberger et al. 2000; Faleiro 2006). The red palm weevil originated from south-

eastern Asia (Faleiro 2006; Shahina et al. 2009), and it has spread to all other continents except North America (Fiaboe et al. 2012). The palmetto weevil is native to Florida (Hunsberger et al. 2000), but its presence has been recorded in a few other states in the southeastern US. It had not been considered a major pest until recently when it caused

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a \$400,000 loss in a single nursery in southern Florida (Hunsberger et al. 2000). Adults of both species can be monitored and trapped with pheromones specific to each species, but the larval stage causes hidden damage inside the tree trunk, and is difficult to detect until it is too late to save the infested palm trees (Giblin-Davis & Howard 1989; Fiaboe et al. 2011; Mankin et al. 2011).

Management strategies for *Rhynchophorus* spp. include chemical control (Giblin-Davis & Howard 1989; El-Ezaby 1997; Abo-El-Saad et al. 2001), mass trapping (Giblin-Davis et al. 1996; Faleiro & Chellapan 1999; Hallett et al. 1999), and biological control (Gindin et al. 2006; Llácer & Martínez de Altube 2009). Unsurprisingly, red palm weevil is now resistant to some classes of insecticides (Al-Ayedh et al. 2016). To develop alternatives to pesticide usage and reduce health hazards from their application to date palms, research has been conducted to develop biological control agents, including entomopathogenic fungi such as *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) (Gindin et al. 2006; Dembilio et al. 2010; Güerri-Agulló et al. 2011; Ricaño et al. 2013; Lo et al. 2015), and entomopathogenic nematodes such as *Steinernema carpocapsae* (Weiser) (Rabbitida: Steinernematidae) (Llácer & Martínez de Altube 2009), *Steinernema abbasi* Elawad Ahmad & Reid (Nematoda: Steinernematidae), and *Heterorhabditis indica* Poinar, Karunakar & David (Rabbitida: Heterorhabditidae) (Abbas et al. 2001).

In studies reported at the 2016 International Congress of Entomology, acoustic technology was used to monitor the effects of different treatments of *B. bassiana* against red palm weevil larvae inside palm trees (Jalinas et al. 2015, 2017). The monitoring of acoustic activity over time after treatment enabled estimation of *B. bassiana* treatment efficacy and red palm weevil mortality in situ. Previously, acoustic technology was demonstrated to detect all larval stages of red palm weevil and palmetto weevil within their host plants (Mankin et al. 2011; Hetzroni et al. 2016), including the early instars of major importance for rapid targeting of hidden infestations (Herrick & Mankin 2012; Dosunmu et al. 2014).

It is worth noting that the acoustic methods and statistical analyses in the studies above had to accommodate considerable variability of larval activity under changes caused by fluctuating environmental conditions, differences in larval physiological state, and differences in the sizes of the palm trees in field experiments under study, and in the spectral characteristics of sounds at different distances between the insect and the acoustic sensor. Fortunately, knowledge of the causes of particular forms of variability, as well as methods to successfully interpret the variability, have significantly improved as acoustic methods have matured over time.

For this report, we describe some of the physical, behavioral, and physiological causes of variability of red palm weevil acoustic activity and consider some of the methods developed to correctly interpret the variability. Our example of physical variability interpretation is a distinctive “squeal” sound frequently detected in field recordings from red palm weevil-infested palms (Rach et al. 2013), but only rarely in laboratory or semi-field studies (Jalinas et al. 2015, 2017). In addition, we discuss how future studies of early detection or biological control treatment efficacy may benefit from continued improvements in acoustic sensor and signal processing technology.

Materials and Methods

Sample red palm weevil squeal sounds were collected from 4 mature palms with visible symptoms of infestation (Güerri-Agulló et al. 2011) in a plantation near Horta de Bernia (Elche, southeast Spain). Records of

180 s duration were collected from each palm with a sensor-preamplifier module (model SP-1 L, Acoustic Emission Consulting, Inc., Sacramento, California, USA) connected by a magnetic attachment to the signal waveguide screw, as in Jalinas et al. (2015) and Dosunmu et al. (2014). The signals were fed from the sensor module through an amplifier (AED-2010, Acoustic Emission Consulting, Inc., Sacramento, California) to a digital audio recorder (model HD-P2, Tascam, Montebello, California, USA) at a 44.1-kHz digitization rate. During collection, signals were monitored with headphones to avoid faulty readings or periods of loud background noise. Precautions were taken to minimize contact by wires and sensors with palm leaves and other surfaces. Oscillograms and spectrograms of the recordings were screened with Raven Pro Software (Charif et al. 2010) to identify relatively noise-free intervals containing red palm weevil signals. Automated analyses of recorded sections containing sounds matching temporal and spectral patterns of known red palm weevil signals were conducted with methods described in Mankin (2011), Dosunmu et al. (2014), and Jalinas et al. (2017).

Results

Signals from a 4.4 s period in a recording from 1 of the infested date palms are displayed in the oscillogram and spectrogram of Fig. 1. Three squeals, i, ii, and iii in the dot-dashed boxes, are representative of squeals detected from all 4 trees. The squeals are in close association with other “typical” signals produced by red palm weevil larval movement and feeding activity, shown in dotted boxes. The “typical” pattern of movement and feeding activity includes 0.1 to 0.3 s trains of short, 1 to 10 ms impulses with broadband spectra produced by weak contacts of larvae scraping or sliding along a tunnel, or by snapping of wood fibers during feeding (Mankin et al. 2011). Such trains, hereafter termed bursts, have been reported previously in studies where larvae and adults of a variety of different insect species had been recovered after recording (Mankin et al. 2011), including palmetto weevil (Dosunmu et al. 2014), red palm weevil (Jalinas et al. 2015, 2017), and *Oryctes elegans* Prell (Coleoptera: Scarabaeidae), with or without red palm weevil recovered from the same tree (Mankin et al. 2016a). In contrast to the commonly observed pattern, each 0.2 to 0.3 s squeal in Figure 1 begins with descending bands of 2 or more loud harmonics, which then are followed by the more typical trains (bursts) of short impulses with broadband spectra.

To incorporate such squeals into further studies to identify insect-infested trees in field environments (Mankin 2011), we constructed spectral profiles (mean spectra) that could be used by signal processing algorithms to automate identification of red palm weevil-produced sounds and discriminate them from background noise (Mankin et al. 2011; Jalinas et al. 2015). Initial screening with the Raven Pro software indicated that 3 different types of squeal occurred frequently in signals from the 4 trees known to be infested. Spectral profiles of the 3 squeal types (Fig. 2) were constructed: SQ-L, a mean of 753 consecutive impulses; SQ-M, a mean of 58 consecutive impulses; and SQ-H, a mean of 1,848 consecutive impulses from 3 different trees. The squeals are highly distinctive, both to the ear and to the red palm weevil signal detection algorithm (Mankin 2011), and thus provide evidence of high likelihood of red palm weevil infestation whenever they are detected in a field recording.

A physical explanation for the occurrence of red palm weevil signals in a tree that alternate in temporal-spectral pattern between bursts of short, broad-band impulses or series of continuous, harmonic squeals of similar duration can be obtained from other documented comparisons of sounds produced by friction of dry and wet surfaces. For example, trains of short, broadband impulses bearing resemblance

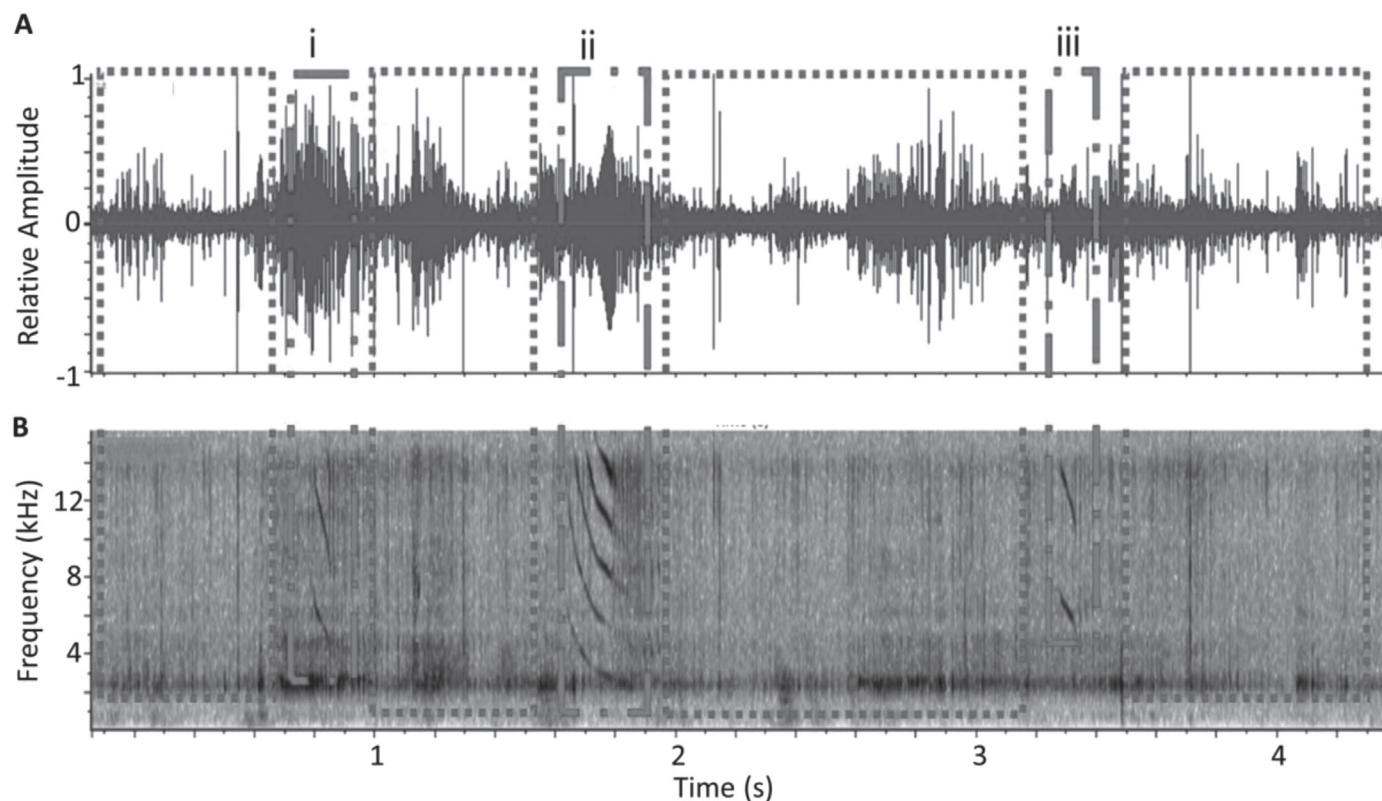


Fig. 1. (A) Oscillogram and (B) spectrogram of feeding sounds (dotted boxes) and squeals (dashed-dotted boxes labeled i, ii, and iii) produced by red palm weevil larvae in a date palm tree. In the spectrogram, darker color indicates greater energy at the specified frequency and time.

to those of typical red palm weevil bursts are commonly observed from sliding sounds in human footsteps, and other weak, sliding contacts between 2 dry surfaces (Ekimov & Sabatier 2006). In contrast, wet skin on glass or other smooth surface, or brakes on a wet road, often produce sounds with loud harmonics resembling red palm weevil squeals resulting from effects of stick-slip friction of rubbing wet surfaces (Patitsas 2010). Indeed, large palm trees are known to ooze fluid from tunnels damaged by red palm weevil larvae (Abraham et al. 1966). As a result, the red palm weevil squeal has opportunity to originate from larval epidermal surfaces that are gliding over a thin film of fluid in a tunnel that the larva has scraped within the palm tree trunk. At points where the larval segments are scraping over dry sections of the tunnel,

the sound pattern is a burst of short impulses as typically expected, but where the larval segments glide over ooze-covered sections of the tunnel, the movement can produce signals with the high-amplitude harmonic bands detectable as squeals. In this case, observation that squeals are more likely to be detected in large trees are consistent also with other observations that a lesser amount of fluid is released in tunnels of small trees.

Discussion

The finding that red palm weevil larval movement can produce 2 distinctively different sounds, likely dependent on wet vs. dry interactions with the substrate, is an example where knowledge of the physical characteristics of the substrate and the larval epicuticle, combined with knowledge of larval behavior, can provide useful guidance for interpretation of sounds produced by red palm weevil larvae in palm trunks. In future field studies, signal analysis algorithms that match spectra of individual signals against profiles of known red palm weevil larval sounds (e.g., Mankin et al. 2016a) may benefit from inclusion of squeal profiles to discriminate red palm weevil signals from background noise, depending on the size of the tree. Additional studies discussed below provide other examples where information about environmental conditions, insect physiology, and behavior together provide helpful cues about the interpretation of insect acoustic recordings.

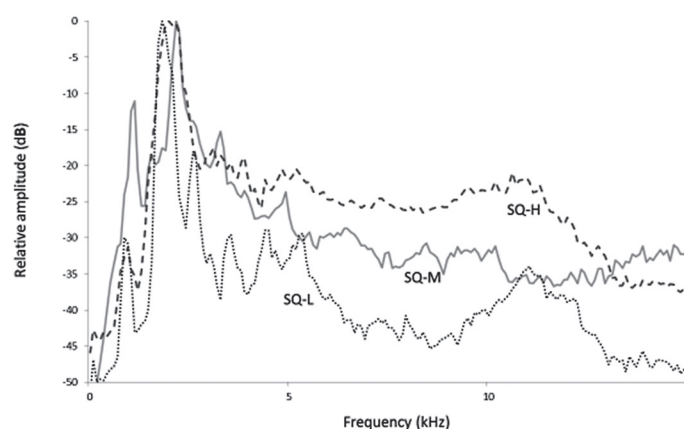


Fig. 2. Spectra of 3 different profiles of red palm weevil squeals: SQ-L, with relatively low energy at frequencies above 7 kHz; SQ-M, with relatively greater energy between 5 to 10 kHz; and SQ-H, with prominent energy over a broad range of frequency up to 12 kHz.

CYCLIC ACTIVITY PATTERNS OF LARVAE PASSING THROUGH MULTIPLE INSTARS

A report presented initially at the 2016 International Congress of Entomology describes an example where larval molting patterns play

an important role in interpretation of insect acoustic activity patterns (Jalinas et al. 2017). In this case, 15-d-old red palm weevil larvae were exposed to different *B. bassiana* treatments and placed into holes drilled into 5-yr-old *Phoenix canariensis* Chabaud (Arecaceae) palms. Unexposed larvae of the same age were used as controls, and the acoustic activities of all larvae were monitored over 40 d in situ. Both the treated and untreated larvae produced bursts of short impulses similar to those observed in the dotted boxes of Figure 1.

Over the course of the experiment, the acoustic activities of the control and treated larvae were modulated by cyclic decreases and increases in burst rates over time after larvae were placed into the palms. The cycles were attributable to the occurrence of molts during which the larvae were quiescent, followed by periods of movement and feeding activity. The larval ages associated with the falls and rises in red palm weevil activity were consistent with molting cycles in previous developmental studies, including Norzainih et al. (2015). Such cycles were not observed in an earlier study of Jalinas et al. (2015) which tested last-instar larvae only.

The activity cycles in Jalinas et al. (2017) were similar to those observed by Dosunmu et al. (2014) in the activity of palmetto weevil larvae monitored in palm fronds over a 56 d period. Shade et al. (1990) also observed cyclic molting activity during acoustic monitoring of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) larvae in cowpea seeds, *Vigna unguiculata* (L.) Walp (Fabaceae).

In Jalinas et al. (2017), acoustic activity of red palm weevil larvae treated with different doses of *B. bassiana* exhibited different patterns of cyclic activity than those in the control. Sound burst rates during active periods between molts were significantly lower in the treated larvae than in the controls, in correlation with other studies where the health status of *B. bassiana*-treated red palm weevil larvae declined over time, resulting in increased mortality (Gindin et al. 2006), weak movements, and reduced appetite (Ekesi 2001).

The experiments of Jalinas et al. (2017) benefited by averaging acoustic measurements obtained from larvae synchronized by age, which is not feasible for experiments in field environments. However, in cases where pest management treatments cause mortality within a few d, such as the cases of hermetic storage treatments of adult *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae) (Njoroge et al. 2017) and *C. maculatus* (Njoroge et al. 2018), visual and acoustic monitoring were able to confirm that mortality and cessation of acoustic activity of randomly collected adults of unknown age occurred within an 8 d period. In these studies, as well as in Jalinas et al. (2017), the use of acoustic monitoring was of benefit partly because it enabled in situ tests.

ENHANCING RELIABILITY OF HIDDEN INSECT INFESTATION DETECTION

Field studies of acoustic detection of hidden insect infestations often must interpret signals collected from insects of unknown physiological state in substrates whose signal-transmission characteristics are not well understood (Mankin et al. 2018). In addition, field-collected signals often contain considerable background noise, including sounds of other insects that may need to be discriminated from sounds of the target insect under study (Mankin et al. 2016a). One successful approach to these problems has been to identify multiple spectral and temporal pattern features of the target insect sounds, and employ ground-truthing to determine which feature combinations provide the most reliable estimates of infestation likelihood (Mankin 2011; Mankin et al. 2018). For example, as noted above, future field studies of red palm weevil larval acoustic activity may benefit from including the spectral features of the squeals characterized in this report. Also, to avoid effects of variability in insect activity, including the variability of sound burst rates produced by red palm weevil and palmetto weevil

larvae between and during molts, it has been useful to construct indicators of infestation likelihood (Mankin et al. 2008), based on ranges of particular types of sounds that are most likely to have been produced by the target insect. This is particularly useful when there are 1 to 2 m distances or more between the insect and the sensor (Mankin et al. 2018).

Finally, considering the effects of insect activity and substrate variability noted above, it is beneficial to monitor a potentially infested tree several times at multiple positions to accommodate effects of insect sound production variability, and the effects of long distances on signal transmission. This can be difficult to accomplish in a timely fashion, given the high cost of commercially available acoustic insect detection systems and labor involved with setting up equipment at multiple sites. Fortunately, microcontroller platform systems are under development that are relatively inexpensive, portable, and simple to operate (Mankin et al. 2016b; Jakhete et al. 2017). Such instruments likely will be used more frequently as red palm weevil and other hidden insect pests continue to expand in range, and contribute to economic and food quality losses throughout the world.

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