



Acoustical Detection of Early Instar *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Canary Island Date Palm, *Phoenix canariensis* (Arecales: Arecaceae)

Authors: Herrick, Nathan J., and Mankin, R. W.

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ACOUSTICAL DETECTION OF EARLY INSTAR *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE) IN CANARY ISLAND DATE PALM, *PHOENIX CANARIENSIS* (ARECALES: ARECACEAE)

NATHAN J. HERRICK^{1*} AND R. W. MANKIN²

¹Florida A&M University, Center for Agriculture and Food Sciences, Center for Biological Control, Tallahassee, Florida 32307, USA

²U.S. Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida 32608, USA

Corresponding author; E-mail: nathan.herrick@famuedu

ABSTRACT

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), recently found in Curaçao and Aruba, has become an economically significant palm tree pest in many tropical and subtropical regions. By the time a palm infested with RPW displays visible damage, larvae have destroyed much of the trunk internal structure, typically resulting in tree mortality. Acoustic technology may enable pest managers to detect and treat early RPW infestations before tree mortality, and to reduce unwanted importation and/or exportation of infested palms. Experiments were conducted in Aruba to determine the detectability of sounds produced by early instars in open, urban environments and in enclosures with ca. 10 dB acoustical shielding. To distinguish RPW signals from background noise, recordings first were analyzed to identify larval sound impulse bursts, trains of 7-199 impulses, 3-30-ms in duration, where impulses within the train were separated by less than 0.25 s. For a burst to be considered a larval sound, it was specified that a majority of its impulses must have spectra that match mean spectra (profiles) of known larval sound impulses more closely than profiles of background noise or known nontargeted sound sources. Based on these analyses, RPW larval bursts were detected in > 80% of palm fronds inoculated with neonates the previous day. There were no significant differences between burst rates in enclosed and open environments, but the shielding provided by the enclosure enabled detection of early instars from greater distances. Thus, there is potential to use acoustic technology to detect early RPW infestation in either minimally shielded or open environments. In addition, because late-instar impulses ranged to higher amplitude and had greater diversity of spectral features than with early instars, it may be possible to identify late-instar infestations based on the amplitudes and the diversity of sound features detected.

Key Words: red palm weevil, invasive species, pest management, Caribbean

RESUMEN

El picudo rojo de las palmas (PRP), *Rhynchophorus ferrugineus* (Olivier), recientemente encontrado en Curazao y Aruba, se ha convertido en una plaga económicamente importante de palmeras en muchas regiones tropicales y subtropicales. Para cuando una palmera infestada de PRP muestra daños visibles, las larvas ya han destruido un gran parte de la estructura interna del tronco, que típicamente resulta en la mortalidad de los árboles. La tecnología acústica puede permitir a las personas que trabajan en el control de plagas a detectar y tratar infestaciones tempranas de PRP antes de que los árboles mueran y reducir la importación y / o exportación de palmeras infestadas no deseadas. Se realizaron los experimentos en Aruba para determinar la capacidad de detectar los sonidos producidos por los estadios tempranos en ambientes urbanos abiertos, y en recintos con un escudo acústico de ca. 10 dB. Se analizaron las señales registradas para identificar ráfagas de impulsos de sonidos de las larvas, secuencias de impulsos de 7-199, 3-30 ms de duración, donde los impulsos dentro de la secuencia fueron separados por menos de 0.25 s. Para una ráfaga ser considerada como un sonido larval, se especifica que la mayoría de sus impulsos deben tener espectros que concuerden con el promedio de los espectros de impulsos de sonido en los perfiles de larvas conocidas más estrechamente que los perfiles de ruido de fondo o de fuentes de sonido conocidos de otros organismos. Se detectaron ráfagas de sonido de larvas de PRP en > 80% de hojas de palmas inoculadas el día anterior con larvas recién nacidas. No hubo diferencias significativas entre la tasa de las ráfagas en los ambientes cerrados y abiertos, pero el escudo proporcionado por el recinto permitió la detección de estadios tempranos de mayores distancias. Por lo tanto, existe el potencial de utilizar la tecnología acústica para

detectar infestaciones tempranas de PRP en ambientes protegidos o mínimamente abiertos. Además, debido a que los impulsos de los estadios tardíos variaron a una mayor amplitud y había una mayor diversidad de características espectrales que en los estadios tempranos, puede ser posible identificar infestaciones de los estadios tardíos basado en la amplitud y la diversidad de características de sonido detectados.

Palabras Clave: picudo rojo de la palma, especies invasoras, manejo de plagas, Caribe

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), is of international concern due to its destructive feeding within palm trees and the threat that it poses to the ornamental and date palm industries, including plantings of *Phoenix dactylifera* L. (Arecales: Areaceae) and *P. canariensis* Chabaud. The economic worth of palm species for ornamental and date production in the United States alone is estimated at \$230,000,000/yr (Roda et al. 2011).

Originating from tropical Asia, RPW was found attacking palms in the Arabian Peninsula in the 1980s. Throughout the late 1980s and early 1990s, RPW spread to the United Arab Emirates, Saudi Arabia, and the Islamic Republic of Iran. El-Sabea et al. (2009) estimated the economic loss due to management and eradication of RPW in a date plantation in the Middle-Eastern-Gulf region to be as much as \$25,920,000 at only 5% infestation.

More recently, RPW entered North Africa, the Sharqiya region of Egypt, Europe, and Australia (Murphy & Biscoe 1999). In 2009, RPW was reported in the Caribbean on the islands of Curaçao and Aruba (Thomas 2010). Its arrival on Curaçao is suspected to be from *Phoenix* spp. imported from Egypt (Roda et al. 2011). Shipments of palm from Curaçao to Aruba, and the lack of phytosanitary regulations, have resulted in a recent establishment of RPW on Aruba.

Adult RPW are attracted to damaged and/or stressed palms. Oviposition at the base of the palm fronds is common. Early instars typically enter the palm through injured or unprotected areas of the tree where they tunnel and feed on the internal structural and meristematic tissues of stems and fronds. Damage to the palm often goes unnoticed during early larval development. By the time the palm displays symptoms of damage, larvae are late in development and it is often too late to prevent tree mortality (Faleiro 2006). Early detection of infested palms would benefit management of RPW because palms could be treated before tree mortality.

Several studies already have demonstrated successful identification of RPW larvae by recording and analyzing sounds produced by their movement and feeding activities in infested palm trees, trunk sections, or fronds in the laboratory (Al-Manie & Alkanhal 2004; Soroker et al. 2004; Pinhas et al. 2008; Mankin et al. 2008a; Potamitis 2009) or in urban field sites (Fiaboe et al. 2011;

Mankin et al. 2011). Although early detection of RPW by acoustic methods is ranked as an important need for improvement of RPW management (Mukhtar et al. 2011), the literature lacks information about the detectability of the earliest RPW instars in field environments. For example, there is a large difference in the sizes of mouthparts and muscles of early and late instars; consequently, early instars are not as powerful and could be more difficult to detect. Dembilio & Jacas (2011) found that the mandible length of first instars was 0.123 mm, while the mandible length of last instars was 2.361 mm, a 20-fold size difference.

The goal of this study was first to detect early instar RPW movement and feeding sounds in a quiet, enclosed environment and then to determine if the signals can be detected reliably in open, urban or field environments where loud ambient sounds may interfere with detection of weak insect sounds. Preliminary studies did confirm that early instars were readily detected in a quiet, enclosed environment using acoustic devices that previously had successfully detected late instar RPW in open, urban environments. Consequently, the focus of the study was on the detectability of early instars under minimally shielded and open, urban conditions.

MATERIALS AND METHODS

Insects, Palm Fronds, and Acoustically Shielded Enclosure

To obtain neonates, 4 male and 4 female field-collected RPW adults were placed in a plastic container (20 × 18 × 14 cm) with an 8-cm-diam hole cut into the lid, covered with mesh screen. Paper towels were placed at the bottom of the container and moistened with a 20% sugar solution to provide moisture and an oviposition substrate. The paper towels were replaced and inspected daily for eggs. Eggs were gently brushed onto moistened filter paper, housed in Petri dishes, and inspected daily for eclosion.

Six uninfested *Phoenix canariensis* Chabaud fronds (12 × 6 cm at base of frond), collected in Aruba (near N 12.53942° W 070.04067°, 21 m asl), were prepared for inoculation by drilling 3 holes approximately 2.54 cm apart into the base of each frond. The holes (3 mm diam) were drilled approximately 5 cm deep. Neonates were placed

gently into each hole using the soft-tipped end of a paintbrush. Holes were plugged with a small piece of paper towel to prevent escape of larvae. Fronds were placed in a plastic bag with 2.54 cm water that was replaced daily to prevent them from drying out. Adults, eggs, and fronds were held under local ambient conditions. Acoustic recordings (see next section) were obtained in enclosed and open environments beginning about 24 h after fronds were inoculated with neonates. The enclosure, set up purposely to be low-cost and easily constructed, was a 3- \times 6-m room with a 2.5-m ceiling, isolated inside a building that provided partial shielding against wind, road noise, bird calls, and other external background sounds. Doors and windows were closed, and circuit breakers and water supply were shut off to reduce background noise levels.

Late instars were collected for acoustic comparisons with early instars by removing a palm frond from a *P. canariensis* tree which had visible signs of infestation, and which displayed drooping and yellowing of the fruiting structures. Three late instars (mandibles ca. 1 mm or larger) were dissected from the frond after the acoustic recordings were complete.

Larval Signal Recording and Analysis

Two sets of 5 (2-min) recordings were made of sounds produced by early instar RPW in the 6 inoculated fronds (replicates). The first set of recordings was made under enclosed conditions, while the second set was made outdoors, where extrinsic environmental conditions were not controlled, and frequent periods of vehicular noise, wind, and bird calls occurred. The recordings were made between 13:00 and 18:00 h.

The experiments were conducted using an AED-2000 amplifier (Acoustic Emission Consulting [AEC], Sacramento, California) with a Model SP-1L sensor-preamplifier module (AEC, Sacramento, California) which had a magnetic attachment at its base, enabling connection to a screw (13 \times 1 cm) inserted into the frond between the second and third inoculation site and approximately 4 cm from the first inoculation site (Fig. 1). The AED-2000 was connected to a digital (44.1 kHz sampling rate) audio recorder (model HD-P2, Tascam, Montebello, California) with headphones that enabled immediate listener assessment of larval signals as they were being recorded. The AED-2000 amplifier filters out signals below 1 kHz where much of the traffic and wind noise occurs (Mankin et al. 2011).

Computer assessments were performed to compare the detectability of early instar RPW in enclosed and open environments. The procedure for conducting assessments was based on previous findings that RPW larval sounds typically are produced as bursts (groups or trains of 7-199 in-

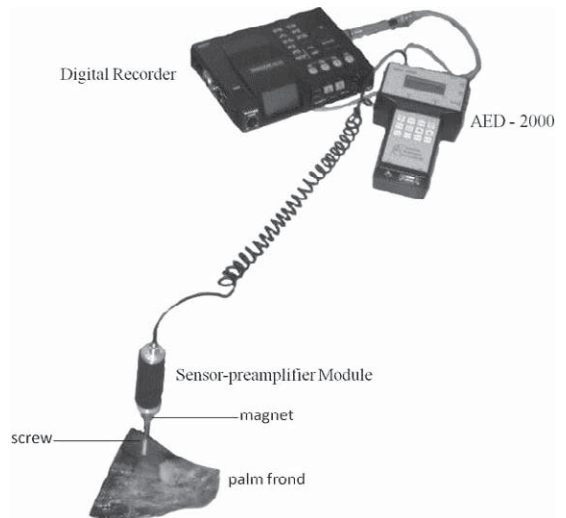


Fig. 1. Experimental set-up of AED-2000, digital recorder, and sensor-preamplifier module magnetically attached to a screw and inserted into the base of a pruned palm frond for use in obtaining digital recordings of *Rhynchophorus ferrugineus* early instar feeding.

dividual, 3-30-ms impulses spaced < 0.25 s apart) interspersed by longer, quiet intervals (Mankin et al. 2008a). Bird noise, vehicular noise, and wind, often contain longer, continuous signals with harmonics rather than the short, broadband impulses typical of insect sounds (Mankin et al. 2011). When wind produces leaf-fluttering and tapping sounds, such signals do contain broadband impulses resembling those produced by insects, but these tend to occur in longer trains with 200 or more impulses (Mankin et al. 2011). Incidental taps or internal tree noises tend to produce shorter trains containing < 7 impulses (Mankin et al. 2008b). In addition, the spectra of vehicular and wind signals often have frequency peaks that are different from insect sound impulses and can be distinguished by comparisons with spectral means (profiles) of impulses in known sounds produced by insects and other sources using DAVIS and other custom-written insect signal analysis programs (Mankin et al. 2000, 2008a). Although these methods of distinguishing insect sounds from background noise are not completely error-free, they have been used successfully in several previous studies to detect RPW (Mankin et al. 2008a; Fiaboe et al. 2011; Mankin et al. 2011) and other insects (Mankin et al. 2008b) in wood when background noise was of low to moderate intensity.

In the initial stage of the insect sound assessment procedure, recordings were screened using the Raven sound analysis program (Charif et al. 2008). Intervals of ca. 40-180-s were selected that separately contained series of insect sounds or background sounds unambiguously

identifiable by a listener with previous field-recording experience. Mean spectral profiles of the insect sound impulses and background noise impulses in the selections were constructed using the DAVIS insect signal analysis program (Mankin et al. 2000). For this experiment, one larval profile was constructed from a relatively noise-free recording with early instars, and a second from a relatively noise-free recording with late-instars (see details in RESULTS). A background noise profile was constructed from a period of vehicle noise and a second profile was used from a previous study in nearby Curacao (Fiaboe 2011) because these islands have similar species in their urban areas. Finally, the complete set of enclosed- and open-environment recordings were analyzed in DAVIS to distinguish whether spectra of individual impulses closely matched a larval sound profile or if they closely matched vehicle or bird noise profiles, based on the relative magnitudes of particular signal features, i.e., by assessing least-square differences among relative spectrum-level magnitudes within specific frequency ranges (Mankin et al. 2000). Impulses that closely matched either the early- or late-instar profile were classified by automated DAVIS subroutines as larval impulses, while impulses that matched either the vehicle noise or bird noise profile were classified as background noise impulses. In this experiment, the threshold for acceptance was an average difference of 5 dB between impulse and profile over the frequency range between 1 and 10 kHz (Mankin et al. 2000). If an impulse failed to closely match any of these profiles, it was discarded as unknown background noise. (It should be noted that comparisons of spectral features of individual impulses with spectral features of insect sound profiles can be done manually for individual impulses using signal analysis programs like Raven, but the manual process is considerably slower than an automated process).

Larval bursts were specified as trains of 7-199 impulses, a majority of which closely matched a larval spectral profile. The rates of occurrences of larval impulses and larval bursts (Mankin et al. 2008b) were calculated, as well as rates of occurrences of background noise impulses and trains of background noise impulses.

For statistical comparisons, the larval-sound-burst rates and larval-sound-impulse rates detected in enclosed and open conditions were compared using two-tailed, paired Student's *t*-tests. In addition, two-tailed, paired Student's *t*-tests were performed on the rates of background noise trains in enclosed and open environments, as well as rates of background noise impulses in enclosed and open environments. Counts of impulses in each recording that matched the two larval impulse profiles were pooled as larval impulses, and counts of impulses in each recording that matched

the vehicle and bird noise impulses were pooled as background noise impulses (see RESULTS).

RESULTS

Early instar RPW produced impulses with a wide range of amplitudes and spectral features, exemplified in the oscillogram and spectrogram of Fig. 2A-B. Late instars (Fig. 2C) produced impulses with amplitudes similar to early-instar impulses, but also produced numerous impulses of greater amplitude than typically observed from the early instars. This difference is reflective of the greater strength and greater range of movement and feeding activity of the late instars. In a series of 582 larval impulses recorded from a frond containing 3 late instars, for example, the mean \pm SE sound pressure level for frequencies $>$ 1 kHz (SPL[$>$ 1kHz]) was 66.45 ± 0.226 dB (for a more complete description of SPL in different frequency ranges see Mankin et al. 2000), while 130 larval impulses recorded from a frond inoculated with three neonates had a lower mean \pm SE SPL[$>$ 1kHz] of 54.8 ± 0.515 dB. The 11.7 dB reduction in SPL was highly significant under a two-tailed Student's *t*-test with unpaired observations and pooled sums of squares ($t = 400$, $P < 0.0001$).

The spectra of impulses produced by late and early instars were similar, with broad-band features primarily between 2 and 14 kHz (Figs 2B, 2D, and 3a-b). The late-instar spectral profile (Mankin et al. 2008a, 2011) in Fig. 3a was calculated as a mean of 44 spectra in a series of impulses recorded from a frond containing 3 late-instar larvae. The early-instar profile in Fig. 3b was calculated as a mean of 42 (512-point) spectra in a series of impulses from a frond inoculated with 3 neonates. The vehicular noise profile in Fig. 3c was calculated as a 5-s mean of 50 consecutive 512-sample spectra of vehicle-produced impulses recorded under open conditions during a period of loud traffic.

The shielding of the enclosed room reduced the sound pressure level of external background noise by ca. 10 dB. For example, in a 50-s period recorded from a palm frond outdoors, 54 impulses classified as background sounds had a mean SPL[$>$ 1kHz] of 62.17 ± 0.336 dB, while 33 background sound impulses had a mean SPL[$>$ 1kHz] of 51.06 ± 0.641 dB when the frond was transferred to the enclosure.

To compare the rates of larval signals and background noise signals in enclosed and open environments, we tested the spectrum of each sound impulse against each of four spectral profiles, a) - d), where profile a) was the late-instar profile in Fig. 3a, profile b) was the early-instar profile in Fig. 3b, profile c) was the vehicle noise profile in Fig. 3c; and profile d) was the bird-calls profile constructed by Fiaboe et al. (2011) from recordings of bird calls in Curaçao. In this ex-

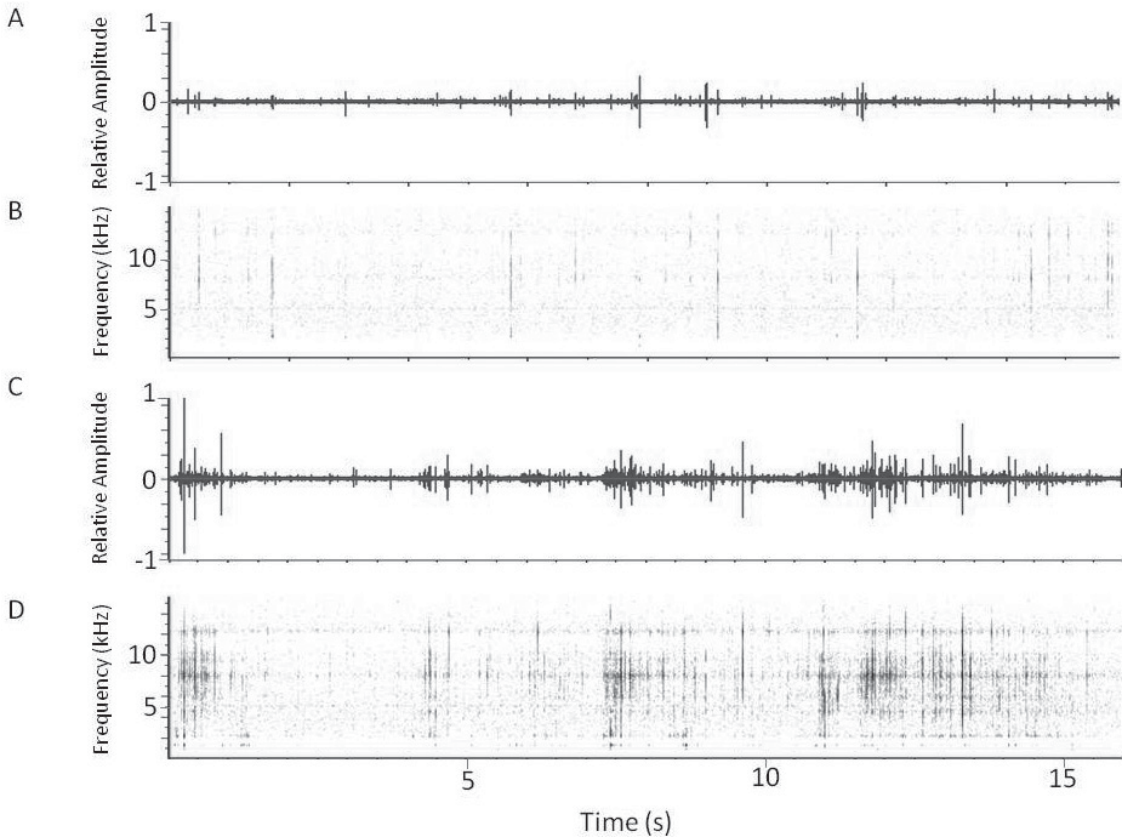


Fig. 2. Oscillogram (A) and spectrogram (B) of sound impulses produced by early-instar *Rhynchophorus ferrugineus* compared with oscillogram (C) and spectrogram (D) of impulses produced by late instars.

periment, it was difficult to find bird calls that did not contain embedded larval sounds, but it

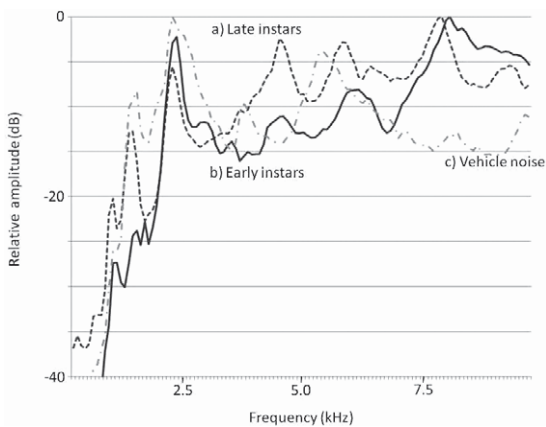


Fig. 3. Mean spectral profiles of sound impulses produced by late-instar *Rhynchophorus ferrugineus* (RPW) larvae (dashed line), early-instar RPW larvae (solid line), and vehicle noise (dot-dashed line) in recordings collected by AED-2000 instrument.

was feasible to use the same bird-call profile that had been constructed from recordings in Curaçao because the bird species are very similar on the Aruba Curaçao, and Bonaire (Prins et al. 2009). Similarity coefficients of 74-78% were measured by Prins et al (2009) for resident species, and 65-73% for migrant species.

DISCUSSION

The recordings in this study from early instar RPW confirm that larvae of all instars can be detected over distances of at least 5-10 cm in either enclosed (shielded) or open (exposed) environments. Based on previous studies with similar sized insects, it is possible that early instars could be detected at distances up to 0.5-1 m in quiet environments, while late instars can be detected at distances of 1-4 m (Mankin et al. 2011). The ~10 dB reduction in background noise afforded by recording in a quiet, enclosed room enables detection of the early instars over a 2-4-fold greater distance than in the open environment (Mankin et al. 2000). It should be noted, however, that background noise can be highly variable, and

frequently could exceed the levels in the current experiment. In the Caribbean region, for example, wind noise can be highly significant in the afternoon and bird calls in the morning. The rate of larval sound production also can be variable. For one early instar tested in Table 1, the rate of production of larval sound bursts varied from 0.01 to 0.23 s⁻¹ in separate recordings and the rate of larval sound impulses varied from 0.84 to 5.13 s⁻¹. Under conditions when the noise levels at a recording position are higher than typically detected larval sound amplitudes, or when larval sound bursts are detected only in brief, widely spaced intervals, the likelihood of obtaining a false negative assessment of infestation can be reduced by recording on multiple occasions or at multiple positions.

In regions where offshoots are a primary method of propagating new trees, the capability to detect early instars provides an opportunity to augment visual inspection with acoustic assessments and reduce the risks of introducing RPW into new plantings or replacement trees. Acoustical technology also affords nursery managers a preventative tool for inspecting palms for RPW that are intended for import or export, the primary pathway of RPW spread.

In addition to our findings of the ability to detect early instar RPW in open environments, the results indicate that early instar RPW produce sounds that are similar to those of late instar RPW. However, late instar RPW produce sounds that are unique to this particular stage of development. These results suggest that distinctions among signals from different instars may be discernible upon analysis of acoustical recordings in the laboratory. The ability to distinguish among instars would be useful in laboratory insect development assays especially when destructive sampling is the only means to estimate develop-

ment time. Future studies should also focus on determining if there is intra-specific variation of acoustical signatures in *Rhynchophorus* spp.

Visual detection of early infestations of RPW is not possible because the tree does not show symptoms (larvae later in development often cause yellowing, drooping, and dieback of the fronds and fruit bodies). However, as demonstrated, early instars can be detected using the equipment we have applied in this study. This is especially useful in instances where palms or palm offshoots are intended for import or export or when there is concern that a tree of high commercial or ornamental value has been exposed to RPW. We recommend the following procedures to practitioners for identifying an RPW infestation in the field:

Step 1. Practitioners should first familiarize themselves with the operation of the instruments. Each day the instrument is used, it should be tested with standardized signals that enable the operator to determine if the systems are powered and functioning properly and if the probe is properly attached and collecting signals of appropriate amplitudes for insect detection.

Step 2. Practitioners should familiarize themselves with amplitudes and spectral profiles of sound impulses produced by RPW in a shielded enclosure, and the amplitudes and spectral profiles of signals present in the open environment. It may be necessary to conduct 0.5 d or more of training that uses recordings of previously verified infestations.

Step 3. If possible, the trees or offshoots should be prescreened for visual signs of infestation, and the probe should be inserted

TABLE 1. COMPARISONS AMONG EARLY-INSTAR *RHYNCHOPHORUS FERRUGINEUS* SOUND RATES AND BACKGROUND NOISE RATES RECORDED IN 6 PALM FRONDS IN ENCLOSED (SHIELDED) AND OPEN (EXPOSED) CONDITIONS IN ARUBA (WITH MEANS \pm STANDARD ERRORS LISTED IN FINAL 2 ROWS)

Larval profiles sound ¹ rate (No. / s)				Background noise ² rate (No. / s)			
Bursts		Impulses		Trains		Impulses	
Shielded	Exposed	Shielded	Exposed	Shielded	Exposed	Shielded	Exposed
0.081	0.178	3.18	0.95	0.162	0.165	0.27	5.76
0.322	0.070	6.14	2.34	0.151	0.241	0.38	0.45
0.000	0.042	0.44	5.75	0.430	0.105	1.24	0.14
0.171	0.110	4.42	4.15	0.150	0.191	0.45	0.53
0.160	0.091	3.39	5.82	0.511	0.314	3.19	9.16
0.231	0.010	5.13	0.84	0.211	0.451	0.58	1.87
0.161	0.084	3.783	3.308	0.269	0.245	1.018	2.985
± 0.046	± 0.024	± 0.806	± 0.923	± 0.065	± 0.050	± 0.456	± 1.501

¹Rates of bursts of sound impulses and rates of sound impulses that matched larval sound profiles Fig. 3a-b).

²Rates of trains of sound impulses and rates of sound impulses that matched either the vehicle noise profile (Fig. 3c) or the bird calls profile (see RESULTS).

into a tree or clamped onto an offshoot or sample at positions most likely to be near a site of infestation. Unless it is very large, an infestation in the crown of the tree is not likely to be detected over distances > 2-4 m. In addition, the signal will be strongly attenuated if the probe is not tightly attached to the sound-conducting structure.

Step 4. A waiting period of 30-120 s after the probe is inserted may be needed to allow larval activity to recover from the effects of disturbance.

Step 5. The signals should be monitored with both headphones and an audio recorder for 30-180 s. Periods with excessive wind or vehicle noise should be discarded. The listener should make notes of specific times of occurrence of salient sounds and events. Often it is helpful to confirm observations with colleagues at the time of recording, and to place greatest reliance on signals that can be verified by multiple listeners at multiple positions. This will provide greater perspective for the results of automated signal analysis.

Step 6. The recorded signals provide an objective, quantitative backup to the subjective listener assessments at the recording sites. If sound impulse bursts that match spectral profiles of known larval signals are detected by the signal analyses and by confirmation of listeners, an infestation has almost always been confirmed by destructive sampling (i.e., by dissection of the trunk or frond) in previous studies. Infestations have been confirmed only infrequently when the automated assessments fail to identify larval sound bursts.

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