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ACOUSTIC DETECTABILITY OF *RHYNCHOPHORUS CRUENTATUS* (COLEOPTERA: DRYOPHTHORIDAE)

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Abstract

The palmetto weevil, *Rhynchophorus cruentatus* (Fabricius), is a pest of cultivated palms in Florida. The larvae of this species and 2 other important *Rhynchophorus* pests, *R. ferrugineus* (Olivier) and *R. palmarum* (L.), feed internally and cause structural damage to palm fronds, trunks, and offshoots. Often, infestations are not detected until the tree suffers irreparable damage. Acoustic methods used previously to detect *R. ferrugineus* in field environments were applied to investigate detectability of early instar *R. cruentatus* larvae. Sounds from neonates inoculated into palm fronds were recorded for 120 s periods at 7-day intervals for 56 days in urban (exposed) and acoustically shielded (enclosed) conditions that might be encountered when screening offshoots for sale or transportation. The sounds were classified by automated spectral analyses into multiple categories, including vehicle noise, bird calls, and broadband, 0.3-3-ms larval sound impulses. Because spectral characteristics alone are not always reliable identifiers of larval signals in wood, the analyses also identified trains of 6 or more closely grouped larval impulses called bursts to help classify fronds as infested or uninfested. Larval bursts were produced at sufficient rates to classify inoculated fronds correctly each day of testing, although molting and resting behaviors resulted in the absence of bursts in 6-50% of individual, 2-min recordings. The rates of larval bursts were not significantly different in paired comparisons of recordings obtained from the same frond on the same day under urban and shielded conditions, which suggests that bursts are useful as indicators of *Rhynchophorus* infestations in a variety of conditions.

Key Words: palmetto weevil, cryptic stem borer, Sabal palm, monitoring

Resumen

El gorgojo del palmeto, *Rhynchophorus cruentatus* Fabricius, es nativo de la Florida y ataca a las palmeras. Al igual que sus congeneres económicamente destructivos, *R. ferrugineus* (Olivier) y *R. palmarum* (L.), *R. cruentatus* se alimenta internamente y, a menudo no se detecta hasta que se produzca un daño irreparable. Los métodos acústicos utilizados con éxito anteriormente para la detección de *R. ferrugineus* en el campo fueron adoptados para investigar la detección de *R. cruentatus* en diferentes condiciones de ruido. Se colocaron los neonatos (recien nacidos) en agujeros perforados en la base de las hojas de palma y se registraron cinco intervalos de 2 min a partir de 10 hojas a intervalos de 7 días por 56 días en un escenario urbano y un espacio acústicamente protegido el cual que sea disponible para la prueba de los vástagos antes de vender y transportarlos. Se clasificaron los sonidos por medio de análisis espectrales automatizados en varias categorías, incluyendo llamadas de aves, ruidos de carros, impulsos de sonidos de larvas de 0.3-3 ms banda ancha. Debido a que las características espectrales por sí solas no siempre son fiables para la identificación de los impulsos de larvas a través de largas distancias en madera, los análisis también identificaron patrones temporales, grupos de 6 o más impulsos estrechamente espaciados categorizados como ruidos de larvas, que se utilizan como indicadores para clasificar las frondas como infestadas o no infestadas. Los ruidos de las larvas fueron producidos a tasas suficientes para clasificar las hojas infestadas correctamente en cada día de la prueba, a pesar del comportamiento de muda y descanso que resultaron en la ausencia de ruidos en el 6-50% de las grabaciones individuales, dependiendo del día de prueba. Las clases de ráfagas de ruido de las larvas no fueron significativamente diferentes en las comparaciones pareadas de grabaciones obtenidas a partir de la misma hoja de palma en el mismo día bajo condiciones urbanas y protegidas, lo que sugiere que las ráfagas de sonido son útiles como indicadores de las infestaciones de *Rhynchophorus* en una variedad de condiciones.

Palabras Clave: gorgojo del palmetto, barrenador críptico del tallo, palma Sabal, monitoreo

Several species of *Rhynchophorus* (Coleoptera: Dryophthoridae) palm weevils attack commercial and ornamental palms, including *R. ferrugineus* (Olivier), originally from Southern Asia, *R. palmarum* (L.) from South America, and *R. cruentatus* (Fabricius)(Wattanapongsiri 1966), native to the southeastern US (Thomas 2010). *Rhynchophorus cruentatus* is a pest of stressed or transplanted cabbage palms, *Sabal palmetto;* saw palmettos, *Serrenoa repens*, and Canary Island date palms, *Phoenix canariensis* (Giblin-Davis et al. 1989). In 2000 for example, *R. cruentatus* caused *P. canariensis* damage estimated at $285,000 - $380,000 in a nursery in South Florida (Hunsberger et al. 2000). Because *R. cruentatus*, *R. ferrugineus*, and *R. palmarum* have similar life cycles (Salama et al. 2009; Miguens et al. 2011), investigations on *R. cruentatus* biology and behavior are of potential interest not only for palm weevil management in Florida but also for *Rhynchophorus* spp. management in other regions where they cause substantial economic damage to cultivated palms.

Female *R. cruentatus* lay eggs singly in leaf bases or in wounds on the exterior of a palm tree trunk (Weissling & Giblin-Davis 1994, 1997). The oval-shaped, cream-colored eggs hatch in approximately 3 days (Hunsberger et al. 2000; Weissling & Giblin-Davis 1997). The larvae crawl into and feed within the trunk, damaging its structural integrity. *Rhynchophorus cruentatus* completes its life cycle from the egg to adult emergence in 84 days (Giblin-Davis & Howard 1989) or less (Hunsberger et al. 2000; Weissling & Giblin-Davis 1995). As with *R. ferrugineus* and *R. palmarum* (Thomas 2010), visible symptoms of *R. cruentatus* larval presence often appear too late to prevent an infested tree from dying (Weissling & Giblin-Davis 1997). Improved methods for earlier detection and treatment of *R. cruentatus* and other *Rhynchophorus* spp. could help prevent economic losses to cultivated palms (Herrick & Mankin 2012).

Because movement and feeding sounds transmit well in wood, early and later stages of all cryptic *Rhynchophorus* larvae are prospects for acoustic detection and monitoring with specialized instrumentation (Herrick et al. 2013). *Rhynchophorus ferrugineus* has been investigated by acoustic methods in both field and laboratory conditions (Soroker et al. 2004; Pinhas et al. 2008; Mankin et al. 2008a; Potamitis et al. 2009; Siriwardena et al. 2010; Fiaboe et al. 2011; Herrick & Mankin 2012; Herrick et al. 2013). In general, acoustic studies of *Rhynchophorus* spp. and other internal feeders have found that detection can be difficult in environments with high background noise, but usually can be achieved in moderate to low background noise. The use of an acoustically shielded enclosure enables detection of early instars over greater distances than in the absence of shielding (Mankin et al. 2011). In a study with *R. ferrugineus* for example, the use of an enclosure resulted in a reduction of background noise from ca. 62 to ca. 51 dB at frequencies above 1 kHz (Herrick & Mankin 2012), where dB is a logarithmic measure of the vibrational acceleration. Finally, it is known that spectral averages or profiles of insect sounds can serve as acoustic indicators of infestation (Mankin et al. 2007), but resonances in wood and plant structures can distort or obscure signals transmitted over long distances from an insect to a sensor (Mankin et al. 2008a). In these cases, it often is necessary to consider the temporal patterns of sounds in addition to their spectral patterns in determining whether they are produced by the target insect or by external noise sources.

Because it has been established already that early-instar *R. cruentatus* can be detected acoustically (Herrick et al. 2013), the focus of this experiment was not on proof of principle, but rather on a more practical consideration of the relative detectability of *R. cruentatus* larvae in urban conditions or in shielded environments that might be used when screening offshoots or small trees before transport to landscapes or commercial groves.

Materials and Methods

Insect Colony

Adults were collected with cruentol (5-methyl-4-octanol, ChemTica Internacional, S.A.) lures in Tallahassee, Florida. Individual male and female pairs were held in tagged glass jars containing an oviposition substrate, cotton balls dosed with 20% honey-water solution (Shahina et al. 2009). One day later, the males were removed (Kaakeh et al. 2001) to prevent interference with the egg-laying process and possible damage to eggs (Giblin-Davis et al. 1989; Weissling & Giblin-Davis 1994). The cotton balls were examined daily for the presence of eggs and replaced. The eggs were placed in petri dishes lined with moistened filter paper. The dishes were sealed with a clear wrap to prevent the larvae from crawling out after eclosion (Weissling & Giblin-Davis 1994). The petri dishes were labeled by oviposition date and female jar number. The total number of eggs laid by each female, the number of eggs that eclosed, and dates of eclosion were recorded.

Thereafter, the petri dishes were examined daily for eclosion of neonates and the filter papers were kept moist. Individual larvae from hatched eggs were introduced into small holes (≈2 mm) drilled into sections of sugarcane stem. The sugarcane stems were tagged with the inoculation date, waxed at both ends to conserve moisture, prevent rapid dehydration, and prevent fungal infection, and incubated in a growth chamber maintained at 30 °C and 70 ± 5% RH and a 13:11 h L:D photoperiod (Weissling & Giblin-Davis 1995).

Acoustic Recordings from Inoculated Palm Fronds

Ten 12-cm-long Sabal palm fronds (replicates) were cut from an uninfested tree in Tallahassee, Florida. Three holes were inserted 1-cm apart at the base of each frond. A screw was inserted at the center of the frond to serve as a connection point for acoustic recordings. Neonates from the laboratory colony were inoculated into the cut base of the palm frond, one per hole. Each hole was plugged with paper towel to prevent larval escape. The fronds were placed into plastic containers with about 25 ml of water to prevent them from drying out and were maintained at ambient environmental conditions with periodic additions of water until the end of the 56-d experiment.

The setup for monitoring and recording larval signals was similar to that described in Herrick & Mankin (2012). A sensor-preamplifier module (model SP-1L Acoustic Emission Consulting [AEC], Sacramento, California) was attached magnetically to the screw in each palm frond and connected to an AED 2000 amplifier (AEC, Sacramento, California). The AED-2000 was connected to a digital audio recorder (model HD-P2, Tascam, Montebello, California) that enabled monitoring with headphones and stored the signals for subsequent computer assessments of the larval sounds (Mankin et al. 2008a, 2011). The digitization rate was 44.1 kHz. The AED-2000 amplifier filters out signals below 1 kHz to reduce traffic and wind noise (Mankin et al. 2011).

For paired comparisons of replicates in enclosed and exposed environments, 2 to 5 recordings of 120 s were collected each day of testing from each frond in each environment. The recordings were collected between 11:00 h and 18:00 h at 7-day intervals over a 56-d period, beginning 1 day after neonate larval inoculation. The acoustically shielded enclosure was a windowless, 6 × 12 m room with a 2.5 m ceiling inside a building. All nearby mechanical equipment was silenced during testing. The exposed environment was in the yard of an apartment complex next to a city street.

Automated Classification of Larval and Background Noise Signals

The 2-min acoustic recordings were prescreened using Raven (Charif et al. 2008) to locate periods of identifiable insect sounds and background noises and discard periods containing electrical interference and other loud signals that masked sounds of interest. As in previous studies with other stem borers (Mankin et al. 2011), larval sounds could be detected as trains (bursts) of short, 1-3 ms broadband impulses, while traffic noise, bird song, and other background noise often occurred as continuous signals with harmonic peaks that could be discriminated from larval sounds either by automated computer analysis or by experienced listeners.

For automated discrimination of larval sounds from noise, 4 spectral profiles (Mankin et al. 2011) were constructed as averages of multiple spectra from recordings of signals identified during prescreening as those most frequently encountered during testing. The profiles were constructed using the custom-written insect signal analysis program: "Digitize, Analyze, View, Insect Sounds" (DAVIS) (Mankin et al. 2000; Herrick et al. 2013). Two larval profiles were average spectra of impulses in trains with distinctive high- or low-frequency characteristics identified from early-instar *R. cruentatus* larvae recorded in this study. A bird-noise profile was constructed as an average spectrum of 10-ms intervals of song recorded over a 15 s period from birds singing in trees near the experimental site. A traffic profile was used that had been constructed from low-frequency vehicular noise described previously in Herrick & Mankin (2012).

To match recorded signals with profiles, a 512-point Fourier transform was performed on each impulse of amplitude greater than a threshold set at 5% of the maximum signal level. Then the spectrum level at each frequency point (i.e., each multiple of 86.13 Hz) between 1 and 10 kHz was matched to the spectrum level of each profile (Mankin et al. 2011). The spectrum was categorized according to the profile to which it had the smallest total mean-square difference. If the smallest total mean square difference was greater than a preset threshold, typically 50 dB, the spectrum was classified as unspecified noise, matching none of the profiles.

The DAVIS program scanned through the complete set of recordings, classifying each impulse as matching one of the 2 larval profiles, a background noise profile, or as a non-match. It saved the time of occurrence and the type of each impulse in a spreadsheet. Consecutive larval impulses separated by < 150 ms were grouped together as impulse trains and classified according to the profile matched by a majority of the impulses. Based on inspection of listener-recognized signals, trains containing ≥ 6 and < 200 larval impulses were classified as larval sound bursts (Mankin et al. 2008a). For each recording, the rates of larval sound impulses, larval bursts, and noise impulses (impulses that matched neither larval sound profile), were calculated for statistical analyses.

Statistical Analyses

Paired 2-tailed *t*-tests were used to compare mean rates of larval impulses and bursts in exposed and enclosed environments recorded from individual fronds on different days after the beginning of testing. Also, a paired 2-tailed *t*-test

was conducted to compare the mean rates of noise impulses in exposed and enclosed conditions over the 56-d duration of the experiment. The mean rates of noise impulses, larval impulses, and larval bursts were calculated for each frond as averages of the rates each day in enclosed and exposed recordings.

In a previous study of bursts produced by *R. ferrugineus* in sugarcane (Mankin et al. 2008a), the likelihood of larval infestation was considered low if the rate of larval bursts was < 1 per 50 s (0.02 bursts / s). For this experiment, it was convenient to set a similar criterion that < 1 larval burst per 120 s recording (0.0083 bursts / s) indicated low likelihood of infestation. In this case, an estimate of low likelihood of infestation would signify a detection error because all of the fronds in the experiment had been inoculated.

Results

Characteristics of Acoustic Recordings from Inoculated Palm Fronds

An example of signals recorded from larvae in frond #6 on day 7 after inoculation (Fig. 1) displays some of the similarities and differences frequently observed with signals recorded in the enclosed and exposed environments. Fig. 1A is an oscillogram of 120 s activity from the enclosed, acoustically shielded environment. The DAVIS program identified 337 larval impulses and 81 bird or traffic noise impulses. Four trains of larval impulses separated by < 150 ms (near 11.5, 19.5, 40 and 97 s) were classified as larval bursts.

One of the larval bursts is expanded in a 30-ms inset (Fig. 1A.a) for additional detail on typical ranges of amplitude and relative timing of impulses. The burst contains 2 larval impulses in the first 2 ms at its beginning, a third near its midpoint (near 13 ms) and 3 larval impulses near its end (20-30 ms).

Signals recorded 4 h earlier from the same frond in the exposed environment are displayed in Fig. 1C. There were 331 larval impulses and 81 noise impulses in this example, and 16 impulse trains met the criterion for larval bursts. The primary difference between the spectrograms in the enclosed (Fig. 1B) and exposed environments (Fig. 1D) was the noticeably higher energy below 4 kHz in the exposed environment. The latter shows a nearly continuous presence of signal between 2 and 4 kHz.

A series of samples recorded once each week from frond #3 over a 56-day period (Fig. 2) is representative of the overall range of amplitudes and temporal patterns of larval and noise impulses recorded in the exposed environment. Two examples of noise include an interval with traffic noise between 20 and 30 s on day 14, and an interval with persons talking between 5 and 15 s on day 28.

Mean Rates of Noise Impulses, Larval Impulses, and Larval Bursts in Enclosed and Exposed Environments

It was expected that the mean rate of noise impulses detected in the exposed environment would be greater than in the enclosed environment but the mean rate of larval bursts would be similar in the 2 environments. Indeed, in paired comparisons where the mean rate of noise impulses from each frond in the enclosed environment each day of testing was subtracted from the mean in the exposed environment, the mean difference ± standard error (SE), 4.213 ± 1.293 impulses / s, indicated a statistically significant difference between the 2 conditions (df = 62, t = 3.26, P = 0.002). Also as expected, in paired comparisons where the mean rate of larval bursts from each frond in the enclosed environment on each day of testing was subtracted from the mean rate in the exposed environment, the mean difference ± SE, 0.022 ± 0.027 bursts / s was not statistically significant (t = 0.823, df = 84, P = 0.412).

However, the mean rates of larval impulses in paired comparisons of recordings obtained from each frond each day under exposed and enclosed conditions were significantly different. The mean difference ± SE between larval sound impulse rates in exposed and enclosed conditions was (1.718 ± 0.849 impulses / s, t = 2.024, df = 84, P = 0.046).

Analysis of signals from different fronds on different days after inoculation (Table 1) indicated that, except on day 21 (Table 1), larval bursts were present in 70–90 percent of individual recordings. Under a criterion that absence of bursts in a 2-min period indicates low infestation likelihood, the tested samples would have been designated incorrectly as uninfested in 6 to 50 percent of the recordings. However, bursts were observed in at least one recording from each frond on each test day, which indicates that the probability of incorrectly classifying an infested sample as uninfested could be reduced by recording signals multiple times each day of testing.

Mean Rates of Larval Bursts from All Fronds on Different Days

Although each frond had different patterns of larval burst activity over the 56-day testing period, there was a trend of greatest activity on day 1 after inoculation and least activity on day 21 (Fig. 3). Otherwise, the mean activity levels remained in the range of 0.09 – 0.15 larval bursts / s. The rates of impulses in the example of Fig. 2 follow

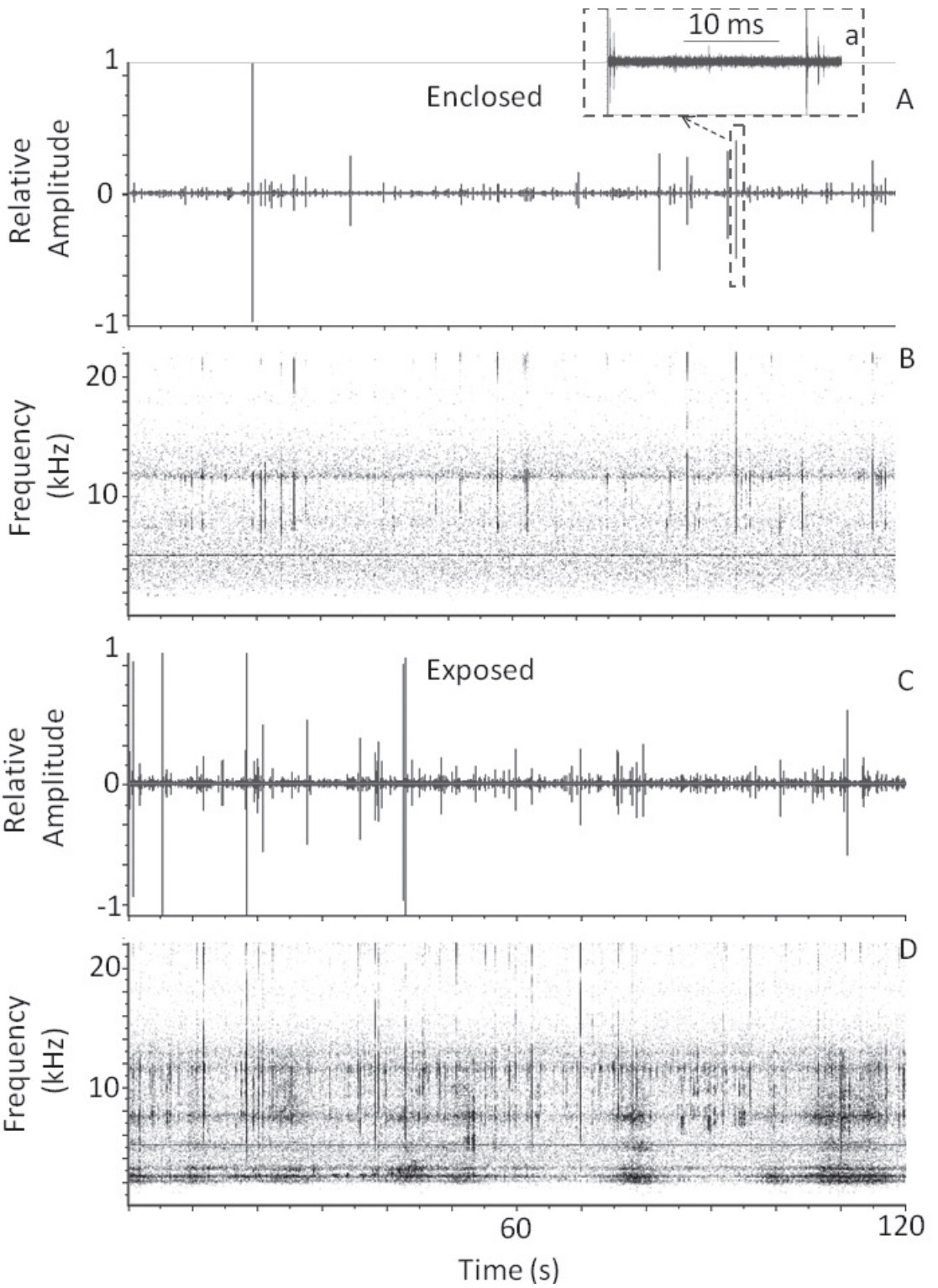

Fig. 1. Examples of oscillograms (A and C) and spectrograms (B and D) of sounds recorded from an inoculated frond on day 7 in enclosed and exposed environments. Inset (a) displays a 30-ms expansion of a larval impulse burst. Darker areas in spectrograms indicate higher relative energy at those frequencies and times.

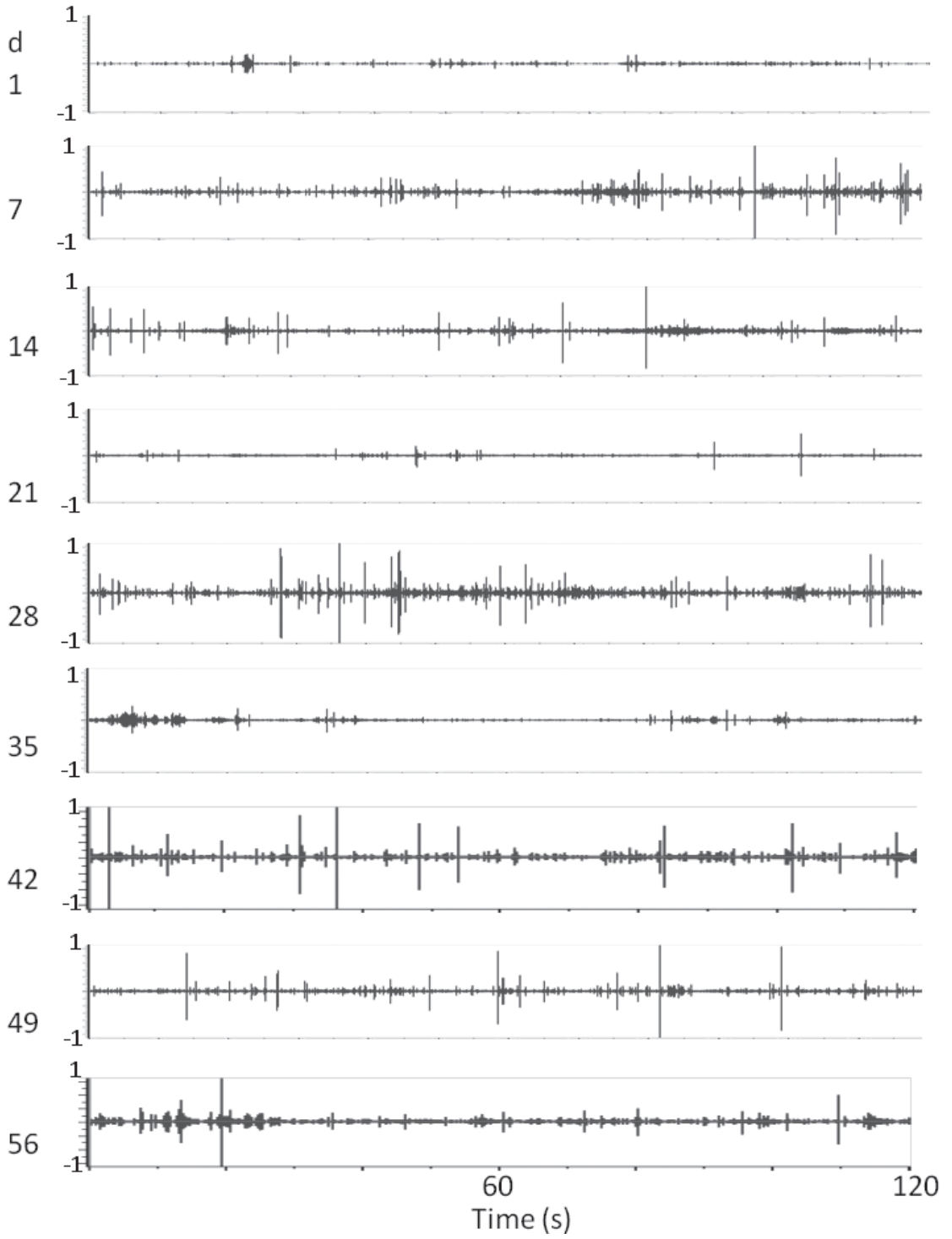

Fig. 2. Oscillograms of sound impulses recorded from an inoculated frond over a 56 d period in the exposed environment. Vertical scales are relative amplitude, held constant for all recordings.

TABLE 1. COMPARISONS OF THE PERCENTAGES OF 2-MIN ACOUSTIC RECORDINGS WITHOUT DETECTABLE *R. CRUENTATUS* LARVAL BURSTS AT DIFFERENT TIMES AFTER NEONATE INOCULATION.

Day after inoculation	Total No. replicates	No. w/o bursts	Detection error (%)
1	32	3	9.4
7	41	4	9.8
14	38	6	15.8
21	35	18	51.4
28	32	5	15.6
35	34	8	23.5
42	28	3	10.7
49	32	2	6.3
56	32	3	9.4

the trend seen in Fig. 3 except for day 1. The rate for this frond on day 1 was 0.134 bursts / s, somewhat less than the overall mean of 0.367 bursts / s for all fronds recorded on the first day (Fig. 3).

DISCUSSION

It was of biological interest that the signals from all fronds on days 14 and 21 were considerably lower than during the rest of the test period. It is possible that the low level of activity on these days coincided with a period when multiple larvae had molted. A similar phenomenon was noticed by Shade et al. (1990) when monitoring acoustic activity of individual *Callosobruchus maculatus* (F.) larvae in cowpea seeds, and by Pittendrigh et al. (1997), who monitored activity of individual *Sitophilus oryzae* (L.) larvae in maize. After the low point of activity on day 21, the activity levels generally trended higher. This result might be expected if older, larger instars produced more frequent, louder sounds than the younger, smaller instars, as was observed by Shade et al. (1990) and Pittendrigh et al. (1997). However, there are numerous factors that could increase or decrease activity rates at any given time; consequently, there is often only a weak relationship between the activity rate and the number or size of insects in a sample (Mankin et al. 2011).

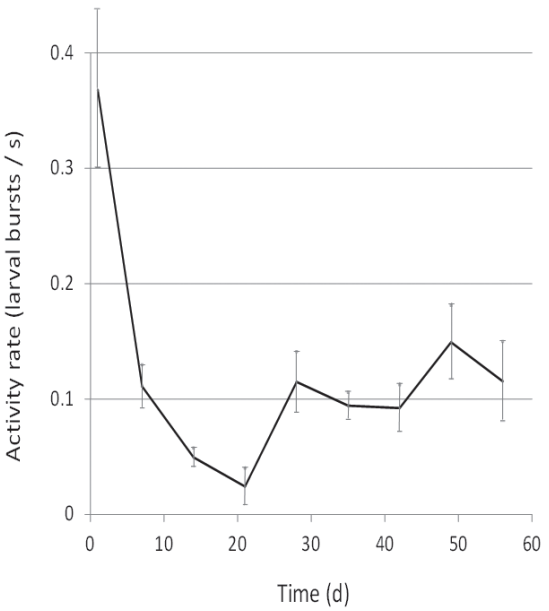

Fig. 3. Mean rates of larval bursts detected each test day in individual samples recorded from ten palm frond replicates. Vertical bars indicate Standard Errors.

An important result of this study was the finding that there were no significant differences between larval burst rates in exposed and enclosed conditions. This result is similar to those in previous investigations of wood-feeding insects, where specification of a temporal pattern of impulses in addition to a spectral pattern enabled improved discrimination of insect sounds from background noise (Mankin et al. (2008b). The result that significant differences were found between rates of larval impulses in exposed and enclosed conditions suggests that some of the background noise impulses in this study had spectra that were similar to the larval profiles and were counted as larval impulses in error. A potential cause of such similarities is resonance within the structure of the frond (Mankin et al. 2008a). A candidate for one of the resonance frequencies is seen as a continuous band between 11.5-12 kHz in the examples of Fig. 1B and 1D.

The results of this experiment combined with results of several investigations on *R. ferrugineus* and other internally feeding beetles (Mankin et al. 2011) suggest that all of the large *Rhynchophorus* weevils can be detected acoustically in host trees and fronds in both exposed and enclosed environments. The capability for early detection of infestation of all the large *Rhynchophorus* offers potential for effective management and reduction of inadvertent spread of these pests through movement of infested offshoots.

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