Acoustic Identification and Measurement of Activity Patterns of White Grubs in Soil

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ABSTRACT Activity patterns of *Phyllophaga crinita* (Burmeister), *Phyllophaga congrua* (LeConte), *Phyllophaga crassissima* (Blanchard), and *Cyclocephala lurida* (Bland) grubs were monitored with acoustic sensors in small pots of bluegrass, *Poa arachnifera* Torr, at varying and constant temperatures over multiple-day periods. Experienced listeners readily distinguished three types of sound with distinct differences in frequency and temporal patterns, intensities, and durations. Of ≈3,000 sounds detected from *P. crinita* larvae, 7% were identifiable as snaps, with large amplitudes and short durations typically associated with root breakage or clipping activity. Approximately 60% were identifiable as rustles, suggestive of surfaces sliding or rubbing past each other during general movement activity. Another 2% of sounds contained patterns of repeated pulses suggestive of surfaces scraping across a pointed ridge. The remaining 31% had spectral or temporal patterns that fell outside the ranges of easily recognizable sound types. Because the behavioral significance of the different sound types has not yet been fully established, the classified and unclassified sounds were pooled together in analyses of the effects of species, temperature, weight, and time of day. Grubs of all four species produced detectable sounds at rates that increased with temperature [0.45 sounds/((min)(°C))] and larval weight [6.3 sounds/((min)(g))]. Mean sound rates were independent of species and time of day. At temperatures <9°C, mean sound rates fell below the typical levels of background noise observed under field conditions. This reduced activity at low temperatures is likely to reduce the effectiveness of acoustic monitoring in the field in cold weather. The consistency of results obtained in these tests over multiple-day periods suggests that acoustic systems have potential as tools for nondestructive monitoring of the efficacy of insect management treatments as well as for biological and ecological studies.

KEY WORDS Coleoptera, Scarabaeidae, sound, soil insects, turfgrass

SEVERAL SPECIES OF WHITE grubs, including *Phyllophaga crinita* Burmeister, *Phyllophaga congrua* (LeConte), *Phyllophaga crassissima* (Blanchard), and *Cyclocephala lurida* (Bland), are root-feeding pests of turfgrass, forage grass, corn, small grains, sugar cane, strawberries, potato tubers, and young nursery trees (Crocker et al. 1996). The behaviors and activity patterns of the economically important, larval stages of these pests are difficult to monitor in either laboratory or field studies because of the laborious destructive nature of commonly available subterranean insect sampling methods (Villani and Wright 1988, Villani et al. 2002).

Acoustic technology has been considered by several investigators as a nondestructive alternative for detection or monitoring of hidden insect infestations. Soon after microphones and recorders were invented, Brain (1924) began using acoustic tools to detect insects hidden in fruit and Adams et al. (1953) used acoustic tools to detect insects in stored grain. Subsequently, acoustic systems were developed to detect and monitor insects hidden in wood (Haack et al. 1988, Fujii et al. 1990, Scheffrahn et al. 1993, Lemaster et al. 1997, Fujii 2001) and cotton bolls (Hickling et al. 1994, 2000). Acoustic methods have recently been adapted for detection of soil insects in turfgrass (Mankin et al. 2000, Brandhorst-Hubbard et al. 2001), but these initial studies were conducted to detect the presence or absence of infestation rather than to identify specific behaviors or activity patterns.

The literature suggests two approaches by which acoustic technology could be used to examine white grub behaviors and activity patterns. First, if a particular behavior produced sounds with identifiable, distinguishing features (e.g., Andrieu and Fleurat-Les-

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sard 1990, Fleurat-Lessard et al. 1994), these signals could be acoustically monitored to estimate the time course of such behaviors. Second, if overall activity was strongly affected by an experimental treatment or an environmental condition, such as temperature, the effects of these treatments or conditions could be assessed by long-term acoustic monitoring (Calkins and Webb 1988, Hagstrum 1993, Mankin et al. 1999).

To assess the utility of acoustic technology for long-term monitoring of white grub activity patterns, we conducted a series of tests over multiple-day periods with *P. crinita*, *P. congrua*, *P. crassissima*, and *C. lurida* larvae of different sizes held under controlled or variable temperatures. Our initial hypotheses were based primarily on the results of previous acoustic studies with stored product insects of several different species (Calkins and Webb 1988; Webb et al. 1988a, 1988b; Hagstrum et al. 1990; Hagstrum 1993; Hagstrum et al. 1996; Mankin et al. 1997; Mankin et al. 1999). In these studies, sound production was strongly affected by temperature and insect size. Consequently, we predicted that: (1) the rate of sounds by white grubs of a given weight and species would be proportional to temperature; and (2) at a given temperature, white grubs of a given species would produce sounds at a rate proportional to weight. However, we did not expect that the effect of temperature would be constant across species but rather that (3) the relationships between sound rate, temperature and weight would be different for different white grub species.

Materials and Methods

Acoustic Equipment. The acoustic sensors were electret microphones placed inside modified 4.5-cm stethoscope heads (Hickling et al. 2000). The sensors and amplifiers were constructed and provided by the National Center for Physical Acoustics, Oxford, MS. The amplified signals were monitored using stereo headphones (Optimus PRO 40, Tandy, Fort Worth, TX) and a digital audio tape recorder (DAT; PCM-M1, Sony, New York, NY).

Monitoring Arenas and Insects. Third instars of *C. lurida* (0.5–0.6 g), *P. congrua* (0.5–0.7 g), and *P. crassissima* (0.4–1.25 g), as well as second (0.1–0.3 g) and third instars (0.4–0.5 g) of *P. crinita* were collected on the Pecan Hollow Golf Course, Plano, TX, and at the Texas A&M University Research and Extension Center at Dallas, TX. Before the experiment, the grubs were kept individually in sand-filled (≈9% moisture, by weight), 4-cm-diameter × 4-cm-height plastic diet cups and fed small chunks of sweet potato, *Ipomoea batatas* (L.) (Convolvulaceae).

The grubs were monitored individually in cages (15-cm-diameter × 18-cm-height plastic flowerpots) containing sandy loam soil with a top layer of bluegrass, *Poa arachnifera* Torr. To monitor a grub, an acoustic sensor was centered just below the surface of the soil at the top of the cage. The cage was placed in an acoustically insulated foam box during the recording sessions and external sources of background noise were minimized. The initial weight of each grub was measured just before it was placed into the cage. The final weight was measured if the grub survived to the end of the experiment.

Monitoring of Activity Patterns. Before testing, a feeding apparently healthy grub was placed in the center of a cage and allowed 1–4 d to adjust to the cage environment. Sounds were monitored with headphones and recorded on the DAT at 4-h intervals. The soil temperature was monitored before each test at a 3-cm depth. When the soil temperature was >13°C, the recording duration was 3 min, except where otherwise noted. When the temperature was ≤13°C, the recording duration was 15 min.

The sounds analyzed in this study were pooled from groups of tests of individual white grubs monitored at ambient outdoor temperatures or at controlled temperatures in the laboratory for periods ranging from 1 to 28 d. Sounds from six third-instar *C. lurida* were monitored for 1 d, and five were monitored for 8 d. Sounds from five third-instar *P. congrua* were monitored for 10 d and five were monitored for 5 d. Sounds from four third-instar *P. crassissima* were monitored for 8 d, five were monitored for 5 d, and six were monitored for 1 d. One second- and one third-instar *P. crinita* were monitored for 28 d. The second instar developed into a third instar during this period. Eight second-instar *P. crinita* were monitored for 7 d, seven second instars for 3 d, and five third instars were monitored for 2 d. A second set of five third instars was monitored at ambient outdoor temperatures for 1 d and then at controlled temperatures in the laboratory for 1 d.

Analysis and Identification of Sounds. For signal analysis, recorded samples were played back from the DAT to the headphones and an oscilloscope (TDB 210, Tektronix, Beaverton, OR). The sounds in each recording were counted, and their durations and amplitudes were noted. To account for signals with peaks of different positive and negative magnitudes, the amplitudes were calculated as one half of the maximum peak-to-peak difference. A sound was considered to have ended when the signal level fell to background levels for at least 4 ms (Mankin et al. 2000). Recordings of particular interest were digitized and further analyzed with a digital signal processing system (Mankin 1994) that provided computer assessment of activity and comparisons with white grub signals in previous experiments (Mankin et al. 2000, Brandhorst-Hubbard et al. 2001). After several hours of practice with the equipment, sounds produced by the grubs could be distinguished reliably from background noises by listening with headphones, observing amplitudes and durations of the oscilloscope traces, and analyzing signal spectra and durations by computer. Because these experiments were conducted in an acoustically shielded box, background noises occurred only infrequently.

Statistical Analyses. Chi-square analyses were used to compare distributions of different types of sounds in recordings from second- and third-instar *P. crinita* at different temperatures. Regression analyses (SAS Institute 1988) were used to examine the effects of soil

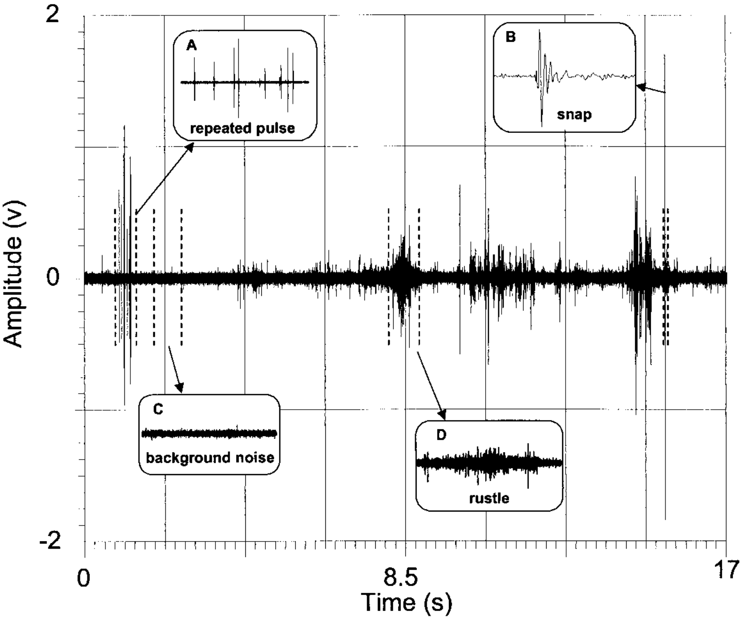

Fig. 1. Sample recording of sounds produced by a third-instar *P. crinita* larva, with insets showing (A) 0.4-s expansion of a repeated pulse sound, (B) 0.02-s expansion of a snap, and (C) 0.6-s expansion of an interval without detectable sounds for comparison with (D), 0.6-s expansion of a rustle.

temperature (SoilTemp), grub initial weight (Weight), and time (TimeofDay) on the pooled rate of production of all grub-produced sounds, SoundRate. The TimeofDay was a class variable with six values (12 a.m., 4 a.m., 8 a.m., 12 p.m., 4 p.m., and 8 p.m.). The effects of weight and instar were correlated and could not be analyzed together in a single regression. Consequently, a separate Proc GLM analysis was conducted with a model containing SoilTemp and Instar. Regression analysis (SAS Institute 1988) was used to examine the effects of SoilTemp, Weight, and Species on grub sound production in the combined set of recordings from all four white grub species.

Results and Discussion

Larvae of the four tested white grub species produced a variety of easily detectable sounds. Several different types of sound were distinguished by differences in amplitude, duration, and periodicity. Usually, amplitude cannot be used as a distinguishing factor, but in this study, the ranges of distances between sensor and grub were small and their effects on amplitude were smaller than the large differences in amplitudes between the different sound types. Examples of the most easily distinguished types of sound are shown in a 17-s sample of activity from a third-instar *P. crinita* in Fig. 1. Snapping sounds (Fig. 1B) had the shortest duration (<10 ms) and highest amplitude. The durations and temporal patterns of the snaps were typical of sounds that are recorded from insects feeding on or cutting vegetation above ground (see e.g., http://cmave.usda.ufl.edu/~rmankin.soundlibrary.html). Frequently occurring weaker and longer (≈ 1-s) rustling sounds were suggestive of general rubbing or dragging movements or digging activity (e.g., the burst of sounds in Fig. 1 between 4 and 13 s, including the expanded segment in Fig. 1D). For comparison, an interval of the same duration but without any sounds is shown in Fig. 1C. A third group of sounds was identified by a distinct pattern of repeated pulses, suggestive of the scraping of a rough surface across a hard ridge (Fig. 1A). Distributions of the amplitudes and durations of 21 snaps, 52 rustles, and 11 repeated pulse sounds produced by third-instar *P. crinita* are shown in Fig. 2. Of ≈3,000 *P. crinita* sounds examined for classification, 7% were identified as snaps, 60% as

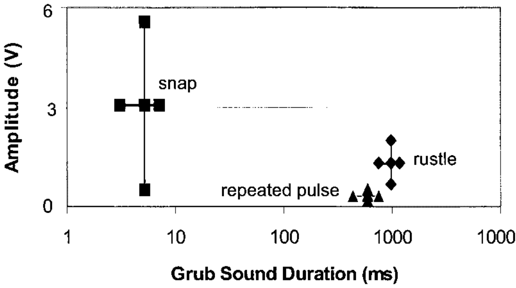

Fig. 2. Distributions of amplitudes and durations of 21 snaps (squares), 52 rustles (diamonds), and 11 repeated pulse sounds (triangles) produced by *P. crinita* larvae in soil. Means for each sound category are in the center of range with standard errors of amplitude and duration on each side.

Table 1. Numbers of recordings containing snaps, rustles, and repeated pulse sounds (see Materials and Methods) in 100 tests from 16 cages holding second-instar *P. crinita* larvae and 113 tests from 11 cages holding third instars, categorized by instar

Type of sound pulse	Second instars	Third instars
Snap	85	61
Rustle	85	85
Repeated pulse	18	23

Distributions of different types of sound pulse from second and third instars were not significantly different ($\chi^2 = 3.55$, df = 2, $P > 0.1$).

rustles, 2% as repeated pulses, and 31% were not classifiable.

No differences were observed in the overall distributions of snaps, rustles, and repeated pulses in different recordings from second-instar and third-instar *P. crinita* larvae, measured by the numbers of recordings containing sounds of each type (Table 1). Consequently, we pooled the counts from both instar categories to examine the effects of temperature on distributions of snaps, rustles, or repeated pulse sounds. Repeated pulse sounds were absent in recordings taken at temperatures <20°C, but they occurred in more than 20% of recordings taken at temperatures >28°C (Table 2). Chi-square analysis revealed significant differences in the relative distributions of snaps, rustles, and repeated pulse sounds in the three temperature ranges, <20°C, 20–28°C, and >28°C. Regression analyses performed in the next section also indicated an effect of temperature on sound production.

We did not distinguish among types of sounds in the regression analyses in the remainder of this report because the behavioral significance of the different sounds has not yet been fully confirmed by visual or other techniques, and a significant fraction of the sounds were not classifiable. However, the presence of these easily distinguishable differences exist suggests that sound classification has potential as a tool for more detailed behavioral analyses in future studies. Andrieu and Fleurat-Lessard (1990) and Fleurat-Lessard et al. (1994) also considered the potential of sound classification for identification of different stored grain insect pests.

Table 2. Numbers of recordings containing snaps, rustles, and repeated pulse sounds (see Materials and Methods) in 100 tests from 16 cages holding second instar *P. crinita* larvae and 113 tests from 11 cages holding third instars, categorized by soil temperature range

Type of sound pulse	Temperature		
	Low (<20°C)	Medium (20–28°C)	High (>28°C)
Snap	26	86	34
Rustle	21	106	43
Repeated pulse	0	19	22

Significant differences were present in the distributions of different sound pulse types at different temperatures ($\chi^2 = 20.65$, df = 4, $P < 0.005$).

Analyses of variance were performed to consider the effects of temperature, time of day, grub size, instar, and species on the total rate of sounds detected. As discussed in separate sections below, the sound rate was affected most strongly by temperature and grub size. The effects of species and time of day were not statistically significant.

Analysis of Factors Affecting Sound Rates of *Phyllophaga crinita* Grubs. The *P. crinita* recordings confirmed two of the experimental hypotheses, i.e., the rate of detected sounds was found to be proportional to temperature and grub weight. We examined the effects of SoilTemp and Weight on SoundRate of second- and third-instar *P. crinita* grubs using Proc GLM (SAS Institute 1988). A four-parameter model, SoundRate = $A + (B)$ (SoilTemp) + (C) (Weight) + (D) (TimeofDay) successfully fit the sound-rate measurements ($F = 9.46$; df = 7, 190; $P < 0.001$; $r^2 = 0.26$; residual mean square error = 20.3), but the mean

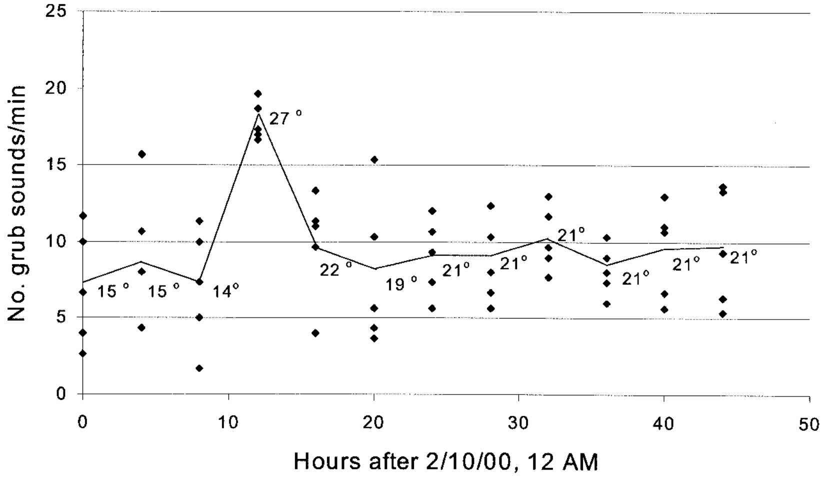

Fig. 3. Activity patterns of five third-instar *P. crinita* exposed to fluctuating ambient temperatures for 24 h and then held at 21°C for 24 h. Individual rates designated by diamonds, means by the solid line, and temperatures in °C.

square for TimeofDay (5.62) was not significant ($F = 0.28$, $P = 0.93$). A graphical representation of the strong effect of temperature relative to the effect of time of day is shown in Fig. 3, plotting the rates of sounds produced by five third-instar *P. crinita* kept 24 h under ambient conditions and then held an additional 24 h at 21°C. The effect of time of day apparently is minimal for these grubs, which were not exposed daily to light that is typically involved in setting a circadian clock (e.g., Panda et al. 2002).

Removing TimeofDay from the analysis, we tested the model, SoundRate = $A + (B)$ (SoilTemp) + (C) (Weight). This three-parameter model (see Table 3) had greater statistical significance ($F = 33.03$; df = 2, 195; $P < 0.001$; $r^2 = 0.25$; residual mean square error = 19.9) than the four-parameter model above with TimeofDay.

Comparison of *Cyclocephala lurida*, *Phyllophaga congrua*, *Phyllophaga crassissima*, and *Phyllophaga crinita* Sound Rates. An analysis of variance was performed on the combined measurements of sounds from grubs of all tested species to consider whether differences among species significantly affected sound production rates. Because time of day was not significant in the antecedent analysis of *P. crinita* sound rates above, the initial model tested was SoundRate = $A + (B)$ (SoilTemp) + (C) (Weight) + (D) (Species). The Species was a class variable with four values (*C. lurida*, *P. congrua*, *P. crassissima*, and *P. crinita*). The model was statistically significant ($F = 22.2$; df = 5, 325; $P < 0.001$; $r^2 = 0.25$; residual mean square error = 22.9), but the mean square for Species (43.89) was not significant ($F = 1.92$, $P = 0.13$). Consequently, we tested the three-parameter model that had successfully fit the measurements from *P. crinita*: SoundRate = $A + (B)$ (SoilTemp) + (C) (Weight). This model provided improved statistical significance ($F = 53.99$; df = 2, 328; $P < 0.001$; $r^2 = 0.25$; residual mean square error = 22.8). The regression coefficients are listed in Table 4. The major difference between the coefficients in the combined measurements (326 observations) and the *P. crinita* measurements (198 observations) was a lower estimate for the effect of Weight on SoundRate.

Although we did not confirm our hypothesis that there would be observable differences in sound rates of different white grub species of a given weight at a given temperature, such differences may be found in future studies with a larger number of individuals of each species. Also, we did not have an opportunity to analyze the different types of sounds of *C. lurida*, *P. congrua*, and *P. crassissima* in comparison with the snapping, rustling, and repeated pulse sounds of *P. crinita*. Additional studies with larger numbers of white grubs of these different species may reveal differences of behavioral or ecological interest.

Factors Affecting Likelihood of White Grub Detection. Because the rate of detectable sounds is proportional to grub size (Tables 3 and 4), further analysis was conducted on the measurements from second- and third-instar *P. crinita* grubs to assess the effect of instar on the likelihood of detection. The model, SoundRate = $A + (B)$ (SoilTemp) + (C) (Instar) was tested under Proc GLM (SAS Institute 1988), where Instar was a class variable with two values (second and third). The model was statistically significant ($F = 24.27$; df = 3, 194; $P < 0.001$; $r^2 = 0.27$; and residual mean square error = 19.5), as was the effect of Instar (mean square = 103.4, $F = 5.30$, $P = 0.022$). The regression coefficients are listed in Table 5. On average, second-instar *P. crinita* larvae produced 3.9 fewer detectable sounds per minute than did third instars at a given temperature.

To place this difference in practical perspective, we can consider the results of previous field studies in which soil insects were acoustically detected (Mankin et al. 2001, Brandhorst-Hubbard et al. 2001). In Mankin et al. (2001), for example, the likelihood of infestation was rated high if the measured rate exceeded 20 sounds/min and low if the rate was below a background noise level of 2/min. Using the regression coefficients for second instars, the temperature would need to exceed 14°C for SoundRate to reach two sounds/min. The third-instar grubs, however, would be predicted to produce detectable sounds at rates >2/min at temperatures >6°C.

Table 3. Regression coefficients for relationship between temperature, weight, and the rate of sounds produced by second and third instar *P. crinita* grubs ($n = 198$) at temperatures between 5 and 34°C

Coefficient for (variable)	Estimate	Standard error	P
A (Intercept)	−5.67 sounds/min	1.73	0.0013
B (SoilTemp)	0.45 sounds/((min) (°C))	0.06	<0.001
C (Weight)	11.21 sounds/((min) (g))	1.88	<0.001

Table 4. Regression coefficients for relationship between temperature, weight, and the rate of sounds produced by second and third instar *C. lurida*, *P. congrua*, *P. crassissima*, and *P. crinita* grubs ($n = 331$) at temperatures between 5 and 34°C

Coefficient for (variable)	Estimate	Standard error	P
A (Intercept)	−4.40 sounds/min	1.27	0.0006
B (SoilTemp)	0.45 sounds/((min) (°C))	0.04	<0.001
C (Weight)	6.37 sounds/((min) (g))	1.08	<0.001

Table 5. Regression coefficients for relationship between temperature, instar, and the rate of sounds produced by *P. crinita* grubs ($n = 198$) at temperatures between 5 and 34°C

Coefficient for (variable)	Estimate	Standard error	P
A (Intercept)	-0.552^a sounds/min	1.35	0.6823
B (SoilTemp)	0.45 sounds/((min) (°C))	0.06	<0.0001
C^b (second instar)	-3.90^a sounds/min	0.76	<0.0001
(third instar)	0.00^a		

[a] The inverse of the independent variable matrix was singular and a generalized inverse was used to solve the normal equations. Superscripted estimates were not uniquely estimable.

[b] Resultant regression equation for *P. crinita* third instars is SoundRate = −.552 + 0.45 SoilTemp, and the equation for second instars is SoundRate = −4.45 + 0.45 SoilTemp.

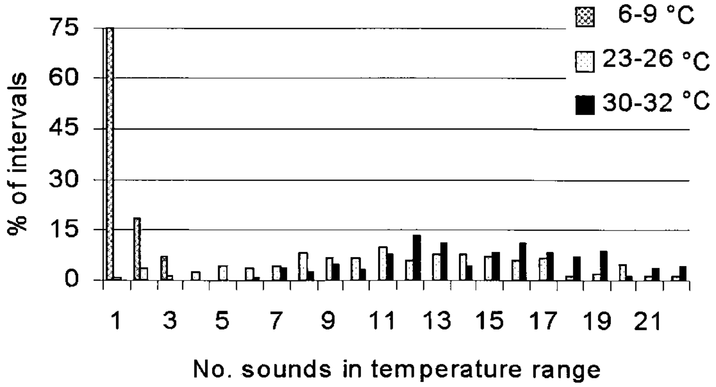

Fig. 4. Percentages of 1-min intervals with specified numbers of sounds recorded from second- and third-instar *P. crinita* at temperatures between 6–9, 23–26, and 30–32°C.

An alternative method of considering the likelihood of detecting a grub during a given monitoring session is to examine the distribution of sound rates across different recordings at different temperatures. Examination of the distributions of the sound rates of six second–third-instar *P. crinita* larvae at different temperatures revealed that the numbers of 1-min intervals with sounds and the rate of sounds increased with temperature (Fig. 4). At or below 9°C, the mean rate was 0.32 sounds/min, and 75% of 1-min recording intervals contained no detectable sounds. Between 23–26°C, the average rate was 10.7/min and 71% of recorded intervals had rates between 7 and 16/min. The average rate increased to 13.6/min between 30–32°C, with 85% of intervals between 8 and 18/min.

The equations in Tables 3 and 4 and the differences in the distributions of the sound rates at different temperatures (Fig. 4) suggest that infestations of white grubs of these four species at field sites may be increasingly difficult to survey by acoustic techniques when temperatures fall below 9°C. If the background noise threshold of two sounds/min in Mankin et al. (2001) is applied to the results in Fig. 4, none of the containers is predicted to contain grubs in the tests in which acoustic measurements were conducted at or below 9°C. All of the tests at higher temperatures successfully predict infestation. The regression equations in Table 3 indicate similarly that the rate of sound production falls below typical background noise levels at low temperatures between 14 and 6°C, depending on the instar.

Potential Applications of Long-term Monitoring. Although acoustic techniques currently are more expensive and time-consuming than traditional visual observation, they have become viable alternatives for nondestructively monitoring pest insects in cryptic environments. Present instrumentation is portable enough for field (Brandhorst-Hubbard et al. 2001, Mankin et al. 2001) and nursery applications (Mankin and Fisher 2002). Rapid, multiple assessments of activity obtained using earphones can be augmented by objective assessments obtained using digital signal processing techniques (Mankin et al. 2000), which increases the likelihood of reliable detection without the precautions taken in this study, where an acoustically shielded box was used to minimize background noise. This suggests that acoustic techniques have potential as nondestructive tools to measure effects of environmental factors on subterranean insects and to assess short- or long-term effects of pest management treatments in a variety of laboratory and field environments. Such techniques already have been used in the laboratory to monitor development of larvae inside wheat grains (e.g., Shade et al. 1990, Pittendrigh et al. 1997) and have potential utility for monitoring other invertebrates and small vertebrates as well.

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