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ACOUSTIC DETECTION OF ARTHROPOD INFESTATION OF GRAPE ROOTS: SCOUTING FOR GRAPE ROOT BORER (LEPIDOPTERA: SESIIDAE)

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Abstract

The grape root borer, *Vitacea polistiformis* Harris, is the principal pest of grapes (*Vitis* spp. L.) in Florida where chlorpyrifos is 1 of the few chemicals registered for its control. However, chlorpyrifos is not an ideal treatment because it is highly toxic to birds, fish, aquatic invertebrates, and honeybees. Also, the recommended timing of application conflicts with harvest dates. There is an effective cultural control method, known as mounding, but this method is currently cost prohibitive for commercial production and is not widely used. If mounding could be applied only to infested plants, the cost of this method would be reduced considerably. This study evaluated the potential of acoustics for detecting the larvae *in-situ*. Human listeners assessed likelihood of arthropod infestation for each site based on live acoustic samples as they were being recorded. Computer software later constructed acoustic indicators from these recordings that were used for computer assessment of infestation likelihood. After recording, the roots of sampled vines were excavated to determine infestation levels. Infestation likelihood predictions of both human listeners and computer software largely reflected infestation condition of tested sites. Consequently, acoustic methods could be developed as tools for growers to employ mounding only at sites most likely to be infested, and thus enable more cost-effective use of this cultural control tactic.

Key Words: IPM, monitoring, mounding, grape pest, *Vitis* spp.

Resumen

El barrenador de la raíz de la uva, *Vitacea polistiformis* Harris, es la principal plaga de la uva (*Vitis* spp. L.) en la Florida, donde clorpirifos es uno de los pocos productos químicos registrados para su control. Sin embargo, el clorpirifos no es un tratamiento ideal, ya que es altamente tóxico para aves, peces, invertebrados acuáticos y abejas. Además, el tiempo recomendado para la aplicación del producto esta en conflicto con la fecha de cosecha. Existe un método eficaz de control cultural, conocido como "el montonar" (agregando la tierra debajo de la vid después de que las larvas se empupan en el suelo, que impeden que los adultos emergen), pero actualmente este método es muy costoso para la producción comercial y no se utiliza ampliamente. Si se aplica el montonar sólo a las plantas infestadas, el costo de este método se reduciría considerablemente. Este estudio evaluó el potencial de la acústica para detectar las larvas *en-sitio*. Oyentes humanos evaluaron la probabilidad de infestación por artrópodos para cada sitio basado en las muestras acústicas en vivo, mientras que fueron grabadas. El programa de computadora más tarde construyeron indicadores acústicos de estas grabaciones que se utilizaron para la evaluación hecha por computadora de la probabilidad de infestación. Después de la grabación, las raíces de la vid muestreadas fueron excavados para determinar los niveles de infestación. Las predicciones de la probabilidad de infestación tanto por los oyentes humanos y por los programas de computadora en gran parte reflejó la condición de infestación de los sitios evaluados. En consecuencia, los métodos acústicos se podrían desarrollar como una herramienta para que los productores empleen el montonar sólo en los sitios de mayor probabilidad de estar infestados y por lo tanto permita un uso más rentable de esta táctica de control cultural.

In Florida, the amount of land devoted to grape (*Vitis* spp. L.) cultivation has steadily increased over the past several years and is now over 400 hectares (Weihman 2005). The number of registered Florida wineries has also increased from 13 to 17 in the past 4 years (FGGA 2009). In 2008, Florida was the fifth largest wine producer of all states in the U.S. with total production equaling 6.6 million liters (Hodgen 2008), and in 2009 Florida was the second largest consuming state (Anderson 2009).

The grape root borer, (GRB) *Vitacea polistiformis* Harris, is the key pest of grapes in Florida (Liburd & Seferina 2004) and Georgia (Weihman 2005) and an important pest in North Carolina (Pearson & Schal 1999) and South Carolina (Pollet 1975). As the Florida grape industry expands, the grape root borer will become a more serious threat to the industry.

Upon hatching, larvae immediately burrow into the soil where they bore into and feed upon grape roots, reducing vine vigor and cold tolerance, increasing susceptibility to pathogens and drought, and hastening vine death (Pearson & Meyer 1996). A low economic injury level (EIL) has been established in Georgia, 0.074 larvae per vine (Dutcher & All 1979). One larva feeding at the root crown can cause as much as 47% decrease in yield. Entire vineyards have been destroyed in Florida, and the grape root borer was cited as the reason for cessation of grape production in South Carolina (Pollet 1975).

The organophosphate chlorpyrifos (Lorsban®) is currently 1 of the few registered chemicals for control of GRB. Chlorpyrifos is applied to the root area as a soil drench but is not ideal for control of GRB because it is toxic to birds, fish, aquatic invertebrates, and honeybees. It is also moderately toxic to pets and livestock and is suspected of being carcinogenic in humans (USCB 1996). Florida vineyards are relatively small, usually 1 to 4 ha, and are typically family owned and operated. Most grape growers live on site with their families so many are reluctant to use chlorpyrifos because of its potential safety and environmental hazards.

A practice known as mounding has shown some promise as an effective control alternative to pesticides. When larvae are ready to pupate, they usually migrate to within 5 cm of the soil surface to form their pupal cells. At this depth, pharate adults are easily able to emerge from the soil. However, placing a mound of soil around the base of the vine after larvae have begun to pupate forces pharate adults to travel farther before reaching the soil surface, and mortality increases with the distance traveled. Sarai (1969) found 100% mortality when mounds were 19 cm high. Once emergence begins to decline for the year, mounds must be removed so that mounding may be done the next year. Mounding is currently labor intensive, which makes the technique cost prohibitive for most growers. The cost of mounding would be greatly decreased if growers were able to determine whether or not a given plant is infested. This would eliminate the cost of unnecessarily mounding vines that are not infested.

This study evaluated the potential of acoustic detection as a means of determining the presence or absence of larvae in an individual grapevine's root system. This detection system will make the sustainable practice of mounding much more attractive to growers, decrease pesticide use, and its associated environmental impact. The acoustic detection method could be implemented wherever the grape root borer is a problem.

Materials and Methods

Acoustic Instruments, Signal Recording, and Soil Sampling Procedures

Acoustic records were collected from 28 root systems at a commercial vineyard near Lithia, Florida, and 8 root systems at a commercial vineyard near Florahome, Florida. Recordings were taken between April 28 and June 9, 2009. Air temperatures ranged between 29 and 35°C during the recordings. Two accelerometer amplifiers and a recorder (details of the instruments are described in Mankin et al. 2009) were set up in the storage bed of an electric cart and transported throughout the vineyard to vines exhibiting symptoms of infestation: wilting, yellowed or dead leaves, and reduced leaf area as compared with neighboring plants of the same variety. A 30 cm nail was inserted into the root system of the selected vine. The accelerometer was attached to the nail head by a magnet. One or more listeners took notes and monitored the signals from potential larval feeding and movement in the roots during a recording period of 3 min or longer. Within 1 to 2 h after recording, the vine was excavated and the contents of the root system were examined to obtain an independent verification of whether a site was uninfested or contained insects.

Listener Assessment of Infestation Likelihood

Subterranean larvae typically produce spectrally distinctive, 3 to 10 ms sound impulses during movement and feeding activities (Mankin et al. 2000, 2009). These sound impulses can be identified and recognized as insect-produced sounds by most listeners after 10 to 20 min practice with the accelerometer and headphones. In this experiment, there were 2 primary listeners and 5 occasional listeners.

Assessments were performed as in Mankin et al. (2007), where l_{ow} indicates detection of no valid, insect-produced sounds or only a few faint sounds during a recording period, m_{edium} indicates detection of sporadic or faint groups of valid sounds, and h_{igh} indicates detection of frequent, easily detectable groups of valid sounds. No attempt was made to distinguish between pest and non-pest species in the assessment. Comparisons between the distributions of assessed infestation likelihoods at infested and uninfested recording sites were performed using the NPAR1WAY procedure in SAS (SAS Institute 2004).

Digital Signal Processing and Classification

Recorded signals were band-pass filtered between 0.2 and 5 kHz to facilitate subsequent analysis, and visualized with audio playback using Raven 1.3 software (Charif et al. 2008). In initial screenings, we confirmed the presence of groups (trains) of discrete, 3 to 10 ms impulses separated by intervals <250 ms that had occurred frequently where insects were recovered in previous studies (Mankin et al. 2009). Trains containing 6 or more impulses were a focus of analysis because they often were identified as insect sounds in playbacks of recordings from infested sites in this and previous studies (Mankin et al. 2009).

The impulses and impulse trains detected in the recordings were analyzed with customized software, DAVIS (Digitize, Analyze, and Visualize Insect Sounds, Mankin et al. 2000), which discarded long-duration, low frequency background noise (Mankin et al. 2007) and then compared the spectrum of a 512-point time-slice centered around the peak of each impulse against averaged spectra (spectral profiles) constructed as described in RESULTS.

The impulse sequences were screened to identify and characterize trains of impulses that listeners typically classify as separate, individual sounds. Each train was labeled according to the spectral profile matched by a plurality of its impulses. The beginning and ending times of impulse trains, their labels, and the number of impulses per train were stored in separate train-sequence spreadsheets for each recording.

RESULTS

The root systems of 25 (of 36 total) recording sites exhibited *V. polistiformis* larval damage, although only 1 live larva was recovered. Collectively, 27 root systems contained 1 or more invertebrates of various species (Tables 1 and 2). Among these were 41 Coleoptera (including 4 *Mycotrupes* (Coleoptera: Geotrupidae), 3 Tenebrionids, 1 Cerambycid, 4 *Phyllophaga* (Coleoptera: Scarabaeidae) larvae and 1 *Anomala* (Coleoptera: Scarabaeidae) larva) 1 Cetoniid larva, 6 *Lepisma saccharina* (L.) (Thysanura: Lepismatidae), and 3 burrowing roaches. Six sites contained *Solenopsis invicta* Buren (Hymenoptera: Formicidae) workers, and 3 had termite workers. Other organisms found in the root systems included 5 unidentified worms, 3 Diplopoda, 3 large spiders, and an earthworm. Only the *V. polistiformis* was to be targeted as a pest (see DISCUSSION), but for purposes of categorizing sites, we considered a site to be infested if the excavated root system contained 1 or more invertebrates capable of producing sounds.

Spectral Profiles

Two types of impulses that could be readily identified by their temporal patterns as insect-produced sounds (Mankin et al. 2000, 2009) appeared frequently in initial screenings of signals detected at recording sites where excavations verified infestation, and a third type appeared at only 9 recording sites. All 3 types of impulses stood out against the background noise because their short durations and distinctive spectral patterns (Mankin et al. 2000, 2007, 2009). Spectral profiles of these impulses, i.e., averaged measurements of their power spectra (Mankin et al. 2000), were calculated to assist in discriminating insect sounds from background noise (Fig. 1). A profile of 1 of the 2 most frequently occurring insect sound impulses, s_{highdB}, was constructed from a series of 128 consecutive impulses in a relatively noise-free recording that contained several sounds identified in previous studies (Mankin et al. 2009) to be indicative of insect burrowing activity. The second profile, s_{middB}, was constructed from a series of 94 consecutive impulses in a recording that

TABLE 1. NUMBERS OF INVERTEBRATES RECOVERED FROM ROOTS, LISTENER ASSESSMENTS, AND RATES OF S_{HIGHDB}, S_{MIDDB}, AND S_{LOWDB} TRAINS AND BURSTS AT SITES WHERE S_{LOWDB} BURSTS WERE DETECTED.

No. recovered				Assessed infest.	Rate (No./min) of					
	beetle		other inv.[3]		s_{highdB}		s_{middB}		s_{lowdB}[4]	
Ants	larvae[1]	adult[2]		likelihood	trains	bursts	trains	bursts	trains	bursts
0	0	1	4	m_{edium}	6.70	0.00	4.69	0.67	15.41	10.72
≥1	0	2	3	h_{igh}	8.31	2.77	2.77	2.77	2.77	2.77
0	0	2	0	h_{igh}	23.39	3.19	8.50	0.53	2.66	1.06
≥1	0	0	0	m_{edium}	10.55	0.00	14.90	0.62	9.93	3.10
0	3	2	1	h_{igh}	3.55	0.00	2.13	0.00	4.97	1.42
0	2	0	1[5]	h_{igh}	7.97	0.61	9.19	0.00	0.61	0.61
0	0	2	1	h_{igh}	0.00	0.00	0.00	0.00	3.20	1.07
0	1	3	8	m_{edium}	1.49	0.00	13.42	0.00	2.24	0.75
0	0	1	0	m_{edium}	2.70	0.00	4.32	0.00	1.08	0.54

[1]Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp.
[2]Including *Mycotrupes* sp.
[3]Other invertebrates included Lumbricid sp., Diplopoda sp., Blattella sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and large spider.
[4]Recording sites arranged in order of the rates of s_{lowdB} bursts
[5]One *V. polistiformis* larva was found in the root system at this recording site.

TABLE 2. NUMBERS OF INVERTEBRATES RECOVERED FROM ROOTS, LISTENER ASSESSMENTS, AND RATES OF S_{HIGHDB} AND S_{MIDDB} TRAINS AND BURSTS AT SITES WHERE S_{LOWDB} BURSTS WERE NOT DETECTED.

No. recovered				Assessed infestation	Rate (No./min) of			
	beetle		other invert.[3]		shighdB		smiddB[4]	
Ants or Termites	larvae[1]	adults[2]		likelihood	trains	bursts	trains	bursts
0	0	0	1	high	61.82	28.17	10.96	0
≥1	0	0	1	high	37.56	14.44	7.22	1.44
0	1	0	0	high	13.38	10.03	1.34	0.67
≥1	2	0	1	high	17.43	2.32	4.65	4.65
≥1	0	4	0	high	3.32	0	17.43	5.81
≥1	0	0	0	medium	26.3	5.58	10.36	0
0	0	1	2	medium	3.16	0	8.2	3.16
0	0	1	0	high	15.77	2.1	5.26	0
0	0	0	1	high	9.71	0.75	11.95	0.75
0	0	1	0	low	1.22	1.22	2.43	0
≥1	0	2	0	medium	7.64	0.69	1.39	0
0	0	1	0	medium	15.83	0.66	9.23	0
≥1	0	2	1	medium	0.55	0	3.3	0.55
0	1	1	0	medium	14.56	0	2.24	0
≥1	0	0	0	medium	3.6	0	8.99	0
0	0	0	0	low	1.33	0	5.33	0
0	0	0	0	low	0	0	5.48	0
0	0	0	0	low	0.65	0	3.92	0
0	0	0	0	medium	1.84	0	1.84	0
0	2	0	0	medium	0.47	0	2.85	0
0	0	3	0	medium	0.47	0	1.41	0
0	0	0	0	low	0.62	0	1.23	0
0	0	0	0	low	0.79	0	0.79	0
0	0	0	2	medium	1.09	0	0	0
0	0	0	0	low	0	0	0.52	0
0	0	0	0	low	0	0	0	0
0	0	0	0	medium	0	0	0	0

[1]Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp., Cetoniid sp. Cerambycid sp.
[2]Including *Mycotrupes* sp.
[3]Including Lumbricid sp., Diplopoda sp., Mutillid sp. *Blattella* sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and a large spider.
[4]Recording sites arranged in order of summed rates of s_{highdB} and s_{middB} bursts.

contained several larval scraping sounds of slightly lower frequency. The third, less frequently occurring profile, s_{lowdB}, was constructed from a 0.1 s period containing 13 consecutive impulses of this distinctive type. The 3 types of impulses had similar temporal patterns but their spectral patterns diverged at frequencies above 2.6 kHz.

Various types of background noise also occurred frequently in all recordings, comprising about 80% of all sounds detected. Continuous noise could be discounted easily because insect sounds usually occur as brief impulse bursts (Mankin et al. 2009), but some low-frequency impulsive noise was discarded by matching it with 1 of 2 noise profiles. To exclude higher-frequency noise impulses, we constructed a noise profile, n_{highdB} (Fig. 1), as an average spectrum of impulses produced during a gust of light wind. A second noise profile, n_{lowdB} (Fig. 1), was constructed as an average spectrum of a 5 s period where impacts of water droplets from an irrigation hose were detected.

Insect Sound-Impulse Bursts

Although isolated s_{highdB}, s_{middB}, and s_{lowdB} impulses occurred frequently in the recordings, most of the signals that listeners interpreted as insect sounds appeared in bursts of more than 6 but less than 50 impulses of a given type, similar to bursts used successfully to construct indicators of insect infestation in other insect acoustic detection studies (Mankin et al. 2007, 2009). In analogy with such studies, we defined trains of type s_{highdB}, s_{middB}, and s_{lowdB} impulses to be a series of impulses of each type, separated by durations <0.25 s. Bursts of type s_{highdB}, s_{middB}, and s_{lowdB} were trains

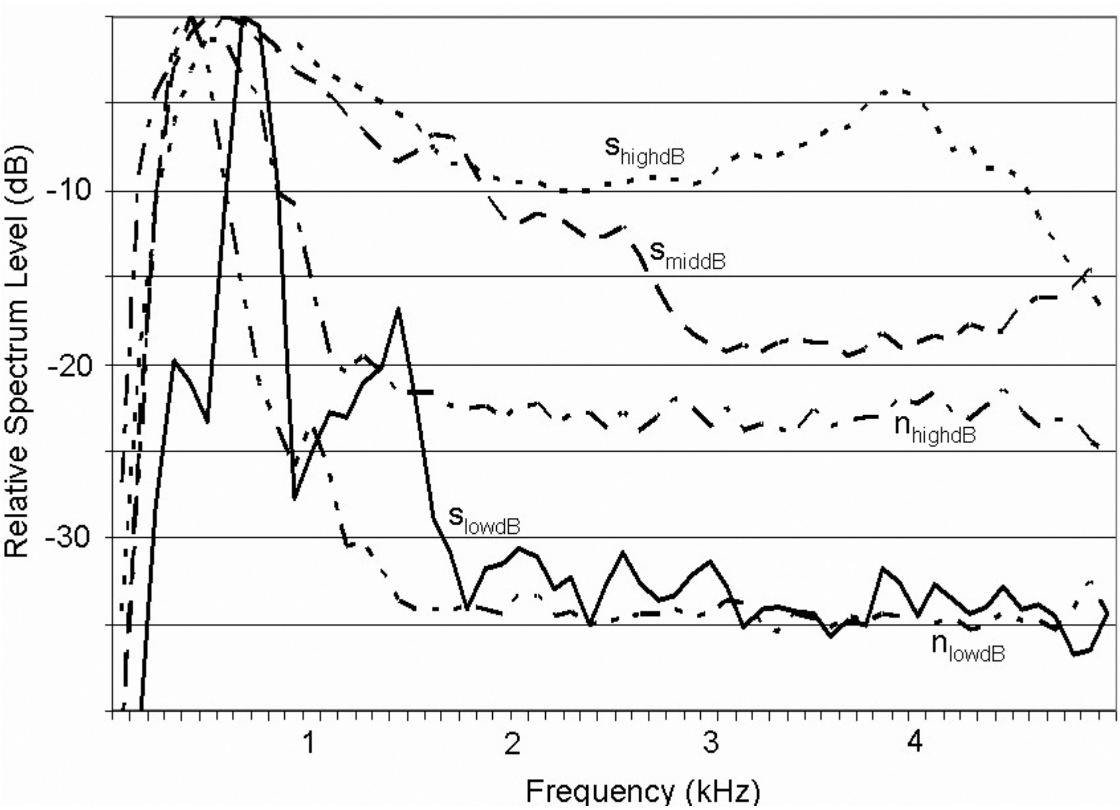

Fig. 1. Spectral profiles of insect-produced sound impulses, s_{highdB}, s_{middB}, and s_{lowdB}, compared with spectral profiles of wind-gust noise, n_{highdB}, and low-frequency background noise, n_{lowdB}, used in analyses to distinguish insect-produced sounds from background noise. The subscripts, highdB, middB, and lowdB, refer to the magnitudes of the relative spectrum levels of these profiles near 2.6 kHz, the midpoint of the 0.2-5 kHz range of frequencies analyzed. Spectrum level is relative to the maximum acceleration measured in the 0.2-5 kHz reference range.

of each impulse type that contained at least 7 but less than 50 impulses. We analyzed recordings from each of the 36 root systems using the DAVIS signal analysis system (Mankin et al. 2000). The DAVIS software calculated a power spectrum for each sound impulse with amplitude above a user-set threshold, and matched it against the spectra of the 3 signal and 2 noise profiles (Fig. 1) by calculating the least-squares difference between the impulse and profile signal levels at each spectrum frequency. The impulse was categorized according to the profile type for which the summed least-squares differences were smallest, unless that smallest least-squares sum exceeded a user set threshold, designating the impulse as uncategorized noise. The burst was categorized then according to the type of profile of its largest fraction of impulses. The rates of detection of trains and bursts in the 9 root systems that contained bursts of type s_{lowdB} are listed in Table 1. One site, assessed by listeners at h_{igh} likelihood of infestation, contained a *V. polistiformis* larva as well as 2 *Phyllophaga* larvae. Bursts of type s_{highdB} also were detected at this site. The rates of detection of trains and bursts in the other root systems are listed in Table 2. As in previous studies (Mankin et al. 2009), the rate of trains was correlated with, but not necessarily proportional to, the rate of bursts at each recording site. There were 14 root systems in which no bursts of any insect-sound profile type were detected. Five of these did contain insects but 9 were found to be uninfested when they were excavated.

Assessments of Infestation Likelihood

The listener assessments of infestation likelihood matched significantly with the presence or absence of insects in the root systems at the recording sites (Table 3). Only 1 infested site was ranked at l_{ow} likelihood of infestation, and all of the sites ranked at h_{igh} likelihood of infestation were infested.

To develop a computer assessment of infestation likelihood, we examined the rates of bursts of different types detected at different infested and

TABLE 3. LISTENER ASSESSMENTS OF RECORDING SITES DETERMINED BY EXCAVATION TO BE UNINFESTED OR INFESTED.

	No. sites	
Assessed likelihood	infested	uninfested
l_{ow}	7	1
m_{edium}	2	14
h_{igh}	0	12

P = 0.0002 that listener assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, *S* = 61.5, *Z* = -4.09).

uninfested sites, and constructed indicators of infestation likelihood as described in Mankin et al. (2007). Sites with rates of bursts of all 3 insect-sound profile types <0.5 / min were considered to have l_{ow} likelihood of infestation, whereas sites with rates of bursts of any insect-sound profile type >1.5/min were assessed at h_{igh} likelihood of infestation. Sites with intermediate rates were assessed at m_{edium} likelihood. Assessments of the results in Tables 1 and 2 based on these criteria are listed in Table 4. The computer assessments, like the listener assessments in Table 3, matched significantly with the presence or absence of insects in the root systems at the recording sites.

DISCUSSION

One of the goals of this acoustic detection study was to develop a method for detecting infestations of *V. polistiformis* within the root system, thereby decreasing the cost and labor of treatments such as mounding. Although it would be helpful to obtain more recordings from *V. polistiformis* larvae, the results of the study are sufficient to provide some insight into how detection might be accomplished. An important finding was that a vineyard contains a large variety of nontarget, sound-producing insects. The signals produced by such insects could easily confound the identification of a targeted pest unless the pest produces a distinctive, easily identifiable sound that distinguished it from nontarget insects.

TABLE 4. COMPUTER ASSESSMENT OF RECORDING SITES DETERMINED BY EXCAVATION TO BE UNINFESTED OR INFESTED.

	No. sites	
Assessed likelihood	infested	uninfested
l_{ow}	9	5
m_{edium}	0	9
h_{igh}	0	13

P = 0.0005 that computer assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, *S* = 67.5, *Z* = -3.83).

A partial solution to this problem would be to include ambiguous signals as positive, i.e. count a false positive as a potential GRB larva. Considering the invertebrates and the burst rates in Table 1, for example, targeting all the sites that contained s_{highdB}, s_{middB}, and s_{lowdB} bursts would result in treatment of 9 out of 36 sites, only 1 of which actually contained a *V. polistiformis*. However, treating ¼ of the sites would be much less costly than treating all of them.

It is common for a vineyard to have approximately 735 vines per hectare. Assuming that the average price for unskilled farm labor is $20 per hour and that it takes 10 minutes to build a mound around a vine and 10 more minutes to remove the soil at the end of the season, the labor to treat 1 hectare would cost approximately $4900. If we assume that our findings of 25% infestation level apply to any vineyard, a farmer would spend approximately $1225 on mounding per hectare. It would therefore need to cost less than $3675 per hectare to acoustically sample all vines for the farmer to break even. It is estimated that the equipment would cost ~$3000 and last for 5 to 10 years. A farmer or scout could perform the assessment after a 15-20 minute training period.

Both human listeners and computer software were able to predict the presence or absence of infestation at statistically significant levels based upon spectral profile and temporal pattern analysis. However, human listeners were more likely to commit type I error whereas the computer was more likely to commit type II error. A type I error will cause the treatment of a vine when it is unnecessary, slightly raising the cost of treatment. However, a type II error will leave an infested site untreated, allowing emergence and reproduction. Without further refining of the spectral profiles or improvement of the software's analysis algorithm, it is recommended for a human listener to assess likelihood of infestation for pest management decisions.

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