Acoustic Detection of Black Vine Weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) Larval Infestations in Nursery Containers[1]

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Abstract

Acoustic detection systems have been developed to locate and target hidden infestations of root weevil larvae in container-grown nursery crops. Tests were conducted in laboratory and field environments with natural and artificial infestations of *Otiorhynchus sulcatus* (Fabricius) in containers with different nursery plants to determine whether the larvae were large enough for acoustic detection during late fall when scouting for infestation often occurs at commercial nurseries in Oregon. The rootballs of tested plants were examined to verify the presence or absence of larvae. All of the containers rated at *high* likelihood of infestation contained *O. sulcatus* larvae (29% of those tested). No larvae were found in any containers rated at *low* likelihood of infestation (20%). Sporadic sounds were detected but failed to exhibit periodicity suggestive of infestation in 51% of the containers (rated at *medium* likelihood). Fifty seven percent of these *medium*-rated containers were infested. Experience with the use of the acoustic system in field environments suggested improvements in user-friendliness and robustness that could improve its utility for early detection and targeting applications in commercial nurseries.

Index words: peppermint, rhododendron, spruce, strawberry, Virginia creeper, yew, subterranean insects.

Species used in this study: Alberta spruce (*Picea glauca* (Moench) Voss 'Albertina'); Norway spruce (*Picea abies* (L.) Karst. 'Mariana Nana'); English yew (*Taxus baccata* L. 'Fastigiata'); peppermint (*Mentha piperita* L.); rhododendron (*Rhododendron* L. 'Sappho'); strawberry, (*Fragraria* x *ananassa* Duch. 'Totem'); Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.).

Significance to the Nursery Industry

Black vine weevil is a major pest of container-grown nursery crops. The nursery industry would benefit from new tools that detect larval infestations in the late summer and fall when temperatures are warm enough for control measures to be effective. Effective curative treatments based on timely detection would reduce the risk of rejection of infested plants and increase the value of nursery plants to consumers. In this study, we used a portable acoustic system adapted for entomological applications to successfully detect natural infestations of black vine weevil larvae in plants in a nursery greenhouse and a research laboratory during mid-November 1999. The success of these tests stimulated additional efforts now in progress to develop robust, user-friendly instrumentation for use in commercial nurseries.

Introduction

Root weevils, including the black vine weevil, *Otiorhynchus sulcatus* (Fabricius), and the strawberry root weevil, *O. ovatus* L., are major insect pests in nurseries in the northern United States (16). Adult *O. sulcatus* feeding causes unsightly leaf notching on broadleaf ornamentals. Larval feeding on the roots of seedlings and potted plants reduces growth and increases mortality (3, 22). There is a need for early, rapid detection of *Otiorhynchus* infestations during the September–November period when temperatures are warm enough to effectively use entomopathogenic nematodes or curative chemical insecticides (5).

Current methods for detecting *O. sulcatus* larvae usually involve destructive sampling of root systems. The inspection process can be extremely time consuming (and wasteful of plant materials) when there are low infestation levels, especially when the plant material has dense, fibrous root systems. The lack of effective monitoring for these insects impedes development of improved management strategies. Pesticide and biological control treatments are expensive and some of the pesticides (organophosphates and carbamates) in current use may soon lose their labels for root weevil control. Tools for early detection may assist in targeting control measures, thereby reducing treatment costs.

Acoustic technology has become a promising candidate in the search for improved insect detection and monitoring methods. Already, several different acoustic systems have been developed for monitoring and detecting hidden infestations. Examples include insect activity monitoring systems (6, 7), the acoustic location fixing insect detector (20, 21), the multiple acoustic sensor system (8), the acoustic emissions detector (4, 18), and the biomonitor (19). Recently, Mankin et al. (11, 12) and Brandhorst-Hubbard et al. (1) conducted laboratory and field studies with a soil insect detection system. Digital signal analysis methods were developed

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to distinguish subterranean larval sounds from incidental environmental noises and sounds made by earthworms and other nonpest organisms (11, 12). The success of such research has fostered further interest in the development of practical acoustic instruments for field applications.

An important concern for entomological applications of acoustics is whether the targeted pests generate sufficient activity to be detected during the time when control measures can be implemented efficiently. Previous experiences with acoustic detection of stored product (e.g., 14) and subterranean insect larvae (11) suggest that, in general, temperatures need to be >10–12C and the larvae need to weigh >30–50 mg for their movement and feeding sounds to be loud enough for detection in moderate noise backgrounds. For this report, we conducted acoustic tests to determine whether *O. sulcatus* larvae are large enough to be detected during late fall when scouting for infestation often occurs at commercial nurseries in Oregon. Tests were conducted in laboratory and field environments with natural and artificial infestations in containers with several different species of nursery plants. The contents of the root systems were examined after testing to verify the acoustic predictions of infestation likelihood and the recovered larvae were counted and weighed.

Materials and Methods

Insects and plants. In studies at the ARS Horticultural Crops Laboratory (HCRL) Corvallis, OR, 2 Alberta spruce, *Picea glauca* (Moench) Voss 'Albertina'; 4 English yew, *Taxus baccata* L. 'Fastigiata'; 2 peppermint, *Mentha piperita* L.; 2 rhododendron, *Rhododendron* L. 'Sappho'; and 7 strawberry, *Fragraria* x *ananassa* Duch. 'Totem' plants in #1 or #5 containers were exposed to natural infestations of *O. sulcatus* in a greenhouse during the summer. To ensure that some plants would contain infestations, an additional group of 6 rhododendrons were artificially infested with 8–50-mg *O. sulcatus* larvae (2 with 10, 2 with 5, and 2 with 2 larvae, each) obtained from a colony at the HCRL using methods similar to those described in (23). Two uninfested rhododendrons were used for an acoustic background control. A field study at the Monrovia, Inc. nursery in Dayton, OR, included 9 Norway spruce, *Picea abies* (L.) Karst. 'Mariana Nana'; and 7 Virginia creeper *Parthenocissus quinquefolia* (L.) Planch. Acoustic testing was done during mid-November 1999. Temperatures were maintained at 20–24C (68–75F) in the laboratory tests but were only 10–15C (50–60F) in the nursery tests.

To acoustically monitor a plant for larval infestation, a 30 cm (11.8 in) nail was inserted near the crown of the root system and an accelerometer (see *Acoustic measurements* below) was attached magnetically to the head. Sounds detected by the accelerometer were monitored with headphones by an experienced listener and simultaneously recorded. The recorded signals were subsequently analyzed in the laboratory with custom-written signal processing software (11, 12). At the nursery, the acoustic tests were conducted inside a greenhouse to reduce background noise. In the laboratory, electrical and air conditioning equipment were turned off while recording.

After acoustic measurements, the roots of each plant were examined and any insects found were identified and weighed. Almost all of the larvae were identified as *O. sulcatus*, but larvae in 2 strawberry plants may have been *O. ovatus*. No attempt was made to distinguish unequivocally between these two species, which also would have been difficult to distinguish acoustically (11).

Ratings of infestation likelihood. Previous comparisons of sounds produced in soil samples where insects had been recovered after testing indicated that experienced listeners and computer analyses could reliably distinguish insect sounds from background noises (1, 11, 12). Listeners were trained in the laboratory to distinguish *O. sulcatus* larval sound pulses from background noises such as vehicles, wind, footsteps, and voices. Training included listening and recording practice with independently verified sources of *O. sulcatus* sounds, and practice with generating and interpreting background noises, including sensor and cable noise. Additional distinguishing features were identified from visual comparisons of spectral and temporal patterns obtained from a computer library of insect sound pulses and background noises (see *Acoustic characteristics of larval sound pulses* section below and from examples at URL: cmave.usda.ufl.edu/~rmankin/soundlibrary.html). Listeners subjectively rated the likelihood of *O. sulcatus* infestation after recording and listening in each container. The rating scale was: *low*, no subterranean sounds or only a few faint sound pulses, easily lost in the noise background; *medium*, sporadic, faint sound pulses, sometimes obscured by background noises, and a lack of temporal patterns typically present with insect sounds; *high*, frequent sound pulses with a high signal level, easily distinguished from background, and temporal patterns suggestive of purposeful movement or chewing activity.

Acoustic measurements. The acoustic system included an accelerometer (Brüel and Kjær [B&K] Nærum, Denmark), sensitivity 10 pC/ms^{-2}, weight 54 g), a charge amplifier (B&K model 2635), and a digital audio tape recorder (DAT). A >180-s period was recorded on the DAT and monitored with headphones at each container.

Signal analysis. The recorded signals were digitized and analyzed with a digital signal processing system (10, 11, 12) that provided computer assessment of activity and distinguished larval sounds from background noise (13). Moving and feeding larvae generated short (0.5–5 ms) pulses that were distinguished from non-insect noises by computer subroutines that analyzed differences in temporal pattern or frequency. Profiles of sound pulses recorded from each plant species in this study were calculated as averaged spectra of representative 10-s samples recorded from each plant (see 11) that can be played at the URL: cmave.usda.ufl.edu/~rmankin/blackvineweevilsounds.html. Vibration Level (VL), a measure of the signal energy (17), was measured as acceleration in dB on a relative scale between specified frequencies (e.g., dB // 0–2 kHz, see also 11).

Results and Discussion

Acoustic identification of black vine weevil infestations. The tests confirmed that natural infestations of *O. sulcatus* larvae could be identified in nursery containers by acoustic techniques during the fall scouting season. However, operation of the acoustic system in the nursery environment identified problems with practical implementation that need to be addressed by future research. A summary of the major results is given in Table 1, including listener ratings of infestation likelihood and the numbers and weights of larvae re-

Table 1. Numbers and weights of larvae recovered from acoustically tested nursery containers, sorted according to total weight in containers rated at *high*, *medium*, or *low* likelihood of infestation.

Container[z]	Listener rating[y]	No. larvae	Weight (mg)					Profile no.[w]
			Mean	S.E.	Max	Min	Total[x]	
Spruce8	*high*	572	49.5	0.9	88.8	1.4	28293.3	
Spruce1	*high*	126	51.6	2.0	85.4	5.4	6503.3	1
Yew12	*high*	53	23.7	2.4	70.7	4.2	1255.9	
Creep23	*high*	24	40.2	4.7	74.7	4.4	963.9	2
Yew11	*high*	19	36.1	3.3	63.4	14.3	685.9	5
Yew31	*high*	16	37.3	6.5	83.6	8.3	596.5	
Yew30	*high*	14	36.6	5.5	66.1	13.6	512.6	
Rhodo2[v]	*high*	10	23.4	3.4	42.4	9.2	234.4	
Alberta9	*high*	7	20.9	3.7	36.4	8.6	146.3	4
Strawb28	*high*	12	10.4	5.8	74.3	2.0	124.8	
Rhodo13	*high*	6	20.8	3.8	31.3	8.8	124.5	
Strawb29	*high*	8	10.3	2.7	24.0	1.0	82.5	
Creep26	*medium*	58	40.3	3.0	89.1	8.5	2338.9	
Creep22	*medium*	28	37.1	4.8	69.1	3.1	1038.7	
Creep25	*medium*	22	37.9	4.8	68.4	2.8	834.2	
Rhodo3[v]	*medium*	3	33.5	9.4	49.1	16.4	100.4	3
Rhodo4[v]	*medium*	4	21.1	6.9	40.9	8.9	84.4	
Strawb16	*medium*	1	62.9	—	62.9	62.9	62.9	
Rhodo5[v]	*medium*	3	20.7	11.5	43.7	8.8	62.2	
Strawb15	*medium*	2	28.9	3.2	32.1	25.7	57.8	6
Rhodo13	*medium*	3	12.7	4.3	19.0	4.3	38.0	
Rhodo6[v]	*medium*	2	17.6	9.9	27.5	7.8	35.3	
Rhodo14	*medium*	2	10.4	5.0	15.4	5.4	20.8	
Strawb19	*medium*	1	13.0	—	13.0	13.0	13.0	

[z] Container designations: Alberta, Alberta spruce; Creep, Virginia creeper; Pmint, peppermint; Rhodo, rhododendron; Spruce, Norway spruce; Strawb, strawberry; Yew, English yew, with number at end to distinguish among containers. Plants without recovered larvae are not shown. These included 9 from the *medium* category (1 Alberta spruce, 2 Virginia creeper, 2 peppermint, 2 Norway spruce, and 2 strawberry) and all 8 from the *low* category (1 Virginia creeper, 5 Norway spruce, and 2 rhododendron).

[y] Rating scale: *low*, no subterranean sounds or only a few faint sound pulses, easily lost in the noise background; *medium*, sporadic, faint sound pulses, sometimes obscured by background noises, and no certainty about presence of infestation; *high*, frequent sound pulses with a high signal level, easily distinguished from background, providing high certainty of infestation.

[x] Total weight of all larvae in container (mg).

[w] No. of spectral profile shown in Fig. 1. (Also corresponds to no. of .wav file in sound library at URL: cmave.usda.ufl.edu/~rmankin/blackvineweevilsounds.html.

[v] Artificially infested with *O. sulcatus* larvae in laboratory.

covered from different containers in both laboratory and field tests. In general, computer ratings of the recordings made in the laboratory matched well with the listener ratings presented in Table 1 (as in Table 5 of Mankin et al. 12), but computer ratings of several nursery recordings were confounded by high background noise so they were not included in the table. The computer analyses are not yet as reliable as the ratings of experienced listeners in the presence of high background noise (e.g., 11). All of the artificially infested rhododendrons (indicated with superscript [v] in Table 1) were rated at *medium* likelihood of infestation except for one with 10 larvae, rated *high*.

The instruments and recorders used in this study, although portable, were designed primarily for laboratory use, and considerable training and care were required to collect and interpret the acoustic signals. Precautions were taken to protect the acoustic instruments during testing that would not be practical for long-term field applications. Consequently, efforts are in progress to develop more robust, user-friendly instrumentation (see URL: cmave.usda.ufl.edu).

Larval size and acoustic detectability. To consider the effect of larval size, the listings in Table 1 were sorted according to the total weight of larvae recovered per container for ratings of *high*, *medium* and *low* infestation likelihood. As in previous studies (11, 12), experienced listeners were most successful at rating infestation likelihood when the insects were highly active during the recording period and large enough to be detected over distances of several cm. The reliability of the ratings decreased but remained above chance when few or no insects were present or when insects were present but produced sounds at low rates (e.g., 3 Virginia creeper plants [Creep22, Creep25, and Creep26]). Creep23 had fewer larvae of lower total weight than Creep26 and Creep22, but a greater rate of sound pulses. A possible explanation for the low rate of sounds in Creep26 is that the most edible roots may have already been consumed. Table 2 lists the numbers of infested and uninfested containers and the mean total weight of larvae recovered from plants rated at *high*, *medium*, and *low* likelihoods of infestation. On average, the containers rated at *high* likelihood of infestation had greater numbers and greater total weight of larvae than those rated *medium*, but these differences were not statistically significant due to the high variance. Larvae as small as 13 mg were detected, but in most cases the weights were at least 30–50 mg.

The relationship between the listener ratings and the independently confirmed infestations was highly significant ($\chi^2 = 19.8$, 2 df, $P < 0.005$). All of the plants rated at *high* likelihood of infestation (29%) contained at least 6 *O. sulcatus* larvae. No larvae were found in any plants rated at *low* likelihood of infestation (20%). Sporadic sounds were detected

Table 2. **Numbers of infested and uninfested containers acoustically rated by listeners at *high*, *medium*, and *low* likelihoods of infestation, and mean ± s.e. total numbers and weights of larvae recovered from containers.**

Rated likelihood	No. containers found		Mean ± s.e. total	
	infested	uninfested	no. larvae	weight (mg)
high	12	0	75 ± 46	3294 ± 2330
medium	12	9	11 ± 5	223 ± 122
low	0	8	0	0

in 51% of the containers. These containers, rated at *medium* likelihood of infestation, would be the most difficult to classify in a screening program. Fifty seven percent of the *medium*-rated containers were confirmed to be infested. If the *medium* ratings are included in the infested category, there was a 100% correct rating of uninfested containers and 78% correctly rated infestations, or 22% incorrectly rated as infested. If the *medium* ratings are included in the uninfested category, there was a 100% correct rating of infestation and 71% correct rating of uninfested containers, or 29% incorrectly rated as uninfested. Containers rated in this category could be retested, or assigned a category based on the relative risks of misclassification.

Acoustic characteristics of larval sound pulses. Spectral profiles of sound pulses produced by *O. sulcatus* larvae in roots of the different plant species in this study are shown in Fig. 1 along with a profile of typical background noise. No profiles are presented from containers with *M. piperita* because they had no detectable larval sounds in the computer analyses, although a few faint sounds were detected by listeners (Pmint17–18). Black vine weevil grow very poorly on mint (2), so it is possible that small larvae were present but they were too small to detect by computer or visual inspection. Black vine weevil larval development is influenced by host plant species and root quality (2), which is likely to cause an interaction between the frequency of detectable sound pulses and host quality.

There were no obvious differences among the profiles from different plants except that the sounds recorded from strawberry had only a small peak between 0.7–1 kHz where most of the other larval profiles contained significant energy. Some of the larvae in these containers were possibly *O. ovatus* rather than *O. sulcatus*.

In many respects, the larval sound profiles resemble the profiles calculated from sound pulses of other subterranean insects (11, 12). Several profiles had peaks near 0.3 kHz as well as between 1.2 and 1.6 kHz. The latter peaks were the signal features most useful to experienced listeners in distinguishing between larval and background noises. There was a 30 dB difference in signal level between the larval pulses and background noises at these frequencies. Listeners can easily recognize and focus on such differences with minimal training. In addition, many background noises have durations

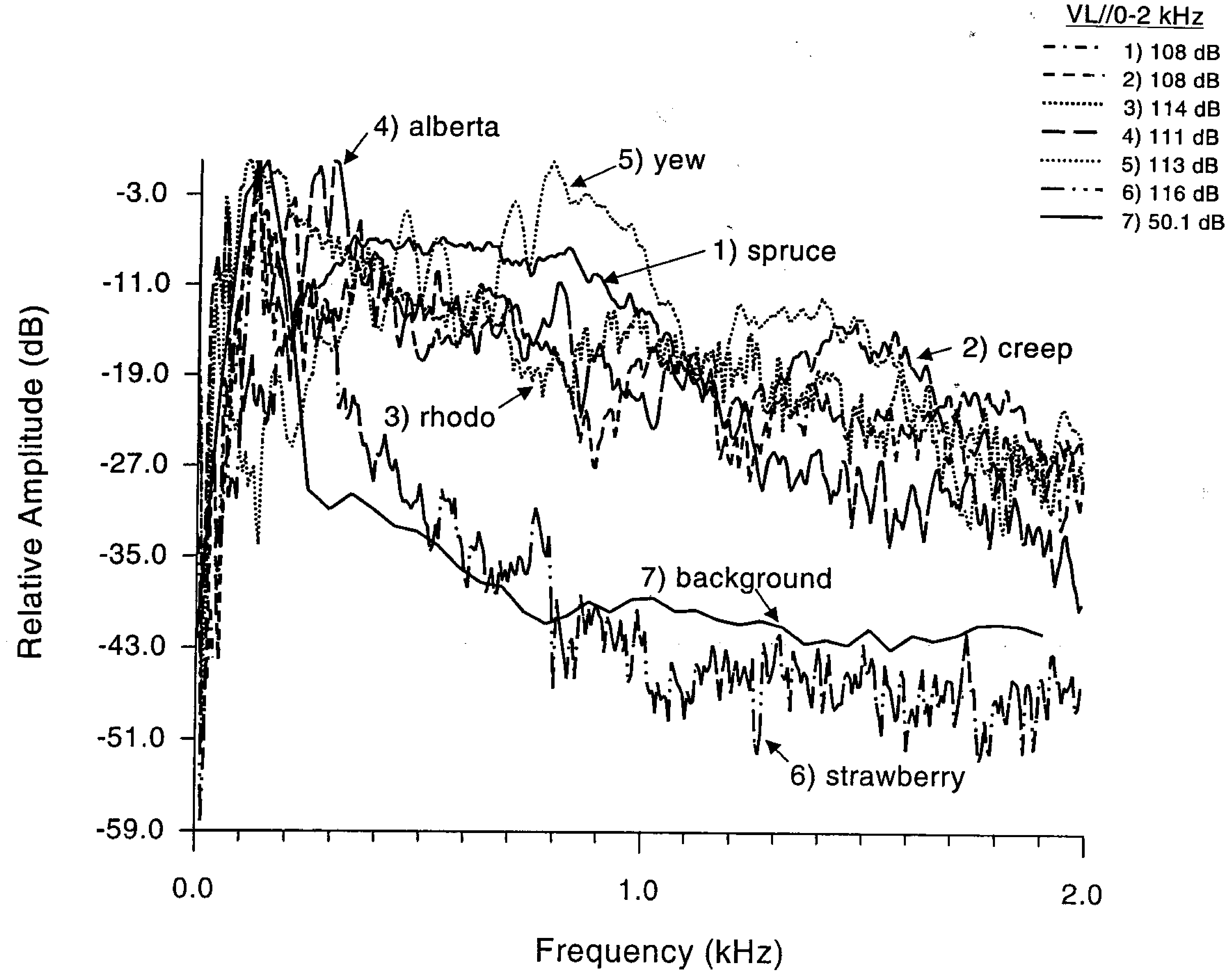

Fig. 1. **Mean spectral profiles of larval sound pulses recorded by accelerometer in containers containing plants of different species: 1) *P. abies*, 2) *P. quinquefolia*, 3) *Rhododendron*, 4) *P. glauca*, 5) *Taxus baccata*, 6) *Fragraria* x *ananassa*.**

of seconds or longer and differ in temporal pattern from the larval sounds. A combination of field and laboratory listening experience and training with computer analyses of spectral profile differences enables listeners to enhance the reliability of their infestation ratings.

Future applications. The results of this study confirm that acoustic monitoring techniques show promise as survey tools for rapid detection of subterranean insect larvae in containers during the critical fall scouting season at commercial nurseries. In reasonable noise backgrounds at temperatures > 10–12C (50–54F), active infestations can be detected in individual containers within 10–100 seconds. Although there are conditions under which acoustic methods would be unreliable, e.g., in cold weather when activity decreases or high noise backgrounds that mask activity, precautions can be taken to avoid operation of acoustic systems at such times. The need for early detection of *O. sulcatus* infestations and the success of these initial results provides impetus for further exploration of acoustic techniques using robust equipment adapted to the needs of entomologists and nursery managers.

In practical applications, acoustic detection methods should enhance the efficiency of the conventional destructive sampling method. Destructive sampling of pots is still necessary to positively identify the species and stage of the infesting insects. However, without acoustic sampling, many uninfested plants would have to be destroyed in order to detect rarely occurring larvae. By using sounds characteristic of insect activity to choose pots for destructive evaluation, fewer plants would need to be sacrificed, and lower levels of infestation could be detected with the same amount of time and effort.

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