Assessment of plant structural characteristics, health, and ecology using bioacoustic tools

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Plants have responded to abiotic stress and biotic threats with evolutionary adaptations resulting in a variety of plant architectures and cell walls with economically important mechanical properties. Bioacoustic tools have been applied to measure mechanical properties of plant structures, optimize mechanical harvesting, and detect the distribution of root systems, as well as to monitor plant health, photosynthesis, and ecology, and to assess the quality of marketable plant products. In addition, acoustic and vibration sensors are used by entomologists to detect hidden infestations of invasive insect species and to monitor insect movement, feeding, and mating activities on host plants. Reliable identification and interpretation of insect-produced sounds and vibrations on and within plants is complicated by physical processes that filter and distort vibrations as they pass through and reflect from different plant structures and the air. This report considers the use of bioacoustic tools to analyze plant health and structural characteristics, and then discusses how combinations of spectral-, temporal-, and spatial-distribution features of signals detected in plants can be interpreted in ways that properly account for plant structural vibrational filtering and distortion processes and enable reliable assessment of hidden pest infestations, including invasive insect species of importance for plant biosecurity.

I. INTRODUCTION

Plants have developed an arsenal of mechanical and chemical defenses (Coley and Barone 1996, Murali Baskaran et al. 2018) to fend off harsh environments and animals seeking food (Kogan 1988) and habitat (Schowalter 2017, Donihue et al. 2018). Plant architectures and cell walls have evolved under these rigors in ways that have contributed to reproductive fitness and also produced structures with a variety of mechanical properties useful to humans as crops, building materials, biomaterials, and even acoustic instruments (Nakata et al. 2018). It is relevant to bioacoustic signaling research, for example, that flower structures have evolved in plants to enhance reflection of bat echolocation signals, encouraging pollination (Schöner et al. 2016) and thereby enhancing reproductive fitness. Researchers have made use of various acoustically important features of plant cells and structures by applying bioacoustic technology to influence plant growth and crop yield (Gagliano et al. 2012, Hassanien et al. 2014, Ghosh et al. 2016), to measure stiffness and other mechanical properties of plants (Niklas and Moon 1988; Zebrowski 1991, 1999), and to grade logs (Tsehaye et al. 2000) and other timber materials (Krause et al. 2014). Agricultural engineers and biologists have used acoustic tools to assess cellular physiological processes (De Roo et al. 2016), measure root growth (Shimotashiro et al. 1997), map root systems (Kalkowski et al. 2018), develop shaking tools for mechanical harvesting (Gupta et al. 2016) and screen for mutants with altered mechanical properties (Nakata et al. 2018). Sound propagation measurements have been used to assess health and monitor underwater plant photosynthesis (Hermand 2006, Kratochvil and Pollirer 2017). Ecologists have monitored soundscapes to estimate populations of plant-pollinating bumble bees (Miller Struttmann et al. 2017) and gain improved understanding of bee-buzzing pollination processes (Rodrigues et al. 2018).
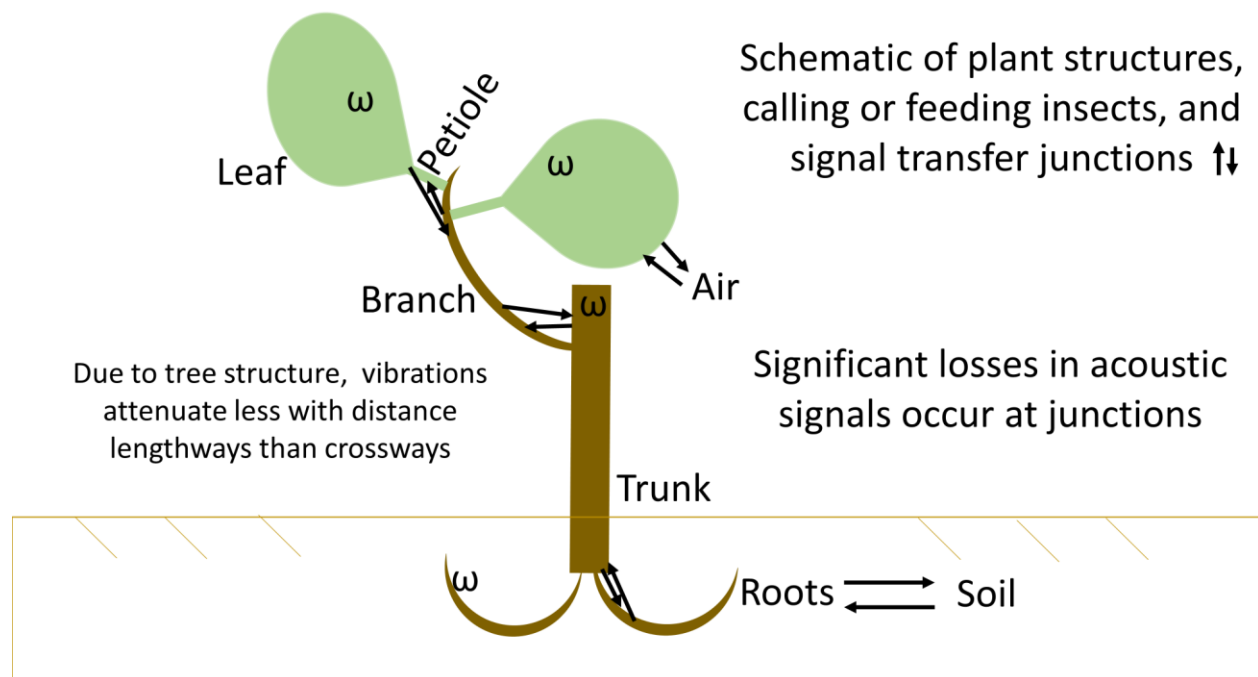

Figure 1. Sheet-like leaves and interconnected, rod-like petiole-, branch-, trunk-, and root-structures transport vibrations induced by insects (ω) and other animals over ca. meter-long distances. Signal amplitude does not decrease uniformly with distance due to resonance effects within individual structural components of different geometries and energy loss at junctions between components. Additional 2-way transfers of signals occur between above-ground structures and air, and between roots and soil.

Of particular relevance to plant health and agriculture is that bioacoustic methods have been applied to monitor pest insect behavior on plants (Cocroft and Rodriguez 2005) and to detect hidden

internal infestations, particularly infestations of invasive species relevant to biosecurity (Mankin et al. 2011, Sutin et al. 2017, Poland and Rassati 2018, Showalter et al. 2018). Insects produce vibrations on or within different plant structures (Figure 1) that can travel long distances as 2-dimensional bending waves (McNett et al. 2006), enabling protective action by pest managers or adaptive responses by the plants against herbivores (Telewski 2006, Appel and Cocroft 2014, Mishra et al. 2016) or by prey insects against predators (Casas et al. 1998).

Vibrational communication is widely used by small insects that cannot transduce mechanical energy efficiently into airborne pressure waves detectable over long distances (Bennet-Clark 1998, Michelsen 2014) but can easily produce and detect vibrations on host plants for social (Yack et al. 2001) or reproductive interactions (Cocroft and Rodríguez 2005, Joyce et al. 2014). Courtship of duetting male and female *Chrysoperla plorabunda* (Neuroptera: Chrysopidae) lacewings has been documented over distances up to a meter (Henry and Wells 2006), and courtship of Neotropical katydids (Pseudophyllinae) has been documented up to four meters (Römer et al. 2010). Methods have been developed to trap or disrupt mating of insects that communicate with vibrations (Polajnar et al. 2016, Mankin et al. 2016b, Gordon et al. 2017).

Although several bioacoustical tools and signal analyses have been developed to detect targeted insects (Mankin et al. 2011) and distinguish their signals from extraneous background noise (Barth et al. 1988; Stölting et al. 2002; Lohrey et al. 2009), the reliability of detection is often complicated by resonances (Polajnar et al. 2012) and other physical processes that filter and distort the vibrational signals as they propagate through and reflect from different plant structures (Hambric 2006, Mortimer 2017) and air (Hambric and Fahnline 2007). This report considers several examples where knowledge of such physical processes has helped inform the interpretation of insect-produced sounds detected over distances up to several meters.

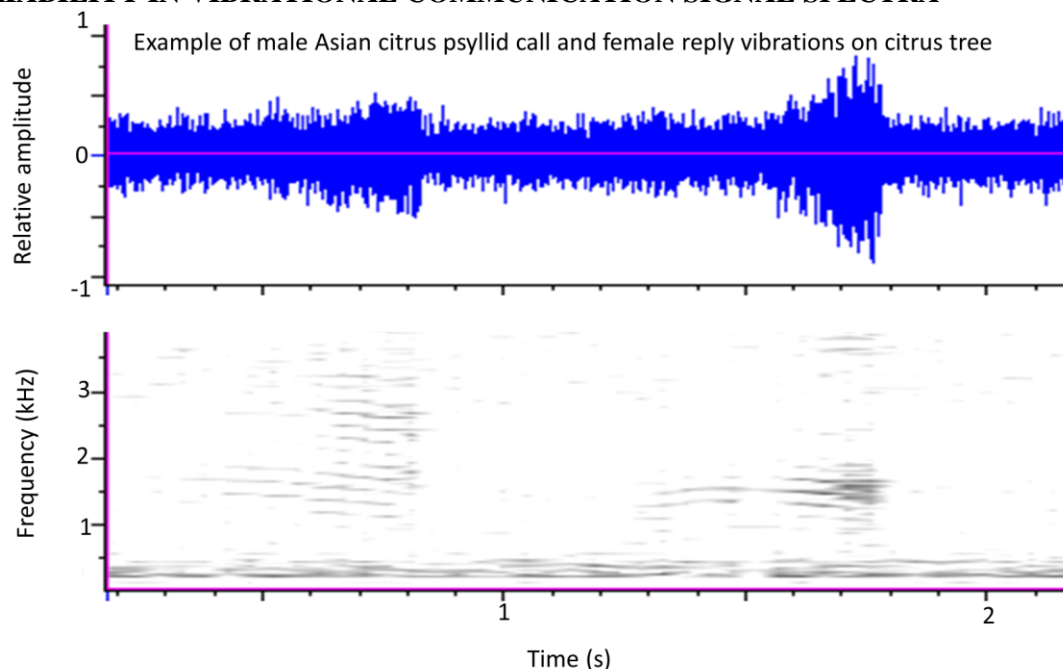## II. VARIABILITY IN VIBRATIONAL COMMUNICATION SIGNAL SPECTRA

Figure 2. Oscillogram (top) and spectrogram (bottom) of a vibrational communication call of a male Asian citrus psyllid followed by a female reply.

Asian citrus psyllids, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), search for mates using vibrational duetting signals (Wenninger et al. 2009) such as those displayed in Figure 2, recorded by an

accelerometer attached to the trunk of a small citrus tree. The first part of the record in Figure 2 includes wing vibrations of a male calling about 15 cm from the end of a small branch and the second part, ca. 0.5 s after the end of the male call, includes the response of a female feeding on a leaf at the branch's tip. Both the call and reply contain multiple harmonics of the approximately 200-Hz wingbeat frequencies. The signals have only a weak airborne component, but the wing vibrations pass through the legs to the branch, and then from the branch to the legs of the duetting partner and the accelerometer (Mankin et al. 2016b). In this example, background noise was present below 200 Hz. Experimental conditions were similar to those described for field recordings in Mankin et al. (2016b).

To assist in management of this devastating citrus pest (Nat. Acad. Sci. 2018), acoustic devices have been developed to trap males (Hartman et al. 2017) and disrupt mating (Lujo et al. 2016). The devices incorporate a contact sensor to detect the vibrations, a microcontroller platform to identify and discriminate calls from background noise, and a piezoelectric buzzer to produce female reply mimics that stimulate male searches. Mimics are produced within 0.4 s after the call, matching the typical interval of a female reply (Lujo et al. 2016). Initially, the microcontroller discriminated each putative male call from background noise using a mean spectrogram template (profile) that identified many but not all male calls (Mankin et al. 2016b). The addition of genetic algorithm methods and inclusion of a greater range of calls in the mean spectrogram improved the identification process; nevertheless, the device continued to discriminate incorrectly a fraction of signals recognizable to psyllids and humans as calls. Analysis of classification errors suggested that amplitudes of harmonics varied significantly among calls at different distances between the male and the microphone, but females responded if 2-3 harmonics were detectable. Consideration of the spectral profile alone was not always sufficient to identify the signal as a male call, but females correctly identified calls in which 2-3 harmonics of the wing-beat frequency were present (Mankin et al. 2016b).

Harmonic amplitude variation results from effects of mass, geometry, stiffness, and damping that differentially affect the speed and amplitude of different frequencies traveling down a tree branch (Mortimer 2017). Fruit-bearing branches of citrus trees have a natural resonance frequency (ω_n) of 3-7.8 Hz, used by mechanical harvesters to facilitate fruit drop (Gupta et al. 2016). In lightly damped systems such as a small citrus tree branch subjected to low-intensity vibrations from a tree shaker, $I(\omega)$, the amplitude of a driven vibration at frequency, ω in units of s^{-1}, is attenuated relative to its amplitude at the natural resonance frequency of the branch via the relationship:

$$I(\omega) \sim ((\omega - \omega_n)^2 + c / (2\, m\, \omega_n))^{-1},$$

where c is a damping coefficient (with units of kg s^{-1}) and m is the mass of the branch in kg (e.g., Siebert 1986, Hambric 2006). Higher-frequency *D. citri* wingbeat harmonics travel faster along the branch than lower harmonics, due to waveguide propagation effects, but higher-frequency harmonics also attenuate more rapidly with distance (e.g., Krugner and Gordon 2018). Future versions of the algorithm for psyllid-call identification are planned to take this into account.

One result of this frequency dependent attenuation with distance from the source is that a listener (receiver) can estimate whether the transmitter is moving towards or away from the sensor by comparing the relative amplitudes of different harmonics over time. Unfortunately, such estimation cannot be accomplished easily if the transmitter, e.g., an insect, produces signals of variable amplitude, as considered in the next section. The relative difference between the times of arrival of high and low frequencies at the receiver, would nevertheless decrease over time if the transmitter were moving closer, and increase if the transmitter were moving away.

III. EFFECTS OF AMPLITUDE VARIATIONS OF INSECT ACTIVITY ON VIBRATIONAL SPECTRA FEATURES

Effects of variation in the amplitude of insect activity on the spectra of vibrations they produce in plants are seen in two 22.67-ms records from different trunks of red maple, *Acer rubrum* L. (Sapindales: Sapindaceae), each infested with a larva of Asian longhorned beetle, *Anaplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Figure 3). The experimental conditions and recording procedures have been described in Mankin et al. (2008b). The wavelet time-frequency and spectral

representations were constructed in MATLAB Release 2012b (The MathWorks Inc. Natick, MA). Both the high- and low-amplitude signals included a 5 ms interval of oscillation after the beginning of the signal, followed by a decay to baseline, but the wavelet representations were considerably different except in the two regions indicated by dashed rectangles. Likewise, the spectra of the high- and low-amplitude signals were considerably different.

Similar effects have been observed with insect-produced vibrations on a spider web, a relatively ideal vibration transmitter compared to a tree trunk because its vibration transmission properties are adjustable by modifying the geometry and tension of individual web elements (Mortimer 2017). In Figure 4, two assassin bugs, *Stenolemus bituberus* Stål (Hemiptera: Reduviidae), hunters of web-building spiders (Wignall and Taylor 2011), plucked different spider webs with high- 4A) or low-4B) amplitude. Wignall and Taylor (2011) found that *S. bituberus* vibrations in spider webs typically ranged between 200-300 Hz, a narrower band of signals than vibrations from prey to which the resident spider was usually attracted. These frequencies elicited spider attraction, enabling the bug to attack the spider, but did not elicit aggressive responses dangerous to the bug.
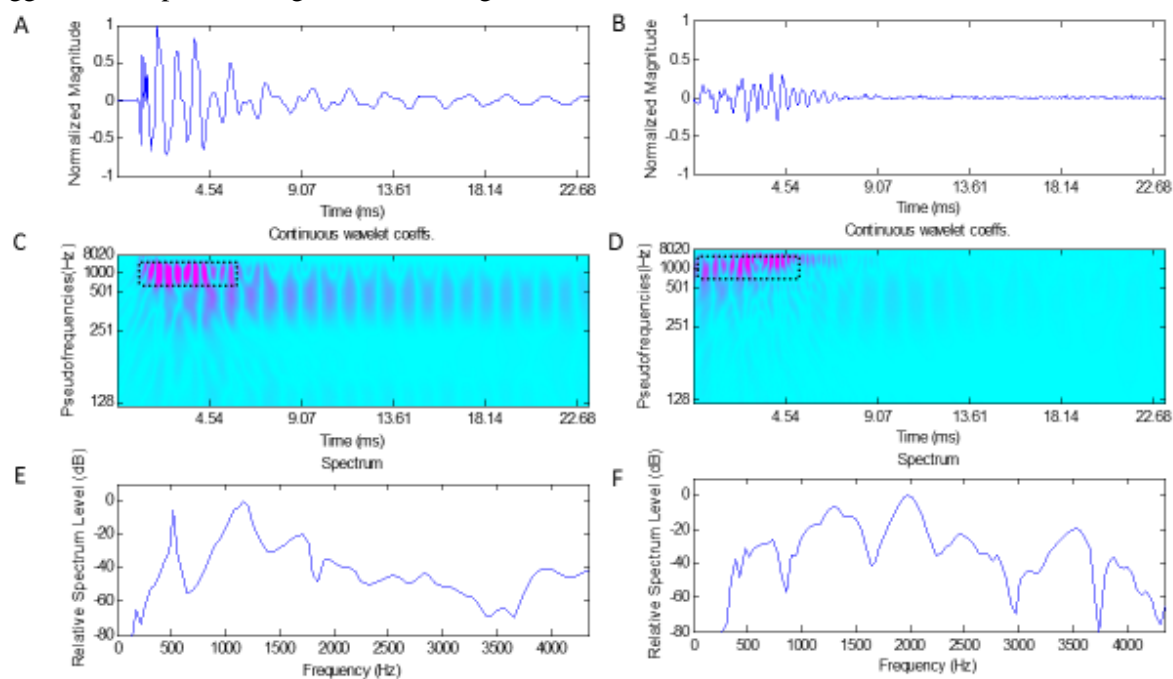

Figure 3. Oscillograms of A) high- and B) low-amplitude vibrations of an Asian longhorned beetle larva in a maple tree trunk, with their corresponding wavelet time-frequency representations, C) and D), and frequency spectra, E) and F). In C) and D), light blue indicates low energy levels and dark pink indicates high energy levels at that time and frequency.
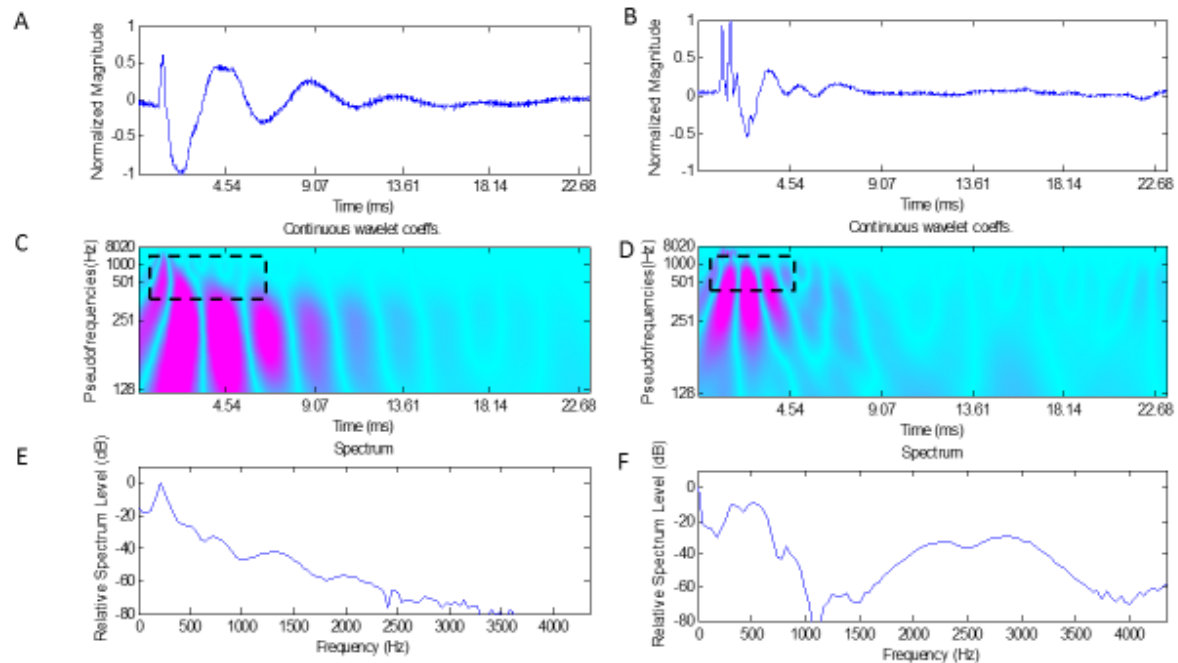
Figure 4. Oscillograms of A) high- and B) low-amplitude vibrations of assassin bug in spider webs, with their corresponding wavelet time-frequency representations, C) and D), and frequency spectra, E) and F). Color schemes for signal energy level in C) and D) are the same as in Figure 3.

The assassin bug recordings in Figure 4 were obtained in a laboratory using experimental procedures described in Wignall and Taylor (2011). A notable result of the experiment was that the spiders responded as if they recognized particular features of vibrations produced by assassin bugs as potential features of prey vibrations, independent of differences in the spectral and temporal features of individual assassin bug plucks (Wignall and Taylor 2011). Signal features that may have contributed to such results could have included the spectral and temporal patterns outlined in the time-frequency rectangles of Figure 4C and D. The patterns were similar inside the outlined ranges but different outside them.

It should also be noted that various abiotic processes, including extreme temperature, weather, and barometric pressure variation (Zagvazdina et al. 2015), as well as physiological processes such as molting (Jalinas et al. 2017), can affect insect activity levels and reduce the reliability of detection by bioacoustic methods. One way to avoid false negatives at individual recording sites in such situations is to sample multiple times over a several-day period, with the specific time span depending on the weather variability and the behavioral cycles of the insect species being targeted. A second method is to set up an index for infestation likelihood that examines a range of signal features to classify the infestation likelihood as high, medium, or low at a particular sampling location, independently of a specific oscillogram or spectrogram feature. This procedure is considered in the next session.

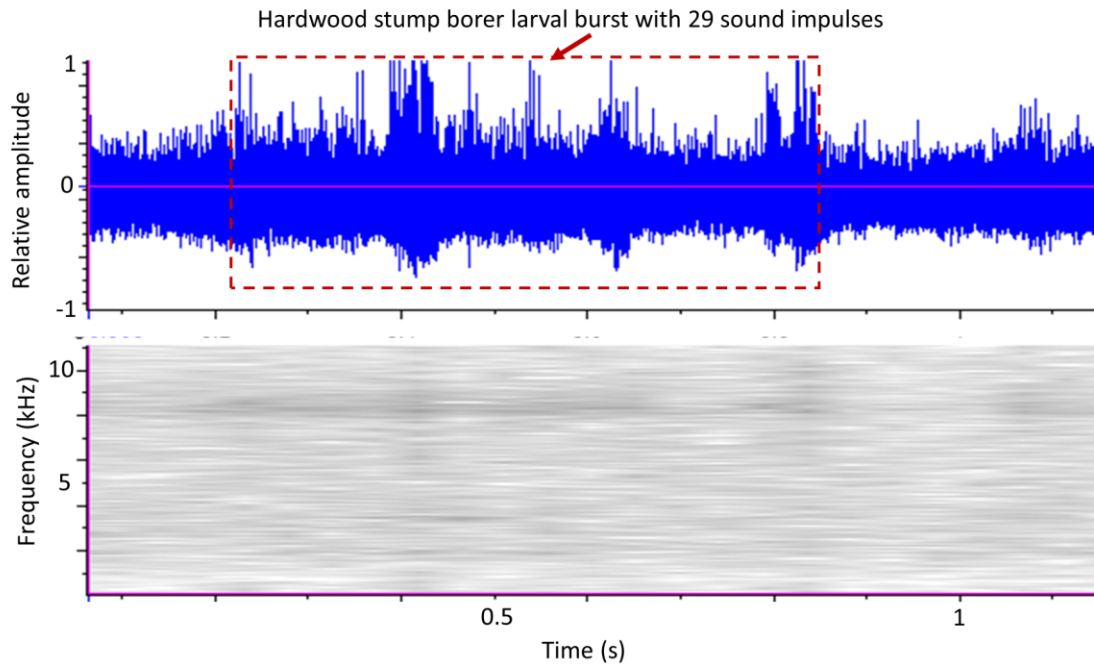## IV. RELIABLE DETECTION OF HIDDEN INSECTS IN FIELD ENVIRONMENTS

Figure 5. Oscillogram (top) and spectrogram (bottom) of a 1.3-s record of sounds produced by a hardwood stump borer larva in an avocado tree. The section of signal outlined in the dashed box contains 29 sound impulses interpreted to be an insect sound burst.

Given the variability of insect activity over time, and the effects of signal filtering, resonances, and distortion that are dependent on the distance between insects and sensors, it is often difficult to interpret without ambiguity the vibrational signals collected from different positions in trees, vines, and other large plants in noisy environments and predict reliably whether the substrate contains a hidden insect infestation. One approach combines together information from multiple features of the spatial distribution of the signals and their temporal and spectral patterns, identifying specific combinations of features as indicators of insect presence (Mankin et al. 2011). Here we consider two examples of signal feature combinations that enabled successful prediction of infestations of Asian longhorned beetle larvae in red maple trees and hardwood stump borer larvae, *Mallodon dasystomus* Say (Coleoptera: Cerambycidae: Prioninae) in avocado trees, *Persea americana* (Laurales: Lauraceae). The Asian longhorned beetle infestation was in an Animal and Plant Health Inspection Quarantine zone where an eradication project was in progress near Amelia, OH, and the hardwood stump borer infestation was in a commercial avocado grove near Homestead, FL.

Insect larvae in tree trunks and other woody substrates produce trains of short, 1-10-ms impulses separated by interpulse intervals of < 200 ms as they break fibers while feeding or scrape the sides of tunnels while moving (Mankin et al. 2008b). The impulses have spectral patterns containing peaks between 2 and 10 kHz that identify them as potential insect sound impulses (Mankin et al. 2011). Such impulses also can be produced by wind and other sources, but experience with insect infestations in laboratory and field studies suggests that particular trains of ≥ 6 but < 200 impulses with spectra of typical insect sounds, designated as insect sound bursts, occur frequently when insects are present but only rarely when they are absent from the monitoring site, and can be used as indicators of insect presence. A typical insect sound burst is displayed in Figure 5, recorded from an avocado tree where a hardwood stump borer subsequently was recovered. The signals were recorded and analyzed using procedures described in Mankin et al. (2018).

Previous investigations comparing the rates of insect sound bursts, r_b in units of s^{-1}, detected in the presence or absence of insects (Mankin et al. 2016a, Mankin et al. 2008a) have established burst rate

ranges that can be used for indicators of High ($r_b \geq 0.06$ s^{-1}), Medium (0.02 s$^{-1} \leq r_b < 0.06$ s^{-1}), and Low ($r_b < 0.02$ s^{-1}) likelihood of insect infestation. An example of how such indicators can be used to estimate whether a specific site contains an insect infestation is shown in Figure 6, which plots insect sound burst rates detected in field studies conducted to detect Asian longhorned beetle (ALB) and hardwood stump borer (HSB) infestations in trees near Amelia, OH and Homestead, FL, respectively. In both surveys, signals had been recorded from an SP1L piezoelectric sensor module (Mankin et al. 2018) at multiple positions on each tree. A single ALB larva was recovered from the red maple tree and a single HSB larva from the avocado tree after the recordings were collected.
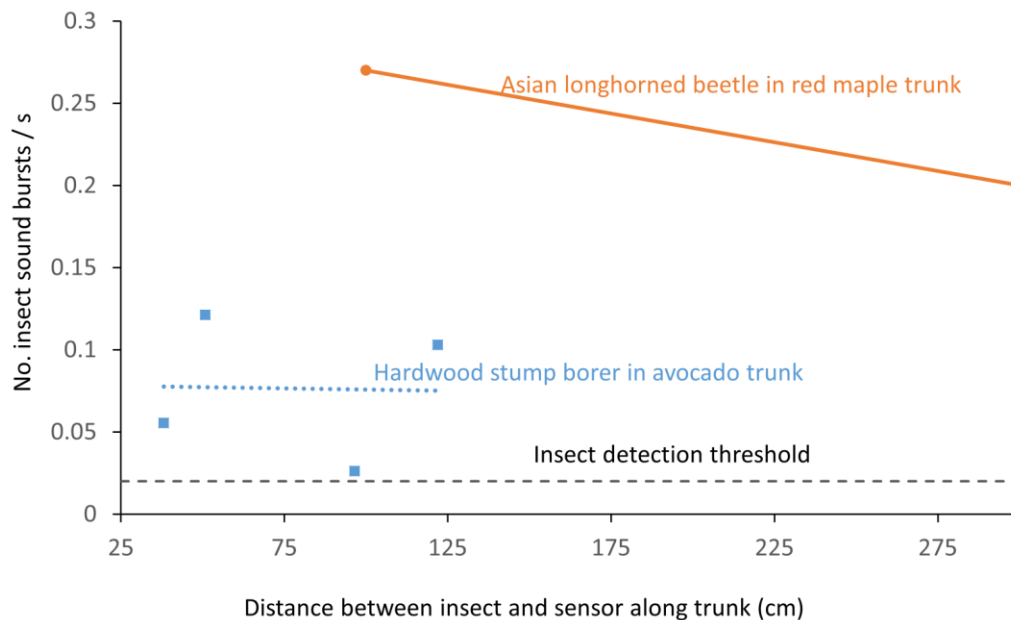

Figure 6. Examples of insect sound burst rates recorded from an Asian longhorned beetle (ALB) larva by a sensor/amplifier device placed at two positions (circles) on a red maple tree, and rates recorded from a hardwood stump borer (HSB) larva at four positions (squares) on an avocado tree. Solid trend line shows the decrease in ALB burst rate and dotted trend line shows the decrease in HSB burst rate with increasing distance between larva and sensor. The threshold for insect detection (dashed line), indicates the insect sound burst rate above which a recording site is predicted to contain an insect infestation.

Although the insect sound burst rates in Figure 6 varied across different recordings, they all were above the insect detection threshold, indicating that both trees were infested. On average, there was a slight decrease in the burst rate with increasing distance between the insect and the sensor, but in the case of the ALB larva in the red maple tree, the larva was detectable over distances > 300 cm. The use of an index for infestation likelihood improves the reliability of detecting an infestation whether or not the recording site is near or several hundred cm away from the insect. The usage of robust acoustic detection indicators and spatial distribution analysis has enabled successful surveys of insect infestation in several previous field studies (Brandhorst-Hubbard et al. 2001, Zhang et al. 2003, Mankin et al. 2007, Fiaboe et al. 2011, Mankin et al. 2016a).

V. CONCLUSION
Plant structures have important mechanical characteristics that facilitate the application of bioacoustic tools for monitoring of insect pests and assessment of plant health. Some of the mechanical properties of plants affect insect-produced sounds and vibrations in ways that may hinder assessment of whether targeted insects or extraneous noises are present at a recording site. These effects can be mitigated by

assessing combinations of spatial distribution, spectral, and temporal patterns when such information is available. Consequently, as the affordability and versatility of bioacoustic tools increase, researchers, managers and regulatory officials likely will continue to increase their use of bioacoustic methods to manage plant health and identify hidden invasive insect pest species for plant biosecurity.

VI. ACKNOWLEDGMENTS

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