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SUBSTRATE-BORNE VIBRATIONAL SIGNALS IN INTRASPECIFIC COMMUNICATION OF THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
Exploitation of vibrational signals for suppressing glassy-winged sharpshooter (GWSS) populations could prove to be a useful tool. However, existing knowledge on GWSS vibrational communication is insufficient to implement a management program for this pest in California. Therefore, the objective of this study is to identify and describe substrate-borne signals associated with intraspecific communication of GWSS. Sound and video recordings of male and female GWSS on plants revealed a complex series of behaviors linked to vibrational signals that lead to mating. Data are currently being analyzed to characterize the spectral and temporal features of signals such as frequency span, dominant frequency, intensity, and pulse repetition time.

LAYPERSON SUMMARY
The goal of this research project is to describe and characterize vibrational signals used in glassy-winged sharpshooter (GWSS) intraspecific communication. A substantial amount of research on other leafhopper species has shown that individuals communicate solely by substrate-borne vibrational signals. To our knowledge, vibrational communication in GWSS has not been investigated in detail. Fundamental understanding of these factors is important for achieving our deliverable: a new management strategy to suppress GWSS populations and Pierce's Disease incidence.

INTRODUCTION
Epidemiological models suggest that vector transmission efficiency, vector population density, and the number of plants visited per vector per unit time are key factors affecting rates of pathogen spread (Jeger et al. 1998). Measures to reduce glassy-winged sharpshooter (GWSS) population density in California include an area-wide insecticide application program and release of natural enemies. Despite such efforts, geographic distribution of GWSS continues to expand. Chemical control of GWSS in urban areas, organic farms, and crops under integrated pest management programs is problematic because insecticides are ineffective, not used, or incompatible with existing practices, respectively. Thus, long-term suppression of GWSS populations will rely heavily on novel methods.

In leafhoppers, mate recognition and localization are mediated exclusively via substrate-borne vibrational signals transmitted through the plant. Exploitation of attractive vibrational signals for trapping leafhoppers or disrupting mating, as well as excluding pests via emission of repellent signals have been considered, but not yet implemented in commercial agricultural landscapes (Polajnar et al. 2014). In Florida, an experimental prototype of a microcontroller-buzzer system attracted the Asian citrus psyllid, *Diaphorina citri*, to branches of citrus trees by playback of insect vibrational signals (Mankin et al. 2013). Recently, small-scale field studies on mating disruption of leafhoppers via playback of vibrational signals through grapevines have demonstrated promising results. Specifically, electromagnetic shakers attached to wires used in vineyard trellis successfully disrupted mating of *Scaphoideus titanus*, vector of the grapevine disease Flavescence dorée in Europe (Eriksson et al. 2012). Exploitation of attractive and/or repellent signals for suppressing GWSS populations could prove to be a useful tool. However, existing knowledge on GWSS vibrational communication is insufficient to implement a management program for this pest in California.

OBJECTIVES
1. To identify and describe substrate-borne signals associated with intraspecific communication of GWSS in the context of mating behavior.

RESULTS AND DISCUSSION

This research project is being conducted at the USDA-ARS San Joaquin Valley Agricultural Sciences Center (SJVASC) in Parlier, California. Discovery in the GWSS behavioral analyses has been bioassay-driven and focused on the identification of signals that initiate natural responses to conspecifics. Insects being used in the experiments are obtained from colonies maintained year-round at the SJVASC. Briefly, late-instar GWSS obtained from colonies are separated by gender in cages to generate virgin adult individuals. Only reproductively active females are being used in the recordings.

A series of laboratory studies are being conducted to describe vibrational signals used in GWSS communication. First, virgin males and females are placed on host plants individually to identify common and unique signals produced by each gender. Second, males and females are paired on host plants to identify signals used in advertisement and species recognition, male-female duetting that result [or not] in oriented movement of one individual to another, and courtship. Third, groups of individuals (males and females together and males and females separately) are placed on plants to identify potential rivalry or distress signals. Insects are monitored via video surveillance. Vibrational signals produced by individuals are recorded and measured using a laser vibrometer (NLV-2500, Polytec) and associated softwares (e.g. Raven, Adobe Audition). Recorded signals have been digitized with 44.1 kHz sample rate and 16 bits resolution. Data from vibrometry are currently being analyzed to characterize the spectral and temporal features of signals such as frequency span, dominant frequency, intensity, and pulse repetition time. Data are analyzed using a window size of 512 samples (124 Hz). Recorded signals are used to perform playback experiments conducted with an electrodynamic mini-shaker (Type 4810, Brüel & Kjær) driven by a computer, where individuals are stimulated with selected signals transmitted to host plants. Together with video analysis, the role of specific signals in GWSS intra- and inter-gender communication are being assessed to identify signals capable of influencing GWSS behavior for applicative purposes (e.g., disruption of mating communication, attraction).

Two different types of GWSS male calls are described in this report: MC1 and MC2 (**Table 1**). MC1 has two distinct parts, whereas MC2 has three distinct parts. In MC2, the first part consists of two or more pulses of comparatively high amplitude. The second part is a modulated signal with dominant frequency within 300 Hz and the third part is a train of pulses with variable length and constant amplitude (**Figure 1a**). The amplitude ratio among the three parts is variable, but the first part has always the highest intensity. MC1 is similar to MC2, except for the absence of the first part of the signal (**Table 2**). A female alone on the plant emitted only one type of signal (FC) with high variability in length. FC is a modulated signal characterized by low frequency. Amplitude peaked in the middle of the call (**Figure 1b**). Recordings of a virgin male and female placed together on the plant revealed a complex series of behaviors linked to vibrational signals that lead to mating, or not. Prior to mating, male and female communication ranges between 6 min to hours. In the example described here, a duet was established after the male replied to the female signal (**Figure 2**). After the duet was established, the male began searching for the female on the plant by alternating a walking behavior and short stops to emit additional signals; likely to maintain communication with the female. The female remained on the same the position on the plant during the mate finding process. Mating began a few minutes after the male found the female. The couple remained in copula for more than six hours. After mating, the female was kept individually on a new plant until fertility was confirmed by deposition of fertilized eggs.

CONCLUSIONS

The project is providing a detailed description of GWSS vibrational communication signals that are relevant for management of Pierce's disease. In the context of GWSS reproduction, descriptions are aiding identification of signals that influence mate recognition, finding, choice, and/or acceptance behaviors. These include signals produced by individuals in duets, trios, and quartets. Outside the context of reproduction, competitive or cooperative interactions may arise to facilitate access to feeding sites. These potential interactions may be mediated by signals used to repel or attract (e.g., aggregational signals) conspecifics to feeding sites. Finally, the research project will provide practical recommendations on the exploitation of GWSS vibrational communication as a novel method to suppress GWSS populations under field conditions.

Table 1. Acoustic parameters of GWSS signals. Pulse repetition time (PRT) is the ratio between the length of the phase call and the number of pulses composing the phase call. MC1 is a male call without part 1, MC2 is a male call with part 1, and FC is a female call. Data are expressed as (mean ± st. dev.) when more than one signal was present, as in the duet, or more pulses of the same part of male's call were analyzed.

Signal		Part	Length (s)	Dominant frequency (Hz)	Number of pulses	PRT
Individual male call		1	0.55	86.10 ± 0.00	6	0.09
		2	0.80	172.30		
		3	1.70	119.62 ± 43.24	18	0.09
		total	3.05			
Individual female			2.67	172.30		
Male and female paired on plant (duet)	MC1	2	0.92 ± 0.07	86.10 ± 0.00		
		3	0.74 ± 0.73	91.49 ± 21.55	7.50 ± 6.36	0.09
	MC2	1	0.40	86.10 ± 0.00	2	0.20
		2	0.82	86.1		
		3	1.14	100.46 ± 49.74	12	0.09
	FC		1.73 ± 0.41	86.1 ± 0.0		

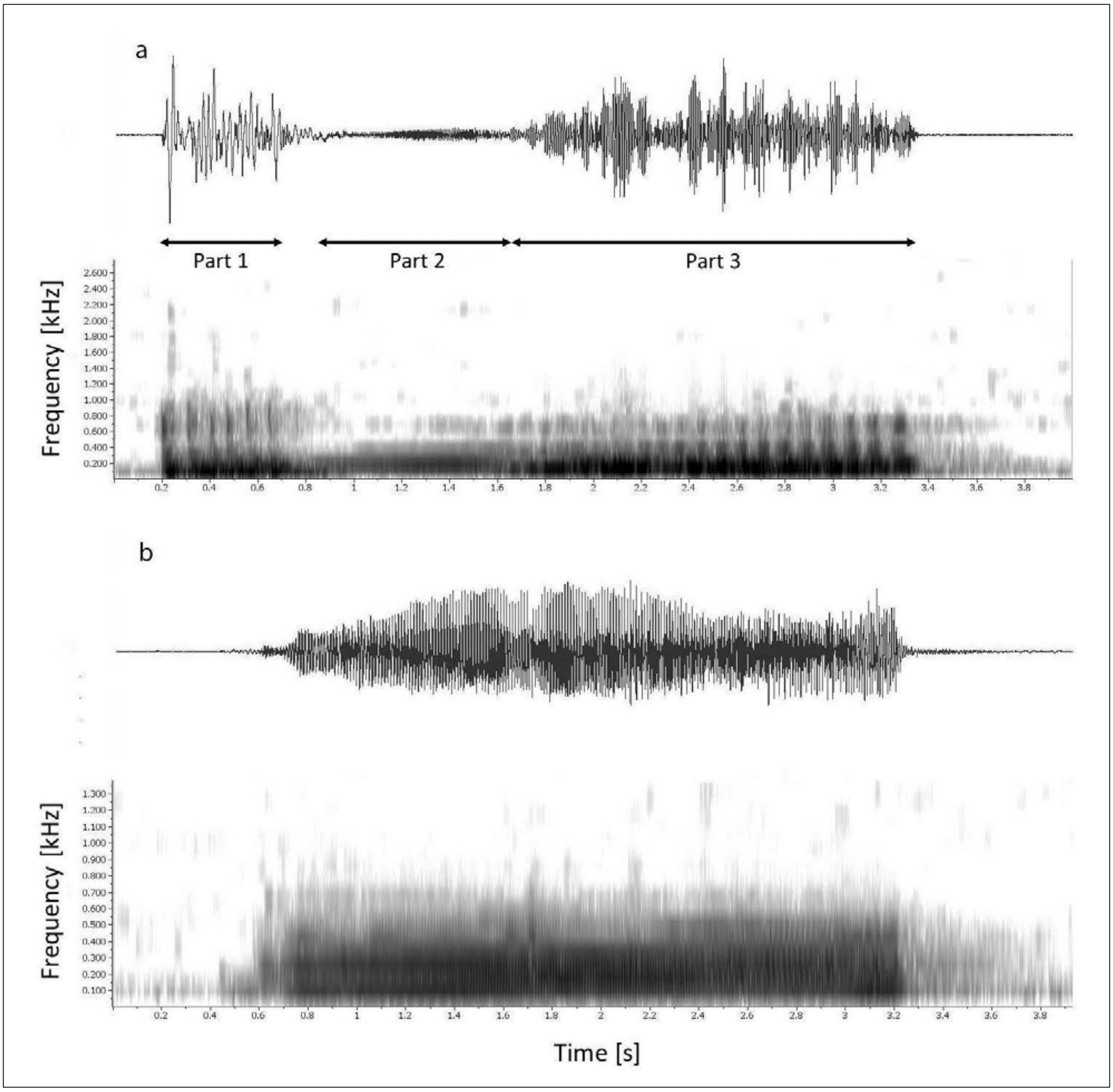

Figure 1. Oscillogram (above) and spectrogram (below) of a male (a) and a female (b) GWSS alone on the plant.

Table 2. Relative amplitude of male and female vibrational signals. Signals were recorded when male and female were together (duet) and alone on the plant. Amplitude is expressed as a relative measure, where the value 1 was assigned to the beginning of the signal. MC1 is a male call without part 1 and MC2 is a male call with part 1.

	Female signal					Male signal			
Position	Alone	With male (duet)			Part	Alone	With female (duet)		
							MC1		MC2
Start	1.00	1.00	1.00	1.00	1	1.00	-	-	1.00
Middle	4.19	4.74	1.27	3.36	2	0.12	1.00	1.00	0.37
End	3.25	4.18	1.41	3.30	3	0.77	8.57	0.88	0.23

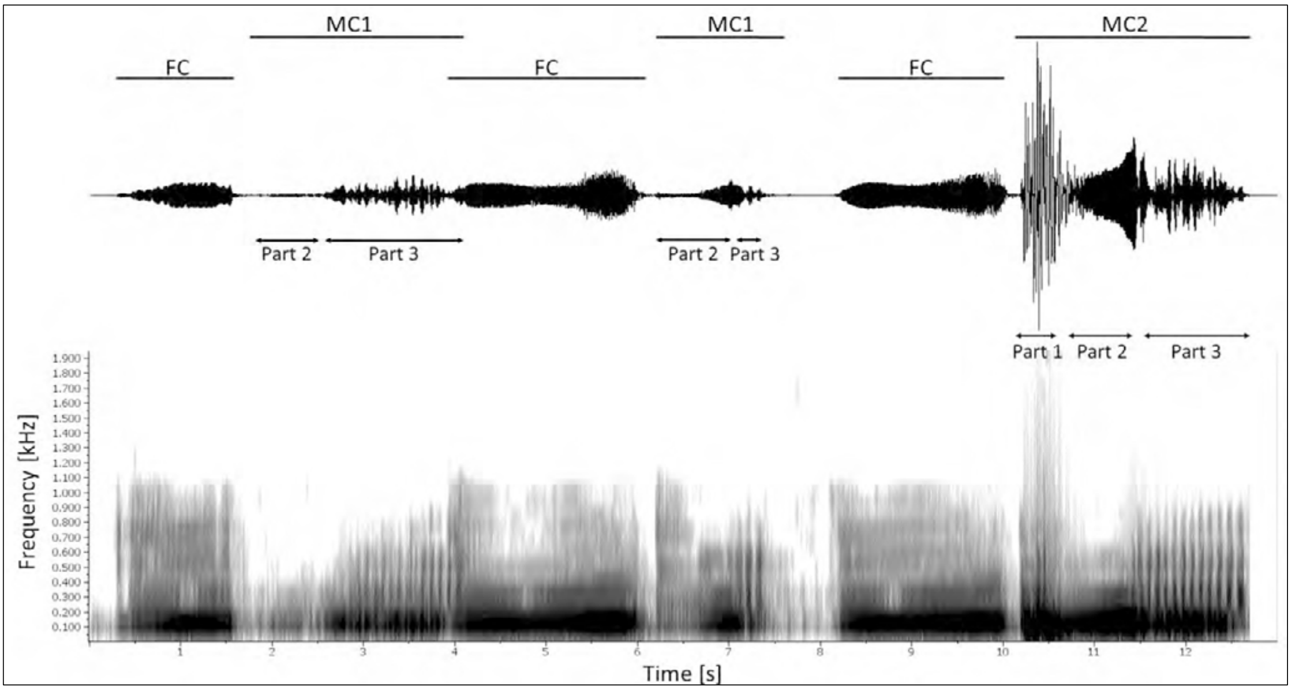

Figure 2. Duet of a male and female GWSS. Female call (FC) followed by a two-part male call (MC1). The third female call at about 8 seconds into the duet was followed by a three-part male call (MC2). Male and female mated shortly thereafter.

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