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SUBSTRATE-BORNE VIBRATIONAL SIGNALS IN INTRASPECIFIC COMMUNICATION OF GWSS

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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) (*Homalodisca vitripennis*) 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. The near-zero tolerance for GWSS in vineyards, particularly in areas where Pierce's disease is endemic, poses a constant challenge for growers and agencies involved in the area-wide program. Thus, long-term suppression of GWSS populations will rely heavily on novel methods.

Vibrational communication is a widespread form of communication in invertebrate and vertebrate animals including fish, amphibians, reptiles, birds, and mammals (Cocroft et al. 2014). Arthropods emit vibratory signals in connection with aggression, distress, calling, courtship, rivalry, searching, and other behaviors associated with finding conspecifics and avoiding predation (Čokl and Virant-Doberlet 2003). In leafhoppers, mate recognition and localization are mediated exclusively via substrate-borne vibrational signals transmitted through the plant. Vibrational signals in leafhoppers are low-frequency bending (or flexural) waves produced by the abdominal tergal plate (aka. Tymbal). Signals are transmitted through the legs to the substrate and travel at a speed of about 100 m/s. Signals are detected by the receiver presumably by subgenual and joint chordotonal organs located in the legs (Čokl and Virant-Doberlet 2003).

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 a phytoplasma that causes the grapevine disease Flavescence dorée in Europe (Eriksson et al. 2012). Exploitation of disruptive, 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.

Objective

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

Description of activities conducted to accomplish the objective

Test insects and plants. This research project is being conducted at the USDA-ARS SJVASC in Parlier, California. Discovery in the GWSS behavioral analyses are bioassay-driven and focus on the identification of signals that initiate natural responses to conspecifics. Test plants used in the bioassays reported here were okra (*Abelmoschus esculentus* (L.) Moench). All plants were kept in an insect-free greenhouse located at the SJVASC until used in the experiments. Insects used in the experiments were obtained from colonies maintained year-round in a containment facility located at the SJVASC. Colonies were established using GWSS originated from Bakersfield, CA and maintained in cages (Bug Dorm-2®, BioQuip Products, Rancho Dominguez, CA) using four plant species: cowpea (*Vigna unguiculata* L. Walp. cv. 'Blackeye', Vermont Bean Seed Co., Randolph, WI, USA), basil (*Ocimum basilicum* L. cv. 'Genovese' Ferry-Morse Seed Co., Fulton, KY, USA), okra, and sunflower (*Helianthus annuus* L. cv. 'American Giant Hybrid', Ferry-Morse Seed Co., Fulton, KY, USA). Late-instar (4th and 5th) GWSS nymphs obtained from colonies were separated by gender in cages to generate virgin adult individuals. After molting to the adult stage, a female was transferred individually to a mesh-screen tube cage (10 cm diameter × 40 cm height) containing a cowpea plant. Reproductive maturity in about 150 individually caged females was determined by oviposition. Virginity of females was confirmed by checking eggs daily for signs of embryo development or nymph emergence. Male insects used in the experiments described below were of the same age as reproductively active females. After female reproductive maturity and virginity was confirmed, test insects were used in the vibrometry experiments described below.

The vibrometer. Experiments were conducted in a transparent experimental arena (60-cm length × 60-cm width × 80-cm height) made of 1-cm thick acrylic walls. The arena was centered inside a chamber formed by 86-cm × 86-cm × 98-cm high black fabric walls. The arena and chamber were placed on an active vibration isolation table (Model 20-561, Technical Manufacturing Corporation, Peabody, MA) to reduce external noise. Four fluorescent lights (Sylvania, Octron®, 4100K, 17W, Danvers, MA) were affixed to the top of each wall of the chamber. Insect behaviors were monitored via video surveillance and recorded to a computer. Vibrational signals produced by individuals were recorded, digitized, and measured using a laser Doppler vibrometer (NLV-2500, Polytec, Inc., Irvine, CA) and associated softwares (Raven Pro 1.5, The Cornell Lab of Ornithology, Ithaca, NY and Adobe Audition® C26, Adobe Systems, Inc., San Jose, CA). Recorded signals were digitized with 44.1 kHz sample rate and 32 bits resolution and analyzed using FFT type Hann with a window size of 560 samples and overlap of 80% to characterize and determine key spectral and temporal features of signals such as frequency span, dominant frequency, intensity, and pulse repetition rate. Recorded signals were used in some preliminary studies to perform playback experiments conducted with an electrodynamic mini-shaker (Type 4810, Brüel & Kjær, Inc., Norcross, GA) driven by a computer, where individuals were stimulated to produce natural behavioral responses to selected signals transmitted to host plants.

Bioassay methodology. Bioassays were conducted between 0800 and 1900 h at 25 ± 0.5°C. Before testing, insects were allowed 15 min to acclimatize to ambient conditions in 130-ml plastic vials placed within the chamber housing the plant. After the acclimatization period, insects introduced into the acrylic arena had free movement to and from a host plant. A series of laboratory studies were conducted to identify and describe GWSS vibrational signals. First, virgin males (n = 21) and females (n = 26) were placed on host plants individually to identify common and unique signals produced by each gender. Second, males and females were paired (n = 32) 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 (two males and one female) (n = 30) were placed on plants to identify potential rivalry or distress signals. Trials consisted of 90-min observations. In cases where mating occurred during the trials, the couple was immediately transferred to a tube cage containing a cowpea plant and kept until copulation ended. After copulation, the male was removed and conserved in alcohol and the female was kept individually on a plant until fertility was confirmed by deposition of fertilized eggs. All four conditions described above (insects alone and in groups) were tested on the same day. The order in which the four conditions were tested was randomized within each day.

Data analysis. Data from vibrometry are currently being analyzed to characterize the spectral and temporal features of signals. After the data set is complete, statistical analysis will be performed to test for differences in frequency span, dominant frequency, intensity, and pulse repetition time of signals produced by individuals tested under different conditions.

Summary of accomplishments and results for each objective

A female alone on the plant emitted only one type of signal (FC, Figure 1) with high variability in length (Table 1). FC was a broadband signal with increasing dominant frequency (Table 1) and a peak in relative amplitude in the middle of the call (Figure 1). Females produced signals by contracting abdominal muscles. When a male was alone on the plant, a male call (MC1) (see figure 3) was sporadically emitted. MC1 was composed of two sections (S2 and S3). S2 was a narrow band frequency signal with increasing frequency (Table

2), whereas S3 was a broadband train of pulses with a low pulse repetition time (Table 2). Among all the trials, in only one case a male alone on the plant emitted a different call (MC2) (Figure 2). MC2 consisted of a drumming signal (S1) followed by S2 and S3. The S1 drumming signal of males was produced during a rapid dorso-ventral movement of the abdomen, whereas S2 and S3 were produced by contraction of abdominal muscles only. The two male calls (MC1 and MC2) were similar, except that S1 was present in MC2 and absent in MC1. In trials with an insect placed individually on the plant, calling activity of females was significantly (G test: G = 15, 61, $P < 0.001$) higher than males. Specifically, females and males tested alone on the plant emitted at least one call in 80.8% and 23.85% of the trials, respectively.

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 ranged between 6 min to hours. Females and males can simultaneously feed on the plant and produce signals, which suggest that stylets inserted in the plant may serve as an additional signal conductor. Male-female duets (Figure 3) were established after an active individual of the opposite gender replied to first caller. After the duet was established, a drumming signal was added to the repertoire of the male (Figure 4). The drumming was a low frequency signal with variable duration. The mechanism of drumming signal production is still unknown. During male-female duets, males used both signals MC1 and MC2. In all cases analyzed, females remained on the same position on the plant during the mate finding process and courtship. However, it is not known whether GWSS female vibrational signals alone provided directionality to searching males or if there is a random component in mate search by GWSS males. Males searched 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. During the final stages of courtship, several short signals (not reported here) have been observed and are being characterized. For example, females produced short “clicking” signals using only the posterior part of the abdomen. Males approached the females from the sides to attempt mating. In many cases, females were not receptive for mating despite long duets with males. To avoid copulation, females lifted the posterior part of the body and stretched the hind legs to keep males away. When mating was successful, the couple remained in copula for more than 6 hours. Females were able to feed during copula. All mating pairs produced viable progeny.

GWSS male-male rivalry signals were recorded during establishment of dominance and subordination between males competing for mates. Different rivalry signals are being characterized. See one example in Figure 5 and table 3). Preliminary data show that visual signals, physical contact, and specific vibrational signals may be used by GWSS to establish male hierarchy and thus, preferential access to mates. In relatively more aggressive situations, a male bended the body by lowering the posterior part of the abdomen forming an arc. However, GWSS females can also be “choosy”, which suggests that both intrasexual (male-male combat) and intersexual (mate choice) may occur in GWSS. While GWSS male rivalry appears to negatively impact courtship behaviors, it is not known if the overall reproductive success of a male can be artificially affected by playbacks.

In conclusion, the project is providing a detailed description of vibrational communication signals that are key for understanding behaviors of GWSS fundamental for mating success. Within the next months, we expect to finalize a “library” of GWSS signals including the identification and characterization of signals produced by individuals under different conditions (insects alone and in groups on the plant). Our work has shown that 1) GWSS uses substrate-borne vibrational signals in intra- and inter-gender communication and 2) specific signals are required for GWSS to achieve mating. Although the role of some signals reported here could be inferred from observations, the role and relevance of individual signals to insect behaviors can be ultimately determined only when insects are stimulated via playback of select signals. Pre-recorded GWSS signals were used to perform preliminary playback experiments, where males and females were artificially stimulated to produce natural responses to signals transmitted to host plants. In these preliminary trials, we were able to establish communication with GWSS males and females using pre-recorded female and male calls, respectively. The ability to establish a communication channel and elicit GWSS response to select signals represents an important step towards our next goal, which is to determine the role of specific signals in GWSS communication and identify signals capable of influencing GWSS behavior for applicative purposes (e.g., disruption of mating communication, attraction).

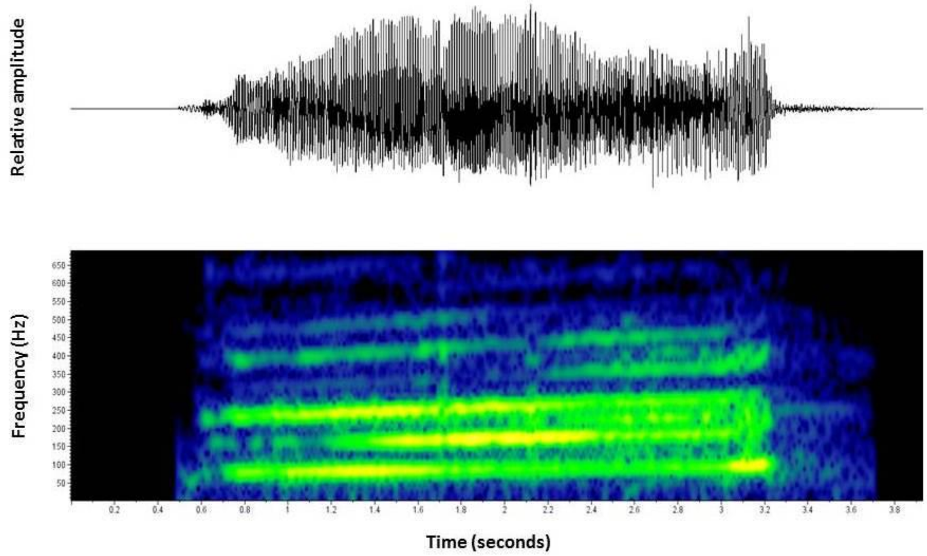

Figure 1. Oscillogram (above) and spectrogram (below) of a GWSS female call (FC).

Table 1. Analysis of spectral and temporal parameters of GWSS female calls. n = number of individuals analyzed, N = number of signals analyzed per individual, Df = dominant frequency, Df2-Df1 = difference between the Df at the end of the signal (Df2) and at the beginning (Df1). Data are expressed as (mean ± st. dev.).

N/n	5/10
Length (sec)	2.00 ± 0.55
Df (Hz)	93.07 ± 17.15
Df2-Df1 (Hz)	16.25 ± 25.34

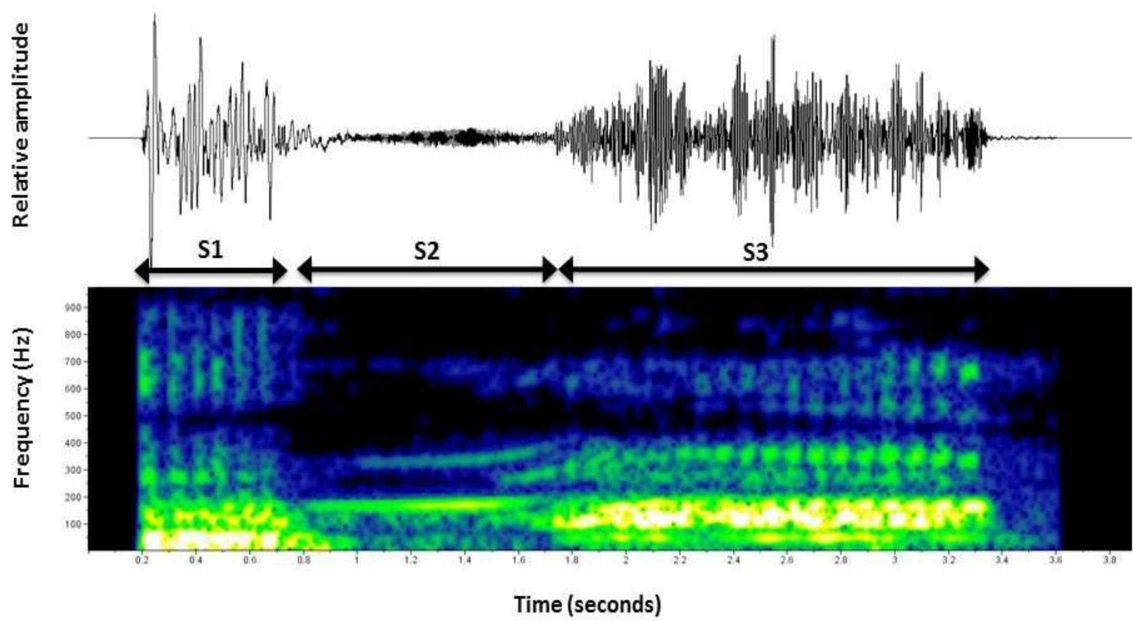

Figure 2. Oscillogram (above) and spectrogram (below) of a GWSS male call (MC2). Three different sections of MC2 are indicated by S1, S2, and S3.

Table 2. Analysis of spectral and temporal parameters of GWSS male calls (MC1 and MC2). n = number of individuals analyzed, N = number of signals analyzed per individual, Df = dominant frequency, Df2-Df1 = difference between the Df at the end of the signal (Df2) and at the beginning (Df1), and PRT = pulse repetition time. Data are expressed as (mean ± st. dev.).

Section	S1	S2	S3
N/n	1/1	1/5	1/5
Length (s)	0.55	0.74 ± 0.14	1.01 ± 0.43
Df (Hz)	86.1	105.5 ±33.15	75.00 ± 26.97
Df2-Df1 (Hz)		23.48 ±11.73	
Number of pulses	6		13.33 ± 5.86
PRT	0.09		0.08 ± 0.01

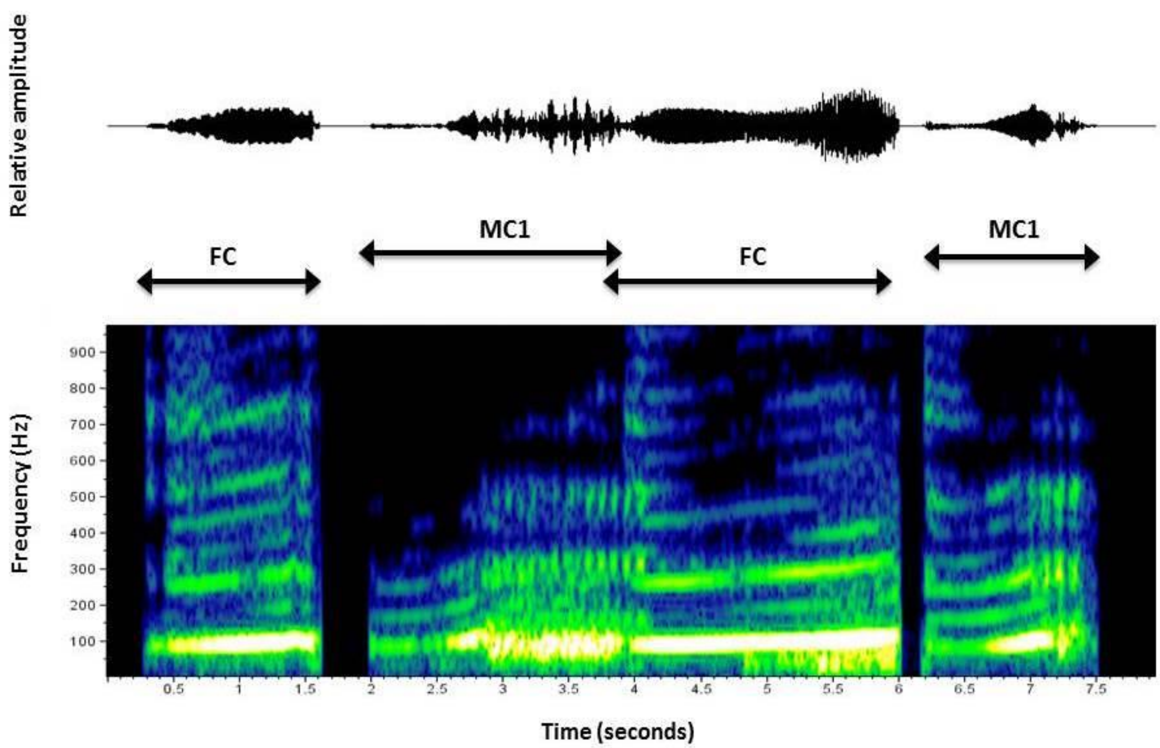

Figure 3. Oscillogram (above) and spectrogram (below) of a duet between a male and female GWSS. FC is a female call and MC1 is a male call.

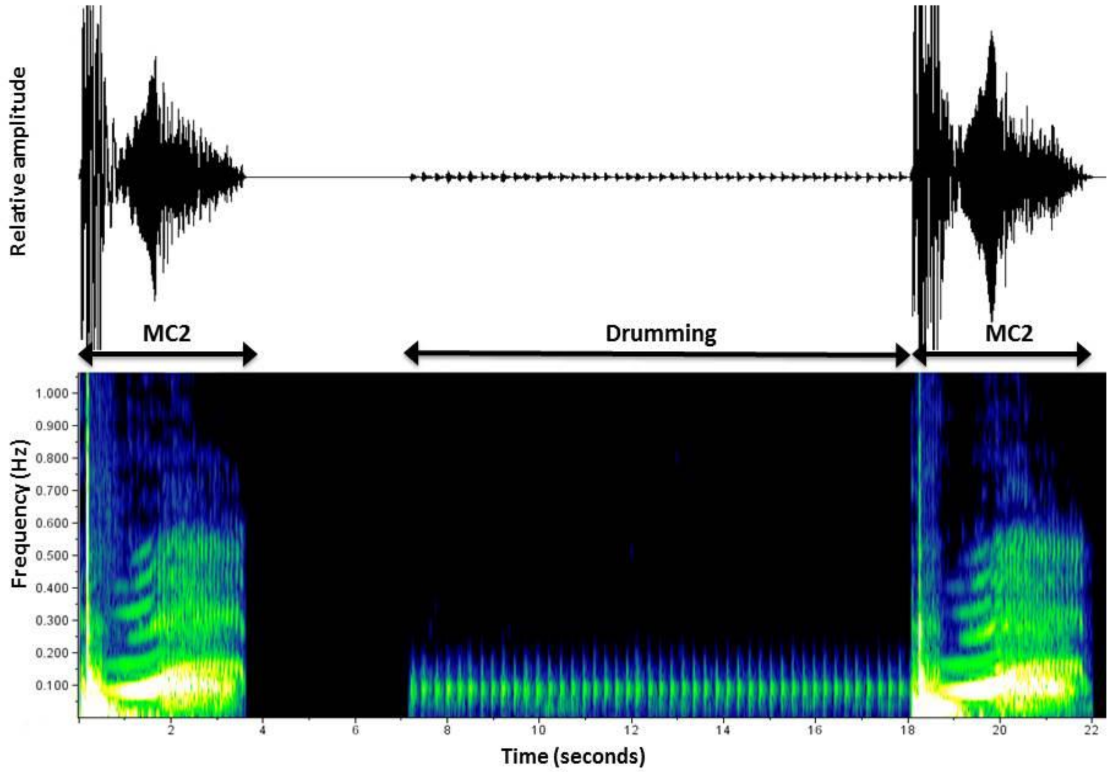

Figure 4. Oscillogram (above) and spectrogram (below) of the male call MC2. The second call was preceded by a drumming signal.

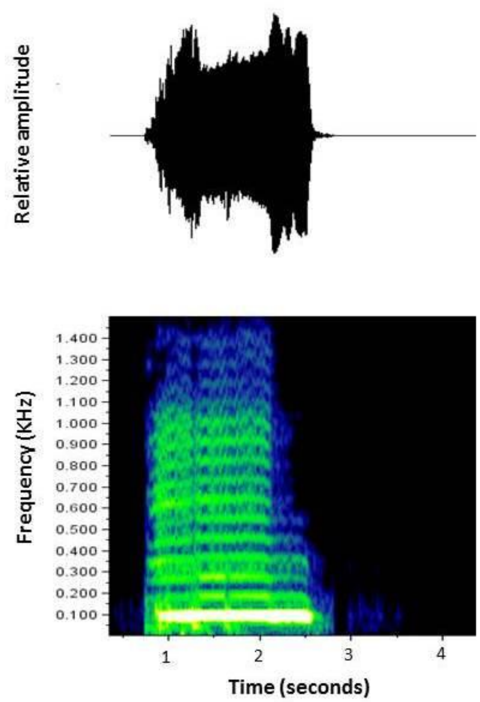

Figure 5. Oscillogram (above) and spectrogram (below) of an aggressive signal of a male GWSS produced during a male-male competition for a female.

Table 3. Analysis of spectral and temporal parameters of a GWSS aggressive signal. n = number of individuals analyzed, N = number of signals analyzed per individual, and Df = dominant frequency. Data are expressed as (mean ± st. dev.).

N/n	5/5
Length (s)	2.18 ± 0.58
Df (Hz)	119.45 ± 55.45

Publications produced and pending, and presentations made that relate to the funded project

Krugner, R. 2014. Substrate-borne Vibrational Signals in Intraspecific Communication of the Glassy-winged Sharpshooter. Oral presentation at the 2014 CDFA Pierce's disease Research Symposium. December 15-17, 2014, Sacramento, California.

Krugner R., Mazzoni V., and Nieri R. 2014. Substrate-borne vibrational signals in intraspecific communication of GWSS. pp. 2-5. In T. Esser and R. Randhawa (eds.), Proceedings, 2014 Pierce's Disease Research Symposium. California Department of Food and Agriculture, 15-17 December, Sacramento, CA. Time Printing, Sacramento, CA.

Nieri, R., Mazzoni, V., and Krugner, R. 2015. Substrate-borne signals involved in the mating behavior of the glassy-winged sharpshooter (GWSS). Poster presentation at the 2015 Entomology Congress of the German Society for General and Applied Entomology. March 2-5, 2015, Frankfurt, Germany.

Research relevance statement, indicating how this research will contribute towards finding solutions to Pierce's disease in California

This project encompasses three compounding phases build on research findings of previous phases: 1) Exploratory Phase – identify and describe the substrate-borne signals associated with intraspecific communication of GWSS; 2) Developmental Phase - determine which signals are effective to attract, repel, and/or disrupt mating of GWSS; and 3) Application Phase - technology transfer for implementation of a sustainable management strategy for GWSS. Our work has shown that 1) GWSS uses substrate-borne vibrational signals in intra- and inter-gender communication and 2) specific signals are required for GWSS to achieve mating. Pre-recorded GWSS signals were used to perform preliminary playback experiments, where males and females were artificially stimulated to produce natural responses to signals transmitted to host plants. In these preliminary trials, we were able to establish communication with GWSS males and females using pre-recorded female and male calls, respectively. The ability to establish a communication channel and elicit GWSS response to select signals represents an important step towards our next goal, which is to determine the role of specific signals in GWSS communication and identify signals capable of influencing GWSS behavior for applicative purposes (e.g., disruption of mating communication, attraction).

Layperson summary of project accomplishments

Vibrational and chemical communications are widespread forms of communication in insects. In leafhoppers, mate recognition and localization are mediated exclusively via vibrational signals transmitted through the plant. Vibrational signals in leafhoppers are low frequency bending waves produced by membranes located in the abdomen. These membranes vibrate rapidly, producing vibrations that are transmitted to the substrate. Exploitation of vibrational 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. The goal of this research project is to describe and characterize vibrational signals used in GWSS communication. Vibrational signals associated with four different stages of GWSS mating behavior were tentatively identified: 1) Advertisement - a reproductively active individual, male or female, spontaneously emitted calls specific of its gender; 2) Species recognition - if another active individual of opposite sex was present on the plant, it replied to the call and a duet was established; 3) Mate location and courtship - the male located the female by walking on the plant, a drumming behavior was added to repertoire and maintained until 4) copula occurred. Vibrational signals produced during the four stages of GWSS mating were recorded and are currently being analyzed. All signals had the dominant frequency under 200 Hz. When two males called on the plant and the female replied, males produced an aggressive signal as part of a competitive behavior that interrupted the duet with the female. Knowledge acquired during this work may contribute to the development of a species-specific mating disruption approach.

Status of funds

Funds were successfully transferred to Fondazione Edmund Mach to support Dr. Mazzoni's visit to the laboratory in Parlier. Funds for salary and benefits to support a post-doctoral researcher are still available.

Summary and status of intellectual property associated with the project

None

Literature cited

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Polajnar J., Eriksson A., Lucchi A., Anfora G., Virant-Doberlet M., and Mazzoni V. 2014. Manipulating behaviour with substrate-borne vibrations ‒ potential for insect pest control. Pest Manag. Sci.. doi: 10.1002/ps.3848.