Development of Vibrational Control Methods for Grapevine Pests in California

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ABSTRACT

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is an important vector of the bacterium *Xylella fastidiosa*, the causal agent of Pierce's disease of grapevine. GWSS communicate by exchanging mating calls that are transmitted through host plants as vibrational signals. Interference with GWSS communication by playback of disruptive signals should lead to reduced population growth, but existing knowledge on mating behavior was insufficient to develop a vibrational control method for this pest. A collaborative research between the United States Department of Agriculture in Parlier, California, and Fondazione Edmund Mach, Italy, led to the description of GWSS mating communication, identification of several candidate disruptive signals for playback interference, and evaluation of the efficacy of a novel vibrational signal playback method in disrupting GWSS mating under field conditions. Results showed that playback of vibrational signals through vineyard trellis significantly reduced mating of GWSS on grapevines compared to control. Although further studies are needed prior to method implementation, data from these studies continue to support application of vibrational mating disruption as a novel method to control GWSS populations.

Keywords: *Xylella fastidiosa*, Pierce's disease, biotremology, mating disruption, vibrational communication

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is a xylem fluid-feeder that transmits *Xylella fastidiosa* (Wells et al. 1983), a xylem-limited bacterium that causes Pierce's disease of grapevines [3]. Grapes are one of the most economically important crops in California ($5.5 billion/year) with over 340,000 ha of vineyards distributed throughout the state [16]. Pierce's disease was first detected in California in 1884 in southern California and the first records of the disease in the San Joaquin Valley occurred in 1917 [7]. Disease management practices have included removal of infected grapevines and control of insect vectors.

A key component in Pierce's disease management has been an area-wide insecticide application program to suppress GWSS populations in vineyards, citrus orchards, and urban areas [1, 8, 13, 17]. The systemic neonicotinoid imidacloprid has been the primary insecticide used to control GWSS populations, but despite constant surveys and insecticide applications re-infestations by GWSS are commonly reported [2]. In addition, GWSS resistance to imidacloprid has been reported from populations under aggressive insecticide treatments [15]. Moreover, insecticide applications to suppress GWSS populations in citrus orchards have the potential to eliminate the GWSS parasitoids [10] and to disrupt biological control of citrus pests such as the cottony cushion scale, *Icerya purchasi* (Williston) (Hemiptera: Margarodidae) [6]. Therefore, novel methods to control GWSS are needed.

On grapevines in California, GWSS reproduce from spring to fall, producing at least two generations per year. Previous research identified vibrational signal playback as a novel pest management strategy for *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) by transmitting a synthetic *S. titanus* male "disturbance noise" through wires of vineyard trellis [4, 14]. In field trials, mating in virgin male-female pairs was suppressed by about 90%. These promising results opened the floodgates for studying the feasibility of this method to disrupt mating of other pests, including the GWSS and other pests of grapevines. For GWSS, the approach encompassed three main objectives: 1) to identify and describe the substrate-borne signals associated with GWSS communication, 2) design and test candidate disruptive signals in laboratory, and 3) evaluate efficacy of a disruptive signal under field conditions.

Identification and description of the substrate-borne signals associated with GWSS communication

Insects used in the experiments described below were obtained from colonies maintained year-round at the USDA-ARS in Parlier, California. Briefly, late-instar

GWSS obtained from colonies were separated by gender in cages to generate virgin adult individuals. A series of laboratory studies were conducted to describe vibrational signals used in GWSS communication. First, virgin males and females were placed on host plants individually to identify common and unique signals produced by each gender. Second, a male and female was paired on a host plant to identify signals used in advertisement and species recognition, male-female duetting that results in oriented movement of one individual to another, and courtship. Third, groups of individuals (males and females together and males and females separately) were placed on plants to identify potential rivalry or distress signals. Insects were monitored via video surveillance and vibrational signals produced by individuals were recorded and measured using laser Doppler vibrometry [12].

Analyses of spectral and temporal characteristics of vibrational signals in parallel with behavioral observations showed that GWSS mating communication involved the emission of three male and two female signals, with specific roles in two distinct phases of mating behavior, identification and courtship. Mating success depended on vibrational duets between genders, which were temporarily interrupted in the presence of male rivalry. Male rivalry behavior involved the emission of three distinct rivalry signals. Two rivalry signals resemble female signals and were associated with replacement of the female in the duet by the rival male. The third rivalry signal was emitted by competing males. Data suggested that rival males used mimicry and hostile signals to interrupt the ongoing duet and gain access to a female. Knowledge acquired from this study was essential to initiate research reported below.

Design and test candidate disruptive signals in laboratory.

Once GWSS mating communication and associated vibrational signals were described, male and female behaviors were targeted separately to identify candidate disruptive signals for each gender. First, because GWSS males search for a stationary female after the onset of signaling by the female, different disruptive signals specifically aimed at interfering with the male search behavior were tested. The frequency pattern of a GWSS female signal was modified to create different versions of the female signal. Signal modification was conducted using the FTT filter function of Adobe Audition to reduce intensity of signal components and remove signal components. Modified signals were transmitted into the plant to 1) identify intrinsic spectral features of female signal that elicit male signaling response, 2) to design disruptive signals that alter male perception and acceptance of a female signal, and (3) to determine the minimum threshold of signal intensity required for an effective application of disruptive signals. Results showed that male responses to playback of modified female signals were significantly reduced by 60-75% when part of the female signal spectral components above or below 400 Hz were deleted. Playback of vibrations to plants showed that transmission of an 80 Hz pure frequency tone completely suppressed male signaling to female signal playback, even if the disruptive signal amplitude was lower than the female signal playback [11].

On the second study, playback of white noise, pre-recorded female signals, and artificial female noise (continuously overlapping female signals) to GWSS male-female pairs were evaluated to determine the efficacy of candidate signals in disrupting mating of GWSS under laboratory conditions. White noise was artificially generated spanning the main frequency range of GWSS mating signal (1 to 1000 Hz) to overpower any natural signal on the plant. Natural female signals were created from a previously recorded female and included a 34-min loop of a natural female signaling pattern; the loop included 120 signals with no repeats and natural spacing of 3 to 61 sec and delivered to plants as an attempt to suppress female signaling activity or confuse males. Female noise was composed of two different female signals overlapped (50% overlap in time) creating a continuous noise (similar to white noise), but with greater signal intensity in the relevant signaling frequencies. Playback of white noise, pre-recorded female signals, and artificial female noise (continuously overlapping female signals) significantly reduced mating of GWSS when compared to silent control mating trials [5].

Evaluate efficacy of a disruptive signal under field conditions

Once candidate signals were identified, designed, and tested under laboratory conditions, the next objective was to evaluate the efficacy of playback of vibrational communication signals for disrupting mating of GWSS in a natural vineyard setting and evaluate spectral properties of signal transmission through vineyard trellis. A modified version of GWSS female signals was used in this experiment. The female signals were transmitted to wires used in the vineyard trellis by a custom-made electronic playback system consisting of a control unit and tuned emitters (CBC

(Europe) Srl - Nova Milanese, Italy) specifically designed for research studies in vibrational mating disruption. At the vineyard, a virgin male and a female were paired and assigned to one of two treatments: silence (control) or disruptive signal (female signal). The pair was kept together on the vine between 0800 and 1630 h and visually inspected every 2 hr to determine whether they mated or not. About 13 male-female pairs were tested per day in each treatment (silence and disruptive playback) using one pair per vine. Trials were repeated in 10 different days totaling 134 male-female pairs per treatment. Results showed that playback reduced mating of GWSS on grapevines (9). A total of 28 (out of 134) male-female pairs mated in the control treatment (silence) and only one (out of 134) pair mated when treated with the vibrational signal playback. Playback of vibrational signals through vineyard trellis was affected by distance from signal source, with frequency composition being the highest at the signal source and lowest on vines positioned away from the source. Frequency composition in canes housing test insects decreased exponentially as distance from the source increased, whereas the relative amplitude of analyzed frequencies decreased linearly.

SUMMARY AND CONCLUSION.

Results showed that GWSS mating communication is vulnerable to playback of vibrational signals, suggesting that mating disruption methods under field conditions may be implemented upon identification of pest-specific disruptive signals that result in little to no non-target effects. In this study, GWSS mating communication and behaviors were described, candidate signals for vibrational disruption were identified, and efficacy of signals in disrupting mating of GWSS was evaluated in laboratory and field conditions. As for delivery of desirable signal characteristics to areas with variable size, vineyard trellis systems, and horticultural practices, the energy requirements for signal transmission has to be assessed. Access to and cost of electricity likely will be determinants when evaluating the efficiency of vibrational disruption compared to other control methods. With that, a reduced-energy signal (described under Objective 2 above) suppressed signaling activity of GWSS males in laboratory, but efficacy of the signal in disrupting mating has not been tested under field conditions. If proven successful, such signal would substantially reduce the energy required to implement playback in the field. While white noise and female noise treatments interfered with GWSS communication and suppressed mating, there is a great likelihood for non-target effects of broadband substrate vibrations on natural enemies that use this frequency range to communicate. Therefore, further research on design of disruptive signals to interfere with pest communication should focus on natural signals or more narrow frequency bands. In conclusion, this research demonstrated that synthetic playback of frequency-specific vibrations through vineyard trellis at intensities above and below the natural signaling of females disrupted mating of GWSS, which ultimately should suppress population growth in vineyards. Although further studies are needed prior to method implementation, data from this study continue to support integration of vibrational mating disruption with current methods to suppress GWSS populations and support expansion to include other grapevine pests that use vibrational communication.

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LITERATURE CITED

1. CDFA, 2006. County agricultural commissioner's data, calendar year 2005. California Department of Food and Agriculture, Sacramento, CA.
2. CDFA, 2016. California Department of Agriculture, Pierce's disease control program, Annual report to the legislature. Available from: www.cdfa.ca.gov.
3. Davis, M. J., Purcell, A. H., and Thompson, S. V. 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199:75-77.
4. Eriksson, A., Anfora, G., Lucchi, A., Lanzo, F., Virant-Doberlet, M., and Mazzoni, V. 2012. Exploitation of insect vibrational signals reveals a new method of pest management. PLoS ONE 7:e32954.

5. Gordon, S. D., Sandoval, N., Mazzoni, V., and Krugner, R. 2017. Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis*. Entomol. Exp. Appl. 164:27-34.
6. Grafton-Cardwell, E. E., and Gu, P. 2003. Conserving vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), in citrus: A continuing challenge as new insecticides gain registration. J. Econ. Entomol. 96:1388-1398.
7. Hewitt, W.B., Frazier, N. W., Freitag, J. H., and Winkler, A. J. 1949. Pierce's disease investigations. Hilgardia 19:207-264.
8. Hix, R. L., Toscano, N., and Gispert, C. 2003. Area-wide management of the glassy-winged sharpshooter in the Temecula and Coachella Valleys. In: Tariq MA, Oswalt S, Blincoe P, Spencer R, Houser L, Ba A, Esser T (eds). Proceedings, 2003 Pierce's Disease Research Symposium. California Department of Food and Agriculture, 8-11 December 2003, Coronado, CA, Copeland Printing, Sacramento, CA, pp 292-294.
9. Krugner, R., and Gordon, S. D. 2018. Mating disruption of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) by playback of vibrational signals in vineyard trellis. Pest Manag. Sci. DOI: 10.1002/ps.4930.
10. Lauziere, I., and Elzen, G. 2007. Effect of formulated insecticides on *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) and its parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). J. Entomol. Sci. 42:11-19.
11. Mazzoni, V., Gordon, S. D., Nieri, R., Krugner, R. 2017. Design of a candidate vibrational signal for mating disruption against the glassy-winged sharpshooter, *Homalodisca vitripennis*. Pest. Manag. Sci. 73:2328-2333.
12. Nieri, R., Mazzoni, V., Gordon, S. D., and Krugner, R. 2017. Mating behavior and vibrational mimicry in the glassy-winged sharpshooter, *Homalodisca vitripennis*. J. Pest. Sci. 90:887-899.
13. Park, Y. L., Perring, T. M., Farrar, C. A., and Gispert, C. 2006. Spatial and temporal distribution of two sympatric *Homalodisca* spp. (Hemiptera: Cicadellidae): Implications for area wide pest management. Agric. Ecosyst. Environ. 113:168-174.
14. Polajnar, J., Eriksson, A., Virant-Doberlet, M., and Mazzoni, V. 2016. Mating disruption of a grapevine pest using mechanical vibrations: From laboratory to the field. J. Pest. Sci. 89:909-92.

15. Redak, R., White, B., and Byrne, F. 2016. Management of insecticide resistance in glassy-winged sharpshooter populations using toxicological, biochemical, and genomic tools. In: Esser, T (ed). Proceedings of the 2016 Pierce's Disease Research Symposium. Time Printing, Sacramento, CA, pp 230-236.
16. USDA-NASS, 2017. California Grape Acreage Report. United States Department of Agriculture, National Agricultural Statistics Service, Pacific Regional Field Office. Available from: www.nass.usda.gov.
17. Wendel, L., Ciomperlik, M., Bartels, D., Luvisi, D., and Elms, D. 2002. The area-wide pest management of glassy-winged sharpshooter in Kern County. In: Tariq MA, Oswalt S, Blincoe P, Esser T (eds). Proceedings, 2002 Pierce's Disease Research Symposium. California Department of Food and Agriculture, 15-18 December 2002, Coronado, CA. Copeland Printing, Sacramento, CA, pp 302-307.