

DIAPHORINA CITRI (HEMIPTERA: LIVIIDAE) RESPONSES TO MICROCONTROLLER-BUZZER COMMUNICATION SIGNALS OF POTENTIAL USE IN VIBRATION TRAPS

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ABSTRACT

Monitoring of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) populations is an important component of efforts to reduce damage caused by Huanglongbing, a devastating disease it vectors in citrus groves. Currently, *D. citri* is monitored primarily by unbaited sticky traps or visual inspection of trees. A potentially more effective method might result from attracting males to vibrational communications produced by females. Males call with wing-buzzing substrate-borne vibrations while searching for females on tree branches and stems. When nearby receptive females detect the calls, they reply immediately in synchronized duets that help direct the males towards them. The spectral and temporal patterns of the duets have been analyzed in previous studies and have been mimicked successfully with computer-operated vibration exciters. Males and females both respond to signals produced by either sex but display different behaviors during duets. To devise practical methods to attract and trap males with vibrational signals in field environments, a microcontroller platform was tested for capability to control inexpensive vibration sensing and output devices. The microcontroller was programmed to send mimics of different *D. citri* signals to a piezo buzzer for substrate-borne broadcast. A mimic that elicited strong female responses was tested in bioassays that jointly compared it with other previously bioassayed signals, and the response to the mimic was found to be statistically comparable to that elicited by a recorded male call. The successful result suggests there is opportunity to develop microcontroller systems further as a means of trapping psyllids.

Key Words: Asian citrus psyllid, behavior, acoustic, detection

RESUMEN

El monitoreo de poblaciones de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) es un componente importante de los esfuerzos para reducir el daño causado por la enfermedad Huanglongbing, una dolencia devastadora transmitida por el psílido en plantaciones de cítricos. Actualmente, se monitorea el *D. citri* principalmente por trampas pegajosas sin cebo o la inspección visual de los árboles. Un método potencialmente más eficaz podría ser del resultado de la atracción de los machos a las comunicaciones de vibraciones producidos por las hembras. Los machos zumban sus alas y transmiten llamadas de vibraciones del sustrato durante la búsqueda de las hembras en las ramas y los tallos. Cuando las hembras receptoras que están cercanas detectan las llamadas, ellas responden inmediatamente en dúos sincronizados que ayudan dirigir los machos hacia ellas. Los patrones espectrales y temporales de los dúos han sido analizados en estudios anteriores y se han imitado con éxito con computadores portátiles que operan excitadores de vibraciones mecánicas. Los machos igual que las hembras responden a las señales producidas por uno u otro sexo, pero muestran diferentes comportamientos durante los duetos. Para idear métodos prácticos para atraer y capturar los machos con señales vibratorias en ambientes de campo, una plataforma de microcontrolador fue probada para su capacidad de controlar los aparatos de bajo costo usados para detectar y enviar las vibraciones. La prueba inicial del microcontrolador involucro la programación de las señales enviadas a un zumbador cargado para la difusión de las diferentes señales que simulan *D. citri*. Una señal imitadora que provocó una fuerte respuesta de las hembras fue probada en un bioensayo que comparó conjuntamente con otras señales de

bioensayos previamente sometidos, y se encontró que la respuesta al imitador fue estadísticamente comparable a la provocada por la llamada grabada del macho. El éxito del imitador sugiere que hay una oportunidad para seguir desarrollando sistemas de microcontroladores como una manera para capturar psílicos.

Palabras Clave: psílido asiático de los cítricos, comportamiento, electrónico, zumbador piezo, sensores

The need to develop improved methods to detect and suppress populations of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Sétamou et al. 2008; Wenninger et al. 2009b; Hall et al. 2012; Grafton-Cardwell et al. 2013), an economically important vector of huanglongbing (Bové 2006; USDA National Invasive Species Information Center 2013; Browning 2013), has stimulated studies to understand and co-opt the vibrational communication and mating behaviors of this pest for detection and trapping (Wenninger et al. 2009a; Rohde et al. 2013). Mate-seeking males produce substrate-vibration calls intermittently while exploring along branches of a host citrus tree. When a receptive female replies back in a synchronous duet, the male performs a directed search to locate her, continuing to call occasionally during the searching process and using her replies to help target his search. Males and females produce vibrational signals with similar spectral and temporal characteristics (Wenninger et al. 2009a). Potentially, a trap that attracts male *D. citri* effectively with vibrations might enable more accurate sampling to better determine when populations begin to increase and treatment is needed (e.g., Stansly et al. 2009).

In a recent proof-of-principle study (Rohde et al. 2013), a laptop-controlled vibration exciter produced *D. citri* signal mimics that attracted males searching on small citrus trees in a laboratory environment. Males respond to signals recorded from either female or male conspecifics, particularly those that contain multiple harmonics of about 200 Hz at frequencies between 1000-2000 Hz. Signals derived from female replies, male calls, and computer program outputs have been used successfully to elicit both male attraction and female replies. Preliminary investigations using the laptop-exciter system suggest that males engage in search behaviors more frequently when the mimics are synchronized to follow immediately after a call than when they are presented at random or are absent.

The demonstration that synchronized mimics of duetting signals are attractive to male *D. citri* in a laboratory setting suggests that such methods might lure mate-seeking males to traps in field environments as well. However, much of the technology associated with detection, analysis, and production of insect vibrational communication signals is difficult or costly to employ in the field (Cocroft & Rodriguez 2005; Mankin et al.

2010, 2011). To transfer this technology into effective trapping systems for *D. citri* males in citrus groves, it would be preferable to substitute low-power, low-cost, compact devices for the laboratory instrumentation. Here, we describe tests of the capability of a battery-powered, low-cost microcontroller platform operating an inexpensive piezoelectric buzzer to produce effective mimics of *D. citri* communicatory vibrations.

A major objective in programming the microcontroller output was to produce multiple harmonics of 200 Hz between 1000 and 2000 Hz, as was observed previously in signals that successfully elicited male attraction (Rohde et al. 2013). In addition, the harmonics usually were 5-10% higher near the middle of the signals than at the beginnings and endings, so this signal characteristic also was incorporated into the mimics. To determine if the output had relevance to *D. citri* behavior, the mimics were tested in bioassays that compared proportional responses of females to mimics with responses to previously bioassayed *D. citri* signals, where proportional response was measured as the fraction of signals presented that elicited a female reply.

MATERIALS AND METHODS

Insects and Experimental Arena

Nymphs obtained from a rearing colony maintained at USDA-ARS-CMAVE, originally collected from citrus in fields near Ft. Pierce, Florida (Hall et al. 2007), were placed individually into isolation chambers, each prepared in advance by filling a 21-cm-long, 3.75-cm-diam cone-tainer (model SC10, Steuwe and Sons, Inc., Tangent, Oregon) with potting soil and adding a small citrus seedling. The isolation chambers were capped with 10-cm-long tubing, screened at the top, with four additional screened holes spaced ca. 1 cm below the top (see additional details in Paris et al. 2013). Soil moisture was maintained by placing the isolation chambers in racks over water-filled trays. The nymphs, kept on a 16:8 light cycle at 25-30 °C, emerged as adults within 2-6 days and females were selected for bioassays 3-9 days after emergence. Bioassayed females were kept in their original isolation chambers, separate from other conspecifics, and remained virgin until testing. They were transferred to the CMAVE rearing colony after the tests.

Bioassays were performed inside a vibration-shielded anechoic chamber, 4-10 h after the beginning of photophase, on a 25-cm-height Duncan grapefruit tree (*Citrus paradisi* Macf.; Rutaceae) or a 30-cm-height Hamlin sweet orange tree (*C. sinensis* L. Osbeck; Rutaceae). Light was supplied by a three 60-W floodlamps from ca. 1 m above the tree. The bioassays were observed remotely using a videocamera (model HDR-SR1, Sony Corp. New York, New York), 0.5 m to the side, that fed its signal to a monitor outside the chamber.

Signals produced in bioassays were detected by an accelerometer (model 4371, Brüel and Kjær [B&K], Naerum, Denmark) that had been attached by an alligator clip near the base of the tree. The signals were fed from the accelerometer through a charge amplifier (model 2635, B&K) to a signal analysis system (model 4300B, Kay Elemetrics Corp, Lincoln Park, New Jersey) outside the anechoic chamber for digitization, recording, and real-time spectral analysis. Additional temporal pattern and spectral analyses of digitized signals were performed using custom-written

(Matlab R2011b, MathWorks, Natick, Massachusetts), Raven (Charif et al. 2008), or Audacity (<http://audacity.sourceforge.net>) software.

Microcontroller-Buzzer and Laptop-Vibration-Exciter Systems for *D. citri* Mimic Broadcasts

The feasibility of low-cost production of *D. citri* vibrational signal mimics was considered by connecting an inexpensive, open-source microcontroller prototyping platform (Arduino Uno, Arduino Inc., Italy <http://arduino.cc/en/Main/ArduinoBoardUno>) to a circuit containing a 6-12 V-DC power source, an on-off switch, and a piezoelectric buzzer (Fig. 1). The microcontroller platform is compact (8 by 5.4 by 3.2 cm) and fits easily into many commonly used insect traps. A standard 8-AA-cell, 6.0 by 3.0 by 6.2-cm battery holder can fit inside the trap if it is not convenient to attach it to a nearby branch or trunk, and a 9-V battery can be used for short-duration applications. Several preliminary laboratory tests were conducted by operating the system with 9-V bat-

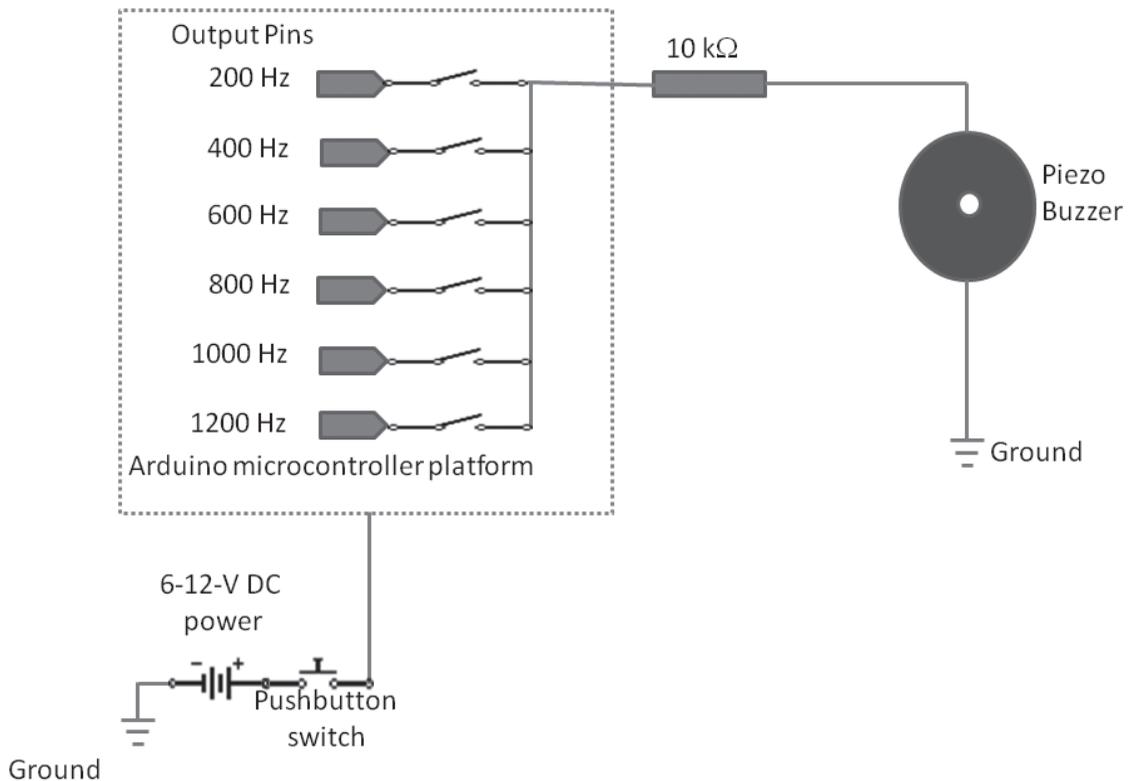


Fig. 1. Diagram of *D. citri* mimic-signal broadcast device, including the output pins on the Arduino microcontroller platform and the manual switches setting the output to the piezo buzzer. During bioassays, the main power switch was controlled remotely (Pushbutton) from outside the anechoic chamber.

teries, and preliminary laboratory and field tests were conducted by operating the system with the AA-cell packs to confirm estimates (see <http://arduino.cc/en/Main/ArduinoBoardUno>) that, at a typical rate of current usage of 50 ma, the 8 AA cells could provide power for periods up to nearly 2 days and a 9-V battery for approximately 6 h. In these cases, the batteries first were connected to the Arduino system to provide continuous power, and the capability of the system to power the buzzer was tested multiple times each hour towards the end of the expected operating period.

Initially, the microcontroller was programmed to oscillate 6 of the 14 digital output pins at frequencies of 200, 400, 600, 800, 1,000, and 1,200 Hz. These 6 frequencies then were combined by adding the signals from the 200-Hz pin to those from one or more of the other output pins to the piezoelectric buzzer. Resistance (e.g., 10 k Ω in Fig. 1) was added to the circuit as needed to reduce or increase signal output to biologically relevant levels (see next section). After the initial tests of system operation, the programming of the output pins was modified slightly to produce a mimic with multiple harmonics that increased and then decreased in frequency during the course of a signal, as had been observed with recorded calls and replies in Weninger et al. (2009) and Rohde et al. (2013) (see RESULTS). The resultant mimic, denoted as mop2_12, was evaluated by attaching the buzzer to a side branch of the tree with an alligator clip and monitoring the responses of females to the microcontroller-buzzer system broadcasts.

For consistency, a 9-V, 17-mm-diam buzzer (9S3164, Taiyo Yuden, Tokyo, Japan) with a resonant frequency of 7.6 kHz was used in all the bioassays and most of the preliminary tests. A variety of inexpensive buzzers of different sizes and resonant frequencies are available from different manufacturers, however, and additional tests were conducted to consider whether differences among buzzers significantly affected characteristics of signals transmitted through the tree. Three other buzzers with different resonant frequencies were tested, manufactured by PUI Audio Inc., Dayton Ohio: 2.0 kHz (No. AB2720B), 3.6 kHz (AB2036B), and 4.6 kHz (AB2746B). Each buzzer was connected to the Arduino system and then attached to a side branch of the tree. The buzzer signals were monitored by the accelerometer and their amplitudes and frequency spectra were analyzed using Audacity software.

Bioassays of Female Response to Microcontroller-Buzzer and Laptop-Vibration-Exciter Signals

The responses of females to the buzzer broadcasts were placed in context by conducting bioassays in which the females were presented with

mop2_12 mimics as well as 4 conventionally produced signals that had elicited a wide range of high to low rates of proportional responses: a recorded male call, a recorded female reply, a synthetic mimic (mh1200), and white noise (Rohde et al. 2013). The conventionally produced signals were played by a laptop computer using QuickTime (Apple, Inc., Cupertino California) to a vibration exciter (Model 4810, B&K) attached with a small push rod near the middle of the main stem of the tree.

In each bioassay, a female was coaxed from a small vial onto a leaf near the top the tree. After she settled on the flush and began feeding, the observers left the anechoic chamber to avoid further disturbance. Thirty examples each of the mop2_12-mimic broadcasts and the 4 signals from the vibration exciter were triggered in random order (generated by the Matlab function, randperm) at intervals of 5-10 s. The responses to each signal were noted. As in previous bioassays, the proportional response to each test signal was measured as the fraction of playbacks to which the female replied. Eleven females were bioassayed.

In preparing for the bioassays, the amplitudes of signals from the microcontroller-buzzer and laptop-vibration-exciter systems were adjusted to approximate the amplitudes of replies from several females in preliminary tests. The mop2_12-mimic amplitude was adjusted by increasing or decreasing the circuit resistance, which typically was set at 10 k Ω . The signals from the vibration-exciter system were adjusted by changing the amplitude of the output from the laptop computer. These settings were used for subsequent bioassays, with occasional adjustments when signal levels of female replies and test signals failed to remain similar in amplitude.

Statistical Analyses

Significance of differences among responses to different signals was confirmed by applying a nonparametric one-way repeated measures Friedman's test (SAS Institute 2004). Nonparametric Wilcoxon signed-ranked tests (SAS Institute 2004) then were applied to compare separately the proportional responses to different signals.

RESULTS

Microcontroller-Buzzer Broadcast Characteristics

An example of a series of signals produced by combinations of output from different pins of the microcontroller platform to the 7.6-kHz piezo buzzer is shown in Fig. 2. The 200 Hz pin operated during each of the series, and multiple har-

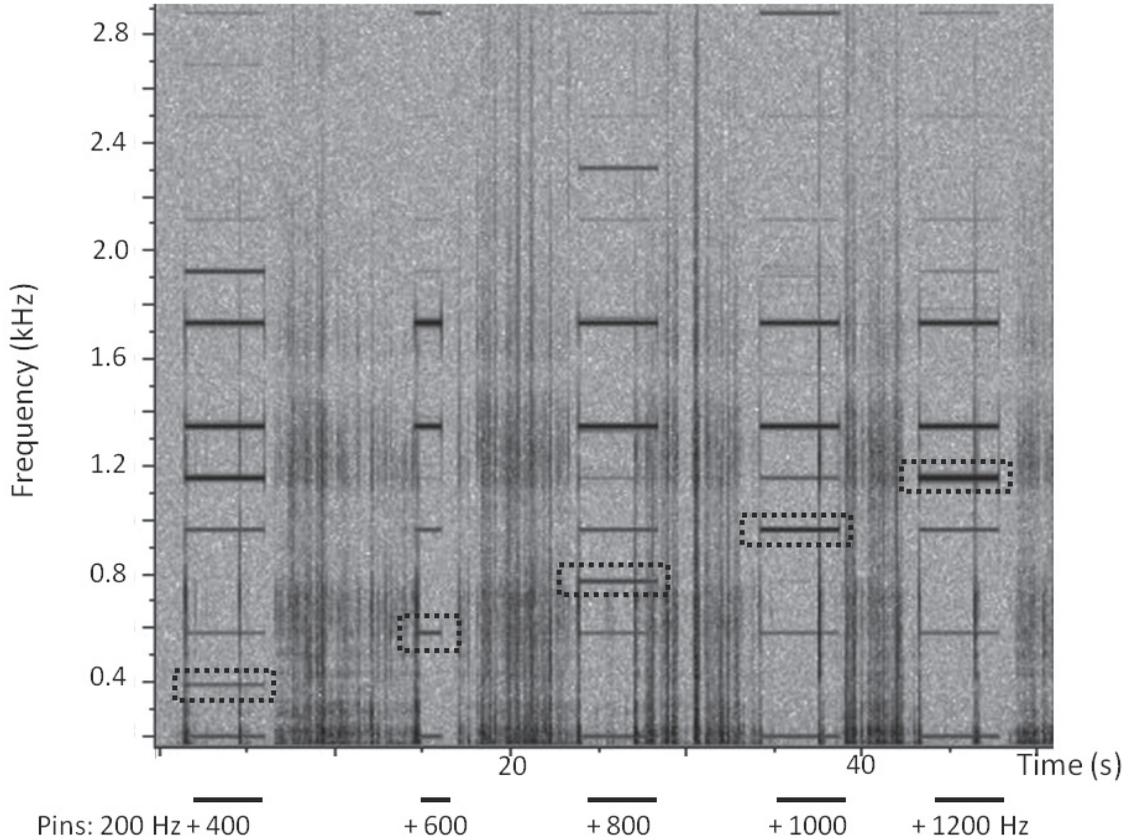


Fig. 2. Spectrogram of a series of signals transmitted to tree by microcontroller output to piezoelectric buzzer. All signals in this series contained output from 200-Hz pin. Solid bars with pin labels below the bottom of the spectrogram mark the times when signals from 400-, 600-, 800-, 1000- or 1200-Hz pins were added to the 200-Hz pin signal. Dotted boxes indicate frequencies where contributions from the added pin were observed. Harmonics of the initial, fundamental frequencies also appear in each signal of the series. Darker lines in the spectrogram indicate relatively higher signal energies; for example, the line near 1 kHz contributed from the 1000-Hz pin, the line near 1.2 kHz contributed from the 1200-Hz pin, and the lines near 1.4 and 1.8 kHz which appeared whenever the 200-Hz pin was turned on.

monics of the 200 Hz fundamental appear clearly. Contributions from operation of the 400, 600, 800, 1,000, and 1,200 Hz pins, respectively, are seen in the dashed boxes. In this example, the output pins were switched manually, and broadband background noise was present during the period when the operator switched the signal from one pin to another.

The combination of 200 and 1,200 Hz output pins (last signal in the series of Fig. 2) was one of several in the series that produced multiple harmonics with strong energy between 1 and 2 kHz, which had been found previously to be important for eliciting high proportions of female replies (Rhode et al. 2003). This signal was selected as the first candidate for bioassay testing. To modify the output so that the frequencies of harmonics increased and then decreased during the course of the signal, the programming of the Arduino

output pins was adjusted so that the 200 Hz and 1,200 Hz fundamental frequencies were increased and then decreased in steps of 0.833 and 5 Hz, respectively, at intervals shown in Table 1. An example of the resultant signal (mop2_12-mimic) and a female reply is shown in Fig. 3, along with examples of other test signals and replies.

The spectra of signals transmitted from buzzers with 2.0-, 3.6-, and 4.6-kHz resonant frequencies to the accelerometer at the base of the tree are shown in Fig. 4. Because each buzzer was driven by the same 200 and 1200 Hz signals from the Arduino microcontroller, it was expected that peak frequencies would occur at multiples of 200 Hz harmonics, but the relative amplitudes might be different. In this case, the 2 kHz buzzer produced the highest signal levels across most of the spectral range, but all of the buzzers produced multiple harmonic peaks between 1-2 kHz.

TABLE 1. FREQUENCIES AND TIMING OF TONES PROGRAMMED TO MICROCONTROLLER OUTPUT PINS FOR RISING AND FALLING PHASES OF *DIAPHORINA CITRI* SIGNAL MIMICS.

Rising phase				Falling phase			
Hpin (Hz)	Lpin (Hz)	Start (ms)	End (ms)	Hpin (Hz)	Lpin (Hz)	Start (ms)	End (ms)
1200	200	0	15	1265	210.83	750	835
1205	200.83	15	35	1260	210	835	915
1210	201.67	35	60	1255	209.167	915	990
1215	202.5	60	90	1250	208.33	990	1060
1220	203.33	90	125	1245	207.5	1060	1125
1225	204.167	125	165	1240	206.67	1125	1185
1230	205	165	210	1235	205.83	1185	1240
1235	205.83	210	260	1230	205	1240	1290
1240	206.67	260	315	1225	204.167	1290	1335
1245	207.5	315	375	1220	203.33	1335	1375
1250	208.33	375	440	1215	202.5	1375	1410
1255	209.167	440	510	1210	201.67	1410	1440
1260	210	510	585	1205	200.83	1440	1475
1265	210.83	585	665	1200	200	1475	1495
1270	211.67	665	750				

Hpin indicates high-frequency output pin.

Lpin, low-frequency output pin; Start and End are times (ms) relative to the beginning of the signal.

Female *D. citri* Proportional Responses

Females replied to a significantly greater fraction of male-call and mop2_12-mimic signals than to female-reply and mh1200-mimic signals, and all of these responses were greater than to white noise signals (Table 2). All but 3 females exhibited a greater proportional response to the male-call signal than to any other. Two of these exhibited a greater proportional response to the mop2_12-mimic signal and one to the female-reply signal. Three females exhibited 0.9 or greater proportional response to the male-call signal but exhibited less than a 0.3 proportional response to any of the other signals. This suggests that *D. citri* females exhibit a range of selectivity to different forms of male calls, as has been observed also with other psyllids (Percy et al. 2006).

DISCUSSION

This study explored the possibility of constructing an inexpensive, low-power device capable of producing synchronized duetting vibrations serving as a lure for trapping male *D. citri* searching for females in the branches of citrus tree canopies. The results above indicate that readily available microcontroller systems can be programmed easily to produce signals with multiple harmonics, confirmed to be of behavioral relevance to male and female *D. citri*. There is evidence also, see last section below, that such systems have satisfactory power usage for short to mid-term trapping durations.

Importance of Multiple Harmonics to Attractiveness of *D. citri* Communication Mimics

The 4 calls and mimics in Table 2 and Fig. 3 were similar in that all of them contained energy at multiple harmonics of 200 Hz between 1-2 kHz, although there many differences in other acoustic characteristics. They all elicited significantly greater behavioral responses than white noise that contained a random distribution of all the stimulatory frequencies. This result is supportive of a hypothesis that the presence of energy at multiple harmonics of about 200 Hz between 1-2 kHz may be important for eliciting female *D. citri* responses, as observed in a previous study (Rohde et al 2013), while differences in relative amplitudes of harmonics and differences in temporal patterns may have lesser effect. Males also may focus on the presence of multiple harmonics rather than specific frequencies or temporal patterns, considering that in Fig. 3, female replies exhibited both spectral and temporal pattern variability. In addition, we observed multiple occurrences of a female beginning her reply before a male call had ended, which suggests that the total duration of the male call is not a significant determinant in a female's decision to produce a reply.

Part of the reason why *D. citri* may exhibit some flexibility in the relative amplitudes of different harmonics in their duets is that these aspects of the vibrational signals can be modified in unpredictable ways as the signals are transmitted between duetting partners along the plant substrate (Cocroft et al. 2006; Hambric 2006; Mankin et al. 2008, 2011). In Figs. 2 and 4 for

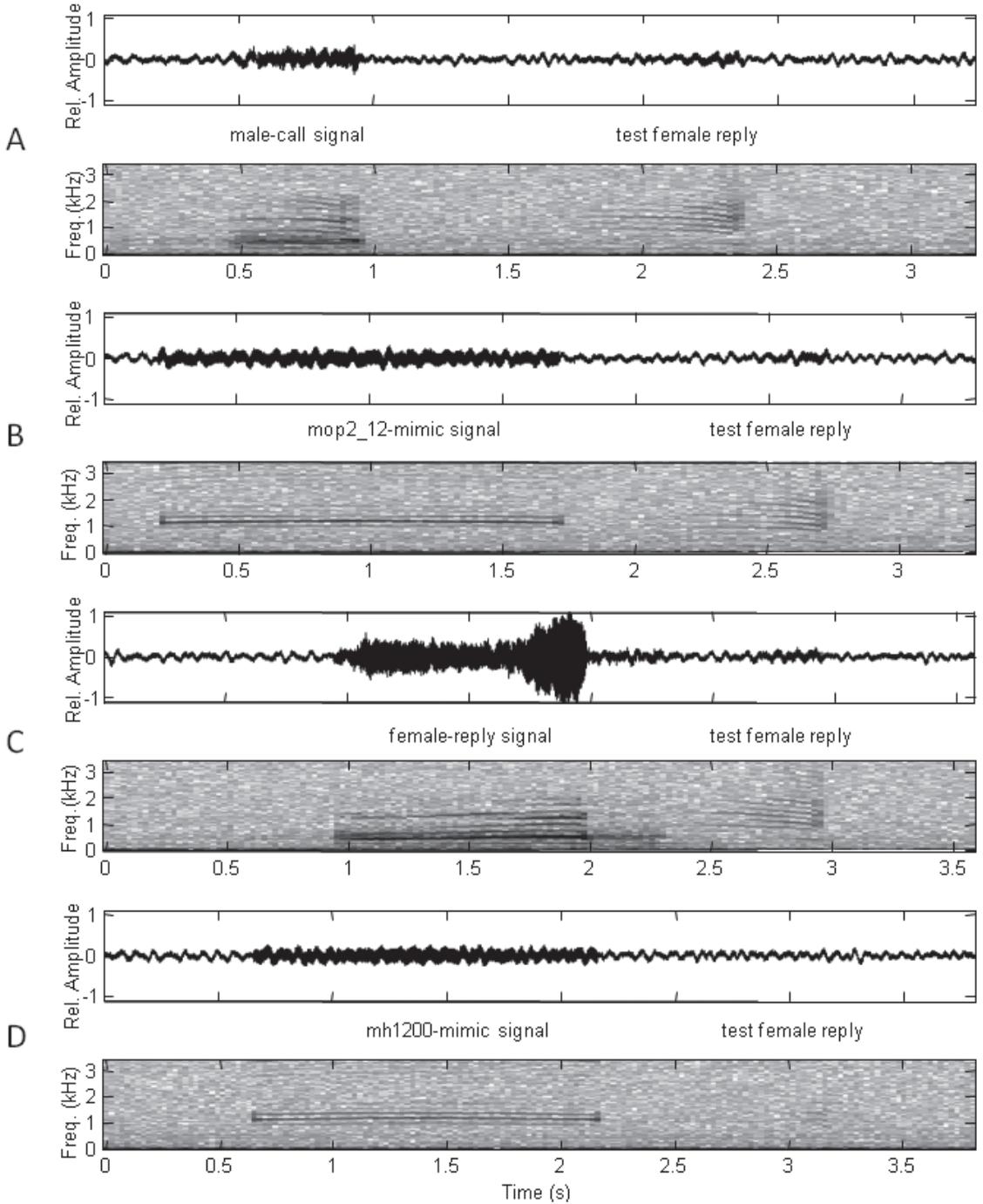


Fig. 3. Oscillograms and spectrograms of microcontroller-buzzer or laptop-vibration-exciter signals of different spectral and temporal patterns and the associated replies from test females: A) male-call, B) mop2_12-mimic, C) female-reply, D) mh1200-mimic. White noise is not shown in this figure, but an example of white noise can be seen in Fig. 3 of Rohde et al. (2013). All oscillograms were set at the same vertical scale relative to the signal received at the accelerometer (relative amplitude), and areas of darker shading in the spectrograms indicate higher relative energies at those frequencies and times.

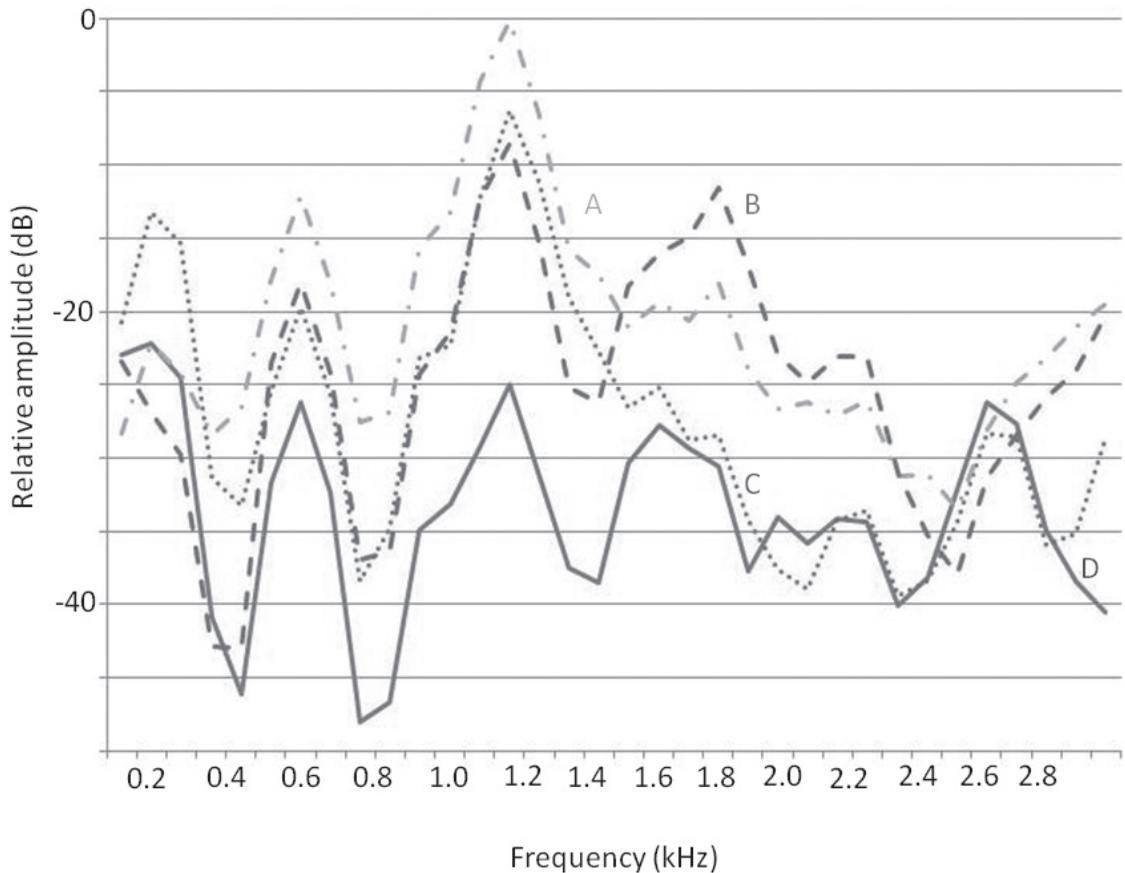


Fig. 4. Spectra of signals produced by Arduino output to A) 2.0-kHz (dash-dot), B) 3.6-kHz (dashed), C) 4.6-kHz (dotted), and D) 7.6-kHz buzzers (solid line), as measured by accelerometer at base of tree. All signals are shown on the same relative amplitude scale.

example, only 2 frequencies are outputted to the piezoelectric buzzers, but multiple harmonics of different relative amplitudes can be detected by the accelerometer at the base of the tree. The amplitudes of the harmonics could be different if the buzzer were attached to a different branch or a differently sized tree (Hambric 2006). Only the presence of energy at multiple harmonics, but not a specific pattern of relative amplitudes or durations was found consistently in successful signals observed in Wenninger et al. (2009a), Rohde et al. (2013), and this report.

D. *citri* Vibration Trap Power Usage and Cost Analysis

Power usage is a practical concern for any electronic system in field environments away from an electrical grid. The Arduino system is specified to draw about 50 ma current under routine conditions (see <http://arduino.cc/en/Main/ArduinoBoardUno>). At this rate, sets of 8 AA cells could power an intermittently operating system for 2 days. A 9-V battery could power the system for

about 6 h. These approximate battery lifetimes were confirmed in several preliminary studies (see METHODS).

According to the instruction manual for the microcontroller chip, reductions in power usage could be achieved by placing parts of the system in sleep mode when not in use. Indeed, recent preliminary tests indicate that the Atmega328 microcontroller chip (Atmel Corp, San Jose, California) on the Arduino Uno system not only has the capability to spectrally modulate the signal output, as with the mop2_12 mimic, but has sufficient capability to perform other tasks concurrently with signal production, such as operation of a light sensor to set the system into sleep mode during darkness. In an initial test of this capability, it has been possible to operate a system for 4 days before loss of power. Also, careful selection of piezo buzzers may increase the effective range of a trap for a given level of power usage. The results in Fig. 4 suggest that, for a given power output, signals from 2-kHz buzzers might be detectable over longer distances than the signals from the

TABLE 2. MEANS \pm SE OF FEMALE *DIAPHORINA CITRI* PROPORTIONAL RESPONSES TO MICROCONTROLLER-BUZZER OR LAPTOP-VIBRATION-EXCITER PRODUCED SIGNALS OF DIFFERENT SPECTRAL AND TEMPORAL PATTERNS, ARRANGED IN ORDER OF HIGH-TO-LOW RESPONSE.

Signal	Proportional response
male-call (Fig. 3A)	0.64 \pm 0.09 a
mop2_12-mimic (Fig. 3B)	0.52 \pm 0.09 a
female-reply (Fig. 3C)	0.31 \pm 0.09 b
mh1200-mimic (Fig. 3D)	0.27 \pm 0.08 b
white noise	0.01 \pm 0.01 c

Significant differences occurred in the mean proportional response to different signals (Friedman's test statistic = 27.77, $df = 4$, $P < 0.001$). Mean proportional responses followed by the same letter are not significantly different at the $P < 0.05$ level under Wilcoxon signed rank test. Specific comparisons were: male-call ranked with mop2_12-mimic, $T_s = 13$, $N = 11$, $P > 0.05$; male-call ranked with female-reply, $T_s = 4.5$, $N = 11$, $P < 0.01$; male-call ranked with mh1200-mimic, $T_s = 0$, $N = 11$, $P < 0.001$; male-call ranked with white noise, $T_s = 0$, $N = 11$, $P < 0.001$; mop2_12-mimic ranked with female-reply, $T_s = 0$, $N = 10$, $P < 0.001$; mop2_12-mimic ranked with mh1200-mimic, $T_s = 10$, $N = 11$, $P < 0.05$; mop2_12-mimic ranked with white noise, $T_s = 0$, $N = 10$, $P < 0.001$; female-reply ranked with mh1200-mimic, $T_s = 13$, $N = 9$, $P > 0.05$; female-reply ranked with white-noise, $T_s = 0$, $N = 9$, $P < 0.001$; mh1200-mimic ranked with white noise, $T_s = 0$, $N = 9$, $P < 0.001$.

higher frequency buzzers because the resonant frequency is closer to the most behaviorally relevant signals.

The \$40-\$60 cost of these microcontroller-sensor systems is greater than the cost of sticky traps but there are potential benefits from improved trapping efficiency and reusability. Certainly the cost is orders of magnitude lower than the cost of the laboratory equipment it replaces. In addition, experience with programming and operation of these systems indicates that sensors to detect vibration, temperature, humidity, and light could be added and controlled at reasonable cost, which could make such devices even more useful as entomological research tools.

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REFERENCES CITED

- BOVÉ, J. M. 2006. Huanglongbing: a destructive, newly emerging, century-old disease of citrus. *J. Plant Pathol.* 88: 7-37.
- BROWNING, H. 2013. A simple math equation to describe HLB research direction. <http://citrusrdf.org/wp-content/uploads/2013/01/CRDF-Jan-2013-Column.pdf>
- CHARIF, R. A., WAACK, A. M., AND STRICKMAN, L. M. 2008. Raven Pro 1.3 user's manual. Cornell Laboratory of Ornithology, Ithaca, NY.
- COCROFT, R. B., AND RODRÍGUEZ, R. L. 2005. The behavioral ecology of insect vibrational communication. *Bioscience* 55: 323-334.
- COCROFT, R. B., SHUGART, H. J., KONRAD, K. T., AND TIBBS, K. 2006. Variation in plant substrates and its consequences for insect vibrational communication. *Ethology* 112: 779-789.
- GRAFTON-CARDWELL, E. E., STELINSKI, L. L., AND STANSLY, P. A. 2013. Biology and management of Asian citrus psyllid, vector of the Huanglongbing pathogens. *Annu. Rev. Entomol.* 58: 413-432.
- HALL, D. G., S. L. LAPOINTE, AND E. J. WENNINGER. 2007. Effects of a particle film on biology and behavior of *Diaphorina citri* (Hemiptera: Psyllidae) and its infestation in citrus. *J. Econ. Entomol.* 100: 847-854.
- HALL, D. G., RICHARDSON, M. L., AMMAR, E.-D., AND HALBERT S. E. 2012. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. *Entomol. Exp. Appl.* 146: 207-223.
- HAMBRIC, S. A. 2006. Structural acoustics tutorial-Part 1: vibrations in structures. *Acoustics Today* 2: 21-33.
- MANKIN, R. W., HAGSTRUM, D. W., SMITH, M. T., RODA, A. L., AND KAIRO, M. T. K. 2011. Perspective and promise: a century of insect acoustic detection and monitoring. *American Entomol.* 57: 30-44.
- MANKIN, R. W., HODGES, R. D., NAGLE, H. T., PEREIRA, R. M., AND KOEHLER, P. G. 2010. Acoustic indicators for targeted detection of stored product and urban insect pests by inexpensive infrared, acoustic and vibrational detection of movement. *J. Econ. Entomol.* 103: 1636-1646.
- MANKIN, R. W., SMITH, M. T., TROPP, J. M., ATKINSON, E. B., AND JONG, D. Y. 2008. Detection of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) larvae in different host trees and tissues by automated analyses of sound-impulse frequency and temporal patterns. *J. Econ. Entomol.* 101: 838-849.
- PARIS, T. M., ROHDE, B. B., ALLAN, S. A., MANKIN, R. W., AND STANSLY, P. A. 2013. Synchronized rearing of mated and unmated *Diaphorina citri* (Hemiptera: Liviidae) of known age. *Florida Entomol.* 69: 1631-1634.
- PERCY, D. M., TAYLOR, G. S., AND KENNEDY, M. 2006. Psyllid communication: acoustic diversity, mate recognition, and phylogenetic signal. *Invertebr. Syst.* 20: 431-445.
- ROHDE, B., PARIS, T. M., HEATHERINGTON, E. M., HALL, D. G., AND MANKIN R. W. 2013. Responses of *Diaphorina citri* (Hemiptera: Psyllidae) to conspecific vibrational signals and synthetic mimics. *Ann. Entomol. Soc. America* 106: 392-399.
- SAS INSTITUTE. 2004. SAS/STAT 9.1 user's guide. SAS Institute, Cary, NC.
- SÉTAMOU M., FLORES D., FRENCH J. V., AND HALL, D. G. 2008. Dispersion patterns and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus. *J. Econ. Entomol.* 101: 1478-1487.

- STANSLY, P., QURESHI, J., AND AREVALO, A. 2009. Why, when and how to monitor and manage Asian citrus psyllid. *Citrus Industry* 90: 24-26.
- USDA NATIONAL INVASIVE SPECIES INFORMATION CENTER 2013. Asian citrus psyllid. <http://www.invasivespeciesinfo.gov/animals/acp.shtml>.
- WENNINGER, E. J., HALL, D. G., AND MANKIN, R. W. 2009a. Vibrational communication between sexes in *Diaphorina citri* (Hemiptera: Psyllidae). *Ann. Entomol. Soc. America* 102: 547-555.
- WENNINGER, E. J., STELINSKI L. L., AND HALL, D. G. 2009b. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. *Environ. Entomol.* 38: 225-234.