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# Vibrational duetting mimics to trap and disrupt mating of the devastating Asian citrus psyllid insect pest.

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the primary vector of a bacterium that causes huanglongbing, a devastating disease of citrus in Florida. Efficient trapping of ACP at low population densities is important for timely pest management decisions. To improve trapping efficiency, there has been interest in coopting the vibrational communication/mating system of this insect. ACP males find mates on tree branches by producing vibrational calls that elicit duetting replies from receptive females. The males then search and continue duetting to locate the replies. To interfere with the duetting process, a device was constructed that incorporates a contact microphone to detect vibrational signals, a microcontroller platform with signal detection and background noise discrimination software, and a piezoelectric buzzer to produce mimics of female reply vibrations. The mimics, produced immediately after a call is detected, stimulate the male to search and find the source of the reply. This report describes the components and operation of the platform and its use in development of methods to interfere with the duetting process and disrupt mating. The objective of these studies is to develop field-worthy systems that target ACP infestations and reduce their populations.



## I. INTRODUCTION

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an insect pest of citrus trees (Hall et al. 2012, Grafton-Cardwell et al. 2013). It causes only minimal damage by itself, but if it feeds on a citrus tree infected with *Candidatus Liberibacter asiaticus* bacteria, it can transmit the bacteria to other trees as it moves through a grove or migrates away. The bacteria reproduce and induce a disease, huanglongbing, which damages and kills its citrus tree host within a few years. Extensive research is being conducted to better understand *D. citri* biology and behavior and develop improved methods to reduce its populations and spread of disease. One of the approaches is to thwart its mating process.

During courtship, males search for females on branches and stems of host trees, broadcasting vibrational signals that elicit duetting replies from receptive females (Wenninger et al. 2009). When a male detects a reply he moves towards the female and calls again. Mating occurs after a series of duetting calls and replies during which the male searches and finds the female. Studies have been conducted to trap males by broadcasting female replies (Rohde et al. 2013, Mankin et al 2013). It was found that multiple harmonics of 200 Hz (in the typical range of *D. citri* wingbeat frequencies and harmonics) were always present when *D. citri* males and females responded behaviorally to either recordings or synthetic mimics of duetting replies. Such results led to exploration of methods to disrupt the duetting process.

The initial hypothesis was that a synthetic mimic of a duetting female reply would be more attractive to the male than the reply of a female if it were broadcast immediately before and at higher amplitude than the actual reply. This report presents preliminary results of bioassays comparing the rates of mating in the presence and absence of a vibrational trap that produced interfering mimics, confirming that *D. citri* mating can be disrupted significantly. Additional studies are described on identification of spectral harmonic features of male calls that could be incorporated into the signal discrimination software to improve the reliability of male call detection in the presence of background noise.

## II. MATERIALS AND METHODS

### A. Bioassay Arena

Bioassays were conducted on 30-cm-height *Citrus macrophylla* trees obtained from a greenhouse at the US Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. Virgin adult males and females were obtained from a colony reared using procedures described in Paris et al. (2013). The experiments were conducted in a vibration-shielded anechoic chamber (Zagvazdina et al 2015), equipped with a video system enabling visual observations of the mating behaviors. Each insect was tested once. To obtain independent, real-time verification of acoustic signals, an accelerometer (Model 4371, Bruel and Kjaer [B&K], Naerum Denmark) was connected to the base of the tree to monitor vibrations. The accelerometer signals were fed to a charge amplifier (Model 2635, B&K), monitored with headphones, and saved on a digital recorder (Model HD-P2, Tascam, TEAC, Montebello, CA) for subsequent signal analyses.

### B. Microcontroller Platform

As in Mankin et al. (2013), synthetic mimic signals were generated using a low-cost microcontroller platform (Arduino Uno, Arduino Inc., Ivrea, Italy) connected to a circuit board (Figure 1A) that included an electret microphone (Model WM-63GNT, Panasonic Corp., Newark, NJ) (Figure 1B) to detect psyllid communication vibrations, and a piezoelectric buzzer (9S3174, Taiyo Yuden, Tokyo, Japan) to produce synthetic mimics of female reply vibrations.

The microphone and buzzer were attached to the tree with small clamps. Examples of signals produced by the piezoelectric buzzer, a male, and a female are shown in Figure 2. Previous bioassays (Mankin et al. 2013) indicated that vibrations produced by the buzzer or by a female had approximately equal behavioral effect on the male at similar amplitudes.

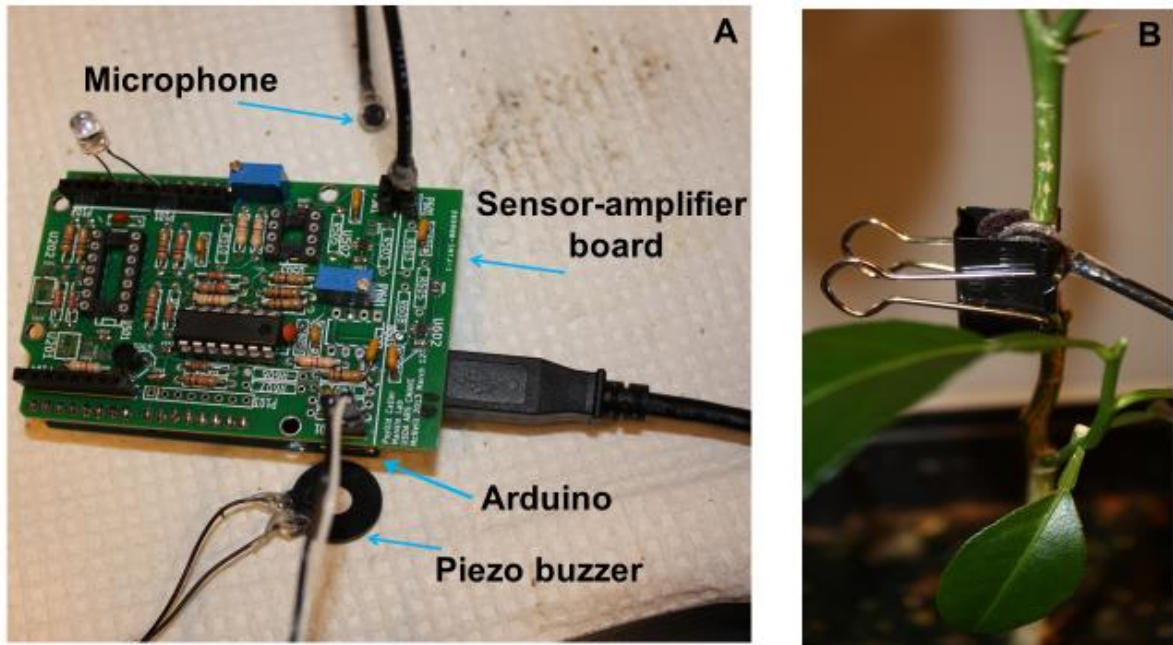


Figure 1. A) Sensor-amplifier board includes an electret microphone to detect ACP signals and operates a piezoelectric buzzer to produce synthetic reply mimics. B) Connection of microphone to tree stem.

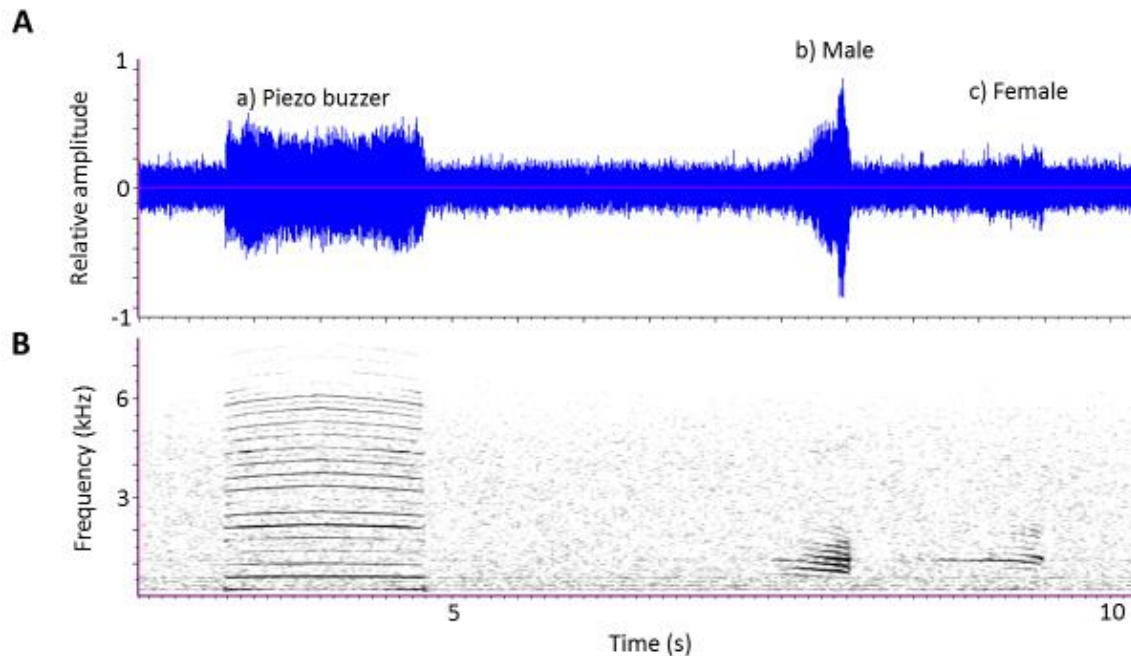
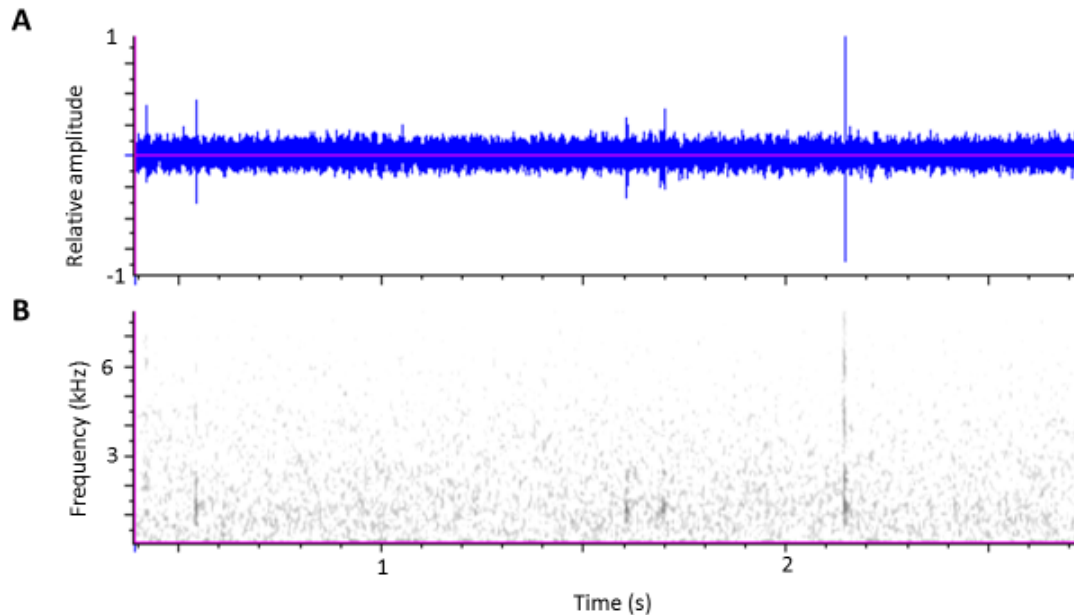


Figure 2. A) Oscillogram and B) spectrogram of vibrations produced by: a) piezoelectric

*buzzer signal, b) male call, and c) female reply over an 8 s interval on a small Citrus macrophylla tree.*

Non-communicatory signals occurred during bioassays, including incidental vibrations caused by psyllid contact impacts or scraping movements on stems or leaves. An example of a short interval with five brief signals of various bandwidths is shown in Figure 3.



*Figure 3. A) Oscillogram and B) spectrogram of incidental movements produced by psyllids over a 2.6 s interval. Darker shading indicates greater relative energy in the spectrogram at the indicated frequencies and times.*

In the anechoic chamber and in field studies, *D. citri* have been observed occasionally to produce wing buzzes that are similar to duetting calls in frequency and harmonics but differ from typical duetting calls (Fig. 2b-c) in their duration and amplitude patterns. In field studies, we also frequently observe louder, longer-duration signals with similar broadband spectral properties caused by wind-induced movement and tapping of leaves and branches, as well as loud bird calls and vehicular background noises with broadband and harmonic spectral components, as noted in Mankin et al. (2011). Consequently, it was of interest to identify specific spectral and temporal patterns of male calls which enabled them to be discriminated from nontarget signals.

To identify spectral features of male *D. citri* calls, the microcontroller was programmed with a Fast Hartley Transform (FHT) algorithm (Bracewell 1984) to calculate a 128 point spectrum every 0.1 s from 256 time points sampled at 8000 Hz. A series of such spectra thereby created a spectrogram of the acoustic environment. To explore the capability of a generalized template to identify male calls, an average spectrogram was constructed from multiple calls in a training data set recorded from six different males (Figure 4). It was expected that, over a spectrogram-matching range of maximum energy, the male calls on average would deviate less from an optimal template than would background noise or nontarget signals. The low and high frequencies of the spectrogram-matching range (Fig. 4) were based on observations that much of the energy of male calls was between 600 and 2000 Hz. For the last 6 samples before the end of

the call, the normalized difference between the spectrum level of a detected signal and the template, summed over all the frequency points in the spectrogram matching range, was used to estimate whether or not the signal was a male call and merited triggering of a reply mimic.

The average-spectrum template was optimized further to discriminate spectrograms of known male calls (selected by a listener) from spectrograms of nontarget background noise in additional laboratory tests as well as two 40-minute field recordings collected from trees known to be infested with psyllids. Overall, about 6% or 280 signals detected from the optimization tests were psyllid calls and 94% were other background signals that rose above a user-set threshold. A Matlab genetic algorithm process was conducted to optimize the template, adapted from Sharma and Gopal (2013). In several iterations, randomly generated test templates competed with the average spectrogram to identify a template which best matched to the correctly labeled spectrograms. During training, the optimal template detected 77% of male calls identified by listeners (true positives) and 26% of its detections were false positive detections of background noises. This was the template used for subsequent trapping and mating disruption bioassays. During mating tests conducted afterwards with new generations of psyllids, however, the true positive rate with this template decreased closer to 50%.

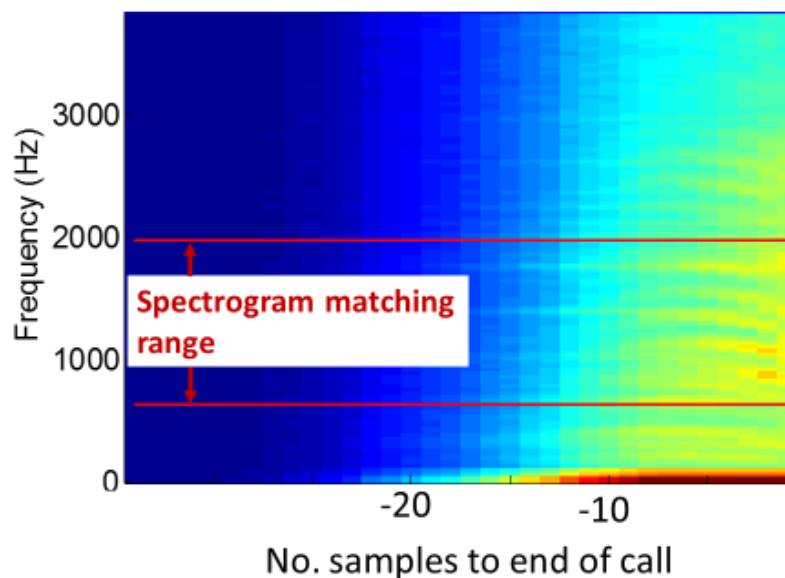


Figure 4. Mean spectrogram (template) of 460 calls recorded from 6 males (signals aligned to end of call). Relative energy is scaled from blue (low energy) to orange (high energy). Call frequencies between upper and lower red bars had the greatest energy and were used for matching against spectrograms of incoming signals.

### C. Mating Disruption Bioassays

Trees with multiple branches were selected for tests that offered each male a “fork” where it could walk either towards a replying female or a piezoelectric buzzer. In all tests, a previously untested male was placed on a leaf near the outer edge of one branch using a walker device described in Pregmon et al. (2016). The buzzer was attached towards the base of the tree and the walker was used to place a previously untested female on a leaf near the top of the other branch.

Video and audio monitoring of the male searching behavior was conducted for 60-minute periods, beginning when the female was placed on her leaf. The mating disruption and control bioassays were different in that whenever the male called, a synthetic mimic reply was triggered immediately in mating disruption tests, either by the microcontroller or manually. In both mating and control bioassays the female usually responded with a duetting reply to the male call. Consequently, the male could search towards either the female or the buzzer in the mating disruption bioassays, or towards the female in the control bioassays.

Because preliminary tests indicated that the originally optimized detection algorithm correctly identified calls from certain males better than others, an observer monitored all bioassays and manually triggered the buzzer to reply in the mating disruption bioassays if a male called and the microcontroller failed to trigger automatically. In both the mating disruption and control tests, if the male did not call within 5 minutes after the test began, an initial synthetic mimic reply was triggered manually. This usually elicited a female reply and initiated male searching behavior. Monitoring continued for 60 minutes or until mating occurred. The duration before mating or end of test was noted, as well as whether or not the male conducted searching behavior, the timing of male and female calls, and whether or not the male made contact with the piezoelectric buzzer.

### III. RESULTS AND DISCUSSION

#### A. Mating Disruption and Control Bioassays

There were observable differences between the behavior of males in the control and mating disruption bioassays. After a reply in either bioassay, males frequently reversed direction, but in the disruption tests, there were more frequent reversals of direction away from the female and toward the piezoelectric buzzer, as well as more frequent contacts with the buzzer. In this preliminary study, such differences in behavior resulted in fewer matings and a longer duration until mating in the mating disruption tests compared to the controls. To take into account tests where no mating occurred, a normalized rate of mating per hour test was calculated as

$$\text{Mating Rate} = (3600 \text{ s} - t) / 3600 \text{ s}, \quad \text{Eq. (1)}$$

where  $t$  is the time until mating in seconds, which yields a rate of 0 when no mating occurs and 1 when mating occurs immediately after the test begins. Only 0.09 matings per test hour occurred on average in the mating disruption bioassay, compared to 0.41 in the control, a difference which was statistically significant under the Wilcoxon rank sum test ( $Z = 2.94$ ,  $P > |Z| = 0.0032$ ) (Table 1).

**Table 1. Comparison of normalized rate of mating per hour in disruption and control tests.**

Bioassay	No. tests	Sum of scores	Mating Rate (Eq. 1)
Mating disruption	21	311	0.09
Control	15	355	0.41

#### B. Automated Identification of Vibrations by Calling Males

During the mating data collection for this study, the algorithm used to identify male *D. citri* calls had limited success (52%) in correctly identifying male calls as opposed to results during algorithm training bioassays. Therefore, it was often necessary for the experimenter to manually

trigger the reply mimic in the mating disruption tests. This contrasts with previous studies where mean spectra of mosquito and fly acoustic calls (Mankin 1994, Mankin et al. 1996, Mankin et al. 2000), mean spectra of insect movement and feeding sounds (Mankin et al. 2011), and classifier systems using Gaussian mixture models (Lampson et al. 2013) or linear prediction cepstral coefficients (Korinšek et al. 2016) have been used more successfully to identify targeted insects and discriminate their signals from background noise or those of non-targeted insects. One possible reason for a reduction in recognition capability relative to previous studies was a noticeable male to male variation in wingbeat frequency. Means of the 512-point spectra of vibrations of calls produced by males in the first 8 tests, recorded from the accelerometer and digitized at 44.1 kHz, were calculated using a custom-written program, DAVIS, as described in Mankin et al. (2011). Plots of the mean spectra (Figure 5) suggested that peaks of different amplitudes occurred at different multiples of the mean wingbeat frequency (the lowest-frequency peak in each test), but because each male had a different mean wingbeat frequency and the peaks of greatest amplitude were different for each male, it was difficult to construct a generalized spectrogram capturing signal features that humans and female *D. citri* used to recognize male calls.

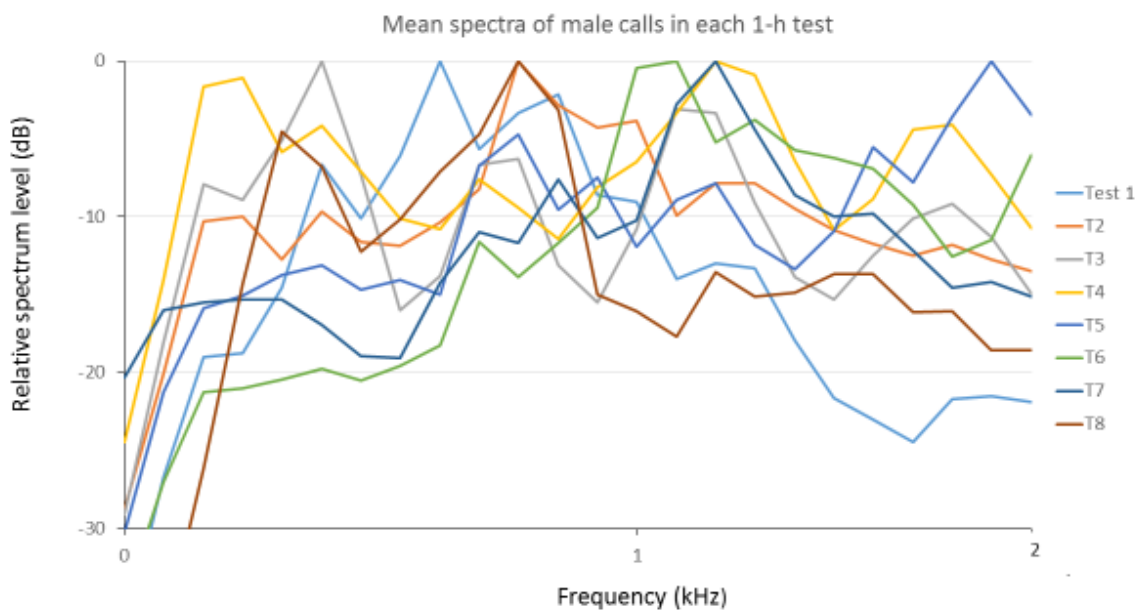


Figure 5. Mean spectra of all calls produced by males in first 8 tests of mating disruption bioassay. Spectrum peaks occurred at harmonics of wingbeat frequency, but locations of peak amplitudes were different among males.

A potential resolution to this dilemma can be seen by replotting the spectra based on units of mean wingbeat frequency (Figure 6). For most males, a peak amplitude occurred between the third and seventh harmonic, and for each male, there were at least two peaks within 10 dB of the peak amplitude. Such information may be useful in devising future iterations of the call identification algorithm.

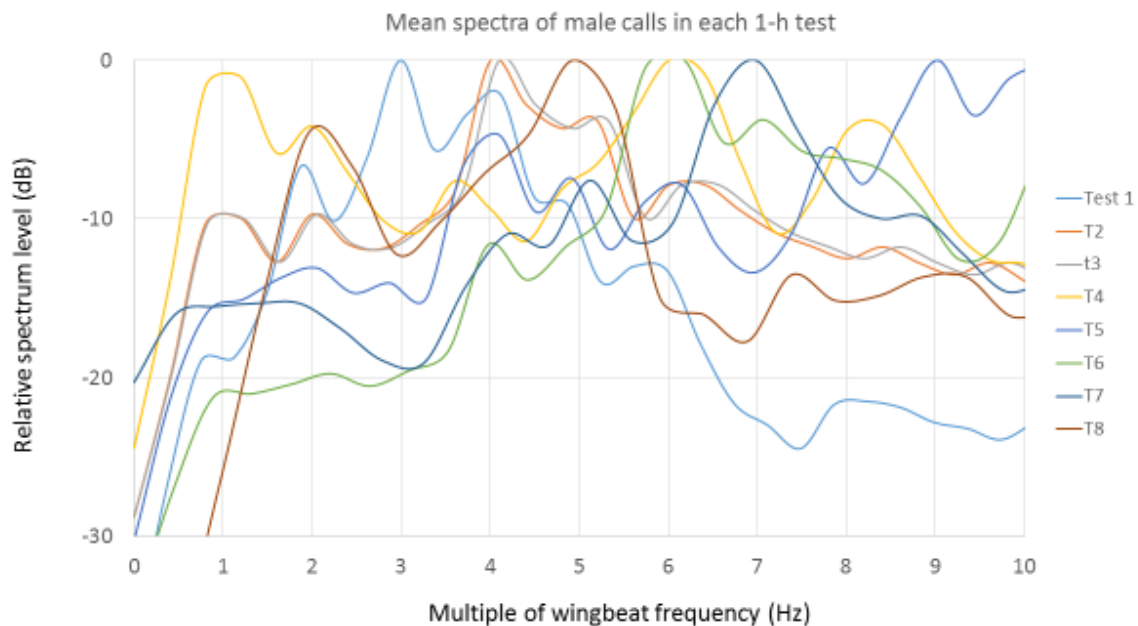


Figure 6. Replot of mean spectra in Fig. 5 of males in the first 8 tests of mating disruption bioassay in terms of multiples of male wingbeat frequency. Signal features evident in this context may be helpful for development of improved algorithms for recognizing male *D. citri* calls.

#### IV. CONCLUSION

Although preliminary, the results presented here on tests of low-cost devices that detect and produce vibrational communication signals to trap male *D. citri* and disrupt mating show promise of future application of such technology in field environments. Successful application of this technology for control of *D. citri* may also lead to additional technology for control of other hemipteran pests (Mankin 2012, Polajnar et al. 2016), especially those that vector important bacterial diseases (Redak et al. 2004, Orlovskis et al. 2015).

#### V. ACKNOWLEDGMENTS

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