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Citrus Industry News

Disrupting Psyllid Mating to Control HLB

(http://citrusindustry.net/wp-content/uploads/2016/04/A9R134d7dx_1sco05m_w8.jpg)**By: R. W. Mankin, B. Rohde and S. McNeill**

The Asian citrus psyllid (ACP) is the primary vector of the devastating huanglongbing (HLB) disease of citrus. Efficient monitoring of ACP at low population densities is essential to conduct management programs with timely effectiveness for protection of Florida groves. Extensive research is being conducted to better understand ACP biology and behavior, and to develop

improved methods to reduce its populations. One relatively new approach to monitoring and managing this pest is to thwart its mating process.

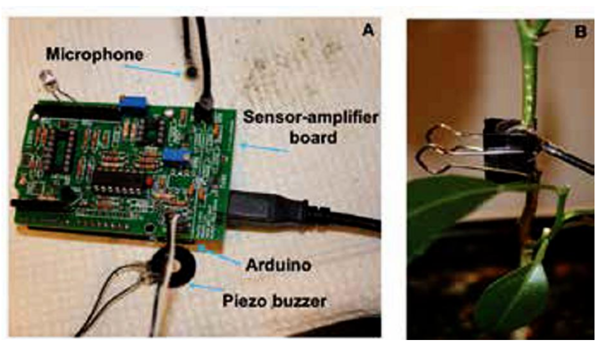
During courtship, ACP males search for females on branches and stems of citrus trees, broadcasting vibrational signals that elicit duetting replies from receptive females feeding sedentarily on tree flush. When a male detects a reply, he moves toward the female and calls again. Mating occurs after a series of duetting calls and replies during which the male searches and finds her.

It is possible to construct an inexpensive trap to monitor males by using a microcontroller with signal detection software, a contact microphone to detect ACP calls and a piezoelectric buzzer to produce calls. The buzzer plays back a female reply when it detects a male call, which stimulates the male to search and be trapped.

In considering ways to disrupt mating, we wondered if a synthetic mimic of a duetting female reply would be more attractive to the male than a replying female, especially if it was broadcast immediately before and at higher amplitude than her reply. A microcontroller platform was constructed that detected male mating communication vibrations and produced synthetic mimics of female replies. We report here on successful mating disruption lab tests and consider the implications for future control of ACP in Florida citrus groves. Our goal is to develop field-worthy systems that target ACP infestations and reduce their populations.

METHODS

Bioassays were conducted on young citrus trees using psyllids from a colony reared at the U.S. Department of Agriculture's Center for Medical, Agricultural and Veterinary Entomology in Gainesville. The experiments were conducted in a sound- and vibration-shielded chamber equipped with a video system to observe the mating behaviors as they occurred. To monitor the psyllid vibrations independently, an accelerometer



(http://citrusindustry.net/wp-content/uploads/2016/04 vibration sensor was connected at the base of the tree.

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FIGURE 1A: A SENSOR-AMPLIFIER BOARD INCLUDES AN ELECTRET MICROPHONE THAT DETECTS THE SIGNALS PRODUCED BY ACP. IT OPERATES A PIEZOELECTRIC BUZZER THAT PRODUCES SYNTHETIC ACP REPLIES. FIGURE 1B: CONNECTION OF MICROPHONE TO TREE STEM.

The synthetic mimic signals were produced by an Arduino Uno microcontroller platform connected to a circuit board (Figure 1A) that included a sensitive contact microphone (Figure 1B) and a piezoelectric buzzer to detect calls and play back synthetic mimics of female reply vibrations. The microphone and buzzer were attached to the tree with small clamps. Previous studies found that buzzer or female vibrations produced at similar amplitudes had approximately equal effect on male behavior.

The microcontroller was programmed to calculate a spectrum every 0.1 second from 256 time points sampled at 8000 hertz. A series of such spectra thereby created a spectrogram of the acoustic environment that was scanned to detect male ACP calls. To identify incoming calls, a template was constructed as an average spectrogram of multiple calls recorded from six different males. The template was compared against incoming signals in a spectrogram range of interest to recognize which signals were male calls.

The low and high frequencies of the spectrogram matching range were based on observations that most of the energy of actual psyllid calls was between 600 and 2000 hertz. The normalized difference between the spectrum level of a detected signal and the template, summed over all the frequency points in the matching range, was used to estimate whether or not the signal was a male call and merited triggering of a reply mimic.

For various reasons, the detection algorithm made some mistakes in identifying some of the calls initially, but we have been working to improve the identification process. For purposes of this proof of principle study, we conducted tests where the experimenter manually triggered the board to reply if it did not reply automatically.

MATING DISRUPTION

Trees with multiple branches were selected for tests that offered each male a ῝forkὸ where it could walk either toward a replying female or toward
a piezoelectric buzzer. In all tests, a previously untested male was placed on a leaf near the outer edge of one branch. The piezoelectric buzzer was attached toward the base of the tree, and a previously untested female was placed on a leaf near the top of the other branch.

Video and audio monitoring of the male searching behavior was conducted for one-hour periods, beginning when the female was placed on her leaf. Whenever the male called, a synthetic mimic reply was triggered immediately in mating disruption trials, either by the microcontroller or manually. No synthetic mimics were triggered in control trials that timed the natural mating process.

In both the mating disruption and control tests, if the male did not call within five 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 one hour or until mating occurred. The duration before mating or the end of the 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.

RESULTS

There were observable differences between the behavior of males in the control and mating disruption trials. In both sets of trials, there were frequent detours along twigs and leaves where the female was not present, but there were more frequent reversals of direction toward the piezoelectric buzzer in the mating disruption tests and frequent contacts with the buzzer.

In this preliminary study, such differences in behavior resulted in fewer matings and a slower rate of mating in the mating disruption trials, compared to the control trials. Only 0.09 matings per hour of testing occurred on average in the mating disruption trial, compared to 0.41 matings per hour in the control, a difference which was statistically significant.

CONCLUSION

The results show promise for future application of
such electronic mating disruption technology in field environments. Successful application of this technology for control of ACP may also lead to additional technology for control of other hemipteran pests that vector important bacterial diseases. Mating disruption of grape vine pests has already been demonstrated by broadcast of disruptive signals on vine trellises. We are considering adaptations of such technology for citrus groves.

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