

# Chapter 20

## Vibrational Trapping and Interference with Mating of *Diaphorina citri*



R. W. Mankin

**Abstract** Microcontroller-platform devices have been constructed that detect *Diaphorina citri* male vibrational communication calls and broadcast mimics of female vibrational replies. The devices successfully interfere with mating of virgin pairs of *Diaphorina citri* in 1-h tests on citrus trees, reducing the mating percentage significantly from 57% in the control to 13% in disruption tests. Video and audio monitoring of searching behaviors in laboratory bioassays indicate that males are attracted to the source of the female reply mimics. The percentage of mating may be reduced by the following: (1) interference of a louder and earlier reply mimic more attractive to the male than the female reply; (2) masking of the female reply by the louder mimic; or (3) reduction of female responsiveness in the presence of other female replies, or combinations thereof. In male *D. citri* trapping bioassays, the device has successfully trapped 45% of males stimulated to initiate search behavior in 1-h tests on citrus trees. Positive and negative effects of social, flush-seeking, and phototactic behaviors of males and females are discussed that may affect the utility of these devices in field applications. In addition, potential applications are discussed for low-cost, modified microcontroller-platform devices that discriminate insect-produced feeding and movement vibrations from background noise in field studies.

### 20.1 Introduction

*Diaphorina citri* (Liviidae) (Hemiptera: Sternorrhyncha) is a recent addition to the increasing count of insects for which biotremology, the study of vibrational communication signals, has been applied in development of trapping or mating disruption applications (Čokl and Millar 2009; Mazzoni et al. 2009, 2017; Mankin

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R. Mankin (✉)

US Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, USA  
e-mail: [Richard.Mankin@ars.usda.gov](mailto:Richard.Mankin@ars.usda.gov)

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2012; Hofstetter et al. 2014, Polajnar et al. 2016; Nieri et al. 2017; Gordon et al. 2017). Vibrational communication has a long history of study in the Sternorrhyncha, including pioneering research by Ossiannilsson (1950), who reported that *Trioza (Bactericera) nigricornis* Förster communicates with wing vibrations. Courtship duets consisting of male vibrational calls followed by female vibrational replies have been characterized for several other Sternorrhynchans in subsequent studies, including those by Tishechkin (2006), Lubanga et al. (2014, 2016), Eben et al. (2015), Liao and Yang (2015, 2017), and Liao et al. (2016).

Because of the urgent need to develop improved methods of detecting and controlling *D. citri* in citrus groves (Sétamou et al. 2008; Hall et al. 2012), the possibility of vibrational communication in *D. citri* was investigated by Wenninger et al. (2009), who recorded and acoustically characterized male and female courtship duets. The recordings (Rohde et al. 2013) as well as synthetic mimics (Mankin et al. 2013) then were considered for their potential to elicit female replies and male searching behavior in citrus trees. These initial studies led to development of prototype signal-mimicking devices that disrupted mating (Lujo et al. 2016; Mankin et al. 2016b) and attracted male *D. citri* to traps (Hartman et al. 2017).

As the technology was being developed, it became apparent that successful deployment of *D. citri* vibrational mating disruption and trapping applications in citrus groves would be highly dependent on the structural features of trees, which affected transmission of courtship signals as substrate vibrations through branches, shoots, and leaves (Mankin et al. 2018a; Krugner and Gordon 2018). In addition, behavioral observations suggested that such applications would benefit not only from understanding and co-opting of mating behavior but also from co-opting of social (Kennedy et al. 1967; Lin 2006), flush-seeking (e.g., Sétamou et al. 2016), and phototactic behaviors (e.g., Sétamou et al. 2011). Finally, it became apparent that mating and dispersal behavior of both sexes are altered by weather patterns, barometric pressure, and other abiotic factors (Zagvazdina et al. 2015; Martini and Stelinski 2017; Udell et al. 2017). Implications of these behavioral and abiotic factors for deployment of mating disruption and trapping applications against *D. citri* and other insects are considered in sections below.

## 20.2 Spectral and Temporal Characteristics of *D. citri* Vibrational Communication Signals

As is frequently observed in the Sternorrhyncha, *D. citri* courtship includes vibrational communication signals produced by wing vibrations in a duetting pattern of male calls and female replies (Wenninger et al. 2009). The spectra of both male and female signals contain multiple harmonics of the 170–250 Hz wingbeat frequency (Wenninger et al. 2009; Mullen et al. 2016). Bae and Moon (2008) suggest that the flapping motion produces the fundamental frequency and the higher harmonics are produced by vortex scattering from the edge of the wing. Due to its small

size, *D. citri* produces only weak acoustic signals, but the wingbeat vibrations are transmitted through the legs to the leaves, stems, and branches of the host tree as substrate vibrations that conspecifics (or commercially available vibration sensors) can detect over distances up to 1–2 m on the same tree (e.g., Ichikawa 1979; Michelsen et al. 1982). Several other members of the Psylloidea have rows of teeth on the axillary cords of the wing mesoscutellum and metascutellum that serve as a stridulatory organ for sound production (Heslop-Harrison 1960; Taylor 1985; Tishechkin 1989). Stridulatory organs are known to produce signals with high-amplitude fundamental frequencies and weak harmonics (e.g., Mankin et al. 2009). Because such spectral patterns are not observed in its signals (Mankin et al. 2016b), *D. citri* likely uses only wing-flapping and not stridulation as a sound-production mechanism.

Male *D. citri* initially signal advertisement calls intermittently to determine whether females are nearby. When a receptive female replies to a male call, typically within 0.3–1.2 s (Wenninger et al. 2009), the male initiates searching behavior, walking toward her and continuing to call at intervals of  $9 \pm 1.4$  s (mean  $\pm$  standard error [SE]) during the  $15.9 \pm 2.38$  min period needed to find her (Lujó et al. 2016). For males actively searching along branches, movement speeds typically are  $>9$  mm/min (Zagvazdina et al. 2015) between bifurcations and other transition points at which they pause, call again, and then move forward or change direction (Lujó et al. 2016; Hartman et al. 2017). The durations of male calls have been observed to range from 148 to 544 ms, and the durations of female replies from 331 to 680 ms (Wenninger et al. 2009).

There has been considerable investigation of the capability of insects to localize the directions and sources of vibrational signals (e.g., Virant-Doberlet et al. 2006). The important factor determining whether a psyllid can localize the direction of the source is whether it can stretch its legs enough apart or place them on different branches in a way that provides enough amplitude and/or time of arrival difference for the central nervous system to distinguish the difference (Virant-Doberlet 2004; Čokl et al. 2006; Virant-Doberlet et al. 2006). The ability to localize direction has been experimentally verified in Hemipterans (Čokl et al. 2006; Virant-Doberlet et al. 2006), sand scorpions, *Paruroctonus mesaensis* (Stahnke) (Scorpiones: Vaejovidae) (Brownell and Farley 1979), and *Macrotermes natalensis* (Haviland) (Isoptera: Macrotermitinae) (Hager and Kirchner 2014).

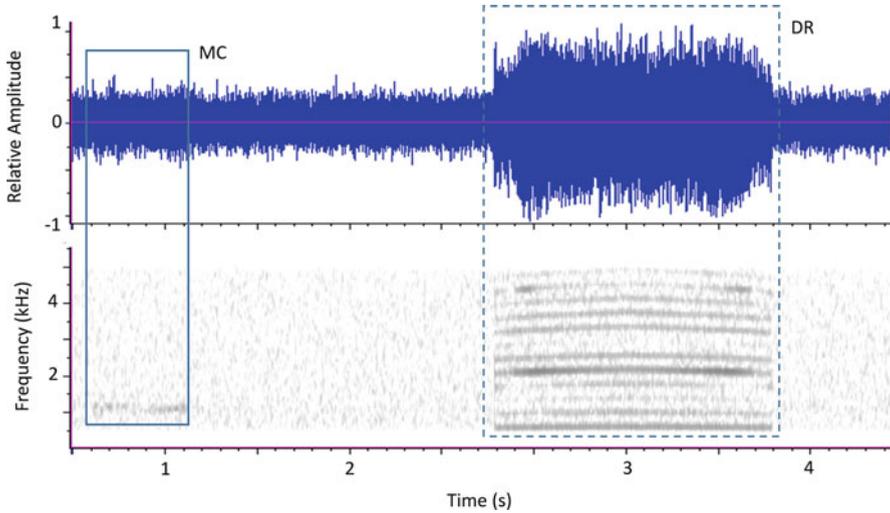
The large variations observed in call and reply durations, as well as in the relative amplitudes of different harmonics in male and female signals, suggest that these parameters are not under selection for species discrimination and that the presence of multiple harmonics of the wingbeat frequency in the duetting signals may be sufficient for species identification (Mankin et al. 2016b). The ability of the *D. citri* central nervous system to identify the fundamental frequency and its harmonics in vibrational signals has not been determined, but it is known that mosquitoes (Mankin 2012; Simões et al. 2016) and mammals (Simmons and Simmons 2011) can do so. The consistently short,  $<1.4$  s, interval between the end of the male call and the female reply (Wenninger et al. 2009) also may be a potential indicator of species recognition. Preliminary studies (Rohde et al. 2013) suggested that the

percentages of male searching in response to female replies decreases as the interval increases above 0.4 s. The duration of the interval between call and reply also has been reported to affect searching behavior in a leafhopper, *Aphrodes makarovi* Zachvatkin (Kuhelj et al. 2015).

### 20.3 Development of a Prototype Device to Mimic and Interfere with Vibrational Communication Signals

The first devices used to produce *D. citri* vibrational communication signals for behavioral studies were vibration exciters controlled by laptop computers that played back recorded or synthetic signals (Rohde et al. 2013). However, laptops, vibration exciters, and many other devices commonly used to assess and broadcast vibrations in the laboratory are difficult or costly to employ in field environments (Cocroft and Rodríguez 2005; Mankin et al. 2010, 2011). A search for portable, low-cost vibrational signal processing and playback devices led to consideration of a battery-powered, 8-bit-microcontroller platform (Arduino Uno, Arduino Inc., Ivrea, Italy) connected to a circuit board with amplifiers for an electret microphone (Model WM-63GNT, Panasonic, Newark, NJ) and a piezoelectric buzzer (9S3174, Taiyo Yuden, Tokyo, Japan). The microphone was clamped to the trunk or branch of a citrus tree to detect male *D. citri* vibrational signals. The piezoelectric buzzer was clamped 10–50 cm away on the tree to broadcast vibrations of synthetic mimics of female replies. It was found that use of either a recorded female reply or a synthetic female reply (Fig. 20.1) usually stimulated the tree structure to produce several additional vibrational harmonics that were not present in the original signal. However, these additional harmonics did not reduce either the male or female responses in comparison to their responses to vibrations produced by conspecifics (Rohde et al. 2013). Indeed, the additional harmonics possibly help mask harmonics of actual female replies.

It was anticipated that the microcontroller could be programmed to discriminate male calls from background noise automatically, and then broadcast a female reply mimic within 0.4 s to optimize the likelihood that the calling male would be attracted to the broadcast source. One way to initiate the process of signal discrimination was to compare spectrograms of incoming microphone signals with average spectrograms (profiles) of known male calls, accepting signals whose squared spectrogram amplitudes sufficiently matched the profile in the call's frequency range of greatest energy (Mankin et al. 2016b). An alternative would have been to adopt a procedure such as that used by Korinšek et al. (2016) that programmed a larger, 32-bit microcontroller platform (ARM Cortex M4, ST Microelectronics, Geneva, Switzerland) using more powerful algorithms, such as those based on linear prediction Cepstral coefficients and multilayer perceptron classifiers to distinguish male calls from background noise.

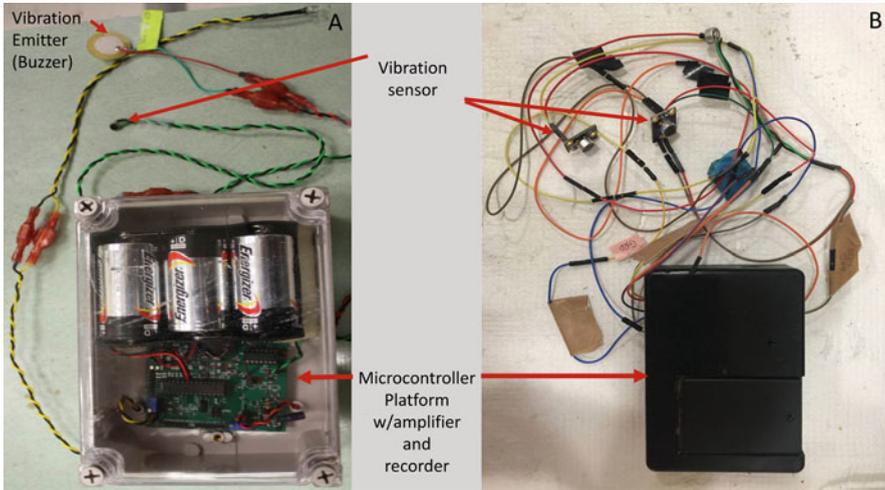


**Fig. 20.1** Example of male call (in solid box, MC) that was detected by the prototype device, which then produced a synthetic female reply (in dashed box, DR)

The microcontroller was programmed to calculate a 128-point spectrum every 0.1 s from 256 time points sampled at 8 kHz. The spectrum was calculated using an open-source, Fast Hartley Transform algorithm (Bracewell 1984). Each time the spectrum amplitude rose above a preset threshold, the program inspected the last six spectra before the spectrum amplitude again fell below threshold. This 0.6 s duration was selected because it slightly exceeded the 0.15–0.59 s range of male calls reported by Wenninger et al. (2009). The six spectra comprised a spectrogram that could be compared against an average spectrogram (profile) of known male calls.

The male-call profile used for matching with incoming signals was constructed as an average spectrogram of a set of 460 calls recorded from six separate males on multiple citrus trees in the laboratory. Most of the non-background energy in these calls appeared between 600 and 2000 Hz, so only these frequencies were used in the spectrogram-matching process in the final version of the noise discrimination algorithm (Mankin et al. 2016b).

Previous experience with vibrational signals in field environments (Mankin et al. 2011), as well as reports about background noise in other field studies (Barth et al. 1988; Cornell and Hawkins 1995; Tishechkin 2007; McNett et al. 2010), suggested that the presence of wind-induced noise, vehicular noise, and bird calls would cause the greatest interference with automated identification of male calls. To consider the background noise in relation to *D. citri* communication vibrations, two 40-min recordings were collected simultaneously with an accelerometer and the prototype device from different *D. citri*-infested trees in an area with moderate interference from traffic and bird noise. Listeners identified individual signals from the record-



**Fig. 20.2** (a) Original field-test version of prototype device showing microcontroller and batteries inside a moisture-proofed container, a microphone used to detect the signal that will be clamped to a branch or trunk of the citrus tree, and a vibration source that is clamped in the upper canopy of the tree; (b) a newer prototype with two sensors to cancel background noise

ings as male calls or noise and compared them with the predictions of the prototype device-discrimination algorithm. The listener vs. algorithm predictions about the above-threshold signals suggested that the algorithm had accepted incorrectly a high percentage of background noise signals (false positives). Consequently, the profile-matching process was optimized further by weighting specific spectral and temporal pattern features using a Matlab genetic algorithm (MathWorks, Natick, MA), described in Mankin et al. (2016b), that maximized the squared differences between the male call profile and the spectrograms of background noise. The optimized procedure was found to identify 77% of listener-identified *D. citri* calls correctly in the 40-min recordings of calls and background noise collected in infested trees, with 26% misidentification of background noise as *D. citri* calls.

The synthetic female reply mimic selected for broadcast by the prototype device was one that had been used successfully to elicit female behavioral responses (Mankin et al. 2013) and had been found also to elicit male searching behavior (Mankin et al. 2016b). The interval between the end of the male call and the initiation of the broadcast was governed primarily by the speed of the signal acquisition and discrimination algorithms and usually was <0.4 s. The signal measured on the tree at 10 cm distance from the buzzer was 1–5-fold greater than the typical amplitude of a female reply (Lujo et al. 2016).

Several devices of this prototype version were tested for efficacy of applications for *D. citri* mating disruption and male trapping. Figure 20.2 shows examples of two devices constructed for field use. The initial study to collect *D. citri* signals and background noise in the field was conducted using the device in Fig. 20.2a.

### 20.3.1 *Mating Disruption Bioassays with Prototype Device*

A series of tests additionally monitored by video and audio equipment was conducted to compare *D. citri* natural courtship behaviors with behaviors in the presence of disruptive signals broadcast by the prototype device (Lujó et al. 2016). Virgin males and females were placed on different branches of small citrus trees and the prototype system platform was attached lower on the trunk, with broadcasting either on or off. Times of calls, replies, and other behaviors were noted for analysis. The mating percentage was significantly lower in 1-h tests where the prototype device broadcast a reply mimic immediately after the male called (Lujó et al. 2016). In control tests without prototype device broadcasts, males were observed searching for replying females by walking rapidly along the branch, pausing, calling, and then walking forward or reversing direction. The pauses usually occurred at the end of a leaf or branch, a bifurcation, or other visually identifiable transition point. After pausing, the male frequently (although not always) walked toward the female reply. The duration before the male reached the female varied considerably, but on average was 15.9 min, and mating occurred in 57% of controls (Lujó et al. 2016).

In contrast, when the prototype device broadcast reply mimics, as in the example of Fig. 20.1, a majority of the males walked toward and remained near the synthetic signal source instead of walking toward the actual female's reply. The duration before reaching the female was significantly greater than in the control, 24.13 min, and mating occurred in only 13% of disruption tests (Lujó et al. 2016). It should be noted that the female often stopped replying after the buzzer had broadcast 1–3 reply mimics in response to the male call, which further reduced the likelihood of the male finding the female (Lujó et al. 2016). This phenomenon was observed also with *D. citri* by Wenninger et al. (2009), and with *Scaphoideus titanus* Ball by Mazzoni et al. (2009). Altogether, the observations suggest that the percentage of mating was reduced by one of the following three factors (or combinations thereof): (1) interference of an earlier and louder signal mimic more attractive to the male than the actual female reply; (2) masking of the female reply by the louder signal mimic; and (3) reduction of female responsiveness in the presence of other female replies.

### 20.3.2 *Vibration Trap Bioassays with Prototype Device*

A second series of 1-h tests using the prototype as a female mimic to attract virgin males was conducted on small citrus trees without a female present (Hartman et al. 2017). As in previous observations of natural courtship (Lujó et al. 2016), males which produced an advertisement call often began searching in response to the female reply mimic. They stopped briefly at transition points, called again, and then continued forward or reversed direction, frequently walking closer to the source of the reply mimic. Forty-five percent of males that initiated searching located the

source (Hartman et al. 2017), only slightly below the percentage that mated in natural courtship (Lujo et al. 2016). In addition, the mean latency before initiation of calling and searching was significantly lower for those males that reached the broadcast source than for those that missed it.

The result that calling and searching latency were lower for successful males supports a hypothesis that the trapping efficiency may be affected by variability in male responsiveness to searching cues. Other reports of male responsiveness variability include Stockton et al. (2017a), who found variability in courtship behaviors of blue and orange color morphs. Variability in searching behaviors of male *D. citri* was observed by Zagvazdina et al. (2015), who noted effects of weather on the proportions of males who moved either  $>$  or  $<$  9 mm/min toward the synthetic female reply from the prototype device. To place this in behavioral context, we note that *D. citri* have been reported to walk toward different types of attractive light sources at rates of 10–80 mm/min (Paris et al. 2017).

Other behavioral factors may affect trapping efficiency also. Several factors are discussed in Sect. 20.5 below.

### ***20.3.3 Extension of Prototype Device Usage to Additional Pest Detection Applications***

A potential use of the prototype device that remains to be addressed is the detection and identification of insect pests without directly trapping them. Early detection of pest presence or identification of particular insect species is often an important use of acoustic technology but the cost and portability of currently available sound and vibration detection devices makes them difficult to use in large-scale field applications (Mankin et al. 2011; Potamitis and Rigakis 2015). In preliminary studies, the low-cost Arduino Uno microcontroller/amplifier platform has been tested in the field to consider its utility for detecting internally feeding stored product insects and hidden infestations of insects in wood. However insect-produced signals detected by device were less easily distinguished from background noise than signals recorded from commercial equipment. For this reason, recent tests also have been conducted using a 32-bit platform operated by an Atmel SAM D20 (Atmel Corp., San Jose, CA) microcontroller (Fig. 20.2b). The system includes a vibration sensor and amplifier to detect the insect feeding and movement vibrations, an SD memory card to record incoming signals, and software to discriminate insect-produced signals from background noise.

A rationale for use of a larger microcontroller platform for detection of insect vibrational signals in field environments is the improved capability to discriminate insect signals from background noise that is provided by the larger memory and the higher resolution analog-digital converter. Identification of particular insect species using either the Arduino or Atmel platform would depend on previous knowledge of empirically determined spectrum profiles of their vibrational signals. However,

the capability provided by the larger microcontroller to employ linear frequency Cepstral coefficients, Gaussian mixture models, probabilistic neural networks, and multilayer perceptron classifiers (Bimbot et al. 2004; Ganchev and Potamitis 2007; Lampson et al. 2013; Korinšek et al. 2016) enables more powerful analysis and interpretation of the spectral and temporal patterns of insect-produced vibrations in trees (Mankin et al. 2016a, 2018a, b), stored products (Njoroge et al. 2016), and soil (Mankin et al. 2007), in addition to the spectral and temporal patterns of *D. citri* vibrational communication signals. Consequently, a third-generation prototype is now under development, designed to reduce costs and improve interpretation of insect-produced vibrations in field environments.

## 20.4 Host Plant and Abiotic Factors That Influence *D. citri* Vibrational Trapping and Mating Disruption

*Diaphorina citri* mate and develop on multiple plant species of different sizes and structural architectures in the rutaceous subfamily Aurantioideae (Halbert and Manjunath 2004) in a wide variety of geographical regions and environmental conditions (Liu and Tsai 2000; Nava et al. 2010; Hall et al. 2011, 2012; Grafton-Cardwell et al. 2013). Differences among these architectures result in considerable variation in vibrational signal amplitudes and in behavioral responses (Cocroft et al. 2006). Transmission of vibratory energy in trees, for example, depends on the natural frequencies, damping ratios, and modal shapes, which are determined by the mass distribution, stiffness, and morphology of the tree (e.g., Castro-Garcia et al. 2008; Mortimer 2017; Mankin et al. 2018a). Consequently, the amplitude of vibrational signals produced by either a female reply or a synthetic reply mimic does not attenuate uniformly with distance from the source. The vibration active space (Mazzoni et al. 2014) is a network of one- or two-dimensional spaces including the trunk, primary limbs, secondary branches, and twigs, which modulate vibration amplitude at each bifurcation point. Improved knowledge of such factors (Du et al. 2014; Gupta et al. 2015) may prove useful when attaching piezoelectric buzzers or waveguides operated by minishakers (Polajnar et al. 2016; Gordon et al. 2017; Krugner and Gordon 2018) to broadcast disruptive signals within individual trees or vineyard rows.

In addition, *D. citri* populations are known to be affected by elevation, and were found to be absent at elevations above 600 m in Puerto Rico, although citrus is found at those elevations (Jenkins et al. 2015). In future studies, it will be worthwhile to consider how environmental factors like elevation and biotic, host plant factors might affect implementation of vibrational trapping and mating disruption applications.

Previous studies of *D. citri* biology have found that adults and nymphs have the highest percentage of survival and greatest reproductive capacity between 11 and 28 °C (Liu and Tsai 2000). It is not certain whether temperatures outside the

ideal range would directly affect mating behavior, or if they would significantly affect physiological processes that result in impairment of mating behavior. It should be noted, however, that extremes of weather have been found to reduce *D. citri* abundance (Catling 1970), as is commonly observed in insects when heavy winds or precipitation reduce foraging success or impair ability to mate (Cornell and Hawkins 1995). Such extremes typically are preceded by strong variation in barometric pressure (Zagvazdina et al. 2015), and it has been reported that mating and phototactic behaviors, as well as dispersal behaviors, are affected by barometric pressure variations. Mating behavior decreased but phototaxis increased with increasing barometric pressure (Zagvazdina et al. 2015), and dispersal increased with increasing barometric pressure (Martini and Stelinski 2017).

## 20.5 Behavioral Factors Influencing *D. citri* Vibrational Trapping and Mating Disruption

Both male and female *D. citri* exhibit phototaxis (Sétamou et al. 2011; Anco and Gottwald 2015) either by walking (Pregmon et al. 2016; Paris et al. 2017) or flying (Paris et al. 2015). Both sexes also are attracted to host plant odors (Hall et al. 2015; Beloti et al. 2017) and new leaf flush (Catling 1970; Hall and Albrigo 2007; Patt and Sétamou 2010; Sule et al. 2012; Sétamou et al. 2016). Mating occurs during photophase, primarily on new leaf flush (Wenninger and Hall 2007), which typically appears on the tree periphery. Part of the attractiveness of flush may be due to the limited ability of nymphs to probe through the thicker structure of leaf veins of older citrus leaves, compared to new flush (Ammar et al. 2013). Placement of traps near attractive phototactic or host odor cues may enhance trap effectiveness. It should be noted, however, that although females are attracted to feeding-damaged plants, they prefer uninfested leaves when they land at the tree (Martini et al. 2014).

Finally, it is relevant to note also that male *D. citri* alter their calling behaviors when they detect female odor (Wenninger et al. 2009) or male calls (unpublished, and observed also in *A. makarovi* Kuhelj and Virant-Doberlet 2017). Males display adaptive plasticity and “learn” to be attracted to female odors after they have experienced mating (Stockton et al. 2017b).

The effects of adaptive learning and biases toward female odor, flush, the tree periphery, and light are likely to be significant factors in the success of efforts to apply vibrational signals to disrupt *D. citri* mating. Such effects should be addressed in detail to optimize placement of vibration traps or sources for mating disruption broadcasts.

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