

Vibrational Communication Between the Sexes in *Diaphorina citri* (Hemiptera: Psyllidae)

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ABSTRACT We examined the substrate-borne vibrational signals used in communication between the sexes in *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a vector of huanglongbing (an economically devastating disease of citrus), in an anechoic chamber and an olfactometer. Males and females both primarily produced simple, low-amplitude vibrational signals at multiples of 170–250 Hz, ranging in duration from 140 to 700 ms. The vibrational frequencies of males and females were negatively correlated with mass, but the correlation was statistically significant only for males. Females replied to male calls within an interval of 0.3–1.2 s. Such signals are within the ranges of signals observed for other psyllids. Female–female interactions were occasionally observed. Intervals between male calls were not significantly different whether or not females replied. In an olfactometer, calling rate did not differ between virgin males exposed to odors from virgin females on citrus versus those exposed only to clean air. However, the latent period for initialization of calling was significantly shorter for males exposed to clean air, suggesting that in the absence of olfactory cues psyllids might be more inclined to use acoustic signals to communicate with conspecifics. Moreover, calling rate and latency to initialization of calling were positively and negatively correlated, respectively, with male age, suggesting that males are more likely to call the longer they remain unmated.

KEY WORDS Asian citrus psyllid, acoustic communication, citrus greening disease, huanglongbing

The psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) vectors three phloem-restricted, noncultured bacteria in the genus *Candidatus Liberibacter*, the causative agents of huanglongbing (citrus greening disease) (Halbert and Manjunath 2004, Hung et al. 2004), one of the world's most important diseases of citrus (Bové 2006). Citrus trees infected by this disease may live only 5 to 8 yr, during which time they produce misshapen, poorly colored, bitter-tasting unmarketable fruit (Halbert and Manjunath 2004, Bové 2006). First found in Florida in June 1998 (Tsai et al. 2000), *D. citri* has since spread throughout the state's citrus-growing regions (Michaud 2004). Huanglongbing was first found in southern Florida in August 2005 (Halbert 2005). In Florida, *Candidatus Liberibacter asiaticus* is the only bacterial agent of the disease and *D. citri* the only competent vector known to occur; *Trioza erytreae* (Del Guercio) is an Old World vector of huanglongbing that is not found in the United States (Bové 2006). Despite the great economic importance of *D. citri* as a vector of huanglongbing, detailed in-

vestigations into the reproductive biology and behavior of this pest have only recently been conducted (Wenninger and Hall 2007, 2008a,b; Wenninger et al. 2008).

Within the Psylloidea little detail is known regarding how reproductively mature individuals attract or locate prospective mates. Female sex pheromone may play a role in the pear-feeding psyllas *Cacopsylla bidens* (Šulc) (Soroker et al. 2004) and *Cacopsylla pyricola* (Förster) (Horton et al. 2007, Horton and Landolt 2007) and in *D. citri* (Wenninger et al. 2008). Given that mating rates in some psyllid species are reduced when held in darkness (Krysan 1990, Wenninger and Hall 2007), visual cues may also be important for orientation to mates—at least at shorter distances. Visual cues, chemical cues, or both from host plants might be involved in orientation to prospective mates as well. For example, males might orient to plants or specific parts of plants before searching locally for females (as in a leafhopper; Hunt and Nault 1991), and feeding by females on plant tissue might alter the odors emitted by plants, resulting in male attraction (as in a scarab beetle; Heath et al. 2002).

Substrate-borne vibrational signaling is involved in mate location within the Auchenorrhyncha and Sternorrhyncha (Kanmiya 2005, Miranda 2006, Percy et al. 2008), including many psyllid species (Tishechkin 1989, 2005, 2007; Percy et al. 2006). A psyllid produces substrate vibrations by orienting its wings in the resting position (held “roof-like” over the body) and rap-

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idly moving them up and down in an almost vertical plane (Tishechkin 2005). In the psyllids studied to date, males are reported to produce vibrations (i.e., call) spontaneously, whereas receptive females are reported to call only in response to calling males (Taylor 1985; Tishechkin 2005, 2006), remaining stationary while the male searches the plant.

Tishechkin (2007) stated that psyllids cannot locate the source of vibrational signals because the amplitude of vibratory oscillations in plant stems do not show a regular pattern of weakening with distance traveled (Michelsen et al. 1982), but Virant-Doberlet and Čokl (2004) argued that the relatively low propagation velocity of low-frequency bending-wave vibrational signals provides sufficient temporal cues to enable even a small insect to identify which leg first receives vibrations. Indeed, many insects have been shown to successfully orient to the source of vibrational signals (Virant-Doberlet et al. 2005).

Preliminary experiments with *D. citri* indicated that males call readily in an olfactometer (Wenninger et al. 2008). In addition, there is mounting evidence that female *D. citri* produce a volatile sex attractant that plays a role in male orientation to females (Wenninger et al. 2008). The use of both acoustic and chemical cues in mate location has been found in a handful of species but across a range of taxa, indicating that the use of multiple signal types might be more common than current data suggest. The southern green stinkbug, *Nezara viridula* (L.), has been found to use a sex pheromone for long-range communication and vibrational signals for short-range communication (Borges et al. 1987, Virant-Doberlet and Čokl 2004). Sex pheromone and vibrational signals also are both involved in mate location in the goldeneye lacewing, *Chrysopa oculata* Say (Zhang et al. 2004). The fact that vibrational signaling in psyllids often does not precede successful pairing (Tishechkin 2005, 2006, 2007; E.J.W., unpublished data) suggests that other cues in addition to acoustic signals may be important for mate location in this group of insects.

For this report, we characterized the substrate-borne vibrational signals produced by *D. citri* and examined the potential role of odors from virgin females on citrus in influencing male calling behavior. We hypothesized that odors from females would decrease the latent period to initialization of calling and increase the calling rate of males. The goal of these studies was to enhance our understanding of the mating behavior of this species, particularly regarding how males locate reproductively mature females.

Materials and Methods

Study Organism. All adult psyllids used in experiments were collected as fifth-instar nymphs from a laboratory colony reared on *Murraya paniculata* (L.) Jack at the United States Horticultural Research Laboratory, as described by Hall et al. (2007). We transferred nymphs individually to 'Duncan' grapefruit (*Citrus paradisi* Macfayden) seedlings (2–3 leaf stage) caged in plastic vial containers, described in Wen-

ninger and Hall (2007). Briefly, each cage consisted of a 52 mm-tall vial, modified as an open-ended cylinder with a foam plug used to stopper the top opening and two ventilation holes on the sides; individual cages were slipped over a seedling grown in a cone-shaped planting container. Until ready for use in experiments, adult psyllids were held in an environmental chamber at 26°C, 60% RH, and a photoperiod of 14:10 (L:D) h, which resulted in 70–80% RH inside the vials. All psyllids used in experiments were between 4 and 8 d posteclosion, with specific age ranges indicated below for each experiment.

Observation Arena. In an initial study, we observed six different male/female pairs in a vibration-shielded anechoic chamber at the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL (Mankin et al. 1996) maintained at 25–26°C and 60% RH. Psyllids were placed on individual small, potted Duncan grapefruit plants (\approx 25 cm tall) with a 3.78-liter clear plastic container inverted over the plant to prevent escape. A subsequent study included 15 different male/female pairs (total $N = 21$ male/female pairs). All psyllids used for recordings were 5–7 d posteclosion, and all individuals were recorded only once. In the second study, after acoustic recordings we froze each individual and recorded the body mass (model UMT2, Mettler-Toledo Corp., Columbus, OH).

For most recordings an individual female was introduced to a plastic container and allowed to settle on the plant in the feeding position before a male was added. Multiple females were placed on one plant, however, for several observations of female/female interactions and interactions among single males with multiple females. All plants used were immature, with a nonwoody stem and a flush shoot (which is the site of oviposition and mating; Wenninger and Hall 2007) at the terminal bud. A 100-W floodlamp was set \approx 1 m above the plant, and the activities of the psyllids were recorded simultaneously with a videocamera (model HDR-SR1, Sony Corp., New York, NY) and a vibration sensor.

Vibrational Communication Recording and Analysis. The vibration sensor was an 11-g accelerometer (model 4371, Brüel and Kjær [B&K], Naerum Denmark), attached by an alligator-clip system (Mankin et al. 2004b) at about the midpoint of the stem of the grapefruit plant. The signals were amplified 40 dB by using a B&K model 2635 charge amplifier and band-pass-filtered between 0.1 and 7 kHz before being digitized (time-sampled) at 44.1 kHz by using a CSL speech analysis system (model 4300B, Kay Elemetrics Corp., Lincoln Park, NJ). The digitized (.wav) files were analyzed using Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY) or a custom-written sound analysis program, DAVIS (Mankin 1994, Mankin et al. 2000). Spectrograms were calculated in Raven by using 4096-sample (92.9 ms) or 512-sample (11.6 ms) Fast Fourier Transforms (FFTs), depending on the duration of the insect sound pulses.

Power spectrum (spectral) profiles (Mankin et al. 2000) of male calls and female replies in duetting bouts

were constructed in DAVIS by calculating 512-sample FFTs of 10 male calls and 10 female replies for each bout and then calculating the mean spectrum level at each FFT frequency. The background spectral profile was calculated in DAVIS as the mean spectrum level at each FFT frequency, averaged over a 10-s period of background noise. Vibration level was measured as acceleration in dB referenced to a threshold of 10^{-6} ms^{-2} (Beranek and Ver 1992) between the frequencies of 0.1 and three kHz (written as dB/0.1–3 kHz). The fundamental frequency (FF) was estimated as the mean difference in the frequencies of the first three spectral peaks, similarly to the procedure used in Raman et al. (2007). More details of spectral analysis procedures, as well as details of the procedures for measuring the times for beginnings and endings of call durations, reply durations, and intervals between calls and replies, are listed in Mankin et al. (2000).

Olfactometer Assay. We compared the calling rate of psyllids in a glass “I-tube” olfactometer (Analytical Research Systems, Gainesville, FL) when exposed to either clean air or to odors from virgin females on grapefruit. The I-tube consisted of a 10-cm-long tube (2 cm i.d.) with a metal screen at the upwind end that prevented psyllids from exiting the tube. A screened glass plug at the downwind end provided entrance into the I-tube. Charcoal-filtered, humidified air was metered at 500 ml/min through the I-tube by using Polytetrafluoroethylene (PTFE) tubing. The air exiting the wire screen plug at the base of the stem was 26–28°C and 70–85% RH; light intensity just above the I-tube was $\approx 7,000$ lux. To introduce odors to the I-tube chamber, we routed the PTFE tubing through a 1-liter glass canning jar that held five to six virgin females age 4–7 d posteclosion on a potted Duncan grapefruit plant with flush. We wrapped Parafilm M (Pechiney Plastic Packaging Inc., Chicago, IL) around the pot up to the base of the stem of the plant to limit soil odors from entering the I-tube. Female psyllids were allowed to settle on the plant for at least 5 h before use in experiments. When clean air was used as the odor source, PTFE tubing was routed through a clean, empty canning jar.

Male psyllids (age 3–8 d posteclosion) were released individually into the glass plug at the base of the I-tube, and the behavior of each male was observed over a 10-min period. We recorded the time to exit the glass plug and the time to first make contact with the metal screen at the upwind end of the I-tube, as well as the time elapsed before calling was observed and the total number of calling events that occurred. The behavior of individual male psyllids was observed in the I-tube in the presence of clean air ($N = 31$ individuals) or odors from virgin females on grapefruit ($N = 30$ individuals). A clean set of glassware was used for each replicate. Between assays, we soaked glassware and tubing in hot soapy water for 30–60 min, rinsed thoroughly with tap water and deionized water, and held all components overnight in a drying oven (150°C) before reuse.

We assessed male responses (time to exit plug at the base of the I-tube, time to contact the screen at the

upwind end of the I-tube, time to start calling, and total number of calls) to the two odor treatments by using analysis of covariance (ANCOVA) with male age as the covariate (PROC GLM, SAS Institute 2002). Data for the time for males to exit the glass plug were log transformed to achieve normality. Data are presented as mean \pm SEM.

Results

Call and Reply Signal Patterns. The calls of male *D. citri* detected in this study primarily were single, ≈ 0.1 –1-s broadband pulses (Fig. 1), similar in structure to calls by several of the *Trioza* species described in Percy et al. (2006) and Tishechkin (2006, 2007). The examples of typical signals, A–E, in Fig. 1 are shown in order of increasing interval between the end of the male call and the beginning of the female reply (Table 1). Male and female signals both had spectral peaks at multiples (harmonics) of a 170–250-Hz FF (Table 1; Fig. 1), which in other insects has been demonstrated to be the wingbeat frequency (see Discussion). The harmonics result from constructive and destructive interference of reflections off the boundaries of the sound-carrying structures (Hambric 2006). In this case, the sound-carrying structures include the vibrating wings and any associated sound-modulating structures on the psyllid body, as well as the plant stem and leaves). Although wing vibrations in isolation usually have “classical” harmonic patterns, with the highest energy (dominant) frequency (DF) at the wingbeat frequency (Mankin et al. 2004, Raman et al. 2007), interactions among different parts of complex sound-transmission structures like the body of a vibrating psyllid on a grapefruit plant can result in inverted harmonic patterns like those in Fig. 1, where the highest-energy (dominant) frequency (DF) occurs one or more harmonics above the FF. Similar inverted patterns also have been observed in vocalizations of birds (e.g., Fletcher et al. 2006) and frogs (e.g., Drewry et al. 1982). In comparisons across 21 different mating pairs, the mean \pm SEM of the median FF in the first 60-s interval recorded with at least five signals from each individual was 205.4 ± 3.6 Hz for the male call and 200.9 ± 3.1 Hz for the female reply. The difference between these frequencies was not significant ($t = 0.945$, $df = 40$, $P = 0.35$). The median frequencies were negatively correlated with mass (Spearman’s rho, $r_s = -0.570$ for 15 males, and $r_s = -0.226$ for 15 females), and the relationship was statistically significant for the males ($P = 0.027$) but not the females ($P = 0.418$) under testing in Jmp 4.0.2 (Sall et al. 2001).

In any given duet, each participant was distinguishable by differences in the order and spectral pattern of the signals. However, there was so much overall variation in male and female signals that it was not always possible to distinguish the signaler’s sex solely on the basis of the signal spectral and temporal properties, and the assignment of signals to individual participants was always checked by reference to the video records. The dominant frequency was usually, but not always, higher for the male call than the female reply

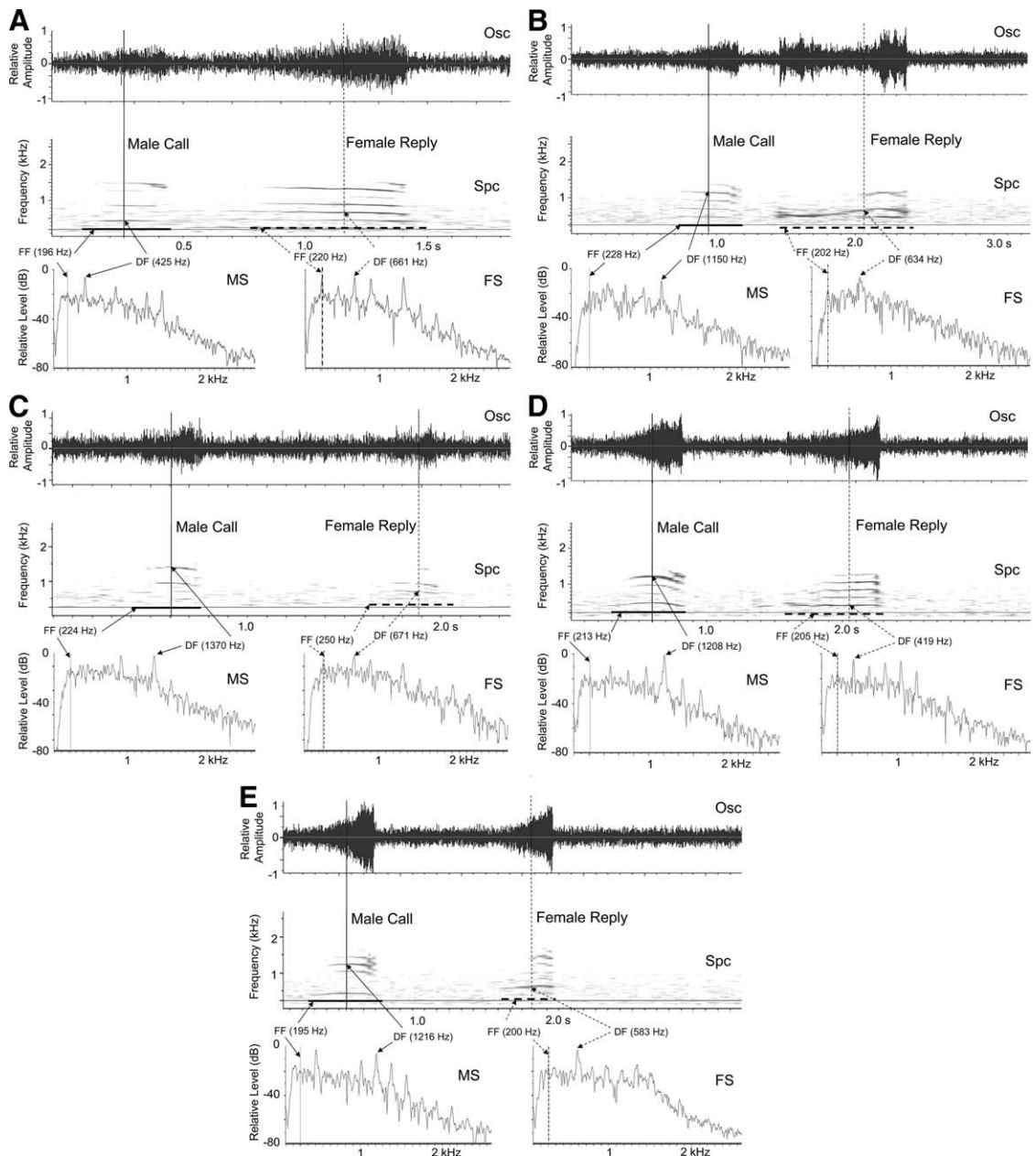


Fig. 1. Oscillograms (Osc), spectrograms (Spc), and spectra (MS, male call; FS, female reply) of signals from five different *D. citri* duetting pairs (A–E; see Table 1). The Osc and Spc are on the same time scale. Areas of darker shading in spectrograms (4096-sample FFTs) indicate higher relative energy at those frequencies and times. Solid and dashed vertical lines in Osc and Spc indicate the midpoint times for calculation of the 4096-sample FFTs MS and FS power spectra, respectively. Male fundamental frequency (wingbeat frequency, FF) is indicated by solid line in MS, and female FF is indicated by dashed line in FS. The FFs also are indicated by solid (for the male) and dashed (for the female) horizontal bars in Spc, with the length of the bars indicating the duration of the call and reply. The dominant frequencies (DFs; i.e., the frequencies of greatest energy) are marked by arrows on the MS and FS power spectrums, and are marked also where they occur on the frequency–time spectrograms (Spc).

(as in all the examples in Fig. 1 except A). The signals of both sexes typically increased in amplitude initially from near background levels to their highest amplitudes near the terminations, which usually were

abrupt (e.g., Fig. 1D and E). The signal-to-noise ratios were greatest in Fig. 1D and E, but multiple harmonics of the fundamental frequency were observable even for the weakest signal (Fig. 1C).

Table 1. Mean \pm SEM of signal durations, intervals between signals, and fundamental frequencies at the midpoints of (*N*) consecutive signals recorded in 60-s intervals of *D. citri* duetting bouts

Duetting pair (<i>N</i>)	Duration (ms)		Reply interval (ms)	Fundamental frequency (Hz)	
	Male call	Female reply		Male	Female
A (5)	170.2 \pm 27.0	680.0 \pm 123.1	290.7 \pm 98.5	212.8 \pm 6.4	220.4 \pm 1.3
B (7)	310.0 \pm 35.5	650.8 \pm 117.0	374.6 \pm 76.4	231.4 \pm 1.7	216.0 \pm 2.6
C (9)	148.4 \pm 61.1	331.2 \pm 71.4	863.0 \pm 73.5	234.6 \pm 5.8	242.0 \pm 7.2
D (8)	379.9 \pm 39.2	460.9 \pm 73.9	917.7 \pm 47.8	245.6 \pm 3.9	208.3 \pm 3.1
E (5)	544.3 \pm 117.1	361.3 \pm 61.7	1170.9 \pm 111.8	202.4 \pm 2.2	213.6 \pm 7.3

There was considerable variation among individual pairs in the durations and intervals of the male calls and female replies. In comparisons of 21 different pairs, the mean \pm SEM of the median duration of male calls in the first 60-s interval recorded with at least five signals from each individual was 0.283 ± 0.021 s, and the median duration of the female replies was 0.408 ± 0.051 s. The mean of the median intervals between the male call and female reply was 0.948 ± 0.089 s, whereas the median of the periods between consecutive male calls had a mean of 8.096 ± 0.410 s.

Because of the large variation in temporal patterns, it was not certain from initial observations of duets whether the intervals between successive calls by males were influenced by the presence or absence of a female reply as noted in Percy et al. (2006). To address this question, we identified the first occurrence in each of 19 bouts with different duetting pairs where two calls with an intervening female reply were followed immediately by a call without a female reply. The mean \pm SEM interval between calls was marginally greater when the female replied (8.44 ± 1.00 s) than when there was no reply (6.76 ± 1.17 s), but the difference between the intervals was not statistically significant (paired *t*-test; $t = -1.01$, *df* = 18, $P = 0.16$).

In addition to the signal variations observed in Figs. 1A–E for typical calls, it should be noted that atypical, multicomponent calls were observed also in several recordings. The multicomponent calls contained either three or four short pulses followed by a longer,

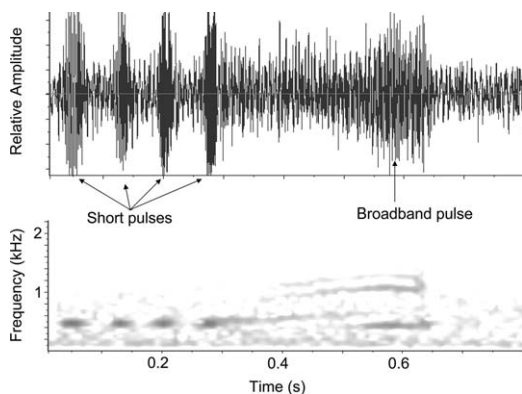


Fig. 2. Expanded oscillogram and spectrogram of a multicomponent male call consisting of four short and one longer, broadband pulse. Areas of darker shading in spectrograms (512-sample FFTs) indicate higher relative energy at those frequencies and times.

broadband pulse. An example of an atypical call is shown in Fig. 2.

Vibrational Communication Behaviors. All pairs were recorded for at least 5 min after initiation of copulation and two pairs were recorded for 15–20 min into copulation, but vibrational signals were never detected while psyllids were in copula. Moreover, no overt vibrational behavior was ever seen in numerous other mating pairs observed (E.J.W., unpublished data).

Females generally called in response to males but were occasionally observed to call spontaneously when not in the presence of males. In the few cases in which multiple individuals of the same sex were observed, calling by an individual elicited calling behavior in other individuals of the same sex. In such cases, calls were made either immediately after a first call or two or more individuals of the same sex almost fully overlapped their calls in a chorus.

Two types of female sounds were recorded in the absence of males (see examples in Fig. 3). In Fig. 3A, two females near each other on a grapefruit plant interacted briefly. A third female further away remained silent. In Fig. 3B and a female made an initial call, a second female replied, and the first female then responded. Both the reply and the response were very

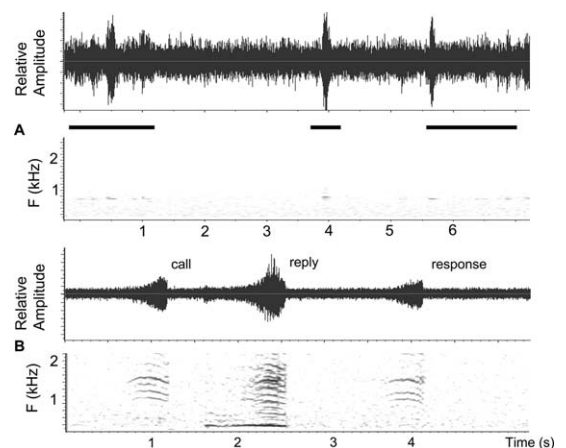


Fig. 3. Oscillograms and spectrograms of representative signals recorded from two separate trios of females on two different grapefruit plants: multiple weak, 700-Hz signals from two interacting females, with solid bars between oscillogram and spectrogram denoting detectable signals (A); and an initial call by a female followed by a reply from a second female and then a response by the first female (B).

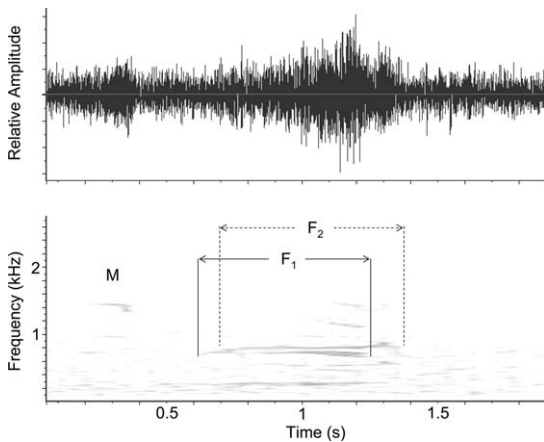


Fig. 4. Oscillogram and spectrogram of male call (M) followed by replies from two females (F_1 and F_2) on the same grapefruit plant.

similar to female replies to male calls in a mating duet. As in Fig. 3A, the third female remained quiet. When a male was present, multiple events occurred where two or more females replied to a male call, as in the example of Fig. 4.

Environmental Context of Duetting Bouts. Because *D. citri* adults are small (3–4-mm in length [Halbert and Manjunath 2004] and ≈ 0.4 – 0.5 mg [Wenninger and Hall 2008a]), their mating duets have very low signal to noise ratios, even in a vibration-shielded anechoic chamber. The small difference from background can be seen in Fig. 5, which displays spectral profiles of 10 male calls and 10 female replies in comparison with mean spectral levels of 10 s of background noise in the recording of the duet sampled in Fig. 1E. Over a 0.1- to 3-kHz range, the vibration level of the male call profile was 65 dB (relative to an acceleration of 10^{-6} ms^{-2}) compared with 63 dB for the female reply profile and 59 dB for background noise recorded from the same file.

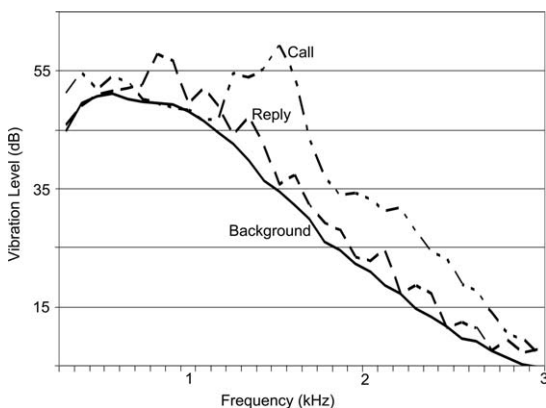


Fig. 5. Power spectrum profiles of 10 male calls, 10 female replies, and background noise from the record of duetting pair in Table 1E.

Olfactometer Assay. Of the 31 males observed for the control treatment, 29 exited the glass plug to enter the I-tube chamber, 24 contacted the screen at the upwind end of the I-tube, and 27 were observed to produce wing vibrations. The corresponding numbers for the 30 males exposed to odors from females on grapefruit were 30, 24, and 30.

The time elapsed before males exited the glass plug to enter the I-tube arena did not differ between males exposed to clean air (1.4 ± 0.4 min) versus those exposed to odors from virgin females on grapefruit (1.4 ± 0.3 min); nor did the time to exit vary according to male age (Table 2). Similarly, the time to first make contact with the metal screen at the upwind end of the I-tube did not differ by male age or between males exposed to clean air (3.9 ± 0.4 min) versus treated air (3.3 ± 0.4 min; Table 2). However, the time elapsed before wing vibration was observed was significantly shorter for males in clean air (2.3 ± 0.4 min) versus males in treated air (3.1 ± 0.4 min) and was also negatively correlated with male age (partial regression slope: -3.71 , $P < 0.001$; Table 2). The number of vibration events observed for each male did not differ by odor treatment (clean air: 18.2 ± 2.8 ; treated air: 20.9 ± 2.3) but was positively correlated with male age (partial regression slope: 3.51 , $P = 0.001$; Table 2).

Discussion

The frequency harmonics patterns of *D. citri* vibrational calls and replies were reminiscent of patterns seen in acoustic recordings of wingbeats of flying insects (Mankin 1994, Caprio et al. 2001, Moore and Miller 2002, Sueur et al. 2005, Raman et al. 2007) and calling fruit flies (Mankin et al. 2004a). The occurrence of such patterns suggests that the signals were generated at least in part by the wingbeats that occurred simultaneously in the video as the recordings were being collected.

The relative amplitudes of different spectral harmonics were often different between males and females. This is consistent with findings by Percy et al. (2006) that acoustic signals in psyllids are typically species and gender specific. However, some of the observed differences may have been due to differences in the orientation of the psyllids during sound production (Sueur et al. 2005) or to the differences in the sound transmission properties of the different paths traversed by the male and female signals (Cocroft and Rodríguez 2005). In the duetting bouts, the female usually was perched in the feeding position on the flush at the top of the main stem, which provided a relatively direct path for transmission of her signal vibrations to the accelerometer, which was clamped ≈ 12 cm below. However, the male often called from a leaf, which provided a less direct signal path to the accelerometer, and the quality of the signal changed noticeably as he moved along the leaf or shifted to a stem. The fundamental frequency of the male call, which is not affected by the signal path, was negatively correlated with male body mass, as has been observed for wingbeat frequency and body mass

Table 2. ANCOVA of each dependent variable examined in the I-tube olfactometer as a function of odor treatment (clean air or virgin females on grapefruit) and male age

Variable	Model				Odor treatment		Male age	
	df	F	P	r ²	F	P	F	P
Time to exit plug	2,58	1.1	0.329	0.039	0.07	0.787	2.3	0.138
Time to touch screen	2,47	0.64	0.532	0.028	1.1	0.297	0.03	0.872
Time to start calling	2,56	8.0	<0.001	0.229	4.4	0.042	13.7	<0.001
Total no. of calls	2,60	6.5	0.003	0.183	0.01	0.938	12.3	<0.001

of other insects (Darveau et al. 2005). Consequently, as has been suggested with tephritid flies (see Mankin et al. 2008), a female may have potential to assess male size by assessing the fundamental frequency of his call. Despite differences between the sexes in the relative amplitudes and spectral harmonics of signals, gender-specific differences observed in *D. citri* were not as pronounced as are often found in the Auchenorrhyncha, in which male signals are more structurally complicated than female signals (Čokl and Virant-Doberlet 2003).

Vibrational signals do not degrade rapidly with distance traveled on a plant stem (Michelsen et al. 1982), but the level of background noise in the natural environment often will be higher than typical insect signal levels due to wind noise (Cocroft and Rodríguez 2005, Mankin and Benshemesh 2006), other natural sources of vibration, and structural vibration caused by incident acoustic waves (Cremer et al. 1988, Hambric 2006). Under these circumstances, it is not surprising that the mating duets occur during photophase when visual cues may assist the male's search for the female or her feeding habitat. One could speculate also that broadcast of high-amplitude broadband sounds between 400 and 1,500 Hz to induce vibrations in the plant leaves and stems might interfere with communication in this species.

In the Psylloidea, receptive females are reported to call only in response to calling males (Taylor 1985; Tishechkin 2005, 2006). However, we recorded females calling in the absence of males, and, although rare, females have been observed to call on several other occasions without initialization from males (E.J.W., unpublished data). Because of the rarity of female initialization of calling in *D. citri*, it is unclear whether the behavior is truly nonexistent in other psyllids or simply has not yet been observed. Within the Auchenorrhyncha, females in some species have been reported to initiate calling, but female initiation is also rare in this group (Claridge et al. 1985, Čokl and Virant-Doberlet 2003, Virant-Doberlet and Žežlina 2007).

The results from our olfactometer assay show that calling rate of virgin males is positively correlated with age (at least for the ages we tested). However, male calling rate was not affected by the presence or absence of odors from females on grapefruit. Thus, we found no evidence to support the hypothesis that male calling behavior is elevated by the detection of female and/or host plant odors. However, latency to calling was significantly shorter for males observed in the presence of clean air. It may be that males are less inclined to call when they detect olfactory cues from

females. In many species that use vibrational signals, including *D. citri* (E.J.W., unpublished data) and other psyllids (Tishechkin 2007), calling by a male elicits a response from receptive females as well as conspecific males that may be competing for access to females. Therefore, a male that delays calling behavior until he has oriented closer to a female using chemical (and/or other) cues might limit intrasexual competition from eavesdropping males. It should also be noted that vibrational communication in insects may have other roles besides mate-location behavior, including locating food resources or a group of conspecifics (Cocroft 2001). Such factors might influence calling behavior for psyllids exposed only to clean air in the olfactometer.

Our simple olfactometer environment precluded the use of acoustic and visual cues that might be important in male orientation behavior to females. Therefore, the possible importance of interactions among psyllids and their host plants that may factor into mate location could not be addressed. For example, mating and oviposition in *D. citri* occurs almost exclusively on the flush of its host plants (Wenninger and Hall 2007); therefore, males in search of a mate might first orient to young shoots before searching locally for females. In the blackfaced leafhopper *Graminella nigrifrons* (Forbes), males use a "call-fly" strategy, and—after establishing a vibrational duet with a receptive female—positive phototaxis guides a male to the top of a plant, which is the preferred perching site of virgin females (Hunt and Nault 1991). Positive phototaxis and/or negative geotaxis in *D. citri* (Yasuda et al. 2005) might facilitate the orientation of males to reproductively mature females in a similar manner. Interestingly, for one duetting pair that we observed, the female settled not on the flush, but on a mature leaf, and—after 60 min of duetting with the original male and a second male that was added—the female was never found by either male.

The research presented here contributes to the understanding of the reproductive biology and behavior of *D. citri*. Studies on the role of a putative female sex attractant in mate location are ongoing (e.g., Wenninger et al. 2008); however, even if a sex pheromone is identified, for a species like *D. citri* in which vibrational communication is an important part of mate location, the use of pheromones for monitoring and/or management may be challenging. For example, possible future efforts to reduce mating rates by sex pheromone-based mating disruption or attract-and-kill might be compromised if the sexes are able to find each other using primarily acoustic cues. More work

is needed to clarify the relative roles of acoustic and chemical cues in mate location in this species as well as how mate location might be influenced by interactions among psyllids and their host plants.

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