

METHODS & TECHNIQUES

Vibrational playback by means of airborne stimuli

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SUMMARY

Substrate-borne vibrational communication is a common form of communication in animals. Current contact-based playback methods limit the number of substrates that can be stimulated simultaneously and potentially change the transmission properties of the substrate. Here, we explore a solution to these limitations by broadcasting airborne stimuli onto plant substrates to impart vibrational playbacks. We demonstrate that one can effectively compensate for the filtering properties of any individual plant across a range of frequencies. We then address how well both compensated broad-band and pure-tone stimuli for one plant individual apply to other individuals across days. Variation within and between plants was similar across the range tested but was quite variable at certain frequencies. Focusing on a subset of this range, at low frequencies, responses were flat across days and pure-tone frequency stimuli in this range were consistently transmitted despite repositioning of plants relative to the loudspeaker. Our results present a potential solution to researchers interested in exposing large samples of individuals to vibrational signals but also highlight the importance of validating the use of airborne stimuli as vibrational playbacks to the particular substrate type and frequency range of interest.

Key words: vibratory signal, seismic signal, transfer function, laser vibrometry.

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INTRODUCTION

As one of the most common forms of communication in animals (Hill, 2008), substrate-borne vibrational signaling is attracting attention from researchers interested in the ecology and evolution of behavior. Animals use a variety of substrates to transmit vibrational signals, and how these signals are transmitted depends on the properties of the substrate (Čokl and Virant-Doberlet, 2003; Cocroft and Rodríguez, 2005; Elias et al., 2010). For example, plants that are used as vibrational substrates vary in their transmission properties among species and individuals (Henry and Wells, 2004; Cocroft et al., 2006; Čokl et al., 2006; McNett et al., 2006; McNett and Cocroft, 2008; Čokl et al., 2009). While these attributes impose interesting challenges for animals to communicate effectively with one another, they pose equal challenges to researchers interested in studying vibrational communication.

Currently, vibrational playback methods are such that the playback device (e.g. shaker, actuator) must be in direct contact with the substrate (Cocroft and Rodríguez, 2005). While these contact-based methods are effective, they have the limitation that only one or a few substrates can be stimulated at any one time. If one wants to isolate individuals so that they experience only the treatment without addition of social interactions, the ability to stimulate several substrates simultaneously would be helpful. Additionally, by contacting the substrate, current vibrational playback methods can alter the transmission features of the substrate (Cocroft and Rodríguez, 2005).

One possible alternative to contact-based methods is to take advantage of the fact that airborne signals are picked up by some substrates, such as the tissues of plants and leaf litter (Saxena and Kumar, 1980; Hill, 2008). Because airborne signals maintain the

same timing and pitch as vibrational signals (Cocroft and Rodríguez, 2005), signals may be effectively and adequately imparted from a loudspeaker onto a substrate. However, the pickup of airborne signals by the substrate introduces a new level of potential variation on top of the variation due to transmission along the substrate. Here, we present a methodological framework to determine if it is appropriate to use airborne induction of vibrational stimuli. We assess this method for broad-band and pure-tone stimuli, representing a range of signals used by vibrationally signaling animals and the types of stimuli used in vibrational playback experiments.

We validate the use of airborne stimuli for vibrational playback in four steps. First, we ask if we can compensate for the differential attenuation of frequencies that occurs due to pickup by a plant substrate and transmission of that stimulus along the plant. We determine if the standard compensation procedure for contact-based playback of stimuli containing a range of frequencies (Cocroft, 1996; Cocroft, 2005) also works for airborne vibrational playback. Second, we ask if the stimulus compensation for one plant individual is adequate for use with multiple individuals of the same species and describe the degree of variation introduced by using this compensated stimulus. Third, we look at how well pure-tone stimuli are picked up by the plants by looking at the waveform's shape, the variation in amplitude due to varying leaf number, and we describe the degree of variation introduced within a plant and across plants by repositioning each plant to the loudspeaker. Fourth, we look at how vibrationally signaling animals interact with the airborne stimuli picked up by the plants and compare these interactions to established vibrational playback techniques.

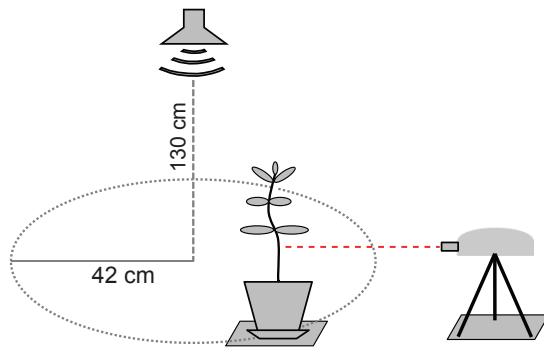


Fig. 1. Depiction of the setup used to broadcast airborne signals and record the signals picked up by the plant. The plant and the laser vibrometer were placed on shock-absorbing sorbothane to isolate them from floor vibrations.

We address both broad-band and pure-tone stimuli in an ecologically relevant case study. We used a member of the *Enchenopa binotata* Say species complex of treehoppers (Hemiptera: Membracidae) and its host plant, *Viburnum lentago* L. (Caprifoliaceae). These plant-feeding insects communicate with plant-borne vibrational signals (Cocroft et al., 2008; Cocroft et al., 2010). Our findings indicate that airborne signals can be used as vibrational playbacks for *Enchenopa* but that validation must be verified for each substrate and species in a case-by-case manner.

MATERIALS AND METHODS

General methods

We used potted exemplars of *Viburnum lentago* plants. Plants were moved to a greenhouse in February 2011 and 2012 to promote leaf growth and remained there until they were used. We selected 11 plants for the following experiments, choosing similarly sized individuals (~30 cm high with 6–8 leaves each). Experiments were performed in an anechoic chamber held at a temperature of $24 \pm 0.5^\circ\text{C}$ during February and March 2011 and 2012.

Our experiments involved creating broad-band and pure-tone stimuli and broadcasting them from a loudspeaker to impart them onto plants. We created and controlled all stimuli using custom-written programs in MATLAB 7.11.0 (m-files courtesy of R. B. Cocroft). We used a Dell Precision T3500 computer (Dell Computer, Round Rock, TX, USA) connected to an Edirol UA-25 USB audio controller (Roland Corp., Los Angeles, CA, USA) connected to a Rokit 8 powered monitor speaker (KRK Systems, Deerfield Beach, FL, USA) to deliver all stimuli. We suspended the speaker from the ceiling of the anechoic chamber such that the speaker cone was parallel to the ground of the chamber (Fig. 1). We placed plants 42 cm away from the center of the cone and isolated them from ground vibrations by placing them on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY, USA). We recorded stimuli picked up by the plants by focusing the beam of a laser vibrometer (Polytec PDV 100; Polytec, Auburn, MA, USA) onto a small (~2 mm) piece of reflective tape placed about halfway up the main plant stem (~14–18 cm high). We sent the vibrational signals from the laser to an HP Pavilion dm4 computer (Hewlett-Packard, Palo Alto, CA, USA) through another Edirol UA-25 USB audio controller and recorded them (44.1 kHz sampling rate) using the program AUDACITY 1.3.13 (<http://audacity.sourceforge.net>). While our use of a single laser vibrometer underestimates maximum signal amplitude variation in the plant stem (McNett et al., 2006), our purpose was to sample variation as would be most often detected in playback experiments.

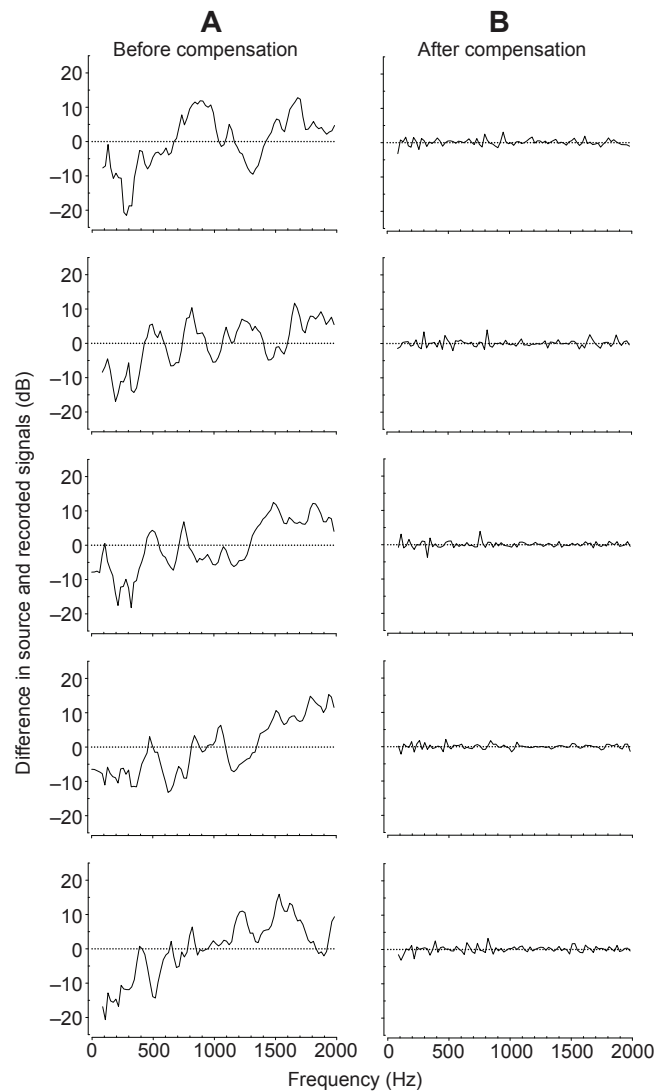


Fig. 2. Transfer functions of five representative plants. (A) Before compensation: filtering as the signal is imparted from air to plant tissue, transmitted within the plant and recorded from the plant stem. (B) After compensation: the same process for the compensated playback stimulus.

Experimental design

Step 1: compensation of broad-band stimuli for individual plants
We asked if we can compensate for each plant, adjusting for its pickup of the airborne stimulus and filtering during stimulus transmission. The goal of the compensation routine is to minimize the difference in dB at any one frequency between the broadcast signal and the signal recorded from the stem (Cocroft, 1996; Cocroft, 2005). We broadcast white noise ranging from 50–2000 Hz through a loudspeaker and recorded it with a laser vibrometer. We obtained a transfer function (fft size=2048) of the recorded and input signals, which described the relative amplitude difference between the signals. This transfer function was used to create a digital filter that we used to create a compensated stimulus (MATLAB m-files courtesy of R. B. Cocroft). We then played back the compensated airborne stimulus and recorded it with the laser vibrometer.

We evaluated the effectiveness of the compensation by looking at the transfer function of the new compensated stimulus and laser recording. We considered compensation successful if the frequency-

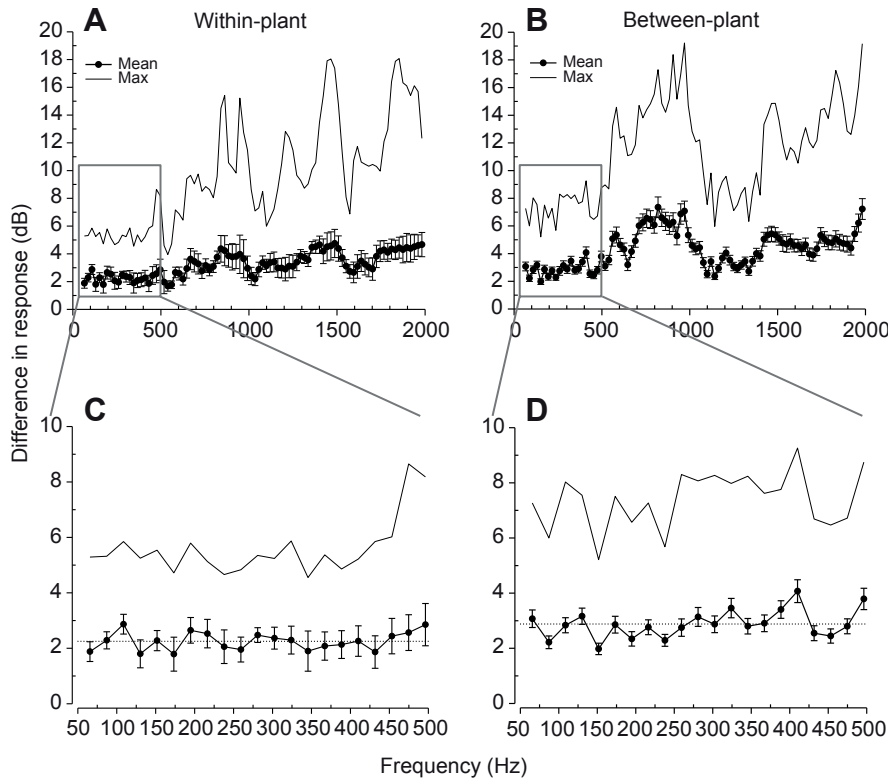


Fig. 3. Transfer functions looking at the effectiveness of a compensated stimulus broadcast to 10 plants. (A) Within-plant shows the mean difference \pm s.e.m. within each plant across the three days tested, with 'max' representing the maximum difference within any plant for that frequency. (B) Between-plant shows the mean difference \pm s.e.m. between the mean response of each plant, with 'max' representing the maximum difference between any two plants for that frequency. Horizontal dotted lines show the mean difference across the 50–500 Hz range for (C) within-plant (2.28 dB) and (D) between-plant (2.88 dB).

response of the plant stem was, on average, within ± 3 dB across the sampled range, which is the standard criterion in acoustic studies (e.g. Gerhardt et al., 2007).

Step 2: generality of compensation of broad-band stimuli across multiple plants

We randomly selected one of the 11 plants and generated a compensated stimulus as above. We broadcast the compensated airborne stimulus and recorded it with the laser vibrometer from each of the other 10 plants. This experiment thus describes how well compensation for one plant applies to other plants. We tested each plant once a day for three consecutive days, randomizing plant order each day.

We analyzed plant variation in two ways. First, we looked at variation due to repositioning of plants by assessing variation within plants over the course of three days of testing. For each day tested, we determined the maximum amplitude in dB for each plant across the frequency range and set that value to 0 dB, adjusting all other amplitudes accordingly. We performed pairwise comparisons in amplitude between the three days at each measured frequency from 50 to 2000 Hz for each plant and then calculated the mean relative amplitude for each plant from those comparisons. We then used plant means to determine the overall mean difference in relative amplitude between the broadcast airborne stimulus and the laser recording for each measured frequency. We focused on variation in the 50–500 Hz range as this encompasses the range of signals used by members of the *E. binotata* species complex (Cocroft et al., 2008; Cocroft et al., 2010). In addition, we noted the maximum amplitude difference within any one plant across the three days of testing for each frequency sampled.

Second, we looked at variation between plants. As before, we adjusted amplitude values for each plant (maximum set to 0 dB) and calculated the mean response across days for each plant. These means were then used to calculate all pairwise differences, resulting

in 45 total comparisons for the 10 plants, again noting the maximum difference.

Step 3: generality of amplitude calibration for pure-tone stimuli

We synthesized five pure-tone stimuli that mimicked signals of males in the *E. binotata* species complex. These stimuli have previously been used to study the communication system of this complex (e.g. Rodríguez et al., 2006; Fowler-Finn and Rodríguez, 2012). Stimuli ranged in frequency from 85 to 285 Hz, which covers 100 Hz above and below the mean signal frequency for males of the *E. binotata* 'V. lentago' species (185 Hz). We randomly selected one of the 11 plants and placed it in the anechoic chamber as previously described. We then calibrated each of the five stimuli to an amplitude of 0.1 mm s^{-1} by using an oscilloscope (Kikusui 5020, Yokohama, Japan). We then broadcast the calibrated stimuli for this plant to each of the remaining 10 plants. Randomizing order, we broadcast each stimulus and recorded it from the plant stem and, in addition, estimated the amplitude of the wave from the oscilloscope each time. We repeated this process two more times so each plant was tested three times for each stimulus.

We also looked at how pickup by plants varies with the numbers of leaves. Using the same five synthetic pure-tone stimuli, we calibrated each stimulus to an amplitude of 0.1 mm s^{-1} and recorded the signal from the plant stem. We then removed half of the leaves and broadcast that same calibrated stimulus, again recording from the stem. We finally removed all the leaves and repeated this process one more time. In total, 10 plants were tested for each stimulus, and each stimulus was calibrated to the plant tested.

We looked at variation due to repositioning the plants by assessing variation within and between plants across the three rounds of testing as in step 2 above. The oscilloscope output was converted to velocity (mm s^{-1}), which was then translated to dB (re. 0.1 mm s^{-1}) for the pairwise comparisons.

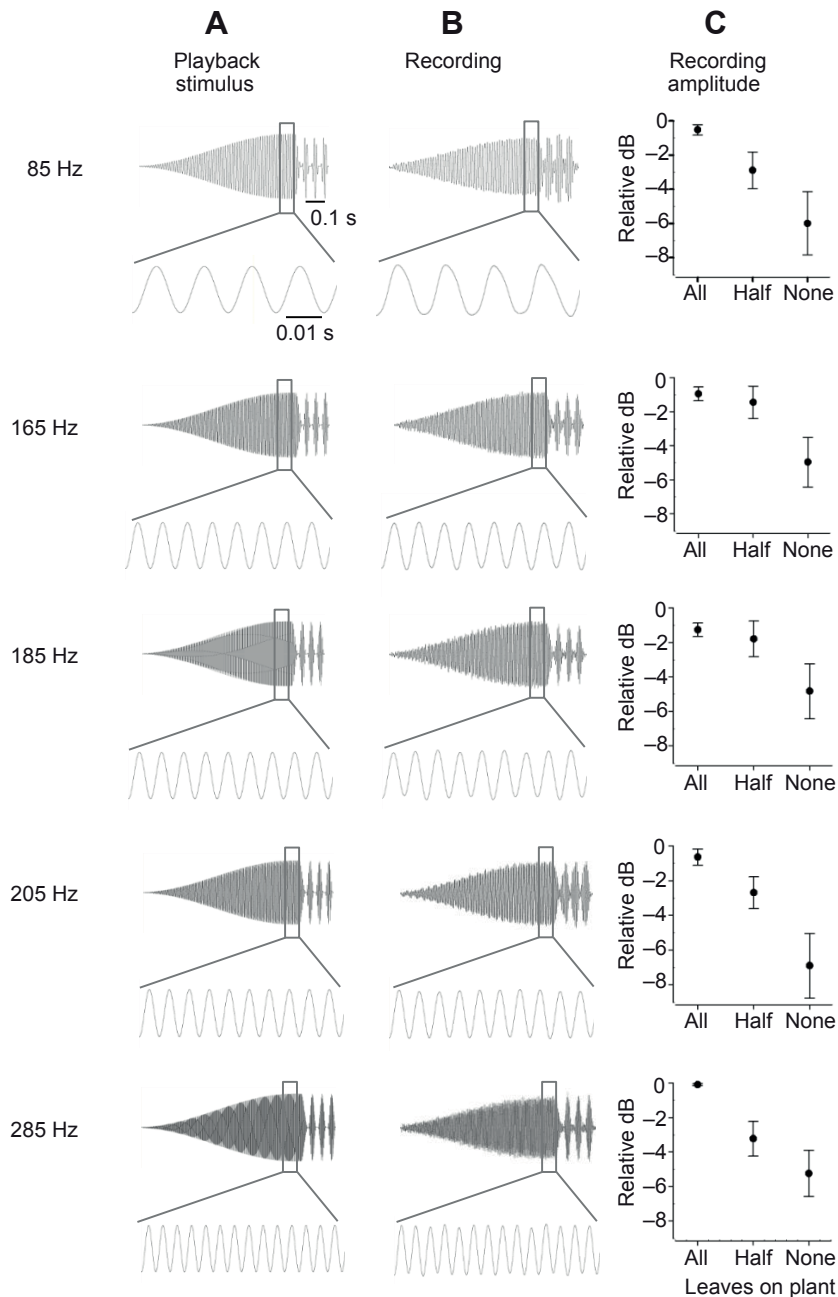


Fig. 4. Five synthetic pure-tone stimuli showing (A) the broadcast airborne stimulus, (B) a recording of the vibrational signal from the stem and (C) the amplitude of the recorded signal. The overall waveform and a zoomed-in portion of the waveform are shown for comparison. The amplitude plots show the mean \pm s.e.m. for each stimulus with all, half or no leaves remaining on the plant ($N=10$).

Step 4: playbacks of pure-tone stimuli to live males and females
We used the 185 Hz synthesized pure-tone stimulus in playbacks to males. We placed an individual male on a plant and allowed him to settle for one minute. We then broadcast the airborne stimulus as a three-call bout, allowing 30 s afterwards to assess if the male was sexually active. This process was repeated a maximum of five times or until the male responded. Males that responded to the stimulus were then broadcast an extended 12-call bout to see if they would engage in chorusing behavior in which their calls alternated with the broadcast stimulus.

For females, we synthesized seven pure-tone stimuli ranging from 145 to 225 Hz (just extending beyond the natural range of male advertisement calls in this species). Following previously established methods (Fowler-Finn and Rodríguez, 2012), we presented a random sequence of seven stimuli to a female, with each stimulus presented as a three-call bout. We tested female preferences through

playbacks using a piezo-electric stack (contact-based method) and by broadcasting airborne stimuli through the aforementioned setup, the assay of preference being the production of duetting signals by females (Rodríguez et al., 2004; Rodríguez et al., 2006; Fowler-Finn and Rodríguez, 2012). We then generated preference functions using the responses of all females from each playback technique by constructing cubic spline regressions (glms40 cubic spline program; <http://www.zoology.ubc.ca/~schluter/wordpress/software>) (Schluter, 1988).

RESULTS

Step 1: compensation of broad-band stimuli for individual plants

We were able to compensate for the pickup and transmission of each plant. The mean difference between the compensated broadcast airborne stimulus and laser recording for any frequency was less than 2 dB and had a maximum difference of 5.4 dB (Fig. 2).

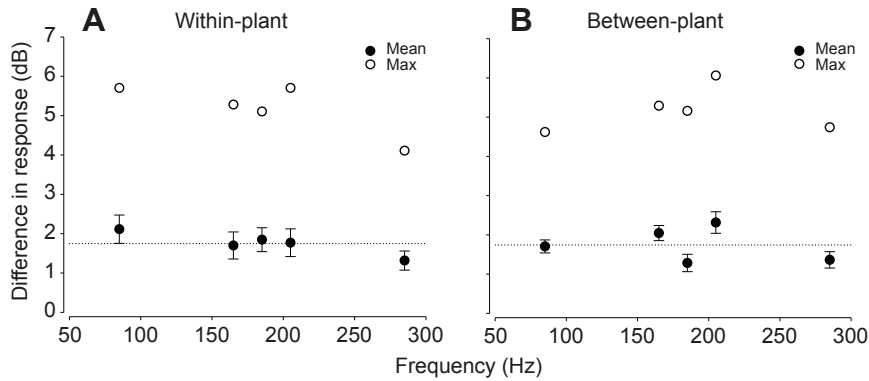


Fig. 5. Variation in signal pickup and transmission by plants due to repositioning. (A) Within-plant shows the mean difference \pm s.e.m. within each plant across three trials, with 'max' representing the maximum difference between trials for each stimulus for any plant. (B) Between-plant shows the mean difference \pm s.e.m. between any two plants for each tested stimulus, with 'max' representing the maximum between any two plants. Horizontal dotted lines show the mean difference across the five stimuli for within-plant (1.75 dB) and between-plant (1.74 dB).

Step 2: generality of compensation of broad-band stimuli across multiple plants

On the whole, average variation within and between plants was similar across the range tested, with a mean difference of 3.2 dB and 4.3 dB for within and between plants, respectively, but there was considerable variability at certain frequencies (Fig. 3A,B). Over the 50–500 Hz range, responses were adequately flat across days, with a mean difference of 2.28 dB and 2.88 dB for within and between plants, respectively (Fig. 3C,D).

Step 3: generality of amplitude calibration for pure-tone stimuli

The structure of pure-tone signals was minimally altered (paired *t*-test comparing frequency of playback stimulus and laser recording, all $P > 0.17$; Fig. 4). These signals appear to be predominantly picked up by the leaves, as removal of all the leaves results in a substantial decrease in signal amplitude (Fig. 4C). Signal pickup and transmission was consistent despite repositioning the plant relative to the loudspeaker, with a mean difference of 1.75 dB and 1.74 dB within and between plants, respectively, for the five signals tested (Fig. 5).

Step 4: playbacks of pure-tone stimuli to live males and females

Eleven of 14 male *Enchenopa* treehoppers called within 30 s of being presented with the airborne stimulus, with six calling after one bout,

three more after the second, and the remaining two after the third bout was presented. Of those that called, two males engaged in chorusing behavior (Fig. 6A).

Females duetted with the airborne stimuli and differentially responded according to their frequency (Fig. 6B). Female preference functions overlapped entirely between playback methods (Fig. 7).

DISCUSSION

We tested whether airborne signals could be adequately imparted onto a substrate as vibrational playbacks. First, we demonstrate that it is possible to compensate effectively for the pickup and transmission properties of a plant stem across a broad frequency range. Second, we describe the variation within and between plants across days, highlighting that in the range used by a group of signaling animals, responses are fairly equal for both measures. Third, we demonstrate that a broadcast pure-tone stimulus is minimally altered by the plant and that these signals are picked up best by the leaves. In addition, repositioning of a plant to the loudspeaker only slightly changes the intensity of a pure-tone stimulus within and between plants across trials. Finally, we show that both male and female *Enchenopa* treehoppers respond similarly to airborne and vibrational stimuli in playback experiments in spite of the multiple inputs into the plant. However, it is unclear how signal localization may be affected, a point that will require further study.

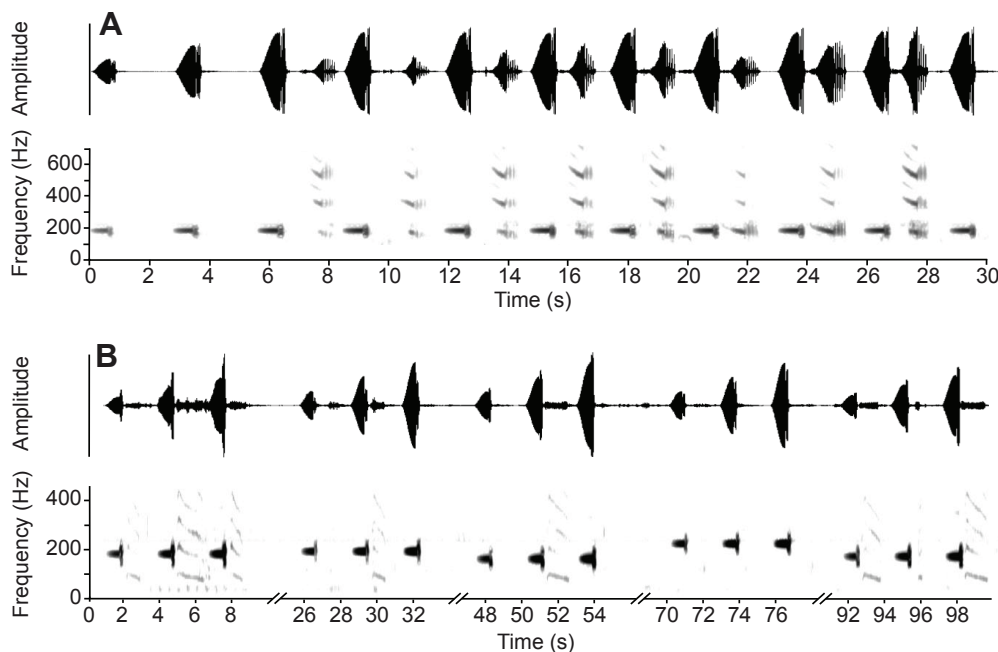


Fig. 6. Spectrogram and waveform of a recording of (A) a male and (B) a female interacting with broadcast synthetic pure-tone stimuli. The male exhibits chorusing behavior (interlaced calls) with the stimulus as if it were another male on the plant. The female responds differentially to the five synthetic pure-tone stimuli spanning a range of male signals. The airborne stimuli appear as pure-tone signals in the spectrogram, and the live individual responses show the harmonics in the signals.

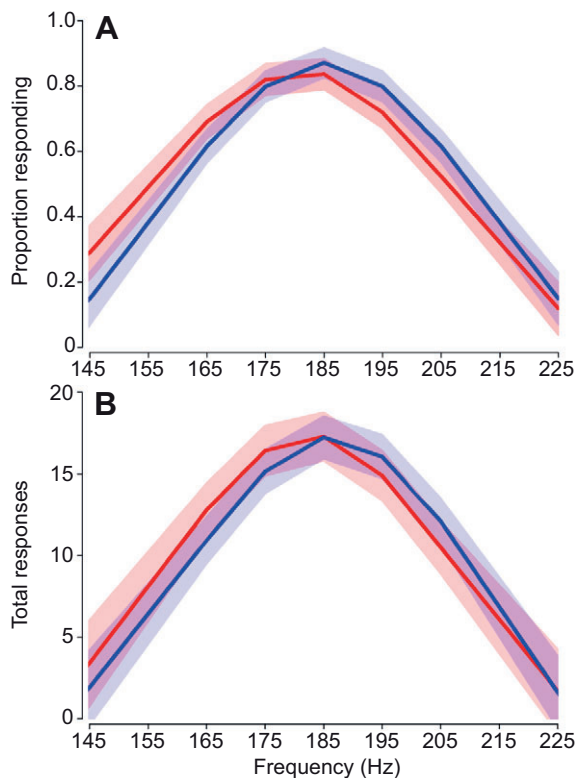


Fig. 7. Preference functions of females ($N=9$) tested across a range of signal frequencies using synthetic pure-tone stimuli. (A) Proportion of females responding to each stimulus; (B) total number of responses of females to each stimulus. The red line corresponds to vibrational playbacks using a piezo-electric stack, and the blue line corresponds to broadcast playbacks. Red and blue shaded regions are the s.e.m. of each preference function, and purple denotes their overlap.

For a single plant, the recorded signals from airborne playbacks were similar to vibrational playbacks. How well this works across plants depends on the frequency range considered. Some frequency ranges are relatively flat with repeatable responses, such as a subset of the range addressed here. The low and similar variation in dB within and between plants shows that individuals exposed to complex signals in this range will be subject to the same stimulus variability. However, the type of stimulus used is important. Broad-band signals may be more problematic than pure-tone signals because of the frequency range they span and the likely increased variability in transmission as the range increases. We stress that this technique needs to be tested for each substrate, frequency range and stimulus type. This technique may be applicable to other types of substrate-borne signals, such as

leaf litter, presenting further opportunities to expand our understanding of vibrational communication.

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REFERENCES

- Cocroft, R. B. (1996). Insect vibrational defence signals. *Nature* **382**, 679-680.
- Cocroft, R. B. (2005). Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *Proc. Biol. Sci.* **272**, 1023-1029.
- 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.
- Cocroft, R. B., Rodríguez, R. L. and Hunt, R. E. (2008). Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* species complex of treehoppers. In *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. K. J. Tilmon), pp. 88-100. Berkeley, CA: University of California Press.
- Cocroft, R. B., Rodríguez, R. L. and Hunt, R. E. (2010). Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol. J. Linn. Soc. Lond.* **99**, 60-72.
- Čokl, A. and Virant-Doberlet, M. (2003). Communication with substrate-borne signals in small plant-dwelling insects. *Annu. Rev. Entomol.* **48**, 29-50.
- Čokl, A., Nardi, C., Simões Bento, J. M., Hirose, E. and Panizzi, A. R. (2006). Transmission of stridulatory signals of the burrower bugs, *Scaptocoris castanea* and *Scaptocoris carvalhoi* (Heteroptera: Cydnidae) through the soil and soybean. *Physiol. Entomol.* **31**, 371-381.
- Čokl, A., Žunič, A. and Millar, J. G. (2009). Transmission of *Podisus maculiventris* tremulatory signals through plants. *Cent. Eur. J. Biol.* **4**, 585-594.
- Elias, D. O., Mason, A. C. and Hebets, E. A. (2010). A signal-substrate match in the substrate-borne component of a multimodal courtship display. *Curr. Zool.* **56**, 370-378.
- Fowler-Finn, K. D. and Rodríguez, R. L. (2012). Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* **66**, 459-468.
- Gerhardt, H. C., Martínez-Rivera, C. C., Schwartz, J. J., Marshall, V. T. and Murphy, C. G. (2007). Preferences based on spectral differences in acoustic signals in four species of treefrogs (Anura: Hylidae). *J. Exp. Biol.* **210**, 2990-2998.
- Henry, C. S. and Wells, M. L. M. (2004). Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). *Anim. Behav.* **68**, 879-895.
- Hill, P. S. M. (2008). *Vibrational Communication in Animals*. Cambridge, MA: Harvard University Press.
- McNett, G. D. and Cocroft, R. B. (2008). Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. *Behav. Ecol.* **19**, 650-656.
- McNett, G. D., Miles, R. N., Homentcovski, D. and Cocroft, R. B. (2006). A method for two-dimensional characterization of animal vibrational signals transmitted along plant stems. *J. Comp. Physiol. A* **192**, 1245-1251.
- Rodríguez, R. L., Sullivan, L. E. and Cocroft, R. B. (2004). Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**, 571-578.
- Rodríguez, R. L., Ramaswamy, K. and Cocroft, R. B. (2006). Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proc. Biol. Sci.* **273**, 2585-2593.
- Saxena, K. and Kumar, H. (1980). Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants. *Experientia* **36**, 933-936.
- Schluter, D. (1988). Estimating the form of natural selection on a quantitative trait. *Evolution* **42**, 849-861.