Vibration-guided mate searching in treehoppers: directional accuracy and sampling strategies in a complex sensory environment

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Summary statement:

This is the first study of how movement decisions of an insect, searching for a vibration source on a plant, are influenced by the complex whirling motion of plant stems.

Abstract

Animal movement decisions involve an action-perception cycle in which sensory flow influences motor output. Key aspects of the action-perception cycle involved in movement decisions can be identified by integrating path information with measurement of environmental cues. We studied mate searching in insects for which the primary sensory cues are mechanical vibrations traveling through the tissues of living plants. We mapped search paths of male thornbug treehoppers locating stationary females through an exchange of vibrational signals. At each of the males' sampling locations, we used two-dimensional laser vibrometry to measure stem motion produced by female vibrational signals. We related properties of the vibrational signals to the males' movement direction, inter-sample distance, and accuracy. Males experienced gradients in signal amplitude and in the whirling motion of the plant stem, and these gradients were influenced to varying degrees by source distance and local stem properties. Males changed their sampling behavior during the search, making longer inter-sample movements farther from the source where uncertainty is higher. The primary directional cue used by searching males was the direction of wave propagation, and males made more accurate decisions when signal amplitude was higher, when time delays were longer between front and back legs, and when female responses were short in duration. The whirling motion of plant stems, including both the eccentricity and the major axes of motion, is a fundamental feature of vibrational environments on living plants, and we show for the first time that it has important influences on the decisions of vibrationally-homing insects.

Introduction

Although understanding the causes and consequences of animal movement is a major goal of ecological research (Nathan et al., 2008; Sutherland et al., 2013), for most species we lack an understanding of the perceptual cues used in movement decisions. Movement decisions involve an integrated process in which sensory flow influences motor output, which in turn determines the nature of the sensory flow (Hofmann et al., 2013). This action-perception cycle and the ways in which it changes with context are well understood for some organisms including echolocating bats (Ulanovsky and Moss, 2008; Yovel et al., 2010), odor-tracking moths and fruit flies (Gaudry et al., 2012; Murlis et al., 1992), visually-tracking insects (Srinivasan, 2011), chemotactic micro-organisms (Berg, 2000) and electroreceptive fish (Hofmann et al., 2013). For the majority of species, for which the sensory system is poorly known and movement decisions are not well understood, key insights into the cues that predict movement decisions come from combining path information with measurements of environmental variables (e.g., Smolka et al., 2011; Dodge et al., 2013). Recent advances including small sensors and global positioning system technologies have made tracking some animals a much easier task (Kays et al., 2015). These technologies allow researchers to not only track movement paths for individuals in real time, but to also gather other data streams simultaneous such as acceleration (Nathan et al., 2012; Resheff et al., 2014; Spiegel et al., 2017), environmental features (Kays et al., 2015) and physiological states (Signer et al., 2010). With the continued miniaturization of these technologies a larger range of animals can be tracked, but small animals such as insects still pose tracking challenges. In such cases, direct observations can be especially powerful when the organism’s movements and sampling behavior can be observed in sufficient detail to reveal the points where reorientation decisions are made, since these are the key points at which to measure environmental information (Gomez-Marin et al., 2011; Smolka et al., 2011). Here we use this approach to understand movement decisions in an environment used by the majority of terrestrial animal species, based on one of the most widespread but least studied information streams: mechanical vibrations traveling through the tissues of living plants.

Many of the social and ecological interactions within the arthropod community on plants depend on the production and perception of plant-borne vibrations. These vibrations are used by some 200,000 insect species to communicate with mates, mutualists, or group members (Cocroft and

Rodríguez, 2005), and by many more insects and other arthropods to locate prey or avoid predators (Barbosa and Castellanos, 2005; Barth, 1998; Casas and Magal, 2006). Plant-borne vibrations are also important in the social and ecological interactions of some arboreal vertebrates (Barnett et al., 1999; Caldwell, 2014; Christensen et al., 2012; Warkentin, 2005).

Many of the vibration-mediated interactions among plant-dwelling animals involve homing in on a vibration source (Cocroft and Rodríguez, 2005). Searches based on plant-borne vibrations are well suited for studying the action-perception cycle that underlies movement decisions. Searches usually occur over distances of less than a meter (Virant-Doberlet and Čokl, 2004). In many cases, mechanical vibrations are the only available search cue, and where other cues such as light are involved, they can be experimentally manipulated (Hunt and Nault, 1991). Because vibration-guided searches are typically based on intermittent locomotion (Kramer and Mclaughlin, 2001) with clearly defined sampling points (Legendre et al., 2012), the key locations for measuring vibrational cues can be identified. The accuracy and efficiency of searching (and likewise the efficacy of signals; Endler 1992) can be quantified precisely, because the available movement paths are constrained by the structure of the plant, and the target location is known. However, although some likely cues for vibration localization have been experimentally tested (reviewed in Hager and Kirchner, 2014; Virant-Doberlet et al., 2006), some search paths quantified (Legendre et al., 2012), and some vibration gradients examined for their effect on male behavior (Polajnar et al., 2014), no studies have integrated entire search paths with measurement of the available vibrational cues at each sampling location to relate sensory input to motor output.

We studied movement decisions during mate searching in the thornbug treehopper (Hemiptera: Membracidae: *Umbonia crassicornis* (Amyot & Serville)). Mate localization in thornbugs occurs through a back-and-forth exchange of plant-borne vibrational signals, or duet, between a searching male and a stationary female. Search efficiency is important for male mating success: thornbugs occur at high densities (Wood and Dowell, 1985), and female thornbugs mate only once, with the first male to arrive during their narrow window of receptivity (Cocroft and McNett, 2006). Male movement decisions are one-dimensional (forward or back along a stem) and are based on identifiable sampling locations: searching males walk along plant stems, periodically stop to signal and elicit a female reply, then continue forward or turn around. Source localization through duetting thus combines features of both echolocation and beacon-guided search: as with echolocation, males obtain information about the target by producing a signal; however rather than relying on physical reflections, the ‘reflection’ is a signal produced by a stationary receiver. Duetting is thus an active sensing strategy in which the sensory flow – transmitted signals from the source – is shaped by the positions at which males decide to stop, the number of signals they produce there, and the signaling decisions of the female.

We analyzed the movement decisions of male thornbugs in three stages. We first characterized their search paths, asking how movement decisions and accuracy changed over the course of the search. We then investigated the vibrational cues correlated with movement decisions, and asked how variation in those cues influenced sampling behavior and accuracy. Finally, we combined the information from all of the searches to get an overview of the vibrational gradients encountered by the searching insects.

Materials and Methods

Study system

Thornbug treehoppers are sap-feeding insects that use vibrational communication throughout their lives (Cocroft, 1999; De Luca and Cocroft, 2008; Hamel and Cocroft, 2012; Ramaswamy and Cocroft, 2009). Their hostplants are woody shrubs and trees in the Mimosaceae. Most individuals grow to adulthood in a sibling aggregation of 50 - 100 individuals (Cocroft, 2002), and when females oviposit near other females the communal aggregations may be much larger. Some females mate before dispersing from the natal aggregation, while others mate after dispersing (De Luca and Cocroft, 2008). Males disperse before females, and either locate and remain with a pre-dispersal aggregation of females, or search for dispersed unmated females. Searching males use a call-fly strategy to locate a host plant with a receptive female, and vibrational duetting to locate the female within the plant. We studied the within-plant phase of mate searching.

Animal care

We drew the study animals from a greenhouse colony at the University of Missouri (Columbia, MO, USA), which we established with collections in and around Miami, FL, USA (USDA APHIS permit P526P-10-03348). Thornbugs have three to four generations per year; in the greenhouse we maintained an outbred colony by mating individuals with non-siblings and by introducing new individuals from the field every two generations. Family groups collected from the field contained late-instar nymphs and/or teneral adults. Each family was maintained on its own potted host plant (Mimosaceae: *Albizia julibrissin* Durazzini or *Lysiloma latisiliquum* (L.) Benth), which was covered with a screen cage. Approximately one week after the adult molt, the sexes were separated to prevent sibling mating. The colony was maintained on a 14:10 L:D cycle, with a temperature range of 27-32°C (day) and 21-24°C (night), at 60-70% humidity.

Experimental design

Our goal was to obtain path data for searching males duetting with stationary females, then annotate the path by obtaining environmental information at each of the males' sampling points (Fig. 1). Obtaining the necessary vibration measurements required several minutes for each point on the plant (see below), so it was not possible to make those measurements in real time during a male's search. For each pair of insects, then, our characterization of search paths proceeded in two phases. First, after introducing the insects onto a plant, we plotted the male's sampling locations on a map of the plant, and videotaped the entire search while recording the duetting signals of the pair using a single, stationary transducer. Second, we removed the male before it reached the female or after 15 minutes, then used two lasers to measure stem vibration at each point sampled by the male during the search. Female signals were evoked as needed by playing back male vibrational signals. The experimental data thus consisted of (1) variables measured from the original duet: signaling behavior (timing, duration) and male movement information (location vs. time); and (2) variables that were a proxy for vibrational signals experienced by the male during its search: signal amplitude, spectral content, and two-dimensional motion of the stem. To ensure that the proxy measurements were as close as possible to the signals experienced by the searching male, we only used trials in which the female was stationary during the entire search and post-search recording period. This criterion ensured that the signal transmission path was virtually identical between the original search and the post-search recordings.

We conducted n=16 trials, each with a unique pair of unrelated individuals drawn from ten family groups. We selected experimental individuals haphazardly from their family group, and did not use individuals more than once. We chose two ~1 m tall potted *Lysiloma latisiliquum* plants (woody hosts with multiple side branches; see Results and Fig. S1 for branching structure) on which to observe searches. Using only two hosts allowed us to estimate the effect of plant differences, while obtaining a detailed map of the structure of the individual plants. We conducted eight trials on each plant, four with the female on a stem near the top of the plant and four with the female on a stem near the bottom of the plant (see Results and Fig. S1 for female locations). Each male was placed at the same starting location on a given plant: ~40 cm from the female (measured along the stem), on a side stem roughly equidistant from both female positions. Males began signaling either at their initial location, or after walking a few cm along the stem.

Treehoppers are not known to use chemical cues during within-plant searches, and playback studies show that localization readily occurs when only vibrational cues are available (e.g., Cocroft, 2005). However, to reduce the influence of potential chemical cues that might have been left on the plant by previous individuals, we conducted trials with the female in the same position (apical or basal) on the same plant at intervals of 1-7 days, alternating with searches for females in the opposite position. Any trials conducted within the same day were separated by at least 1.25 h.

We know little about the role of vision in movement decisions of thornbugs, but visual cues appear to be important within 10 cm on the same plant stem. Females defending offspring walk directly toward a moving predator in their line of sight (Wood 1976; Cocroft 2002), but do not do so if their compound eyes are covered (Wood 1976). Searching males sometimes climb onto and court a vibration actuator playing back female signals, but males may also walk past a stationary female that is on the same stem and clearly in their line of sight (authors, pers. obs.). In this study, we minimized the potential influence of close-range visual homing by ending the trial when the male was within 5 cm of the female. However, future studies should address the contribution of visual cues to movement decisions (see Verdeny-Vilalta et al., 2015), ideally in combination with vibrational cues.

For each trial we placed a receptive female at the assigned location, waited five minutes, then introduced a male at the starting location. If the male did not begin producing advertisement signals within 2 min, we elicited signaling by playing back a signal exchange from a pre-recorded male-female duet. The trial started when the male began signaling and initiated a duet with the female. The trial ended when the male approached within 5 cm of the female or after 15 minutes, whichever came first. During the trial, the male was videotaped using a stationary video camera (Exilim EX-F1; Casio, Dover, New Jersey, USA) and the duet was recorded using a piezo film sensor attached near the base of the plant (1/2” piezo film tab and General Purpose Amplifier model 1007214; Measurement Specialties, Hampton, Virginia, USA). We ended the searches by removing each male from the plant when it came within 5 cm of the female.

We identified the sampling locations visually during each search using a map of the plant, yielding a precision of ± 0.5 cm, as determined by comparison with the video recordings. We then used vibrational playback of male signals to induce the female to continue signaling from her original location, and measured the signals at each location where the male stopped, signaled, and received a reply from the female.

Evoking female signals for measurement

To evoke continued female signals for measurement, we used a piezoelectric stack actuator (ThorLabs: AE0505D18F, Newton, New Jersey, USA) mounted on a positioning arm and contacting the plant 5 cm above the base, with the contact secured using accelerometer wax. The actuator was driven by an open loop controller (ThorLabs: MDT694A), custom DC-offset box, Dell computer and Audacity (version 1.3) software.

Vibration recording and analysis

We measured stem motion using two laser Doppler vibrometers (CLV 1000 lasers, CLV M030 decoder modules; Polytec Inc., Auburn, Massachusetts, USA) oriented perpendicularly to the long axis of the stem and to each other (see McNett et al., 2006). We attached two ~1 mm^2 pieces of reflective tape at the recording locations to increase laser reflectance. The laser output was high-pass filtered at 30 Hz, which is below the frequencies in the female signals (Filter model 3202; Krohn-Hite Corp., Brockton, Massachusetts, USA), and digitized using a data acquisition system (CA-1000 board; National Instruments, Austin, Texas, USA) with a Dell PC computer running LabView software v. 5.1 (National Instruments, Austin, Texas, USA).

The two simultaneous laser signals were analyzed using custom-written scripts in MatLab (version 10: Mathworks, Natick, Massachusetts, USA) based on equations from McNett et al. (2006). Because stem motion can occur along any axis within a plane perpendicular to the long axis of the stem. measuring motion along a single axis (as with use of a single laser or accelerometer) will typically result in underestimation of the signal amplitude (McNett et al., 2006). Furthermore, when a vibration is transmitted along a plant stem, any point on the stem will move in an ellipse whose shape can vary from linear to circular, and this motion cannot be captured with a single transducer (McNett et al., 2006). Use of two orthogonal transducers allows measurement of the amplitude of the major axis of stem motion (Fig. 2A), the relative amplitude of the major and minor axes of motion Fig. 2A), and the angle of rotation of the major axis of motion with respect to a reference axis (the male's dorsoventral axis at the time the female signal was produced; Fig. 2B). Each of these properties of motion can vary between different frequencies in the same signal (Fig. 1). Because we could not precisely score the male's orientation when it was hidden from the camera by the plant stem, we characterized the male's orientation around the stem to the nearest 90°. We thus had four possible reference axes, depending on the male's position: 0° when the male's dorsoventral axis was visible and aligned with the video camera lens; 180 ° when it was on the opposite side of the stem; and 90° when the male's dorsoventral axis was perpendicular to the 0 ° axis.

The information contained in two-sensor measurements of the signal is distributed across three spectra (Fig. 1): an amplitude spectrum (representing the major axis of stem motion at each frequency); an eccentricity spectrum (a unitless ratio of the amplitudes of the minor axis: major axis at each frequency); and a rotation spectrum (the angle of rotation of the major axis at each frequency, relative to the searching male's dorsoventral axis).

Wave propagation velocity

Plant-borne vibrations are transmitted in the form of bending waves under most conditions (Casas et al., 2007; Hill and Wessel, 2016; Miles, 2016), including those experienced by the insects in this study. Bending wave velocity is proportional to the square root of frequency, and an important feature of the sensory world of insects on plants is that this overall frequency-velocity curve varies among plant substrates depending on properties including stiffness and diameter (Cremer et al., 2005). To estimate the wave propagation velocity at each location on the plants used in this study, we played back broadband noise using a piezo actuator, as above, and recorded the noise simultaneously using two lasers oriented in parallel and focused on two points 5 cm apart and 3-5 cm from the actuator (see Cocroft et al., 2000). We made these measurements at 12 locations spanning the range of variation in stem diameter within a plant. Frequency-velocity curves have the form *k* times the square root of frequency, where *k* is a constant that varies among substrates. We estimated this constant for each measured location by curve-fitting, and found a strong, positive relationship between the velocity constant and stem diameter (Fig. S2). The expected relationship between diameter and velocity cannot be predicted precisely without also measuring stem properties including the mass per unit volume and the elastic modulus (Michelsen et al, 1982), whose relationship to stem diameter can vary along the length of the stem (Niklas, 1992). Although we investigate only one type of wave (bending waves), other wave types are possible in plants, including surface waves (Casas et al., 2007; Michelsen, 2014) whose propagation velocity depends neither on diameter nor frequency, and longitudinal waves, whose role in plant-borne vibrational communication has not been investigated, but which might be generated at junctions (Michelsen, 2014). In this study, however, we encountered only waves whose dispersive propagation is consistent with bending waves; and we did not attempt to measure longitudinal waves.

To estimate the propagation velocity at each location sampled by males, we measured stem diameter throughout the plant, interpolated the value of the constant based on the curve obtained above, and calculated the propagation velocity at an arbitrary frequency in the signal (200 Hz; the frequency with the highest amplitude is usually between 150 and 200 Hz). For statistical analysis we converted this measure into an estimate of the time delay between front and back legs, which on a male thornbug are separated by ~5 mm.

Note that we did not similarly estimate differences in amplitude between front and back legs. For insects obtaining a 5 mm spatial sample along a vibrating stem, Δ amplitude is near zero (Virant-Doberlet et al., 2006). Furthermore, for thornbug treehoppers, any differences in the amplitude of stem motion between front and back legs will be far smaller than the amplitude differences (up to

20dB) generated between the front and back of the body by the mechanical response of the insects' body to time-of-arrival differences between the front and back legs (Cocroft et al., 2000).

Statistical analysis of movement decisions

We analyzed the data with SAS v. 9.4 (SAS Institute, 2017). We used PROC GLIMMIX, unless otherwise noted; this module supports general linear mixed models with response variables that have non-normal distributions, as was the case with most of the variables we measured.

Vibration gradients

To further understand the insects' sensory environment and their use of vibrational cues, we examined the effect of distance-to-source on four signal features (amplitude, spectral shape, eccentricity of stem motion, and variation among frequencies in the major axis of stem motion) that could provide directional information through comparisons of sequential samples along a gradient. Furthermore, because plants are highly heterogeneous substrates for signal propagation, some gradients may occur as a consequence of plant structure rather than source distance *per se*. Consequently, we looked at variation in these signal features not only in relation to source distance, but also in relation to a local property of the plant substrate: stem diameter.

Amplitude:

The response signals of the female thornbugs measured in this study consisted primarily of a harmonic series without amplitude modulation, and we characterized amplitude by averaging the RMS amplitude over the entire signal. Female signals can also contain broadband clicks under some circumstances, but there were few or no clicks in most of the female signals in our recordings. Measurements based on the waveform obtained by a single laser would, on average, underestimate the signal amplitude, as explained above. Accordingly, we used the two-laser recordings to calculate the amplitude spectrum of the major axis of vibration at each frequency, then used an inverse FFT to construct a new signal waveform with this amplitude spectrum. This waveform does not by itself represent the 'true' signal (i.e., the one experienced by the insect), but rather the maximum amplitude of stem motion for each frequency in the signal, regardless of the axis along which the stem was vibrating. There is additional signal energy along the minor axis of stem motion, and in principle the energy in major and minor axes could be summed; here we accounted for energy along the minor axis in a separate variable, the eccentricity of the elliptical path of stem motion (see *Eccentricity*, below).

Spectral shape: Because attenuation of bending waves is greater at higher frequencies (Markl 1983; Aicher et al., 1983; Barth et al., 1988), as a signal propagates farther from the source (other things being equal) there should be increasing differences between the amplitudes of lower and higher frequencies. As an index of spectral content, we calculated the ratio of energy in higher: lower frequencies using a cutoff frequency of 300 Hz, an approximate mid-point of the bandwidth for most female signals. To make this measure comparable to our measure of overall signal amplitude, we used the 'major-axis' waveform described in the previous paragraph. To calculate the RMS energy in higher vs. lower frequencies in the signal, we filtered out the frequencies above or below the cutoff frequency, generated the respective higher and lower frequency waveforms using an inverse FFT, and obtained the ratio of the average RMS in higher frequencies to that of lower frequencies (Fig. S3).

Eccentricity:

Stems move with a whirling motion during propagation of bending waves (McNett et al., 2006), and this motions varies from nearly linear to circular. As an index of the shape of the ellipse through which the stem travels at each frequency in a signal, we use the ratio of the amplitudes of the two axes of the ellipse (minor axis / major axis). To exclude values from frequencies with little energy, we used the amplitude spectrogram of the signal as a mask, and averaged only those frequencies with amplitudes within 20 dB of the maximum amplitude in the signal (Fig. S4). To avoid assuming *a priori* that the eccentricity of motion at a given frequency would remain constant during the signal -- and thus to account for potential variation in eccentricity as a function of not only frequency, but also time -- we averaged values from the masked spectrogram (a time series of spectra).

Angle of rotation:

A description of the whirling motion of the stem at a given signal frequency includes not only the shape of the elliptical path (the eccentricity) but also the orientation of the major axis of motion relative to a reference axis. We used the searching male's dorsoventral axis at the location where it received the female's signal as a reference axis (an angle of rotation of 0^{o} is aligned with this axis, while an angle of 90^{o} is perpendicular to this axis). Because the major axis of stem motion can vary among different frequencies in the signal, we estimated an average angle of rotation. As with eccentricity, we first generated a rotation spectrogram (Fig. 1), used the corresponding amplitude spectrogram as a mask, and included only frequencies with amplitudes within 20 dB of the maximum amplitude. Because the measurements are angles, we used circular statistics to estimate the average angle and the vector length, which a measure of how correlated the axis is among frequencies in the signal, where a vector length of 1 would indicate that all the frequencies in the signal share the same major axis of stem motion.

Results

Search behavior

Males began their search on the stem on which they were placed. Before leaving this stem they made one to seven samples, where a 'sample' involved stopping, signaling, and receiving female vibrational responses. Males then walked onto the main stem of the plant, where about a third (5/16) made their first sample while oriented across the stem (i.e., with the male's long axis perpendicular to the long axis of the stem; virtually all of the other samples we observed were made with the male's long axis parallel to the stem on which it was standing). Most of the males (13/16) walked upward after first encountering the main stem.

Males then continued to duet with the stationary female, stopping to make another sample every 5.2 ± 5.02 cm (x±SD; range 1 - 34 cm). Males sampled side stems by walking a few cm onto them and signaling; they did not sample branching points by simultaneously touching both branches at a branching point, as seen in some other insects during vibrational homing (Cokl et al., 1999). Time vs distance-to-source plots for all of the searches are provided in the supplementary material (Figs. S5 and S6).

Males stayed at a sampling location for 1.3 - 14.2 sec, depending on the number of signals they exchanged with the female (grand mean = 3.2 ± 0.74 sec; N=16 pairs, 236 samples). Stationary samples constituted, on average, about 25% of the total search time (range 11% - 39%).

Most males located the female within 2-3 min (range 54 sec to 9 min 33 sec), but one male failed to locate the female within 15 min. Males located females in both locations (apical location, 8 out of 8 males; basal location, 7 out of 8 males), but males took significantly less time and made more accurate directional decisions when the female was in the apical location (Fig. 3A, B). The average movement efficiency (minimum distance between male and female along the plant surface / total distance traveled) when the female was in the apical location was 80%, and 4 of the 8 males traveled the minimum possible distance. In contrast, the average movement efficiency when the female was in the basal location was 43% (including only the 7 males that located the female within 15 min). In the 'basal position' searches, only one male traveled on a direct path between starting position and the female, while others searched more of the plant; e.g., of two males that started 39 cm from the female, one walked 1.5 m before locating the female, and the other walked over 3 m in 15 min without locating the female.

Distance-to-source and the accuracy of movement decisions

The directional accuracy of the males' movement decisions (i.e., whether the movement was toward or away from the female) was lower when males were farther from the female (Fig. 3C). Most of the inaccurate decisions occurred when males searched for females that were near the base of the plant, and errors typically occurred in runs (see the time-vs-distance-to-source plots in Figs. S4 and S5). Of the ten searches in which males made at least two directional errors, four searches had significantly more erroneous decisions in a row than expected by chance (runs tests, all $p<0.05$ after adjusting for multiple comparisons using the False Discovery Rate procedure (Benjamini and Hochberg, 1995)). During such runs, which occurred both on the main stem and on side stems, males continued to walk forward through 2 – 6 samples, moving farther from the female each time, before finally reversing direction.

Males also sampled differently at different distances from the female. The farther males were from the female, the greater distance they moved between samples (GLMM, DF 1,219, F =13.83, $P<0.001$).

Observations and video analysis revealed an additional, previously unrecognized sampling behavior, which we have termed ‘cryptic samples’. After leaving a stationary sampling point, males sometimes continued to signal while walking, making brief (<1 sec) interruptions in their forward motion during the female’s reply. Males often made 2 - 4 cryptic samples in succession, about 1.5 cm apart, before making another stationary sample. These brief samples constituted about 30% (± 11% SE) of all sampling points. The time and location of all cryptic samples was mapped from the videos (see Figs. S4 and S5); however, female signals were only recorded from 12 cryptic samples that were obvious enough to be detectable in real time. The accuracy of directional decisions did not differ between stationary samples and cryptic samples (GLMM, DF 1,324, F = 0.28, P=0.59).

Vibrational cues and movement decisions

Because there was substantial variation in directional accuracy and sampling behavior within and among searches, we next asked which vibrational cues were correlated with accuracy, forward / reverse decisions, and the distance moved between samples. In these analyses, we included only the ‘stationary’ samples and not the 12 cryptic samples for which we obtained signal data.

Forward / reverse decisions. The main predictor of turning decisions was the direction of wave propagation. Males were more likely to walk forward if the propagating waves came from in front of them, and to turn around if the waves came from behind them (Fig. 4A). Among the potential gradients (i.e., differences between successive sampling points in the amplitude or two-dimensional properties of the signal), the only variable correlated with male decisions was the average angle of rotation of the signal, relative to the male’s dorsoventral axis. Males were more likely to reverse direction if they first sampled a signal whose average angle of rotation was closely aligned with their dorsoventral axis, and at the next sample encountered a signal whose average angle of rotation that was more nearly perpendicular to the male’s dorsoventral axis (side-to-side with respect to the male; Fig. 4B). Between-sample differences in signal amplitude, eccentricity, ratio of high and low frequencies, and female signal duration, did not significantly predict turning decisions (Table 2).

Directional accuracy: Males made more accurate decisions (i.e., they were more likely to move in the direction of the female after a sample) when signal amplitude was higher (Fig. 4C; Table 1), which occurred closer to the source (Fig. 5A). Males also made more accurate decisions when they experienced a longer time delay between front and back legs – i.e., when signal propagation speed was slower (Fig. 4B). This property of signal transmission varied with stem diameter (Fig. 6B), and during a search, males sometimes experienced very different signal propagation speeds between one sample and the next. The duration of the female's reply signal was also correlated with male directional accuracy: males made more accurate decisions when the female's reply was shorter (Fig. 4C).

Distance moved between samples: Two aspects of the whirling motion of the stem were correlated with how far males moved between samples. Males moved farther after sampling a signal for which the axis of stem motion was highly variable among frequencies; and males moved farther after sampling a signal for which the average path of stem motion was more linear (Table 3).

Vibration gradients

Amplitude: Signal amplitude decreased with distance from the source, on both plants and in both apical and basal female locations (Fig. 6, Table 4). However, amplitude gradients were local, with amplitude decreasing consistently only within 10-20 cm of the signal source (Fig. 5A).

Amplitude is also strongly dependent on stem diameter: for a given signal, smaller stems vibrate at a higher amplitude than larger stems. The influences of diameter and source distance on amplitude were independent of each other (Fig. 6; Table 4).

Eccentricity: Signal eccentricity was not reliably correlated with distance from the source (Fig. 6; Table 4). Instead, there was a three-way interaction between diameter, female location, and distance to the female; overall, eccentricity increased toward the top of the plant and the outer stems in both female location treatments.

Angle of rotation: Within-signal variability in the angle of rotation increased with distance from the female (Fig. 6; Table 4).

Spectral shape: The relative amplitude of higher and lower frequencies changed as a function of distance from the source, with higher frequencies becoming increasingly attenuated, as predicted. In addition to this gradient, however, the relative amplitude of higher and lower frequencies was also influenced by the stem diameter (Fig. 6; Table 4).

Discussion

Plant-dwelling insects make up a large fraction of terrestrial biodiversity, perhaps 40% of animal species (Bush and Butlin, 2004), and many of their social and ecological interactions are mediated by plant-borne vibrations (Cocroft and Rodríguez, 2005). In this study we have gained insights into both the nature of vibrational environments on living plants, and the search strategies of male thornbugs that allow them to navigate a complex environment using highly variable and often uncertain sensory information. Vibration transmission in living plants presents challenges for localization: waves travel at very different speeds on different parts of the plant, and at different frequencies; adding to that complexity, at any given location the stem moves in a whirling path whose properties also vary among different frequencies in the signal. These difficulties are especially great for small insects whose vibration sensors are separated by only a few millimeters. Nonetheless, nearly all of the male thornbugs in this study located the stationary, signaling female. Because the insects' directional decisions are made at identifiable sampling locations, we were able to compare behavioral output to sensory input to identify cues that are important in movement decisions. This study is the first to annotate complete within-plant searches based on plant-borne vibrations by measuring the motion of the substrate at sampling locations, and the first study to investigate the influence of the two-dimensional motion of the substrate on behavior. The whirling motion of plant stems is a fundamental feature of the vibrational world of insects on plants, and differences in this motion have an important influence on behavior.

We identified two mechanisms by which males can determine the direction of the signal source. The most important cue was the direction of wave propagation, with higher accuracy at lower transmission speeds and correspondingly higher time-of-arrival differences at front and back legs. Desert scorpions and termites also use time-of-arrival differences between spatially separated legs to assess direction, and likewise lost directional accuracy at high transmission speeds (Brownell and Farley, 1979; Hager and Kirchner 2014). Although the vibration receptors in the legs of male thornbugs are separated by 5 mm or less, the mechanical resonance of the thornbug's body (Cocroft et al., 2000) converts small time delays into large amplitude differences at the front and back of the body, potentially providing an additional source of information about wave propagation direction. Importantly, there was no evidence of the standing waves that can occur during vibration transmission on plants (Michelsen et al., 1982; Polajnar et al., 2012); standing waves provide no information about source direction. Instead, vibration transmission on these woody plants was dominated by one-way, transient wave propagation. Males also compared signals between sequential samples: males were more likely to walk forward if the axis of stem motion became more closely aligned with their own dorsoventral axis, and more likely to turn around if the axis became less closely aligned with their dorsoventral axis. In honeybees, substrate motion along the long axis of the leg stimulated higher output from the subgenual organ (the principal vibration receptor in the legs of many insects) than did motion in a direction perpendicular to the leg. This difference in the axis of motion was equivalent to an amplitude difference of 10 dB (Kilpinen and Storm, 1997). If the same is true for male thornbugs, the axis of substrate motion relative to a searching male may translate into a substantial difference in perceived amplitude, such that males perceived an increase in amplitude between samples.

We identified a previously unnoticed type of sampling behavior in thornbugs, which we termed "cryptic sampling." In contrast to stationary samples that typically last 3-4 seconds, cryptic samples take only a fraction of a second. They occur when males continue to signal while walking, but pause briefly during the female reply. Males evidently gained information from cryptic samples: the proportion of reversals of direction, and the influence of distance from the female on accuracy and step length, did not differ between sample types. We hypothesize that cryptic samples are a competitive strategy used by males to reduce search time. In this study there was only one male on the plant at a time, but playback of a male-female duet to initiate signaling may have caused males to behave as though a competitor were present. Thornbugs often occur at high densities; because females mate only once and will duet with more than one male at a time (Gibson, per. obs.), there is likely sexual selection for rapid localization. Males spent about a quarter of their search time in stationary sampling, so individuals may be able to reduce their sampling time through these short samples. Although data from this study did not reveal a speed vs. accuracy tradeoff in sampling behavior, study of search behavior in other contexts (such as the presence of other males) may reveal why males usually engaged in the more time-consuming stationary samples. The discovery that males can obtain brief samples while moving from one stationary sample to another is relevant to studies of other species with intermittent locomotion; closer examination may reveal that individuals are obtaining information not just during the stationary phase, but also during the movement phase.

Thornbugs often experience substantial uncertainty about source location. Accuracy can be unambiguously quantified during these searches, because both the target location and the decision points are known (see Legendre et al., 2012; Polajnar et al., 2014). The accuracy of movement decisions was lower when males were farther from the signaling female. Errors occurred in runs, with males making several incorrect decisions in a row before correcting course; shorter runs of errors were also seen during vibrational homing in a leafhopper (Polajnar et al., 2014). Note that we only considered a decision to be inaccurate if the male turned in the incorrect direction after a sample; we did not consider it an error if the male took the wrong turn at a branching point without stopping to sample. The relationship between distance to the source and accuracy was mirrored by a relationship between distance and sampling behavior. The distance males moved between samples became successively smaller as males approached the female. The longer inter-sample movements made at greater distances may allow individuals to leave areas where source direction was difficult to assess (Bartumeus, 2009; Hein and McKinley, 2012).

The observation that the sampling behavior of searching males changed predictably with distance from the source indicates that males experienced some form of distance-dependent vibrational gradient. We examined four potential vibrational gradients, including overall signal amplitude; the relative amplitude of higher and lower frequencies; and within-signal variation in both the axis of motion and the elliptical shape of stem motion at different frequencies. We investigated how each of these gradients was influenced by both distance to the source, and by the stem diameter at the males’ location.

The searching males in this study encountered amplitude gradients: within 10-20 cm of the source, signal amplitude increased reliably as males approached the source, while at greater distances there was no relationship between amplitude and distance from the source (see Fig. 5A). These short-range gradients occurred only on the side stem on which the female was located. The steep increase in amplitude close to the source may be due at least in part to vibrational near-field effects (Miles, 2016). The existence of a local amplitude gradient on side stems where females were located provides reliable information about whether to explore a side stem: on the 'correct' side stem the amplitude will increase relative to the main stem, while on the 'incorrect' side stems, the amplitude will decrease or stay the same relative to the main stem. Similarly, searching leafhoppers (Polajnar et al. 2014) make rapid corrections after walking from the stem onto the petioles of leaves not containing the female. The leafhopper males encountered amplitude increases once they walked onto the leaf from which the stationary female was signaling (Mazzoni et al. 2014), and the amplitude increases are correlated with changes in the searching male's behavior.

At any one frequency produced by a vibration source on a plant, there is unlikely to be a gradient of monotonically decreasing amplitude with distance from the source (Mazzoni et al., 2014; Michelsen, 2014; Michelsen et al., 1982; Mortimer, 2017; Virant-Doberlet et al., 2006); this is because of changes in the properties of the transmission channel, or because of reflections that create standing waves with local minima and maxima (Polajnar et al., 2012). However, signal amplitude must decrease with distance due to frictional loss during transmission (Mortimer, 2017), and for signals with a broad band of frequencies the amplitude does fall off reliably with distance (this study; Polajnar et al 2014). Michelsen et al. (1982) point out that in their measurements of signal propagation along plant stems, there were monotonic decreases in signal amplitude with distance for frequencies above 2kHz. Furthermore, in that study, the average amplitude across the entire spectrum measured (based on area under the curve from image analysis in ImageJ (Schindelin et al., 2015), also decreased monotonically: at 3, 7 and 17 cm from the source, the average amplitude was approximately -2dB, -5dB, and -12dB relative to the amplitude at the source. Amplitude gradients thus appear to provide a reliable cue for insects homing in on signals with a wide frequency range, such as insect songs with broadband components (Polajnar et al., 2014) or herbivore feeding vibrations (Pfannenstiel et al., 1995).

The signal amplitude perceived by a searching insect will also be influenced by changes in the two-dimensional nature of stem motion. We already mentioned the influence of the major axis of stem motion, and its degree of alignment with the axis of greatest sensitivity of the vibration receptors in the legs. This effect may be further modified by the eccentricity of stem motion. For a given amplitude of stem motion, there is twice as much energy in the signal if the stem moves in a circular path than it moves in a linear path (i.e., there is an equal amplitude of motion along two orthogonal axes). Furthermore, if there is considerable energy in the minor axis of the elliptical path of stem motion, this may compensate for a misalignment of the major axis of stem motion with the axis of greatest sensitivity of the leg vibration sensors. Searching male thornbugs provide behavioral evidence that both aspects of stem motion are important. Males in situations of high uncertainty (such as when far from the female) tended to walk farther between samples. The signal traits correlated with these longer inter-sample distances were the signal eccentricity (males walked farther when motion was more linear) and the axis of stem motion (males walked farther when there was greater variation in the axis of stem motion among different frequencies in the signal).

Signal amplitude is influenced not only by the transmission path, but also by the local properties at the sampling location, in particular the stem diameter. Whether the insects make use of the strong inverse relationship between amplitude and stem diameter is unknown, but there are at least three ways in which this relationship could shape communicative interactions. First, because distance and diameter have independent effects on signal amplitude, there will be a steeper amplitude gradient relative to a signal source at the narrow end of a tapering stem than at the wider end. Depending on whether the fitness of the vibration-producing individual is increased by being located (as with a signaling female) or decreased (as with a feeding herbivore), it may be an advantage to be at the narrower or wider end of a tapering stem. Second, detection of low-amplitude signals may be enhanced when insects ‘listen’ on small-diameter stems.

In addition to amplitude gradients, searching male thornbugs also experienced distance-to-source gradients in the nature of stem motion. In particular, there was a distance-to-source gradient in the major axis of stem motion: with distance, the axes of motion within the signal became increasingly uncorrelated among different frequencies in the signal. In contrast, for the plants in this study the eccentricity of stem motion was primarily a function of plant structure rather than source distance, increasing toward the less anchored parts of the plant including the top of the plant, and the outer parts of the side stems. Accordingly, eccentricity gradients were unreliable indicators of source direction; instead there was a coincidental increase or decrease in eccentricity with distance, depending on whether the signaler was near the bottom or the top of the plant. This finding does not support the hypothesis proposed by Virant-Doberlet et al. (2006), that changes from more linear to more circular motion provide a cue of source distance. The two studies that support the 'eccentricity gradient' hypothesis were limited in scope – one was based on single-sensor measurements around the stem of a sedge vibrated with an actuator, at two distances from the source (Virant-Doberlet et al. 2006); the other was based on two-sensor measurements as in this study, at three distances from signaling male thornbugs on a woody plant (McNett et al. 2006) – and cannot separate out the effects of distance from those of plant structure or the possible anchoring effect of a vibration actuator.

Frequency-dependent attenuation of bending waves has also been proposed as a ranging mechanism for vibrational signals on plants (Aicher et al., 1983; Barth et al., 1988), because higher frequencies have shorter wavelengths and thus lose more energy to friction per unit distance than lower frequencies (Mortimer 2017). In the present study there was indeed a distance-dependent change in the spectral shape of female signals, with the predicted loss of higher frequencies at greater distances; however, there was no evidence that males used this vibrational gradient. Polajnar et al. (2014) looked for an influence of such attenuation on the behavior of searching male leafhoppers, but in that study there was no consistent relationship between spectral shape of the female reply signals and either distance from the female, or male behavior.

The picture emerging from consideration of these three gradients — amplitude, whirling motion of the stem, spectral shape -- is that as a male travels farther from the signaling female, the plant stems move in an increasingly complex and disordered fashion. This increasing disorder of the signal is associated with the males' decisions to travel farther before stopping to obtain another sample. In thornbugs, then, the action-perception cycle responds to the quality of the available cues of source location. These properties of stem motion – the axes along which the stem moves, the eccentricity of its elliptical path, and the variation of these features among different frequencies in the signal – should be taken into account in future studies of vibration-guided searching.

The behavior of the stationary female may provide another source of directional information, in addition to the transmission-dependent changes in vibrational signals. In many insect duetting systems there is mutual behavioral adjustment between signalers (Rodríguez and Barbosa, 2014). Both individuals may be able to assess the distance between them, and if the interaction is cooperative, the stationary individual could in principle change its signaling behavior to provide the searcher with additional information. In this study, females varied the duration of their signals, and male search accuracy was lower when the female signals were longer. The cause and effect relationship here is unclear, and the most we can say is that the behavior of the searching and stationary individuals were correlated such that not only males, but also females, were altering their behavior in predictable ways over the course of the search. It would be useful to examine the relationship between sensory input and behavioral output in females, for example by measuring signals at the female's location and relating properties of male signals to female signaling behavior.

The efficacy of female signals, i.e., their transmission and detectability (Endler 1992; Endler and Basolo 1998) was maximized when females were near the top of the plant. Although male-female distance was the same at the start of all trials, the transmission path between female and male consisted of larger-diameter stems when the female was at the bottom of the plant. As stem diameter increases, signal amplitude decreases and wave propagation speed increases; thus as males search on larger-diameter stems they will encounter lower signal amplitudes and smaller time delays between sensors in front and back legs, and both of these features will reduce accuracy. Furthermore, stem properties such as xylem vessel size, stiffness, and mass per unit volume also differ at different locations on a plant, further influencing vibration propagation (Niklas, 1992). A female's choice of a signaling site within a plant will therefore have a large effect on signal efficacy.

Further studies will likely reveal additional adaptations that allow insects to navigate such a complex environment, and will be especially fruitful when combined with experimental tests of the hierarchy of cues used by searching insects. Furthermore, laboratory conditions such as those in this study represent an ideal case for search efficiency. In nature, vibrational environments are noisy, with plant tissues set into vibration by wind, rain, airborne sound, and movement or signaling by multiple species on the same plant (Cocroft and Rodríguez, 2005; McNett et al., 2010; Tishechkin, 2007; Virant-Doberlet et al., 2014). Sociality will also influence biotic noise levels: male thornbugs are most likely to encounter relatively low-noise conditions like those in this study when searching for solitary, dispersed females. In contrast, other mate-finding mechanisms may be required when searching for females still in the natal aggregation, where other signaling males are typically present and there may be dozens of females in close proximity, most of which are non-receptive (De Luca and Cocroft, 2011).” In any case, search efficiency is likely to be under strong selection, especially during competitive mate searching (Legendre et al., 2012) or during recruitment to a feeding site (Cocroft, 2005). The strategies used by insects during vibrational homing on plants are thus an excellent model system for understanding how search strategies evolve to exploit uncertain sensory cues in a complex environment (Giuggioli and Bartumeus, 2010).

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Figures

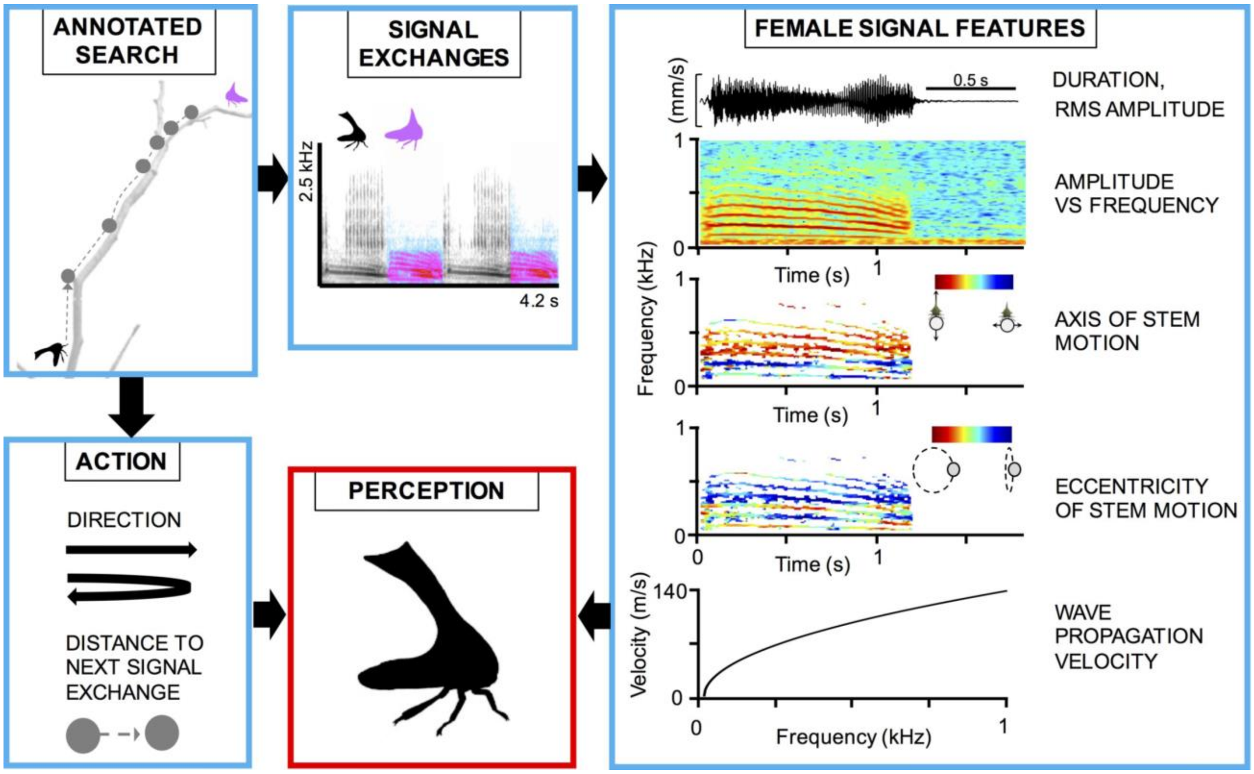

Figure 1: **Study overview:** We first annotated male searches, mapping each decision point (dots, top left panel) where the searching male ( ) stopped and exchanged signals with the stationary female ( ). We used two-dimensional laser vibrometry to measure the female's vibrational signals at each of the male's decision points. We then extracted multiple features of the signals, including traditional features such as signal amplitude and duration as well as previously unmeasured features including the major axis and the eccentricity of stem motion at each frequency in the signal (signal shown is from the search in the top left panel). Finally, we used a general linear mixed model to relate sensory input to subsequent action, to infer how the searching males perceived and weighted female signal features when making movement decisions. Blue boxes represent the 'knowns' at each decision point and the red box represents the 'unknown' estimated by the statistical model

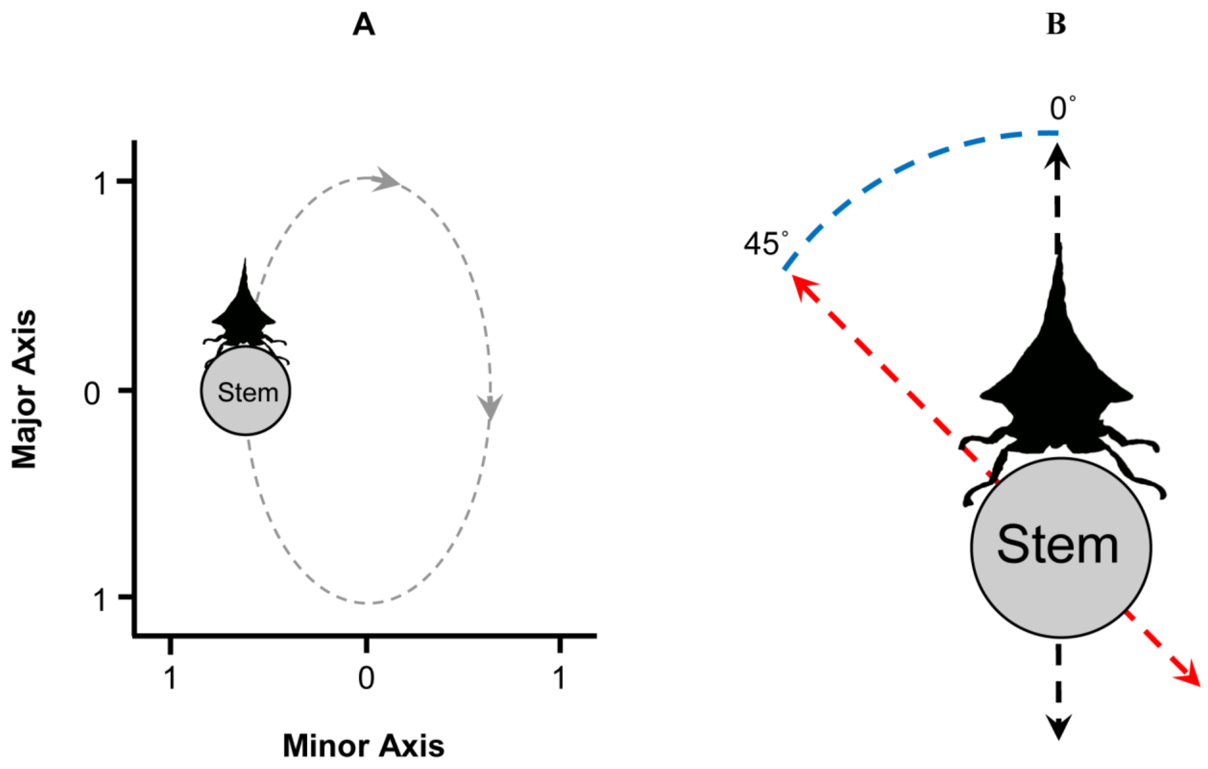

Figure 2: **Two features of the two-dimensional motion of a plant stem during transmission of vibrational signals.** A) Eccentricity (minor axis / major axis, in this case eccentricity = 0.5), magnitude of motion is exaggerated for illustration. B) Angle of rotation (in this case 45˚; the black dotted line at 0˚ represents the male’s dorsoventral axis and the red dotted line the major axis of stem motion).

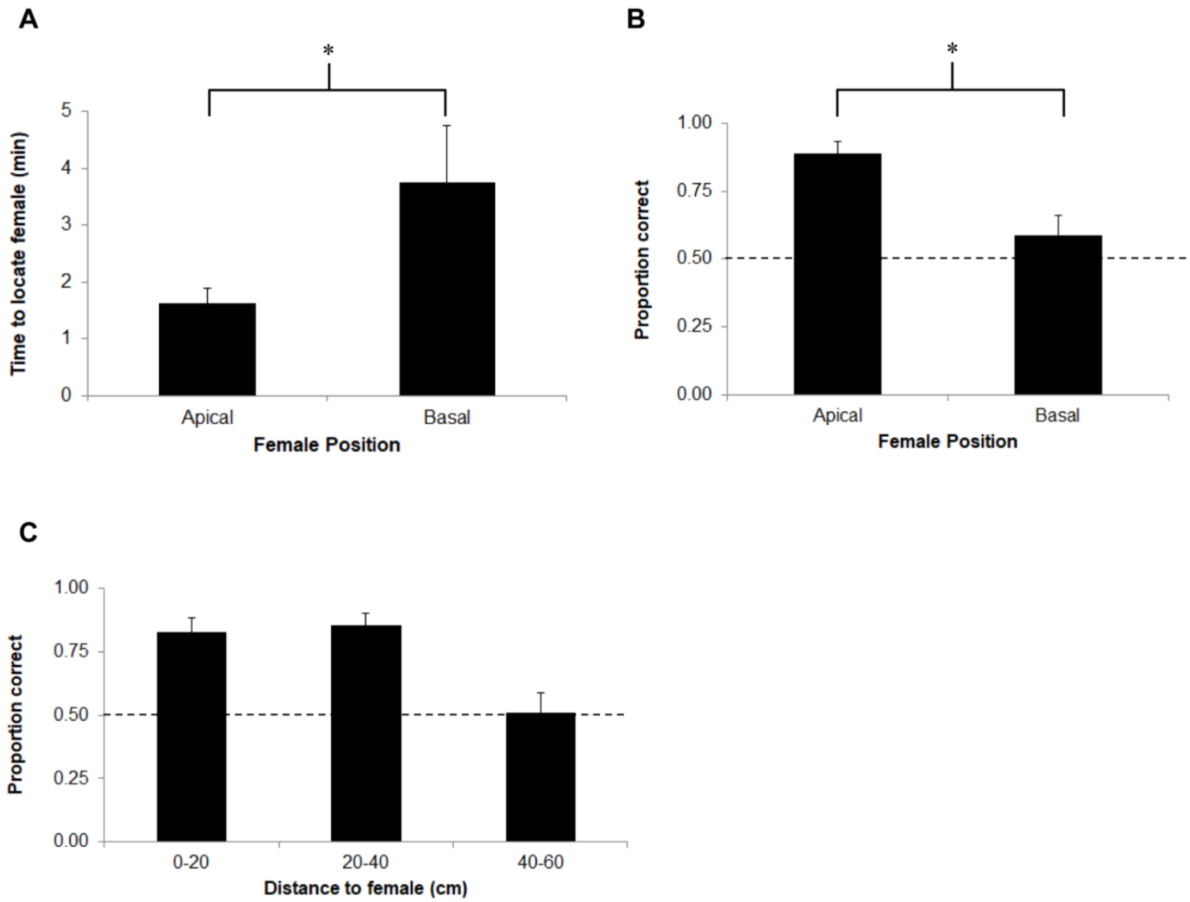

Figure 3: **The influence of female location on male search time and accuracy:** males located females more quickly (A) and made fewer directional errors. (B) when the female was in the apical location on the plant (16 male-female pairs). (C) Males made more accurate decisions when closer to the female (grand means, 16 males. 360 directional decisions). * p = < 0.05. Error bars represent standard error of the mean.

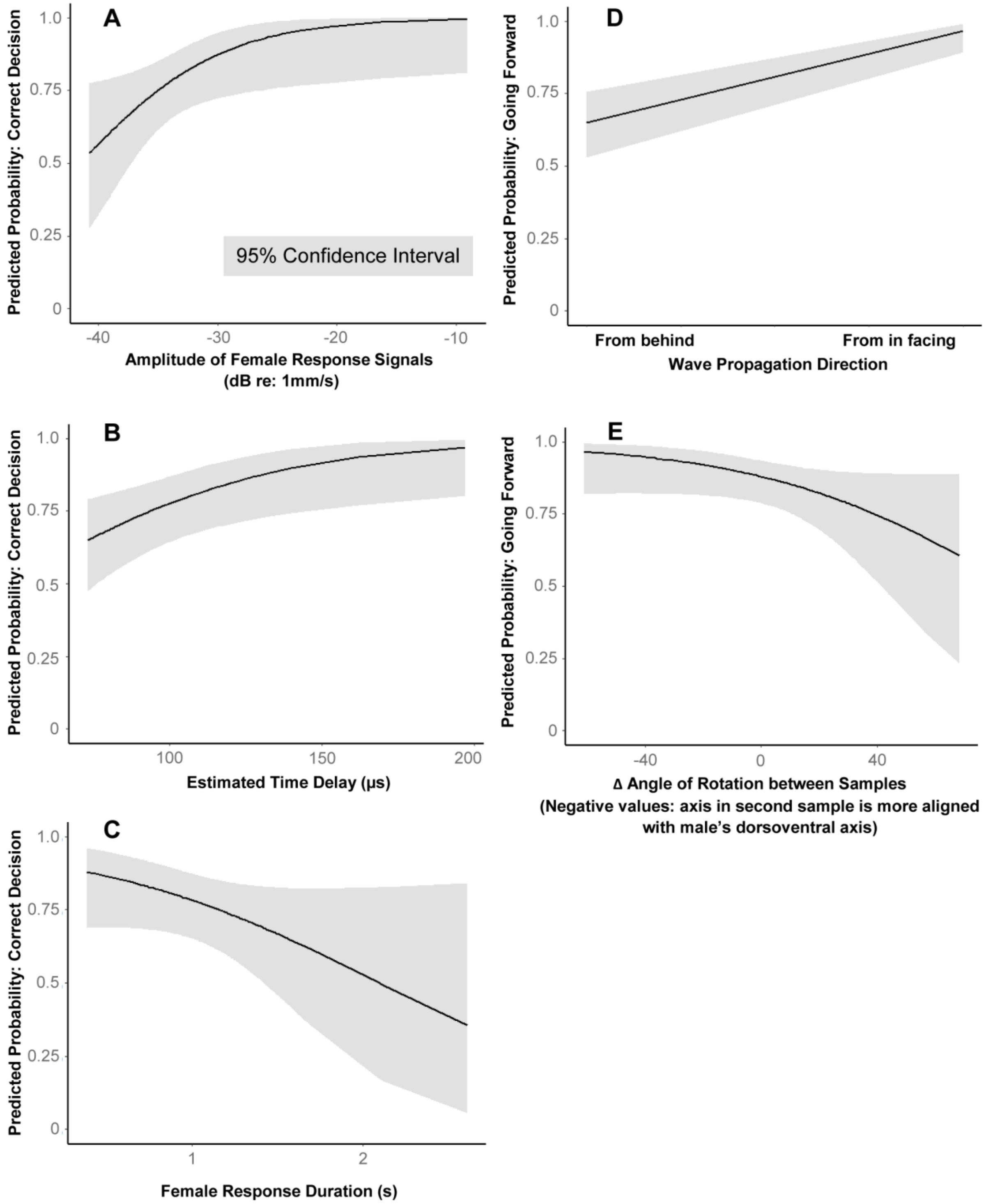

Figure 4: Relationship between sensory input and motor output for male thornbugs. During their search, males make more accurate decisions when the female response signal at the current sampling location (A) is greater in amplitude; (B) has a lower propagation speed and correspondingly greater time-of-arrival difference between vibration sensors in front and back

legs; and (C) is shorter in duration. Males are more likely to walk forward rather than turn around when (D) the propagating wave arrives from in front rather than from behind; and (E) when the male experiences a between-sample change in the axis of stem motion, such that the stem motion during the second signal is more closely aligned with the male’s dorsoventral axis.

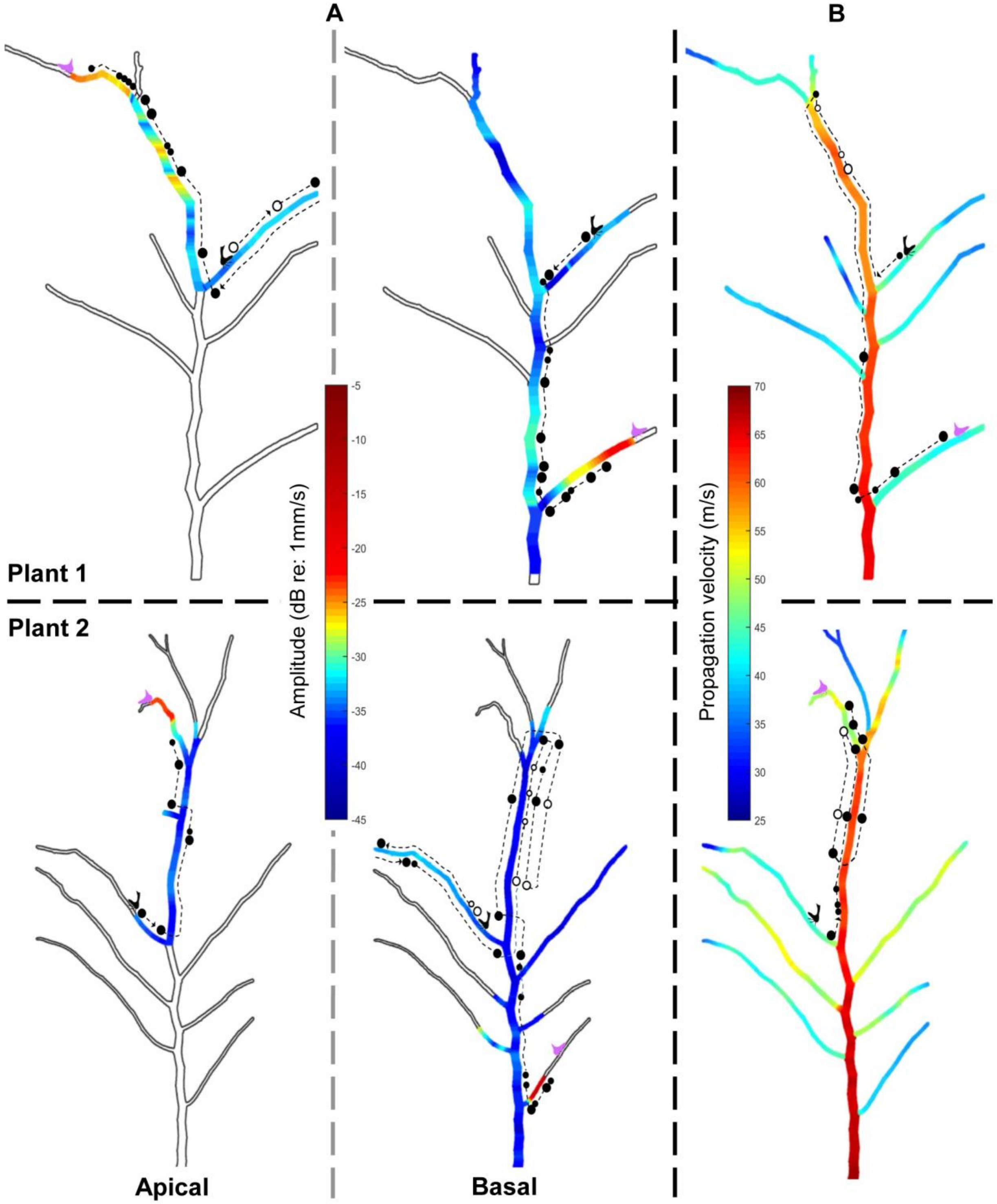

Figure 5: **Within-plant spatial variation in vibrational signal properties encountered by searching male thornbugs:** silhouettes represent female ( ) location and male ( ) starting location. A) Amplitude gradients for both female positions within each plant; heat maps represent measurements at all locations sampled by males for that female position on that plant,

plus interpolated values. B) Wave propagation speed at 200Hz for all locations on each plant, estimated from the measured relationship between stem diameter and propagation speed. One search path is superimposed on each heat map, to illustrate the changing vibrational environment encountered by searching males; circles show decision points where males stopped and exchanged signals with stationary females. Closed circles = correct decisions; open circles = incorrect decisions; larger circles = longer, stationary samples; smaller circles = brief, cryptic samples.

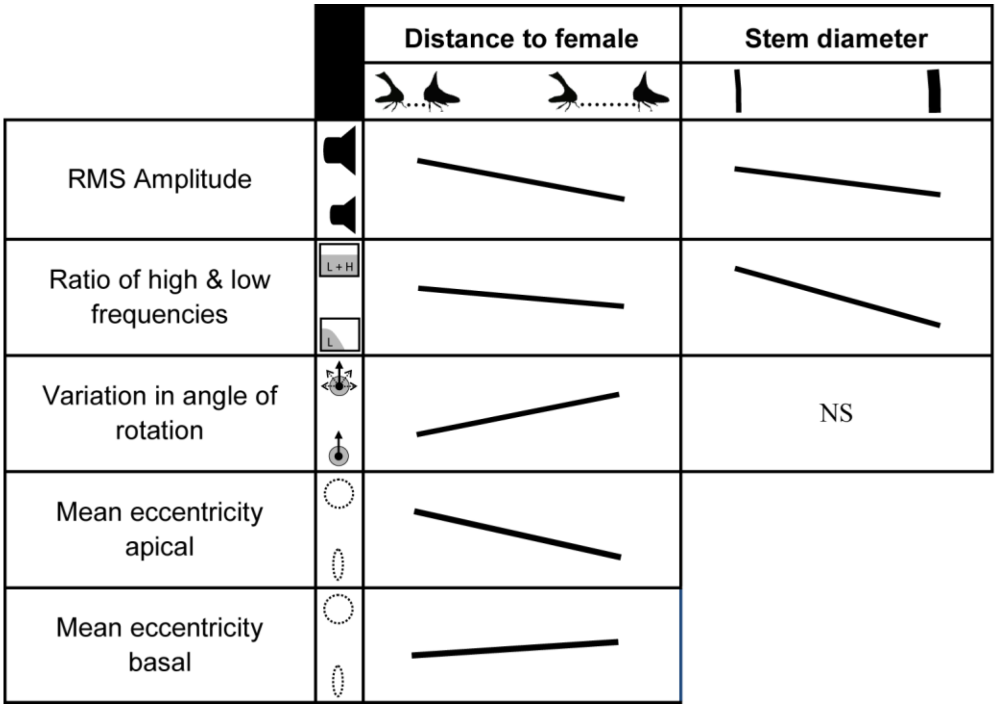

Figure 6: **The relationship between vibrational signal properties and distance along the plant structure from the signaling female, and the stem diameter at the sampled location.** Solid lines indicate the slope of significant relationships, based on linear regression.

Tables

Table 1: Signal features that influence the accuracy of movement decisions by searching male thornbugs. Random effect (controlling for differences among male-female pairs) not included in tables.

Effects	**Numerator DF /Denominator DF**	**F-Value**	**P-Value**
Amplitude RMS	1/199	8.74	**0.0035**
Mean eccentricity	1/199	0.00	0.9956
Mean angle of rotation	1/199	2.05	0.1541
Estimated time delay	1/199	9.65	**0.0022**
Ratio of high & low frequencies	1/199	0.62	0.4336
Female response duration	1/199	6.28	**0.0130**

Table 2: Signal features that influence the forward / reverse decisions of searching male thornbugs.

Effects	**Numerator DF /Denominator DF**	**F-Value**	**P-Value**
Wave propagation direction	1/139	15.33	**0.0001**
Δ Amplitude RMS	1/139	0.28	0.5989
Δ Angle of rotation	1/139	4.63	**0.0332**
Δ Mean eccentricity	1/139	1.45	0.2299
Δ Ratio of high & low frequencies	1/139	0.05	0.8209
Δ Female response duration	1/139	1.85	0.1763

Table 3: Signal features that influence the distance that searching male thornbugs walked between sampling locations.

Effects	**Numerator DF /Denominator DF**	**F-Value**	**P-Value**
Amplitude RMS	1/192	1.76	0.1862
Mean eccentricity	1/192	9.12	**0.0029**
Mean angle of rotation	1/192	0.34	0.5629
Variation in angle of rotation	1/192	9.80	**0.0020**
Estimated time delay	1/192	0.05	0.8206
Ratio of high & low frequencies	1/192	0.42	0.5184
Female response duration	1/192	3.64	0.0579

Table 4: The relationship between signal features, distance to the signal source, and stem diameter. Non-significant interaction terms removed from models. *** indicates significant three-way interaction between distance to female, diameter, and female position (see Table S1 for details).

	Distance to Female			**Stem Diameter**		
	Num DF / Den DF	**F-Value**	**P-Value**	**Num DF / Den DF**	**F-Value**	**P-Value**
Amplitude RMS	1/230	65.09	**<0.0001**	1/230	59.94	**<0.0001**
Ratio of high & low frequencies	1/226	21.37	**<0.0001**	1/226	21.14	**<0.0001**
Variation in angle of rotation	1/230	72.30	**<0.0001**	1/230	1.07	0.3016
Mean eccentricity***	1/226	7.02	**0.0086**	1/226	1.99	0.1602

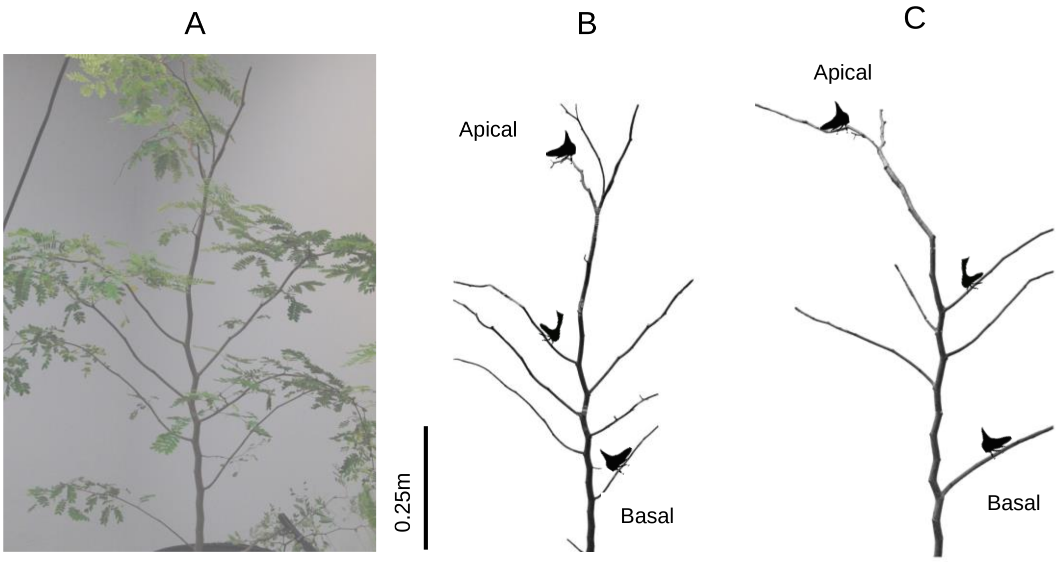

Figure S1. Host plants used in the experiment. (A) one of the plants, with leaves as used in the experiment; (B,C) Branching structure of the two plants, with the two possible female locations ( ) and the starting male location ( );

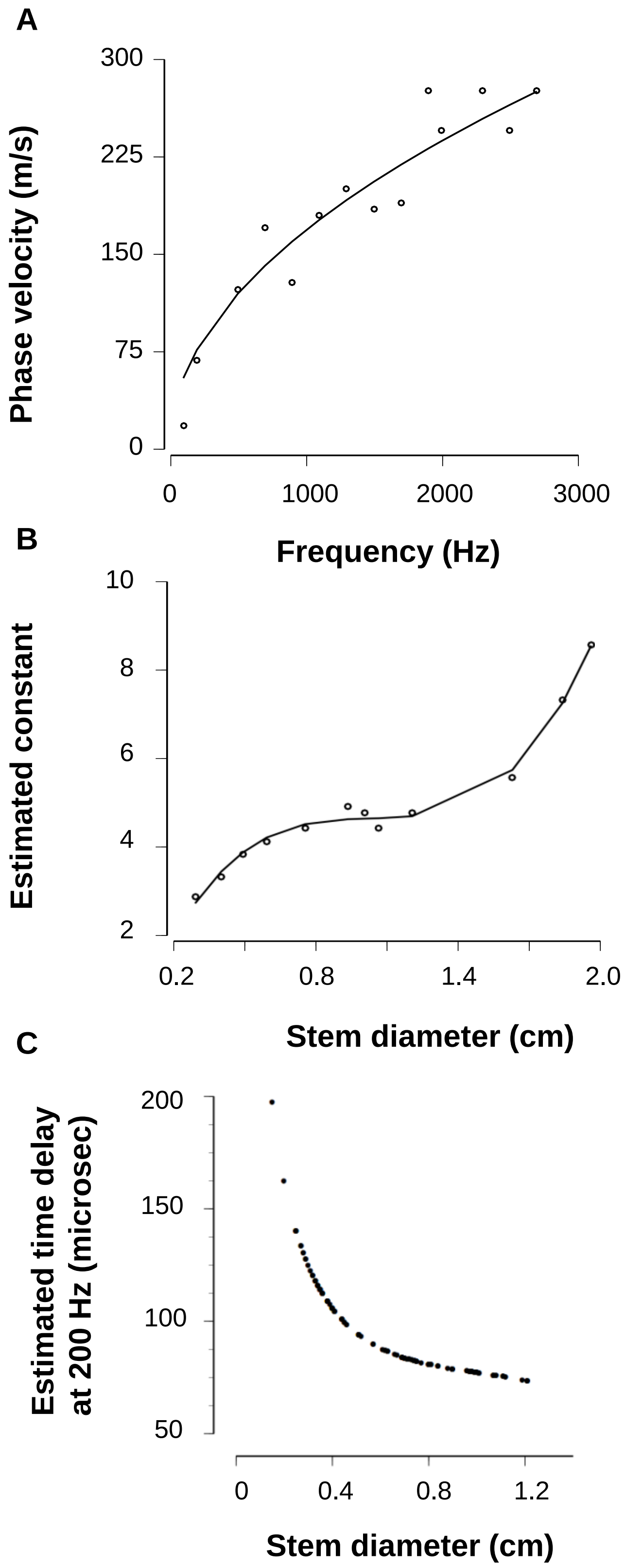

Figure S2. Wave propagation velocity as a function of stem diameter. A. Example of phase propagation measurement at one location with a diameter of 1.63 cm, with curve = constant (5.3) * square root of frequency. Note that measured points will deviate from predicted velocity when there has been a change in the major axis of stem movement. B. Stem diameter vs. estimated constants, with fitted curve used to estimate phase velocity at plant locations sampled by males. C. Estimated time-of-arrival delay at 200 Hz between front and back legs of a male thornbug (distance ~5 mm).

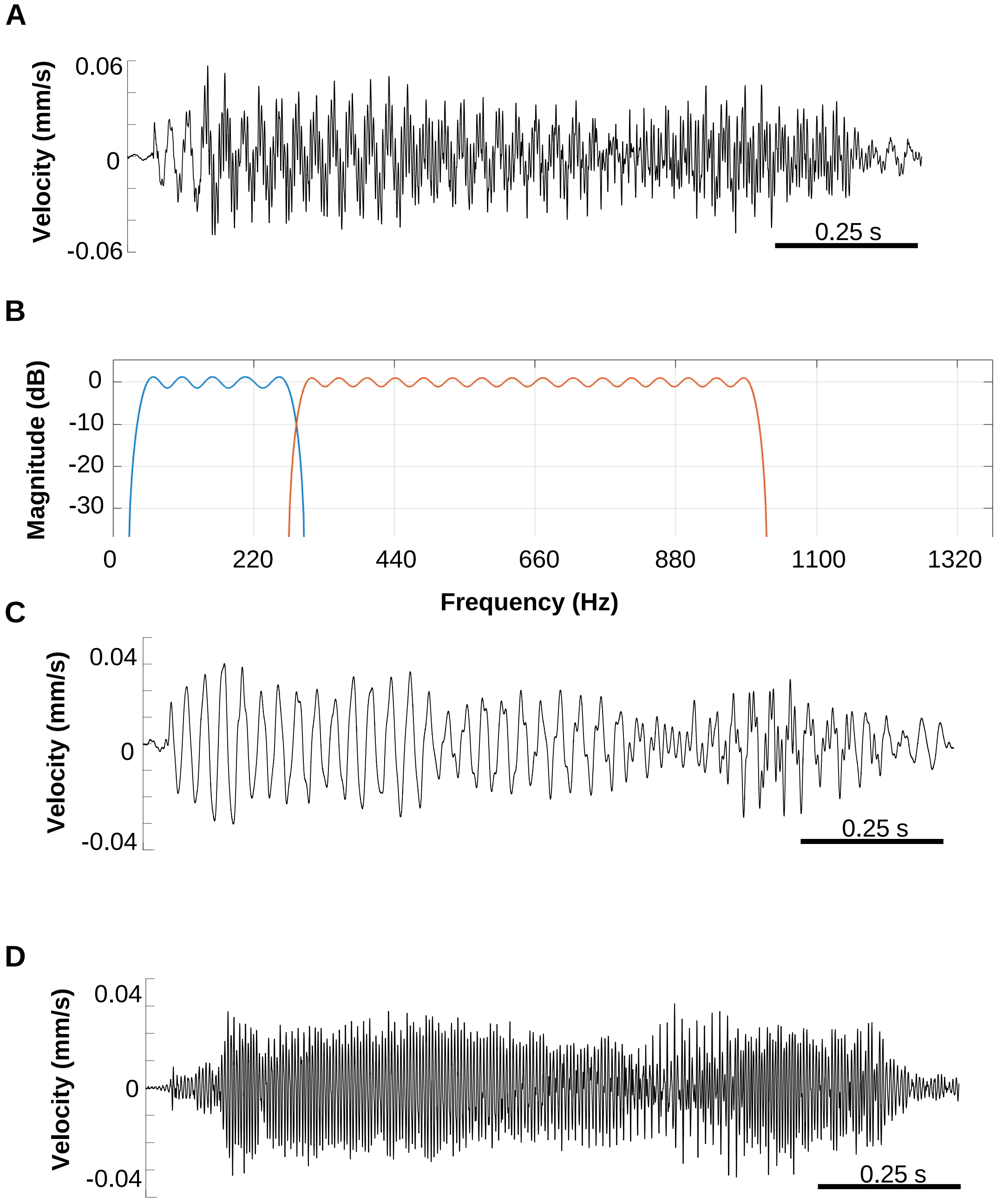

Figure S3. Example measurement of the relative amplitude of higher and lower frequencies in a female response signal, recorded at a location sampled by a searching male. A. Waveform of the female signal; B. Bandpass filters used to generate waveforms of lower and higher frequency bands in the signal shown in (A). C. Waveform of lower frequency band; D. Waveform of higher frequency band. In this example, the RMS amplitude of C is 0.0124 mm/s, the RMS amplitude of D is 0.0121 mm/s, and the relative amplitude of higher and lower frequency bands is 20*log10(.0121/.0124) = -0.2 dB.

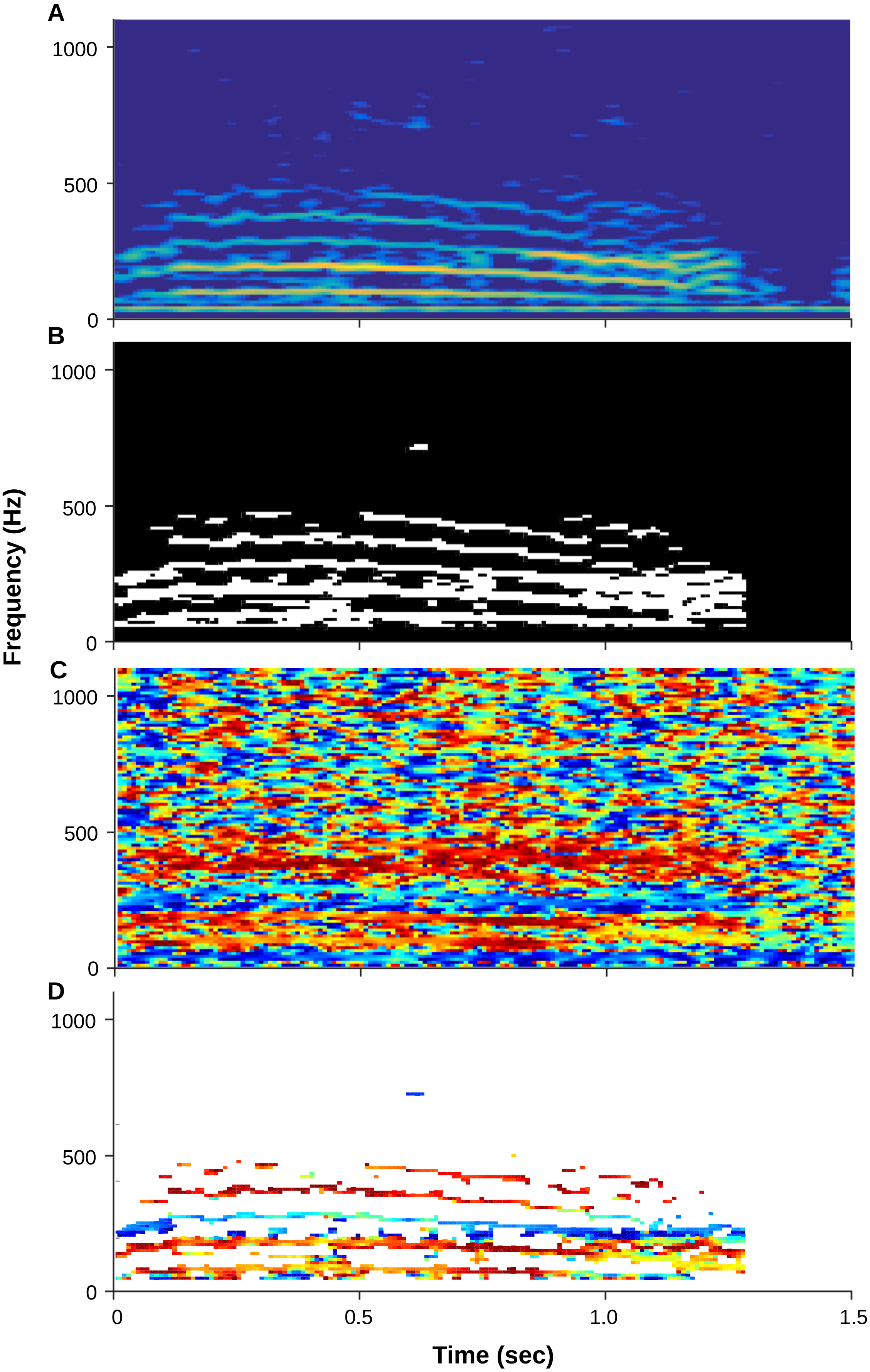

Figure S4. Generation of a spectrogram for angle of rotation. A. Amplitude spectrum; B. Mask generated from amplitude spectrum, excluding values not within 24 dB of peak; C. Spectrogram of angle of rotation; D. Masked spectrogram of angle of rotation, where only the time-frequency bins with signal energy w/in 24 dB of peak are included.

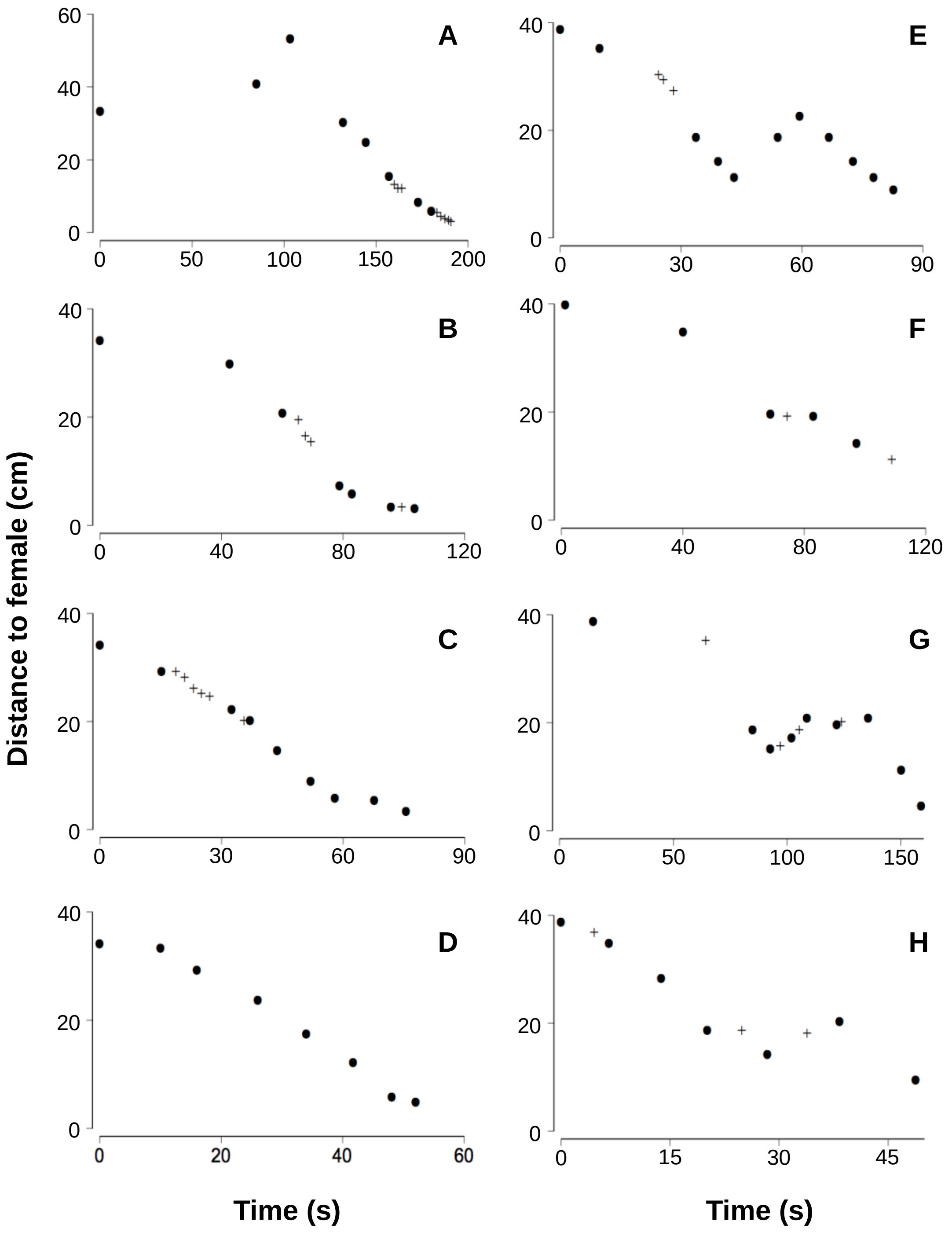

Figure S5. Search paths of male *U. crassicornis*, when the female was in the apical location in the plant. Searches on one plant (A-D); searches on other plant (E-H). ● = stationary samples, during which male signaled and elicited a female response; + = microsamples, during which male signaled while walking, and paused briefly during the female response.

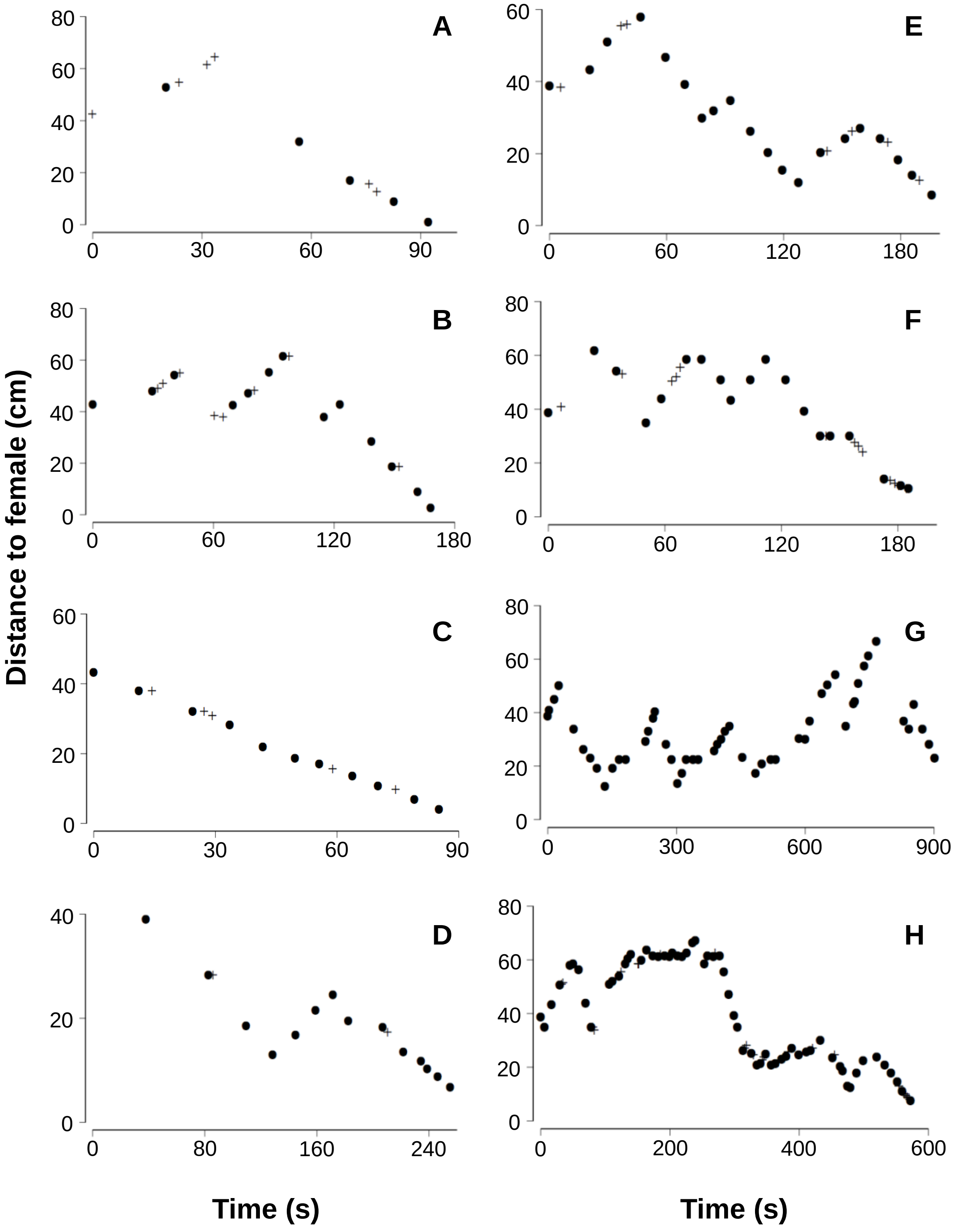

Figure S6. Search paths of male *U. crassicornis*, when the female was in the basal location in the plant. Searches on one plant (A-D); searches on other plant (E-H). ●= stationary samples, during which male signaled and elicited a female response; + = microsamples, during which male signaled while walking, and paused briefly during the female response.

Table S1: The relationship between mean eccentricity, distance to the signal source, stem diameter, and female position.

Mean eccentricity **Effects**	**Num DF / Den DF**	**F-Value**	**P-Value**
Distance to female	1/226	7.02	**0.0086**
Stem diameter	1/226	1.99	0.1602
Female position	1/226	22.48	**<0.0001**
Distance to female * diameter	1/226	3.00	0.0848
Distance to female * female position	1/226	16.80	**<0.0001**
Diameter * female position	1/226	6.45	**0.0117**
Distance to female * diameter * female position	1/226	4.83	**0.0290**