

Green stink bug *Nezara viridula* detects differences in amplitude between courtship song vibrations at stem and petiolus

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Abstract Green stink bug *Nezara viridula* courtship songs are transmitted through plants as substrate vibrations. The amplitude of the vibrations is different at different distances from the source of vibration and at different locations on the plant. Amplitudes of the local vibration were measured on stem and petioli of the bean plant (*Phaseolus vulgaris*) with a Laser-Doppler vibrometer. Differences of the amplitudes of vibration between adjacent points around the nodes were large enough to release differential nerve activities of vibration receptor cells of different legs. There was no correlation between the signal amplitude and the distance from the singing bug, however; the differences in amplitudes of vibrations between the stem and the adjacent petioli of leaves potentially permitted direction finding in the green stink bug males.

Key words: insects · vibroreception · electrophysiology · neuroethology · mating behavior

Introduction

Sexual partners of the green stink bug *Nezara viridula* communicate with low frequency vibrations that are transmitted through host plants [3]. In the mating period these species-specific signals enable stink bug to locate a mate on the plant. Insects and chelicerates use different cues to locate sources of vibration: amplitude, vibration arrival times and/or phase differences between the vibrations at adjacent points. The signals are detected with vibroreceptors located in different legs [5]. Approach towards the source of vibrations was demonstrated in scorpions [1] and spiders [6]. Scorpions detect the time lags of the signals, which can be as small as 0,2 ms [1]. A green stink bug male also uses substrate vibrations to locate a singing female [8]. The

amplitude of plant tissue vibration changes irregularly with the distance from its source [4,7], making it impossible to predict frequency and amplitude of the signal for a specified location on the plant. The aim of the present study was to measure amplitudes of the arriving vibrational signals at different distances from the ramification of the stem and petioli of the green stink bug host plant.

Materials and methods

Artificially induced vibrations of plant tissue were measured on bean plant (*Phaseolus vulgaris*) in an anechoic and sound isolated chamber. The vibrations (natural songs of *N. viridula*) were delivered to the top of the plant with a rod of a minishaker (Bruel&Kjaer type 4810) that was coupled to the plant with a double-sided Scotch tape. Vibrations of the plant surface were measured with a Laser-Doppler vibrometer (Polytec, OFV-303 sensor head and OFV-3001 controller) at distances of 10 cm and 35 cm from the source of vibration, at the stem and leaf petioli. The two points of measurements at the stem and petiolus were 0,5 cm - 2 cm apart (Fig.1). Amplitude of vibration was calculated in decibels (dB) from the equation $\text{dB} = 20 \times \log(x/\text{ref.})$, where x is the measured value and ref. is the reference measured at the node (Fig.1).

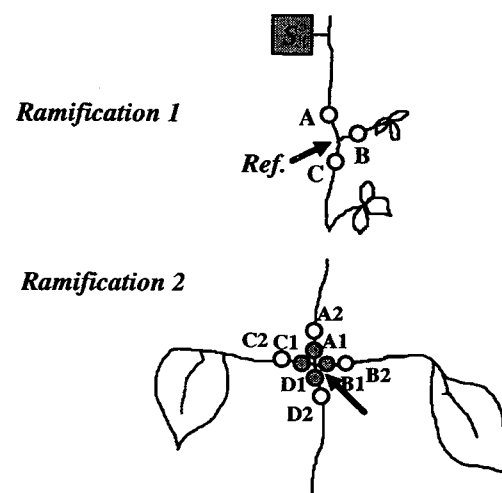


Fig 1: Vibration measurement points on bean plant were located on node (0.0 dB reference point), stem and petiolus, 0.5 and 1 cm away from the node.

Results

Ramification 1: Signal amplitudes were measured at three points located 1 cm from the node (Fig.1). Their values relative to the reference at the node are shown in Table 1. The largest amplitude of the male calling song (MCS) and of the pre-pulse of the female calling song (FCS pre-pulse) were detected on the stem, 1 cm below the node (Fig.1, C). The differences between amplitudes of vibration of the stem above and below the node (Fig.1, A, C) were 0.8 dB (MCS) and 5.1 dB (FCS pre-pulse). The amplitudes of the vibration of the stem above the node were 7.4 dB (MCS) and 2.6 dB (FCS pre-pulse) larger than those on the petiolus (Fig.1, B). Amplitudes of the main FCS pulse were less than 0 on all tested points; the amplitude measured on the stem below the node (Fig.1, C) was 1.3 dB larger than that measured on petiolus (Fig.1, B) and the amplitude on the petiolus was 2.3 dB larger than the one measured on the stem above the node (Fig.1, A).

	MCS	FCS pre-pulse	FCS main pulse
A	8.8	1.9	-4
B	1.4	-0.7	-1.7
C	9.6	7	-0.4

Table 1: Amplitudes (in dB relative to the node amplitude) of the *N. viridula* calling songs, measured at three points (A-C) of the ramification 1.

Ramification 2: Amplitudes of substrate vibrations were measured at eight points around the ramification, at distances 0.5 cm and 1cm away from the reference point at the node (Fig.1, Table 2). The largest amplitude of the MCS chirp was detected on the stem above the node (Fig.1, A2). The amplitudes of the MCS plant tissue vibrations on the leave petioli (Fig.1, B1, B2, C1, and C2) were smaller than at the stem. There was 5.2 dB difference in amplitude of vibration between points B1 and A2. The smallest amplitude of MCS vibrations was measured at the stem below the node; the difference of 6.5 dB was detected between points D1 and C2. The amplitude of the FCS pre-pulse plant vibrations was 7 dB larger on the stem below the node than on the right petiolus (Fig.1, D2 and B2). On the stem above the node the amplitude of the FCS pre-pulse was 6.3 dB larger than on the right petiolus (Fig.1, A2, B2) and 5.6 dB smaller than on the left petiolus (Fig.1, A1, C1). The largest amplitude of the main pulse of FCS chirp was measured on the stem below the node (Fig. 1, D2); it was 9 dB larger than on the right petiolus (D2 and B2). The amplitude of FCS vibratory signal was the smallest on the stem above the node; there was a 7.3 dB difference between points A2 and C1.

	MCS	FCS pre-pulse	FCS main pulse
A1	4.6	-3.6	-2.1
A2	7.3	2.1	-2.5
B1	2.1	-1	0.3
B2	3.5	-4.2	0
C1	3.8	1.8	4.7
C2	4.6	0	4.4
D1	-1.9	0	6.4
D2	2.1	2.8	9

Table 2: Amplitudes (in dB relative to the node amplitude) of the *N. viridula* calling songs, measured at eight points (A1-D2) of ramification 2.

Discussion

The differences in parameters of plant tissue vibrations potentially enable the green stink bug males to locate the direction of the singing female: the difference in amplitude of the signal between stem and petiolus and the difference in signal arrival times. Green stink bug female calling song that propagates through host plant tissue releases the appetitive phase of the male sexual behavior that includes singing and female searching. The male searching behavior is the same on different plants [8]. The searching male halts at ramification, straddles legs across the bifurcation and proceeds walking after it detects the next female signal. Amplitude differences of plant tissue vibration between adjacent points around the ramification were large enough to release differential neuronal responses at legs positioned at stem and petiolus [2]. Thus the detection of the amplitude difference of the vibratory signals between stem and petiolus potentially enables the male to locate the direction of the incoming vibratory stimuli.

In addition, green stink bug possibly detects differences in arrival times of the incoming vibrations at different legs. Time lags of the substrate vibration arrival times at different legs of the green stink bug were calculated for known propagation velocities of the 200 Hz vibration [7] and distances of 1-1.5 cm. These time lags are at the threshold of the arrival time discrimination as determined for scorpions in behavioral experiment [1]. The 100 Hz vibrations of the green stink bug female calling song yield even larger differences in vibration arrival times. The substrate vibration arrival time difference might supplement the direction finding data in the green stink bug males.

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