

Tuning of host plants with vibratory songs of *Nezara viridula* L (Heteroptera: Pentatomidae)

Andrej Čokl*, Maja Zorović, Alenka Žunič and Meta Virant-Doberlet

Department of Entomology, National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia

*Author for correspondence (e-mail: andrej.cokl@nib.si)

Accepted 23 February 2005

Summary

Songs of the southern green stink bug *Nezara viridula* L, recorded on a non-resonant loudspeaker membrane, reflect frequency characteristics of body vibrations. The song dominant frequency directly depends on the repetition rate of potentials recorded from synchronously contracting muscles that vibrate the abdomen during singing. Spectra of naturally emitted signals recorded on the pronotum of a singing bug or on a plant contain peaks characteristic of plant resonant spectra. The dominant resonant frequency of sound-induced vibrations in bean and other stink bug host plants ranges between 160 and 215 Hz and subdominant peaks do not exceed 600 Hz. There is no correlation between spectral peak positions

and recording points on a plant. The dominant resonance peak corresponds to the best frequency sensitivity of *N. viridula* middle frequency subgenual receptor cell. Subdominant peaks around and below 100 Hz lie close to the dominant frequency of body vibrations during singing and to the range of best frequency sensitivity of low frequency receptor cells. Tuning of plant resonant frequencies with spectral properties of songs and frequency sensitivity of sensory organs is discussed in the context of stink bug substrate-borne communication.

Key words: vibratory communication, vibratory song, resonance, host plant, *Nezara viridula*, southern green stink bug.

Introduction

Substrate-borne vibrational communication plays an important role during long-range calling and short-range courtship communication in stink bugs (Fish and Alcock, 1973; Harris and Todd, 1980; Borges et al., 1987; Kon et al., 1988; Čokl and Virant-Doberlet, 2003). All songs described so far were recorded either as airborne signals (Čokl et al., 1972; Kon et al., 1988) or as vibrations of a non-resonant loudspeaker membrane (Čokl and Virant-Doberlet, 2003). Spectra of songs recorded by the latter technique have uniformly low frequency and narrow band characteristics: the dominant frequency ranges between 60 and 150 Hz and a different number of higher harmonics do not exceed 600 Hz. The dominant frequency of emitted vibratory signals in the southern green stink bug *Nezara viridula* L corresponds to the frequency of body vibration as well as to the repetition rate of potentials recorded simultaneously from muscles vibrating abdomen during singing (Kuštor, 1989; Amon, 1990). Spectral differences between songs of different type or species are mainly expressed in the number of higher harmonics and in the presence or absence of frequency modulated parts. For example, spectra of *N. viridula* courtship and calling songs differ by the presence or absence of the first harmonic peak (Čokl et al., 2000); most of male and female songs of *Thyanta pallidovirens* (Stal) and *T. custator accerra* McAtee are characterized by distinct dominant and several higher harmonic peaks (McBrien et al., 2002); and extensive

frequency modulation was demonstrated in some songs of *Euschistus conspersus* Uhler (McBrien and Millar, 2003).

All species of the subfamily Pentatominae are entirely plant feeders (Panizzi et al., 2000). On branched dycotyledonous plants with different impedance characteristics one can expect high distortion of low frequency and narrow-band signals, due to reflections and frequency-dependent standing wave patterns (Michelsen et al., 1982; Barth, 1998). In such conditions, songs of *N. viridula* should be less suitable for communication through plants. Furthermore, vibratory sensory organs of *N. viridula* are not precisely tuned with the spectral properties of loudspeaker recorded songs (Čokl, 1983). Nevertheless, behavioural experiments prove that male and female *N. viridula* communicate efficiently with vibratory songs on the same plant. Female calling-song signals, for example, mediate vibrational directionality of males on stem/stalk crossings (Čokl et al., 1999) and mates differentiate the conspecific song from those of other stink bug species at long distances (Hrabar et al., 2004).

The impact of substrate type on vibratory communication was first shown in *N. viridula*: males differentiate temporally different female calling-song pulse trains on a non-resonant loudspeaker membrane but not on a plant (Miklas et al., 2001). The leaf and its structural components play an important role in the propagation of the short transient signals produced by insects (Magal et al., 2000). Significantly higher

attenuation of the harklequin bug vibratory signals was demonstrated in leaf lamina than in leaf vein (Čokl et al., 2004). In several examples of lacewing species confined to conifers vs those on herbaceous vegetation, song phenotype showed a correlation with substrate independent of phylogeny; nevertheless the pattern suggestive of environmental adaptation of songs to their substrates was not experimentally supported (Henry and Martinez Wells, 2004). The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider was recently investigated (Elias et al., 2004). It has been demonstrated that despite different filtering properties, the male courtship behaviour was not modified on different substrates, but the proportion of males mating successfully on leaf litter was significantly higher than on rocks or desert sand.

The aim of our study was to elucidate the apparent inconsistency between spectral properties of loudspeaker-recorded songs and tuning of vibrational receptors on the one hand and efficient substrate-borne communication through plants on the other. We propose the hypothesis that singing stink bugs induce resonant vibration of a plant with frequency characteristics reflected in the spectra of the transmitted signals. To confirm this hypothesis we measured resonant frequency characteristics of stink bug host plants and compared them with the spectra of naturally or artificially induced vibrations, measured simultaneously from the body of a singing bug and different parts on a plant. To avoid potential impact on the mechanical properties of plants, we used non-contact stimulation and recording techniques.

Materials and methods

Animals and plants

All experiments were conducted in an anechoic room (Amplifon Type E-Large, Amplaid, Italy) at the Department of Entomology of the National Institute of Biology (Ljubljana, Slovenia), at 22–26°C ambient temperature, 65–75% relative humidity, between 09:00 h and 14:00 h (i.e. 2 h after the start of the photophase). Laboratory grown adult (14–30 days after the final moult) and sexually mature (Brennan et al., 1977) male and female southern green stink bugs *Nezara viridula* L were used in all experiments. The Slovene colony started from wild adults collected at the North Adriatic coast and the French one from the colony grown at INRA Centre Versailles (France). Animals were reared in plastic cages (38 cm long, 23 cm wide and 23 cm high), at 22–26°C, relative humidity 70–80%, 16 h:8 h L:D cycle, and on a diet of green beans (*Phaesolus vulgaris* L), mung beans (*Vigna mungo* L) hepper, raw peanuts (*Arachis hypogaea* L) and sunflower seeds (*Helianthus annuus* L).

Vibratory signals were recorded from different points on the surface of fresh green bean, grown in the laboratory and harvested fresh that day. Each bean plant had a 15–24 cm long stem and two 4–7 cm long stalks, each with a 5–9 cm long and 4–8 cm wide leaf at its distal end. Resonant properties were determined in detail for ten green bean plants (leaf length

6.1±0.9 cm, leaf width 5.9±0.9 cm, stalk length 4.3±0.7 cm, stem length 24.2±2.2 cm). For comparative reasons resonance was tested for single brussels sprouts (*Brassica oleracea* var. *gemmifera* L), turnip cabbage (*Brassica oleracea* var. *caulorapa* L) and tomato (*Lycopersicon esculentum* Mill.) as well as for two cyperus (*Cyperus alternifolius* L), broccoli (*Brassica oleracea* var. *italica* L), strawberry (*Fragaria chiloensis* L) and cauliflower (*Brassica oleracea* var. *botrytis* L) plants.

Induction and recording of vibrations in plants and loudspeaker membrane

Plant vibrations were induced naturally by females of a Slovene population, singing on the dorsal surface of a bean leaf 1–3 cm from the insertion to the stalk. Bugs were triggered to sing by a male who was presented to a female over the air and removed immediately after the first emitted female calls. Spectra were determined from signals of four females, each singing on a different plant.

Plant vibrations were induced artificially by a magnet glued to the upper surface of a bean leaf at the place from where females are usually calling. The magnet weight of 26 mg corresponded approximately to the weight of adult females. The magnet was vibrated by an electromagnet positioned 0.5–1.0 cm away. Stimulus sequences were synthesized from loudspeaker-recorded calling songs of *N. viridula* females of Slovene and French populations. Stimulus pulse trains of both populations differed in their mean dominant frequency (83±1 Hz, *N*=10 and 102±4 Hz, *N*=10 for the Slovene and French population, respectively), pulse train duration (1962±125 ms, *N*=10 for the Slovene and 988±72 ms, *N*=10 for the French population) and repetition time (5379±117 ms, *N*=10 for the Slovene and 3732±174 ms, *N*=10 for the French population). Each stimulus song was composed of 10 pulse trains.

Airborne sound stimuli (380 Hz, 5 s duration, random repetition rate) were used to induce resonance of a loudspeaker membrane (2r=10 cm, 40–6000 Hz frequency response, impedance 8 Ω, #WS 13 BF, Visaton, Germany) or plants. Acoustic stimuli were synthesized using Sound Forge (Sonic Foundry, Madison, WI, USA) software, amplified over an amplifier (PM 5175, Philips, Holland) and applied by a middle-tone loudspeaker positioned 1–2 cm from the investigated substrate. Sound evoked vibrations of velocity values of 18 mm s⁻¹ in a loudspeaker membrane and between 0.2 and 4.1 mm s⁻¹ in leaves, 0.1 and 1.9 mm s⁻¹ in stalks and between 0.1 and 2.5 mm s⁻¹ in the bean stems.

Substrate vibrations were recorded using a laser vibrometer (Polytec, Waldbronn, Germany; OFV-353 sensor head and OFV-2200 controller). Two identical laser vibrometers were used for simultaneous recording from different points. Recorded signals were stored directly on a computer for later analyses by Cool EditPro (Adobe Systems Incorporate, San Jose, CA, USA) and Sound Forge software. To obtain better reflection small reflective flags (ca. 1 mm²) were attached to plant surfaces at a measuring point and small areas (ca. 1 mm²)

on the bug surface were painted using white correction fluid (Tipp-Ex).

Naturally emitted signals were recorded simultaneously with the reference laser vibrometer from the pronotum of a singing bug and with the measuring one from (a) the leaf immediately below, (b) from ipsi- or (c) contralateral stalks 1 cm from the crossing with the stem, or (d) from the middle of the stem. Artificially induced vibrations were measured simultaneously on the bean with the reference laser vibrometer from the ipsilateral stalk about 0.5 cm from the junction with the leaf and about 1 cm from the vibration source, and with the measuring laser vibrometer from (a) ipsi- and (b) contralateral stalks about 1 cm from the crossing with the stem, (c) at the distal end of the contralateral stalk as well as (d) on the stem about 1 cm below the crossing or (e) approximately at stem middle. Sound-induced vibrations were recorded from the surface of a loudspeaker membrane or from plant leaves, stalks and stem.

Terminology and statistics

Pulses are defined as unitary homogeneous parcels of sound waves of finite duration, and pulse trains as repeatable and temporally distinct groups of pulses (Broughton, 1963). In spectra of vibratory-induced vibrations the positions of the dominant and subdominant peaks were determined together with relative amplitude, as the difference between amplitudes of the dominant (0 dB) and subdominant peaks. Position and amplitude values were calculated for peaks whose amplitudes were at least 15 dB above the resonant spectra baseline. Values of spectral peak positions and amplitudes were averaged in frequency ranges of 50–100 Hz, 100–150 Hz, 150–250 Hz and 250–400 Hz. Two-tailed Student's *t*-test and analysis of variance (ANOVA) were used for statistical data processing.

Results

Spectral properties of naturally emitted vibratory signals

The calling song of females singing on the dorsal surface of a bean leaf was recorded simultaneously from the body (pronotum) and substrate (plant). Spectra of body- and plant-recorded signals are characterized by the dominant and several subdominant peaks (Fig. 1). Within each frequency range peak positions and relative amplitudes differed significantly ($P < 0.05$) between recordings from different points. We could find no correlation between spectral properties and place of recording. Spectra of signals recorded on the ipsilateral stalk lack subdominant peaks in the frequency range above 250 Hz and those of stem-recorded signals have no distinct peaks in the frequency range 100–150 Hz.

Spectra of signals recorded on different parts of a plant differ. Comparing only spectra of body- and leaf-recorded signals (Fig. 1F), we could find no significant difference in the position of the dominant and subdominant spectral peaks. By contrast, spectra of leaf-recorded signals show a significant ($P < 0.05$) amplitude decrease of subdominant peaks and a general damping of frequencies below 70 Hz (Fig. 1F). The

amplitude difference between the dominant and 40–50 Hz subdominant peaks increased from 8 ± 3 dB ($N = 20$) as measured in body-recorded signals to 28 ± 4 dB ($N = 20$) in signals recorded on the leaf. Mean subdominant peak frequencies between 150 and 250 Hz are not the double of mean dominant frequencies, indicating that they do not represent their first harmonics.

Spectral properties of artificially induced vibratory signals

Spectra of loudspeaker-recorded stimulus songs differ from spectra of plant-recorded signals (Figs 1E, 2). Mean dominant frequencies of plant-recorded signals were about 1 Hz above the mean value (83 ± 1 Hz, $N = 10$) of the stimulus song of the Slovene population; the difference was significant ($P < 0.05$) only for signals recorded on the ipsilateral stalk.

The mean dominant frequency of the French population stimulus song (102 ± 4 Hz, $N = 10$) ranged in plant-recorded signals between 101 ± 11 Hz ($N = 11$) and 110 ± 2 Hz ($N = 16$); a significant ($P < 0.05$) difference between stimulus song and plant-recorded signals could be shown only on the ipsilateral stalk. Spectra of artificially induced vibrations lack peaks above 250 Hz and the main subdominant peak of the Slovene population stimulus song at 169 ± 3 Hz ($N = 10$) does not differ significantly from values of plant-recorded signals. The corresponding subdominant peak in plant-recorded French female calling-song signals could be shown only in spectra of signals recorded on the ipsilateral stalk and stem close to the crossing (Fig. 2A,C). Subdominant spectral peaks not characteristic for the stimulus songs appear in plant-recorded signals in the frequency range below 150 Hz (Fig. 2). Their peak position corresponds to values measured for naturally emitted signals in frequency ranges below 100 Hz and between 100 and 150 Hz (Figs 1, 2). Mean values differ significantly ($P < 0.05$) between populations and between recordings from different points on a plant within each frequency range, except for the Slovene female calling-song signals in the 100–150 Hz frequency range.

Resonant properties of plants

Spectra of loudspeaker- or plant-recorded environmental noise contained peaks around 15, 50, 150 and 250 Hz (Fig. 3). Velocity of sound-induced vibrations of a loudspeaker membrane or plant was comparable with values of signals recorded on the surface of a singing bug. Resonant peaks could be shown in spectra of vibrations recorded from plants (Fig. 3A) but not from the loudspeaker (Fig. 3B). The main resonant peak of leaf-recorded signals in 10 different bean plants was 194 ± 33 Hz and subdominant peaks were detected around 81 Hz, 118 and 290 Hz (Table 1). No significant difference could be shown for the position of resonant peaks in spectra of signals recorded from leaves and stalks. By contrast, significantly different ($P < 0.05$) values were measured for resonant peaks of stem-recorded vibrations in the frequency range 100–150 Hz. In the frequency ranges below 100 and above 150 Hz the plant resonant peaks correspond to the spectral peaks of naturally emitted female calling-song signals

recorded on the body or the plant (Fig. 3C). Comparable spectral peak values of naturally and artificially induced signals were recorded in the frequency range 150–250 Hz (Figs 1F, 2; Table 1). For comparative reasons we tested resonant properties in other *N. viridula* host plants. Resonant spectra are similar to those of bean (Fig. 4), with the dominant peak for leaf-recorded vibrations at 201 ± 31 Hz ($N=2$) for cyperus, 189 ± 30 Hz ($N=2$) for strawberry, 174 ± 23 Hz ($N=2$) for cauliflower, 170 ± 13 Hz ($N=2$) for broccoli, 190 Hz for brussels sprouts and turnip/cabbage and 160 Hz for tomato.

Discussion

The fused first and second abdominal tergites of the southern green stink bug *N. viridula* are joined into the tymbal tergal plate (Kuštór, 1989; Gogala, 1984). Two pairs of tergal

longitudinal and lateral compressor muscles with a pair of depressor tymbali muscles are attached to the plate (Maluf, 1932; Kuštór, 1989) and contract synchronously during singing (Kuštór, 1989). Their muscle potentials are in-phase with vibration cycles recorded either directly from the body surface or indirectly as vibrations of a loudspeaker membrane on which the bugs were singing (Kuštór, 1989; Amon, 1990). Spectra of most stink bug songs investigated to date were determined for loudspeaker-recorded signals and revealed low frequency and narrow band characteristics (Ryan et al., 1995; Čokl et al., 2000; Pavlovčič and Čokl, 2001; Čokl et al., 2001; McBrien et al., 2002; McBrien and Millar, 2003; Čokl et al., 2004). The dominant frequency of the female calling song of *N. viridula* varies within (Miklas et al., 2003) and between (Čokl et al., 2000) geographically isolated populations in the range 94–123 Hz (Čokl et al., 2000). The dominant frequency

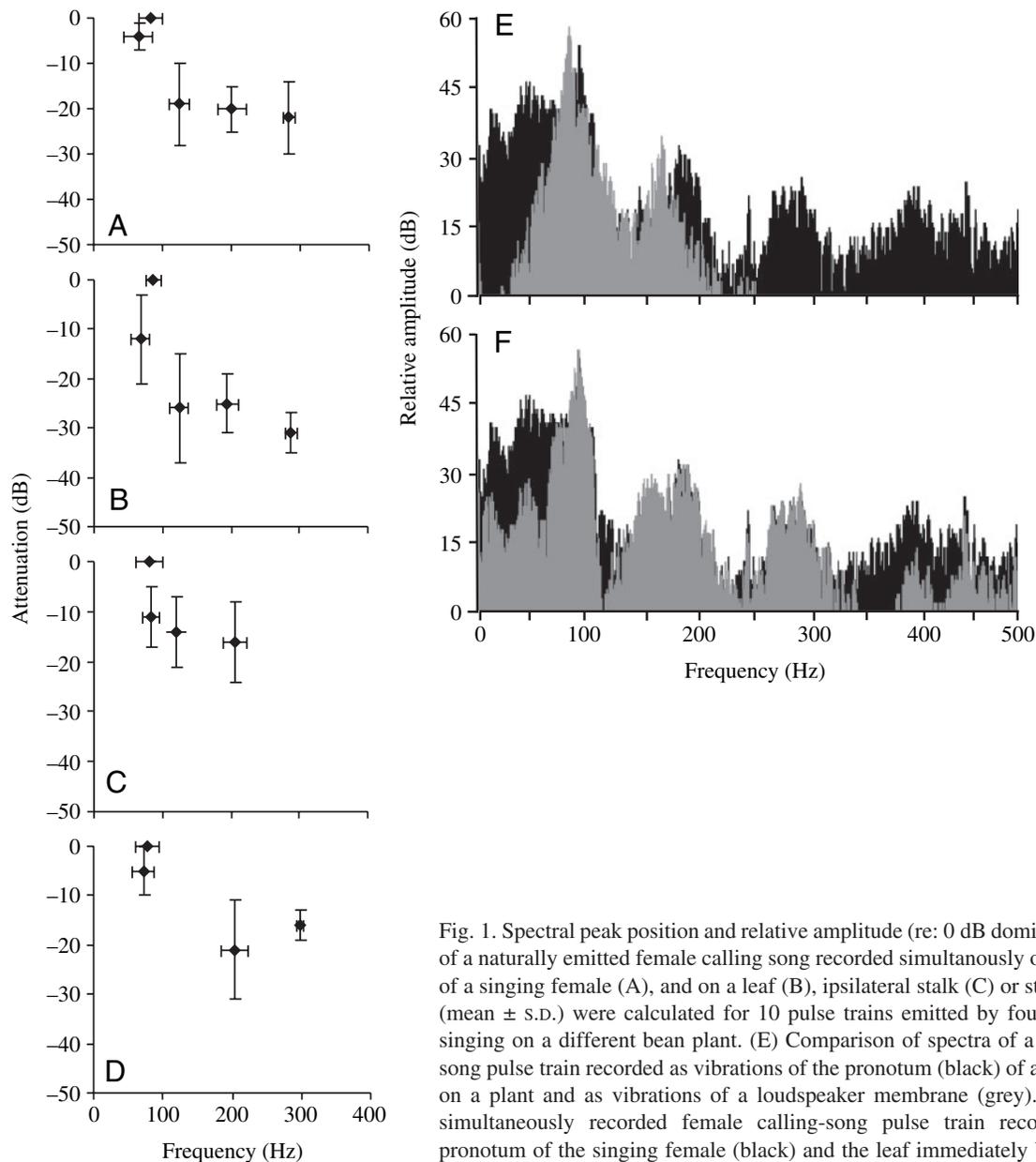


Fig. 1. Spectral peak position and relative amplitude (re: 0 dB dominant frequency) of a naturally emitted female calling song recorded simultaneously on the pronotum of a singing female (A), and on a leaf (B), ipsilateral stalk (C) or stem (D). Values (mean \pm S.D.) were calculated for 10 pulse trains emitted by four females, each singing on a different bean plant. (E) Comparison of spectra of a female calling-song pulse train recorded as vibrations of the pronotum (black) of a female singing on a plant and as vibrations of a loudspeaker membrane (grey). (F) Spectra of simultaneously recorded female calling-song pulse train recorded from the pronotum of the singing female (black) and the leaf immediately below (grey).

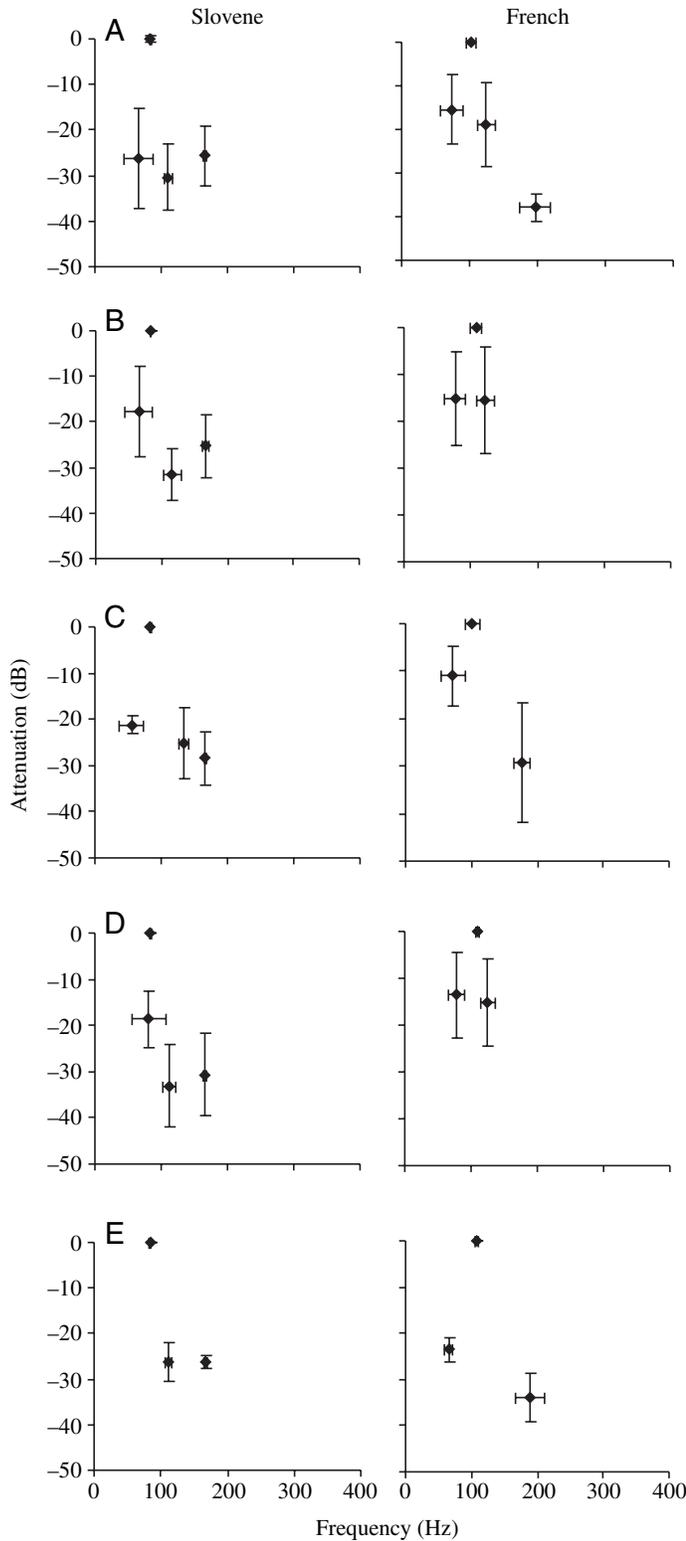


Fig. 2. Spectral peak position (mean \pm S.D.) and relative amplitude (re: 0 dB dominant frequency) of artificially induced female calling-song pulse trains recorded from a bean ipsilateral stalk (A), contralateral stalk (B), stem close to the crossing (C) and middle of the stem (D). Spectral peak positions of the female calling-song stimulus (reference) signals of the Slovene and French population (left and right, respectively) are shown in (E).

of the female calling song recorded as airborne sound is 110 Hz (Čokl et al., 1972).

Loudspeaker-recorded vibratory signals reflect characteristics of body vibrations without any feed-back from the substrate. This recording technique is relevant for comparison of spectral properties of different songs or species, but does not reveal anything about the possible effect of substrate vibration on the spectral properties of transmitted signals. Panizzi (1997) summarized data on plants on which polyphagous stink bugs feed and mate; no special plants preferred for mating have been identified. *N. viridula*, for example, concentrates in Paraná on soybean plants during summer but is also found on common bean *P. vulgaris*. During

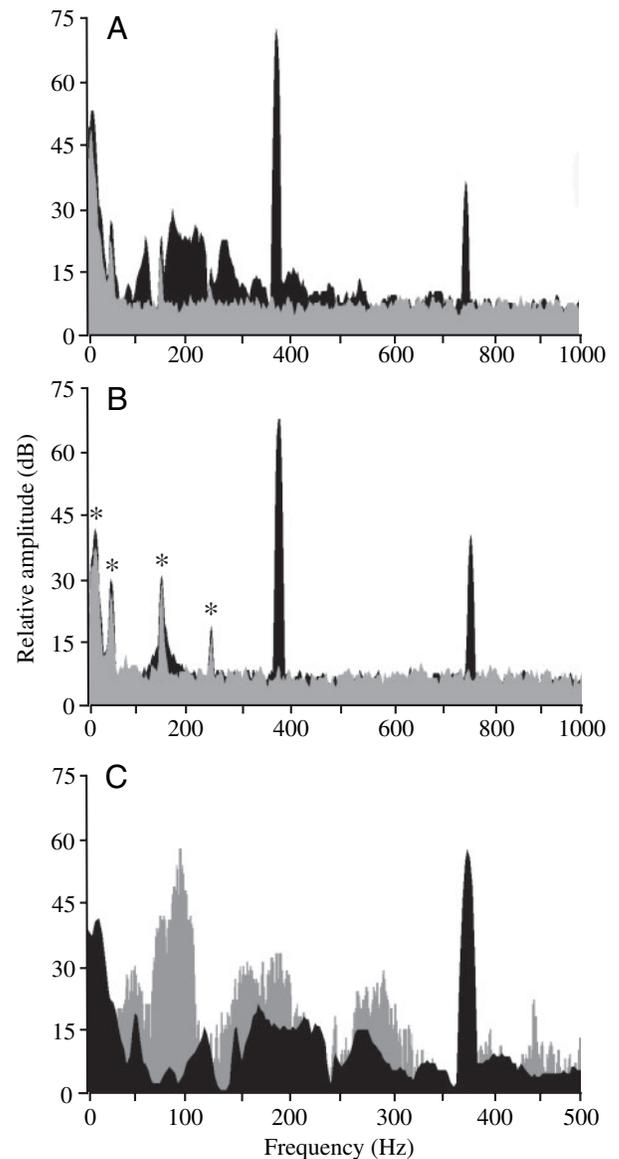


Fig. 3. Spectra of non-vibrated (grey) and sound-vibrated (black) bean leaf (A) or loudspeaker membrane (B). (C) Comparison of a leaf resonant spectrum with spectrum of a naturally emitted female calling-song pulse train recorded on a leaf. Peaks of environmental noise in non-stimulus conditions are marked with asterisks in B.

Table 1. Mean positions and relative amplitudes of the dominant and subdominant peaks in spectra of vibrations induced acoustically and recorded on bean leaf, stalk and stem

	Frequency range (Hz)			
	50–100	100–150	150–250	250–400
Leaf				
Frequency (Hz)	81±14	118±6	194±33	290±29
Relative amplitude (dB)	24±3	27±7	29±7	26±7
Stalk				
Frequency (Hz)	81±14	116±11	182±26	284±18
Relative amplitude (dB)	24±4	28±5	32±5	20±4
Stem				
Frequency (Hz)	81±12	129±13	187±30	290±2
Relative amplitude (dB)	22±3	25±6	29±46	23±5

Values are means ± s.d. obtained from 10 tested plants.

Mean of subdominant peaks are shown in frequency ranges below 100 Hz, 100–150 Hz, 150–250 Hz and 250–400 Hz.

autumn, adults feed, reproduce and complete the fourth generation on wild legumes but also move to wild hosts like star bristle and castor bean to feed. During late fall and early winter the species is found on radish, mustards and wheat. During spring a sixth generation is completed on Siberian motherwort. Although polyphagous, *N. viridula* prefers legumes and brassicas in Brazil, pods of green bean *P. vulgaris* in India, individuals from United States prefer pods of soybean *G. max*, and nymphs from South Carolina but not those from Florida survive on *Cassia fasciculata* L. At the North Adriatic coast *N. viridula* feeds and reproduces during spring and summer on different legumes and brassicas but in autumn it gathers on *Clematis* plants, upon which reproduction was not observed. Green bean *P. vulgaris* is one of the common host plants of *N. viridula* on which feeding and reproduction was observed in nature.

The mechanical properties of plants as transmission channels for vibratory signals have been studied in the context of communication in insects (Michelsen et al., 1982) and spiders (Barth, 2002). Arthropods communicating through plants use bending waves, and in standing wave conditions it would not be a good strategy to use pure tone vibratory signals for communication (Michelsen et al., 1982). In fact pure tone vibratory signals are not emitted by arthropods and broad-band stridulatory together with narrow band low frequency components are characteristic for signals of some insect groups like planthoppers, leafhoppers and cydnide bugs (Gogala, 1984; Claridge, 1985; Čokl and Virant-Doberlet, 2003). On the other hand several spider and insect species communicate successfully via plants using only low frequency and narrow band signals. The frequency spectrum of male wandering spider *Cupiennius salei* (Keyserling) opisthosomal signals has its prominent peak between 75 and 100 Hz and the main frequency components of female vibrations are at ca. 20–40 Hz (Barth, 2002). Stink bugs emit only low frequency narrow band songs

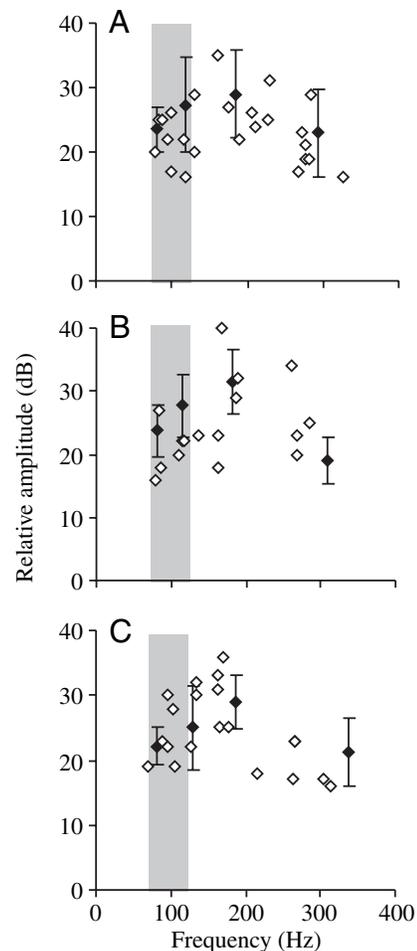


Fig. 4. Resonant peak positions and amplitudes above the baseline of vibrations induced by sound stimuli in bean (black diamonds) and other tested plants (open diamonds; see Materials and methods), recorded on a leaf (A), stalk (B) or stem (C). Values (mean ± s.d.) are shown for bean and single values for other tested plants. Grey areas show the range of variation of the female calling-song dominant frequency.

(Čokl and Virant-Doberlet, 2003). Even in combined low frequency and broad-band stridulatory signals the main energy is emitted at the lower frequencies of the substrate-borne component. For example, the vibration component of *Euides speciosa* Boh. signals shows its maximum between 150 and 200 Hz, whereas the maximum for airborne sound is at about 550 Hz (Traue, 1978). The main energy emitted by cydnide bugs is in most cases below 500 Hz, although sound energy extends in some species at least to 12 kHz (Gogala et al., 1974).

The mean dominant frequency of the *N. viridula* female calling song was only occasionally measured below 90 Hz in bugs singing on a loudspeaker membrane. In our experiments the mean dominant frequency value was regularly below 90 Hz in spectra of signals recorded from bugs singing on a plant. Although one cannot exclude the possibility that the 80–90 Hz dominant frequency in the latter case can be attributed to inter-individual differences, several data indicate that the position of

the dominant frequency peak is potentially determined by the resonant properties of plants. The 80–90 Hz component dominates in spectra of naturally emitted signals recorded on both body and plant, and represents one of the resonant peaks in the frequency range below 100 Hz. The mean dominant frequency of plant-recorded song varies by just 1 Hz when the song with 83 Hz dominant frequency was used to induce vibrations and by almost 10 Hz in the case of stimulus song of higher dominant frequency. Early experiments on transmission of *N. viridula* male calling song through cyperus (Čokl, 1988) demonstrated that increasing the distance from the source results in a decrease of the amount of spectral components above 200 Hz and shift of the dominant frequency to values around 90 Hz. The 80–90 Hz peak also becomes prominent in spectra of stem-recorded vibrations that were artificially induced by 124 Hz pure tone signals.

Spectra of plant-recorded female calling songs differ in some respect when induced naturally by singing females or artificially by electromagnetic vibration of a magnet glued to the upper surface of the leaf. The dominant frequency of the French female stimulus calling song did not fall below 90 Hz in plant-recorded signals and no peaks above 250 Hz could be recorded. We have no explanation for differences in naturally emitted signals except that vibrations in the latter case are transferred from the body to the substrate over spatially separated legs and that loading of a plant even with a light magnet potentially modifies its mechanical properties. The stimulation technique using a magnet glued to the plant surface needs a critical application when used to mimic natural conditions.

Spectra of loudspeaker-recorded vibratory signals differ among different species, sex and song types, mainly in the number of higher harmonics and in the amounts of frequency modulation. In this respect the female calling song of *N. viridula* represents a simple example with narrow dominant and first harmonic spectral peaks, without any pronounced frequency modulation. Spectra of body- and plant-recorded signals contain subdominant peaks that are not present in loudspeaker-recorded vibrations. The dominant resonant peak of bean between 180 and 200 Hz corresponds to the subdominant spectral peak of laser-recorded songs from the pronotum or different parts of a plant. The new added peaks outside the range of the first harmonic support the hypothesis that spectral properties of signals transmitted through plant are at least partly determined by feed-back from vibrated plants.

Similar resonant properties of different plants indicate that frequency filtering of transmitted vibratory signals is of a rather similar nature. The propagation velocity at a particular frequency is largely independent of a plant's mechanical properties (Michelsen et al., 1982). Attenuation of only 0.3 dB cm⁻¹ was measured for 75 Hz signals transmitted in the banana leaf and signals of frequencies around 100 Hz show amplification (and not attenuation) when recorded at a distance of several cm from the source on *Thesium bavarum* Schrank (Michelsen et al., 1982). *N. viridula* mates were observed to alternate at a distance above 2 m through different cyperus stems in mechanical contact only by their roots and

surrounding earth, and the naturally emitted female calling song of the species was attenuated for less than 5 dB at a distance of 1 m from the source on a cyperus stem (A. Čokl, unpublished). Such low attenuation can be explained by signals tuned with the plant resonance.

Plant resonant peaks that determine spectra of transmitted signals fit well with tuning of leg vibratory receptors in *N. viridula* (Čokl, 1983). The middle frequency receptor cell of the subgenual organ responds with highest sensitivity and with prolonged responses to frequencies around 200 Hz (Čokl, 1983). In the frequency range above 200 Hz the sensitivity of the subgenual middle frequency receptor cell decreases but that of the high frequency receptor cell increases, so that spectral components above 250 Hz can be detected efficiently. Low frequency receptor cells show best sensitivity around 70 Hz and the shift of the signal dominant frequency below 100 Hz enables better cycle-by-cycle analysis of time of arrival differences as the peripheral neuronal basis for male-expressed vibratory directionality.

We can conclude that efficient substrate-borne communication of stink bugs is based on optimal tuning between frequency characteristics of vibratory songs, frequency sensitivity of vibratory sensory organs and resonant properties of green plants. Experimental data on the efficiency of transmission of broad-band stridulatory signals through plants is needed to determine their role in vibratory communication between insects.

This work was financially supported by the Slovene Ministry of Education, Science and Sports (Program P1-0255). The authors are grateful to Dr Michel Renou from INRA Centre Versailles (France) for providing stink bugs from France. We are indebted to Fa. Politec (Germany) for loaning the laser vibrometer. The authors wish to thank anonymous reviewers for helpful suggestions to improve the original manuscript.

References

- Amon, T. (1990). Electrical brain stimulation elicits singing in the bug *Nezara viridula*. *Naturwissenschaften* **77**, 291-292.
- Barth, F. G. (1998). The vibrational sense of spiders. In *Comparative Hearing: Insects* (ed. R. R. Hoy, A. N. Popper and R. R. Fay), pp. 228-278. New York: Springer-Verlag.
- Barth, F. G. (2002). Courtship and vibratory communication. In *A Spider's World: Senses and Behavior* (ed. F. G. Barth), pp. 268-302. New York: Springer Verlag.
- Borges, M., Jepson, P. C. and Howse, P. E. (1987). Long-range mate location and close-range courtship behavior of the green stink bug, *Nezara viridula* and its mediation by sex pheromones. *Entomol. Exp. Appl.* **44**, 205-212.
- Brennan, B. M., Chang, F. and Mitchel, W. C. (1977). Physiological effects on sex pheromone communication in the southern green stink bug, *Nezara viridula*. *Environ. Entomol.* **6**, 169-172.
- Broughton, W. B. (1963). Method in bioacoustic terminology. In *Acoustic Behaviour of Animals* (ed. R.-G. Busnel), pp. 3-24. Amsterdam, London, New York: Elsevier Publishing Company.
- Claridge, M. F. (1985). Acoustic signals in the Homoptera: behavior, taxonomy and evolution. *Annu. Rev. Entomol.* **30**, 297-317.
- Čokl, A. (1983). Functional properties of vibroreceptors in the legs of *Nezara viridula* (L.) (Heteroptera, Pentatomidae). *J. Comp. Physiol. A* **150**, 261-269.

- Čokl, A. (1988). Vibratory signal transmission in plants as measured by laser vibrometry. *Periodicum biologorum* **90**, 193-196.
- Čokl, A., Gogala, M. and Jež, M. (1972). The analysis of the acoustic signals of the bug *Nezara viridula* (L.). *Biol. Vestnik* **20**, 47-53.
- Čokl, A., McBrien, H. L. and Millar, J. G. (2001). Comparison of substrate-borne vibrational signals of two stink bug species, *Acrosternum hilare* and *Nezara viridula* (Heteroptera: Pentatomidae). *Ann. Entomol. Soc. Am.* **94**, 471-479.
- Čokl, A., Prešern, J., Virant-Doberlet, M., Bagwell, G. J. and Millar, J. G. (2004). Vibratory signals of the harlequin bug and their transmission through plants. *Physiol. Entomol.* **29**, 372-380.
- 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., Virant-Doberlet, M. and McDowell, A. (1999). Vibrational directionality in the southern green stink bug *Nezara viridula* is mediated by female song. *Anim. Behav.* **58**, 1277-1283.
- Čokl, A., Virant-Doberlet, M. and Stritih, N. (2000). The structure and function of songs emitted by southern green stink bugs from Brazil, Florida, Italy and Slovenia. *Physiol. Entomol.* **25**, 196-205.
- Elias, D. O., Mason, A. and Hoy, R. R. (2004). The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dosseus* (Aranea, Salticidae). *J. Exp. Biol.* **207**, 4105-4110.
- Fish, J. and Alcock, J. (1973). The behavior of *Chlorochroa ligata* (Say) and *Cosmopepla bimaculata* (Thomas) (Hemiptera: Pentatomidae). *Entomol. News* **84**, 260-268.
- Gogala, M. (1984). Vibration producing structures and songs of terrestrial Heteroptera as systematic character. *Biol. vestnik* **32**, 19-36.
- Gogala, M., Čokl, A., Drašlar, K. and Blažević, A. (1974). Substrate-borne sound communication in Cydnidae (Heteroptera). *J. Comp. Physiol.* **94**, 25-31.
- Harris, V. E. and Todd, J. W. (1980). Temporal and numerical patterns of reproduction behavior in the southern green stink bug, *Nezara viridula* (Hemiptera: Pentatomidae). *Entomol. Exp. Appl.* **27**, 105-116.
- Henry, S. C. and Martinez Wells, M. L. (2004). The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). *Anim. Behav.* **68**, 879-895.
- Hrabar, N., Virant-Doberlet, M. and Čokl, A. (2004). Species specificity of male southern green stink bug *Nezara viridula* (L.) reactions to the female calling song. *Acta. Zool Sinica* **50**, 566-575.
- Kon, M., Oe, A., Numata, H. and Hidaka, T. (1988). Comparison of the mating behaviour between two sympatric species *Nezara antennata* and *Nezara viridula* (Heteroptera: Pentatomidae) with special reference to sound emission. *J. Ethol.* **6**, 91-98.
- Kuštor, V. (1989). Activity of vibratory organ muscles in the bug *Nezara viridula* (L.). MsD thesis, University of Ljubljana, 68pp.
- Magal, C., Schöller, M., Tautz, J. and Casas, J. (2000). The role of leaf structure in vibration propagation. *J. Acoust. Soc. Am.* **108**, 2412-2418.
- Maluf, N. S. R. (1932). The skeletal motor mechanisms of the thorax of the 'stink bug' *Nezara viridula* L. *Bull. Soc. R. Ent. Egypte* **16**, 161-203.
- McBrien, H. L., Čokl, A. and Millar, J. G. (2002). Comparison of substrate-borne vibrational signals of two congeneric stink bug species, *Thyanta pallidovirens* and *T. custator accerra* (Heteroptera: Pentatomidae). *J. Insect Behav.* **15**, 715-738.
- McBrien, H. L. and Millar, J. G. (2003). Substrate-borne vibrational signals of the consperse stink bug (Hemiptera: Pentatomidae). *Can. Entomol.* **135**, 555-567.
- Michelsen, A., Fink, F., Gogala, M. and Traue, D. (1982). Plants as transmission channels for insect vibrational songs. *Behav. Ecol. Sociobiol.* **11**, 269-281.
- Miklas, N., Čokl, A., Renou, M. and Virant-Doberlet, M. (2003). Variability of vibratory signals and mate choice selectivity in the southern green stink bug. *Behav. Processes* **61**, 131-142.
- Miklas, N., Stritih, N., Čokl, A., Virant-Doberlet, M. and Renou, M. (2001). The influence of substrate on male responsiveness to the female calling song in *Nezara viridula*. *J. Insect Behav.* **14**, 313-332.
- Panizzi, R. A. (1997). Wild hosts of pentatomids: ecological significance and role in their pest status on crops. *Annu. Rev. Entomol.* **42**, 99-122.
- Panizzi, R. A., McPherson, J. E., James, D. G., Javahery, M. and McPherson, R. M. (2000). Stink bugs (Pentatomidae). In *Heteroptera of Economic Importance* (ed. C. W. Schaefer and A. R. Panizzi), pp. 421-474. Boca Raton, London, New York, Washington DC: CRC Press.
- Pavlovčič, P. and Čokl, A. (1991). Songs of *Holcostethus strictus* (Fabricius): a different repertoire among landbugs (Heteroptera: Pentatomidae). *Behav. Processes* **53**, 65-73.
- Ryan, M. A., Čokl, A. and Walter, G. H. (1995). Differences in vibratory sound communication between a Slovenian and an Australian population of *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *Behav. Processes* **36**, 183-193.
- Traue, D. (1978). Vibrationskommunikation bei *Euides speciosa* Boh (Homoptera-Cicadina: Delphacidae). *Verh. Dtsch. Zool. Ges.* **1978**, 167.