Cent. Eur. J. Biol. • 9(5) • 2014 • 543-549
DOI: 10.2478/s11535-014-0285-2

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Central European Journal of **Biology**

Percussion signals of *Lygus rugulipennis* Poppius (Heteroptera: Miridae)

Research Article

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Received 03 September 2013; Accepted 19 October 2013

Abstract: The European tarnished plant bug (*Lygus rugulipennis* Poppius) is among the most serious pests in the family Miridae, and therefore there is increasing interest in understanding the behaviour of this species. In the present study, laboratory recordings were taken using a laser vibrometer on adult males and females to ascertain whether acoustic signals are involved in intraspecific communication. Recordings were both carried out on plant and loudspeaker membrane substrates. Males and females emitted vibratory signals and the present results indicate that these signals are important during courtship. The basic signal characteristics measured were the dominant frequency, pulse duration, repetition time and number of pulses per group within the signal. Male and female signals did not differ in respect to any of these characteristics. Plant recorded signals were longer because of different mechanical properties of substrates. Additionally, the high frequency components were attenuated due to the low-pass filtering properties of plants. As this is the first study on vibratory communication of the European tarnished plant bug, we believe these findings may contribute considerably to the better understanding of the mating behavior of this important pest species.

Keywords: *European tarnished plant bug • Acoustic communication • Laser vibrometer • Plant • Substrate • Loudspeaker membrane*

1. Introduction

Miridae (plant bugs) with about 10000 species represent the largest heteropteran family. Their economic importance has been underestimated, although some species are serious pests of several crops [1]. Despite the importance of the family within Heteroptera, there is relatively little information to our knowledge on their mating behavior and associated communication processes.

Although semiochemicals probably provide an important means of intraspecific communication within this family, there is only limited knowledge on mirid bug pheromones. For instance in the Nearctic *Lygus* species the presence of the sex pheromone has been reported [2,3], however even after decades of research, compounds identified from adult bugs have failed to evoke behavioral responses in the field [4]. For *L. rugulipennis* however, the same compounds were identified and were found to be attractive to males [5].

Communication with vibratory signals transmitted through the substrate is characteristic for most representatives of Heteroptera (reviewed by Gogala [6]). Contrary to well studied communication at different levels in the pentatomid subfamily Pentatominae (reviewed by Čokl [7]), there are only scarce data on stridulatory structures in some African mirid bugs in the subfamilies Mirinae and Hyaliodinae [8,9]. Among the closely related species, the Nearctic *Lygus hesperus* was reported to tap on the surface during courtship behavior [3] and vibratory signals were recorded from male *Lygocoris pabulinus* [10].

Within Miridae, the European tarnished plant bug (*Lygus rugulipennis* Poppius) is one of the most serious crop pests in the Palaearctic, as it was reported to damage several economically-important food crops,

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e.g. strawberry [11,12], alfalfa [13,14], sugarbeet [15], wheat [16] and glasshouse cucumber [17].

In the present study we aimed to ascertain whether acoustic signals were involved in the communication process during courtship behavior of *Lygus rugulipennis*. In order to do this, we recorded any vibratory signals produced by European tarnished plant bugs on a host plant surface. Because plants represent a medium that changes vibratory signals during their transmission [7,18-21], we also recorded the bugs' vibratory emissions on a loudspeaker membrane substrate. The aim of this study was to acquire basic information on signal production and signal characteristics to allow us to have a greater understanding of mating behavior in the European tarnished plant bug.

2. Experimental Procedures

2.1 Insects

Recordings were conducted both on field-collected and laboratory-reared individuals. Since *Lygus* are known to mate multiple times during a mating season, and previous studies indicated that 5 days after copulation females are ready to mate again [3], field-collected adults were kept separated by sex at least for 8-10 days before the recordings.

In order that naïve (virgin) individuals could be used in trials, we collected nymphs using classic sweep-netting techniques from a barley and an alfalfa field at Halásztelek and Julianna-major (Pest county, Hungary) respectively. Animals were kept in cylindrical glass vials (diameter 14.5 cm, height 18 cm) and were provided fresh green beans, water and also bands of filter paper to allow them sufficient walking surface. Rearing conditions were the following: 18:6 light:dark period, 26°C, and ca. 40% relative humidity. As a rule, vials were checked at least every second day for freshly moulted adults. Freshly-moulted individuals were identified to species using a stereomicroscope, based on previously-published studies [22]. Young (1-2 days post-moult) *L. rugulipennis* adults were sexed and reared separately to prevent copulation prior to trials. All adults used in the experiments were older than 8 days, to ensure all individuals were sexually mature.

2.2 Vibratory signal recording and analysis

All experiments were conducted between 08:00 and 17:00 under normal laboratory room conditions (temperature 20-24°C, 60-80% humidity). To record vibrations from a routinely used 'non-resonant' substrate we placed a male and a female *L. rugulipennis* bug on the the membrane (10 cm diameter) of a loudspeaker (40-6000 Hz frequency response, 8 Ω impedance; Radio Shack, Taipei, Taiwan) and covered the loudspeaker with a 2 mm thick Perspex sheet without contacting the membrane to prevent their escape. The loudspeaker was placed in a sound-insulated box (1x1x1 m) on a shock-proof table to reduce any extraneous environmental noise. A 0.5x0.5 cm piece of special laser reflecting sheet was fixed to the centre of the loudspeaker membrane, as a reference point during measurements.

L. rugulipennis vibrations emitted on a plant surface were recorded from bean (*Phaseolus vulgaris* L.) stems. A 15 cm stem section from a live bean plant was cut and put into humid soil in a plastic pot (diameter 10 cm, height 20 cm), to provide fresh plant material as a substrate. The plant was positioned in a way so that it did not touch the wall of the pot, to avoid any confounding effect on the signals. A 0.5x0.5 cm piece of special laser reflecting sheet was fixed to the stem, as a reference point during the measurements.

Vibratory signals of *L. rugulipennis* emitted on both plant and loudspeaker membrane substrate were recorded using a laser vibrometer (PDV 100, Polytec, Waldbronn, Germany). Vibrations were digitized (24 bit, 96 kHz, 100 dB signal-to-noise ratio; Sound Blaster Extigy, Creative Laboratories Inc., Milpitas, CA) and stored in a computer by the Cool Edit Pro version 2.0 software (Adobe Systems Inc., San Jose, CA). Further analysis was performed by the Sound Forge Version 6.0 (Sonic Foundry, Madison, WI, U.S.A) software.

2.3 Experimental protocol

Vibratory signals were recorded from couples of European tarnished plant bugs placed on a plant or on a loudspeaker membrane. Recordings were performed for 60 different male-female combinations of *L. rugulipennis* on each substrate during the experiments. If for a period of 15-20 minutes a pair was found inactive either the male, the female or both were replaced. Recordings lasted 30-60 minutes depending on the activity of the bugs. After the recordings, signaling individuals (either male, female or both) were removed and not used in further recordings. Copulating pairs were also removed after copulation and omitted from further experiments. In addition to the recordings, behavior on a loudspeaker membrane and on plant was visually observed.

2.4 Signal analysis and statistics

When tapping onto the substrate with their abdomen, insects produce 'pulses' of substrate vibrations, a mechanism called percussion [23]. 'Pulses' are defined as 'unitary homogenous parcels of vibrations of finite duration' [24]. Pulses were described by their duration, repetition time (time between onsets of two consecutive pulses) and by their dominant frequency (Hz). Pulse duration was defined as the time difference between signal onset and its end, determined as the point where the wave amplitude decreased below the noise level. Pulse groups (pulse trains) were defined as pulses arranged with a steady pulse repetition rate separated with the first and last pulse of the group with intervals outside the normal range of pulse repetition rate values (Figure 1). The repetition time was calculated only for monotonously repeated pulses, time between single, individual pulses was not included in this analysis. Monotonously-repeated pulses were all repeated within 1 second on both the plant and artificial surfaces.

For each individual a mean for each of the measured signal characteristics was calculated, representing one replication. Statistical differences of signal characteristics between sexes and substrates were evaluated using the Wilcoxon rank sum test. All statistical procedures were conducted using the software R [25].

3. Results

During observations, no specific calling posture of the females was observed. When in the close vicinity (*ca*. 1 cm) of the female, motivated field or laboratory-reared males tapped on the surface with the tip of their abdomen. Signals were produced on both bean plant and loudspeaker membrane substrates (Figure 2). Pulses emitted within the same sequence as sub-sequences of single or grouped ones showed no regularly repeated pattern either in the same or in different individuals. Number of pulses per pulse train varied from 1 to 19, however most signal groups only consisted of a few pulses (Figure 3D, 4D).

Most of the time females moved away from the male, who usually followed and kept on signaling to the female. However if females moved more than a few centimeters distance from the males, males usually stopped

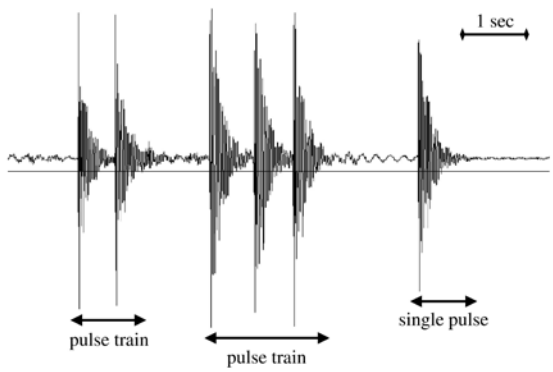

Figure 1. Single pulses and pulse trains of male *Lygus rugulipennis* on a plant substrate.

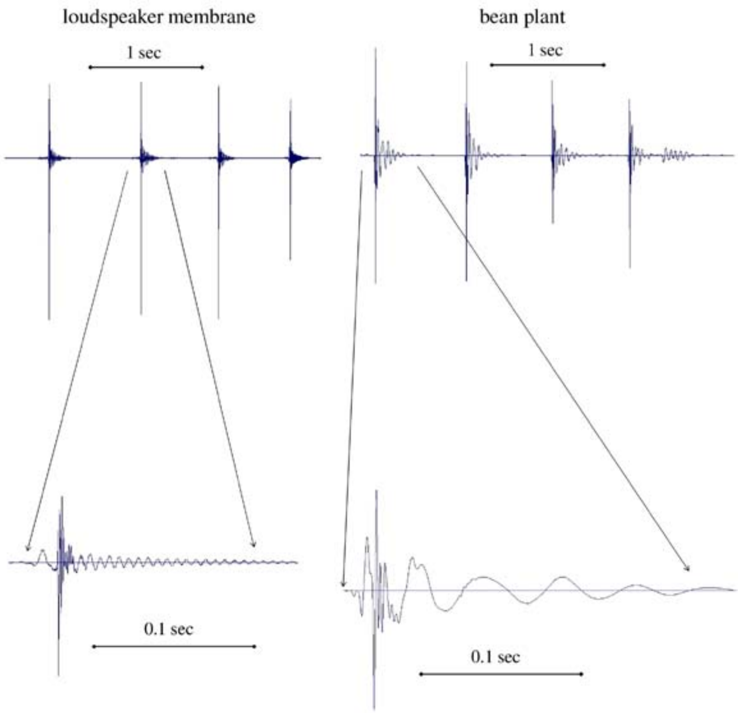

Figure 2. Percussion signals of male *Lygus rugulipennis* on loudspeaker membrane and plant substrate.

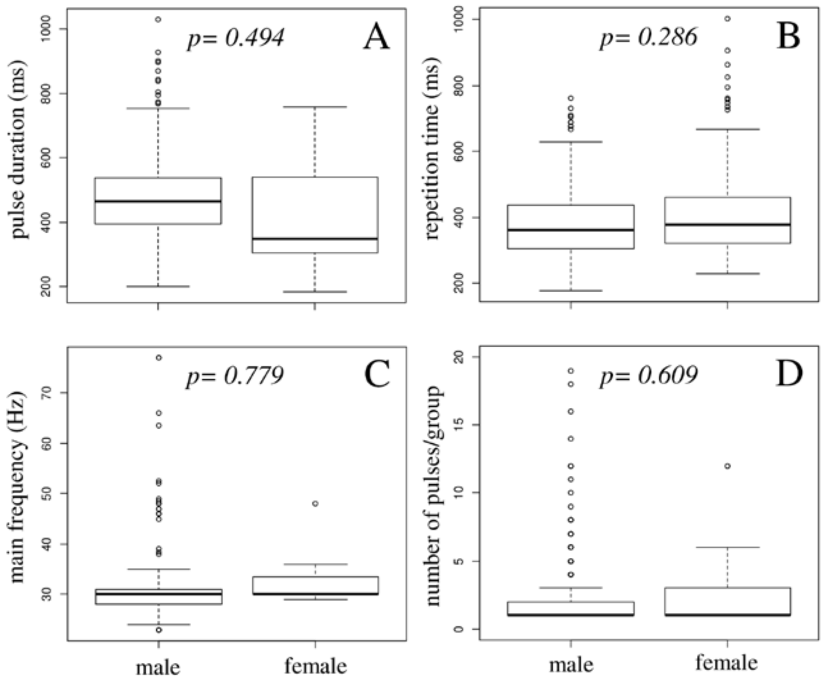

Figure 3. Percussion signals of *Lygus rugulipennis* males (N=17) and females (N=6) on bean plant shown on box-plot graphs showing median, minimum, maximum, upper and lower quartiles and outliers. A: pulse duration [ms]; B: repetition time [ms]; C: dominant frequency component [Hz]; D: number of pulses per signal group. Statistical comparisons were performed for averages of individual values by Wilcoxon rank sum test.

signaling. Some females were occasionally observed to tap on the surface (6 females on plant surface and 1 on loudspeaker membrane), however females were also observed to copulate without forming a duet.

Male and female plant recorded signals did not differ significantly in any measured signal characteristics, including pulse duration (W = 38, P= 0.494; Figure 3A), repetition time (W = 35, P= 0.286; Figure 3B), main frequency component (W = 46.5; P= 0.779; Figure 3C) and number of pulses/pulse train (W = 43, P= 0.609; Figure 3D).

Interestingly, however, male signals recorded on the two different substrates differed markedly (Figures 2, 4, 5) in their dominant frequency (W = 221, P < 0.0001; Figure 4C) and pulse duration (W = 0, P < 0.0001; Figure 4A). Repetition time (W = 81, P= 0.229; Figure 4B) and number of pulses/group was similar on both substrates (W = 99, P= 0.645; Figure 4D).

4. Discussion

In the present study we have shown that vibrations are involved in the mating behavior of the European tarnished plant bug. Although males of *L. rugulipennis* were reported to be attracted to female sex pheromones [5], in our studies no specific female calling posture was observed, as in other taxa that use sex pheromones (*e.g.* moths [26]). These results support other work in a closely-related mirid species, *Lygocoris pabulinus* as well [10]. In our experiments, both males and females were observed to emit percussion signals similar to another species, the Nearctic *L. hesperus*, where females have been reported to show similar behaviors toward males [3]. Contrary to *L. rugulipennis*, in *L. pabulinus* only male signals were reported, elicited by abdominal vibration rather than percussion [10]. The fact that males of *L. rugulipennis* started signaling when the female was in their close vicinity suggests the presence of the female provided key stimuli which evoked male signaling. Beside visual and possibly tactile stimuli, close-range chemical signals may also be involved in *L. rugulipennis* courtship behavior, as it was found in the closely related *L. pabulinus* [27]. Our observations also imply that the detected vibrations are not used for mate location as in case of many stink bug species (*e.g.* [28]), since males only started emitting signals when already in the close vicinity of the female. This suggests that male percussion signals produced during courting may provide information on species identity and possibly inform the female on male quality. For instance, the amplitude of signals (not measured

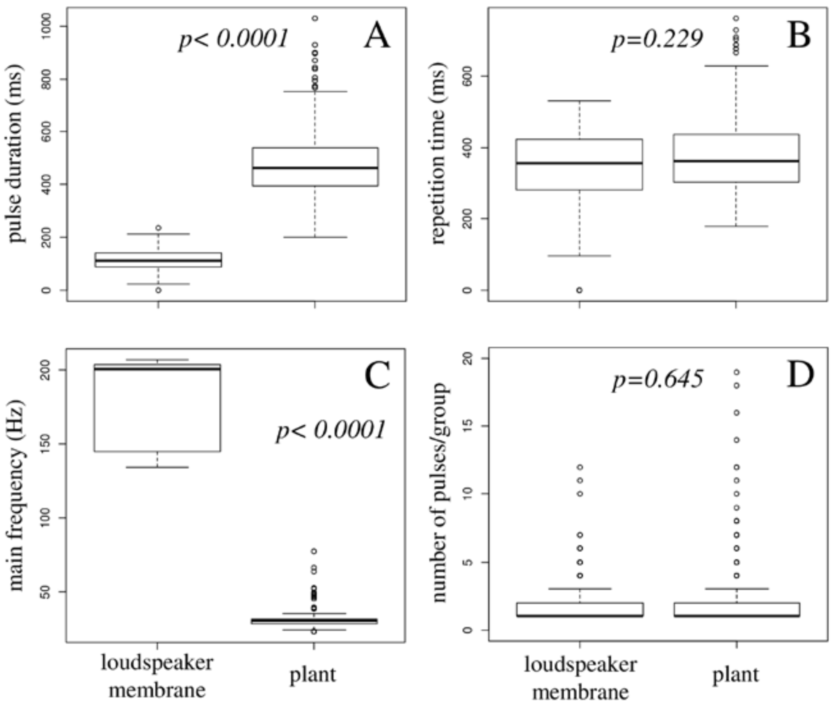

Figure 4. Percussion signals of male *Lygus rugulipennis* on loudspeaker membrane (N=13) and plant substrate (N=17) shown on box-plot graphs showing median, minimum, maximum, upper and lower quartiles and outliers. A: pulse duration [ms]; B: repetition time [ms]; C: dominant frequency component [Hz]; D: number of pulses per signal group. Statistical comparisons were performed for averages of individual values by Wilcoxon rank sum test.

in our experiments), the repetition rate of pulses or pulse groups might give the female information about male's size or fitness. On the other hand visual cues may also tell the female about male's size or activity in close vicinity. This question needs attention and experimentation that was not the subject of our present study.

Males signaled both on the membrane and on the plant, however signals were markedly different on the two different substrates. Pulse duration was markedly elongated on the plant, and frequency characteristics also differed greatly on bean plant and loudspeaker membrane substrates. Whereas signals on the loudspeaker membrane substrate contained high frequency components as well, pulses emitted on the plant were almost exclusively dominated by low frequency components. These differences are probably due to different mechanical properties of substrates including resonance and low-pass filtering properties of experimental plants. Our results are in accordance with previous findings on signals of other insect species [7,18-20].

As far as repetition rate is concerned, no conservative pattern of pulses was observed: pulses on the artificial membrane, and the live plant tissue were emitted individually and in groups of multiple pulses as well.

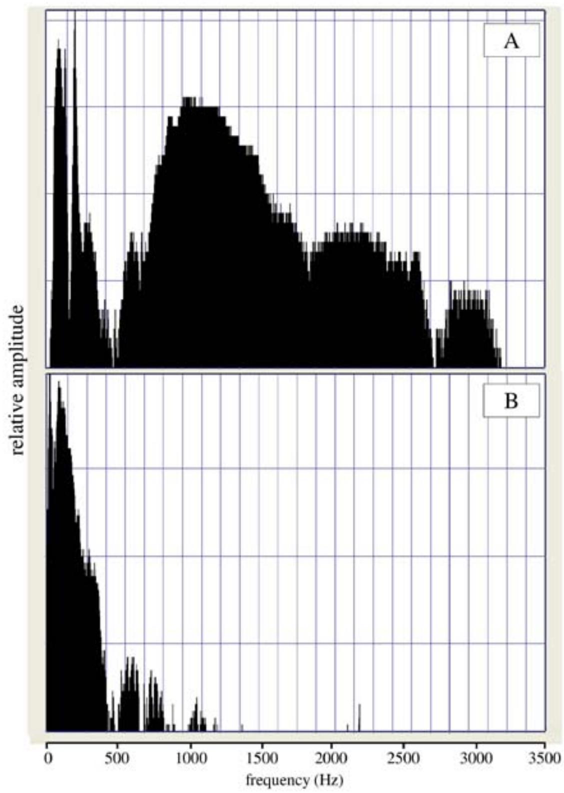

Figure 5. Frequency characteristics of *Lygus rugulipennis* signals. A: loudspeaker membrane and B: plant substrate.

Females also emitted percussion signals by tapping with the abdomen on the surface. These signals had similar characteristics to those produced by males in respect of all measured characteristics. Therefore it seems likely that percussion signals themselves may not provide information on the sex of the emitter.

To our best knowledge this is the first report of acoustic signals produced by the European tarnished plant bug, and we believe that these findings may contribute to the better understanding of the mating behavior of this important pest.

Acknowledgements

The authors are grateful for the kind support of the interacademic cooperation arrangement between the Hungarian and Slovenian Academy of Sciences, without which these experiments could not have been performed. The study was financially supported by the ARRS Program No. P1-0255-0105. The authors are also grateful for anonymous reviewers who helped considerably with their suggestions and comments.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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