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SHORT COMMUNICATION

Do *Scaphoideus titanus* (Hemiptera: Cicadellidae) nymphs use vibrational communication?

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Abstract Small Auchenorrhyncha use substrate-borne vibrations to communicate. Although this behaviour is well known in adult leafhoppers, so far no studies have been published on nymphs. Here we checked the occurrence of vibrational communication in *Scaphoideus titanus* (Hemiptera: Cicadellidae) nymphs as a possible explanation of their aggregative distributions on host plants. We studied possible vibratory emissions of isolated and grouped nymphs, as well as their behavioural responses to vibration stimuli that simulated presence of conspecifics, to disturbance noise, white noise and predator spiders. None of our synthetic stimuli or pre-recorded substrate vibrations from nymphs elicited specific vibration responses and only those due to grooming or mechanical contacts of the insect with the leaf were recorded. Thus, *S. titanus* nymphs showed to not use species-specific vibrations neither for intra- nor interspecific communication and also did not produce alarm vibrations when facing potential predators. We conclude that their aggregative behaviour is independent from a vibrational communication.

Keywords Substrate-borne signals · Intraspecific interaction · Host–predatory interaction · Playback stimulation

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Introduction

In Auchenorrhyncha (Hemiptera: Fulgoromorpha and Cicadomorpha), intraspecific communication is mainly mediated by the emission of substrate-borne vibrations. In particular, adults emit species-specific signals associated to mating behaviour (reviewed in Čokl and Virant-Doberlet 2003). On the other hand, in some groups also, nymphs can emit vibrational signals in association with defensive behaviour against potential predators, either by eliciting mutualism from ants (Morales et al. 2008) or by alerting the defending mother (Cocroft 1996).

The grapevine leafhopper *Scaphoideus titanus* Ball, vector of the flavescence dorée phytoplasma, has a reproductive behaviour driven by vibrational signals (Mazzoni et al. 2009a). In vineyards, adults (Bosco et al. 1997) and nymphs (Lessio and Alma 2006) exhibit aggregated patterns of distribution on grapevines. In general, grouping can result either from the attractiveness of a site without "social" interaction between the conspecifics or from attraction by the group members by means of conspecific stimuli (Parrish et al. 1997). Field observations showed that *S. titanus* nymphs aggregate on young developing organs on basal parts of grapevine (Schvester et al. 1962; Posenato et al. 2001), and a recent study (Chuche et al. 2009) attributed such an aggregation partly to the leaf development stage and colour. Also host plant volatiles were suggested to play a role in plant selection (Mazzoni et al., 2009b). However, to our knowledge, no study dealt with the implication of an intraspecific communication mediated by vibrations in this species, as a possible synergism with other stimuli. The present study aimed to check whether vibrational communication occurs in *S. titanus* nymphs. We thus tested whether vibrational signals can be emitted by nymphs spontaneously, as possible grouping elicitor, or in response to conspecifics, predators or generic noise.

Materials and methods

Insects

Third to fifth instar nymphs of *S. titanus* were collected in a vineyard from Trentino region (northern Italy) in June 2010. Nymphs were kept per group of five in cylindrical plastic containers (height 10 cm, diameter 5 cm) placed in a controlled environment chamber (25±1°C; 16:8, L:D; 75±1% r.h.). On the floor of each container, a grapevine leaf disk was laid over a 1-cm layer of technical agar solution [0.8% (wt/vol)] and replaced twice a week.

Recording vibrational signals and behaviour

Vibrational signals were detected from substrate by laser vibrometer (Ometron, VQ-500-D-V, Harpenden, UK). Signals were digitized with 48 kHz sample rate and 16-bit depth and stored directly onto a hard drive of a computer using Sound Blaster Audigy 4 sound card (Creative Labs Inc.) and Adobe Audition 1.0 (Adobe Systems Inc.). Signal recordings were analyzed using the computer software program Raven 1.2.1 (Charif et al. 2004). The behaviour of *S. titanus* was recorded with a video Panasonic HDC-TM700K camera together with vibrational signals to identify individuals emitting the signals and to associate vibrational signals with particular behaviour.

Leafhoppers were placed either on a small grapevine cutting with two main leaves or a cut grapevine stem with a leaf according to the goal of the experiment. The bottom of the stem was put into a vial filled with water to prevent withering. To prevent insects from escaping, a Plexiglas cylinder (height 50 cm, diameter 30 cm) with a small opening for laser beam was put over the jar. Preliminary observations of larval activity done along the day (09:00 a.m. to 08:00 p.m.) did not show any difference, and thus all experiments were similarly done during this period.

Control tests

To determine whether the nymphs spontaneously emit vibrational signals, individuals were placed on a vine leaf and vibrational signals and behaviour were recorded for 20 min. These experiments were conducted with single individuals ($n=12$) and groups of nymphs (three to five individuals, 11 groups, $n=44$).

Responses of leafhoppers to vibrational stimulations

Leafhoppers were stimulated either with pre-recorded signals of *S. titanus* or with artificially synthesized signals (Table 1). The surface of the grapevine leaf was vibrated with the conical tip of a 5-cm aluminium rod (diameter 4 mm) screwed firmly into the head of a minishaker (Type 4810; Bruël and Kjær, Nærum, Denmark), driven from the computer via Adobe Audition 1.0 with stimulatory signals. The following signals played back in loop mode for the duration of the test were used for stimulation: grooming noise (GN), male disturbance noise (DN) and white noise (WN). The GN (Fig. 1a) was obtained by recording nymphs during grooming. The DN (Fig. 1b) is a signal used by adult males during intra-sexual competition that was chosen from a library of *S. titanus* signals previously recorded (Mazzoni at al. 2009a, b). We applied two DN levels: one adjusted on leafhopper natural intensity and the other amplified of 20 dB. The WN, with a flat spectrum over a 0–8,000-Hz frequency band and created with Adobe Audition 1.0, was used at natural, increase of 10 and of 20 dB intensities.

Original files were taken from the leaf lamina (surface 6×10 cm) from points 2–3 cm distant from the border of the leaf by laser vibrometer. Intensity was measured by means of Pulse 14 (Brüel and Kjær Sound & Vibration A/S) in terms of

Table 1 Summary of stimuli used in playback experiments

Stimulus	Duration (s)	Device	Nymphs per test	Recording time (min)	No. of repetitions	No. of insects
Grooming noise	3	Isolated leaf/cutting	1	15	22	22
Disturbance noise	2	Cutting	5–8	15	8	54
Disturbance noise+20 dB	2	Cutting	5–8	15	10	62
White noise	3	Cutting	5–8	30	10	58
White noise+10 dB	3	Cutting	5–8	30	10	60
White noise+20 dB	3	Cutting	5–8	30	10	60
Predators (spiders)	–	Isolated leaf	5–8	15	15	109

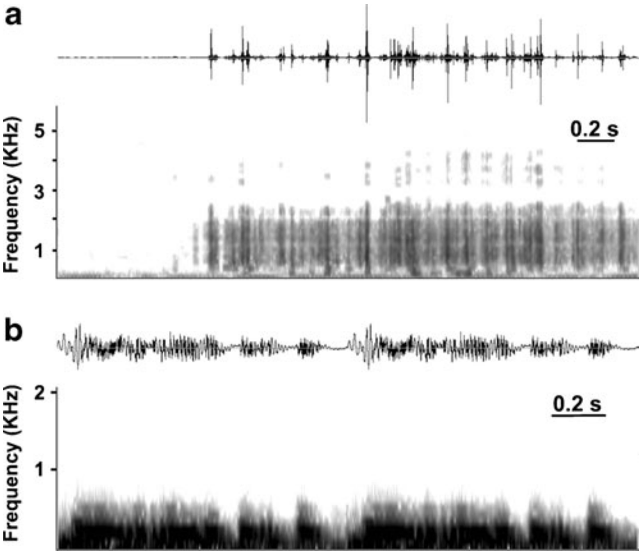

Fig. 1 Oscillogram (*above*) and spectrogram corresponding (*below*) of grooming noise (**a**) and male disturbance noise (**b**) used as stimulations

substrate velocity (in millimetres per second). DN signals present in the library ranged between 0.01 and 0.05 mm/s. We chose one DN and then we adjusted the minishaker in order to let the leaf maximal vibration velocity be approximately 0.03 mm/s. To calibrate the intensity, we took measurements from two distinct points of the leaf lamina, at least 2 cm distant from both the tip of the shaker and the leaf margin. Instead, for GN playbacks, we chose one representative sequence from the control test. Since substrate velocity due to GN peaks never exceeded 0.005 mm/s, we adjusted the playback sequence having 0.005 mm/s as peak of maximum value.

Responses of leafhoppers to predators

We presented immature and adult spiders (Tables 1 and 2) collected in vineyard and on surrounding vegetation to our test insects to determine whether *S. titanus* emit alarm vibrational signals when exposed to potential predators. To increase the probability of predator/prey encounters, five to ten nymphs were inserted together with a spider on a cutting leaf into a smaller cylindrical plastic box (height 16 cm, diameter on base 8 cm, on top 5.5 cm) with a small opening for the laser beam for a maximal period of 15 min.

Results and discussion

S. titanus nymphs did not show any vibrational activity. No vibrational signals were spontaneously emitted by nymphs neither alone nor when grouped, even if very close or in actual contact. In fact, we did not detect any other vibration than those due to mechanical contacts of the insect with the leaf (walking, jumping) or to body scratches (grooming, brochosome anointing). Also the repeated playback of such mechanical playback (GN) did not elicit any relevant response in nymphs. This fact would suggest that the distribution on the plant of *S. titanus* nymphs is not driven or conditioned by the presence of conspecifics, or at least it is not mediated by the emission of vibrational signals. Nymph aggregation seems likely to be due to host plant characteristics (Schvester et al. 1962; Posenato et al. 2001; Lessio and Alma 2006; Chuche et al. 2009; Mazzoni et al., 2009b).

Another possible factor influencing spatial distribution, dynamics and behaviour is the presence of predators (Cronin et al. 2004). In this way, we explored whether spiders could elicit in nymphs the emission of defensive, deterrent or alarm signals. The presentation of spiders offered the leafhopper nymphs a variety of vibrational, tactile and visual stimuli, but none of these elicited vibrational signals from the nymphs. Spider species 4 and 10 were observed feeding on *S. titanus*, and species 6 tried unsuccessfully to catch some nymphs without inducing any reaction neither from the prey nor from neighbouring nymphs. Spiders that did not attack nymphs were even active and generated

Table 2 Arachnids used as stimulation source

Number	Instar	Sex	Family	Species	Predation
1	Immature	–	Clubionidae	ND	Hunting
2	Adult	Male	Gnaphosidae	ND	Web
3	Adult	Female	Phylodromidae	*Phylodromus rufus*	Hunting
4	Immature	–	Phylodromidae	ND	Hunting
5	Adult	Female	Salticidae	*Salticus* sp.	Hunting
6	Adult	Female	Salticidae	*Salticus* sp.	Hunting
7	Adult	Female	Salticidae	ND	Hunting
8	Adult	Female	Salticidae	ND	Hunting
9	Immature	–	Thomisidae	*Misumenops tricuspidatus*	Hunting
10	Adult	Female	Thomisidae	ND	Hunting
11	Immature	–	Opilionidae	ND	Hunting

ND not determined

numerous vibration and physical contacts with nymphs without causing any relevant effect on them. We did not observe nymphs avoiding contact with the spiders in any of our experiments, even if leafhoppers sometimes jumped after physical contact. The only visible observed nymph reaction was a dissuasive posture when facing the spider by head and abdomen raising. The face-to-face ended either with a spider attack or by nymph escape (either jumping or walking). Any voluntary emission of vibrations would possibly have represented an alarm signal for neighbours and consequently induced emigration. Animals living in group may benefit from the behavioural reaction of an attacked conspecific. We find the examples closest to *S. titanus* in subsocial species like the Nearctic membracids *Umbonia crassicornis* and *Publilia concava*. In *U. crassicornis*, aggregated nymphs respond to predatory wasps by emitting substrate-borne vibrational signals aimed at soliciting parental care (Cocroft 1999). Conversely, *P. concava* emit interspecific vibrations when attacked by a ladybeetle (Morales et al. 2008). In this case the alarming signal is directed to mutualist ants that provide defence to the nymphs. It indicates that interspecific communication can play a role in Auchenorrhyncha although it is likely limited to species with a certain degree of sociality. Thus, we can conclude that each *S. titanus* nymph on a plant seems to be not connected to the others: the total absence of any kind of volunteer vibration and also of any behavioural response to different stimuli would suggest the lack of interactions among individuals, which consequently would live an isolated life, unaware of the presence of other individuals, at least via vibrational communication. Indeed, we cannot exclude the use of other cues (e.g. olfaction and vision) in determining such interactions.

As for noises, again either the use of the species-specific disturbance noise or the generalist white noise in the range 0–8,000 Hz did not induce any nymph reaction. In very similar experimental conditions, the same signals were sufficient to interrupt the mating vibrational duet of *S. titanus* adults (Mazzoni et al. 2009c). By covering both in frequency and intensity the mating signals, these signals effectively masked the communication between both genders and then prevented the female location by the male. That shows how the adults are sensitive to substrate-borne vibrations contrary to nymphs that do not react to such vibrations; at least they do not in the time (30 min) of the trials. Indeed, we cannot assert that those vibrations do not affect other aspects, like feeding behaviour. If true, that would elicit delayed effects on the nymphs and probably their pull out from the vibrating tissues. It would be worth to further investigate this hypothesis since it could open new perspectives in the field of the pest control. We conclude that *S. titanus* nymphs do not use species-specific vibrations either for intra- or interspecific communication.

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