**Entomologia
Experimentalis et Applicata**

DOI: 10.1111/eea.12394

Disruption of reproductive behavior of grapevine cicada, *Cicadatra alhageos*, by acoustic signals playback

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Accepted: 7 October 2015

Key words: acoustic communication, mating disruption, bioacoustic signal processing, oviposition, pest control, Hemiptera, Cicadidae, Vitaceae

Abstract

The grapevine cicada, *Cicadatra alhageos* (Kolenati) (Hemiptera: Cicadidae), is a key pest of grapevine (*Vitis* spp., Vitaceae) in the Middle East. The main damage is caused by nymphs that feed on root sap, and adults that oviposit on branches. As males produce sound to attract females, one of the control methods can be disruption of sexual communication. Disruptive effects of acoustic playbacks on singing males were studied. Signals (0.5–10 kHz) were broadcast to disrupt male calling behavior. Playback of acoustic signals interrupted male sexual signalling. To reduce female oviposition behavior in the field, an experiment was conducted based on a completely randomized design with two treatments (control vs. acoustic broadcasting) and four replications over a period of 3 years. Playback of disruption signals in the field reduced female oviposition on grapevine branches that were close to the signal source. Therefore, application of acoustic stimuli may be an effective and low-cost control method against grapevine cicada.

Introduction

The grapevine cicada, *Cicadatra alhageos* (Kolenati) (Hemiptera: Cicadidae), is a key pest of grape vineyards in the Middle East (Babaei, 1967; Beheshti, 1980; Behdad, 1984; Esmaeili, 1991). The nymphs feed on roots of grapevine (*Vitis* spp., Vitaceae), apple, almond, pitch, cherry, pomegranate, quince, walnut, and pear (Babaei, 1967; Mirzayans et al., 1976; Behdad, 1984; Rajabi, 1989), which reduces the growth of branches, leaves, and fruits. Moreover, oviposition on newly-grown branches leads to branch wilt and death (Babaei, 1967). In Iran, adults emerge during June and July (Babaei, 1967; Behdad, 1984; Shekaryan & Rezvani, 2000). While keeping their heads down, males generate a calling song by tymbals to attract females (Esmaeili, 1991; Zamanian et al., 2008). After mating, females oviposit on young branches (Shekaryan & Rezvani, 2000). Control methods include removing damaged branches and applying imidaclopridin soil against nymphs (Valizadeh & Farazmand, 2009).

Disruption of intersexual communication to control agricultural pests has been studied for the past few decades (Haynes & Birch, 1983; Palaniswamy & Underhill, 1988; Bengtsson et al., 1994; Suckling & Burnip, 1996; Howse et al., 1998). Although these studies were on disruption of airborne or substrate-borne chemical signals of lepidopterans and dipterans, interfering sound communication for interruption of mating has been promising (Hunt & Morton, 2001; Mazzoni et al., 2009; McNett et al., 2010). The idea of using sound to disrupt mating processes is based on biomimetics. Males of some insect species, for example, *Ennya chrysura* Fairmaire, interfere in courtship calls of males (Miranda, 2006), through masking or disrupting the conspecific male's courtship signals. Male *Scaphoideus titanus* Ball leafhoppers reply to playbacks of male–female duets with specific competitive behavior and produce disruption signals (Mazzoni et al., 2009). The disruptive signals influence female acoustic replies and inhibit mating. Stridulation playback disrupts tunnelling and mating of pine bark beetles (*Dendroctonus* spp.) (Hofstetter et al., 2014). These studies indicate that vibrational

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communication in insects is affected by abiotic environmental noises and by signals produced by sexual competitors or heterospecifics (Mazzoni et al., 2009).

Mating interruption by induced vibrations has been rarely considered from a theoretical and applied viewpoint (Cocroft & Rodríguez, 2005; Čokl & Millar, 2009; Mazzoni et al., 2009), and due to technical constraints, it has not been implemented in the field (Virant-Doberlet & Čokl, 2004; Mankin, 2012). Field studies by Zamanian et al. (2008) showed that synthetic signals (1–6 kHz) affected the calling song of and disrupted mating in grapevine cicada. Therefore, application of acoustic signals against grapevine cicada might be a useful control method which needs more research to be effective and to be incorporated in integrated pest management (IPM) programs. Our objectives were to study the effect of (1) frequency and timing of signals on calling behavior of male *C. alhageos* and (2) sound on oviposition behavior of *C. alhageos* females in the field.

Materials and methods

The research was conducted in grape vineyards (*Vitis vinifera* L. var. Asgari) in Tiran (Esfahan, Iran) (32°38′N, 51°21′E, 1799 m a.s.l.) in June 2010 (20–35 °C and 13–20% r.h.). Grape is the main product in this region; other products include walnut, cherry, and apple.

Calling songs of 12 cicadas were recorded in the vineyards. Next, disruptive signals (feedback amplifier signal) were broadcast while cicadas were producing calling songs. The feedback amplifier signal occurs when a sound loop exists between an audio input (e.g., a microphone) and an audio output (e.g., a loudspeaker). The bandwidth of the feedback amplifier signal was similar to that of cicada signals, that is, 500 Hz to 10 kHz, which contained a four-peak frequency (2, 3, 5, and 8 kHz). Each cicada was used once in the experiment. Six cicadas were selected to analyze the song. Playback signals were produced by a computer using PROTEUS v.7.6 software (Labcenter Electronics, Grassington, UK). The sounds were played for each insect with a Model 50 W CHANG amplifier and an electromagnetic speaker (both Echochange, Tehran, Iran) at maximum 70 dB. The amplifier and the speaker were connected to the headphone jack of the computer. Sound pressure level of disruptive signal was measured by a Sound Level Meter (model 2232; Brüel & Kjær, Nærum, Denmark). Disruptive sounds were broadcast at a distance of 2 m from each male. We evaluated the effect of all frequencies of disruptive signals on reduction of male calling activity by comparing the characteristics of male calling behavior. These characteristics were as follows: echeme, interecheme interval, and dominant frequency.

Disruptive signals and male response sounds were recorded with a ZOOM-H4 audio recorder (Sound Laboratory ZOOM, Tokyo, Japan) through an internal microphone at a sampling rate of 44 Kbps. The recorder was placed at 2 m from each male in the warmest hours of the day, during which cicadas were most active. The recorded sounds were transferred to an Acer computer and analysed by Cool Record Edit Deluxe v.7.8.6 (Syntrillium Software, Phoenix, AZ, USA) and MATLAB (MathWorks, Natick, MA, USA).

To study the effect of sound on oviposition behavior, sound was broadcast from June (beginning of flight of cicadas) until August (the end of the reproductive season), every day from sunrise to sunset. The experiment was conducted based on a completely randomized design with two treatments (control vs. acoustic broadcasting) and four replications over a period of 3 years (2010–2012). The size of each plot was 1 ha. To generate the disruptive signal, a broadcast system was designed based on an AVR-ATmega-32 microcontroller (Atmel, San Jose, CA, USA) which could stimulate feedback amplifier noise. The broadcast systems were installed in each vineyard.

The total number of branches in the plots and the number of branches infected with cicada eggs were recorded at four distances (5, 10, 15, and 20 m) from the sound source, at the end of the flight period of adult cicadas. Oviposition data were analysed using paired t-test (treatment vs. control) and ANOVA (i.e., among distances) in SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Broadcasting an airborne signal could disrupt the calling behavior of male cicadas in the field. The calling

Table 1 Time and spectral characteristics of the male calling song and response signal of *Cicadatra alhageos* (n = 6)

	Variables	Mean ± SD	Range
Calling song	Dominant frequency (kHz)	10.1 ± 0.28	9.75–10.5
	Phrase duration (s)	2.75 ± 2.50	0.188–15.43
	Inter-phrase (ms)	56.045 ± 59.392	6–623
	Echeme (ms)	75.77 ± 130.17	7–773
	Interecheme interval (ms)	69.81 ± 103.67	4–567
	No. echemes	29.75 ± 10.37	21–43
Response signal	Echeme (s)	0.012 ± 0.001	0.008–0.013
	Interecheme intervals (s)	1.66 ± 2.96	0.045–10.21
	Dominant frequency (kHz)	8.84 ± 0.98	7.6–10.8

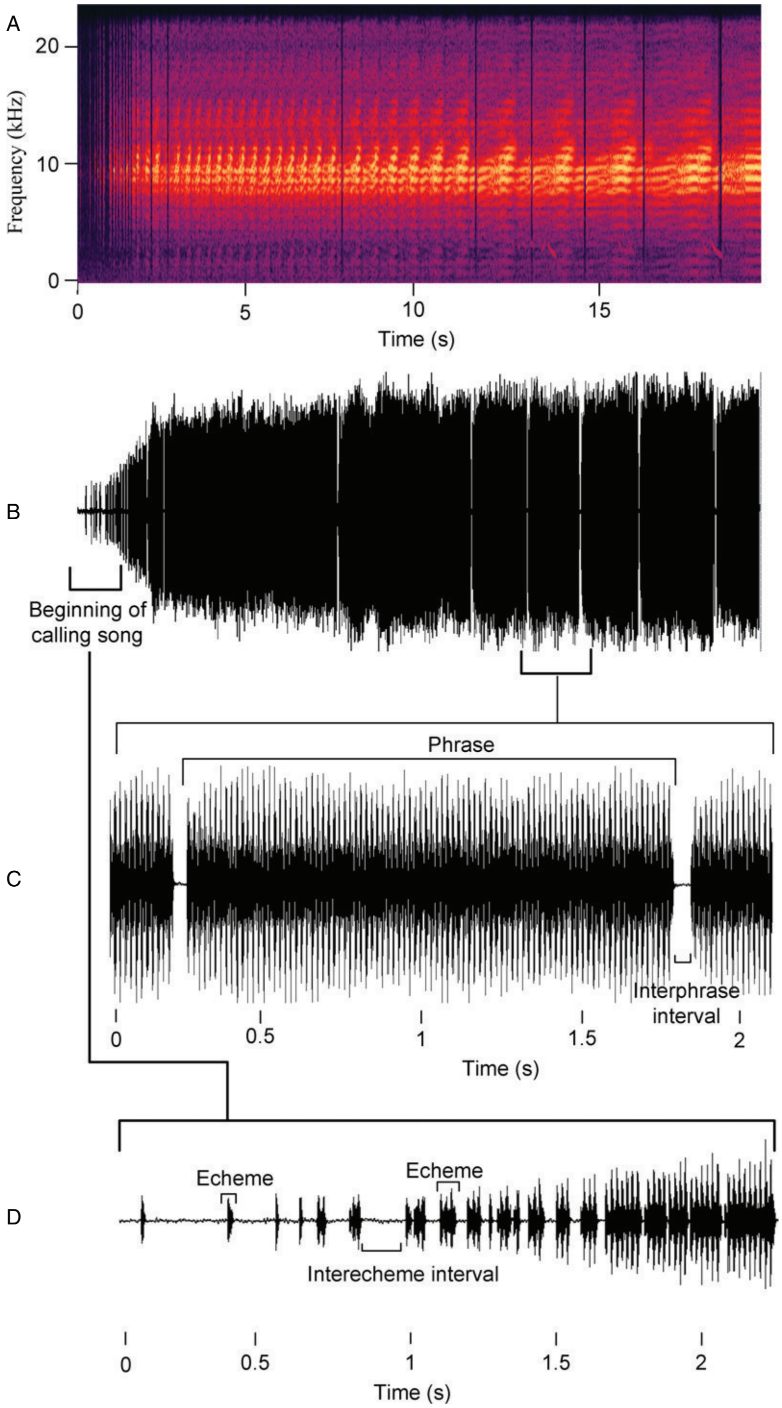

Figure 1 Calling song of *Cicadatra alhageos*. (A) Spectrum; (B) oscillogram of the calling song that included echeme, continuous phrase, and interecheme intervals; (C) the beginning part of a calling song that included echeme and interecheme intervals; (D) a phrase and interecheme intervals.

song consisted of a repeating phase (0.188–15.43 s) with irregular interruptions (6–623 ms) (Table 1, Figure 1A and B). Each phase was composed of a continuous series of pulses (ca. 0.001 s) (Figure 1C). At the beginning of a calling song, there were some irregular short echeme (7–773 ms) and interecheme intervals (4–567 ms) (Table 1, Figure 1D). In the dominant frequency, the power spectrum was characterized by a frequency bandwidth of about 6 kHz with a peak frequency of 10.1 kHz (Figure 2).

After playing the disruptive signal, male cicadas responded by stopping their calling song and produced a response signal with short echemes (0.008–0.013 s) and interecheme (0.045–10.21 s) with a dominant frequency

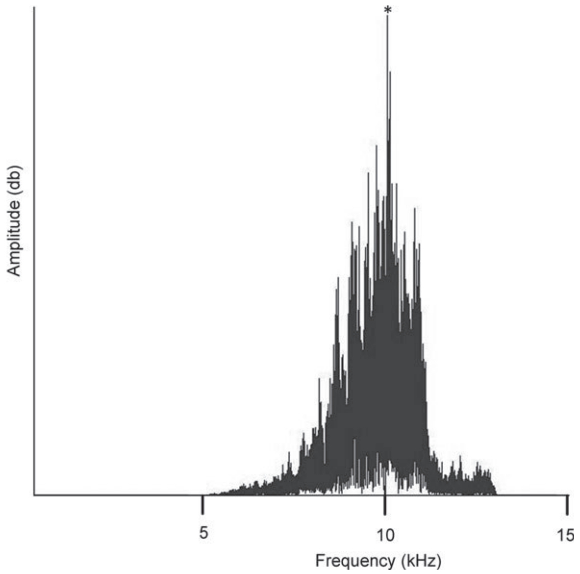

Figure 2 Example of peak frequency determination for the calling song of a *Cicadatra alhageos* male in the range of 9.75–10.5 kHz.

range between 7.6 and 10.8 kHz (Table 1, Figure 3B and C). None of the male cicadas left its position during playing of the disruptive signal. The cicadas stopped the calling song after 3–8 s of noise playback. The time between the end of noise and the beginning of a calling song was 35–60 s. The maximum time that a cicada ceased its calling song due to the disruptive signal was chosen as disruptive signal duration. The minimum time between the end of the disruptive signal and the beginning of the calling song was considered as 'silence time'. Therefore, disruptive signal duration was 8 s, followed by 35 s of silence. The cyclic repetition of disruptive signal broadcasting did not allow male cicada to produce the calling song.

The number of infested branches per vine differed between control and treated sites during the reproductive season (t = 4.38, d.f. = 15, P<0.001). The mean (± SD) number of eggs per branch was 2.48 ± 0.38 in the control and 0.33 ± 0.21, 0.33 ± 0.21, 0.67 ± 0.21, and 1.33 ± 0.49 in the treated vineyard at 5, 10, 15, and 20 m from the sound source, respectively. Distance to the sound source had no effect on the number of infested branches (ANOVA: $F_{3,15}$ = 2.11, P = 0.14).

Discussion

The calling song of *C. alhageos* is continuous and consists of irregular intervals. Zamanian et al. (2008) described that it is divided into two sections: 'start sound' and 'continued sound'. The irregular echemes during the first seconds of the calling song belong to the start sound section. The continued sound section includes phrase and inter-phrase intervals. According to Zamanian et al. (2008), the dominant frequency of *C. alhageos* is 10.2 kHz, which is similar to our results. Playback of acoustic signals in the field interrupted the calling behavior of male cicadas and disrupted mate recognition by females. This reduced female oviposition in the field. Our results corroborated with other studies that indicate broadcasting sound can cause a disruption in behavior of singing insects. For example, external sounds with particular frequencies disrupted mating in the leafhopper *Amrasca devastans* (Distant), the planthopper *Nilaparvata lugens* (Stål) (Saxena & Kumar, 1980), and the leafhopper *Graminella nigrifrons* Forbes (Hunt & Morton, 2001). The number of matings was reduced when the leafhopper *S. titanus* was subjected to playback of intra-specific and synthesized vibrational signals (60 and 200 Hz; Mazzoni et al., 2009). In the cicada *Tibicina haematodes* (Scopoli), males stopped responding when the signal was shifted down or up by 2 kHz (Sueur & Aubin, 2002).

The cessation of sound production is related to a variety of environmental factors which can affect communication among singing insects. Wind can change calling behavior in, for example, the leafhopper *G. nigrifrons* (Hunt & Morton, 2001), the cicada *C. alhageos* (Zamanian et al., 2008), and the treehopper *Enchenopa binotata* Say (McNett et al., 2010). Human activity like walking is another environmental factor which regulates calling songs of insects, for example, *C. alhageos* (Zamanian et al., 2008). Heterospecific vibrational signals disrupt the mating of insects, for example, *S. titanus* (Brumm & Slabbekoorn, 2005; Cocroft & Rodríguez, 2005). Zamanian et al. (2008) showed that heterospecific females did not affect the calling song of male *C. alhageos*, whereas they significantly decreased vibrational signals in *Nezara viridula* (L.) (Miklas et al., 2003). Predators such as spiders might change calling songs and induce a so-called anti-predator response (Barth et al., 1988). Singing insects stop calling signals until the environment is safe and free of interference (Hunt & Morton, 2001).

Artificial vibration signals may disrupt the natural behavior of pest species and beneficial arthropods, including the natural enemies of pests (Polajnar et al., 2015). Predators and parasitoids use vibrational signals to locate their prey, for example, the spider *Enoplognatha ovate* (Clerck), the stinkbug *Podisus maculiventris* (Say) (Pfannenstiel et al., 1995), the predatory katydid *Chlorobalius leucoviridis* Tepper (Marshall & Hill, 2009), and several parasitoid wasp species (Casas et al., 1998).

It is important to synthesize a disruptive signal that on the one hand controls pests, while not disturbing the bene-

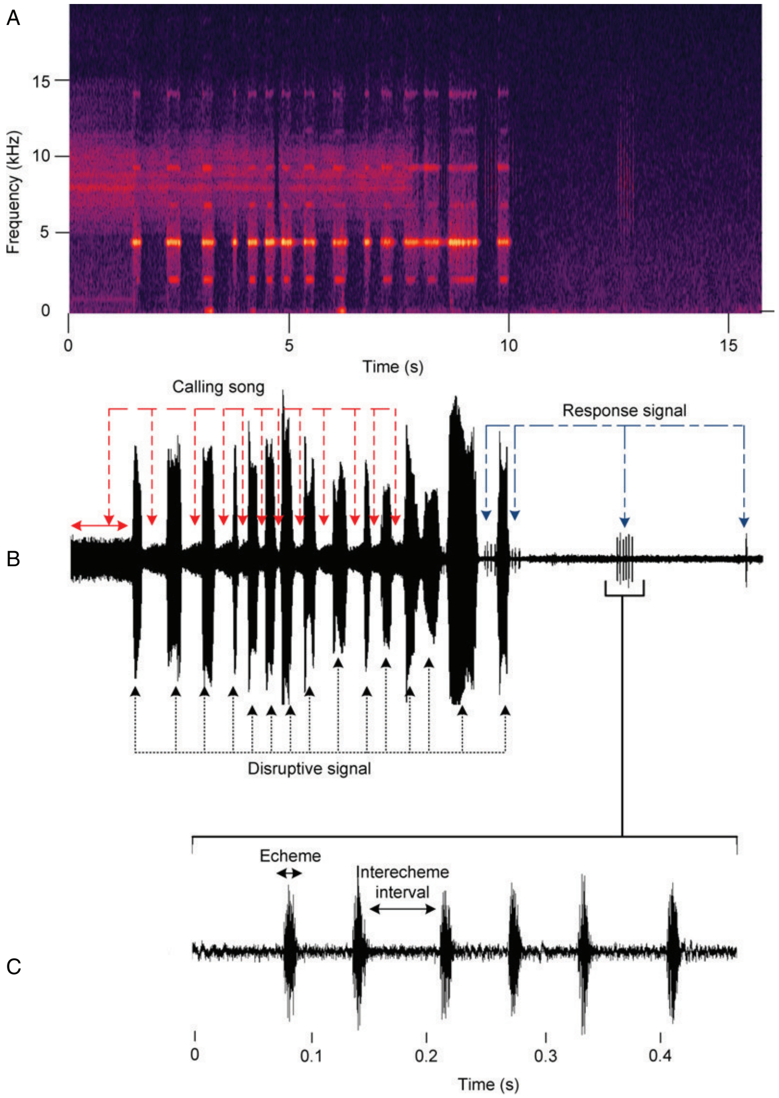

Figure 3 Calling song, disruptive signal, and response signal of *Cicadatra alhageos*. (A) Spectrum; (B) oscillogram of the calling song, disruptive signal, and response signal (singing male cicadas responded to the disruptive signal by stopping their calling song and producing a response signal); (C) response signal that included echeme and interecheme intervals.

ficial fauna in the field. Therefore, detailed investigation is needed to determine the suitable spectral characteristics, that is, the amplitude and temporal pattern of signal broadcasting (Polajnar et al., 2015). More research is needed to explain why the calling behavior of male *C. alhageos* was changed by the disruptive signals. The playback may have been perceived as a predatory threat or a competitor.

Our results indicated a negative relation between presence of sound and number of infected grapevine branches. Reduced oviposition might be due to fewer females on vine branches, which may have resulted from reduced calling behavior of males due to disruptive signal broadcasting. A female cicada flies from a long distance toward a male based on the frequency of the calling song and selects a particular male to mate with based on the temporal parameters of the call after she has become close to the male (Doolan & Young, 1989; Mehdipour et al., 2014). Thus, disruptive signals that stop a calling song form a barrier for male-searching females.

In conclusion, our study demonstrated that field acoustic playbacks can reduce branch damage by ca. 3.5×. The results support initial hypotheses that airborne sound can be used to alter male acoustic signals and female oviposition behavior. Although our study does not address the function or mechanisms of disturbance behavior, it does provide preliminary support for the potential use of sound as a low-cost complement to the commonly used chemical control agents. Acoustic methods may control pests in the field similar to pheromone application (i.e., attract-andkill or mating disruption) and may, therefore, reduce chemical application. More research is needed on the effect of disruptive signals against *C. alhageos* and on interactions between disruptive signal and natural enemies. Moreover, the efficacy of this technique over larger temporal and spatial scales must be established, before it may be used in IPM programs.

Acknowledgment

We thank Dr. Daniel Howard for his valuable advices.

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