Acoustic Playback Stimulus Experiment to Study Mating Behavioral Responses of *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae)

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**ABSTRACT**

The melon fly, *Bactrocera cucurbitae* is a serious pest attacks fruits and vegetables. The manipulation of male mating behavior that displays acoustic signals is one of the steps in the behavior-based control strategy of *B. cucurbitae*. The first study period determines the courtship song characteristics, frequency, and occurrence time. The recording was carried out when *B. cucurbitae* was mated using recording devices at 04.00–07.00 pm. The results show three sounds produced before copulation, namely, calling, courtship, and stimulating, with frequencies 274.30 ± 3.29 Hz, 284.65 ± 2.00 Hz, and 304.36 ± 1.82 Hz. *B. cucurbitae* courtship song began at 04.00–06.26 pm. The second study was performed to determine the effect of experimental acoustic playback stimulus on the selection of mating pairs by females. The acoustic playback stimulus is made synthetically by playback of two channels, namely, calling and courtship sounds, with frequencies of 274 and 284 Hz at a sound pressure level (SPL) of 85 dB. The experimental results of acoustic playback stimulus show that the sexual behavior of female *B. cucurbitae* was disrupted and marked by the decreased number of copulations during SPL is high, resulting in the overlapping calling and courtship sounds frequencies that impair signal detection.

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**INTRODUCTION**

The modern ability to record and manipulate acoustic signals has made acoustic behavior one of ethology's most active and advanced fields. Playback studies are the hallmark of ecology and animal communication studies, allowing the observation of subjects' behavioral responses to signals while eliminating behavioral variations in other issues (Hopp & Morton, 1998; McGregor et al., 1992). Signal playback has been extensively studied in animal communication. It enables a precise understanding of the function of a particular call by investigating the significance of animal signals through an analytical approach and by replaying natural or synthetic signals, which make it potentially the most powerful experimental tool available (McGregor et al., 1992). Synthetic signal playback is particularly useful for testing focused hypotheses regarding the specific properties used in the assessment of signals by receivers that initiate appropriate behavioral responses and locate signalers, thereby enabling complete control of signal parameters (Gerhardt & Huber, 2002).

Deep acoustic signal playback has been studied for vocal communication in birds and anurans and has also been widely used in mammals, fish, and insects (Rosenthal, 2019). The use of acoustic signal recording and playback technology in pest insect control applications has increased rapidly since the early 1990s (Mankin, Hagstrum, Smith, Roda, & Kairo, 2011) to attract or trap pests (Walker, 1988; 1996). It is also used to manipulate mating behavior, disrupt intraspecific communication using sound (Klappert, Samarra, Miller, & Brumm, 2009), or interfere with mating behavior toward...
mate recognition and mate localization through the transmitted vibrational signals in the host plant (Čokl & Millar, 2009). Information from acoustic signal playback stimulus experiments addresses sexual selection by female preference. In conjunction with signal forecasting among males, it can predict signal selection patterns by female mate choice (Gerhardt, 1991). An acoustic signal playback stimulus experiment was performed in this study to investigate the fruit fly species Bactrocera cucurbitae.

The melon fly, B. cucurbitae Coquillet (Diptera: Tephritidae), is an important pest that attacks fruits and vegetables with high economic value (White & Elson-Harris, 1992). B. cucurbitae attacks can directly damage fruits and vegetables, causing decrease in quality and quantity. McQuate, Liquido, & Nakamichi (2017) report that B. cucurbitae attacks 136 plant taxa from 62 plant genera and 30 plant families. The loss rate from B. cucurbitae varies from 30% to 100%, depending on the cucurbit species and season (Dhillon, Singh, Naresh, & Sharma, 2005). The attack of B. cucurbitae causes indirect quarantine restrictions, almost disrupting the international market. B. cucurbitae has been found in more than 40 countries and has been registered as a quarantine pest in many countries with large invasive status (CABI, 2022). B. cucurbitae has been designated a category A pest species of Bactrocera, with highly destructive polyphagous characteristics (Vargas, Piñero, & Leblanc, 2015). The study of mating behavior is one of the steps in developing strategies for managing B. cucurbitae (Benelli et al., 2014).

Male B. cucurbitae has a lek mating system (group of males visited by females) for mating (Bradbury, 1981; Iwahashi & Majima, 1986). At dusk, lazy B. cucurbitae defends its leaf territory by fighting with head blows or grappling against male intruders (Kuba & Koyama, 1985). The mating behavior of male B. cucurbitae uses visual stimulus (wing patterns and movements), chemical (sex pheromones), and acoustic signals (wing vibrations) to attract females (Iwahashi & Majima, 1986; Kuba & Koyama, 1985; Kuba, Koyama, & Prokopy, 1984). Acoustic signals are calls during mating activity and sexually important communication in species recognition, presence of females, or means through which females can distinguish males (Svinski, Burk, & Webb, 1984). Species-specific acoustic signals are produced by vibrations of the male wings accompanied by sound production. The male wing vibration is from the behavior of keeping the wings close to the abdomen. It vibrates them rapidly in a dorso-ventral fashion. It rolls them along the longitudinal axis while manifesting a stridulation behavior (during wing vibration, the posterior portion of the male wing repeatedly touches the abdomen, rubbing the cubital microtrichia against the abdomen pecten) (Keiser, Kobayashi, Chambers, & Schneider, 1973). Fruit flies produce acoustic signals at low frequencies and within close ranges by generating air currents that can be used to transmit specific sex pheromones (Bennet-Clark, 1998).

This study conducted two studies. The first study determined the courtship song characteristics, frequency, and occurrence time. The mating behavior of B. cucurbitae can contribute to controlling by interfering with sexual communication and has generally been carried out using pheromones (Campion, Critchley, & McVeigh, 1989) which are considered environmentally friendly biological control agents. In addition, to control using pheromones, alternative control strategies by interfering with acoustic signal communication can contribute to the development of new control devices. The second study determined the effect of acoustic playback stimulus on a female’s selection of mating pairs, with pairing and copulation activity as signs of successful mating. Stimulus acoustic playback is a potential approach to managing species-specific pests, and it is environmentally friendly and effective as a part of Integrated Pest Management (IPM). Therefore, this study examined the acoustic playback stimulus required to manipulate females to lead them to similar calls.

**MATERIALS AND METHODS**

**Experimental Site**

The research was carried out from January to December 2021 at the Laboratory of Basic Entomology and the Laboratory of Applied Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada (UGM).

**Mass Rearing Procedure**

B. cucurbitae pupae were obtained from the Laboratory of Basic Entomology, Department of Plant Protection, Faculty of Agriculture, UGM. The mass rearing procedure was carried out at the
Hawaiian Department of Agriculture (Toto Himawan, personal communication) and later developed at the laboratory was substituted protein hydrolysates using yeast extract as an adult diet to reduce diet costs. *B. cucurbitae* in adult stages were reared in cages measuring 30 × 30 × 30 cm³ and given an artificial diet in the form of a mixture of sugar and yeast extract at a ratio of 4:1 in a petri dish (9 cm diameter, 1.5 cm high). A wet sponge was placed into the top cage. The mass rearing of *B. cucurbitae* in the laboratory was performed under conditions of natural light (12 h light:12 h dark cycle), at room temperature 28 ± 1 °C, and with relative humidity of 60%−70%.

**Acoustic Signal Recordings**

The mating sounds of sexually mature males and females *B. cucurbitae* were recorded on the eight day after emergence (DAE). The population included 1000 male and female individuals (1:1 sex ratio) in a cage measuring 30 × 30 × 30 cm³. Recordings were conducted for 24 h and repeated thrice on different days to determine the mating time of *B. cucurbitae*. The recordings showed that the courtship song appeared at 04.00–07.00 pm. Therefore, the recording was performed with 10 repetitions on different days. The sound acquisition devices used in the recordings were the Zoom H6 Handy Recorder 96 kHz/24-bit digital audio recorder and Sony Xperia M4 Aqua mobile phone with the Arbimon Touch application installed and connected to a Dayton Audio microphone. The files were saved in WAV format. The use of the Arbimon Touch application aimed to sample one minute recordings for every five minutes interval for a total of 360 recording samples.

**Acoustic Signal Analysis**

Analysis of the courtship song of *B. cucurbitae* was conducted using Raven Pro software v.1.6.2 (Charif, Waack, & Strickman, 2010; K. Lisa Yang Center for Conservation Bioacoustics, 2022), which displays acoustic signals and performs frequency and temporal pattern analysis in the form of waveforms and spectrograms. Spectrograms were generated with the Hann Window type, with a window size of 548 samples, 50% overlap, hop size of 274 samples, DFT size of 1024 samples, and grid spacing of 43.1 Hz.

Various acoustic signal parameters, namely, PT duration (PTD), PT interval (PTI), PT period (PTP), and fundamental frequency (FF), were studied. The start, middle, and endpoints of each pulse, the sound sequence, and the start and end of each pulse courtship song sequence were noted on a spreadsheet. The PTD was calculated as the time between the start and end of each train. The PTI is the time difference between the end of a train and the start of the next. The PTP is the time difference between the start of two consecutive trains (= PTD + PTI). The first harmonic is the FF (lowest frequency of the sound wave) at each pulse’s start, midpoint, and end (Mankin, Lemon, Harmer, Evans, & Taylor, 2008). The first harmonic is the frequency of the fruit fly wing vibrations (Webb, Sharp, Chambers, McDow, & Benner, 1976).

**Acoustic Playback Stimulus Experiment**

The experiment using *B. cucurbitae* pupae of the same size were selected to eliminate the effect of size on male competitiveness during mating (Sivinski, 1993). Sex separation was carried out after the first 1–3 days of adult appearance to obtain virgin specimens of the same age, with sexual maturity around the period of 8–10 days (Vargas, Miyashita, & Nishida, 1984; Wong, McInnis, & Nishimoto, 1986). The separated adult stage was then placed in cages measuring 24 × 24 × 24 cm³ and containing 20 pairs of males and females, 60–90 minutes before treatment as a species introduction. The acoustic playback stimulus was performed on pairs aged 12, 13, and 14 days.

The first step in the acoustic playback stimulus experiment is the creation of a synthetic that is comparable to the typical natural in the behavioral effects of *B. cucurbitae* during mating. A synthetic acoustic playback stimulus was created using a waveform generator (Rigol DG1022Z Series Function/Arbitrary) connected to loudspeakers (Creative Labs GigaWorks T40 Series II Speakers) 10 cm apart near the enclosure. The stimulus for acoustic playback was in the form of a pulse connected to loudspeakers in a low-noise room. The acoustic playback stimulus experiment was conducted with sound exposure for 30 minutes before mating and after mating *B. cucurbitae*. The experiment consisted of five replications, acoustic playback stimulus with two channels on calling and courtship sounds at frequencies of 274 and 284 Hz. The magnitude of acoustic signals is usually measured as the spectrum level at a certain frequency (Beranek, 1988). The amplitude was set to 20,000 Vpp at SPL measured.
at 85 dB and 10 cm from the loudspeaker. The sound level was expressed as a decibel equivalent peak SPL (0 dB = 20 Pa) (Stapells, Picton, & Smith, 1982).

During the experiment, the behavior of males and females *B. cucurbitae* was recorded using Canon EOS 70D 18–135 mm and Fujifilm X–A10 16–50 mm camera. Additional lights at dusk using red light-emitting diode (LED) lights were used to observe the mating behavior of fruit flies. The red light was selected because it corresponds to the maximum absorption frequency of the camera and does not damage the visual apparatus of insects that lack receptors for that wavelength (Briscoe & Chittka, 2001).

**Data Analysis**

This research used Sigma Plot v.14.0 software (Systat Software, Inc., San Jose, CA, USA) to analyze the occurrence of sounds in graphical form at every 30 minutes interval. It also calculated the mean and standard error (SE) values of PTD, PTI, PTP, and FF from PT in every calling, courtship, and stimulating sounds. Meanwhile, the data obtained from the observations were analyzed by variance (ANOVA; 5%). The ANOVA data was analyzed using JMP v.16 and SAS® OnDemand for Academics to access SAS Studio via a web browser (https://welcome.oda.sas.com/home). When there was a significant difference, the least significant difference (LSD) test was carried out at the 5% level as a post-hoc test continued with analysis using figure design for mean ± SE.

**RESULTS AND DISCUSSION**

**Character Courtship Song Bactrocera cucurbitae**

Courtship is a series of behaviors sequentially carried out between males and females, leading to the recognition and selection of suitable mating pairs (Clarke, 2019). Three different male courtship song were produced before copulation. The first sound (Fig. 1 and Fig. 2) was a calling sound with very short pulses and rather irregular intervals, heard when a male was first orienting toward a female. The second sound (Fig. 3 and Fig. 4) was a courtship sound with a train of short pulses at regular intervals, heard when a male was facing a female. The third sound (Fig. 5 and Fig. 6) started stimulating sound with long continuous bursts. It was heard when a male sprayed pheromone clouds, and a high-pitched premating sound with fluctuating amplitude was produced just before mounting the female (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987; Miyatake & Kanmiya, 2004).

![Fig. 1. Calling sound waveform in the form of a series with very short pulses and rather irregular intervals](https://welcome.oda.sas.com/home)
Fig. 2. Calling sound spectrogram in the form of a series with very short pulses and rather irregular intervals

Fig. 3. Courtship sound waveform in the form a train with short pulses at regular intervals
Several male fruit flies of Tephritidae, including *B. cucurbitae*, produce several types of sound in a sexual context; these sounds consist of repeated bursts of a group of pulses with similar characteristics. Pulse trains (PTs) are short or long bursts (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987; Miyatake & Kanmiya, 2004; Sivinski, Burk, & Webb, 1984; Webb, Sharp, Chambers, McDow, & Benner, 1976; Webb, Sivinski, & Litzkow, 1984) in a series of calling and courtship sounds of the fruit fly. Early calling and courtship sounds begin after the female approaches the male (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987). Table 1 shows that the frequency and temporal characteristics of the series of wing vibrations produced by male *B. cucurbitae* during mating had a calling sound with a frequency of 274.30 ± 3.29 Hz, PTD 0.13 ± 0.00 s, PTI 0.22 ± 0.01 s, and PTP 0.32 ± 0.01 s. Courtship sound with frequency of 284.65 ± 2.00 Hz, PTD 0.51 ± 0.02 s, PTI 0.29 ± 0.02

**Fig. 4.** Courtship sound spectrogram in the form a train with short pulses at regular intervals

**Fig. 5.** Stimulating sound waveform for long continuous bursts, and high-pitched premating sound with fluctuating amplitude
s, and PTP 0.74 ± 0.04 s. Stimulating sound with a frequency of 304.36 ± 1.82 Hz, PTD 3.03 ± 0.10 s, PTI 0.31 ± 0.04 s, and PTP 3.04 ± 0.15 s. Based on Kanmiya (1988) research, the basic frequencies of short and long bursts by males in mating songs are 282 ± 24.30 and 290 ± 28.03 Hz, respectively, on the mean. *B. cucurbitae* has a longer PTD in the older age group than in the younger age group (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987; Miyatake & Kanmiya, 2004). PTI exhibits geographic variations (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987). Sivinski & Webb (1986) reported male *Anastrepha suspensa* prolongs the pulse duration with a higher baseline frequency when near other males, shortening the pulse interval in the presence of females. Thus, a longer PT plays an important role in aggression against sexual rivals, and a shorter PT plays a role in attracting female attention (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987).

The acoustic parameters observed during the mating of male fruit flies were FF, sound amplitude, and harmonics, which have a fairly wide range due to differences in courtship behavior (Sivinski & Webb, 1986; Webb, Calkins, Chambers, Schwinbacher, & Russ, 1983; Webb, Sharp, Chambers, McDow, & Benner, 1976) or movements made by females (Kanmiya, Tanaka, Kamiwada, Nakagawa, & Nishioka, 1987). The frequency produced was limited by phylogeny, body size, biotic and abiotic sounds, and habitat structure (Morton, 1975; Ryan & Brenowitz, 1985; Wilkins, Seddon, & Safran, 2013). The amplitude of dipteran wing vibrations is determined in part by the placement of pleural sclerites, which act as mechanical stops to the movement of the structures that move the wings (Boettiger & Furshpan, 1952). Suppose the behavior that produces the calling and courtship sounds involves setting the pleural sclerite to a fixed stop, which limits the wing vibration amplitude to a small and narrow range. In that case, wing loading becomes negligible, and the vibration frequency will approach the maximal level of an undamped flight (King & Buchmann, 1996). Thus, each male’s frequency of calling and courtship sounds is close to the resonant frequency of its flying motor.

**Fig. 6.** Stimulating sound spectrogram for long continuous bursts, and high-pitched premating sound with fluctuating amplitude
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Table 1. Frequency and temporal characteristics of the series of wing vibrations produced by male B. cucurbitae during courtship

| Pulse-train parameters | Mean ± standard error (SE) (n = 1000, sex ratio 1:1) |
|------------------------|----------------------------------------------------|
| **Calling sound**      |                                                    |
| Fundamental Frequency (FF) (Hz) | 274.30 ± 3.29                                    |
| Pulse Train Duration (PTD) (s) | 0.13 ± 0.00                                      |
| Pulse Train Interval (PTI) (s) | 0.22 ± 0.01                                      |
| Pulse Train Period (PTP) (s) | 0.32 ± 0.01                                      |
| **Courtship sound**    |                                                    |
| Fundamental Frequency (FF) (Hz) | 284.65 ± 2.00                                    |
| Pulse Train Duration (PTD) (s) | 0.51 ± 0.02                                      |
| Pulse Train Interval (PTI) (s) | 0.29 ± 0.02                                      |
| Pulse Train Period (PTP) (s) | 0.74 ± 0.04                                      |
| **Stimulating sound**  |                                                    |
| Fundamental Frequency (FF) (Hz) | 304.36 ± 1.82                                    |
| Pulse Train Duration (PTD) (s) | 3.03 ± 0.10                                      |
| Pulse Train Interval (PTI) (s) | 0.31 ± 0.04                                      |
| Pulse Train Period (PTP) (s) | 3.04 ± 0.15                                      |

Remarks: *FF at the midpoint is the fundamental frequency at the midpoint of the PT
Fig. 7. Mean occurrence of sounds at every 30 min interval of *B. cucurbitae* courtship song

Fig. 8. Mating achieved (mean ± SE) and tested on 12, 13, and 14 days
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Acoustic Playback Stimulus Experiment

The second study aimed to determine the effect of acoustic playback stimulus on the selection of a mating pair by females, with pairing and copulation activities as signs of successful mating. The experiment was conducted with species-specific frequencies of 274 and 284 Hz and high amplitude at a SPL of 85 dB. The study observed the mating SPL of fruit flies at 45–50 dB. The results showed that the acoustic playback stimulus affected the selection of mating pairs (Fig. 8), marked by a decrease in the number of copulations tested on day 12 with a mean control of 14.20 mating pairs. The treatment of the acoustic playback stimulus contained 10.00 mating pairs, on day 13 with a mean control of 13.20 mating pairs, and in the treatment of the acoustic playback stimulus, there were 11.80 mating pairs. On day 14 with the control mean of 12.80 mating pairs and the acoustic playback stimulus treatment, there was 11.20 mating pairs.

A significant decrease in the response of B. cucurbitae females is indicated by the inferior capability of females to detect acoustic signals by male B. cucurbitae when exposed to acoustic playback stimulus at high SPL. Based on the research by Sivinski & Webb (1985), female virgin Toxotrypana curvicauda (Diptera: Tephritidae) became less active with the sound of flapping wings exposed to a SPL of 90 dB. Therefore, the mating sounds of males play a major role in sexual arousal. The female B. cucurbitae showed a significant response to the acoustic playback stimulus at high SPL but overlapped with the mating sound frequency, thus impairing signal detection. Thus, high-amplitude acoustic playback stimulus can influence mate selection and have the potential for environmentally friendly and effective species-specific pest management as a part of IPM.

CONCLUSION

Three different sounds were produced by male Bactrocera cucurbitae before copulation namely calling, courtship, and stimulating sounds, which began at 04:00–06:26 pm. In addition, the selection of female B. cucurbitae pairs was influenced by the acoustic playback stimulus experiment with frequencies of 274 and 284 Hz, an amplitude of 20,000 Vpp, and a sound pressure level (SPL) of 85 dB. It was characterized by a decrease in the number of mating in pairs aged 12, 13, and 14 days.

ACKNOWLEDGEMENT

The authors thank Michael Pitzrick, K. Lisa Yang Center for Conservation Bioacoustics, Cornell Lab of Ornithology, Cornell University at Ithaca, New York, USA for granting the license for use of the Raven Pro software 1.6.2 version. The authors also thank the Publishers and Publications Board, Universitas Gadjah Mada for assistance in language editing and proofreading.

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