# ELECTROANTENNOGRAM (EAG) RESPONSE OF THREE FRUIT FLY SPECIES (*Bactrocera dorsalis, B. occipitalis* AND *Zeugodacus cucurbitae)* (DIPTERA: TEPHRITIDAE)) TOWARDS PLANT VOLATILE γ-OCTALACTONE

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## ABSTRACT

**Electroantennogram (EAG) device is a useful instrument utilized for measuring the perception capability of sensory organs toward a known stimulus. It gives valuation into designated expression generated by stimulated receptor cells. For insects specifically, it aids in a deeper understanding of herbivore-host plant interaction. It provides a new paradigm in studying host-searching behavior. In this research, electroantennography was conducted in three local fruit fly species: *Bactrocera dorsalis, B. occipitalis* and *Zeugodacus cucurbitae.* Each species was assigned with a representative of different sex, age, sexual maturity, and host origin. The chemical used was γ- octalactone, a volatile compound in fruits such as mango (*Mangifera indica*) that signals the ripening stage. The antenna of the test insect was mounted into EAG probe and exposed into to the increasing concentration of compound (0, 0.5, 1.0 and 5.0 ppm of 2 mL volume). For all the fruit fly species and populations, a linear relationship was observed between γ-octalactone dosage and EAG response. The majority of EAG responses had negative polarity. Both maless and females perceived the cue and correspondingly distinguished its intensity. However, the mated female group showed the most consistent response. Among the three species, *B. dorsalis* was the most responsive tothe γ-octalactone. This information implies that γ-octalactone triggers sensory neurons of mature fruit fly at significant intensity. The role of this chemical in the oviposition behavior of *B. dorsalis* was discussed.**

**Keywords:** Electroantennogram, fruit fly, γ- octalactone, EAG response, oviposition

## INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most economically important pest groups attacking fruit crops. They are known to cause significant yield losses and reduce the value and marketability of horticultural products. Yield loss due to fruit flies was estimated to be between 30-100 percent depending on fruit species and season (Dhillon et al., 2005). The export potential of produce is also negatively affected due to the fear of incursion to new areas or further infestation of the inhabited areas (Serem, 2010).

Among the species, *Bactrocera dorsalis* is the most prevalent in the Southeast Asian region. It is considered a polyphagous frugivore, recorded to utilize around 300 plant species in 14 genera and 40 families (Clarke et al., 2005). In the Philippines, where it is native, *B. dorsalis* is most damaging in fruits such as mango (*Mangifera indica*), guava (*Psidium guajava*), papaya (*Carica papaya*) and breadfruit (*Artocarpus altilis*) (Leblanc, 2021). It is considered a target pest of high quarantine importance, cited in the Plant Protection Regulation 1998 of the Queensland State Government of Australia and Japanese Plant Quarantine Law (Iwaizumi, 2004).

Currently, the focus of fruit fly control is the use of chemicals which contributes to rapid insecticide resistance of pest through indiscriminate application. Development of para-pheromone traps is also practiced (Sumathi et al., 2019). However, these are mostly targeting male flies which indirectly impacts fruit infestation. Mechanical control such as fruit bagging is also practiced but the materials and labor for these results to increase on input cost. Focus on the female flies is about the synthesis of protein-based bait, which provides varying results. One integral aspect that lacks exposure is oviposition attractant and stimulant. Understanding the external cues utilized by *B. dorsalis* to locate and assess hosts may have greater potential in trapping gravid females and reducing possible contact within the fruit (Ponnusamy et al., 2008). Host searching would then serve as important biological concept to consider.

Host searching pattern is utilized by insects to find an appropriate partner for mating, resource for feeding or site use to establish offspring (Aluja & Mangan, 2008). It is considered as a trait with adaptive value for its significance in increasing th survival and reproductive probability of an individual, eventually translating to persistence within the natural habitat. An effective host-searching pattern requires matching condition of physiology, morphology, and behavior of organism with the outside environment. For fruit flies, oviposition site selection determines the success of generation due to the less mobile characteristic of larvae. Fruits at the most suitable phenological stage are selected, which would provide nourishment and habitat for future immatures.

Vinson (1976) broke down host-searching behavior into sequential steps, which are host habitat location, host finding, host acceptance, host suitability, and host regulation. In terms of insect proximity toward the host, it can be dissected into pre-alighting, alighting, and post-alighting actions. Each component is mediated through accurate perception of environmental cues by the insect using its sensory organs (Bell, 1990). However, physiological status must match with the external conditions to provoke the desired response.

Phytophagous insects like fruit flies are broadly classified in terms of host range- monophagous or specialist (single host) and polyphagous or generalist (multiple hosts). However, this characterization of organisms is usually falsely equated to innate adaptive trait itself. Butbeing a “monophagous” or “polyphagous” is only the consequence of exposure of individuals to multiple environmental cues that lead them to different set of favorable resources.

Being considered s polyphagous fruit fly, *B. dorsalis* is commonly used as a subject in further understanding host-searching behavior pattern of insect. Several studies revealed that female fruit flies utilized fruit volatile blends for attraction to locate possible hosts (Siderhurst & Jang, 2006; Biasazin, 2017; Jayanthi et al., 2014). Damodaram et al. (2014) meanwhile introduced the concept of Innate Recognition Template (IRT) of *B. dorsalis* towards fruit volatile γ-octalactone. Innate Recognition Template refers to the embedded biological connection of individual response to specific internal or external stimulus. This genetic adaptation contributes to prolonged survival of adults and successful proliferation of succeeding generations. In the case of a gravid female, it showed distinctive bias towards material emitting γ-octalactone, referred as hardwire preference. Concentration invariance was also exhibited, indicating that even a small amount of γ-octalactone existence would elicit an oviposition response. This chemical cue perception phenomenon was connected to the preference-performance concept of phytophagous species. Lactones are attributed to resources capable of sustaining nourishment and providing protection for developing fruit fly larvae. The idea of IRT provides an evolutionary mechanism by which host searching behavior of *B. dorsalis* came about.

To understand how insects like fruit flies detect external cues such as γ-octalactone, a device called electroantennogram (EAG) can be utilized. It is an instrument used to quantify neural response of insect organs (Light et al., 1987). It is composed of a monitor, signal connection box, stimulus provider containing an air vent, micromanipulator sensory assembly and air pump. The general idea is that a freshly dissected sensory organ (antenna, proboscis, and ovipositor) retains a significant number of electronic impulses. Thus, it can be used to read insect perception capability towards a specific stimulus. Electroantennogram provides data in the form of a continuous graph showing neural activity. This information would then be interpreted to determine whether the insect can detect cues, to what extent and overall pattern.

In this study, the main question that was addressed is: How do three different fruit fly species (*Bactrocera dorsalis, B. occipitalis and Zeugodacus cucurbitae*) respond towards γ-octalactone with focus on different sex, age and host origin? It has the objective of determining the perception capability of the three fruit fly species; two of which are known to utilize γ-octalactone on host searching behavior (*B. dorsalis* and *B. occipitalis*) while the other one is not (*Z. cucurbitae*).

## MATERIALS AND METHODS

### Test insect

**Collection and mass rearing.** Wild population was obtained from fruit fly infested fruit crop field. In this study, the starting population of *B. dorsalis* was collected from San Ildefonso, Bulacan. Samal Island, Davao, was set to be the source of *B. occipitalis*, and Los Banos, Laguna, for *Z. cucurbitae*. Collected fruits were stored in plastic containers covered with mesh cloth until larvae emerged and pupated on the bottom surface. Fine sterilized sawdust was placed inside the container to aid in pupation. Upon pupal formation within two days, pupae were separated from sawdust using chicken wire. Adults were allowed to emerge inside a cubical fiberglass cage (30 x 30 x 30 cm). Water and an artificial diet composed of table sugar and yeast hydrolysate (3:1 ratio) were provided. The protein source was necessary for sexual and ovarian maturation of females. Adult flies were kept inside the cage until the oviposition period.

**Taxonomic identification.** To ensure homology between the sibling species *B. dorsalis and B. occipitalis* being reared, fruit fly was sorted using the reversed scoring system modified by Iwahashi (1999) (**Table 1**). With this, *Bactrocera dorsalis* individuals were maintained while its sibling species *B. occipitalis* was separated. For each character, a score of 1 corresponds to a typical *B. dorsalis,* while a score of 0 signifies *B. occipitalis* (**Figure 1**). The total of scores for characters served as basis for classification. Score of 0-2 means the insect is *B. occipitalis,* while score of 4-6 means *B. dorsalis.* A score of 3 represents the intermediate.

**Table 1.** The reversed rating system for distinguishing *B. occipitalis* from *B. dorsalis* (Iwahashi, 1999)

| Character | Scoring and corresponding description | |
|---|---|---|
| | **0** | **1** |
| Medial line on abdominal tergite 4 | Broad | Narrow |
| Transverse dark band on abdominal tergite 3 | Very broad down sides | At most narrow down |
| Lateral marks on abdominal tergite 4 | Broad and rectangular | Absent or narrow triangle |
| Ground color around pre-scutellar setae | Red | Black |
| Ground color around frontal setae | Yellow | Black |
| Costal band on wings | Deep clearly extending below vein $R_{2+3}$ | Narrow and not below vein $R_{2+3}$ |

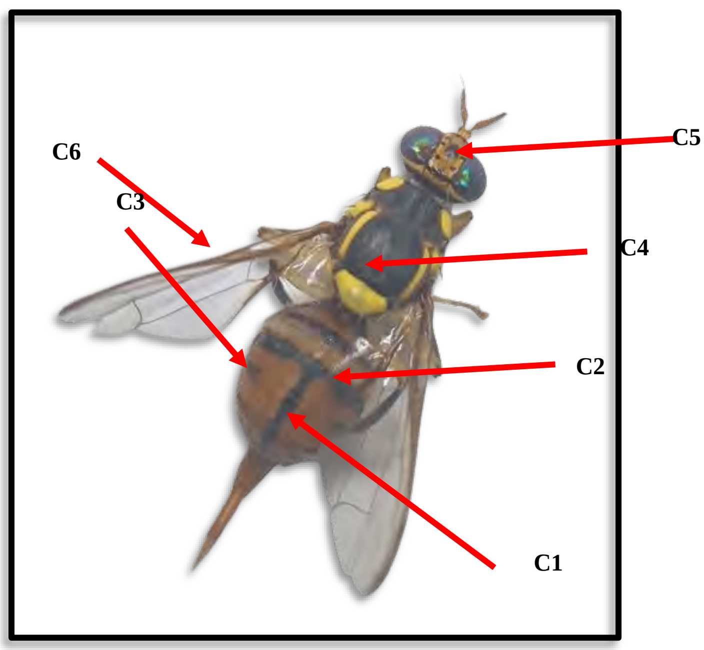


**Figure 1.** Fruit fly with a total score of 4 using the reverse scoring method (Iwahashi, 1999); C1- 1, C2- 0; C3- 1; C4- 1; C5- 0; C6- 1, specimen identified. as *B. dorsalis*

**Oviposition.** Adult *B. dorsalis* was allowed to feed inside the cage to complete their maturation. After 15 days (pre-oviposition period), various oviposition host fruits were offered per cage. These were mango (*Mangifera indica*), papaya (*Carica papaya*), guava (*Psidium guajava*) and starfruit (*Averrhoa carambola*). The first two were known to release γ-octalactone, while the latter are not (Pino et al., 2005). Fruit peels were placed in the cage for 2 days to maximize egg deposition. After this, hosts were collected and separately cultured inside the plastic container. Site of oviposition dictated what fruit to feed for the offspring. Same rearing procedure was followed until adult stage of fruit fly. However, strict regulation of host utilized by succeeding generation was observed to ensure exclusivity of food upon where the fruit fly was exposed into.

For *B. occipitalis* adults, mango peel was provided as an oviposition site, while cucumber peel which was provided for *Z. cucurbitae* adults.

**Electroantennogram study**

For this experiment, adult fruit flies of varying condition and population were utilized for the experiment to compare the responsiveness of different physiological status of individuals to the γ-octalactone. As perception is a product of external and internal environment, it is hypothesized that differently status individuals might response to distinct signal variably.

- Species: *B. dorsalis, B. occipitalis* and *Z. cucurbitae*
- Sex: Female and male
- Maturity: Young (1-3-day old) and mature adult (15- day old)
- Generation: Two for *B. dorsalis* (3$^{rd}$ and 4th), One for *B. occipitalis* and *Z. cucurbitae* (1$^{st}$)
- Developmental host: Two sets for *B. dorsalis;* one from non-γ-octalactone host (guava) and one from γ-octalactone- producing hosts (mango)

Fruit fly was anesthetized by placing it inside the freezer (0°C) for 5 minutes. Antenna of the insect was then immediately dissected starting from the scape and mounted dorsally on the electrode holder. The surface of the holder was lined with electroconductive gel to ensure maximum signal perception. Then the electrode holder was connected to Universal Single ended probe attached to Channel Acquisition controller of SYNTECH © 2015 Electroantennogram (**Figure 2**).

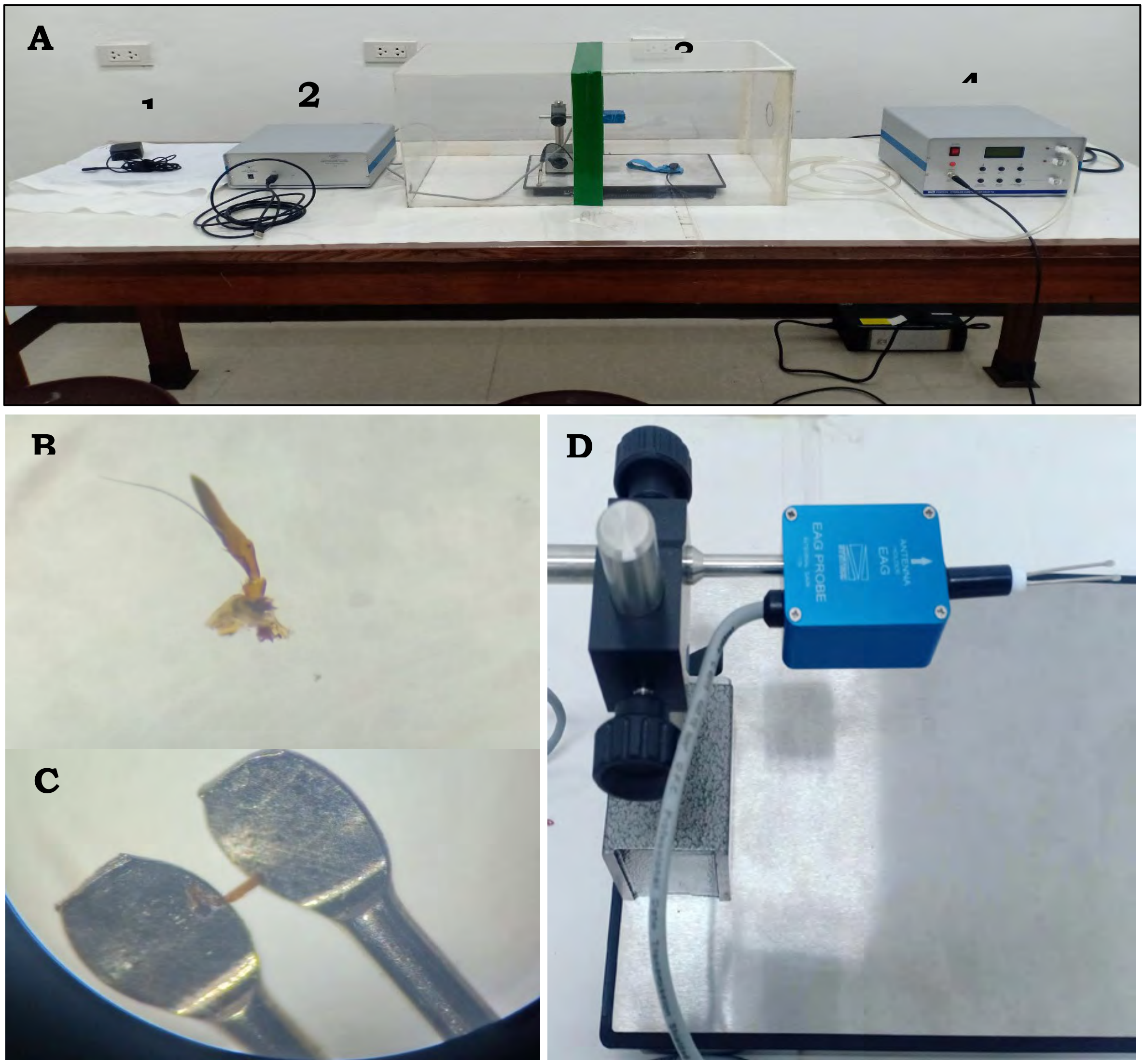


**Figure 2.** The electroantennogram (EAG) setup with test specimen. (A) Complete set up: 1. computer for recording, 2. channel acquisition controller, 3. EAG probe, 4. stimulus controller. (B) Freshly dissected fruit fly antenna. (C) Antenna on electrode probe holder. (D) EAG probe with probe holder.

For the stimuli, laboratory grade γ-octalactone (natural, ≥97%, density of 0.981 g/mL at 25 °C, Fragrance Grade) was diluted to paraffin oil to attain different concentration. Signals offered to the antenna were 0, 0.5, 1.0 and 5 parts per million (ppm). Pure puff of air was also utilized to serve as standard. Total volume of paraffin oil used is 10 mL per treatment. This accounts for the total γ-octalactone prepared for each treatment to 0, 1,000 and 5,000 nanoliter.

Upon completion of antennal setup, stimulus was offered by dropping about 2 mL of each treatment to a Pasteur pipette lined inside with filter paper.

In terms of γ-octalactone dosage, 0, 196.2 and 981 microgram (µg) were used for each treatment. Signals were displaced using Stimulus controller with built-in air pumps. Air flow was regulated at 50 ml/s to induce standard release of fumes. Exposure of antenna for each treatment was done for 2 minutes. Sequence of stimulus provision followed an increasing concentration of γ-octalactone. Interval of 30 seconds was utilized after each exposure to prevent acclimation of sensory cells to stimulus presented.

Perception capability of antenna was recorded through SYNTECH software GcEad 2022 v1.3.0 (2022-01-30) which showed data in linear wavelength pattern (time in seconds vs EAG value in millivolts). Fruit fly antennal exposure is replicated three (3) times per treatment, and repeated on 3 individual fruit flies. Linear trend produced by the software would then be extracted and twenty peaks indicating sensory organ excitation would be measured.

Perception capability of fruit fly antenna was observed by measuring the absolute maximum electroantennogram (EAG) values of linear wavelength produced. Linear trend produced by the software was extracted and twenty peaks indicating sensory organ excitation were measured. Linear regression analysis was performed to determine the existence of relationship between γ-octalactone concentration and EAG values. Electroantennogram values from fruit fly were also compared among treatments, species, sex, maturity, generation, and developmental host. The overall experiment was laid out into Randomized Complete Block Design (RCBD), treating different fruit fly condition and population as blocking factors. Data were analyzed using Analysis of Variance (ANOVA) and comparison among means were done using Tukey’s test.

## RESULTS

### Response pattern and magnitude

*Treatments (x): γ-octalactone concentration (0, 0.5, 1.0, 5.0 ppm)*
*Response variable (y): Electroantennogram response, mV (Absolute values)*

Prior to the assessing response of different fruit fly population to γ-octalactone, a negative standard was initially offered in the form of pure air. This is to establish that the excitation observed in EAG trend line is caused by γ-octalactone. Results showed that there is no significant difference between antennal response of all specimens when exposed to both pure air and pure paraffin oil (solvent) (**Figure 3)**.

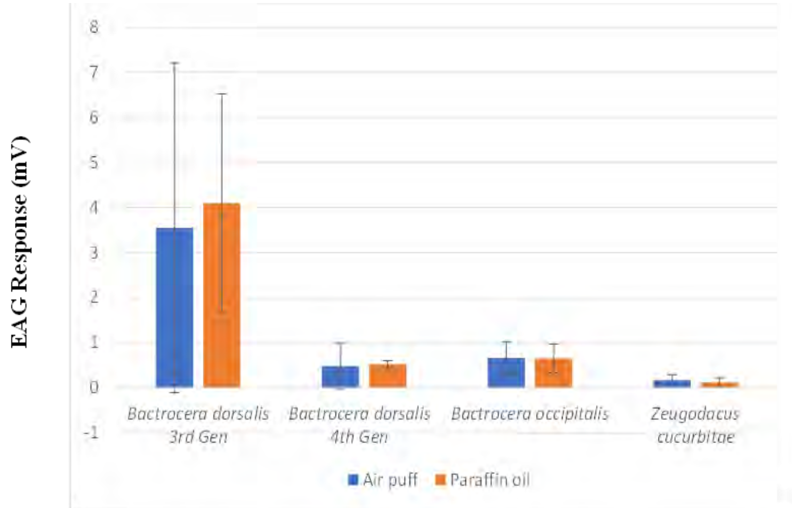


**Figure 3.** Comparison of mean (±SD) (mV) of EAG signal emitted by different fruit fly population exposed at air only and paraffin oil. Values shown are not significantly different (t-test; α=0.05).

For all the representative samples of the fruit fly species and population, linear relationship was observed between γ-octalactone concentration and electroantennogram (EAG) response of antenna (**Figures 4-7**). This indicates that the sensitivity of the sensory organ of fruit flies toward γ-octalactone was directly related to the stimulus concentration. Both male and female fruit flies could perceive this signal and correspondingly distinguish its intensity. However, between the sexes, it is the female fly that showed consistent direct linear regression response for γ-octalactone for all species. This is by virtue of having more positive slope coefficient across all species for females than males.

With regards to the mean EAG response value, *B. dorsalis* and *B. occipitalis* have relatively higher sensitivity for γ-octalactone than *Z. cucurbitae* (**Figure 8**). This is consistent with all the concentrations provided. High variation within *B. dorsalis* response was observed due to differences in EAG value among sex, maturity, and population origin of test insects.

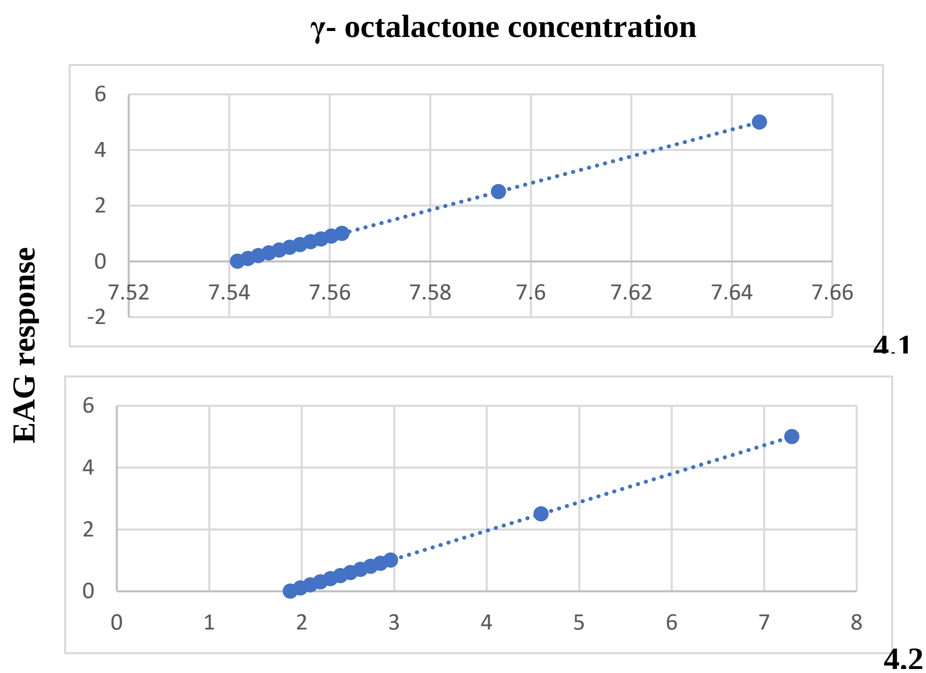


**Figure 4.** Linear regression graph of 3rd generation *Bactrocera dorsalis* to varying concentration of γ-octalactone; 3.1- Reared on non- γ-octalactone host, 3.2- Reared on γ-octalactone host; r= correlation coefficient.

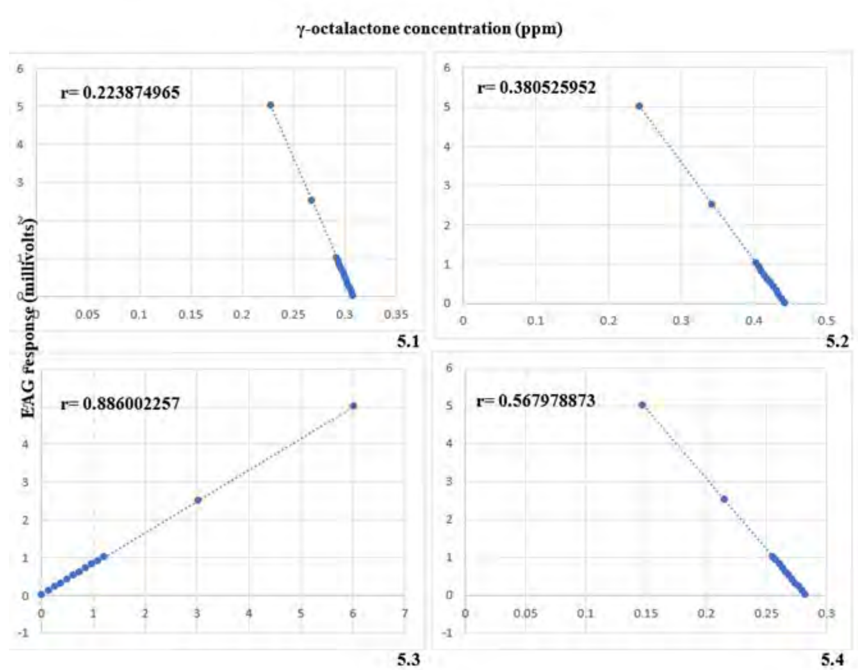


**Figure 5.** Linear regression graph of 4th generation *Bactrocera dorsalis* reared on non- γ-octalactone host to varying concentration of γ-octalactone; 5.1- Young male, 5.2- Old male, 5.3- Virgin female, 5.4- Mature female; r= correlation coefficient.

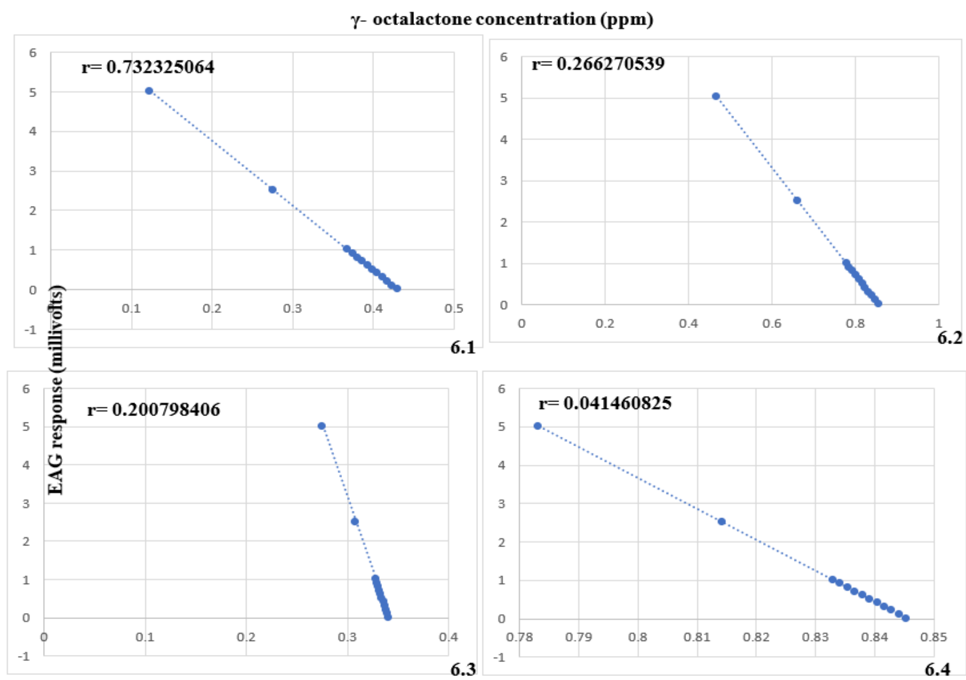


**Figure 6.** Linear regression graph of 4th generation *Bactrocera dorsalis* reared γ-octalactone host to varying concentration of γ-octalactone; 6.1- Young male, 6.2- Old male, 6.3- Virgin female, 6.4- Mature female; r= correlation coefficient.

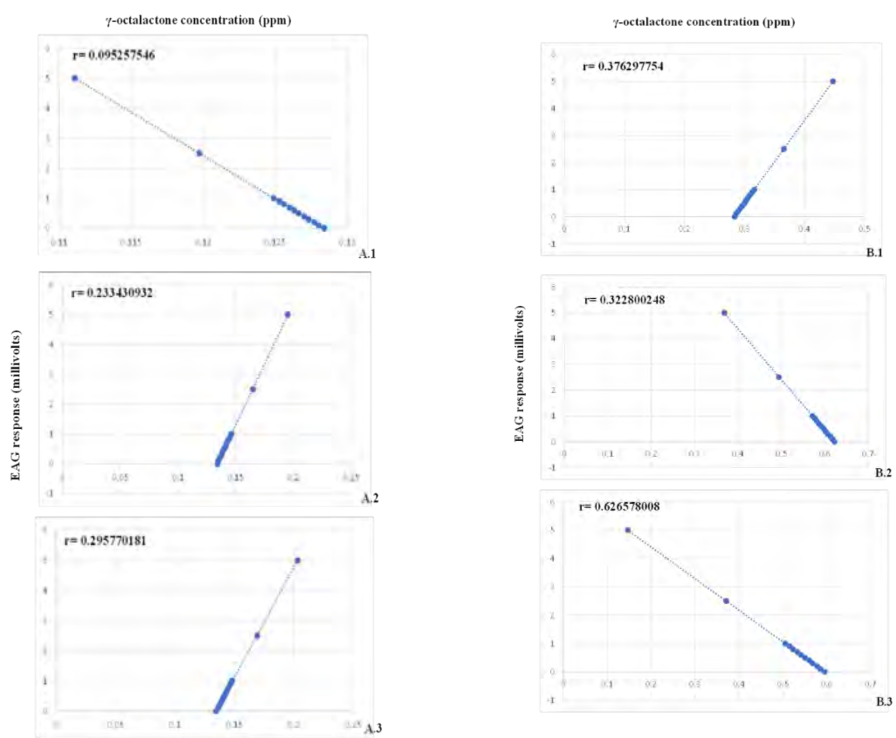


**Figure 7**. **A**. Linear regression graph of *Zeugodacus cucurbitae* to varying concentration of γ-octalactone; A.1- Male, A.2- Virgin female, A.3- Mature female. **B**. Linear regression graph of *B. occipitalis* to varying concentration of γ-octalactone; 7.1- Male, 7.2- Virgin female, 7.3- Mature female; r= correlation

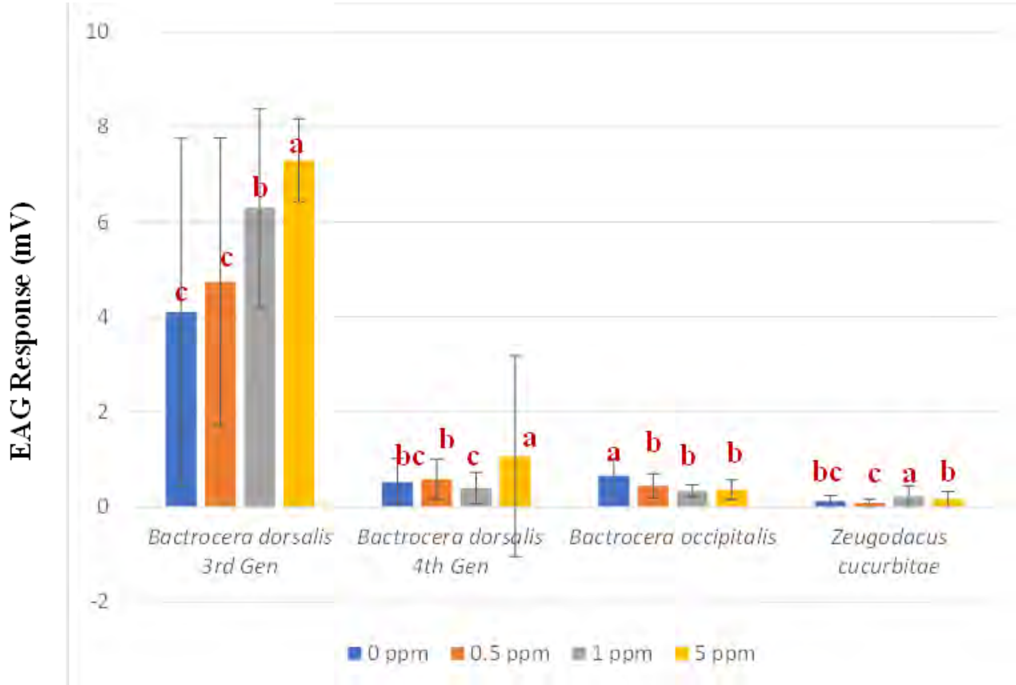


**Figure 8.** Mean (±SD) (mV) of EAG signal emitted by different fruit fly population exposed at varying γ-octalactone concentration. Values in a column followed with the same letter are not significantly different (Tukey's test; α=0.05) n=40.

The mean EAG responses for varying γ-octalactone concentrations were compared. Fruit fly population was utilized as a blocking factor. For *B. dorsalis* 3$^{rd}$ generation population, the concentration of γ-octalactone at highest level (5 ppm) elicited the highest response (**Tables 2 and 3**). The trend of EAG signal and concentration is directly related. In the case of *B. dorsalis* from 4$^{th}$ generation population, the same case as that of 3$^{rd}$ generation was observed. Highest γ-octalactone concentration instigated significantly strongest response (**Table 3**).

**Table 2.** Mean (±SD) (mV) of EAG signal of *Bactrocera dorsalis* 3$^{rd}$ generation.

| Block | Mean ± Sd (mV) | | | |
|---|---|---|---|---|
| Non- γ-octalactone Mature Female | 7.575425 | ± | 0.645632 | **a** |
| γ-octalactone Mature Female | 3.6393 | ± | 2.896529 | **b** |

Values in a column followed by the same letter are not significantly different (Tukey's test; α=0.05) n=40.

**Table 3.** Mean (±SD) of EAG signal of *Bactrocera dorsalis* 4th generation.

| Block | MEAN ± SD (mV) | | | |
|---|---|---|---|---|
| Non- γ-octalactone Young Male | 0.2826 | ± | 0.143822 | **c** |
| Non- γ-octalactone Old Male | 0.378525 | ± | 0.163961 | **c** |
| Non- γ-octalactone Virgin Female | 1.9776 | ± | 2.715345 | **a** |
| Non- γ-octalactone Mature Female | 0.238925 | ± | 0.09529 | **c** |
| γ-octalactone Young Male | 0.330475 | ± | 0.167981 | **c** |
| γ-octalactone Old Male | 0.730875 | ± | 0.583656 | **b** |
| γ-octalactone Virgin Female | 0.32015 | ± | 0.131204 | **c** |
| γ-octalactone Mature Female | 0.825275 | ± | 0.601985 | **b** |

Values in a column followed by the same letter are not significantly different (Tukey's test; α=0.05) n=40.

Among different host origins, sex and maturity, the trend is inconclusive. For fruit flies reared from non- γ-octalactone resource, virgin females produced the highest response. In the case of γ-octalactone resource origin, mature fruit flies elicited the highest response. Nevertheless, it was still consistently observed in female population where highest EAG response was observed.

Fruit fly of *Z. cucurbitae* also has the same response as that of *B. dorsalis*. As concentration was increased, perception was also monitored at highest level (**Table 4**). Maturity and sex were not significant factors with regards to EAG response.

**Table 4.** Mean (±SD) of EAG signal of *Zeugodacus cucurbitae.*

| Block | Mean ± Sd (mV) | | | |
|---|---|---|---|---|
| Male | 0.243167 | ± | 0.285712 | **a** |
| Virgin Female | 0.181556 | ± | 0.127787 | **a** |
| Mature Female | 0.184472 | ± | 0.137596 | **a** |

Values in a column followed by the same letter are not significantly different (Tukey's test; α=0.05).

Finally, in the case of *B. occipitalis,* it was shown to perceive γ-octalactone, but the concentration did not elicit a significant effect (**Table 5**). Mature female elicited significantly highest EAG response.

**Table 5.** Mean (±SD) of EAG signal of *Bactrocera occipitalis.*

| Block | Mean ± Sd (mV) |
|---|---|
| Male | 0.400417 ± 0.281843 **ab** |
| Virgin Female | 0.580806 ± 0.321253 **a** |
| Mature Female | 0.812139 ± 0.599685 **b** |

Values in a column followed by the same letter are not significantly different\ (Tukey's test; α=0.05).

Overall, it was found that *B. dorsalis* is the most responsive toward γ-octalactone among the three species. Female fruit flies were observed to respond at significantly higher intensity to γ-octalactone than males. Between sexes, it was mated female individuals which showed higher EAG response. This was like the pattern observed by Mas et. Al. (2020), where mated *B. tryoni* was also more responsive towards fruit odor complexes. This is because plant volatiles are the signals more necessarily required by gravid female fruit flies to be obtained for suitable host-searching behavior. Higher γ-octalactone concentration almost certainly yields higher EAG values. This indicates a positive linear relationship between γ-octalactone level and signal perception. Overall, γ-octalactone was a sensitive stimulus for all fruit fly species at varying degrees.

**EAG Polarity**

Together with magnitude of the EAG response, another good indicator of perception capability of a particular receptor is through the EAG response polarity. It describes how the sensory organ reacts to the continuous supply of stimulus. It can be classified by how the response line graph is presented upon introduction to a representative stimulus (**Figure 9)**).

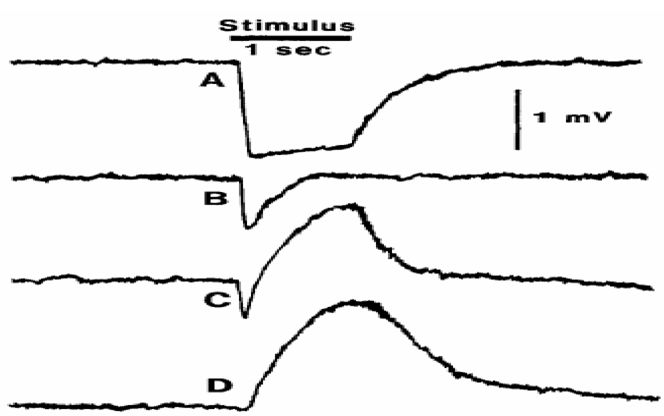


**Figure 9.** Different types of Electroantennogram response polarity; (A) typical negative-polarity, (B) early recovery, (C) biphasic negative-positive and (D) positive polarity (Light et al., 1987).

Most of the EAG recordings have purely negative voltage polarities thus the utilization of absolute values. Upon presentation of γ-octalactone, majority of *B. dorsalis* and *Z. cucurbitatae* individuals recorded negative voltage deflection, followed by early recovery at the optimization of stimulus exposure (**Figures 10.1, 10.2 and 10.4**). The trend continued to have a stabilizing phase until γ-octalactone was removed. There were certain negative deflections at latter period of some exposures, which were immediately recovered to follow the flat trend.

In the case of *B. occipitalis,* there certainly was negative voltage deflection upon exposure to γ-octalactone at the beginning, but some models eventually exhibited positive polarity (**Figure 10.3**). This is shown as an upward trend of the line, followed by a decreasing trend upon reaching climax. In the latter part, stabilization was also achieved.

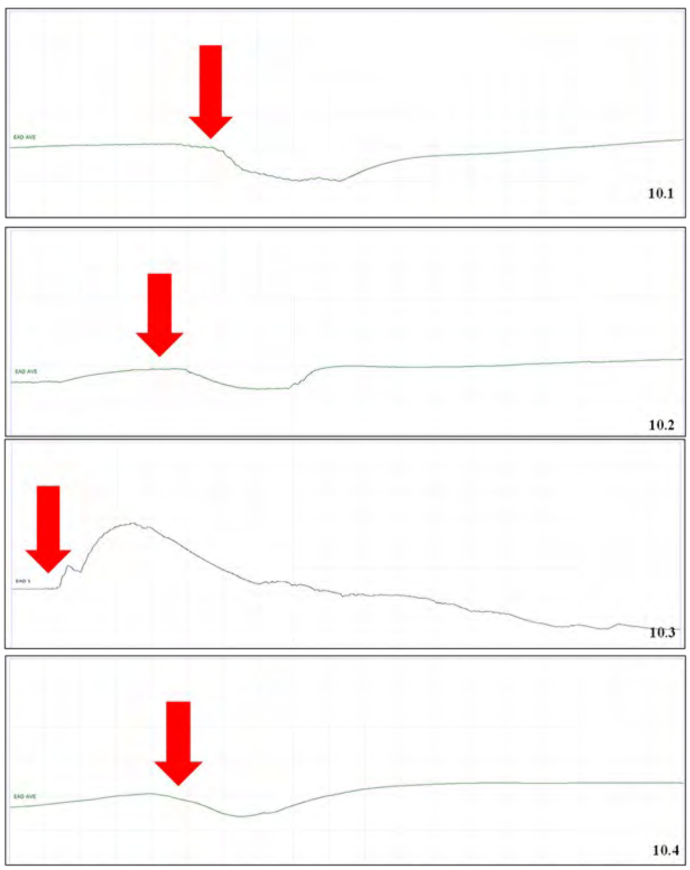


**Figure 10**. Sample EAG recording of four mated fruit flies from different populations; **10.1**- 3rd Generation *B. dorsalis* (Type A), **10.2-** 4th Generation *B. dorsalis* (Type A); **10.3-** *B. occipitalis* (Type B); **10.4**- *Z. cucurbitae* (Type A); arrows point the period of initial reaction towards stimulus.

Electroantennogram responses have abundant negative amplitude in the wave per se, which designates the number of relative acceptors responding (Dickens & Payne, 1977). Waveform has a typical stable trend, especially for both generations of *B. dorsalis* and *B. occipitalis*. As γ-octalactone is introduced to the fresh antenna, rapid depolarization ensues followed by slow recovery and finally stabilization. This is typical of negative polarity status, which signals excitation towards the stimulus in focus (Light et al., 1987). This is most probable whenever the stimulus utilized belongs to that of a chemical with a reactive functional group, which includes aldehyde, alcohol moieties, acetate and lactone. In the case of γ-octalactone, it belongs to the lactone group attributed to fruit ripening volatiles present in the host utilized by fruit flies. This plant volatile is considered as a crucial cue for gravid fruit fly to search and assess a potential resource for oviposition (Damodaram et al., 2014). Thus, it is important for γ-octalactone to create a strong and direct excitation on insect's sensory organ.

Electroantennogram tests showed that all fruit fly species can significantly detect γ-octalactone. *Bactrocera dorsalis* and *B. occipitalis* had a more stable EAG response pattern than *Z. cucurbitae*, translating to the possible necessity of the stimulus offered towards a particular response. This is observed through production of higher EAG values by the antennae of these two species. However, this is insufficient to prove that γ-octalactone is a major player in the oviposition response of *B. dorsalis*. This can be further proven through oviposition preference tests, which includes the stimulus γ-octalactone as naturally emitted and artificially embedded.

## DISCUSSION

As shown by the EAG experiments, all the tested populations of the 3 fruit fly species showed a linear response relationship with γ-octalactone. This means that they are all capable of identifying the presence of this stimulus in the environment. However, female individuals at mated status were significantly more responsive. This implies that γ-octalactone triggers sensory neurons of gravid fruit fly at significantly highest intensity, providing a clue to its importance in oviposition behavior. In the case of host origin of insect, there was no significant effect shown with EAG level. Regardless of resource utilized by the larvae of fruit fly, adults would react similarly to the γ-octalactone signal.

Among species, *B. dorsalis* and *B. occipitalis* had relatively more stable EAG s toward γ-octalactone. They are more discriminatory for its presence specifically for female individuals. Given the strong linear relationship, it is reasonable to assume that γ-octalactone can serve as an indicator of most preferential host for gravid females regardless of host origin. The occurrence of γ-octalactone on fruits may indicate close relationship with fruit fly. This was best exemplified through behavioral study.

All the results point to a strong response *of B. dorsalis toward* γ-octalactone. This was evidenced by distinct perception and consistent preference for resources containing this volatile. Concentration invariance was also observed, as γ-octalactone level is insignificant in terms of number of eggs deposited (Zhang et al., 2013). In short, γ-octalactone is an important component among the chemical blends utilized by *B. dorsalis* that directly influences oviposition stimulation (Webster et al., 2010). It possesses the highest priority status as host acceptance cue among all chemical signals, as stated by sequential preference (Silva & Clarke, 2020). The presence of this compound is directly connected to fruit fly reproduction build-up over intimate evolutionary relationship.

Insect olfaction is extremely sensitive and specific for volatiles (Bruce & Pickett, 2011). In the case of fruit flies, olfactory perception of γ-octalactone revealed a strong connection towards behavioral response. Minor compounds are also observed to elicit stronger behavioral responses than major attractants (Siderhurst & Jang, 2006). Fruit fly species *Ceratitis capitata*, *Rhagolotis pomonella* and *B. tryoni* have exhibited significant electrophysiological activity to at least one of the following minor compounds released by *Terminalia catappa*: ethanol, ethyl acetate, (E)-2-hexenal, isopentyl acetate, isopentenyl acetate, ethyl hexanoate, hexyl acetate, linalyl acetate, 2- phenylethyl acetate, geranyl acetate, nonyl acetate, and the isomers of farnesene. Finally, sex and physiological status significantly affect the magnitude of perception capability of insects (Anton et al., 2007). Mated female fruit fly individuals are more responsive towards fruit compounds which can be attributed to their innate requirement of seeking potential oviposition sites (Mas et al., 2020). This was exemplified by females of *Bactrocera tryoni* as they responded significantly higher to chemical blends of orange, cherry, guava, feijoa and banana than male. Mated female individuals especially showed higher EAG response intensity.

This study further showed that *B. dorsalis* has the most consistent response towards γ-octalactone. Electroantennogram experiments revealed that this species has a direct linear relationship response with γ-octalactone concentration. Mated females were significantly more reactive. With regards to host origin, it did not influence fruit fly response among all species with respect to EAG magnitude and pattern. Finally, the majority of EAG responses have negative polarity, signaling a common detective response of insect sensory organs. In addition, γ-octalactone triggers sensory neurons of gravid fruit flies at significantly high intensity, giving a clue to its importance towards oviposition behavior.

As one of the priority olfactory cues utilized by fruit flies, γ-octalactone serves numerous biological functions. For one, it signals the ripeness and the sweet flavor of a potential host (Pandit et al., 2009). It also gives the impression of suitable skin color and texture. In terms of protection, its presence means the absence of resin duct that might cause mortality for potential offspring (Rattanapun et al., 2009). Lastly, γ-octalactone has known antimicrobial properties against fungal pathogens, which ensures safety of fruit fly egg once deposited on suitable host plant (Kishimoto et al., 2005; Mohamed et al., 2017).

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