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Ovipositional Behaviour Preference of Oriental Fruit Fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) on Different Host Fruits

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ABSTRACT

The oriental fruit fly *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a polyphagous insect pest that gives severe damage to orchard fruits when the female adults lay their eggs into the fruits. However, the damage can be reduced by manipulating the behavior of this female insect. Thus, this study aimed to determine the ovipositional preference behavior of *B. dorsalis* on different host fruits. The experiment set up was laboratory-based 'no-choice' and 'choice' of oviposition preference behavior by the insect. The parameter recorded were the number of fruit visit, duration of fruit visit, number of attempted to oviposit, number of oviposition and duration of oviposition completed. Guava (*Psidium guajava* L), mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.) were used as host fruits. In 'no-choice' experiments, the host fruits significantly influenced the number of fruit visit and duration of the visit by *B. dorsalis* female. The most visited fruit by *B. dorsalis* female was mango whilst papaya shows the longest duration of the host fruit visit by *B. dorsalis*. Although the characteristics of host fruits differs, the number and duration of oviposition by *B. dorsalis* shows no significant difference among the host fruits and no activity of oviposition was recorded on guava fruit. When given a choice, the duration of fruit visit and number of attempt to oviposit by *B. dorsalis* significantly differed among host fruits. However, the host fruits do not influenced the number of fruit visit, number of oviposition and duration of oviposition completed. However, guava fruit shows the egg oviposition activity by *B. dorsalis* females whilst no oviposition behaviour was observed on mango and papaya. Results obtained in this experiment will benefit the study of insect pest behaviour which then can be use in biological control program in order to reduce the agriculture damage.

Keywords: *Bactrocera dorsalis,* host fruits, ovipositional preference

ABSTRAK

Lalat buah oriental *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) adalah sejenis serangga perosak pelbagai tanaman buah-buahan yang boleh memberi kerosakan yang teruk terhadap sesebuah kebun apabila lalat buah betina dewasa bertelur di dalam buah perumah. Namun, kerosakan itu boleh dikurangkan dengan cara memanipulasi kelakuan serangga betina ini. Maka, kajian ini dijalankan bertujuan untuk menentukan keutamaan kelakuan *B. dorsalis* mencucuk telur terhadap buah perumah yang berbeza. Eksperimen yang dijalankan adalah di dalam makmal secara 'tanpa pilihan' dan 'pilihan' melalui kelakuan mencucuk telur oleh serangga tersebut. Parameter yang direkodkan adalah bilangan lawatan buah, tempoh lawatan buah, bilangan percubaan mencucuk telur, bilangan mencucuk telur dan tempoh mencucuk telur selesai. Jambu batu (*Psidium guajava* L), manga (*Mangifera indica* L.) dan betik (*Carica papaya* L.) telah digunakan sebagai buah-buahan perumah. Dalam eksperimen tanpa pilihan, buah-buahan perumah mempengaruhi secara signifikan terhadap bilangan lawatan buah dan tempoh lawatan buah oleh *B. dorsalis* betina. Buah yang paling dilawati oleh *B. dorsalis* betina adalah mangga manakala betik menunjukkan tempoh lawatan buah terpanjang oleh *B. dorsalis*. Walaupun ciri-ciri buah-buahan perumah adalah berbeza, bilangan dan tempoh mencucuk telur oleh *B. dorsalis* menunjukkan tiada perbezaan yang signifikan dikalangan perumah dan tiada aktiviti mencucuk telur direkodkan ke atas jambu batu. Apabila diberi pilihan , tempoh lawatan buah dan bilangan percubaan mencucuk telur oleh *B. dorsalis* berbeza secara signifikan dikalangan buah-buahan perumah. Namun, buah-buahan perumah tidak mempengaruhi bilangan lawatan buah, bilangan mencucuk telur dan tempoh mencucuk telur selesai. Walau bagaimanapun, jambu batu menunjukkan aktiviti mencucuk telur oleh *B. dorsalis* betina manakala tiada kelakuan mencucuk telur diperhatikan ke atas mangga dan betik. Hasil yang didapati dalam kajian ini akan memberi manfaat kepada kajian kelakuan serangga perosak di mana ia kemudiannya boleh digunakan dalam program kawalan biologi bertujuan untuk mengurangkan kerosakan dalam pertanian.

Kata Kunci: *Bactrocera dorsalis,* buah perumah, keutamaan mencucuk telur

INTRODUCTION

Tephritid fruit flies (Diptera: Tephritidae) is the most influential insect pest on global agricultural products. This insect pest is the most tenacious pest of fruits and vegetables in the world causing direct and indirect economic loss due to their injury (Sarwar, 2006). This insect also considered as a serious threat to the horticultural crops because of the wide host range of its species (Clarke et al., 2005) which can reduce the yield; and drop the value and marketability of the crops (Kumar et al., 2011). An Oriental fruit fly, *Bactrocera dorsalis* (Hendel) in family Tephritidae is one of the related species of *B. dorsalis* complex. This pest is a native insect species of Malaysia and was considered as the most virulent fruit fly species. This polyphagous insect attacks about 209 plant species from 51 different families (Chua, 1991; Drew and Romig, 1997; White and Elson-Haris, 1992). In Peninsular Malaysia, this insect pest was reported attack many species of commercial fruit crops such as mango (*Mangifera indica* L.), guava (*Psidium guajava* L), and papaya (*Carica papaya* L.) (Allwood et al., 1999).

Bactrocera dorsalis is capable in damaging fruit crops at the severe stage. The damage to fruits vary from a few per cent loss up to 90% or even worst at 100% depending on the fruit fly population, season, variety and area (Kumar et al., 2011). For instance, José et al. (2013) observed that the damage caused by *B. dorsalis* in Nothern Mozambique was the highest in guava (92.5% of fruits) followed by tropical almond (67.3%) and mango (56.5%). Ali et al. (2014) found that the highest infestation level caused by tephritid fruit flies was on guava (67%) and followed by mango (31%) and grapefruit (18%) in Abugubeiha, Sudan. Latest, Salmah et al. (2017a) reported that *B. dorsalis* was the most abundance species on mango in Peninsular Malaysia and the damage up to 91%.

Adult female of fruit flies finds the host to oviposit using olfactory, visual and contact cues such as the color, size, shape and smell of host fruit, and all these factors influence a female fruit fly's response to choose the best host for their progeny (Drew and Romig, 2013). Decisions about into which fruit to oviposit normally are based on the suitability of the fruit for their offspring's performance (Joachim-Bravo et al., 2001). For example, Salmah et al. (2017b) investigated oviposition preference of *B. dorsalis* on Chokanan mango at different ripening stage on choice and no-choice experiments in laboratory studies. The findings demonstrated an oviposition preference hierarchy of *B. dorsalis* among the three-different ripening stage of mango host tested.

Typically, *B. dorsalis* attack mature and ripe fruits by laying their eggs under the skin of the fruit (Vargas et al., 1995). The eggs hatch into larvae which feed on the fruit tissue resulting the rotting of the fruit and premature fruit drop (Ambele et al., 2012). The oviposition preference behaviour is crucial as females of *B. dorsalis* need to determine the most suitable host to lay their eggs in order to ensure the development of their offspring at the maximum level and the larvae get enough essential nutrients (Salmah et al., 2017b). The determination of suitable host normally based on the physical characters of the fruit such as fruit hardness, fruit firmness, fruit chewiness and gumminess (Am et al., 2017) whilst fruit odour, fruit colour and degree of fruit ripeness also contribute to the ovipositional preference of fruit fly (Rattanapun, 2009). Therefore, the objective of this study was to determine the ovipositional behavior of *B. dorsalis* when there is no choice or given a choice of different host fruits to oviposit.

MATERIAL AND METHODS

Adults of *Bactrocera dorsalis*

The adults of *B. dorsalis* used in this experiment were obtained from the fruit fly colonies cultured and maintained in the laboratory (28 ± 2ºC, 70-80% RH, 12:12 h L:D) for five generation. Fifty pairs of newly emerged *B. dorsalis* adults were sexed and released into a rearing cage (30 x 30 x 30 cm). The adults were fed with water soaked on sponge and sugar cubes with a mixture of yeast extract and sugar at ratio 3:1. Only female adults of 21-days-old were used in the experiment as this is the optimum age for them to oviposit eggs (Rattanapun, 2009).

Host Fruits

Guava (*Psidium guajava*), mango (*Mangifera indica*) and papaya (*Carica papaya*) were used a host fruit in this experiment. Those fruit were reported to be as a major fruit host for *B. dorsalis* in Peninsular Malaysia (Allwood et al., 1999). All fruit samples were bought from the local market in Besut, Terengganu and only the ripe fruits were used in the experiments as a *B. dorsalis* preferred to oviposit their eggs into the ripe fruits (Vargas et al., 1995). For each fruit types, five replicates were conducted for each experiment (i.e. choice and no-choice experiment).

Determination of Host Fruits Characteristics

Physical Measurements

All fruit samples (10 fruits from each type) were weighed individually by using a kitchen scale (Cook Master, Malaysia). The diameter of the same fruit samples was measured by using a Mitutoyo Vernier Calliper (Series 533, Japan). The means for all the measurements of the fruits were calculated and recorded.

Total Soluble Solids (TSS) and Firmness Determination

The same fruits then were tested for total soluble solids (TSS = °Brix) and firmness. The TSS of the flesh of the fruit was determined from the juice of the squeezed fresh fruit samples using a digital pocket refractometer Pal – 1 (Atago, Japan). Two drops of juice sample were applied on the measuring surface of the prism and kept in the dark for 15 seconds to avoid light disturbance during the reading of TSS. The results recorded in °Brix are displayed on the LCD panel. This test was repeated for three times for every sample of fruits.

The firmness of the fruits was measured in the equatorial position of the fruits by using penetrometer (Instron 5543, USA) with 5 mm diameter stainless steel probe. Ten fruits for each type were sliced 15 mm thick horizontally at the middle part of the fruits and the data was taken at three points on each sample fruit. Then, the average firmness of each type of fruits was calculated. The firmness of the fruits is based on the maximum value recorded by the probe while passing through the fruit to a depth of 10 mm, in Newton (N) (Pauziah and Wan Mohd Reza Ikhwan, 2014).

Determination of *Bactocera dorsalis* Ovipositional Preference

The *B. dorsalis* ovipositional preference behaviour on three different host fruits were determined through no-choice and choice experiments. The methods were adopted and modified from Rattanapun (2009) and Salmah et al. (2017b).

No-choice Experiment

For the no-choice experiment, fruits (guava, mango and papaya) were placed individually in a transparent round plastic container (24 x 10 cm). A gravid female of *B. dorsalis* from established rearing culture was released into each aquarium box through the transparent lid on the top of the aquarium cover.

Female fruit fly behaviour was observed for two hours (from 0900 to 1100 h). This time period was chosen as the activity of the flies (i.e: visiting and ovipositing eggs) is maximum during this period (Hee and Toh, 2016). The parameter for ovipositional behaviour of *B. dorsalis* recorded were; 1) the number of fruit visit, 2) duration of fruit visit, 3) number of attempted to oviposit, 4) number of ovipositing and 5) duration of oviposition completed. The frequency of the fruit fly to visit, attempt to oviposit and oviposit fruit were recorded using an electronic hand counter (LINE™, Japan) while the duration of fruit visit and oviposition process completed (starting from inserting the ovipositor until the ovipositor out of the host fruit) were recorded using a stopwatch (Diamond, China).

Choice Experiment

For the choice experiment, fruits were placed together in a rearing cage (30 x 30 x 30 cm) with the distance of 10 cm between the fruits. A gravid female of *B. dorsalis* from established rearing culture was released into the aquarium box for two hours. The ovipositional behaviour preference of *B. dorsalis* female was observed from 0900 to 1100 hours. The parameters recorded were the same as for the no-choice experiment. Experiments (no-choice and choice) were repeated five times and conducted under laboratory conditions (28 ± 2°C, 60-70% RH, 12:12 h L:D).

Data Analysis

The experimental design was based on Completely Randomized Design (CRD) with five replications per treatment. The data of the fruit characteristic (physical measurements, TSS, and firmness), and flies ovipositional behaviour (number of fruit visit, duration of visit, number of attempted to oviposit, number of oviposit, duration of oviposition completed) obtained from choice and no-choice experiment was subjected to One-way Analysis of Variance (ANOVA) for comparison between three different types of fruits. Means were separated with Tukey's Range (HSD) test at 0.05 level of significance. All data were performed using MINITAB® 17 software (MINITAB, 2017).

RESULTS AND DISCUSSION

Host Fruits Characteristics

Table 1 showed the fruit characteristics of guava, mango and papaya. The weight of the host fruits shows that there was a significant difference (P<0.05) among the fruits. Papaya shows the highest weight (0.99±0.11) followed by mango (0.43±0.02) and guava (0.15±0.01). Whilst for the diameter of the fruits, guava was significantly smaller compared to mango and papaya. The diameter of mango and papaya is nearly similar (P>0.05).

Table 1 The properties of host fruit characteristics

Host Fruit	Fruit Characteristics			
	Weight (kg)	Diameter(cm)	TSS (°Brix)	Firmness (N)
Guava	0.15±0.01a	4.47±0.09a	9.53±0.32a	69.30±1.43a
Mango	0.43±0.02b	8.20±0.20b	12.30±0.35b	6.54±0.33b
Papaya	0.99±0.11c	7.97±0.35b	11.13±0.15ab	11.44±0.65c

Means with same letters within columns were not significantly different (P>0.05) by Tukey's (HSD) test

Results showed that the TSS content was the lowest in guava fruits (9.53±0.32ºBrix) compared to mango (12.30±0.35 ºBrix) and papaya (11.13±0.15 ºBrix). There is no significant different observed in term of TSS content for mango and papaya. This indicated that guava is less sweet than mango and papaya. However, firmness shows a significant difference (P<0.05) among the three fruits which mango was the softest (6.54 ± 0.33 N), followed by papaya (11.44±0.65 N). Guava has the highest firmness (69.30±1.43 N) compared to mango and papaya.

Oviposition Behavior Preference of *Bactrocera dorsalis* on Different Host Fruits

No-choice Experiment

The results obtained shows there was a significant difference (P<0.05) on the number of fruit visit, duration of fruit visit and the frequency of attempted to oviposit (Table 2). No significant difference (P>0.05) was observed on the number of oviposit and duration of oviposition completed. The female of *B. dorsalis* visited mango more frequent (30.70±17.50 times) compared to guava (13.00±4.04 times) and papaya (2.33±0.88 times) (Table 2). However, *B. dorsalis* spend longer on papaya (55.20±2.60 min) compared to guava (15.90±14.80 min) and mango (2.81±1.67 min) (Table 2). It was noted that mango fruit has the highest value of sugar content and the least firm (Table 1). Therefore, the sweetness and softer pulp of ripe mango fruits may attract the flies to visit more for ovoposition. Rattanapun (2009) stated that the volatiles of host fruit are believed to influence the preference of *B. dorsalis* females. In general, insects use an array of volatile compounds as cues to locate food, mates and oviposition sites (Kamala Jayanthi et al., 2014). Volatile chemical cues from the host plant play a major role in the orientation of gravid females to their hosts from a distance (Kamala Jayanthi et al., 2015).

The frequency of attempted to oviposit was not significantly different (P>0.05) between papaya and guava but the attempted to oviposit was significantly higher on mango (Table 2). The attempt to oviposit by *B. dorsalis* is a typical early detection action of females in order to locate the suitable oviposition spot before inserting their eggs and this behavior known as probing (Hernandez et al., 1996; Rattanapun, 2009). In natural environments, the females of *B. dorsalis* were always attracted to the bruises, wounds or cracks in the fruit; that may cause either by the previous activities of oviposition by other female flies (Liu and Huang, 1990) or as the result of feeding by other insects, farming practices such as harvesting and pruning, plant diseases or fruit over-ripeness (Rattanapun et al., 2009).

Nonetheless, although there was no significant difference in a number of oviposition and duration of oviposition completed among the fruits, both parameters shows slightly higher on mango which was 1.33±0.67

times and 3.34±2.42 minute (Table 2), respectively. Again, this might be due to a softer pulp in mango fruits that can help and ease the *B. dorsalis* females to insert ovipositor and ensure larvae to move easily while feeding (Pena et al., 1998). Am et al. (2017) recorded that the fruits have less hardness and firmness has a higher infestation and higher preference for oviposition. Guava which had the highest value of firmness (Table 1) shows no activity of eggs oviposition (Table 2) indicated that it was not favourable fruit for *B. dorsalis* females to lay eggs. According to Seo et al. (1982), Messina and Jones (1990) and Balagawi et al. (2005), firmness was considered as a limiting factor that can influence adult of *Bactrocera* oviposition preference.

Table 2 Oviposition behaviour parameter of *B. dorsalis* under no-choice experiment

Fruit Host	Oviposition Behaviour Parameter				
	No. of fruit visit	Duration of visit (min)	No. of attempted to oviposit	No. of oviposit	Duration of oviposition completed (min)
Guava	13.00±4.04a	15.90±14.80a	1.07±0.67a	0.00±0.00a	0.00±0.00a
Mango	30.70±17.50b	2.81±1.67b	8.67±1.86b	1.33±0.58a	3.34±2.42a
Papaya	2.33±0.88c	55.20±2.60c	2.00±1.15a	1.00±0.67a	1.24±0.63a

Means with same letters within columns were not significantly different (P>0.05) by Tukey's (HSD) test

Choice experiment

Overall, there was a significant difference (P<0.05) of duration for fruit visit and the number of attempted to oviposit. However, there are no significant difference (P>0.05) was recorded on number of fruit visit, number of oviposit and duration of oviposition completed (Table 3). However, guava is the only host fruit that shows the egg oviposition activity by *B. dorsalis* females whilst no oviposition behavior was observed on mango and papaya (Table 3). This findings was in contrast with the results in a no-choice experiment which guava no oviposition activity recorded (Table 2). In all oviposition behavioral parameters observed, guava shows significantly the highest value even though guava was the hardest fruit (Table 1). However, no significant difference (P>0.05) was observed in a number of fruit visit among the host fruits (Table 3). This is might be due to the other factors such as fruit volatiles (Jang and Light, 1991), wound or crack on the fruits (Papaj et al., 1989) and oviposition holes of conspecifics (Papaj and Alonso-Pimentel, 1997) that may influence the preferences of female flies to oviposit.

Table 3 Oviposition behaviour parameters of *B. dorsalis* under choice experiment

Host Fruit	Oviposition Behavior Parameter				
	No. of fruit visit	Duration of visit (min)	No. of attempted to oviposit	No. of oviposit	Duration of oviposition completed (min)
Guava	2.33±1.33a	45.50±34.60a	2.67±1.76a	0.67± 0.67a	1.16±1.16a
Mango	1.00±1.00a	0.17±0.17b	0.00±0.00b	0.00±0.00a	0.00±0.00a
Papaya	0.67±0.67a	1.41±1.41b	0.00±0.00b	0.00±0.00a	0.00±0.00a

Means with same letters within columns were not significantly different (P>0.05) by Tukey's (HSD) test

In general, the females of *B. dorsalis* usually screen the host fruit by touching the fruit surface with their antennae and mouthparts and probing the fruit skin with their ovipositor before determining the fruit host that is appropriate for eggs laying and larval survival (Hernandez et al., 1996; Rattanapun, 2009). For this purpose, visual such as color, shape, and size, and olfactory cues are generally used by fruit flies to recognize and select a suitable host for their offspring (Dalby-Ball and Meats, 2000; Piñero et al., 2006). Prokopy (1968) has suggested that the most attractive shape was a sphere because of the fact that it can be seen flies from all directions and in this case guava fruits had met the criteria. Thus, Ambele et al. (2012) showed that peel firmness and thickness of the fruit host was not the only factor influenced the *B. dorsalis* ovipositional behavior preference which indicating that there might be other factors that contribute to host acceptability and susceptibility.

The female flies used in no-choice and choice experiments emerged from the laboratory-reared colonies which have no prior experience in oviposition. Thus, it was believed that lacking of prior experience of newly emerged females affect the behaviour of *B. dorsalis* females to choose a better host for their offspring (Yu, 2013). According to Rattanapun (2009), females with prior experience with host fruit is an important factor influencing host fruit acceptance. As reported by Aluja and Mangan (2008), the ovipositional preference of *B. dorsalis* is basically influenced by the fruit quality such as through the size, color, penetrability and degree of ripeness.

CONCLUSIONS

This study shows that fruit characteristics can influence the oviposition preference behaviour of *B. dorsalis*. In the no-choice experiment, guava shows the highest value of firmness, therefore it has the lowest number of attempted to oviposit and no oviposition activity was recorded (i.e. number of oviposit). Whilst, mango has the highest value in TSS and least firmness, thus, it has the highest number of fruit fly behaviour to oviposit and a number of attempted to oviposit. However, in choice experiment, guava shows the highest value in oviposition behavioural preference of *B. dorsalis* females. This indicated that the ovipositional behaviour preference of *B. dorsalis* female is influenced by many factors and not just from the size, color, penetrability and degree of ripeness of the host fruits. Thus, as a future work, it is suggested that the intensive study should include the study of *B. dorsalis* ovipositional behaviour in field conditions to obtain a comprehensive data on its host fruit preferences. It is hoped that the results from the study could enhance the knowledge of the behaviour of *B. dorsalis* in order to develop a better monitoring and management control practices.

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