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DETERMINATION OF LARVAL INSTAR OF *BACTROCERA PAPAYAE* (DIPTERA : TEPHRITIDAE) ON GUAVA, *PSIDIUM GUAJAVA*, LINN. BASED ON MORPHOMETRIC CHARACTERS

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ABSTRACT

Morphometric characters such as length and width of the *Bactrocera papayae* larvae could be used to determine the larval instars at their immature stage. Observation in this study indicated that *B. papayae* underwent three larval instars. The duration for the first instar, second and third instar was 4,4 and 7 days respectively. There was a significant difference in the length and width within larval instars of *B. papayae*. After hatching, the length of the larvae was 1.04 mm and the longest could reach up to 8.32 mm while the width starts from 0.18 mm and could reach up to 2.08 mm before completing all instars during the immature stage. Means length of the larvae were 1.27 ± 0.03, 4.33 ± 0.05 and 7.84 ± 0.07 mm whilst means width were 0.23 ± 0.01, 1.04 ± 0.01 and 1.85 ± 0.03 mm

for the first instar, second instar and third instar respectively. Moulting process of the larvae occurred twice between day-4 and day-5 and also between day- 8 and day-9.

Keywords: *Bactrocera papayae*, instars, moulting, length, width and larvae.

ABSTRAK

Ciri morfometrik seperti panjang dan lebar larva *Bactrocera papayae* larva boleh digunakan untuk menentukan instar larva pada peringkat matang mereka. Pemerhatian dalam kajian ini menunjukkan bahawa *B. papayae* menjalani tiga instar larva. Tempoh untuk instar pertama, kedua dan instar ketiga masing masing 4, 4 dan 7 hari. Terdapat perbezaan yang signifikan dalam panjang dan lebar dalam instar larva *B. papayae*. Selepas penetasan, panjang larva adalah 1.04 mm dan yang paling lama boleh mencecah sehingga 8.32 mm manakala lebar bermula dari 0.18 mm dan boleh mencapai sehingga 2.08 mm sebelum melengkapkan semua instar pada peringkat matang. Purata panjang larva adalah 1.27 ± 0.03, 4.33 ± 0.05 dan 7.84 ± 0.07 mm manakala lebar adalah 0.23 ± 0.01, 1.04 ± 0.01 dan 1.85 ± 0.03 mm bagi instar pertama, kedua dan instar instar ketiga. Proses penyalinan kulit larva itu berlaku dua kali antara hari-4 dan hari-5 dan juga antara hari-8 dan hari-9.

Kata kunci: *Bactrocera papayae*, instar, penyalinan kulit, panjang, lebar and larva.

INTRODUCTION

Insect pests cause damage to their hosts at two stages in their life. Firstly in the adult stage when they are foraging for food or searching suitable surface for oviposition process, and secondly during the immature stage when the larvae are feeding on pulp or fleshy parts of the fruits and vegetables. The importance of determining the number of instars in insect biology is crucial in order to understand the pest and conduct pest control program (Nascimento et al., 1993; Esperk et al., 2007 and Calvo & Molina, 2008). The basic knowledge helps in the development of the phenology or refinement of the existing models and explains the reasons for failures of the treatment (Godin et al., 2002 and Calvo & Molina, 2008). Esperk et al. (2007) reported that there were a total of 27 insect species that have been studied. Sixteen species were from the order Lepidoptera and 11 remaining species include the orders of Dictyoptera, Orthoptera, Coleoptera and Diptera. Calvo and Molina (2008) reported that there were brief descriptions of larval instar stages but no morphometric studies have been conducted on the Lepidopteran insects. There have been a number of studies on Dipteran larval instars of the family Sciaridae, Simuliidae, Tabanidae and Tephritidae (Berg, 2000; Esperk et al., 2007 and Berni et al., 2009). In the immature stage, there is a time when the larvae aggressively feed. The movement when tunneling and feeding indirectly affects the morphometric measurements of the larvaes' bodies. Since there have been no detailed studies on the morphometric measurements of larvae *B. papayae*, this study was conducted with the objective to determine the number of larval instars based on length and width of the larvae's body.

METHODOLOGY

The techniques of mass rearing of *B. papayae* in this study were adopted and modified from Chua (1991), Vargas et al. (2000) , Kaspi et al. (2001), Carey et al. (2005), Hee and Tan (2006), Chuang and Hou, (2008), Wang et al. (2009).

Preliminary Rearing and Insect Species Identification

Fifty rotten pink guavas, *Psidium guajava*, Linn var beaumont fruits were collected randomly from the farm of Golden Hope and Beverages Sdn. Bhd., Sitiawan, Perak, Malaysia (N 4° 20' E 100° 50'). The fruits were kept in five 30.0 x 30.0 x 30.0 cm rearing cages lined with 4.0 cm thick of sterilized vermiculite until the emergence of the adults, where 10 fruits in each cage under laboratory conditions at 23.92 ± 0.16°C (Min: 21°C ; Max: 29°C) and 61.14 ± 0.33% (Min: 51% ; Max: 70%) relative humidity at the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (N 3° 2' E 101° 43').After emerged, ten females from each cage were caught by using 2.0 cm diameters with 5.0 cm length vials. Each individual caught was paralyzed in 16℃ cooler for three minutes and immediately morphology study conducted under light microscope.

Mass Rearing and Establishing *B. papayae* Colony

Fifty rotten pink guavas, *P. guajava*, Linn var beaumont fruits were collected randomly and each rotten fruit was kept individually in 24.5 x 13.5 x 13.0 cm plastic containers lined with 4.0 cm thick of sterilized vermiculite until the emergence of the adults as at the place and environment condition mentioned in 2.1 above

Emerged adults were collected and placed into 30.0 x 30.0 x 30.0 cm rearing cage lined with4.0 cm thick of sterilized vermiculite. Two ends of tissue were soaked in guava juice in a container perforated in two places to allow the tissue to pass through. The flies feed by sucking the juice from the middle part of the tissue. A mixture solution of honey and yeast extract in 3 : 1 (Rattanapun et al., 2009) ratio was prepared. A piece of tissue was soaked in the solution and placed on the floor of the rearing cage. The solution acted as supplement for *B. papayae* reared. The diets were changed every two days.

Six non-infested guava fruits (approximately 100 – 200 g) placed individually on conical flasks in the cage were introduced to the cage as semi natural egging-devices for eggs laying. The egging-devices were kept for five days. These infested fruits were then removed into24.5 x 13.5 x 13.0 cm plastic containers lined with 4.0 cm thick of sterilized vermiculite to avoid contamination of microbes in the rearing cage. After one week, pupae found in the vermiculite were collected daily by sieving the vermiculite (Somta et al., 2010). Collected pupae were placed back into the cage prepared earlier and kept until the emergence of the adults.

Every five months, 50 rotten guava fruits were obtained and kept in different cages until the emergence of the adults. These adults were then introduced into the established cage prepared to maintain the wilderness characteristics in the cage colony used in this study. Dead *B. papayae* adults were removed from the cage every days and feeding devices (food container andtissues) were cleaned every two days to avoid contamination from fungus and bacteria.

Egg-collecting-device and egg collection

The artificial-egging-devices were prepared by adopting techniques established by Chua (1991) and Kaspi et al. (2001). Small holes with 1.0 mm in size were made on 500 ml drinking water bottle. The distance between holes were 0.5 cm vertically and 1.0 cm horizontally. The bottle was first wrapped with tissue then wrapped again with parafilm. The bottle was then fully filled up with pink guava juice (Plate 1). The tissue will absorb the juice and produce smell that will attract the adult female to oviposit in the holes. After 24 hours, the parafilm was removed and eggs were collected using fine brush.

The technique employed to collect *B. papayae* eggs in this study was adopted fromVargas et al. (2000) which some modifications to suit the laboratory condition and the equipments available. Artificial-egging-devicewas kept in the mass rearing cage for two hours. All eggs were laid by the females on the parafilm and collected using a fine brush (Vargas et al., 2000) and soaked with distilled water to determine the viability of the eggs. The immerted eggs were viable while the floated ones were unviable. All the viable eggs were placed on black fine mesh (soaked in guava juice earlier) and kept in 90.0 mm diameter petri dishes. The petri dishes were sealed with parafilm to avoid larvae moving out or outside pests getting in. After 24 hours, the petri dishes were observed and first instar larvae were collected and reared in the laboratory condition until the last larva moult (Godin et al., 2002).

Morphometric measurement study

Ten larvae were taken out daily and they were dipped in hot water (± 95°C) for one minute. The killed larvae were put on tissue paper for drying for two minutes before their bodies' morphometric measurement were taken under the light

microscope which was calibrated before. The morphometric characters measured in this study were:

1. Length (mm): Each larva was measured from the edge of tapered head until the edge of the posterior end (Plate 2).

2. Width (mm): Each larva was measured at the point of two largest diameter along the length of the larva (Plate 3).

The means for length and width were recorded. The remaining larvae were then put on 5.0 g diced pulp of the guava individually, kept in 50.0 mm diameter petri dishes. The media (5.0 g guava pulp) that provided to the larvae were changed daily.

RESULTS AND DISCUSSION

Figure 1.0 showed daily changes of means of length and width in the immature stage of *B. papayae*. The changes were shown by the gradients plotted. The means of length and width on day 1 were 1.12 ± 0.04 and 0.20 ± 0.00 mm respectively. There was a small increment in the means of length and width of the larvae up to day-4. The length were 1.22 ± 0.07, 1.33 ± 0.06 and 1.40 ± 0.04 mm whilst the width were 0.21 ± 0.01, 0.26 ± 0.01 and 0.27 ± 0.01 mm forday-2, day-3 and day-4 respectively. However, there was a significant increment ($p<0.05$) in the means of length and width from day-4 to day-5. The increment of the length from day 4 (1.40 ± 0.04 mm) to day 5 (4.06 ± 0.04 mm) was 2.66 mm where else for the width, the increment from day-4 (0.27 ± 0.01 mm) to day-5 (0.95 ± 0.01 mm) was 0.68 mm. It is suggested that the first moulting took place within this

period. Hence the time duration first instar stage was estimated for four days.

The means of length and width continued to slowly increase between day-5 to days-8 until significant increment ($p<0.05$) occurred between day-8 and day-9. This was resulted by the second moulting of the larvae. The increment of the length from day 8 (4.65 ± 0.07 mm) to day 9 (7.12 ± 0.07 mm) was 2.47 mm whilst the increment of the width from day 8 (1.12 ± 0.01 mm) to day 9 (1.56 ± 0.01 mm) was 0.44 mm. Insignificant increment in means of length and width of the larvae was observed from day-9 until day-15 since they were ready to pupate. All the larvae pupated by day-15.

The first, second and third larval instars took three, three and six days respectively during their growth and development. The steep gradients which were presented by (a), (b), (c) and (d) determined the moulting interval of the larvae studied. The occurrence of moulting process was rapid and this took one day before the larval instar changed from one instar to another and before the third larval instar entered the pupal stage.

The length of larvae was more suitable to be used as an indicator to determine the instars compared to the width (Fig. 1). The length of the larva's body usually is influenced by the movement when it reacts and adapts to the surrounding areas, for example tunneling while foraging for food or avoiding from unsuitable condition as well as competition among the larvae. The width of the body is influenced by the food which the larva ingests. The gut of the larva would increase in size so that the width of the body will increase.

There were difference in the length and width within larval instars of *B. papayae* (Table 1 and 2). After hatching, the length of the larvae was 1.04 mm and the longest could reach up to 8.32 mm while the width starts from 0.18 mm and could reach up to 2.08 mm before completing all instars during the immature stage. Means length of the larvae were 1.27 ± 0.03, 4.33 ± 0.05 and 7.84 ± 0.07 mm whilst means width were 0.23 ± 0.01, 1.04 ± 0.01 and 1.85 ± 0.03 mm for the first instar (Plate 4), second instar (Plate 5) and third instar (Plate 6) respectively.

Several factors contribute to the growth rates and morphometrics measurement of the larvae. The factors that may influence the larval instar are the quantity of the diets, temperature and rearing regimes (Chua, 1991; Godin et al., 2002 and Esperk et al., 2007). The techniques that were employed in measuring the length and width of the larvae will also affect the results (Godin et al., 2002). Since there were not many studies conducted previously on the larval instars of Diptera in general, specifically on the tephritids fruit flies, some discussions in this study were based on information from lepidopteran and coleopteran immature stages to represent and support the results obtained (Godin et al., 2002).

The result showed that the larvae of *B. papayae* in this study fed more than *C. capitata* which was studied by Couldato and Zucoloto (1997). However, the quantity of the diet provided does not affect the growth of the larvae but it will affect the feeding behavior depending on the selection and regulation of the food ingested involved. Indirectly this situation could be the reason as to why the change in the third instar between day 8 and day 9 in widths was not as steep as the graph of the length of the larvae.

The flavour and odour from the flesh of the guavas might attract the larvae but the fresh guavas flesh provided in this study may be low in nutrients due to postharvest process or when the fruits were cut or diced to feed the larvae. Couldato and Zucoloto (1997) reported that there was a relationship between the diets and growth rates. Chua (1991) discussed in brief the techniques employed to sustain the nutrients acquired by *B. papayae* larvae. He stated that, to ensure the larvae have enough diet, pieces of fresh fruits were put daily on top of the old one for the larvae to move, while the old flesh pieces without larvae in them were discarded later. Therefore in this study, the similar technique was adopted where larvae were transferred to a new fresh piece of guava to ensure sufficient nutrients were provided.

Genc and Nation (2008) stated that the *B. oleae* larval growth is influenced by temperature. Both *B. oleae* and *B. papayae* are multivoltine species and that the larvae development could be affected by low temperature (Genc & Nation, 2008). However, a different result was obtained in this study. Although the temperature in this study was maintained at optimum condition, the number of larvae survived to the next instar stage decreased. This indicates that there are other factors that influence the survival of the larvae. Indirect factors such as moisture content in the guava flesh and contamination of guava flesh by microorganisms when handling the apparatus were probably the main reason why the number of larvae decreased. However,this reasons were not discussed in detail by Chua (1991), Couldato and Zucoloto (1997) and Genc and Nation (2008).

In this study, the length and width of the larvae were taken after the larvae were killed. In the observation conducted,

some larvae extended and stretched their bodies to the fullest in hot water but some did not although they were larger in size.

The results for the measurement may not be accurate due to error when the larvae were measured. The occurrence of stretching or body movement in this study might be affected by the treatment of the hot water before the larvae dead. The length and width of insects' larvae corresponded positively to their movement. According to Berg (2000), a difference in body stretching of larvae might occur due to the different methods used on extracted larvae to measure body length.

The dead larvae were measured manually using the ocular micrometer and errors may affect the measurements. Godin et al. (2002) reported that errors in head capsules measurement for lepidopteran could be due to variation in the operator's ability to align the ocular micrometer repeatedly across the widest point of the head capsules or from the transcription or conversion of ocular units to metric system equivalents.

CONCLUSION

Means length and width of the larvae's bodies could be used to determine the number of larval instars of *B. papayae* and it is observed that this species underwent three larval instars. The length of the larvae is a better indicator to determine the larval instars especially in the third instar as compared to the width. Even though temperature is crucial in tephritid behaviour, in this study it could be concluded that *B. papayae* larvae was not affected much by this factor. Changes in larval instars measurements for *B. papayae* were demonstrated by their length and width as it corresponds positively to the movements of the larvae.

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Table 1. Means of comparison of larvae's length (mm) at different instar larval

Larval length (mm)	Min	Max	Means±S.E.
1st instar	1.04	1.56	1.27 ± 0.03a
2nd instar	3.90	4.94	4.33 ± 0.05b
3rd instar	6.76	8.32	7.84 ± 0.07c

Value within column with different letter indicates significantly different (LSD at P< 0.05)

Table 2. Means of comparison of larvae's width (mm) at different instar larval

Larval length (mm)	Min	Max	Means±S.E.
1st instar	0.18	0.29	0.23 ± 0.01a
2nd instar	0.91	1.14	1.04 ± 0.01b
3rd instar	1.53	2.08	1.85 ± 0.03c

Value within column with different letter indicates significantly different (LSD at P< 0.05)

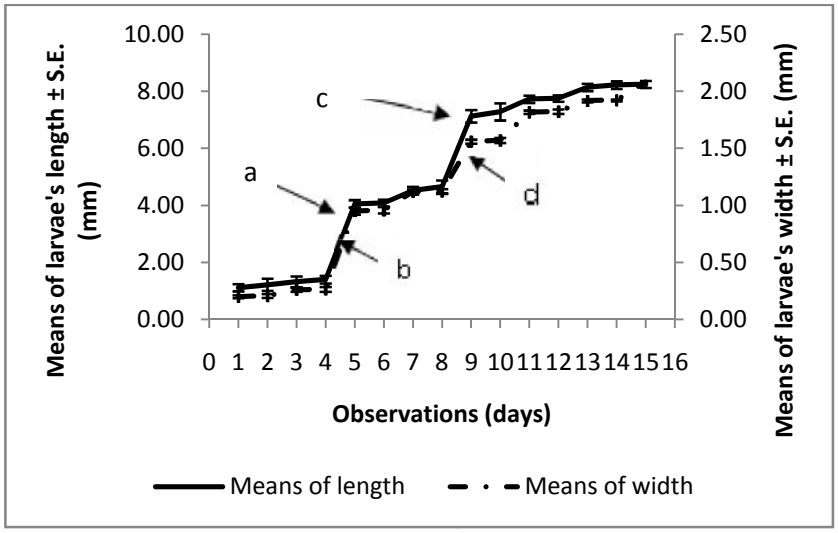

Fig. 1. Graph of means length and width for determination of larval instars. a-d indicates moulting phase.

Plate 1. Artificial-egging-devices made for eggs collecting

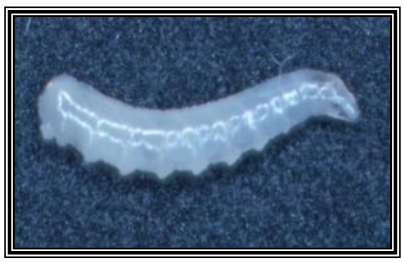

Plate 2. Larval length measurement

Plate 3. Larval width measurement

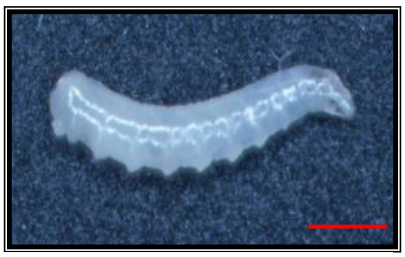

Plate 4. 1st instar larva; Scale bar 0.22 mm

Plate 5. 2nd instar larva; Scale bar 0.73 mm

Plate 6. 3rd instar larva; Scale bar 0.73 mm