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Identification of Pest Species in Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) Species Complex

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Abstract.- Different identification methods to differentiate pest species *B. carambolae* from *B. occipitalis, B. papayae* from *B. philippinensis and B. dorsalis* from *B. papayae,* and *B. philippinensis* (in oriental fruit fly complex) are presented. These methods / techniques are based on aculeus length, aedeagus length, discal cell length, and scanning electron microscopy of tomentum pattern on prescutum. The character "scales on the distal end of eversible membrane of ovipositor" is reviewed. However, morphological characters could not differentiate the two species *B. papayae* and *B. philippinensis*.

Key Words: Oriental fruit fly, *Bactrocera dorsalis* complex

INTRODUCTION

The fruit flies are the classical international pests. They are not only the production deterrent to fresh fruits and vegetables, but indeed a formidable export barrier limiting a potential multimillion dollar worldwide trade. The major hurdle in the export of fruits and vegetables was plant quarantine restrictions due to the fruit flies in the Philippines (Rejesus *et al.,* 1991).

In Malaysia fruit flies incur severe damage to certain potential fruit crops like starfruit and guava. Fruit crops may suffer 100% damage if not protected owing to the fact that these are the polyphagous pests and losses can run several million dollars annually. Apart from direct damage, Malaysian fruit cannot be freely exported to lucrative markets like USA, Japan and Australia, because of strict quarantine regulations in these countries, which prohibit entry of fresh fruits from the fruit flies infested countries unless proper disinfestation treatments are carried out. Therefore, the fruit flies are the major obstacles in development of the fruit industry in Malaysia (Singh, 1991). Other countries like Thailand, Indonesia, India, Taiwan and China are also facing similar problems.

In addition to the other taxonomic applications, the fruit fly identification has its application in the development of the plant quarantine guidelines, post-harvest regulations, and the use of correctly identified cultures in sterile insect release method eradication programmes (Drew, 1991).

The Oriental fruit fly *Bactrocera dorsalis* (Hendel) complex was indicated by Bezzi (1914) who described four varieties of *Chaetodacus ferrugineus* Fabricius [a synonym of *Bactrocera dorsalis* (Hendel)], from the Indian subcontinent. Later, Bezzi (1919) clearly stated that "*Dacus ferrugineus* is evidently a complex of several species" and he described four varieties of *Chaetodacus ferrugineus* from the Philippines, two of which were included in the present definition of the Oriental fruit fly complex by Drew and Hancock (1994). Hardy (1969) included sixteen species in this complex, although only eight of these were included by Drew and Hancock (1994) who used a more restricted definition of the group. Hardy (1969) also discussed the nomenclature of *B. dorsalis*. Drew and Hancock (1994) revised the Oriental fruit fly complex in Asia with fifty-two species. Although this was a good quality scientific work, it is extremely difficult to use it without lot of experience in studying the complex and access to a good reference collection. Moreover, they dealt with all the species without regard to economic importance, which makes the work confusing and difficult for inexperienced users. Usually, the requirement of the local workers is to identify a

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couple of locally occurring economically important species. It is difficult to use the key with more than fifty couplets and closely related species, and the chances of making a wrong identification are high.

According to Drew and Hancock (1994), there were eight pest species (that have been recorded from economically important fruits) in the Oriental fruit fly complex, namely: *B. (B.) carambolae* Drew and Hancock, *B. (B.) caryeae* (Kapoor), *B. (B.) dorsalis* (Hendel), *B. (B.) kandiensis* Drew and Hancock, *B. (B.) occipitalis* (Bezzi), *B. (B.) papayae* Drew and Hancock, *B. (B.) philippinensis* Drew and Hancock and *B. (B.) pyrifoliae* Drew and Hancock. *B. pyrifoliae* has tapered vittae and can be easily separated from the other pest species. Specimens of *B. caryeae* and *B. kandiensis* can easily be separated from the other species by the presence of a black spot on the postpronotal lobe and on each femur, and from each other by the lateral markings on abdominal tergites III-V (*B. caryeae* has broad lateral markings on abdominal tergites III- V and *B. kandiensis* has narrow markings). Mahmood (2004) gave different identification methods for separating *B. carambolae, B. papayae* and *B. occipitalis, B. philippinensis*, respectively.

Different identification methods for 1) *B. occipitalis* and *B. carambolae,* 2) *B. papayae* and *B. philippinensis* and 3) *B. dorsalis* and *B. philippinensis*, *B. papayae* are described here.

MATERIALS AND METHODS

Specimens of the following species from the following localities present in the Department of Entomology, Natural History Museum, London were available for this study: *B. occipitalis* from the Philippines and Brunei; *B. philippinensis* from the Philippines; *B. papayae* from Brunei, Indonesia, Malaysia and Singapore; *B. carambolae* from French Guiana, Indonesia, Malaysia and Singapore; and *B. dorsalis* from Taiwan.

The procedure for different identification techniques were same as described in Mahmood (2002).The results and discussion are presented in a manner that compares pair of species likely to be of practical interest to field entomologists.

RESULTS

Bactrocera carambolae *and* Bactrocera occipitalis

Aculeus and discal cell length

Both species share a common range of aculeus length: *B. carambolae* 1.36 - 1.68mm (mean 1.51mm); *B. occipitalis* 1.34 - 1.60mm (mean 1.50mm). There was no separation between these two species (Fig. 1). The value of aculeus length when expressed as a ratio to discal cell length was higher for *B. carambolae* (83% specimens had more than 0.66) and was lower for *B. occipitalis* (50% specimens had more than 0.66).

The mean for aculeus length to discal cell length ratio was significantly different between the two species.

Aedeagus length and discal cell length

Both species largely share a common range of aedeagus length: *B. carambolae* 2.55 - 3.15mm, mean 2.93mm; *B. occipitalis* 2.69 - 3.25mm, mean 3.08mm. There was no separation between these two species (Fig. 2). However, the mean aedeagus length was bigger in *B. occipitalis* than *B. carambolae*.

Tomentum pattern on prescutum

These two species were separated by difference in the tomentum pattern on the prescutum. *B. occipitalis* has a clear wide central longitudinal gap in the prescutum tomentum (Fig. 3). In *B. carambolae* the central longitudinal gap in tomentum pattern on prescutum was sometimes present and sometimes absent. It was very narrow, when it was present (Fig.4).

Bactrocera philippinensis *and* Bactrocera papayae

Aculeus length and discal cell length

Both species share a common range of aculeus length: *B. philippinensis* 1.65 - 2.14mm (mean 1.89mm); *B. papayae* 1.59 – 2.02mm (mean 1.85mm). There was no separation between these two species (Fig. 5). The value of aculeus length when expressed as a ratio to discal cell length was higher for *B. papayae* and was lower for *B. philippinensis*.

Aedeagus length and discal cell length

When aedeagus length was plotted against discal

Figs. 1-2. Relationship of aculeus length (1), and aedeagus length (2) to discal cell length between *B. carambolae* and *B. occipitalis*

cell length, there was some overlap of these two species, with *B. philippinensis* having the higher values (Fig. 6).

Tomentum pattern on prescutum

There was no difference in the tomentum pattern of *B. philippinensis* and *B. papayae*, both the species lack a longitudinal gap in the centre of the prescutum (Fig. 7 and 8, respectively).

Scales on the distal end of the eversible membrane of ovipositor

The scales on the eversible membrane (Fig. 9) of the ovipositor were different at different places. At the

Figs. 3-4. Tomentum pattern on prescutum *B. occipitalis* (3) and *B. carambolae* (4). Magnification 3, 100X, 4, 140X.

base the scales were broad, blunt and without dentation (Fig. 10). The width of scales and tooth - like structure on them increased from the basal end to the distal end. The scales in the middle were narrow with a fewer (6-10) dentations (Fig. 11) and on the distal end were wide with a maximum of 10-14 dentations (Fig. 12). The scales on the distal end of the eversible membrane of the ovipositor of both species were observed in the SEM. There were no differences in the length and width of the scale- like structures, between *B. philippinensis* (Fig. 13) and *B. papayae* (Fig. 14).

Separation of B. dorsalis *from* B. philippinensis *and* B. papayae

Aculeus length and discal cell length

When aculeus length was plotted against discal cell length, there was a clear separation of *B. dorsalis* from *B. papayae* (Fig. 15) and *B. philippinensis* (Fig. 16). The aculeus length in *B. dorsalis* was smaller (1.28 - 1.50mm, 1.40mm mean), compared to other two species.

The difference in the means for aculeus length was highly significant between *B. dorsalis* and *B. papayae* or *B. philippinensis*.

The difference in the means for aculeus length to discal cell length was highly significant between *B. dorsalis* and *B. papayae* or *B. philippinensis*.

Tomentum pattern on prescutum

The tomentum pattern on prescutum in *B. philippinensis*, *B. papayae* and *B. dorsalis* was similar in lacking the central longitudinal gap (Figs 7, 8, 17, respectively).

DISCUSSION

Bactrocera carambolae *and* Bactrocera occipitalis

According to Drew (1991), Malaysian A (*B. carambolae*) has a black spot on the fore femur but *B. occipitalis* does not. This character was found to be variabe in both the species. Drew and Hancock (1994) separated these species as follows:

35 Costal band broadening across apex of wing; abdominal tergite III usually with outer 1/3 darkened; usually with red-brown around *prsc*. setae; usually without dark spots around bases of orbital setae; never with a dark spot on the apices of fore femora; major pest species; attracted to methyl eugenol... *B. occipitalis*

Figs. 5-6. Relationship of aculeus length (5) and aedeagus length (6) to discal cell length between *B. papayae* and *B. philippinensis.*

- Costal band with a pale overlap of R_{2+3} and broadening only around apex of R_{4+5}; abdominal tergite III without broad dark lateral margins; without pale coloration around *prsc*. setae; dark markings present around bases of orbital setae; usually with a dark fuscous spot on the outer apical surface of fore femora; major pest species; attracted to methyl eugenol. ....*B. carambolae*

The present study revealed that in some Malaysian specimens of *B. carambolae,* the costal band was broadened around the apex of R_{4+5}, otherwise there was broadening of the wing costal band at the wing apex. The costal band overlap with vein R_{2+3} was also similar in the two species (78%

overlapping in *B. carambolae* and 85% in *B. occipitalis*). The basal transverse black band on abdominal tergite III was also almost similar in the two species. The colour around the frontal setae was variable. Similarly the patch around the prescutellar acrostichal setae was also a variable character in both species. It was impossible to separate these two species by these characters, especially where these are in sympatry (Borneo).

Although, the mean for aculeus length in these species was not significantly different (t = 0.46, P = 0.64), however the mean for aculeus length when expressed as a ratio to the discal cell length was significantly different (t = 3.59, P = 0.01) between the two species. This difference was due to bigger discal cell length in *B. occipitalis*. The difference in the means for aedeagus length was highly significant (t = 6.52, P < 0.01). The aedeagus was longer in *B. occipitalis* than in *B. carambolae*. The aedeagus length when expressed as a ratio to discal cell length ratio was not significantly different (t=1.91, P = 0.06).

The tomentum pattern on the prescutum was the most important character for differentiating these two species. Some specimens from Borneo, were with deep black lateral markings on tergites III-V (like some *B. occipitalis*), while other were only with anterolateral rectangular black marking on tergites IV and V like mostly (*B. carambolae*). In such situations it was impossible to identify the specimens. The tomentum pattern on the prescutum has not only made the identification easy but also confirmed the specific status of *B. carambolae*. According to Drew and Hancock (1994) both species are sympatric in Borneo. In the present study all specimens from Borneo (Sabah), were found to be *B. carambolae*, and all those from Brunei, were *B. occipitalis*. Similarly the record of *B. occipitalis* from India (Andaman Is.) by Kapoor (1993) was based on mis-identification of *B. carambolae*.

Bactrocera philippinensis *and* Bactrocera papayae

Drew (1991) and Drew and Hancock (1994) separated *B. philippinensis* and *B. papayae* by the length of the scales on the distal end of the eversible membrane of the ovipositor. Their key couplet separating these two species was:

36 Scales on the distal end of eversible membrane of ovipositor long and narrow; major pest species; attracted to methyl eugenol;.. *B. papayae*

Figs. 7-8. Tomentum pattern on prescutum, *B. philippinensis* (7) and *B. papayae* (8). Magnification: 100 X.

Figs. 9-12. Ovipositor, eversible membrane (9), basal end of eversible membrane (10), middle part of eversible membrane (11), distal end eversible membrane (12). Magnification: 9, 110X; 10, 3.5 KX, 11, 12, 4.0 K.

Scales on the distal end of eversible membrane of ovipositor short; major pest species; attracted to methyl eugenol;... ....*B. philippinensis*

B. papayae was believed to have elongate and narrow scales on the distal end of the eversible membrane with 10 - 24 small tooth - like structures while *B. philippinensis* had only 6 - 10 broad tooth - like structures (Drew and Hancock, 1994). In the present study twelve specimens of *B. papayae* were observed in the SEM and only one long scale on one specimen was observed. Eight specimens of *B. philippinensis* were dissected for examination under the scanning electron microscope but there was no difference in the scales on the distal end of the eversible membrane between these two species. Both the species had similar scales of medium size with 10 - 14 tooth - like structures. Similarly in *B. occipitalis*, the scales at the distal end of eversible membrane are long with 10-16 dentations (Fig.18), however, according to Drew and Hancock (1994) these scales were of medium width, with 5 - 10 small dentations (Fig 19). It is assumed that Drew and Hancock (1994) observed either an inadequate or a typical sample, or that they were inconsistent about the area of the membrane observed. All the seven species of economic importance in the Oriental fruit fly complex of species were with similar scales at the base, middle and the distal end of eversible membrane of ovipositor.

Aculeus length, aedeagus length and their ratios to discal cell length could not separate these two species. The means were significantly different for aculeus length ($t = 2.71$, $P = 0.01$), aculeus length to the discal cell length ratio ($t = 4.08$, $P = 0.01$), aedeagus length ($t = 6.43$, $P = 0.01$) and aedeagus length to discal cell length ($t = 4.07$, $P = 0.01$). The tomentum pattern on prescutum was also similar in two species. So these two species were not differentiated by morphological means which differentiated other closely related species.

These two species are the best example of cryptic species in the Oriental fruit fly complex. The revision of the Oriental fruit fly complex by Drew and Hancock (1994) was mainly based on the morphological species concept. *B. papayae* is now found from Thailand to Australia. *B. philippinensis* and *B. papayae* occurring in neighbouring countries

Figs. 13-14. Scales on the distal end of the eversible membrane of ovipositor, *B. philippinensis* (13), and *B. papayde* (14). Magnifications: X4.0K.

Figs. 15-16. Relationship of aculeus length to discal cell length between *B. dorsalis* and *B. papayae* (15), and *B. dorsalis* and *B. philippinensis* (16).

were similar in morphological characters, but the male pheromones (Perkins *et al.*, 1990) and allozyme electrophoresis (Mahmood, 1999) showed differences between these two species. There is some evidence of more undescribed cryptic species, if some advanced molecular systematic techniques be employed, e.g. *B. papayae* in Indonesia has a shorter aculeus and discal cell length than *B. papayae* from Malaysia and Thailand, and so the smaller flies.

Separation of B. dorsalis *from* B. philippinensis *and* B. papayae

No tenable method has been found to separate specimens of *B. dorsalis* from *B. papayae* and *B.*

philippinensis using external morphological characters. The key couplet (Drew and Hancock, 1994) to separate *B. dorsalis* and *B. papayae* was as follows:

13 Pleural area immediately below postpronotal lobe brown (*i.e.* pale); aculeus short (1.4 - 1.6mm); major economic pest; attracted to methyl eugenol .... *B. dorsalis*

\- Pleural area immediately below postpronotal lobe brown to fuscous (*i.e.* dark); aculeus long (1.77 - 2.12mm); major economic pest; attracted to methyl eugenol .... *B. papayae*

Fig. 17. *B. dorsalis*: tomentum pattern on prescutum (X70)

Drew and Hancock (1994) separated *B. dorsalis* from *B. papayae* and *B. philippinensis* by aculeus length, *i.e. B. dorsalis* have a shorter aculeus (1.4 - 1.6 mm) than the other two species (1.77 - 2.12 mm). It was only possible to separate *B. dorsalis* from Taiwan (type locality) using aculeus length. In the present study it was found that 50 percent of specimens of *B. dorsalis* has a dark area below the postpronotal lobe. Similarly, there were some specimens of *B. dorsalis* from India and Hawaii that had an aculeus length of up to 1.8mm. The aculeus length of *B. dorsalis*

Figs. 18-19. *B. occipitalis*, scales on the distal end of the eversible membrane of ovipositor in this study (18), and according to Drew & Hancock (1994) (19). Magnification: 18, X4.0K.

Table I.- t-test tables.

Species		Cases	Mean	Std. Dev.	Std. Err.	t-value	2-tailpr
a)	**Aculeus length**						
	B. occipitalis	22	1.50	0.067	0.014	0.464	0.644
	B. carambolae	53	1.51	0.072	0.010		
b)	**Aculeus length to discal cell length ratio**						
	B. carambolae	53	0.70	0.037	0.005	3.59***	0.001
	B. occipitalis	22	0.66	0.030	0.006		
c)	**Aedeagus length**						
	B. carambolae	55	2.93	0.136	0.018	6.52***	<0.001
	B. occipitalis	61	3.08	0.121	0.015		
d)	**Aedeagus length to discal cell length ratio**						
	B. carambolae	55	1.44	0.078	0.010	1.91	0.058
	B. occipitalis	61	1.41	0.089	0.011		
e)	**Aculeus length**						
	B. philippinensis	76	1.89	0.099	0.011	2.72**	0.007
	B. papayae	49	1.84	0.108	0.015		
f)	**Aculeus length to discal cell length ratio**						
	B. papayae	49	0.87	0.038	0.005	4.08***	0.001
	B. philippinensis	76	0.84	0.040	0.004		
g)	**Aedeagus length**						
	B. philippinensis	65	3.49	0.160	0.020	6.73***	<0.001
	B. papayae	49	3.30	0.145	0.021		
h)	**Aedeagus length to discal cell length ratio**						
	B. papayae	49	1.70	0.128	0.018	5.75***	<0.001
	B. philippinensis	65	1.59	0.083	0.010		

Runs = a,b = 73, P<0.001; c = 114, P<0.001
Degrees of freedom = 124, P<0.001
The differences between the means for total scores (a), for aculeus length (b), for aculeus length to discal cell length ratio (c), for aedeagus length (d), and for aedeagus length to discal cell length ratio (e) were highly significant.

(1.8mm), therefore slightly overlapped with the aculeus length of *B. papayae* and *B. philippinensis* (1.65 - 2.12 mm). However, *B. dorsalis* from Taiwan has a shorter aculeus (1.28 - 1.5mm). The difference in the mean was highly significant for aculeus length and the aculeus length when expressed as a ratio to discal cell length between *B. dorsalis* and *B. papayae* ($t = 16.25$, $P < 0.01$ and $t = 24.64$, $P < 0.01$ respectively) or *B. philippinensis* ($t = 20.09$, $P < 0.01$ and $t = 21.91$, $P < 0.01$ respectively). Although male specimens of *B. dorsalis* from Taiwan were not available for dissection, specimens from Hawaii, India, Thailand, Pakistan and Sri Lanka were found to have shorter aedeagi. These three species could not be differentiated by the tomentum pattern on the prescutum.

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