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Research Article

Interspecific Mating Effect of *Bactrocera carambolae* and *Bactrocera dorsalis* on its Fecundity, Survival and Morphologies

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Abstract

Background and Objective: Worldwide, *Bactrocera carambolae* and *Bactrocera dorsalis* are important pests in horticultural commodities. Based on the trapping with methyl eugenol attractant, it was found that the intermediate morphology between *B. carambolae* and *B. dorsalis*. This study observed the comparative biology and survivability of the interspecific and intraspecific hybrids *B. carambolae* and *B. dorsalis*. **Materials and Methods:** This study was conducted at the Indonesian Center of Forecasting Plant Pest Organisms (BBPOPT) and Plant Pest Laboratory, Faculty of Agriculture, Universitas Padjadjaran, Indonesia. The method is an experimental method with a randomized block design that involves parental fecundity and survival test (four treatments and six replications) and fecundity and fertility tests (eight treatments and four replications). **Results:** The results showed a bigger reduction in the fecundity of hybrid parents of *B. carambolae* and *B. dorsalis* on interspecific rather than an intraspecific hybrid. The longevity of survival and development of eggs and larvae stages hybrid *B. carambolae* (♂). Moreover, the fecundity of F1 hybrids was increased compared to the hybrid parents and the fertility was in the range of 79.00-96.75%. **Conclusion:** Interspecific mating of *B. carambolae* and *B. dorsalis* have the potential to survive in nature.

Key words: Back-cross, fruit-fly, fertility, hybrid, self-cross, larvae, pheromone analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bactrocera carambolae Drew and Hancock and *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) are the important pests that are responsible for fruit and vegetable crop losses. Great yield losses due to rot and dropping of the vegetable or fruits occur mostly because of invading at the larvae stage of Bactrocera. Moreover, the presence of eggs and larvae causes quarantine restrictions to prevent the spread of fruit flies. The highest abundance of *B. dorsalis* and *B. carambolae* was found in Thailand, Malaysia and Indonesia[1]. *Bactrocera carambolae* has 77 host plants from 27 families, while *B. dorsalis* has 209 host plants from 51 families[1]. They also could live together in the same host plants, such as mango, guava, avocado, hair, tomato, sapodilla, mangosteen, orange, lemon, lime, papaya and star fruit.

Both *B. carambolae* and *B. dorsalis* are included in the *B. dorsalis* complex which can be detected by the dark spots, wing pattern, abdominal pattern and forefoot femora dark spots[2,3]. The distinctive differences between the two species are also found in the glandular pheromone components found in males *B. carambolae* which produces (E)-coniferyl alcohol, whereas, *B. dorsalis* produces (E)-coniferyl alcohol and 2-Allyl-4,5-dimethoxyphenol[4]. On the other hand, the species has been identified morphologically from wild fruit flies in Malaysia and Indonesia[5,6]. The natural hybridization speculation has been demonstrated based on pheromone analysis[4]. Based on[7], *B. carambolae* and *B. dorsalis* mating affinity are about 40%.

The important role of the invasive populations of hybridization between species encompasses the distribution, formation and impact[8]. The tendency from *B. neohumeralis* to *B. tryoni* allowed their hybrids to expand their host range and geographic area. Additionally, the interspecific hybrid mating time between *B. neohumeralis* and *B. tryoni* can be done at any time, although inherently the intraspecific hybrid mating time of *B. neohumeralis* during the daytime and *B. tryoni* during twilight[9]. The ability of hybrids to mate can be used for controlling fruit flies using the Sterile Insect Technique (SIT) method[10].

Therefore, this study evaluates the postzygotic compatibility between *B. carambolae* and *B. dorsalis*. The survival rates and time of development in the egg, larval and pupa stages of the hybrids were also evaluated to determine the hybrids.

MATERIALS AND METHODS

Study site: The experiments were conducted in Vapor Heat Treatment Laboratory at the Indonesian Center of Forecasting Plant Pest Organisms (BBPOPT) (6°23'06.5"S 107°30'26.2"E) and Plant Pest Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran, Indonesia (6°55'33.0" S & 107°46'24.6" E) from 2016-2018.

Samples collection and rearing: Fruit flies *B. carambolae* and *B. dorsalis* male and female were collected and reared in a biotron chamber (STH-19p, Sanshu Sankyou Co. Ltd.) with a temperature of 27-30°C, 70-80% humidity and a lighting period of 12:12 hrs bright and dark. The fruit flies were reproduced and used at the egg, larva and pupae stages. In the spawning preparation of *Bactrocera* spp., required mango extract which is the host of test insects. The fruit is placed in a plastic cup with a diameter of 8 cm and a height of 13 cm. The plastic cups are given 240 holes with a hole spacing of 1 cm with a hole size of 0.5 mm for *B. dorsalis* and *B. carambolae*.

Separation of males and females is carried out at the age of 3-4 days after the pupal phase before mating occurs[4]. The feed from a solution of wheat husks (175 g of wheat husks mixed with 150 mL hot water). The solution of drained wheat husks is added and mashed in a blender with HCl 3.5% 2 mL, 50 g of granulated sugar, 35 g of yeast, 0.75 g of sodium benzoate and 25 g of tissue paper and 500 mL of water. Afterwards, the feed is mixed with AY-65 (Autolyzed Yeast, Asahi Food and Health Care C, Ltd.) and sugar with a weight ratio of 1:4 in Petri dishes. Finally, the feeds were ready to put in the propagation cage. Along with the feed, the provision of drinking (from tap water) was replaced every week with a particular bottle with a wick made from tissue to make it easier for the imago to absorb the water.

Fecundity test of hybrid ancestry and survival: In each treatment, the survival rate and development duration of the egg, larva and pupa stages were observed: Interspecific mating *B. carambolae* males with *B. dorsalis* females as F_1(A), interspecific mating *B. carambolae* females with males *B. dorsalis* as F_1(B) (Fig. a), (Bd) intraspecific mating males *B. dorsalis* (♂ Bd) with females *B. dorsalis* (♀ Bd) and (Bc) males *B. carambolae* (♂ Bc) and females *B. carambolae* (♀Bc) (Fig. 1b).

Hybrid fecundity and fertility test: The treatments tested in this study were self-cross hybrid, back-cross hybrid and intraspecific mating (Fig. 2a-d). The fecundity test calculated the average number of eggs produced from 40 female ancestors on every treatment[11]. On the other hand, the fertility test was carried out by observing the percentage of hatching from the 400 eggs produced.

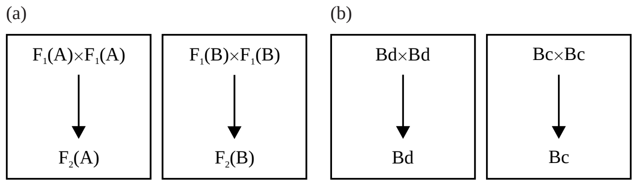

Fig. 1(a-b): Mating diagram, (a) Interspecific mating and (b) Intraspecific mating
Bc: *B. carambolae* and Bd: *B. dorsalis*

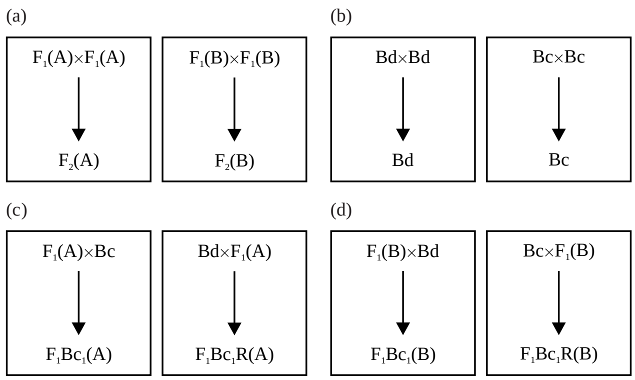

Fig. 2(a-d): Mating diagram, (a) Self-cross hybrid, (b) Intraspecific mating, (c) Back-cross hybrid F_1(A) and (d) Back-cross hybrid F_1(B)
F_1(A): Offspring of Bc×Bd and F_1(B): Offspring of Bd×Bc

Data processing and analyses: The analyzed data were survival, development time, fertility, pupa size, adult size, wing and sex ratio. The experiment method used an experiment randomized design and the four treatments were done with six replications. Data were analyzed statistically by ANOVA using the IBM SPSS® 106 software ver. 21.0 for Windows. The analysis was followed by Duncan Multiple Range Test at the 5% confidence level to determine the difference in the average treatment.

RESULTS

The result showed the fecundity of interspecific mated *B. carambolae* was slightly higher than that of *B. dorsalis* (Table 1). On the other hand, intraspecific mated *B. carambolae* females x *B. dorsalis* males showed a significant difference compared to *B. dorsalis* females x *B. carambolae* males. The statistical result indicates there was a significant decrease in the fecundity between interspecific and intraspecific mating. Moreover, the fecundity decrease of *B. dorsalis* fruit flies was greater than that of *B. carambolae* (25.13% compared to 10.11%).

The number of eggs produced by the interspecific hybrids has decreased *B. carambolae* and *B. dorsalis* which indicates that mating causes the potential for reproductive disorders. After the self-cross and back-cross were carried out on the interspecific hybrid offspring of *B. carambolae* and *B. dorsalis*, the difference was only significant in the interspecific self-cross hybrid fecundities of *B. carambolae* and *B. dorsalis* (Table 2). The fecundity of F_1(B) hybrids in self-cross mating were higher than F_1(A) hybrids, however, the fecundity of F_1(A) and F_1(B) hybrids back-cross was not significantly different.

Nevertheless, the hybrid fertility percentages at the back-cross were well over than at the self-cross (Table 2). Female hybrids F_1(A) self-cross, F_1(B) self-cross, F_1(A) back-cross reciprocal and *B. dorsalis* had the lowest fertility ranging from 79.00±2.71-88.00±4.55%. While the hybrids F_1(A) back-cross, F_1(B) back-cross and *B. carambolae* have intermediate fertility ranging from 90.50±3.11-96.75±2.63%. The highest fertility was shown by the F_1(B) reciprocal back-cross for about 96.75±2.63%.

Based on the results of survival in the laboratory, *B. carambolae* and *B. dorsalis* have type III survival curves. This study showed that there were a higher survival rate for interspecific mating F_1(A) than F_1(B) egg, larvae and pupae (Table 3). The reason was that F_1(A) has a high birth rate during the egg phase with a value of 72.67%. However, there was a difference in intraspecific mating results. There was a slight difference between the amount of survival and development time between intraspecific mating *B. carambolae* which was higher than *B. dorsalis* in the egg phase. Nevertheless, in the larvae and pupae phase, a bit of change in the count results showed *B. dorsalis* became higher than *B. carambolae*.

Regarding the morphometric parameters test study, interspecific mating of *B. carambolae* and *B. dorsalis* causes variations in the length of the aculeus, aedeagus and black markings of the fore femora in hybrids (Table 4, Fig. 3a-d, 4a-d, 5a-d). The average length of aculeus hybrids shows a tendency to approach *B. carambolae*. The average length of aculeus *B. carambolae* and *B. dorsalis* were 1.73±0.08 mm and respectively 1.45±0.11 mm, while the F_1(A) and F_1(B) hybrids are 1.45±0.18 and 1.47±0.15 mm. The trend of hybrid morphology approaching *B. carambolae* was also found in the average length of the aedeagus hybrid F_1(B).

The results of observations of the length of the aedeagus hybrids showed a difference, the average length of the aedeagus hybrids F_1(A) was in the middle of the two parental species which was 2.63±0.21 mm, while the average length

Table 1: Fecundity means of *B. carambolae* and *B. dorsalis*

Mated species	Fecundity (eggs)
Bd ♀×Bc ♂	63.58 ± 6.80^{a}
Bc ♀×Bd ♂	88.02 ± 22.87^{b}
Bd ♀×Bd ♂	84.92 ± 10.85^{ab}
Bc ♀×Bc ♂	97.92 ± 12.79^{c}

Bd: *B. dorsalis*, Bc: *B. carambolae*, the mean value (Mean±Standard error) followed by the same letter shows no significant difference according to the Duncan Multiple Range Test at the 5% level

Table 2: Fecundity and fertility mean percentage of *B. carambolae* and *B. dorsalis* in self-cross and back-cross

Mated species	Fecundity (eggs)	Percentage of fertility (%)
F_1(A)×F_1(A)	74.23 ± 8.39^{a}	83.25 ± 1.89^{a}
F_1(B)×F_1(B)	89.90 ± 11.30^{b}	80.75 ± 3.20^{a}
F_1(A)×Bc	83.43 ± 11.58^{abc}	90.50 ± 3.11^{b}
Bd×F_1(A)	78.75 ± 4.27^{ab}	79.00 ± 2.71^{a}
F_1(B)×Bd	75.93 ± 3.70^{ab}	92.25 ± 1.26^{b}
Bc×F_1(B)	74.93 ± 4.32^{ab}	96.75 ± 2.63^{c}
Bd ♀×Bd ♂	83.05 ± 5.76^{abc}	88.00 ± 4.55^{a}
Bc ♀×Bc ♂	94.21 ± 13.40^{c}	90.75 ± 2.22^{b}

Interspecific mating *B. carambolae* ♂ with *B. dorsalis* ♀ as F_1(A), interspecific mating *B. carambolae* ♀ with *B. dorsalis* ♂ as F_1(B), *B. dorsalis* (Bd), *B. carambolae* (Bc), the mean value (Mean±Standard error) followed by the same letter shows no significant difference according to the Duncan Multiple Range Test at the 5% level

Table 3: Percentage of survival (%) and development time of *B. carambolae* and *B. dorsalis*

	Egg		Larvae		Pupae	
Mated species	Survival	DT	Survival	DT	Survival	DT
F_1(A) (Bc ♀×Bd ♂)	72.67 ± 4.37^{b}	1.29 ± 0.01^{a}	89.16 ± 8.99^{b}	4.25 ± 0.09^{a}	92.94 ± 3.10^{b}	8.37 ± 0.17^{b}
F_1(B) (Bd ♀×Bc ♂)	43.00 ± 7.82^{a}	1.31 ± 0.01^{b}	80.71 ± 9.06^{a}	5.42 ± 0.14^{b}	84.22 ± 13.11^{a}	8.10 ± 0.18^{a}
Bc (Bc ♀×Bc ♂)	80.83 ± 3.13^{c}	1.33 ± 0.01^{c}	84.89 ± 3.58^{b}	4.25 ± 0.09^{a}	93.60 ± 3.48^{b}	8.35 ± 0.09^{b}
Bd (Bd ♀×Bd ♂)	75.67 ± 7.00^{bc}	1.30 ± 0.01^{ab}	90.23 ± 2.91^{b}	4.34 ± 0.15^{a}	95.39 ± 2.70^{c}	8.93 ± 0.05^{c}

DT: Development time, interspecific mating *B. carambolae* ♂ with *B. dorsalis* ♀ as F_1(A), interspecific mating *B. carambolae* ♀ with *B. dorsalis* ♂ as F_1(B), Bd: *B. dorsalis*, Bc: *B. carambolae*, the mean value (Mean±Standard error) followed by the same letter shows no significant difference according to the Duncan Multiple Range Test at the 5% level

Table 4: Morphometric parameters of interspecific and intraspecific mating

	Morphological length (mm±SD)		
Species mated	Aculeus	Aedeagus	Blackmark of femora
F_1(A) (Bc ♀×Bd ♂)	1.45 ± 0.18^{a}	2.63 ± 0.21^{b}	0.20 ± 0.12^{b}
F_1(B) (Bd ♀×Bc ♂)	1.47 ± 0.15^{b}	2.56 ± 0.21^{a}	0.24 ± 0.12^{c}
Bc (Bc ♀×Bc ♂)	1.73 ± 0.08^{c}	2.84 ± 0.14^{c}	0.00 ± 0.00^{a}
Bd (Bd ♀×Bd ♂)	1.45 ± 0.11^{a}	2.56 ± 0.17^{a}	0.31 ± 0.08^{d}

SD: Standard deviation, interspecific mating *B. carambolae* ♂ with *B. dorsalis* ♀ as F_1(A), interspecific mating *B. carambolae* ♀ with *B. dorsalis* ♂ as F_1(B), *B. dorsalis* (Bd), *B. carambolae* (Bc), the numbers in the column followed by the same letter are not significantly different at the 5% BNT level

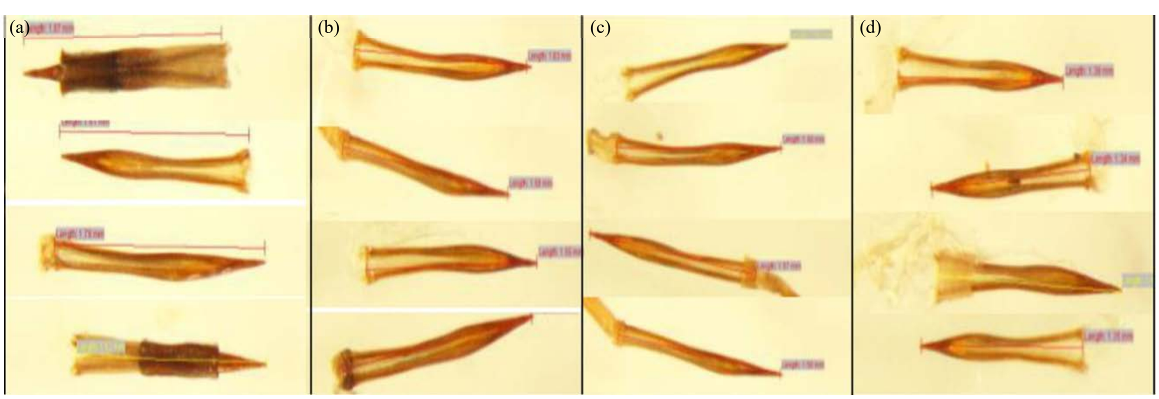

Fig. 3(a-d): Aculeus (female genitalia) morphology, (a) F_1(A), (b) F_1(B), (c) *B. dorsalis* and (d) *B. carambolae*

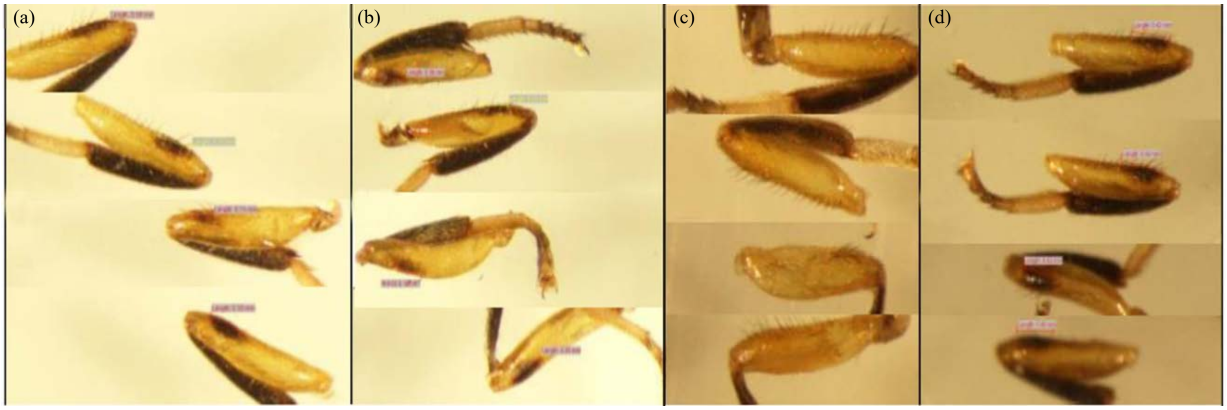

Fig. 4(a-d): Aedeagus (male genitalia) morphology, (a) F_1(A), (b) F_1(B), (c) *B. dorsalis* and (d) *B. carambolae*

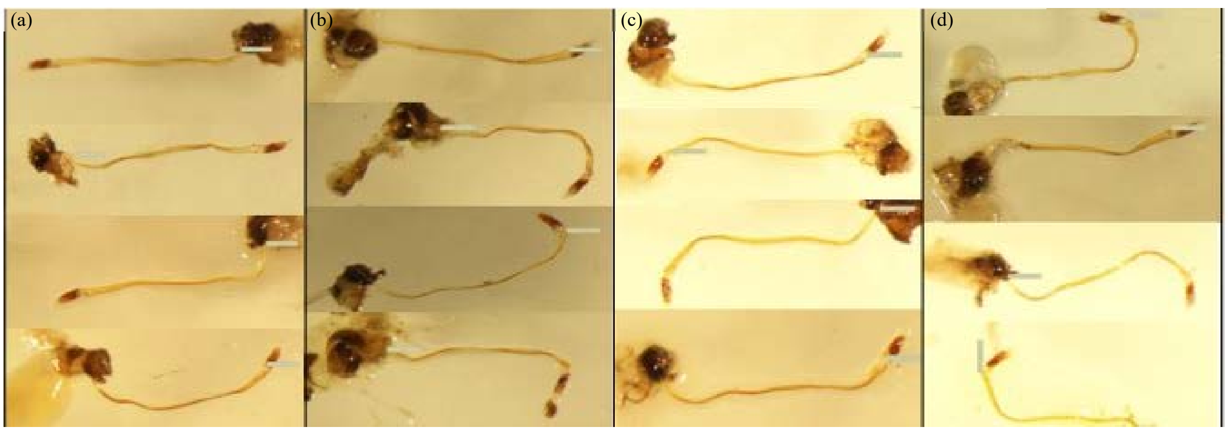

Fig. 5(a-d): Morphology of the black mark of femora, (a) F_1(A), (b) F_1(B), (c) *B. dorsalis* and (d) *B. carambolae*

of the aedeagus hybrids F_1(B) showed a tendency to follow *B. carambolae* i.e., 2.56±0.21 mm. The results of the mean length of the front femora black markings showed a significant difference between the hybrids with the two parental species. The length of the black markings of the front femora of the two parental species (*B. carambolae* and *B. dorsalis*) was significantly different at 0.00±0.00 and 0.31±0.08 mm, respectively. Observation of the length of the black mark of the front femora of the F_1(A) and F_1(B) hybrids showed results of 0.24±0.12 and 0.20±0.12 mm.

DISCUSSION

This study showed that interspecific marriage between *B. carambolae* and *B. dorsalis* could produce offspring yet decreased fecundity. This degradation presumed was influenced by post-zygotic reproductive isolation. The genetic differences between the two different species will have an impact on the maturation process of the egg cell in hybridization[11]. The F_1 hybrid parent B. carambolae female and male *B. dorsalis* had the highest fecundity compared to other combinations. This was supported by the research of previous authors[4,7] which showed that the mating of female *B. carambolae* with male *B. dorsalis* has higher compatibility than reciprocal hybrid mating, which is 39 and 15%. The ability to have high compatibility of *B. dorsalis* males toward females of *B. carambolae* can be caused by the behaviour of males in consuming ME. Male interest in consuming ME is well-correlated with the sexual maturity of fruit flies. *B. carambolae* began to mate at the age of 2 weeks after "emergence" from the pupa stage and started consuming ME on the first day of mating. In contrast to *B. dorsalis*, which had experienced sexual maturity 2 weeks before mating, consuming ME was faster than *B. carambolae*[12]. Male imago attracted more female imago when they consume ME, therefore, *B. dorsalis* males have a higher population than *B. carambolae*[13]. Interspecific mating might happen if *B. carambolae* females are attracted to the presence of *B. dorsalis* naturally.

Interspecific mating of *B. carambolae* and *B. dorsalis* causes variations in the length of the aculeus, aedeagus and black markings of the fore femora in the hybrids. The length of hybrid aculeus with parent species was significantly different because *B. carambolae* and *B. dorsalis* did not have long slices on the aedeagus and aculeus[14]. The presence of long slices in the results of the observation resulted in no clear classification of length. This grouping is useful for viewing Mendel's law ratios. The length of the interspecific hybrid aculeus *B. carambolae* and *B. dorsalis* was divided F_2 into three groups, namely 1.621 ± 0.042 (*B. carambolae* zone), 1.777 ± 0.039 (hybrid zone) and 1.915 ± 0.022 mm (zone *B. dorsalis*) with a ratio of 10:7:25[14]. Therefore, it is known that morphological inheritance is influenced by a single locus. These data could be a reference for species identification, distribution and rearing methods for experiment purposes. But the data is still limited on the biology of interspecific hybrid species, but it is also necessary to know how to control these interspecific hybrid species, especially in agricultural quarantine treatment.

CONCLUSION

Current findings provide conclusive evidence that the *B. carambolae* and *B. dorsalis* hybrids have the potential to live in nature. The fecundity of F_1 hybrids increased compared to their hybrid parents and the fertility of the hybrids was in the range of 79.00-96.75%.

SIGNIFICANCE STATEMENT

These findings provide conclusive evidence as a claimed novelty that the interspecific of *Bactrocera* sp., potentially survives which contributes to a new species and the possibility of becoming a new pest in nature.

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