RESEARCH PAPER

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Suparman Suparman¹, Rafika Oktarida², Harman Hamidson¹, & Arsi Arsi¹

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ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of Banana Bunchy Top Virus (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and become important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, it was shorter than the one that of lived on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is a major pest of banana (Biale et al., 2017) and is the main vector of *Banana bunchy top virus* (BBTV). The role of the aphid as the vector of BBTV make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen & Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic

Corresponding author: Suparman Suparman (suparmanshk@gmail.com) areas appear on leaf margins (Elayabalan et al., 2015).

P. nigronervosa generally lives in association with banana plants worldwide, including tropical and subtropical regions (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 min and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant

¹Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir, South Sumatra, Indonesia 30662 ²Crop Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia 30662

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conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* due to parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25 °C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could influence morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

BBTV is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid, *P. nigronervosa* was taken from banana plant belong to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a $50 \times 50 \times 150$ cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and

successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of P. nigronervosa was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (Xanthosoma sagittifolium (L.) H.W. Schott & Endl), Bogor taro (Colocasia esculenta L. Schott), Pontianak taro (C. esculenta (L.) Schott), Japanese taro (C. esculenta var. antiquorum), Ornamental caladium (Caladium bicolor Vent), Wild taro (Colocasia sp.) and Rodent tuber (Typhonium flagelliforme (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa.* The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length and head width were measured accordingly to the method proposed by Bagariang et al. (2019). Body length was measured from the front to the cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of *Pentalonia nigronervosa*. Morphological characteristics of *P. nigronervosa* lived on Araceous species and banana were not significantly different from each other in the first instar, they started to vary from second instar. The body length of the aphid lived on araceous plants ranged from 0.70 to 0.84 mm in the second instar, 1.04 to 1.16 mm in the third instar and 1.23 to 1.30 mm in the fourth instar (Table 1). In the second and third instars, the average body length of the aphid in Bogor taro and rodent tuber were significantly different from that in banana. However, in the fourth instar, only the body length of the aphid lived in Belitung taro was significantly different from that in banana.

The measurement of head width of the aphid showed that the average head width of the aphid lived in some Araceous species were significantly different from the one that lived in banana (Table 2). In the second instar, the Head width in Bogor taro (0.43 mm) and Japanese taro (0.45 mm) were significantly different from that in banana (0.54 mm). In the third instar, the characteristics of aphid lived in Bogor taro (0.62 mm), Pontianak taro (0.62 mm), rodent tuber (0.67 mm), and ornamental caladium (0.67 mm) were significantly different from the one that lived in banana (0.79 mm). When the aphid reached the fourth instar, only the one that lived in Bogor taro (0.79 mm) and Pontianak taro (0,84 mm) that has different characteristics from the one that lived in banana.

The measurement of the aphid antennae also showed that significant differences of the average length of the antennae among host species was not consistent from instar to instar (Table 3). In the second instar, the aphid length of the antennae in Bogor taro (0.72 mm) and rodent tuber (0.72 mm) were significantly different from that in banana (0.84 mm). In the third instar, the length of the antennae in Bogor taro (1.07 mm), Japanese taro (1.08 mm) and rodent tuber (1.07 mm) were significantly different from that in banana (1.16 mm). In the fourth instar, the length of the antennae in Belitung taro (1.28 mm) and Pontianak taro (1.28 mm) were significantly different from that in banana (1.34 mm).

P. nigronervosa is a hemimetabolous insect

Table 1. Body length measurements of Pentalonia nigronervosa lived on various host plants

Heat analisa	Body length (mm)					
nost species	First instar ^{ns}	Second instar	Third instar	Fourth instar		
Bogor taro	0.37 ± 0.01	$0.70\pm0.02\ b$	$1.04\pm0.03\ b$	$1.25\pm0.01\ ab$		
Belitung taro	0.37 ± 0.01	$0.73\pm0.02 \text{ ab}$	$1.16\pm0.02 \text{ ab}$	$1.23\pm0.01\ b$		
Pontianak taro	0.36 ± 0.02	$0.81\pm0.02~a$	$1.12\pm0.01\ ab$	$1.24\pm0.005\ ab$		
Japanese taro	0.36 ± 0.02	$0.72\pm0.03\ ab$	$1.06\pm0.01\ b$	$1.27\pm0.01\ ab$		
Wild taro	0.39 ± 0.01	$0.72\pm0.04\ ab$	$1.16\pm0.02~a$	$1.30\pm0.02~a$		
Rodent tuber	0.38 ± 0.02	$0.70\pm0.02\ b$	$1.04\pm0.04\ b$	$1.24\pm0.03\ ab$		
Ornamental caladium	0.38 ± 0.01	$0.79\pm0.01\ ab$	$1.10\pm0.03~ab$	$1.25\pm0.02\ ab$		
Lady finger banana	0.38 ± 0.01	$0.84\pm0.04~a$	$1.16\pm0.01~a$	$1.30\pm0.02~a$		

^{ns}= not significant; Numbers followed by the same letter are not significantly different according to LSD 5%.

undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into adult stage. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor seemed to be the Araceous host causing significant difference on body length and head width size of the aphid compared to those living on banana, even though the difference of body length was only significant in second and third instar. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

The head width of *P. nigronervosa* was measured

as the representative of the aphid body width. Unlike the aphid abdomen which is elastic and getting bigger after feeding, the head of the aphid is rigid and its size does not change after feeding. In order to obtain the maximum head width, the measurement was taken across the aphid eyes. As presented in Table 2, the head width of *P. nigronervosa* lived on Araceous species varied in every instar except in the first instar, and the variation differed from instar to instar. In Araceous species, the head width of *P. nigronervosa* tent to be smaller than that in banana. Foottit et al. (2010) reported that the head width of *P. nigronervosa* was wider when it lived on Musaceae plants than when it lived on other hosts.

P. nigronervosa that lived on Araceous plant has similar or smaller body length and head width than those of the aphid lived on banana, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical

Table 2. Head width measurements of Pentalonia nigronervosa lived on various host plants

Host spacies	Head width (mm)						
flost species	First instar ^{ns}	Second instar	Third instar	Fourth instar			
Bogor taro	0.21 ± 0.01	$0.43\pm0.01\;b$	$0.62\pm0.03\ b$	$0.79\pm0.03\ b$			
Belitung taro	0.20 ± 0.01	$0.47\pm0.03\ ab$	$0.75\pm0.01\ ab$	$0.87\pm0.02\ ab$			
Pontianak taro	0.20 ± 0.01	$0.47\pm0.02\ ab$	$0.62\pm0.02\;b$	$0.84\pm0.03~\text{b}$			
Japanese taro	0.19 ± 0.02	$0.45\pm0.03\ b$	$0.74\pm0.03\ ab$	$0.98\pm0.03\ ab$			
Wild taro	0.21 ± 0.02	$0.46\pm0.03\ ab$	$0.76\pm0.04~a$	$1.01 \pm 0.05 \ a$			
Rodent tuber	0.23 ± 0.02	$0.38\pm0.02 \ ab$	$0.67\pm0.02\;b$	$0.93\pm0.07\;ab$			
Ornamental caladium	0.22 ± 0.02	$0.49\pm0.02\ ab$	$0.67\pm0.02\;b$	$0.97\pm004 \; ab$			
Lady finger banana	0.21 ± 0.02	0.54 ± 0.04 a	0.79 ± 0.04 a	1.00 ± 0.05 a			

^{ns}= not significant; Numbers followed by the same letter are not significantly different according to LSD 5%.

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Heat an anion	Length of antenna (mm)					
Host species –	First instar ^{ns}	Second instar	Third instar	Fourth instar ^{ns}		
Bogor taro	0.40 ± 0.02	$0.72\pm0.02\;b$	$1.07\pm0.03\ b$	1.31 ± 0.02		
Belitung taro	0.39 ± 0.02	$0.74\pm0.02\ ab$	$1.17\pm0.01~a$	1.28 ± 0.004		
Pontianak taro	0.37 ± 0.02	$0.82\pm0.02~a$	$1.14\pm0.01~ab$	1.28 ± 0.01		
Japanese taro	0.38 ± 0.02	$0.77\pm0.03~ab$	$1.08\pm0.01\ b$	1.31 ± 0.01		
Wild taro	0.42 ± 0.02	$0.75\pm0.05\;ab$	$1.18\pm0.01~a$	1.33 ± 0.01		
Rodent tuber	0.41 ± 0.02	$0.72\pm0.02\;b$	$1.07\pm0.03\ b$	1.29 ± 0.03		
Ornamental caladium	0.39 ± 0.01	$0.80\pm0.01\ ab$	$1.14\pm0.03\ ab$	1.30 ± 0.02		
Lady finger banana	0.39 ± 0.01	0.84 ± 0.04 a	1.16 ± 0.01 ab	1.34 ± 0.02		

¹⁵= not significant; Numbers followed by the same letter are not significantly different according to LSD 5%.

factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was assumed to have less influence to the aphid colony members.

The length of the antennae of P. nigronervosa was measured by using Milimeter v2.3.0 developed by Vis Tech. Project LLC after calibration. The antenna was removed from the aphid and put on the screen and and bound the length to see the result of measurement. The aphid antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of P. nigronervosa living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The length of antennae and body length of *P. nigronervosa* lived on Bogor taro and rodent tuber was shorter than that of the aphid lived on banana, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of P. nigronervosa living on various Araceous plants species was significantly different between-some species (Table 1, 2 and 3), but the other morphological characteristics such body shape was relatively the same (Figure 1). Based on visual observation during the aphid development, the color of the aphid bodies was getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to Tsuchida (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (Aphis craccivora) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on Pterochloroides persicae living on different hosts. The first instar of P. nigronervosa was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar antennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of P. *nigronervosa* antennae was about 1.01 to $1.35 \times its$ body length.

Biological Characteristics. Biological characteristics measured in the experiment were instar period, adult longevity, life span, and fecundity. Instar periods almost similar for each instar and there was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar period was 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana was always significantly longer than the period of the aphid living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition and more suitable to the need of banana aphid. Both nymph and adult of *P. nigronervosa* required

similar nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. *P. nigronervosa* actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 38.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of *P. nigronervosa* was reported to produce 33.4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous



Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. A. Bogor taro; B. Belitung taro; C. Pontianak taro; D. Japanese taro; E. Wild taro; F. Rodent tuber; G. Ornamental caladium; H. Banana.



Figure 2. The development of the antennae *Pentalonia nigronervosa*. A. First instar; B. Second instar; C. Third instar; D. Fourth instar.

plants, an adult female of *P. nigronervosa* could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of P. nigronervosa (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for *P. nigronervosa*, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support P. nigronervosa even better than banana plant. The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25 °C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of *P. nigronervosa* living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female (Table 5). The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different

Table 4. Nymph instar period of Pentalonia nigronervosa lived on various host plants

Hast Spacios	Instar period (days)					
nost species	First instar	Second instar	Third instar	Fourth instar		
Bogor Taro	$2.20\pm0.12\ b$	$2.10\pm0.09\ b$	$2.20\pm0.12\ b$	$3.10\pm0.09\ bc$		
Belitung Taro	$2.10\pm0.09\;b$	$2.10\pm0.09\ b$	$2.30\pm0.14\ b$	$3.50\pm0.15\ b$		
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09\ b$	$2.50\pm0.15\ b$	$3.00\pm0.20\ bc$		
Japanese Taro	$2.30\pm0.14\ b$	$2.50\pm0.15~a$	$2.90\pm0.09\;a$	$3.40\pm0.15\ b$		
Wild Taro	$2.10\pm0.09\ b$	$2.00\pm0.00\;b$	$2.30\pm0.14\ b$	$3.40\pm0.20\ b$		
Rodent Tuber	$2.20\pm0.12\ b$	$2.00\pm0.00\;b$	$2.40\pm0.15\ b$	$3.60\pm0.20\ b$		
Ornamental Caladium	$2.30\pm0.14\ b$	$2.50\pm0.15~a$	$2.10\pm0.00\;b$	$2.70\pm0.14\;c$		
Lady Finger Banana	$3.00 \pm 0.00 \text{ a}$	2.80 ± 0.12 a	3.00 ± 0.00 a	4.20 ± 0.25 a		

Number followed by the same letter are not significantly different according to LSD 5%.

Tał	bl	e	5.	The	repro	ductive	period	of	P	Pental	lonia	nigronervosa	on	various	host p	lants
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Host species	The reproductive period (days)	Life span (days)	Fecundity (nymph/female)
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a
Belitung Taro	$26.30\pm2.80\ b$	$36.50\pm2.94~b$	$37.70 \pm 3.49 \text{ bc}$
Pontianak Taro	$23.60\pm1.79~b$	$33.50\pm1.83~\text{b}$	$40.10\pm4.60\ bc$
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10~\text{b}$	$26.40 \pm 3.45 \text{ cd}$
Wild Taro	$23.90\pm1.85\ b$	$33.70\pm1.78~b$	33.60 ± 3.44 c
Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46~\text{b}$	$19.60 \pm 1.34 \text{ d}$
Ornamental Caladium	$21.90\pm1.17~b$	$31.20\pm1.16~\text{b}$	$40.10 \pm 3.92 \text{ bc}$
Lady Finger Banana	$32.70\pm0.63~ab$	44.60 ± 1.45 a	$51.50\pm1.36~b$

Numbers followed by the same letters are not significantly different according to LSD 5%.

temperature or rearing method as suggested by Xie et al. (2020). Data on the table Table 5 also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of P. nigronervosa has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of P. nigronervosa in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* was able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants were abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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AUTHORS' CONTRIBUTIONS

SS was the main contributor of this manuscript. SS and HH concepted and designed the experiment. RO and AA conducted survey for banana aphid collection. All authors involved in the laboratory and field experiments. SS prepared the manuscript, and all authors read and approved the final manuscript.

COMPETING INTERESTS

The Authors declare that there is no competing interest in the publication of this manuscript.

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[JHPTT:JTPPD] Revision from editor > Inbox ×



jhpt tropika <jhpt.tropika@fp.unila.ac.id>

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Dear Mr.Suparman

Berikut kami kirimkan naskah atas artikel bapak, ada beberapa perbaikan yang harus dilakukan pada naskah bapak

Atas perhatian dan kerjasama yang baik, kami sampaikan terimakasih.

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COVER LETTER

Dear Editor-in-Chief,

I am enclosing herewith a manuscript,

Title:

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Author(s) name:

Suparman Suparman, Rafika Oktarida, Harman Hamidson, Arsi Arsi

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya Phone 081532736903, enail suparmanshk@gmail.com

Type of article <u>: a. Research Paper</u> b. Short Communication c. Review

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For publication in the Jurnal Hama dan Penyakit Tumbuhan Tropika: Jurnal of Tropical Plant Pests and Diseases

Novelty:

(state your claimed novelty of the findings versus current knowledge)

Pentalonia nigronervosa has been known to have various alternative hosts, however the morphometric and biological characteristics of the aphid in Araceous plants species as its alternative hosts have not been studied. In this researched, both characteristics were studied and reported.

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Title page

Type of manuscript: research paper (research paper, short communication, review)

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Suparman Suparman", Rafika Oktarida², Harman Hamidson¹, Arsi Arsi¹

Department of Plant Protection, Faculty of Agriculture, Universitas Lampung. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir, South Sumatra, Indonesia 30662.

Crop Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia 30662.

Corresponding author: Suparman Suparman (suparmanshk@gmail.com)

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AUTHORS' CONTRIBUTIONS

SS was the main contributor of this manuscript. SS and HH concepted and designed the experiment. RO and AA conducted survey for banana aphid collection. All authors involved in the laboratory and field experiments. SS prepared the manuscript, and all authors read and approved the final manuscript.

COMPETING INTEREST

The Authors declare that there is no competing interest in the publication of this manuscript

Main text

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is not seriously damaging to banana plant even though the aphid is considered as a major pest of banana (Biale et al., 2017). The role of the aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen and Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants in tropical areas, but the aphid had also been widely distributed in tropical and subtropical regions worldwide (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25°C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days

and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro (*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia* sp.) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the

cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect that undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into imago. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. Aphid imagoes do not molt. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor taro and Rodent tuber seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by

inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar natennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar periods as 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana and those living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of P. nigronervosa could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of *P. nigronervosa* (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support *P. nigronervosa* even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of P. nigronervosa living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female. The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of P. nigronervosa has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Hast Spacios	Body Length					
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar		
Bogor Taro	$0,\!37\pm0,\!01$	$0{,}70\pm0{,}02~b$	$1,04 \pm 0,03 \text{ b}$	$1,25 \pm 0,01$ ab		
Belitung Taro	$\textbf{0,37} \pm \textbf{0,01}$	$0{,}73\pm0{,}02ab$	$1,\!16\pm0,\!02$ ab	$1{,}23\pm0{,}01\text{ b}$		
Pontianak Taro	$0,\!36\pm0,\!02$	$0{,}81\pm0{,}02a$	$1,\!12\pm0,\!01$ ab	$1{,}24\pm0{,}005~ab$		
Japanese Taro	$0,\!36\pm0,\!02$	$0,\!72\pm0,\!03ab$	$\textbf{1,06} \pm \textbf{0,01} \text{ b}$	$1,\!27\pm0,\!01~ab$		
Wild Taro	$0,\!39\pm0,\!01$	0,72±0,04 ab	$1,16 \pm 0,02$ a	$1{,}30\pm0{,}02~a$		
Rodent Tuber	$0,\!38\pm0,\!02$	$0{,}70\pm0{,}02~b$	$1{,}04\pm0{,}04~b$	$1{,}24\pm0{,}03~ab$		
Ornamental Caladium	$0,\!38\pm0,\!01$	$0{,}79\pm0{,}01ab$	$1,\!10\pm0,\!03$ ab	$1{,}25\pm0{,}02 \text{ ab}$		
Lady Finger Banana	$0{,}38\pm0{,}01$	$0,\!84\pm0,\!04a$	$1,16 \pm 0,01$ a	$1{,}30\pm0{,}02~a$		

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 2. Head width measurements of Pentalonia nigronervosa lived on various host plants

Host Spacios	Head width					
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar		
Bogor Taro	$0,\!21\pm0,\!01$	$0{,}43\pm0{,}01\text{ b}$	$0{,}62\pm0{,}03\text{ b}$	$0{,}79\pm0{,}03\text{ b}$		
Belitung Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}03~ab$	$0{,}75\pm0{,}01~ab$	$0,\!87\pm0,\!02ab$		
Pontianak Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}02~ab$	$0{,}62\pm0{,}02\text{ b}$	$0{,}84\pm0{,}03\text{ b}$		
Japanese Taro	$0,\!19\pm0,\!02$	$0{,}45\pm0{,}03\text{ b}$	$0{,}74\pm0{,}03$ ab	$0{,}98\pm0{,}03~ab$		
Wild Taro	$0{,}21\pm0{,}02$	$0{,}46\pm0{,}03~ab$	$0,76\pm0,04~a$	$1{,}01\pm0{,}05~a$		
Rodent Tuber	$0{,}23\pm0{,}02$	$0{,}38\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}93\pm0{,}07~ab$		
Ornamental Caladium	$0{,}22\pm0{,}02$	$0{,}49\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}97\pm0{,}04~ab$		
Lady Finger Banana	$0{,}21\pm0{,}02$	$0{,}54\pm0{,}04~a$	$0,\!79\pm0,\!04~a$	$1{,}00\pm0{,}05~a$		

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Heat Species	Length of antenna (mm)						
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar			
Bogor Taro	$0{,}40\pm0{,}02$	$0{,}72\pm0{,}02~\mathrm{b}$	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$			
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,\!28 \pm 0,\!004$			
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28 \pm 0,\!01$			
Japanese Taro	$0,\!38\pm0,\!02$	$0,77 \pm 0,03$ ab	$1,08 \pm 0,01$ b	$1,31 \pm 0,01$			
Wild Taro	$0,\!42 \pm 0,\!02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,33 \pm 0,01$			
Rodent Tuber	$0,\!41 \pm 0,\!02$	$0,72 \pm 0,02 \text{ b}$	$1,07 \pm 0,03$ b	$1,\!29 \pm 0,\!03$			
Ornamental Caladium	$0,\!39\pm0,\!01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,30 \pm 0,02$			
Lady Finger Banana	$0,\!39\pm0,\!01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,\!34\pm0,\!02$			
Note: figures followed by the same letter are not significantly different according to LSD 5%							

Table 4. Nymph instar period of *Pentalonia nigronervosa* lived on various host plants

Instar period (day)						
1 st instar	2 nd instar	3 rd instar	4 th instar			
$2.20\pm0.12\ b$	$2.10\pm0.09~b$	$2.20\pm0.12\ b$	$3.10\pm0.09~bc$			
$2.10\pm0.09\ b$	$2.10\pm0.09~b$	$2.30\pm0.14\ b$	$3.50\pm0.15\ b$			
$2.30\pm0.14\ b$	$2.10\pm0.09~b$	$2.50\pm0.15\ b$	$3.00\pm0.20~bc$			
$2.30\pm0.14\ b$	2.50 ± 0.15 a	2.90 ± 0.09 a	$3.40\pm0.15\ b$			
$2.10\pm0.09\ b$	$2.00\pm0.00\;b$	$2.30\pm0.14\ b$	$3.40\pm0.20\ b$			
$2.20\pm0.12\ b$	$2.00\pm0.00\;b$	$2.40\pm0.15\ b$	$3.60\pm0.20\ b$			
$2.30\pm0.14\ b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	$2.70\pm0.14~\mathrm{c}$			
$3.00\pm0.00\;a$	2.80 ± 0.12 a	$3.00\pm0.00\ a$	$4.20\pm0.25~a$			
	$\begin{array}{c} 1^{\rm st} {\rm instar} \\ 2.20 \pm 0.12 \ {\rm b} \\ 2.10 \pm 0.09 \ {\rm b} \\ 2.30 \pm 0.14 \ {\rm b} \\ 2.30 \pm 0.14 \ {\rm b} \\ 2.10 \pm 0.09 \ {\rm b} \\ 2.20 \pm 0.12 \ {\rm b} \\ 2.30 \pm 0.14 \ {\rm b} \\ 3.00 \pm 0.00 \ {\rm a} \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $			

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 5. The reproductive period of Pentalonia nigronervosa on various host plants

Host spacios	The reproductive period	Life span	Fecundity
riost species	(day)	(day)	(nymph/female)
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a
Belitung Taro	$26.30\pm2.80~b$	$36.50\pm2.94~b$	37.70 ± 3.49 bc
Pontianak Taro	$23.60\pm1.79~b$	$33.50\pm1.83~b$	$40.10\pm4.60~bc$
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10~b$	$26.40 \pm 3.45 \text{ cd}$
Wild Taro	$23.90\pm1.85~b$	$33.70\pm1.78~b$	33.60 ± 3.44 c
Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46~b$	$19.60 \pm 1.34 \text{ d}$
Ornamental Caladium	$21.90\pm1.17~b$	$31.20\pm1.16~b$	40.10 ± 3.92 bc
Lady Finger Banana	32.70 ± 0.63 ab	44.60 ± 1.45 a	$51.50\pm1.36~\text{b}$

Note: figures followed by the same letters are not significantly different according to LSD 5%



Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. [A] Bogor taro, [B] Belitung taro, [C] Pontianak taro, [D] Japanese taro, [E] Wild taro, [F] Rodent tuber, [G] Ornamental caladium, [H] Banana.



Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.



COVER LETTER

Dear Editor-in-Chief,

I am enclosing herewith a manuscript,

Title:

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Author(s) name:

Suparman Suparman, Rafika Oktarida, Harman Hamidson, Arsi Arsi

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya Phone 081532736903, email suparmanshk@gmail.com

Type of article <u>: a. Research Paper</u> b. Short Communication c. Review

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For publication in the Jurnal Hama dan Penyakit Tumbuhan Tropika: Jurnal of Tropical Plant Pests and Diseases

Novelty:

(state your claimed novelty of the findings versus current knowledge)

Pentalonia nigronervosa has been known to have various alternative hosts, however the morphometric and biological characteristics of the aphid in Araceous plants species as its alternative hosts have not been studied. In this researched, both characteristics were studied and reported.

Statements:

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Suparman Suparman

Title page

Type of manuscript: research paper (research paper, short communication, review)

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Suparman Suparman", Rafika Oktarida², Harman Hamidson¹, Arsi Arsi¹

Department of Plant Protection, Faculty of Agriculture, Universitas Lampung. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir, South Sumatra, Indonesia 30662.

Crop Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia 30662.

Corresponding author: Suparman Suparman (suparmanshk@gmail.com)

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AUTHORS' CONTRIBUTIONS

SS was the main contributor of this manuscript. SS and HH concepted and designed the experiment. RO and AA conducted survey for banana aphid collection. All authors involved in the laboratory and field experiments. SS prepared the manuscript, and all authors read and approved the final manuscript.

COMPETING INTEREST

The Authors declare that there is no competing interest in the publication of this manuscript

Main text

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is not seriously damaging to banana plant even though the aphid is considered as a major pest of banana (Biale et al., 2017). The role of the aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen and Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants in tropical areas, but the aphid had also been widely distributed in tropical and subtropical regions worldwide (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25°C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days

and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro (*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia* sp.) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the

cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect that undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into imago. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. Aphid imagoes do not molt. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor taro and Rodent tuber seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by

inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar natennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar periods as 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana and those living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of P. nigronervosa could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of *P. nigronervosa* (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support *P. nigronervosa* even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of P. nigronervosa living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female. The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of P. nigronervosa has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Table

Table 1. Body length measurements of Pentalonia nigronervosa lived on various host plants

Heat Species	Body Length			
Host species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0,\!37\pm0,\!01$	$0{,}70\pm0{,}02~b$	$1{,}04\pm0{,}03~b$	$1{,}25\pm0{,}01~ab$
Belitung Taro	$0,\!37\pm0,\!01$	$0{,}73\pm0{,}02ab$	$1,\!16\pm0,\!02$ ab	$1{,}23\pm0{,}01\text{ b}$
Pontianak Taro	$0,\!36\pm0,\!02$	$0,\!81\pm0,\!02a$	$1,\!12\pm0,\!01~ab$	$1{,}24\pm0{,}005 \text{ ab}$
Japanese Taro	$0,\!36\pm0,\!02$	$0{,}72\pm0{,}03ab$	$1{,}06\pm0{,}01~b$	$1{,}27\pm0{,}01~ab$
Wild Taro	$0,\!39\pm0,\!01$	0,72±0,04 ab	$1,16 \pm 0,02$ a	$1{,}30\pm0{,}02~a$
Rodent Tuber	$0{,}38\pm0{,}02$	$0{,}70\pm0{,}02~b$	$1{,}04\pm0{,}04~b$	$1{,}24\pm0{,}03~ab$
Ornamental Caladium	$0{,}38\pm0{,}01$	$0{,}79\pm0{,}01ab$	$1,\!10\pm0,\!03$ ab	$1{,}25\pm0{,}02 \text{ ab}$
Lady Finger Banana	$\textbf{0,38} \pm \textbf{0,01}$	$0,\!84\pm0,\!04a$	$1,16 \pm 0,01$ a	$1,\!30\pm0,\!02~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 2. Head width measurements of Pentalonia nigronervosa lived on various host plants

Hast Spacios	Head width			
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0{,}21\pm0{,}01$	$0{,}43\pm0{,}01\text{ b}$	$0{,}62\pm0{,}03\text{ b}$	$0{,}79\pm0{,}03\text{ b}$
Belitung Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}03~ab$	$0,75\pm0,01~ab$	$0,\!87\pm0,\!02ab$
Pontianak Taro	$0,\!20\pm0,\!01$	$0,\!47\pm0,\!02~ab$	$0{,}62\pm0{,}02~b$	$0{,}84\pm0{,}03\text{ b}$
Japanese Taro	$0,\!19\pm0,\!02$	$0{,}45\pm0{,}03\text{ b}$	$0,74\pm0,03$ ab	$0{,}98 \pm 0{,}03 \text{ ab}$
Wild Taro	$0{,}21\pm0{,}02$	$0{,}46\pm0{,}03~ab$	$0,76\pm0,04~a$	$1{,}01\pm0{,}05~a$
Rodent Tuber	$0{,}23\pm0{,}02$	$0{,}38\pm0{,}02~ab$	$0{,}67\pm0{,}02~b$	$0{,}93\pm0{,}07~ab$
Ornamental Caladium	$0{,}22\pm0{,}02$	$0{,}49\pm0{,}02~ab$	$0{,}67\pm0{,}02~b$	$0{,}97\pm0{,}04~ab$
Lady Finger Banana	$0{,}21\pm0{,}02$	$0{,}54\pm0{,}04~a$	$0,\!79\pm0,\!04~a$	$1{,}00\pm0{,}05~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Heat Species	Length of antenna (mm)			
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0{,}40\pm0{,}02$	$0{,}72\pm0{,}02~\mathrm{b}$	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,\!28 \pm 0,\!004$
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28 \pm 0,\!01$
Japanese Taro	$0,\!38\pm0,\!02$	$0,77 \pm 0,03$ ab	$1,08 \pm 0,01$ b	$1,31 \pm 0,01$
Wild Taro	$0,\!42 \pm 0,\!02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,33 \pm 0,01$
Rodent Tuber	$0,\!41 \pm 0,\!02$	$0,72 \pm 0,02 \text{ b}$	$1,07 \pm 0,03$ b	$1,29 \pm 0,03$
Ornamental Caladium	$0,\!39\pm0,\!01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,30 \pm 0,02$
Lady Finger Banana	$0,\!39\pm0,\!01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,\!34\pm0,\!02$
Note: figures followed by the same letter are not significantly different according to LSD 5%				

Table 4. Nymph instar period of *Pentalonia nigronervosa* lived on various host plants

II+ C	Instar period (day)			
Host species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$2.20\pm0.12\ b$	$2.10\pm0.09~b$	$2.20\pm0.12~\text{b}$	$3.10\pm0.09~bc$
Belitung Taro	$2.10\pm0.09~b$	$2.10\pm0.09~b$	$2.30\pm0.14\ b$	$3.50\pm0.15\ b$
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09~b$	$2.50\pm0.15~b$	$3.00\pm0.20\ bc$
Japanese Taro	$2.30\pm0.14~b$	2.50 ± 0.15 a	2.90 ± 0.09 a	$3.40\pm0.15~b$
Wild Taro	$2.10\pm0.09~b$	$2.00\pm0.00\ b$	$2.30\pm0.14\ b$	$3.40\pm0.20\ b$
Rodent Tuber	$2.20\pm0.12~b$	$2.00\pm0.00\ b$	$2.40\pm0.15~b$	$3.60\pm0.20\ b$
Ornamental Caladium	$2.30\pm0.14~b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	$2.70\pm0.14~\mathrm{c}$
Lady Finger Banana	$3.00\pm0.00~a$	2.80 ± 0.12 a	$3.00\pm0.00\ a$	4.20 ± 0.25 a

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 5. The reproductive period of Pentalonia nigronervosa on various host plants

Host species	The reproductive period	Life span	Fecundity
	(day)	(day)	(nymph/female)
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a
Belitung Taro	$26.30 \pm 2.80 \text{ b}$	$36.50\pm2.94~b$	37.70 ± 3.49 bc
Pontianak Taro	$23.60 \pm 1.79 \text{ b}$	$33.50\pm1.83~b$	$40.10 \pm 4.60 \text{ bc}$
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10\ b$	$26.40 \pm 3.45 \text{ cd}$
Wild Taro	$23.90 \pm 1.85 \text{ b}$	$33.70\pm1.78~b$	33.60 ± 3.44 c
Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46~b$	$19.60 \pm 1.34 \text{ d}$
Ornamental Caladium	$21.90 \pm 1.17 \text{ b}$	$31.20\pm1.16~b$	$40.10 \pm 3.92 \text{ bc}$
Lady Finger Banana	32.70 ± 0.63 ab	44.60 ± 1.45 a	$51.50\pm1.36~\text{b}$

Note: figures followed by the same letters are not significantly different according to LSD 5%


Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. [A] Bogor taro, [B] Belitung taro, [C] Pontianak taro, [D] Japanese taro, [E] Wild taro, [F] Rodent tuber, [G] Ornamental caladium, [H] Banana.



Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.



Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

Suparman Suparman¹, Rafika Oktarida², Harman Hamidson¹, Arsi Arsi¹

Department of Plant Protection, Faculty of Agriculture, Universitas Lampung. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir, South Sumatra, Indonesia 30662.

Crop Sciences Graduate Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia 30662.

Corresponding author: Suparman Suparman (suparmanshk@gmail.com)

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AUTHORS' CONTRIBUTIONS

SS was the main contributor of this manuscript. SS and HH concepted and designed the experiment. RO and AA conducted survey for banana aphid collection. All authors involved in the laboratory and field experiments. SS prepared the manuscript, and all authors read and approved the final manuscript.

COMPETING INTEREST

The Authors declare that there is no competing interest in the publication of this manuscript

Main text

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is not seriously damaging to banana plant even though the aphid is considered as a major pest of banana (Biale et al., 2017). The role of the aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen and Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants in tropical areas, but the aphid had also been widely distributed in tropical and subtropical regions worldwide (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25°C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days

and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro (*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia* sp.) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the

cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect that undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into imago. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. Aphid imagoes do not molt. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor taro and Rodent tuber seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by

inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar natennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar periods as 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana and those living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of P. nigronervosa could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of *P. nigronervosa* (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support *P. nigronervosa* even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of P. nigronervosa living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female. The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of P. nigronervosa has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is not seriously damaging to banana plant even though the aphid is considered as a major pest of banana (Biale et al., 2017). The role of the

aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen and Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants in tropical areas, but the aphid had also been widely distributed in tropical and subtropical regions worldwide (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25°C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a $50 \times 50 \times 150 \text{ cm}^3$ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered

with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro (*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia esculenta* var. antiquorum), Gramental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia esculenta* var. antiquorum), and egenotype of banana vas collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect that undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into imago. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. Aphid imagoes do not molt. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor taro and Rodent tuber seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the

nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar instar antennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar period was 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araccous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* longer than the period of the aphid living on Araceous host. The difference of instar period between banana aphids living on banana and those living on

Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition and more suitable to the need of banana aphid. Both nymph and adult of *P. nigronervosa* required similar nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of P. nigronervosa could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of *P. nigronervosa* (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support P. nigronervosa even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of *P. nigronervosa* living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female. The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of *P. nigronervosa* living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of *P. nigronervosa* has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Table

Table 1. Body length measurements of Pentalonia nigronervosa lived on various host plants

Host Spacios		Body	Length	
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0,\!37\pm0,\!01$	$0{,}70\pm0{,}02\text{ b}$	$1{,}04\pm0{,}03\text{ b}$	$1{,}25\pm0{,}01~ab$
Belitung Taro	$0,\!37\pm0,\!01$	$0{,}73\pm0{,}02ab$	$1,\!16\pm0,\!02$ ab	$1{,}23\pm0{,}01\text{ b}$
Pontianak Taro	$0{,}36\pm0{,}02$	$0,\!81 \pm 0,\!02a$	$1,12 \pm 0,01 \text{ ab}$	$1{,}24\pm0{,}005~ab$
Japanese Taro	$0{,}36\pm0{,}02$	$0{,}72\pm0{,}03ab$	$1{,}06\pm0{,}01\text{ b}$	$1,27 \pm 0,01 \text{ ab}$
Wild Taro	$0{,}39\pm0{,}01$	$0{,}72{\pm}0{,}04$ ab	$1,16 \pm 0,02$ a	$1{,}30\pm0{,}02~a$
Rodent Tuber	$0{,}38\pm0{,}02$	$0{,}70\pm0{,}02\text{ b}$	$1{,}04\pm0{,}04~b$	$1{,}24\pm0{,}03$ ab
Ornamental Caladium	$0{,}38 \pm 0{,}01$	$0,\!79\pm0,\!01ab$	$1,\!10\pm0,\!03$ ab	$1{,}25\pm0{,}02~ab$
Lady Finger Banana	$\textbf{0,38} \pm \textbf{0,01}$	$0,\!84\pm0,\!04a$	$1,16 \pm 0,01$ a	$1,\!30\pm0,\!02~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

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Table 7	Head	width	measurements	ot.	Pontalonia	nigranervasa	lived	on	Various	host	nlants
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Heat Straging	Head width				
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar	
Bogor Taro	$0{,}21\pm0{,}01$	$0{,}43\pm0{,}01\text{ b}$	$0{,}62\pm0{,}03\text{ b}$	$0{,}79\pm0{,}03\text{ b}$	
Belitung Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}03~ab$	$0{,}75\pm0{,}01~ab$	$0,\!87\pm0,\!02ab$	
Pontianak Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}02~ab$	$0{,}62\pm0{,}02\text{ b}$	$0{,}84\pm0{,}03\text{ b}$	
Japanese Taro	$0,\!19\pm0,\!02$	$0{,}45\pm0{,}03\text{ b}$	$0{,}74\pm0{,}03$ ab	$0{,}98\pm0{,}03~ab$	
Wild Taro	$0{,}21\pm0{,}02$	$0{,}46\pm0{,}03~ab$	$0{,}76\pm0{,}04~a$	$1{,}01\pm0{,}05~a$	
Rodent Tuber	$0{,}23\pm0{,}02$	$0{,}38\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}93\pm0{,}07~ab$	
Ornamental Caladium	$0{,}22\pm0{,}02$	$0{,}49\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}97\pm0{,}04~ab$	
Lady Finger Banana	$0{,}21\pm0{,}02$	$0{,}54\pm0{,}04~a$	$0{,}79\pm0{,}04~a$	$1{,}00\pm0{,}05~a$	

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Heat Spacing	Length of antenna (mm)				
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar	
Bogor Taro	$0{,}40\pm0{,}02$	$0,72\pm0,02$ b	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$	
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,\!28 \pm 0,\!004$	
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28\pm0,\!01$	
Japanese Taro	$0,\!38\pm0,\!02$	$0,77 \pm 0,03$ ab	$1,08 \pm 0,01 \text{ b}$	$1,31 \pm 0,01$	
Wild Taro	$0{,}42\pm0{,}02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,33 \pm 0,01$	
Rodent Tuber	$0{,}41\pm0{,}02$	$0,72\pm0,02~\mathrm{b}$	$1,07 \pm 0,03$ b	$1,\!29 \pm 0,\!03$	
Ornamental Caladium	$0,\!39\pm0,\!01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,30 \pm 0,02$	
Lady Finger Banana	$0,\!39\pm0,\!01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,34 \pm 0,02$	

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 4. Nymph instar period of Pentalonia nigronervosa lived on various host plants

Hard Caralian	Instar period (day)				
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar	
Bogor Taro	$2.20\pm0.12\ b$	$2.10\pm0.09\ b$	$2.20\pm0.12\ b$	$3.10\pm0.09~bc$	
Belitung Taro	$2.10\pm0.09~b$	$2.10\pm0.09\ b$	$2.30\pm0.14\ b$	$3.50\pm0.15\ b$	
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09\ b$	$2.50\pm0.15\ b$	$3.00\pm0.20~bc$	
Japanese Taro	$2.30\pm0.14\ b$	2.50 ± 0.15 a	$2.90\pm0.09~a$	$3.40\pm0.15\ b$	
Wild Taro	$2.10\pm0.09~b$	$2.00\pm0.00\ b$	$2.30\pm0.14\ b$	$3.40\pm0.20\ b$	
Rodent Tuber	$2.20\pm0.12~\text{b}$	$2.00\pm0.00\ b$	$2.40\pm0.15~b$	$3.60\pm0.20\ b$	
Ornamental Caladium	$2.30\pm0.14~b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	$2.70\pm0.14~\mathrm{c}$	
Lady Finger Banana	$3.00\pm0.00\;a$	$2.80\pm0.12\ a$	$3.00\pm0.00\ a$	$4.20\pm0.25\ a$	
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Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 5. The reproductive period of Pentalonia nigronervosa on various host plants

Host species	The reproductive period	Life span	Fecundity
riost species	(day)	(day)	(nymph/female)
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a
Belitung Taro	$26.30\pm2.80\ b$	$36.50\pm2.94~b$	37.70 ± 3.49 bc
Pontianak Taro	$23.60 \pm 1.79 \text{ b}$	$33.50\pm1.83~b$	$40.10 \pm 4.60 \text{ bc}$
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10~\text{b}$	26.40 ± 3.45 cd
Wild Taro	$23.90\pm1.85~\text{b}$	$33.70\pm1.78\ b$	33.60 ± 3.44 c

Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46~\text{b}$	$19.60 \pm 1.34 \text{ d}$
Ornamental Caladium	$21.90\pm1.17~b$	$31.20\pm1.16~b$	$40.10 \pm 3.92 \text{ bc}$
Lady Finger Banana	$32.70\pm0.63~ab$	44.60 ± 1.45 a	$51.50\pm1.36~b$
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Note: figures followed by the same letters are not significantly different according to LSD 5%



Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. [A] Bogor taro, [B] Belitung taro, [C] Pontianak taro, [D] Japanese taro, [E] Wild taro, [F] Rodent tuber, [G] Ornamental caladium, [H] Banana.



Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.



Manuscript Evaluation Report- Referee 1

Manuscript ID	: 613
Manuscript Title	:Morphometrics and biological characteristics of <i>Pentalonia nigronervosa</i> , the vector of <i>Banana Bunchy Top Virus</i> , living on various Araceous plants spe

 Type of Article
 : Short Communication
 Research Paper

PART A: Comments per Section of Manuscript:

Abstract	The conclusion "The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV." is not supported by sufficient data shown in this
Introduction	Ok with minor editorial revision. All references cited were listed in REFERENCES, but 2 ref listed on
Methodology	Sufficient.

Results	Morphometrics of P. nigronervosa: Paragraph 2 need more description based on data on Table 1 including the description/note that significant difference occurred on data of 2 nd 3 rd and 4 th instars. In addition to Bogor taro and Rodent tuber, Japanese taro also reduced body length, but not mentioned. Table 2 has not been explained in the taxt. Biological characteristics: 2 nd line: it was writted " no significant difference." but the data a Table 4 shows in jifeget difference.
Discussion and Conclusion	Need more thorough discussion in order to make more meaning out of the data obtained from this research.

	Conclusion : 2 nd line: it was writted " were not significant different from".
	But data on Table 1, 2, 3, 4, and 5 show significant difference.
	This part needs also major revision.
References	All references cited were listed in REFERENCES, but 2 ref listed on REFERENCES (Braendle et al, 2006 and Moran, 1992) were not cited on the text.

PART B: Re commend ation (Kindly Mark With A "V")

Acceptable	
Acceptable with Minor Revision	\checkmark
Reconsidered after Major Revision	
Rejected	

PART C: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

This manuscript could be considered to be published in this journal as data documented here are valuable.

Minor editorial revision on

Attachment: Table 1 and 2 need unit

of measurements.

Figure 1: the symbols and letter are too small, unclear.

-

Main text

Morphometrics and biological characteristics of *Pentalonia* nigronervosa, the vector of Banana Bunchy Top Virus, living on various Araceous plants species

ABSTRACT

Banana aphid Pentalonia nigronervosa Coquerel (Hemiptera: Aphididae) is widely known as the main vector of Banana Bunchy Top Virus (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of P. nigronervosa living on Araceous plants was shorter than that of the aphids living on a banana plants. P. nigronervosa was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of P. nigronervosa indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBIV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid Pentalonia nigronervosa Coquerel (Hemiptera: Aphididae) is not seriously damaging to banana plant even though the aphid is considered as a major pest of banana (Biale et al., 2012). The role of the aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen and Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants in tropical areas, by the aphid had also been widely distributed in tropical and subtropical regions worldwide (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P*. nigronervosa could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017) *P. nigronervosal* ansmit BBTV in a circulative manner through the feeding activity (Myongere et

al., 2013; Jebakupar et al., 2018; Jekayinoluva et al., 2021). The virus particles enter the aphid body

together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25°C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015). Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmite BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The

Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro (*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia sp.*) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the treat to the cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect that undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the term into imago. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphopass 4 nymph instars. Aphid imagoes do not molt. The measurement of body length (Table 1) and heat width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first moltary produces and there instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymons living on banana and those living on some other hosts was found in the next three instars. Bogut taro and Rodent tuber seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those

living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Randarishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was ferr reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aplie color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar antennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length bit *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar period was 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of P. nigronervosa live on banana were in accordance with the report by Padmanaban (2018) that *P/nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of P. nigronervosa living on banana was always significantly longer than the period of the aphid living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition and more suitable to the need of banana aphid. Both nymph and adult of P. nigronervosa required similar nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of *P. nigronervosa* was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of *P. nigronervosa* could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of *P. nigronervosa* (Suparman et al., 2017), even though the fecunatty of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to *P*. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fectuality, *P. nigronervosa* living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support P. nigronervosa even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 300 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasabramanian 2013; Chakraborty et al., 2021).

The averages life span of P. nigronervosa living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to \$8.30 nymphs per female. The results were in wider ranges compared to those reported by Padmanaban (2018) that the life spin of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of *P. nigronervosa* has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Table				
Table 1. Body length measuren	nents of Pentalonia	a nigronervosa live	ed on various host pl	ants
		Body Le	ength (MM	
Host Species —	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0,\!37\pm0,\!01$	0,70 ± 0,02 b	$1,04 \pm 0,03 \text{ b}$	$1,25 \pm 0,01$ ab
Belitung Taro	$0,\!37\pm0,\!01$	0,73 ± 0.02ab	1,16 \pm 0,02 ab	1.22 ± 0.01 b
Pontianak Taro	$0,\!36\pm0,\!02$	0,81 ± 0,02a	$1,12 \pm 0,01$ ab	$1,24 \pm 0,005$ ab
Japanese Taro	$0,\!36\pm0,\!02$	$0,\!72\pm0,\!03ab$	$1,\!06\pm0,\!01~b$	$1,\!27\pm0,\!01~ab$
Wild Taro	$0{,}39\pm0{,}01$	0,72± 0,04 ab	$1,16 \pm 0,02$ a	$1,\!30\pm0,\!02~a$
Rodent Tuber	$\textbf{0,38} \pm \textbf{0,02}$	$0{,}70\pm0{,}02\text{ b}$	$1,\!04\pm0,\!04~b$	$1{,}24\pm0{,}03~ab$
Ornamental Caladium	$0{,}38\pm0{,}01$	$0{,}79\pm0{,}01ab$	$1{,}10\pm0{,}03~ab$	$1{,}25\pm0{,}02~ab$
Lady Finger Banana	$0,\!38\pm0,\!01$	$\textbf{0,84} \pm \textbf{0,04a}$	$1,16 \pm 0,01$ a	$1,\!30\pm0,\!02~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 2. Head width measur	ements of Pentalo	onia nigronervosta liv	ved on various host I	plants	
Host Species		Head width			
	1 st instar	2 nd instar	3 rd instar	4 th instar	
Bogor Taro	$0,\!21\pm0,\!01$	$0,43 \pm 0,01$ b	$0,62 \pm 0,03$ b	$0,79 \pm 0,03$ b	
Belitung Taro	$0{,}20\pm0{,}01$	$0,\!47 \pm 0,\!03$ ab	0,75 ± 0,01 ab	$0,87\pm0,02$ ab	
Pontianak Taro	$0{,}20\pm0{,}01$	$0,\!47\pm0,\!02~ab$	$0{,}62\pm0{,}02\text{ b}$	$0{,}84\pm0{,}03~b$	
Japanese Taro	$0,\!19\pm0,\!02$	$0{,}45\pm0{,}03\text{ b}$	$0{,}74\pm0{,}03~ab$	$0{,}98\pm0{,}03~ab$	
Wild Taro	$0{,}21\pm0{,}02$	$0,\!46\pm0,\!03$ ab	$0{,}76\pm0{,}04~\mathrm{a}$	$1{,}01\pm0{,}05~a$	
Rodent Tuber	$0{,}23\pm0{,}02$	$0{,}38\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}93\pm0{,}07~ab$	
Ornamental Caladium	$0,\!22\pm0,\!02$	$0{,}49\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0,\!97\pm0,\!04$ ab	
Lady Finger Banana	$0{,}21\pm0{,}02$	$0{,}54\pm0{,}04~a$	$0{,}79\pm0{,}04~a$	$1{,}00\pm0{,}05~a$	

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

II. (C		Length of ant	enna (mm)	
Host Species –	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0{,}40\pm0{,}02$	$0,72 \pm 0,02$ b	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,\!28\pm0,\!004$
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28\pm0,\!01$
Japanese Taro	$0,\!38\pm0,\!02$	$0,77\pm0,03$ ab	$1,\!08\pm0,\!01~\mathrm{b}$	$1,\!31\pm0,\!01$

Wild Taro	$0,\!42 \pm 0,\!02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,33 \pm 0,01$
Rodent Tuber	$0{,}41\pm0{,}02$	$0,72 \pm 0,02 \text{ b}$	$1,07 \pm 0,03$ b	$1,\!29\pm0,\!03$
Ornamental Caladium	$0,39 \pm 0,01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,30 \pm 0,02$
Lady Finger Banana	$0,39 \pm 0,01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,34 \pm 0,02$
Note: figures followed by the same letter are not significantly different according to LSD 5%				

Table 4. Nymph instar period of Pentalonia nigronervosa lived on various host plants

Host Species -	Instar period (day)			
	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$2.20\pm0.12~b$	$2.10\pm0.09~b$	$2.20\pm0.12~b$	$3.10\pm0.09~bc$
Belitung Taro	$2.10\pm0.09~b$	$2.10\pm0.09~b$	$2.30\pm0.14~b$	$3.50\pm0.15~b$
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09~b$	$2.50\pm0.15~b$	$3.00\pm0.20~bc$
Japanese Taro	$2.30\pm0.14~b$	2.50 ± 0.15 a	$2.90\pm0.09~a$	$3.40\pm0.15\ b$
Wild Taro	$2.10\pm0.09~b$	$2.00\pm0.00\ b$	$2.30\pm0.14~b$	$3.40\pm0.20\ b$
Rodent Tuber	$2.20\pm0.12\ b$	$2.00\pm0.00\ b$	$2.40\pm0.15~b$	$3.60\pm0.20\ b$
Ornamental Caladium	$2.30\pm0.14~b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	$2.70 \pm 0.14 \text{ c}$
Lady Finger Banana	$3.00\pm0.00\ a$	$2.80\pm0.12~\text{a}$	$3.00\pm0.00\ a$	$4.20\pm0.25~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 5. The reproductive period of Pentalonia nigronervosa on various host plants

Host species	The reproductive period (day)	Life span (day)	Fecundity (nymph/female)
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a
Belitung Taro	$26.30\pm2.80\ b$	$36.50 \pm 2.94 \text{ b}$	37.70 ± 3.49 bc
Pontianak Taro	23.60 ± 1.79 b	$33.50\pm1.83~b$	$40.10 \pm 4.60 \text{ bc}$
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10\ b$	$26.40 \pm 3.45 \text{ cd}$
Wild Taro	23.90 ± 1.85 b	$33.70\pm1.78~b$	33.60 ± 3.44 c
Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46\ b$	$19.60 \pm 1.34 \text{ d}$
Ornamental Caladium	$21.90 \pm 1.17 \text{ b}$	$31.20\pm1.16~b$	$40.10 \pm 3.92 \text{ bc}$
Lady Finger Banana	32.70 ± 0.63 ab	44.60 ± 1.45 a	51.50 ± 1.36 b

Note: figures followed by the same letters are not significantly different according to LSD 5%



Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. [A] Bogor taro, [B] Belitung taro, [C] Pontianak taro, [D] Japanese taro, [E] Wild taro, [F] Rodent tuber, [G] Ornamental caladium, [H] Banana.



Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.



Manuscript Evaluation Report- Referee 2

Manuscript ID : 613

Manuscript Title :Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants spe

Type of Article : Short Communication Research Paper

PART A: Comments per Section of Manuscript:

Abstract	The abstract it clear and concise
Introduction	The introduction is good enough understandable
Methodology	The methodology is clear
Results	The data in Table 1 Table 3 did not mention in the result Some of the explanation was not supported by data Picture in Figure 1 is not clear to show morphometric characteristics All comments were stated in the reviewed article
Discussion and Conclusion	Discussions & conclusion has to be related to the reviewed results
References	The references is in great quantities.
PART B: Recommendation (Kindly Mark With A "V")

Acceptable	
Acceptable with Minor	"V"
Revision	
Reconsidered after Major	
Revision	
Rejected	

PART C: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

Main text

Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is considered as a major pest of banana (Biale et al., 2017). The role of the aphid as the main vector of banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen & Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants worldwide including tropical and subtropical regions (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move

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from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25° C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x 150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro

(*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia* sp.) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

P. nigronervosa is a hemimetabolous insect undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into adult stage. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor taro and Rodent tuber

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seemed to be the Araceous hosts causing significant difference on body size of the aphid compared to those living on banana. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

P. nigronervosa could live and feed on Bogor taro and rodent tuber but with smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigronervosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid fecundity, so no aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemies, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemies.

The length of *P. nigronervosa* antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The significant difference of the length of *P. nigronervosa* antennae only occurred between the aphid living on banana and those living on Bogor taro and Rodent tuber, which also occurred between their body length, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between certain species, but the other morphological characteristics such body shape and body color were relatively the same (Figure 1). The color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

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In forth instar, Belitang Taro was the smallest one (Check Table 1)

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2. How do you get to this conclusion, what kind morphometric characteristic to support this statement 3. While your data in Figure 1 only body shape and color, which are the same

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The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar antennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar period was 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of P. nigronervosa live on banana were in accordance with the report by Padmanaban (2018) that P. nigronervosa had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana was always significantly longer than the period of the aphid living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition and more suitable to the need of banana aphid. Both nymph and adult of P. nigronervosa required similar nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 8.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33,4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of *P. nigronervosa* could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of P. nigronervosa (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for *P. nigronervosa*, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support P. nigronervosa even better than banana plant.

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The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of P. nigronervosa living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female (Table 5). The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table also show that the fecundity of P. nigronervosa living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of P. nigronervosa has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Table

Table 1. Body length measurements of Pentalonia nigronervosa lived on various host plants

		Body	Commented [A28]: There is no unit measuren		
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar	(Cmmm??)
Bogor Taro	$0,\!37\pm0,\!01$	$0{,}70\pm0{,}02~b$	$1{,}04\pm0{,}03\text{ b}$	$1{,}25\pm0{,}01~ab$	
Belitung Taro	$\textbf{0,}\textbf{37} \pm \textbf{0,}\textbf{01}$	$0,\!73\pm0,\!02ab$	$1,\!16\pm0,\!02 \text{ ab}$	$1{,}23\pm0{,}01~b$	
Pontianak Taro	$\textbf{0,}36\pm\textbf{0,}02$	$0,\!81\pm0,\!02a$	$1,\!12\pm0,\!01~ab$	$1,\!24\pm0,\!005$ ab	
Japanese Taro	$0,\!36\pm0,\!02$	$0{,}72\pm0{,}03ab$	$1{,}06\pm0{,}01\text{ b}$	$1,\!27\pm0,\!01~ab$	
Wild Taro	$\textbf{0,}39\pm\textbf{0,}01$	$0{,}72{\pm}~0{,}04$ ab	$1,16 \pm 0,02$ a	$1,\!30\pm0,\!02~a$	
Rodent Tuber	$0,\!38\pm0,\!02$	$0{,}70\pm0{,}02\text{ b}$	$1,\!04\pm0,\!04~b$	$1{,}24\pm0{,}03~ab$	
Ornamental Caladium	$\textbf{0,}38 \pm \textbf{0,}01$	$0{,}79\pm0{,}01ab$	$1{,}10\pm0{,}03~ab$	$1{,}25\pm0{,}02~ab$	
Lady Finger Banana	$\textbf{0,}38 \pm \textbf{0,}01$	$0,\!84\pm0,\!04a$	$1,16 \pm 0,01$ a	$1{,}30\pm0{,}02~a$	
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Table 2. Head width measurements of Pentalonia nigronervosa lived on various host plants

Host Species		Head	l width	
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar
Bogor Taro	$0{,}21\pm0{,}01$	$0{,}43\pm0{,}01~b$	$0{,}62\pm0{,}03~\mathrm{b}$	$0,79\pm0,03~b$
Belitung Taro	$0,\!20\pm0,\!01$	$0{,}47\pm0{,}03~ab$	$0{,}75\pm0{,}01~ab$	$0,\!87\pm0,\!02ab$
Pontianak Taro	$0{,}20\pm0{,}01$	$0{,}47\pm0{,}02~ab$	$0{,}62\pm0{,}02~b$	$0,\!84\pm0,\!03~b$
Japanese Taro	$0,\!19\pm0,\!02$	$0{,}45\pm0{,}03\text{ b}$	$0,74\pm0,03~ab$	$0{,}98\pm0{,}03~ab$
Wild Taro	$0{,}21\pm0{,}02$	$0{,}46\pm0{,}03~ab$	$0{,}76\pm0{,}04~a$	$1{,}01\pm0{,}05~a$
Rodent Tuber	$0{,}23\pm0{,}02$	$0{,}38\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}93\pm0{,}07\text{ ab}$
Ornamental Caladium	$0{,}22\pm0{,}02$	$0{,}49\pm0{,}02~ab$	$0{,}67\pm0{,}02\text{ b}$	$0{,}97\pm0{,}04~ab$
Lady Finger Banana	$0{,}21\pm0{,}02$	$0{,}54\pm0{,}04~a$	$0{,}79\pm0{,}04~a$	$1{,}00\pm0{,}05~a$

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Hast Spacing		Length of antenna (mm)							
nost species	1 st instar	2 nd instar	3 rd instar	4 th instar					
Bogor Taro	$0,\!40 \pm 0,\!02$	$0,72 \pm 0,02$ b	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$					
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,28 \pm 0,004$					
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28 \pm 0,\!01$					
Japanese Taro	$0,\!38\pm0,\!02$	$0,\!77\pm0,\!03~ab$	$1,\!08\pm0,\!01~\mathrm{b}$	$1,\!31\pm0,\!01$					

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Wild Taro	$0,\!42 \pm 0,\!02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,33 \pm 0,01$			
Rodent Tuber	$0{,}41\pm0{,}02$	$0,72 \pm 0,02 \text{ b}$	$1,07 \pm 0,03$ b	$1,\!29\pm0,\!03$			
Ornamental Caladium	$0,39 \pm 0,01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,30 \pm 0,02$			
Lady Finger Banana	$0,39 \pm 0,01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,34 \pm 0,02$			
Note: figures followed by the same letter are not significantly different according to LSD 5%							

Table 4. Nymph instar period of Pentalonia nigronervosa lived on various host plants

Hant Consider	Instar period (day)								
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar					
Bogor Taro	$2.20\pm0.12\ b$	$2.10\pm0.09~b$	$2.20\pm0.12\ b$	$3.10\pm0.09~bc$					
Belitung Taro	$2.10\pm0.09\ b$	$2.10\pm0.09\ b$	$2.30\pm0.14\ b$	$3.50\pm0.15~b$					
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09~b$	2.50 ± 0.15 b	3.00 ± 0.20 bc					
Japanese Taro	$2.30\pm0.14\ b$	2.50 ± 0.15 a	$2.90\pm0.09~a$	$3.40\pm0.15~b$					
Wild Taro	$2.10\pm0.09\ b$	$2.00\pm0.00\ b$	$2.30\pm0.14~b$	$3.40\pm0.20\ b$					
Rodent Tuber	$2.20\pm0.12\ b$	$2.00\pm0.00\ b$	2.40 ± 0.15 b	$3.60\pm0.20\ b$					
Ornamental Caladium	$2.30\pm0.14\ b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	2.70 ± 0.14 c					
Lady Finger Banana	$3.00\pm0.00\ a$	2.80 ± 0.12 a	$3.00\pm0.00\ a$	$4.20\pm0.25~a$					

Note: figures followed by the same letter are not significantly different according to LSD 5%

Table 5. The reproductive period of Pentalonia nigronervosa on various host plants

Host species	The reproductive period (day)	Life span (day)	Fecundity (nymph/female)				
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a				
Belitung Taro	$26.30\pm2.80\ b$	$36.50\pm2.94~b$	$37.70 \pm 3.49 \text{ bc}$				
Pontianak Taro	$23.60 \pm 1.79 \text{ b}$	$33.50\pm1.83~b$	$40.10 \pm 4.60 \text{ bc}$				
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10~b$	$26.40 \pm 3.45 \text{ cd}$				
Wild Taro	$23.90\pm1.85~b$	$33.70\pm1.78~b$	33.60 ± 3.44 c				
Rodent Tuber	12.60 ± 1.53 c	$22.80\pm1.46~b$	$19.60 \pm 1.34 \text{ d}$				
Ornamental Caladium	$21.90 \pm 1.17 \text{ b}$	$31.20\pm1.16~\text{b}$	$40.10 \pm 3.92 \text{ bc}$				
Lady Finger Banana	32.70 ± 0.63 ab	44.60 ± 1.45 a	$51.50\pm1.36~b$				
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Figure 1. Morphological development of *P. nigronervosa* nymphs living on Araceous hosts and banana. [A] Bogor taro, [B] Belitung taro, [C] Pontianak taro, [D] Japanese taro, [E] Wild taro, [F] Rodent tuber, [G] Ornamental caladium, [H] Banana



Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.

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Morphometrics and biological characteristics of *Pentalonia nigronervosa*, the vector of *Banana Bunchy Top Virus*, living on various Araceous plants species

ABSTRACT

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is widely known as the main vector of *Banana Bunchy Top Virus* (BBTV). It had been reported that banana aphid could live on alternative hosts belong to Family Araceae, and this is an important issue since Araceous plants are commonly found surrounding banana cultivations. Research had been conducted to study the morphometrics and biological characteristics of banana aphids living on various species of Araceous plants. The results showed that Araceous plant species could influence the morphometric and biological characteristic of banana aphids, indicated by the significant difference in some biological characteristics such as life cycle, life span, reproductive period, and fecundity. The average life span of banana aphids living on Araceous plants ranged from 22.80 to 47.90 days, and the average life cycle of *P. nigronervosa* living on Araceous plants was shorter than that of the aphids living on a banana plants. *P. nigronervosa* was able to develop a bigger colony when the aphid lived on Araceous plants, especially when they were transferred to Bogor taro where their fecundity reached 63 nymphs per female. Araceous plants also affected the morphometric of *P. nigronervosa* indicated by smaller size of the aphids, compared to those living on banana plant. The ability of banana aphid to live and reproduce in various Araceous plants would be beneficial to the development of control strategies of BBTV.

Key words: Banana aphid, life cycle, life span, fecundity

INTRODUCTION

Banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is a major pest of banana (Biale et al., 2017) and is the main vector of Banana Bunchy Top Virus (BBTV). is considered as a major pest of banana (Biale et al., 2017). The role of the aphid as the vector of BBTV banana Bunchy Top Virus (BBTV) make the aphid more dangerous to banana cultivation (Watanabe et al., 2013). The virus is considered to be the most important pathogen of bananas which might cause yield losses up to 100% (Qazi, 2016). Infected banana produces no fruit, but if the plant is infected at later stage, small fruits might be produced but not marketable (Jekayinoluwa et al., 2020). The first symptom of BBTV infection is the appearance of dark green broken lines on infected plant leaves (Chen & Hu, 2013). At the later stage of infection, newly formed leaves are shorter and narrower with chlorotic areas appear on leaf margins (Elayabalan et al., 2014).

P. nigronervosa generally lives in association with banana plants worldwide, including tropical and subtropical regions (Foottit & Maw, 2019). Banana aphids prefer to stay on the lower part of the host plant where they can build an association with ants, from which they get protection from predators (Biale et al., 2017). Instead of being the major pest of banana, *P. nigronervosa* could also live on various alternative hosts belonging to Families Araceae and Zingiberaceae (Suparman et al., 2017).

P. nigronervosa transmit BBTV in a circulative manner through the feeding activity (Niyongere et al., 2013; Jebakumar et al., 2018; Jekayinoluwa et al., 2021). The virus particles enter the aphid body together with plant liquid sucked by the aphid from the phloem of infected banana. The virus particles move

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from foregut to midgut and hemocoel before reaching salivary gland, from where the virus particles are transmitted to healthy plants through the aphid stylet (Watanabe & Bressan, 2013). BBTV particles were acquired by *P. nigronervosa* after an acquisition feeding period of 15 minutes, and a single aphid was able to acquire 861 virus particles after 24 h of feeding period (Jebakumar et al., 2018). In the transmission of the virus, the vector is influenced by many factors such as host plant conditions, temperature, and the life stage of the vector itself (Bragard et al., 2013). Environmental conditions, especially temperature, could affect the population of *P. nigronervosa* through its effect on parthenogenetic reproduction of the aphid which reached the highest rate of population development at the temperature of 25° C. At the same temperature, the life cycle of *P. nigronervosa* ranged from 10 to 14 days and reproduction period ranged from 9 to 12 days with fecundity 20 to 34 nymphs per female (Basak et al., 2015).

Host species could be a factor influencing morphological and physiological change of aphids (Mdellel & Kamel, 2015). Different host species could provide different food and environment for aphid colonization (Le Guigo et al., 2012), and *P. nigronervosa* showed different reproduction capacities when lived on different alternative host species (Suparman et al., 2017).

Banana Bunchy Top Virus is very difficult to control due to the virus itself and the vector involved. The virus is able to persist for quite long time in cut infected banana pseudo stem and still transmittable to healthy banana (Tricahyati et al., 2022), and persists for even longer in the infected corm. The vector has many alternative hosts and shows different performance on different host species and some host species are able to reduce transmission efficiency of the virus (Oktarida et al., 2022). Therefore, understanding the morphometric and biological characteristics of *P. nigronervosa* on each alternative host might be valuable in the design of control measures of the vector and the disease.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology and experimental garden, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from May to December 2021.

Banana Aphid Isolation and Rearing. Banana aphid *P. nigronervosa* was taken from banana plant belonging to farmers in Ogan Ilir Regency, South Sumatra. Single aphid was then bred parthenogenetically on banana sucker in a 50 x 50 x 150 cm³ wire mesh rearing cage for three weeks to produce a relatively big colony. Thirty individual aphids were used in this rearing and the biggest colony developed was selected for further works. The colony was then further reared strictly on banana suckers to guarantee that the research used only aphids purely reproduced from single aphid originally from banana host. Numbers of nymphs and adults of the colony had also been used to experimentally transmit BBTV and successfully transmitted the virus at high efficiency. The strict isolation and rearing of the banana aphid were aimed at preventing the mixing of *P. nigronervosa* and *P. caladii* which was morphologically and behaviorally similar to the banana aphid (Duay et al., 2014).

The banana aphids were also bred on Araceous plants prior to the conduction of the biological assay experiment, to make the aphids familiar with the alternative host where they would live and feed on during the experiment. The aphids were only being reared on the same plant species as they would be assayed during the experiment. The Araceous plant suckers used to rear the banana aphis were grown in 20-cm diameter pots covered with a plastic transparent cylinder with a cheese cloth window for aeration. The pots were placed in a shade house and the aphids were allowed to breed and produce enough imagoes for the biological assay experiment.

Araceous Plant Preparation. The experiment on the morphometric and biological characteristics of *P. nigronervosa* was conducted by using 7 species of Araceous plants and one genotype of banana. The Araceous plants were Belitung taro (*Xanthosoma sagittifolium* (L.) H.W. Schott & Endl), Bogor taro

(*Colocasia esculenta* L. Schott), Pontianak taro (*C. esculenta* (L.) Schott), Japanese taro (*Colocasia esculenta* var. antiquorum), Ornamental caladium (*Caladium bicolor* Vent), Wild taro (*Colocasia* sp.) and Rodent tuber (*Typhonium flagelliforme* (L.) Bl.). The Araceous plants which were commonly found surrounding banana cultivation areas and one genotype of banana was collected from farmers' fields. The banana genotype used in the experiment was the Lady Finger banana (AAA), the most widely cultivated banana cultivar in South Sumatera. All experimental plants were planted individually to produce more than 10 equal-size suckers to facilitate the experiment which needed 10 suckers of each host species for replication.

Biological Assay of *P. nigronervosa*. The experiment to obtain morphometric and biological characteristics of *P. nigronervosa* on Araceous plants was arranged in a completely randomized design with 8 treatments and 10 replications. The treatment was *P. nigronervosa* host species, comprised of 7 Araceous plant species and 1 banana genotype. Ten suckers of equal size were used for each host species and planted separately in 10-cm diameter transparent-plastic bottles filled with 5-cm high soil at the bottom. A newly born *P. nigronervosa* nymph was carefully transferred to the sucker in each bottle by using wetted tip of fine-paint brush. Each bottle was then covered with cheese cloth to facilitate air movement and protect the nymph from predators. The observation and measurement were made daily to collect morphometric and biological data of each nymph until the nymph became imago. The morphometrics of *P. nigronervosa* such as body length was measured from the front to the cauda apex and head width was measured across the eyes. All measurement was taken for every instar of the aphid.

Number of instars was recorded based on the number of molting that occurred from hatching until the formation of the aphid imago. The instar period was measured as a period between two successive molts of a nymph. The life cycle was measured from the aphid was born until its adult gave birth for the first time. The reproductive period was a period between the first and the last time an adult gave birth to its progenies. The life span of an aphid was a period from the aphid was born until died, and it could also be calculated by summing up all instar periods and adult period. Fecundity was measured as the number of nymphs delivered by an adult female during its reproductive period. During the counting of fecundity, a newly born nymph was taken out from the bottle after being counted (Gao et al., 2021). The counting of the progenies was conducted every 24 hours.

RESULTS AND DISCUSSION

Morphometric of Pentalonia nigronervosa

Morphological characteristics of *P. nigronervosa* lived on Araceous species and banana were not significantly different from each other in the first instar, they started to vary from second instar. The body length of the aphid lived on araceous plants ranged from 0.70 to 0.84 mm in the second instar, 1.04 to 1.16 mm in the third instar and 1.23 to 1.30 mm in the fourth instar (Table 1). In the second and third instars, the average body length of the aphid in Bogor taro and rodent tuber were significantly different from that in banana. However, in the fourth instar, only the characteristic in Belitung taro was significantly different from that in banana. The measurement of head width of the aphid showed that the average head width of the aphid in some Araceous species were significantly different from that in banana (Table 2). In the second instar, the characteristic in Bogor taro (0.43 mm) and Japanese taro (0.45 mm) were significantly different from that in banana (0.54 mm). In the third instar, the characteristic in Bogor taro (0.62 mm), Pontianak taro (0.62 mm), rodent tuber (0.67 mm) and ornamental caladium (0.67 mm) were significantly different from the characteristic in banana (0.79 mm). When the aphid reached the fourth instar, only the characteristic in Bogor taro (0.79 mm) and Pontianak taro (0,84 mm) were different from that in banana.

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The measurement of the aphid antennae also showed that significant differences of the average length of the antennae among host species was not consistent from instar to instar (Table 3). In the second instar, the characteristic in Bogor taro (0.72 mm) and rodent tuber (0.72 mm) were significantly different from that in banana (0.84 mm). In the third instar, the characteristic in Bogor taro (1.07 mm), Japanese taro (1.08 mm) and rodent tuber (1.07 mm) were significantly different from that in banana (1.16 mm). In the fourth instar, the characteristic in Belitung taro (1.28 mm) and Pontianak taro (1.28 mm) were significantly different from that in banana (1.34 mm).

P. nigronervosa is a hemimetabolous insect undergoes incomplete metamorphosis. The insect reproduces parthenogenetically and has only 2 distinct life stages because there is no egg stage in their life cycle. Nymphs of *P. nigronervosa* are born live and they pass 4 instars before the turn into adult stage. The first instar nymph begins at born until the first molt, and the next instars are a stage period between two successive molts. *P. nigronervosa* undergoes 4 successive molts and therefore the aphid nymphs pass 4 nymph instars. The measurement of body length (Table 1) and head width (Table 2) of the aphid living on Araceous and banana were not significantly different for the first instar, but there were some differences for the next three instars.

As presented in Table 1, there was no difference of the size of the first instar of *P. nigronervosa* living on different host species. The significant difference of body size between the aphid nymphs living on banana and those living on some other hosts was found in the next three instars. Bogor tare and Rodent tuber seemed to be the Araceous host causing significant difference on body length and head width size of the aphid compared to those living on banana, even though the difference of body length was only significant in second and third instar. The difference of body size indicated the different growth rate of the insect, which could be caused by different quality of nutrition intake (Martínez & Costamagna, 2018).

The head width of *P. nigronervosa* was measured as the representative of the aphid body width. Unlike the aphid abdomen which is elastic and getting bigger after feeding, the head of the aphid is rigid and its size does not change after feeding. In order to obtain the maximum head width, the measurement was taken across the aphid eyes. As presented in Table 2, the head width of *P. nigronervosa* lived on Araceous species varied in every instar except in the first instar, and the variation differed from instar to instar. In Araceous species, the head width of *P. nigronervosa* tent to be smaller than that in banana. Foottit et al. (2010) reported that the head width of *P. nigronervosa* was wider when it lived on Musaceae plants than when it lived on other hosts.

P. nigronervosa could live and feed on Araceous plant species Bogor taro and rodent tuber but with body length and head width similar to or smaller than those of the aphid lived on banana smaller body size, indicating that the plants contained appropriate nutrition suitable to the aphids, but the quality of the nutrition contained was lower than that of other hosts, especially when being compared to the nutritional value of banana, the main host of the aphid. However, there was a report that the morphological differences among aphid living on different hosts were more likely to be caused by genetical factors rather than by environmental factors, including the nutritional quality of the hosts (Aqueel et al., 2013). In this experiment we used aphid colony built up from single female, so genetical factor was supposed to be not too much different among the aphid colony members.

One of distinctive characteristics of *P. nigroncrvosa* is the venation of its wings. The veins of the aphid wings are clearly thick and black (Savory & Ramakrishnan, 2015). The middle part of the radial sector of the forewings is fused with the media vein, the hind wings are smaller and have only one oblique vein. However, no alate or winged adult was produced in the experiment but we found them produced in the rearing cages. The absence of alate imagoes in the biological assay experiment was very reasonable since the wing formation is normally triggered by the aphid population density (Shang et al., 2020). The formation of wings is also highly influenced by crowding and predation (Hu et al., 2019). In this experiment, every newly born progeny was removed and counted to determine the aphid focundity, so no

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In forth instar, Belitang Taro was the smallest one (Check Table 1)

Commented [A51R50]: The sentence has been rewritten to be more general covering all araceous plant species used in the research and in accordance with the data presented in Table 1 and Table 2. aphid colony was developed on the experimental hosts which might lead to crowded situation. There was also no threat from natural enemics, especially predator, in the experiment, because all experimental aphids were placed individually in a pot designed to be free from natural enemics.

The length of the antennae of *P. nigronervosa* was measured by using Milimeter v2.3.0 developed by Vis Tech. Project LLC after calibration. The antenna was removed from the aphid and put on the screen and and bound the length to see the result of measurement. The aphid antennae varied only in the second and third instar, but there was no significant difference of antennal size in the first and fourth instar. Aphid antennal length correlate with the body length, shorter or longer than their body length, and *P. nigronervosa* had been reported to have antennae longer than their body length (Bagariang et al., 2019). This results of the aphid antennal measurement confirmed that the length of antennae of *P. nigronervosa* living in all experimental host plants were longer than their body length (Table 3). The measurement of body length and head width of the aphid was in the range of the measurement results reported by Bagariang et al. (2019).

The length of antennae and body length of *P. nigronervosa* lived on Bogor taro and rodent tuber was shorter than that of the aphid lived on banana, confirming the correlation between antennal length and body length of aphid species. The function of aphid antennae is also related to the safety of the aphid. The antennal sensors response to chemical and visual signal by inducing the formation of wings so the aphid has ability to avoid dangerous enemies or unfavorable conditions (Hu et al., 2019).

The morphometric of *P. nigronervosa* living on various Araceous plants species was significantly different between -some species (Table 1,2 and 3), but the other morphological characteristics such body shape was relatively the same (Figure 1). Based on visual observation during the aphid development, the color of the aphid bodies was increasingly getting darker, from reddish brown in the first instar to blackish brown in the fourth instar. Host species did not affect the color of banana aphid. The color of the first instar was slightly transparent reddish brown, turned to greenish brown in the next two instars, getting darker in the fourth instar, and shiny black when reached the adult stage. Aphid color can be species-specific and is not influenced by nutritional quality of the host. According to <u>Tsuchida</u> (2016), aphid color is influenced by environment, especially the presence of natural enemies which may trigger physiological response in the form of color polymorphisms. In this experiment, the color of banana aphid used in the experiment was the same as that of the aphid in the rearing cage, because there was no natural enemy in both sites.

The body shape development from the first to the fourth instar also similar amongst the aphid nymphs feeding on different hosts. This was not in accordance to the report by Mehrparvar et al. (2012) who found different morphology of black legume aphid (*Aphis craccivora*) living on different hosts, and Mdellel & Kamel (2015) who found the same phenomenon on *Pterochloroides persicae* living on different hosts. The first instar of *P. nigronervosa* was slightly angular in shape and becoming more oval along with the nymphal development to second, third and fourth instar. The segmentation of the antennae also noticeable from instar to instar. The first instar antennae consisted of 4 segments, the second and third instar had 5 segment antennae, and the fourth instar had the full length of six segment antennae (Figure 2). The length and segmentation of the aphid antennae were in accordance to the measurement of the aphid antennae reported by Bhadra & Agarwala (2012) that the length of *P. nigronervosa* antennae was about 1.01 to 1.35 x its body length.

Biological Characteristics

Biological characteristics measured in the experiment were instar period, adult longevity, life span and fecundity. Instar periods almost similar for each instar and was no significant difference amongst host species (Table 4). The shortest instar period was 2.0 days, found in the second instar of the aphid living on wild taro and rodent tuber, while the longest instar period was 4.20 days found in the fourth instar of aphid nymph living on banana. The ranges of the four instar periods of *P. nigronervosa* live on banana were in accordance with the report by Padmanaban (2018) that *P. nigronervosa* had four nymphal instars and each instar was completed in 2-4 days. In all experimental hosts, nymphal period of the banana aphid was always longer in the fourth instar, while in the three previous instars the average instar periods almost the same, except that of Japanese taro which had second and third instar periods longer than those of other Araceous **Commented [A52]:** This research does not cover the venation of wing.

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5. How do you get to this conclusion, what kind morphometric characteristic to support this statement 6. While your data in Figure 1 only body shape and color, which are the same

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Commented [A61R60]: I am sorry for the poor picture but I cannot replace with the better picture because ata the moment I don't have various instar of the aphid on various Araceous plant species. hosts. It is also noticeable that nymphal instar periods of *P. nigronervosa* living on banana was always significantly longer than the period of the aphid living on Araceous host. The difference of instar period between banana aphids living on banana and those living on Araceous plant might be in relation with nutritional quality of the hosts, where banana has better quality of nutrition and more suitable to the need of banana aphid. Both nymph and adult of *P. nigronervosa* required similar nutrition, and good quality of nutrition contained in their host would support nymphal growth better than the hosts with less nutritional quality (Awmack & Leather, 2002).

Adult females started to reproduce parthenogenetically a day after the last molting of the fourth instar and keep reproducing for the rest of their life. P. nigronervosa actively gave birth to their offspring. The reproduction period differed significantly among host plant species, ranging from 12 to 38 days with an average of 24 days. The longest reproduction period was found in Bogor taro with an average of 38.3 days, while the shortest was found in Rodent tuber with an average of 12.6 days (Table 5). The difference in the reproduction period had resulted in the difference in the number of offspring produced by adult females who lived on different host plants. The process of giving birth to the first instar nymph by an adult female was very slow and only one nymph was borne in each delivery. One adult female of P. nigronervosa was reported to produce 33.4 nymphs during its adult life (Basak et al., 2015). However, this research found that, on Araceous plants, an adult female of P. nigronervosa could give birth to a wider range of progenies, from 19.60 to 68.30 nymphs per adult female. The smallest fecundity was found in Rodent tuber, while the highest was found in Bogor taro (Table 5). Rodent tuber was reported to be one of the preferable alternative hosts of P. nigronervosa (Suparman et al., 2017), even though the fecundity of the aphid in this host was relatively low. Bogor taro was more preferable alternative host to P. nigronervosa, and its fecundity on this host was the highest. Host preference seems likely to relate to food quality contained in the host (Clissold & Simpson, 2015). Instead of having the highest fecundity, P. nigronervosa living on Bogor taro also had the longer average life span (47.9 days) and even longer than the aphid life span on banana plant as the main host, where the aphid had life span only 44.6 days (Table 5). All Araceous species used in this research were suitable as alternative host for P. nigronervosa, indicated by the aphid ability to feed on the plant and got nutrition to support their life and reproduction. Some species such as Bogor taro and Belitung taro could support P. nigronervosa even better than banana plant.

The average reproductive period in the experiment ranged from 13.10 to 38.30 (Table 5) and was wider than that reported by Basak et al. (2015) who reported that reproductive period of *P. nigronervosa* in winter time ranged from 20.4 to 30.0 days. This difference could be caused by different temperature, because in this experiment the temperature was set at 25°C, the temperature at which the aphid was at its best performance on adult longevity and fecundity. At the same temperature, the aphid was reported to reached its maximum efficiency in transmitting BBTV with incubation period about 35.32 days. Lower temperature reduced life span of the aphid and reduced transmission efficiency with longer incubation period, up to 189 days (Selvarajan & Balasubramanian 2013; Chakraborty et al., 2021).

The averages life span of *P. nigronervosa* living on Araceous plants ranged from 22.80 to 47.90 days, and the fecundity ranged from 26.40 to 68.30 nymphs per female (Table 5). The results were in wider ranges compared to those reported by Padmanaban (2018) that the life span of the aphid ranged from 27 to 37 days and the fecundity ranged from 35 to 50 nymphs per female. This difference could be caused by different temperature or rearing method as suggested by Xie et al. (2020). Data on the table [Table 5 also show that the fecundity of *P. nigronervosa* living on banana had significantly higher than the aphid living on Araceous plants, except Bogor taro where the aphid had the highest fecundity, even significantly higher than banana itself. The biological performance of banana aphid on Bogor taro was outstanding with longer reproductive period, longer life span and higher fecundity. This is an indication that banana and Bogor taro had better host quality for the aphid, and the aphid itself is able to live on good as well as on poor-quality hosts. Banana as the main host of *P. nigronervosa* has had long good interaction with the aphid, indicating that the interaction is supported by good quality of nutrition provided by the host. A poor-quality host plant would cause a female insect produce only few good-quality progenies or a large number of poor-quality progenies (Gilkeson, 2012). In this experiment, we used young suckers to facilitate the aphid with better nutrient quality, since nymphal nutrition intake had strong influence to the aphid fecundity. The main food

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of aphids is phloem sap with high content of sugar and low content of amino acids (Shaaban et al., 2020) and to obtain enough amino acid, aphids suck a lot of phloem sap and secrete the sugar in the form of honey dew. The amino acid content of phloem sap was also very important for aphid to infest new plant host (Liu et al., 2016).

Even though many insects had considerable plasticity to their host plant quality (Awmack and Leather, 2002), the quality of the host might affect the quality of the insect progenies but not the quantity. In this research, the progenies were removed from the reproducing female so the quality of the progenies was not intensively studied. However, the aphid had been reared in Araceous plants prior to its used in the biological assay experiment, and the aphid could produce numbers of progenies needed by the experiment. Furthermore, we prefer to measure fecundity of the aphid without considering the viability of the progenies which determine their fertility. The difference between fecundity and fertility of *P. nigronervosa* in relation to host quality was clearly reported by Safari et al. (2022) that banana plants infected by BBTV reduced fertility of the aphid but increases its fecundity.

CONCLUSION

Banana aphid *P. nigronervosa* is able to live, feed and reproduce on Araceous plants with most morphometric and biological characteristics were not significantly different from those of the aphids living on banana. However, the aphids living on Bogor taro showed significantly longer life span and higher fecundity compared to those living on other hosts, including banana. Since Araceous plants are abundantly available surrounding banana cultivation, the interaction between banana aphid and Araceous plants would have implication on BBTD epidemiology. The long persistence of BBTV in the body of viruliferous aphid and the tendency of the aphid to leave infected banana will encourage the viruliferous aphid to migrate to Araceous plants which have ability to reduce the transmission efficiency of BBTV by the aphid.

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Table

Table 1. Body length measurements of *Pentalonia nigronervosa* lived on various host plants

Heat Species	Body Length (mm)							
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar				
Bogor Taro	$0,\!37\pm0,\!01$	$0{,}70\pm0{,}02\text{ b}$	$1,\!04\pm0,\!03\text{ b}$	$1{,}25\pm0{,}01~ab$				
Belitung Taro	$\textbf{0,}\textbf{37} \pm \textbf{0,}\textbf{01}$	$0{,}73\pm0{,}02ab$	$1{,}16\pm0{,}02\text{ ab}$	$1{,}23\pm0{,}01\text{ b}$				
Pontianak Taro	$0{,}36\pm0{,}02$	$0{,}81\pm0{,}02a$	$1{,}12\pm0{,}01~ab$	$1{,}24\pm0{,}005~ab$				
Japanese Taro	$0{,}36\pm0{,}02$	$0{,}72\pm0{,}03ab$	$1,\!06\pm0,\!01~b$	$1,\!27\pm0,\!01~ab$				
Wild Taro	$0{,}39\pm0{,}01$	$0,72\pm0,04$ ab	$1,\!16\pm0,\!02~a$	$1,30 \pm 0,02$ a				
Rodent Tuber	$0,\!38\pm0,\!02$	$0{,}70\pm0{,}02\text{ b}$	$1,\!04\pm0,\!04~b$	$1,\!24\pm0,\!03$ ab				

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Ornamental Caladium	$\textbf{0,38} \pm \textbf{0,01}$	$0{,}79\pm0{,}01ab$	$1{,}10\pm0{,}03~ab$	$1{,}25\pm0{,}02 \text{ ab}$
Lady Finger Banana	$0,\!38\pm0,\!01$	$0,\!84\pm0,\!04a$	$1,16 \pm 0,01$ a	$1{,}30\pm0{,}02~a$

Note: figures numbers followed by the same letter are not significantly different according to LSD 5%

Table 2. Head width measurements of Pentalonia nigronervosa lived on various host plants

Tiost species 1st the and the ard the state of the state	octor
¹ I st instar 2 nd instar 4 nd in	istai
Bogor Taro $0,21 \pm 0,01$ $0,43 \pm 0,01$ b $0,62 \pm 0,03$ b $0,79 \pm 0,01$ c	0,03 b
Belitung Taro $0,20 \pm 0,01$ $0,47 \pm 0,03$ ab $0,75 \pm 0,01$ ab $0,87 \pm 0,01$	0,02ab
Pontianak Taro $0,20 \pm 0,01$ $0,47 \pm 0,02$ ab $0,62 \pm 0,02$ b $0,84 \pm 0,02$ b	0,03 b
Japanese Taro $0,19 \pm 0,02$ $0,45 \pm 0,03$ b $0,74 \pm 0,03$ ab $0,98 \pm 0.02$	0,03 ab
Wild Taro $0,21 \pm 0,02$ $0,46 \pm 0,03$ ab $0,76 \pm 0,04$ a $1,01 \pm 0.01$	0,05 a
Rodent Tuber $0,23 \pm 0,02$ $0,38 \pm 0,02$ ab $0,67 \pm 0,02$ b $0,93 \pm 0,02$ ab	0,07 ab
Ornamental Caladium $0,22 \pm 0,02$ $0,49 \pm 0,02$ ab $0,67 \pm 0,02$ b $0,97 \pm 0,02$ b	0,04 ab
Lady Finger Banana $0,21 \pm 0,02$ $0,54 \pm 0,04$ a $0,79 \pm 0,04$ a $1,00 \pm 0,04$ a	0,05 a

Note: figures numbers followed by the same letter are not significantly different according to LSD 5%

Table 3. Length of antenna measurements of Pentalonia nigronervosa lived on various host plants

Hast Spacios	Length of antenna (mm)							
Host Species	1 st instar	2 nd instar	3 rd instar	4 th instar				
Bogor Taro	$0,40 \pm 0,02$	$0,72 \pm 0,02$ b	$1,07 \pm 0,03$ b	$1,31 \pm 0,02$				
Belitung Taro	$0,\!39\pm0,\!02$	$0,74 \pm 0,02$ ab	$1,17 \pm 0,01$ a	$1,\!28\pm0,\!004$				
Pontianak Taro	$0,\!37\pm0,\!02$	$0,82 \pm 0,02$ a	$1,14 \pm 0,01$ ab	$1,\!28\pm0,\!01$				
Japanese Taro	$0,38 \pm 0,02$	$0,77 \pm 0,03$ ab	$1,08 \pm 0,01 \text{ b}$	$1,31 \pm 0,01$				
Wild Taro	$0,\!42\pm0,\!02$	$0,75 \pm 0,05$ ab	$1,18 \pm 0,01$ a	$1,\!33\pm0,\!01$				
Rodent Tuber	$0,\!41 \pm 0,\!02$	$0,72 \pm 0,02 \text{ b}$	$1,07 \pm 0,03$ b	$1,\!29\pm0,\!03$				
Ornamental Caladium	$0,\!39\pm0,\!01$	$0,80 \pm 0,01$ ab	$1,14 \pm 0,03$ ab	$1,\!30\pm0,\!02$				
Lady Finger Banana	$0,\!39\pm0,\!01$	$0,84 \pm 0,04$ a	$1,16 \pm 0,01$ ab	$1,\!34\pm0,\!02$				
Note: figures numbers followed by the same letter are not significantly different according to LSD 5%								

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Table 4. Nymph instar period of *Pentalonia nigronervosa* lived on various host plants

Uast Spanias	Instar period (day)									
nost species	1 st instar	2 nd instar	3 rd instar	4 th instar						
Bogor Taro	$2.20\pm0.12~b$	$2.10\pm0.09~b$	$2.20\pm0.12\;b$	$3.10\pm0.09~bc$						
Belitung Taro	$2.10\pm0.09\ b$	$2.10\pm0.09\ b$	$2.30\pm0.14\ b$	$3.50\pm0.15~b$						
Pontianak Taro	$2.30\pm0.14\ b$	$2.10\pm0.09\ b$	$2.50\pm0.15~b$	$3.00\pm0.20~bc$						
Japanese Taro	$2.30\pm0.14\ b$	2.50 ± 0.15 a	2.90 ± 0.09 a	$3.40\pm0.15~b$						
Wild Taro	$2.10\pm0.09\ b$	$2.00\pm0.00\;b$	$2.30\pm0.14\ b$	$3.40\pm0.20\ b$						

Commented [A68]: There is no figure in Table 1, correct it Commented [A69R68]: 'figure' has been replaced with 'number'

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Rodent Tuber	$2.20\pm0.12\ b$	$2.00\pm0.00\ b$	$2.40\pm0.15\ b$	$3.60\pm0.20\ b$
Ornamental Caladium	$2.30\pm0.14\ b$	2.50 ± 0.15 a	$2.10\pm0.00\ b$	$2.70\pm0.14~\mathrm{c}$
Lady Finger Banana	3.00 ± 0.00 a	2.80 ± 0.12 a	$3.00\pm0.00\ a$	4.20 ± 0.25 a
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Table 5. The reproductive period of *Pentalonia nigronervosa* on various host plants

Host species	The reproductive period (day)	Life span (day)	Fecundity (nymph/female)					
	(au)	(au))	(11)111111111111					
Bogor Taro	38.30 ± 3.64 a	47.90 ± 3.75 a	68.30 ± 7.78 a					
Belitung Taro	$26.30\pm2.80\ b$	$36.50 \pm 2.94 \text{ b}$	37.70 ± 3.49 bc					
Pontianak Taro	$23.60 \pm 1.79 \text{ b}$	$33.50 \pm 1.83 \text{ b}$	$40.10 \pm 4.60 \text{ bc}$					
Japanese Taro	$13.10 \pm 1.30 \text{ c}$	$23.20\pm1.10~b$	$26.40 \pm 3.45 \text{ cd}$					
Wild Taro	$23.90\pm1.85~b$	$33.70 \pm 1.78 \text{ b}$	33.60 ± 3.44 c					
Rodent Tuber	12.60 ± 1.53 c	22.80 ± 1.46 b	$19.60 \pm 1.34 \text{ d}$					
Ornamental Caladium	$21.90\pm1.17~b$	$31.20\pm1.16~b$	40.10 ± 3.92 bc					
Lady Finger Banana	$32.70 \pm 0.63 \text{ ab}$	44.60 ± 1.45 a	$51.50\pm1.36~b$					
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Figure 2. The development of the antennae Pentalonia nigronervosa. [A] instar 1, [B] instar 2, [C] instar 3, [D] instar 4.

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