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Transmission of Banana Bunchy Top Virus (BBTV) by *Hysteroneura setariae* (Thomas) aphids from *Paspalum conjugatum* Bergius weeds

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This research was conducted to determine the ability of *H. setariae* aphids living on the weeds to transmit the dwarf disease.Cavendish bananas were placed in aphids-free gauze box with a length of 120 cm, a width of 120 cm and a height of 110 cm. Then, *P. conjugatum* weeds inhabited by 14-16 aphids were picked and transmitted to the healthy Cavendish bananas. When infested, the aphids were seen away from the bananas. The second transmission was done by placing a tray filled with water and then Cavendish bananas were placed in the middle of the tray to prevent the movement of aphids from the banana plant and *P. conjugatum* cultivation around the Cavendish as a temporary source of food for the aphids. After 10 – 20 days, the *P. conjugatum* weeds were removed and cleaned around the banana so that the aphids moved to and sucked on the Cavendish bananas.The symptoms of dwarf on the Cavendish banana plant appeared on 41 days after the transmission, including the shrunken, stiff, and degenerated leaves. Additionally, the edges of the leaves turned yellow, gradually discoloring into brown. The plant became stunted and experienced stagnant growth. If seen from the lower portion of the leaf lamina and midrib, there were rather dark green lines dotted like “Morse code”. Finding research, decline in the growth of Cavendish banana plants due to the dwarf disease was very significant, reaching 45.8% of the plant height, 47.7% of the leaf length, 47.1% of the leaf width and 26.1% of the canopy. The DAS-Elisa test on wild dwarf banana plants showed a positive result of BBTV with an absorbance value of 0.248 nm (the K^- value of 0.118 and K^+ of 1.267 nm).

Keywords: Aphid, *Hysteroneura setariae* (Thomas), *Banana Bunchy Top Virus*, Cavendish Banana and DAS-ELISA.

INTRODUCTION

Dwarf diseases have been identified in *Mauli* banana plants in several districts in Kutai Kartanegara Regency. *Pentalonia nigronervosa Coquerel* aphids as a vector transmitting dwarf diseases (Jebakumar et al., 2018; Dela et al., 2014; Foottit et al., 2010; Robson et al., 2006; Bhadra et al., 2010; Hooks et al., 2006; and Magee, 1927) are difficult to find on the lower portion of the leaf or inside the midrib. The presence of predators such as spiders and fire ants is quite a lot, making it difficult to find aphids naturally in dwarf *Mauli* bananas.

A dwarf disease caused by BBTV (Banana Bunchy Top Virus) is dispersed by *Pentalonia nigronervosa Coquerel* aphids and cannot be transmitted mechanically (Selvarajan et al., 2018; Manohar and Selvarajan, 2018; Hull, 2018; Das & Banerjee, 2018; Grigoras et al., 2018; Halbert et al., 2018; and Magee, 1927). Based on the results of the observation in the field, a quite large number of *Hysteroneura setariae* aphid colonies was found on *Paspalum conjugatum* weeds growing around *Mauli* bananas that showed dwarf

symptoms. Aphids are often seen on leaves and flowers of weeds (spikelets). Aphids are symbiotic with black ants to get honey.

Figure 1a shows dwarf *Mauli* bananas found in a mixed banana plantation with an area of 3 Ha which poorly managed while Figure 1b depicts a colony of *H. setariae* aphids inhabited on *P.conjugatum* weeds. There were several types of bananas planted, such as *Mauli*, *Kepok*, *Ambon*, and *Raja* bananas. Furthermore, there were also other fruit plants, including *Rambutan, Durian, Lai,* Avocado*,* and *Cempedak.* On the base of the plantation, there were growing many shrubs, broadleaf weeds, and other types of weeds such as *Cynodon dactylon* and *Paspalum conjugatum*.

MATERIALS AND METHODS

Research Location

This research was conducted from December 2017 – February 2018 in a banana plantation in Badak Mekar Village Km-44 on the axis road of Samarinda-Bontang, Muara Badak District, Kutai Kartanegara Regency, East Kalimantan Province.

Research Methods

Healthy Cavendish bananas were planted in plastic pots with a diameter of 50 cm and height of 40 cm to be then put into a gauze box with a length of 120 cm, a width of 120 cm, and a height of 110 cm. After that, *Hysteroneura setariae* aphid colonies consisted of 14-16 aphids (imago and nympha) were taken along with the *P. conjugatum* weeds growing around the dwarf *Mauli* bananas. The weeds were then planted around the Cavendish bananas. The weed planting was aimed as a temporary source of food for the aphids before being moved to the Cavendish bananas.

A tray with a diameter of 60 cm and height of 15 cm was filled with water and the centre of the tray was given a brick as a base to place the plastic pots of Cavendish bananas. The tray filled with water was intended to prevent the *Hysteroneura setariae* aphids leaving the Cavendish banana plant and avoiding the presence of fire ants to prey on the aphids.

After 10 – 20 days of the weed planting around the Cavendish banana plant, the weeds were gradually removed. The removal of the *P. conjugatum* weeds aimed to move the aphids to the Cavendish bananas as well as eliminate the main food of the aphids.

The DAS (*Double Antibody Sandwich*) - ELISA method was applied to detect the causes of dwarf disease in the Cavendish banana plants. The BBTV antibody and Carbonate Coating Buffer, PBST Wash Buffer, ECI Buffer, PNP Substrate Buffer, PNP Substrate Tablets and General Extract Buffer (GEB) derived from www.agdia.com. The carbonate coating buffer solution was added to the BBTV antibody with a ratio of 1:100. After that, 100 µl of the antibody solution was put into the microplate well and then incubated for 4 hours at room temperature. After incubation, the microplate was emptied by knocking it on towel paper, then washed 4x with PBST and dried. The banana plant samples were weighed 1 g, then added with 10 ml of GEB buffer and crushed until smooth. Furthermore, the microplate well was filled with 100 µl of the sample and given 1x GEB solution for both negative and positive controls. As for the overnight incubation, the microplate was washed 4x with PBST solution and the conjugate was dissolved 1x in ECI buffer with a ratio of 1:10. Each test well was added with 100 µl of it and incubated at room temperature for 2 hours. After incubation, the microplate was again washed 8x. Furthermore, PNP solution was prepared by inserting 1 PNP tablet (5mg) in 5 ml of PNP buffer with a ratio of 1:1. The microplate was then filled with 100 µl of the PNP buffer and incubated at room temperature for 30-60 minutes. Observation of discoloration and readings of Elisa reader was at a wavelength of 405 nm.

Statistical analysis

Data analysis was conducted by descriptive method and assisted by Figures, Graphs, and Tables.

RESULTS AND DISCUSSION

The transmission of *Hysteroneura setariae* aphids in healthy Cavendish banana plants was carried out through *P.conjugatum* weeds planted around the banana trees (Hapsari& Masrum, 2012, and Paparu, 2008).The planting was a gradual effort to replace the main food source for the aphids from the weeds to the Cavendish banana plant (Figure 2).

By transmitting the dwarf disease to 10 healthy Cavendish banana plants using 14-16 *Hysteroneura setariae* aphids obtained from *P. conjugatum* weeds growing around the dwarf *Mauli* bananas, it was obtained 3 dwarf Cavendish banana plants with positive BBTV while the rest 7 were still in healthy condition.

(1a) (1b)

Figure 1. (1a) Growth Condition of Dwarf *Mauli* Banana and (1b) *Paspalum conjugatum* Aphid Colony

The 7 Cavendish banana plants were suspected to remain healthy because the body of the *H.setariae* aphids which alighted upon the plants was negative of BBTV.

Based on the results of this research, the symptoms of dwarfism in the Cavendish banana plants appeared on 41 days after the transmission, including the shrunken, stiff, and degenerated leaves. Besides, the edges of the leaves turned yellow and the young leaves looked getting smaller and experienced a stagnant growth. The petiole did not come out like a normal banana leaf (Figure 3a). If seen from the veins and the midrib, there were rather dark green lines dotted like “Morse code” and green J-hook lines near the midrib (3c). According to Hull (2018); Jacobsen et al., (2018); Wei et al., (2009); Selvarajan& Balasubramanian (2014); Islam et al., (2010); Fu et al., (2009); Niu at al., (2009); Kumar et al., (2009); Anhalt &Almeida (2008); and Dale (1987) and Nelson (2004), BBTV-infected banana leaves can be identified from the dark green lines resembling “Morse code” on the veins as well as the symptom of green J-hook lines emerging along the midrib and petiole. The differences of dwarfism symptoms in the Cavendish bananas transmitted by *Hysteroneura setariae* and *P. nigronervosa* vectors can be seen in Figure 4.

The symptoms of dwarfism transmitted by *Pentalonia nigronervosa* in Cavendish banana plants include the stagnant growth of the plants, the shrunken and stiff leaves, the wavy leaf surface, the edges of the leaves turning yellow. As seen in the figure above, the petiole is still clearly visible if compared to the dwarfism symptoms transmitted by *Hysteroneura setariae* in which the petiole is not seen.

Blackman and Eastop (2006) explained that *Hysteroneura setariae* has a body length of 1.3 to 2.1 mm and belongs to aptera aphids that are slightly blackish with very pale antenna and tibia (lower leg), dark siphunculi and pale long cauda (Figure 5). According to Blackman and Eastop (1994) and Anonym (?), the body of *H. setariae* is oval in shape. Moreover, the siphunculi tip without an apical zone is polygonal in shape and the pale-colored long cauda has 4 hairs.

According to Blackman and Eastop (1994), *H,* the host plants for *H. setariae* in North America are Prunus domestica. Meanwhile, Noordam (2004) stated that the host plants for *H. setariae* include several weeds that have been known, such as *Paspalum commersonii* Lamk*, Paspalum conjugatim* Berg*, Setaria geniculatum* (Lam) Beauv., *Eleusine indica* (L.) Gaertn., *Cyperus compressus* L., *Gomphrena celosioides* Mart., *Pennisetum purpureum* Schumach., and *Panicum repens* L.

Pentalonia nigronervosa *Coquerel* has a body length of 1.2 – 1.9 mm. The aptera (wingless) aphid is reddish brown to almost black with a black antenna tip. *P. nigronervosa* isoften found in Musaceae family and lives under the base of leaves. Usually, there are ants centring around the aphid. This aphid is an important vector of bunchy top virus (Blackman dan Eastop, 2006). Both wingless and winged adult *P. nigronervosa* aphids can be seen in Figure 6.

The existence of Hysteroneura *setariae* aphids (Hemiptera: Aphididae) in Indonesia was first reported by Nasruddin (2013) on rice plants in South Sulawesi Province. *Hysteroneura setariae* cannot survive on 7-year-old rice plants and preferably makes a living by the panicle stems and spikelets, not rice leaves.

Figure 2. Disease Transmission to Cavendish Banana Plant by planting *Paspalum conjugatum* weed(arrows) whose flowers are inhabited by *Hysteroneura setariae* aphids. Insert: *P.conjugatum* flowers with aphid colonies.

Figure 3. Dwarfism Symptoms of BBTV Transmitted by *Hysteroneura setariae* to Cavendish Banana Plant (3a and 3b) and Morse Codes and J-Hook Lines on the Lower Portion of Cavendish Leaves (3c)

Figure 4. Symptoms of BBTV Transmitted by *Pentalonia nigronervosa* to Cavendish Banana Plant: (4a) Symptoms seen from the side; (4b) *Pentalonia nigronervosa* aphids seen from the shoot; and (4c) Symptoms seen from the top.

(5a) (5b)

Figure 5. *Hysteroneura setariae*(Thomas) Aphid Infested to Cavendish Banana Plant: (5a) wingless aphid and (5b) winged aphid.

Figure 6. Adult *Pentalonia nigronervosa* Aphids: (6a) Wingless Aphid, and (6b) Winged Aphid

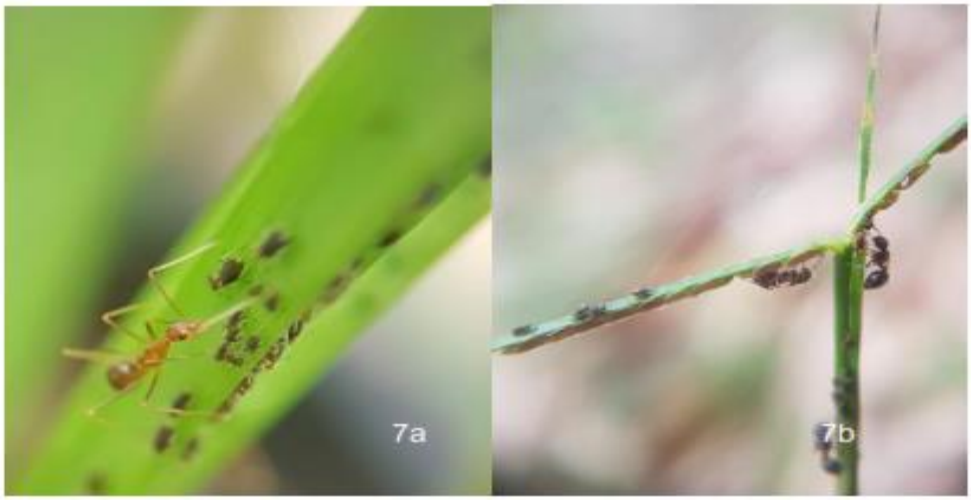

Figure 7. *Hysteroneura setariae* Aphids on (7a) the Leaf and (7b) the Seed Stem of *P. conjugatum* Weed

In *Paspalum conjugatum* weeds in the research field, *Hysteroneura setariae* aphids were found on the leaves, seed stems and blowing flowers (Figure 7).

Based on the growth measurements of healthy and dwarf Cavendish banana plants in Table 1, it can be seen that the dwarf disease significantly declined some growth parameters of Cavendish bananas, including the plant height by 45.8%, the leaf width by 47.1%, the leaf length by 47.7%, the stem diameter by 3.8%, and the canopy by 26.1%.The decline in the Cavendish banana plants due to dwarfism was quite significant. The banana plants could not flower and bear fruit, eventually dying.

The DAS-Elisa test conducted on the samples of dwarf Cavendish banana plants showed a positive transmission of Banana Bunchy Top Virus (BBTV), indicated by pale yellow discoloration occurred on the plate (Figure 8) and the absorbance value was 0.248 nm (the K^- value of 0.118 and K^+ value of 1.267 nm).

The results of the DAS-Elisa test showing a relatively pale discoloration and an absorbance value of 0.248 nm indicate that the BBTV virus concentration was relatively small in the dwarf Cavendish banana plants.

The role of *Hysteroneura setariae* aphids as a disease-causing virus vector has been known through several studies. It turns out that *Hysteroneura setariae* aphids are able to disperse Sugarcane Mosaic Virus (SMV) in addition to *maidis* aphids which have been initially known as the vector of the disease (Ingram, 1936). Meanwhile, according to Masumi et al., (2011), *Bermuda grass Mosaic Virus* (BgMV) can be dispersed by *Sipha elegans* and *Hysteroneura* sp.

Furthermore, Quimio and Calilung (1993) found that *Hysteroneura setariae* was able to transmit *Soybean Mosaic Virus* (SMV) to soybeans by 70% in the laboratory. Furthermore, their study suggested that 7 of 18 aphid species caught in a net were responsible for dispersing the viral disease by 84% in soybean cultivation.

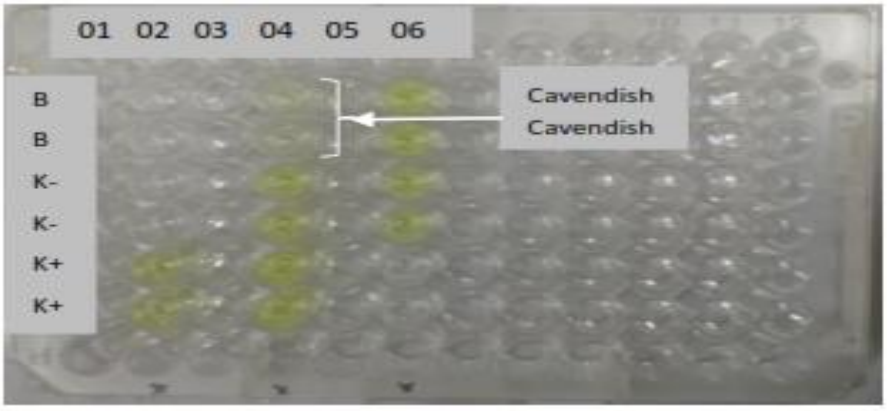

Figure 8. Pale Yellow Discoloration on the Microplate of Cavendish Banana Samples Indicating Dwarfism Symptoms

Table 1. Growth Measurement of Healthy and Dwarf Cavendish Banana Plants

No.	Parameter	Average (Cm)		Growth Decline (%)
		Healthy	Dwarf	
1.	**Plant Height**	192	104	45.8
2.	**Leaf Width**	32.7	17.3	47.1
3.	**Leaf Length**	72.7	38	47.7
4.	**Canopy**	161	119	26.1
5.	**Stem Diameter**	5.2	5	3.8
6.	**Petiole Diameter**	2	1.7	15
7.	**Number of Leaves**	5	4	20

Source: Primary Data (2018)

CONCLUSION

The results of the DAS-Elisa showed a pale yellow discoloration and absorbance value of 0.248 nm (the K^- value of 0.118 and K^+ value of 1.267 nm). Thus, it can be concluded that *Hysteroneura setariae* aphids obtained from *Paspalum conjugatum* weeds growing around dwarf *Mauli* banana plants can transmit *Banana Bunchy Top Virus* (BBTV) to Cavendish banana plants.

The differences in dwarfism symptoms identified from Cavendish banana transmitted by *Hysteroneura setariae* (Thomas) and *Pentalonia nigronervosa Coquerel* aphids are basically influenced by BBTV's genetic material travel that is quite long after going through *Paspalum conjugatum* weeds, *Hysteroneura setariae* (Thomas) and ending in Cavendish banana.

The low absorbance value read in the Elisa reader indicates that the concentration of *Bunchy Top* virus in *Hysteroneura setariae* (Thomas) is very low. This is because banana plants are not the main host of the aphids and not all *Hysteroneura setariae* (Thomas) aphids infested in Cavendish banana plants contain BBTV virus.

This initial research of *Hysteroneura setariae* (Thomas) needs to be further developed to determine the presence of BBTV in the body of *Hysteroneura setariae* (Thomas) aphids.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The article is part of the Dissertation of Doctoral and all the authors have contributed: SS conducted experiments, data collection, data analysis and writing manuscript, Dr. ALA contributed to the experimental design, the determination of the research treatment and the field survey, Prof. GM and Prof THA contributes to experimental design, determination of research treatment and review of manuscripts.

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