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The potential of *Pentalonia nigronervosa* Coq. and *Pentalonia caladii* van der Goot as vectors of *Banana bunchy top virus*

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Abstract The potential of *Pentalonia caladii*, also known as a taro aphid as vector of *Banana bunchy top virus* in comparison to banana aphid, *P. nigronervosa* was determined**.** A specific DNA fragment of BBTV was successfully amplified after acquisition feeding period from single and pooled samples of *P. nigronervosa*, but only from pooled samples of *P. caladii* (5, 10, 15, and 20 aphids). Differences in transmission efficiency were based on the disease incidence and severity, i.e., 70% and 58.3% by *P. nigronervosa* and 25% and 25% by *P. caladii*, respectively. Furthermore, the serial transmission assay indicated that *P. nigronervosa* retained BBTV longer than *P. caladii* (96 hours and 24 hours, respectively). Although further studies are needed, *P. caladii* showed potential as a BBTV vector, meaning it should be considered as part of disease management strategies.

Keywords: Acquisition feeding period, Banana aphid, Retention period, Taro aphid, Transmission efficiency

Introduction

Banana (*Musa* sp.) is the fourth most important food commodity in the world after rice, wheat, and corn (FAO, 2019). One of the challenges in banana cultivation is banana bunchy top disease (BBTD) caused by *Banana bunchy top virus* (BBTV), which is a member of the genus Babuvirus and family Nanoviridae (Burns *et al*., 1995). The distribution of BBTD has been reported in various countries in Asia, Africa, Australia, and the South Pacific. The incidence of BBTD has been reported ranging from 1 – 18 % in Republic of Congo (Mukwa *et al.*, 2014); 50 – 75% in Pakistan (Rao *et al*., 2002); and 5 – 90% in Australia (Smith *et al.*, 2007). The distribution of BBTD in Indonesia were found in the province of Yogyakarta, Central Java, East Java, North Sulawesi, West Sumatra, Lampung, Bali, Papua, East Nusa Tenggara, West Nusa Tenggara (Rahayuniati *et al*., 2021), Bengkulu (Sutrawati and Ginting, 2020), and West Java (Latifah *et al.*, 2021).

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BBTV infection is easily recognized by its characteristic symptoms, including the appearance of dark green dotted lines in the lower veins of the midrib, narrow leaves, chlorosis, necrosis, and stunted plants. BBTV infection in the vegetative phase may render plants incapable of producing banana bunches. If a plant is infected before the reproductive phase, it will still produce bunches; however, the fruits will be small and distorted and thus abnormal. As a result, the bananas are not eligible for market (Nelson, 2004). It has also been reported that BBTV infection in banana-producing countries such as Cameroon has become the main cause of decreased yields, ranging between 40 and 80% in cv. Cavendish Grande Nain and 100% in cv. Cavendish Williams (Ngatat *et al.,* 2017).

BBTV transmission occurs through infected vegetative propagation material and aphids as insect vector. The banana aphid, *Pentalonia nigronervosa* Coq. (Hemiptera: Aphididae), is an efficient vector that is known to transmit BBTV in a circulative non-propagative manner (Anhalt and Almeida, 2008; Watanabe *et al.*, 2013). Aside from *P. nigronervosa*, other aphids belonging to the same genus have also been linked to banana plants, i.e., *P. caladii*, also known as a taro aphid. The host range of *P. caladii* has been shown to involve plants from the Araceae such as *Colocasia* sp., *Xanthosoma* sp., *Dieffenbachia* sp., and *Caladium* sp. and the Zingiberales such as *Costus* sp. (Costaceae), *Canna* sp. (Cannaceae*), Elettaria* sp., *Hedycium* and *Zingiber* sp. (Zingiberaceae) (Foottit and Maw, 2019). Foottit *et al.*, (2010), and Miller *et al.*, (2014) reported that based on various morphometric and morphological characteristics observed, these two *Pentalonia* species shared many similarities, which led to them being declared cryptic species.

Furthermore, Greenwell (2012) reported that *P. caladii* was capable of transmitting BBTV under certain conditions, although its effectiveness was lower than that of *P. nigronervosa*. Therefore, the potential of *P. caladii* as a BBTV insect vector needs to be studied to obtain important information related to banana dwarf disease control. The study was conducted to test the potential of *P. caladii* as an insect vector of BBTV by confirming the presence of the virus in the aphids after the acquisition feeding period, the ability of the aphids to transmit BBTV, and the ability of the virus to survive in the aphids (retention period). In this case, *P. nigronervosa*, which is a known vector of BBTV, acted as a check comparison.

Materials and methods

Aphid propagation

Two species of aphids, *P. nigronervosa* and *P. caladii*, were propagated in healthy plants of banana cv. Cavendish and taro, respectively. Each plant was placed in an insect-free cage. Screenhouse were maintained in growth room condition at 25 - 30˚ providing 50 - 65% relative humidity and photoperiod of 12:12 hours. The aphids were maintained to reproduce until a sufficient amount had been obtained for further use.

Preparation of virus inoculum

The initial BBTV isolate was provided by the Plant Virology Laboratory, IPB University. The identity of the BBTV isolate was confirmed through DNA sequence analysis (GenBank no. ON081513) and the isolate was maintained in banana cv. Cavendish. The BBTV isolate was then propagated by transmission using *P. nigronervosa*. Adult aphids were given an acquisition feeding period of 24 hours on the initial inoculum source plant. After passing the acquisition feeding period, as many as 20 aphids were transferred to healthy banana plants for an inoculation feeding period of 48 hours. Typical symptoms of BBTV developed and the infected plants were then used as a source of BBTV inoculum.

BBTV acquisition, transmission, and retention assay

The ability of *P. nigronervosa* and *P. caladii* to acquire BBTV was examined by detecting BBTV from aphids immediately after they were given a 24-hour acquisition feeding period on infected plants. The polymerase chain reaction (PCR) method (Foottit *et al.*, 2010) was used to detect BBTV from the aphids. Aphid samples from each species were differentiated based on the number of insects, i.e. a single aphid and composite or pooled aphid samples (5, 10, 15, and 20 aphids) with five repetitions for each sample. BBTV detection began with the extraction of total DNA according to the method by Goodwin *et al.*, (1994), followed by the amplification of the DNA-R portion of the BBTV genome using specific primers, i.e., BBT-F(5'-GCGTGAAACGCACAAAAGGCC-3') and BBT-R (5'-GCATACGTTGTCAAACCTTCTCCTC-3') (Mansoor *et al.*, 2005). DNA amplification was conducted using a thermal cycler (GeneAmp PCR system 9700) at 94 ℃ for 5 minutes for pre-denaturation, followed by 35 cycles consisting of denaturation (94 ℃ for 30 seconds), annealing (55 ℃ for 45 seconds), extension (72 ℃ for 1 min), and final extension (72 ℃ for 5 min). The amplification products were visualized by electrophoresis using 1% agarose gel in 0.5x TBE buffer.

BBTV transmission was carried out as mentioned earlier; the aphids were given a 24-hour acquisition feeding period and a 48-hour inoculation feeding period, with 20 aphids per plant. The experiment was arranged using a completely randomized design with the aphid species, i.e. *P. nigronervosa* and *P. caladii*, as the treatment. Each treatment was repeated five times and each replication consisted of four plants. The banana plants were maintained for eight weeks and observed at two-week intervals. Observations included incubation period, type of symptoms, incidence, and severity of disease, plant height, number, and width of leaves. Disease incidence (DI) was calculated by the formula below (Townsend and Heuberger, 1943):

$$DI = \frac{n}{N} \times 100\%$$

Where n is number of symptomatic plants, and N is sum of all plants observed

Symptom severity of each plant was scored as described in Table 1, then disease severity (DS) was measured by the formula below (Townsend and Heuberger, 1943):

$$DS = \frac{\sum n_i v_i}{Z \times N} \times 100\%$$

Where n_i is number of assessed plants in each score of disease symptom, v_i is specific symptom score, Z is the highest score, and N is sum of all plants observed.

Table 1. Symptoms scoring for banana bunchy top disease (Brooks *et al.*, 1999)

Score	Disease Severity	Symptom description
0	Healthy plant	There were no symptoms
1	Mild infection	Vein clearing, there was a dark green dotted line on the underside of the lamina. There was no significant reduction in leaf blade width.
2	Moderate infection	There was clearing of leaf veins, chlorosis, and sometimes uneven leaf edges. Stem length, leaf spacing, and width decreased.
3	Severe infection	Plants were dwarf, leaf blades had severe chlorosis, leaf margins were uneven, there were symptoms of necrosis, and shrinking leaves were arranged at the top of the plant.

The BBTV retention assay was conducted through serial inoculation to determine the period in which the aphids retained the ability to transmit BBTV (i.e., they were viruliferous) to new plants after the first inoculation. Wingless adult aphids were given an acquisition feeding period on the virus source plant for 24 hours. Up to 20 aphids were transferred to healthy banana plants for 24 hours for the first inoculation feeding period. The second inoculation was carried out by transferring the aphids used in the first inoculation to new healthy banana plants and giving them an inoculation feeding period of 24 hours. The same steps were carried out until the sixth day of inoculation.

The experiment was conducted using a completely randomized design with the aphid species as treatments (*P. nigronervosa* and *P. caladii*). Each treatment set consisted of six plants and was repeated five times to give a total of 30 experimental units. Observations were made on the incubation period and the incidence and severity of the disease, as described previously.

Data analysis

The experimental data were statistically analyzed using SAS software with analysis of variance (ANOVA). If there was a significant effect of the tested factors, then further tests were carried out by Tukey test at a 95% confidence interval.

Results

Acquisition and transmission of BBTV by aphids

BBTV-specific DNA bands measuring 240 bp were successfully amplified from all samples of *P. nigronervosa*, from single to pooled aphids (5, 10, 15, and 20 aphids) (Figure 1A). BBTV-specific DNA band amplification was also obtained from pooled samples (5 to 20 aphids) of *P. caladii*, but it was not amplified from single aphid samples (Figure 1B).

The banana plants inoculated by BBTV through aphids showed typical symptoms of BBTD. The first symptoms appeared 26 – 45 and 29 – 31 days after virus inoculation through *P. nigronervosa* and *P. caladii*, respectively. The initial symptoms usually showed as chlorosis on the young leaf buds, followed by the development of a dark green line on the veins at the bottom of the leaf midrib. The leaf margins were necrotic at further symptom development. Severe infection was characterized by the stunting of plants, which had more erect, shorter, and narrower leaves compared to healthy plants (Figure 2). There were no differences in the types of symptoms between plants

inoculated with the virus through *P. nigronervosa* and plants inoculated through *P. caladii*.

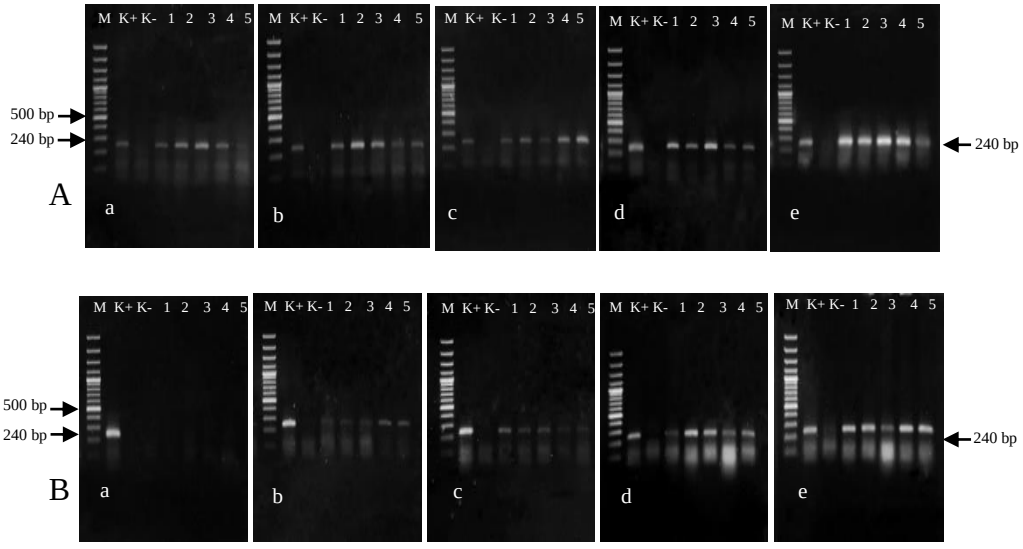

Figure 1. Visualization of specific DNA of *Banana bunchy top virus* from *P. nigronervosa* (A) and *P. caladii* (B). (a) single aphid; (b) to (e) pooled 5, 10, 15, and 20 aphids, respectively. (M) 100 bp DNA marker; (K+) BBTV-infected plant; (K-) healthy plant. Column 1 to 5, amplification was carried out using 5 different samples for each treatment of the number of aphids

Figure 2. Disease symptoms on banana plants inoculated with *Banana bunchy top virus* through *P. nigronervosa* or *P. caladii* aphids. (a) chlorosis of young leaves; (b) necrosis of the leaf margins; (c) dwarf plants; (d) Heights differences betweenhealthy (left) and diseased (right) plants

Although the types of symptoms were similar, when examining plant growth assessments, differences were observed between the symptoms of BBTV infection transmitted by *P. nigronervosa* and those transmitted by *P. caladii* (Table 2). Inhibition of plant height occurred faster in plants inoculated with the virus through *P. nigronervosa*. However, It found no significant difference at the end of the observation (eight weeks after inoculation). Similarly, plants inoculated with the virus through *P. nigronervosa* showed more restrained growth in leaf width.

There was an increase in both the number of symptomatic plants and symptom severity during the observation period from two to eight weeks after inoculation (Figure 3). At the end of the observation, virus inoculation through *P. nigronervosa* resulted in 70% DI with 58.3% DS while inoculation through *P. caladii* produced only 25% DI with 25% DS. Furthermore, detection by PCR confirmed BBTV infection in some asymptomatic plants, resulting in DI reaching 90% in plants inoculated with *P. nigronervosa* (data not shown).

Table 2. Effect of *Banana bunchy top virus* inoculation through aphids on plant height, number of leaves, and leaf width

Treatment	Observation Time (Weeks after Inoculation) *			
	2	4	6	8
	Plant Height (cm)			
Control	35.26 ± 5.28a	50.33 ± 5.69a	64.90 ± 7.56a	69.40 ± 8.14a
P. caladii	29.43 ± 4.26b	40.65 ± 10.33b	48.75 ± 12.27b	53.28 ± 13.20b
P. nigronervosa	25.99 ± 3.21c	31.90 ± 7.06c	38.90 ± 11.47c	47.55 ± 17.42b
	Number of Leaves			
Control	8.95 ± 1.32	9.60 ± 0.88	10.20 ± 0.89	10.40 ± 0.88
P. caladii	8.20 ± 1.47	9.55 ± 1.50	10.20 ± 1.28	10.25 ± 1.29
P. nigronervosa	8.70 ± 0.87	8.75 ± 1.16	9.50 ± 1.39	9.65 ± 1.50
	Leaf Width (cm)			
Control	6.16 ± 0.97a	7.73 ± 0.86a	10.21 ± 1.31a	12.20 ± 1.03a
P. caladii	5.13 ± 1.06b	7.35 ± 1.36a	9.03 ± 2.21a	10.34 ± 2.36b
P. nigronervosa	4.41 ± 0.58c	6.28 ± 1.08b	7.15 ± 1.39b	8.54 ± 2.30c

*Numbers in the same column followed by the same letter were not significantly different based on the Tukey Test at the 5% level

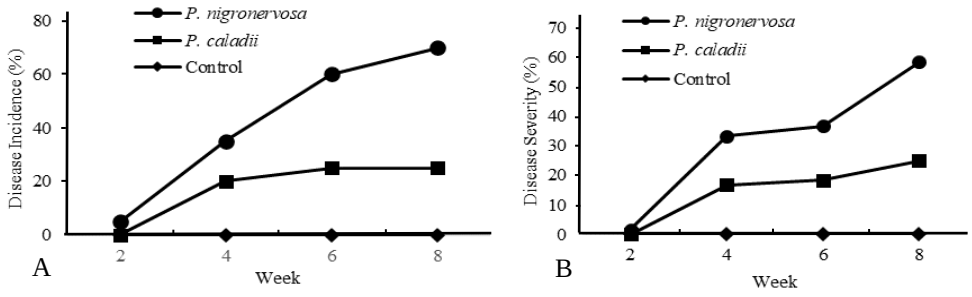

Figure 3. Disease progress in banana cv. Cavendish inoculated with *Banana bunchy top virus* through *P. nigronervosa* and *P. caladii*: (A) Disease incidence and (B) Disease severity

BBTV retention

The BBTV retention assay indicated differences between the two aphid species. While *P. nigronervosa* was able to transmit BBTV up to day 4 with DI 60 – 100%, *P. caladii* was only able to transmit BBTV on day 1 with DI 60%, with no transmission (0%) the following day (Table 3).

Tabel 3. The incubation period, disease incidence (DI), disease severity (DS), and disease symptoms in the retention assay

Day	Incubation period (dai)[1]	DI (%)[2]	DS (%)	Symptoms[3]
		P. nigronervosa		
1	21 – 24	5/5 (100)	100	dw, nl, cl, rs, nc
2	31 – 35	5/5 (100)	80,0	dw, nl, cl, rs, nc
3	53 – 57	5/5 (100)	46,7	nl, cl, lc, rs
4	69 – 75	3/5 (60,0)	20,0	lc, rs
5	-	0/5 (0)	0	-
6	-	0/5 (0)	0	-
		P. caladii		
1	22 – 41	3/5 (60,0)	46,7	dw, nl, cl, rs
2	-	0/5 (0)	0	-
3	-	0/5 (0)	0	-
4	-	0/5 (0)	0	-
5	-	0/5 (0)	0	-
6	-	0/5 (0)	0	-

[1]dai: days after inoculation, [2]n/N: Number of infected plants/total plant, [3]Symptoms: dwarf (dw), narrow leaf (nl), chlorosis (cl), rosette (rs), necrotic (nc), leaf curl (lc)

The longer the transmission time the longer the incubation period was shown in Table 3. However, the most infected plants showed a highly typical BBTV infection. Furthermore, DI decreased with increasing transmission time, which was thought to be influenced by the concentration of the virus in the insect's body. *P. nigronervosa* could retain BBTV up to day 4, or 96 hours after the acquisition feeding period. In contrast, *P. caladii* retained BBTV only until the end of day 1, or 24 hours after the acquisition feeding period. Therefore, both species of aphids, *P. nigronervosa* and *P. caladii* can acquire and transmit BBTV but with different efficiencies.

Discussion

BBTD was reported for the first time in Fiji in 1889 and is now widely distributed in Africa (Angola, Benin, Burundi, Cameroon, Gabon, Mozambique, Zambia, Egypt, Congo, Burundi, Nigeria, Malawi, Rwanda), Asia (China, Hongkong, India, Indonesia, Iran, Jepang, Laos, Malaysia, Myanmar, Pakistan, Philipines, Sri Lanka, Taiwan, Thailand, Vietnam), Oceania (Australia, Kiribati, Mariana Islands, New Caledonia, Tonga, Tuvalu, Samoa) (Diekman and Putter, 1996; Thomas *et al.*, 2015). The disease spreads through the transfer or exchange of infected planting material such as corm, sucker, and plantlet from one place to another and through the banana aphid, *P. nigronervosa*, from one plant to another (Thomas *et al.*, 2003; Robson *et al.*, 2006; Kumar *et al.*, 2011). *P. nigronervosa* has a high degree of host specificity to *Musa* spp. for its propagation. This aphid species is found in almost all banana-growing countries such as Pakistan (Yasmin *et al.*, 2001); Angola, Congo, Cameroon, Gabon, Malawi (Kumar *et al.*, 2011), Indonesia (Suparman, *et al.*, 2017). While it has been reported that *P. nigronervosa* has also been found in several plants from the Zingiberaceae and Araceae families, further studies based on morphological and morphometric analysis confirmed that the aphids found on the non-Musa plants were of a different species, namely *P. caladii* (Blackman and Eastop, 2000; Foottit *et al.*, 2010). In Indonesia, Bagariang *et al.* (2019) reported that *P. nigronervosa* colonies were found to infest more bananas (*Musa* spp.) than non-Musa. By contrast, *P. caladii* has been found to infest *Colocasia esculenta*, *Curcuma longa*, *Costus* sp., and *Dieffenbachia* sp., but rarely bananas. Therefore, further study is required on the potential for *P. caladii* to transmit BBTV from one plant to another.

P. nigronervosa acquires the virus at least four hours after feeding on infected plants. BBTV, meanwhile, persists throughout the life of the aphids (i.e., 15 – 20 days) but is not transmitted to the progeny (Anhalt and Almeida, 2008; Watanabe *et al.*, 2013). In our study, the ability of *P. nigronervosa* and *P. caladii* to acquire the virus was determined by giving aphids a 24-hour

acquisition feeding period on infected plants. BBTV-specific DNA bands measuring 240 bp were successfully amplified from *P. nigronervosa*, both single and pooled samples. This indicated the high ability of *P. nigronervosa* to acquire the virus, with each individual than having the opportunity to transmit BBTV to other plants. This is in contrast to *P. caladii*, in which BBTV was only detected in pooled samples. Thus, *P. caladii* can also acquire the virus, although not all individuals have the opportunity to transmit BBTV. The low acquisition ability of *P. caladii* further affects the efficiency of its transmission. In transmission using *P. caladii*, the incidence of disease reached 25%, while transmission using *P. nigronervosa* resulted in a higher DI (70%). The suitability of the host plant determines the efficiency with which the virus is transmitted by insect vectors. *P. nigronervosa*, known asthe banana aphid, easily feeds and probes on the banana plant. Meanwhile, *P. caladii* is known as a taro aphid, which meant it had to adapt to the banana plant during the experiment. Based on a free-choice test, Rahmah *et al*. (2021) reported that *P. caladii* prefers plants from the Araceae family, while *P. nigronervosa* prefers Musaceae.

While circulative viruses generally do not replicate in their insect vectors but traverse the insect gut, hemolymph, and salivary tissue membranes to reach the salivary glands for transmission. They need to feed for extensive periods of time to facilitate efficient transmissions and they do exhibit a persistent pattern of transmission that can last from a few days to several weeks (Gray and Gildow, 2003; Whitfield *et al.*, 2015; Dietzgen *et al.*, 2016). This indicates that the virion persists in the vector body and does not undergo minimal degradation. For example, whitefly-transmitted begomoviruses have been found in high concentrations in the vector gastrointestinal tract (Ghanim *et al.*, 2009; Wang *et al.*, 2020). Similarly, *Potato leafroll virus* (Polerovirus) accumulates in the posterior midgut of its vector aphids (Garret *et al.*, 1993; Rouze-Jouan*et al.*, 2001). Besides the gastrointestinal tract, hemolymph is also a reservoir of virions as significant amounts of both viruses have been detected (van den Heuvel *et al.*, 1994; Gray and Gildow, 2003).

The retention experiment in our study was carried out by transferring viruliferous aphids to healthy banana plants, which were replaced daily. The results showed that *P. nigronervosa* was able to transmit BBTV up to day four. Watanabe and Bressan (2013) reported that *P. nigronervosa* retained the ability to transmit the virus for up to 16 days, however the probability of BBTV transmission decreased over time. While *P. caladii* was only able to transmit BBTV on day one. The same phenomenon has been found to occur in *Tomato yellow leaf curl virus* (TYLCV), which survived in the non-vector *Trialeuroides vaporariorum* for a shorter time than in the *Bemisia tabaci* vector (Czosnek *et al.*, 2002). It was described in detail that TYLCV was detected in

the salivary glands of *B. tabaci* but not in *T. vaporariorum*. Interestingly, TYLCV was detected in low levels in the midgut of *T. vaporariorum*. This indicates that TYLCV cannot cross the gut-hemolymph barrier of *T. vaporariorum* andis thus associated with the inability of *T. vaporariorum* to transmit TYLCV. In this case, further research using *P. caladii* and BBTV is required to study the translocation of BBTV in aphid vectors.

All of the data presented in this work, along with previous research (Watanabe *et al.*, 2013; Greenwell, 2012), confirmed the potential of *P. caladii* to transmit BBTV, albeit less effectively than *P. nigronervosa*. Further study on the distribution of *P. caladii* in banana-growing areas and its host range might be important in determining its role in virus spread. If this aphid species is proven to have the potential to be an efficient virus vector, then this must be taken into account in disease management strategy.

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