Alternative Hosts of Banana Aphid Pentalonia nigronervosa Coq. (Hemiptera: Aphididae), the Vector Transmitting Banana Bunchy Top Virus

by Dr.ir.yulia Pujiastuti, Ms.

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Alternative Hosts of Banana Aphid *Pentalonia nigronervosa* Coq. (Hemiptera: Aphididae), the Vector Transmitting Banana Bunchy Top Virus

Suparman, Bambang Gunawan, Yulia Pujiastuti, Arsi, and Rizky Randal Cameron
Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia
Email: {suparman, ypujiastuti, arsyarsy}@unsri.ac.id, {bgsyailendra, rizky.be.hha}@gmail.com

***Abstract*—Banana Bunchy Top Virus cause very destructive disease on banana. The virus is transmitted by *Pentalonia nigronervosa* in circulative manner. Plants infected show progressive dwarfing, upright crowded leaves and produce no fruit. Surveys conducted in 2016 in South Sumatra, Indonesia, showed that the disease distribution was affected by human activities and the vector, but the vector was scarcely found in the field, suggested that there must be alternative hosts for the vector. There was a report that the aphid can live on members of zingiberaceous and araceous plants. In this research, 11 zingiberaceous and araceous plants frequently found in banana planting areas were tested for their suitability as alternative host of *P. nigronerosa*. The infestation of 4 wingless adults of *P. nigronervosa* on the plants showed that the aphid could live on *Alpinia galanga*, *Kamferia galangal*, *Curcuma domestica*, *Colocasia esculenta*, *Xanthosoma sagittifolium*, *Caladium bicolor* and *Typhonium flagelliform* with different levels of growth rate.**

***Index Terms*—banana bunchy top virus, *Pentalonia nigronervosa*, alternative host**

I. Introduction

Banana and plantain (*Musa* spp.) are commonly cultivated as income-generating fruit crop in tropical and subtropical countries. The fruit, especially banana, has become part of daily diet of people from all over the world. Plantain is important staple food for millions of people in the tropical regions of the world, especially in African countries [1]. Therefore, banana is considered as a fruit of great socio-economic [2]. The crop is grown everywhere in low altitude until 1800 m above sea level. There are many banana and plantain cultivars available, but farmers tend to cultivate only common and marketable cultivars. Common cultivars are those having preferable flavor, good pulp taste, and high juice quality and, therefore, have better price [3]. Banana and plantain are subjected to various diseases, particularly viral diseases, which cause a major disturbance to the crop worldwide. Among viral diseases, Banana Bunchy Top Disease (BBTD) caused by Banana Bunchy Top Virus (BBTV) is known to be the most destructive disease of the crops. BBTD indiscriminately infects banana and plantain cultivated in the low altitude areas [3].

The early symptoms of BBTV infection are obvious, easily observable and quite distinct from other known banana virus symptoms. These symptoms include development of morse code streaking of variable length in the leaf veins, midribs and petioles, correlate internally with modification of the phloem and surrounding tissue of the vascular bundles [4]. The very specific symptom of BBTD is the upright and crowded leaves at the apex of the plant for which the disease is named bunchy top. In certain banana cultivars, the yellowing of leaf margins frequently turn into necrotic zones. The incubation period of BBTD or appearance of visual symptoms ranged from 25 to 85 days after inoculation by its vector. However, PCR assays might provide earlier detection of the presence of BBTV in banana plants before the disease produces early visual symptoms [5]. Banana plants infected early by BBTV do not bear fruit, and fruits of later infected plants are typically stunted and inconsumable. After producing initial infection in the locus of inoculation, the virus then spreads to suckers through the rhizome and thus the entire banana mat eventually becomes infected [6].

Banana bunchy top disease is persistently transmitted by banana aphid *Pentalonia nigronervosa* Coq. and is not transmitted mechanically or by any other common modus of virus transmission [7], [8]. Banana aphids are the only known vectors of BBTV and is widely distributed and found in tropical and subtropical regions worldwide [9]. The aphids acquire the virus from infected plants, and no transovarial infection occurs even though the aphid retains the virus after molting. Besides living on banana plant, *P. nogronervosa* has been frequently found living on some species of zingiberaceous and araceous species [10].

P. nigronerosa transmit BBTV in a persistent and circulative manner. The virus may be acquired and transmitted within a minimum acquisition access period of 4 hours and inoculation access period of 15 min [11], [12]. Circulative viruses do not replicate in their vectors [13]. However, they show a persistent pattern of transmission that generally lasts for several days to weeks [14]. During the process of transmission, circulative viruses are ingested by the aphid with the sap of the

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infected plants. The viruses are then internalized and cross the insect's gut cells, from which they are translocated into the vector's haemocoel. After internalization within the salivary glands, the viruses can then be discharged into plant tissues along with the saliva produced during the feeding process [15]. The virus particles are retained in the aphids' body and encountering no degradation. Haemolymph appears to be an important reservoir of the virus because the virus can be detected at significant amounts [16].

P. nigronervosa reproduces parthenogenetically, no male is required for reproduction. The reproductive period ranged from 20.4 to 30.0 days with pre-nymphoposition period ranged from 3.0 to 6.0 days and fecundity ranged from 20.0 to 33.4 nymphs per female [17]. Under favorable condition, the aphid has high rate of reproduction with 26 -36 overlapping generation a year [18]. However, the aphid development and reproduction seem to be significantly influenced by environmental or geographical conditions. Under favorable laboratory conditions, the aphid nymphal development took 9-13 days, adult longevity varying between 9.9 to 12.5 days and fecundities of 15-20 nymphs per female [19]. In the Philippine, it was reported that the development period of *P. nigronervosa* ranged from 6 to12 days with generation times varied from 20 to 28 days [20], In China, the banana aphid was reported to have only four generations a year with highest population occurred in April, September and October [21].

All stadia of *P. nigronervosa* are able to transmit BBTV, but adult aphids transmit the virus more efficiently than their nymphs. The virus transmission by the aphid is affected by temperature, aphid life stage, and plant access period [22]. High temperature is more favorable for *P. nigronervosa* to transmit BBTV than low temperature. Under normal conditions, the virus may be acquired and transmitted within a minimum acquisition access period of 4 hours and inoculation access period of 15 min, and a latent period of 20 to 28 hours [11]. Furthermore, plant access periods may increase viral acquisition and inoculation efficiencies in a range of 60 min to 24 h. In general, BBTV acquisition and inoculation efficiencies peaked after 18 h of plant access period [22]. Under 16^{o}C the banana aphid cannot transmit BBTV. This may be the reason of the absence of BBTD in high altitude of tropical areas where temperature mostly under 16°C, as transmission rate of BBTV was reported to directly correlate with the number of viruliferous aphids feeding on the healthy hosts and is inversely correlated with age of the host [23]. They found that 53% of single viruliferous aphid could transmit BBTV to one month old plant with incubation period of 15 days.

Population of *P. nigronervosa* in the field fluctuates due to many factors. The key factor is rainfall which cause the reduction of *P. nigronervosa* population, especially in the plantation with less dense canopies [24]. Under high rainfall the sheaths of the leaves became inundated with water and drowned many aphids. Dense canopy significantly reduce the amount of rainfall penetrating the leaf whorls of banana suckers where aphid colonies stay and develop. Moreover, heavy rainfall may reduce banana aphid due to the growth of the entomopathogenous fungi, *Verticillium intertextum* [18]. Colonies of *P. nigronervosa* mostly develop around the base of pseudostems of banana sucker, and frequently attended by ants, which feed on the large quantity of honeydew produced by the aphids. The colonies of ant might considerably reduce the density of indigenous predators of *P. nigronervosa* by covering the aphid colonies with its nest. Ants also transport aphids from plant to plant and establish new infestations [25], [26].

P. nigronervosa lives on banana (*Musa paradisiaca)* and manila hemp (*Musa textilis*) as its major hosts and is reported to have minor host such as gingerlily (*Alpinia purpurata*), taro (*Colocasia esculenta*), dumbcanes (*Dieffenbachia*), cardamom (*Elettaria cardamomum*), cocoyam (*Xanthosoma*), ornamental banana (*Heliconia*), and ginger (*Zingiber officinale*) [27].

The result of BBTD survey in South Sumatra, Indonesia conducted in 2016 showed that the disease incidence was increasing, covering wider areas and involving more banana cultivars. The interesting as well as surprising finding was that the banana aphid *P. nigronervosa* was hardly found both in diseased and healthy banana plants. The fact that the vector nearly disappeared among the abundance of infected banana mats has led to the supposition of the presence of local alternative hosts for the aphid. In this research, some zingiberaceous and araceous plant frequently found in the surrounding areas of banana mats were tested for their role as alternative host for *P. nigronervosa*. Many and various cultivated zingiberacous and wild araceous plants were found in the banana planting areas.

II. Materials and Methods

This study was conducted at the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Indonesia.

A. Aphid Preparations

Single wingless aphid was transferred from infested banana and was reared on true-horn banana sucker kept in aphid proof plastic box (30 x 30 x 30 cm) covered on all sides with cheese cloth, except the bottom which was kept open. True horn banana cultivar was selected to rear the aphid because banana aphid breeds at highest rate on this cultivar. All plastic boxes containing banana sucker infested with single banana aphid were kept in an air conditioned room where the temperature was maintained at 25°C ± 5°C.

B. Banana Plant Preparations

Six species of zingiberaceous plants and five species of araceous plants frequently found in the banana planting areas were collected and planted in the screen house for regeneration before being used as experiment plants. The six zingeberaceous plants selected were: greater galangal (*Alpinia galanga* (L.) Willd.), bitter ginger (*Zingiber zerumbet* (L.) Smith), galangal (*Kaempferia galanga* L.),

turmeric (*Curcuma domestica* Val.), ginger (*Zingiber officinale* Roxb. var Rubra.) and Javanese ginger (*Curcuma zanthorrhiza* Roxb.). Five species of araceous plants were: taro (*Colocasia* esculenta L.), blue taro (*Xanthosoma sagittifolium* (L.) Schott), angel wings (*Caladium bicolor* Vent.), elephant ear *Alocasia macrorrhizos* (L.) G.Don Vent.) and rodent tuber (*Typhonium flagelliforme* (Lood) Bl. All plants were planted individually in a 30 cm diameter plastic pot filled with sterilized mixture of soil and compost (1:1) and were kept in an insect proof screen house until produced enough shoot for the experiment.

C. Aphid Infestations

Young shoots of zingiberaceous plants and young petioles of araceous plants were used to assess the plants' suitability as alternative hosts of *P. nigronervosa* in a detached organ experiment. A 25 cm young shoot with no leaf was cut from each zingiberaceous plant, and a 25 cm young petiole was cut from each araceous plant. The basal end of each cutting was inserted into watered cotton and wrapped with aluminium foil. The cutting was then placed in the middle of a plastic cup in such a way that the cutting could stand in the centre of the cup. Pre starved apterate adult of banana aphids were introduced into the cutting, 4 aphids per cutting. Only 5 dult aphids were used for the experiment. Adult banana aphids were recognized by the development of black coloration on their legs. Aphids were transferred using a water moistened paintbrush. Individual cup containing test plant cutting was placed inside a 25 cm diameter and 40 cm height plastic cylinder, the top of the cylinder was covered with cheese cloth. 20 cuttings of shoot or petiole, depended on the plant species, of each plant species were used as replication. All cups with shot or petiole infested with 4 banana aphid, 9gether with aphid proof plastic cylinder, were place in an air conditioned room with temperatures of 25°C ± 5°C.

D. Observation

Population counts of the aphids were taken on interval of 24 hours until aphid population on all cuttings us in the experime2 finished due to the deterioration of the cuttings. Population growth rate, the increase in num2er of *P. nigronervosa* per day per plant cutting was calculated by the following formula.

$$GR = \frac{Nt - No}{\Delta t}$$

where Nt is the number of aphids present at the maximum count of the population on a plant, No is the number of aphids initially released on a plant cutting, and Δt is the difference of time between No and Nt (Odum, 1971) [28]. From these values, the mean of population growth was computed and analyzed.

III. Results and Discussion

P. nigronervosa infested on zingiberaceous and araceous plant cuttings showed significant differences in behavior and performance. Both behavior and performance were observed during 15 days after aphid infestation on the plant cuttings. This was to guarantee that all additional aphis found on the plants were of second generation, because banana aphid needs 10 to 15 days to reach adult stage [18] and 6 days for pre-nymphoposition [17]. Being infested on detached plant organ, shoot or petiole, the performance of the aphids might be different from those infested on living plants, and the aphids' performance could be significantly affected by the wilting process of the detached organs. Nevertheless, the behavior and performance of the aphids under controlled laboratory conditions should reflect their natural life.

The different behavior of the aphids on the given hosts seemed to be influenced by the suitability of the plant species to the aphids. *P. nigronervosa* settled down more quickly, less than 5 minutes, on greater galangal, galangal, angle wing, blue taro, and rodent tuber (Fig. 1). At the time some aphids had settled down on those considered as suitable host plants, the rest were still moving around and some tried to leave the plant cuttings. This happened for 10 to 20 minutes before eventually all aphids got settle down. When all aphids finally settled down, most of them were found close to the base of the plant cuttings. It seemed likely that they tried to find a place to hide. Under natural conditions, *P. nigronervosa* tends to stay and breed in enclosed places as it was shown that the banana aphid occurs more often between sheaths of older leaves and the petiole [29], or on underground plant parts [30], [31].

The strange behaviour of *P. nigronerosa*, moving around without probing for several hours, on *Z. zerumbet*, *C. domestica, Z. officinale*, *C. zanthorrhiza, C. esculenta,* and *A. macrorrhizos* could be an indication that the aphid could feel that the plants were unsuitable for them. This was consistent with the finding of Givovi13 Weibull, and Pettersson [32] that on unsuitable host, aphids waited a significantly longer time before making their first test probe. In general and under natural conditions, aphids tend to test the plant by probing using both olfactory and gustatory stimuli soon after arriving on the new host [33].

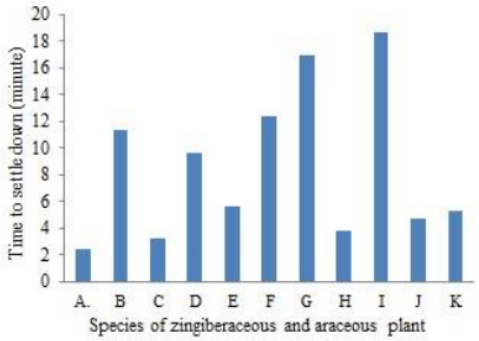

Figure 1. Time required by *Pentalonia nigronervosa* to settle down on zingiberaaceous and araceous plant cuttings. (A) *A. galanga,* (B) *C. domestica*, (C) *K. galanga*, (D) *C. zerumbet*, (E) *Z. officinale*, (F) *C. zanthorrhiza*, (G) *C. esculenta*, (H) *C. bicolor*, (I) *A. macrorrhizos*, (J) *X. sagittifolium*, (K) *T. flaglliforme*.

In this experiment, the plant cuttings were not of the same size due to the characteristic of each plant. The texture and hardness of the surface also different among the cuttings which could affect the aphids behavior. According to Goffreda, Mutschler, and Tingey [34], aphid uses chemical and morphological characteristic of the plant to assess the suitability of the plant. The morphological characteristic that can influence plant suitability include trichome [34] and epicuticular waxes [35], [36]. Aphids can also use plant allelochemicals [37] to assess the suitability of a plant. All plants tested in this experiment had no trichome. Therefore, the aphid strange behavior of keeping moving around and postponing the first test probe, could be related to the epicuticular wax and allelochemicals possessed by the plant. Bhadra and Agarwala [38] confirmed that different plant species provided different food environments for colonization by aphids [38] even though they belong to the same family.

The performance of *P. nigronervosa* on all plant cuttings showed significant difference. The banana aphid displayed its ability to reproduce only on few species of the given plant, but failed to do so on others. The highest population reached by the aphid varied considerably. Time to reach the population peak also noticeably varied (Fig. 2), indicated that the aphid failed to reproduce continuously on some species of given plants, and successfully reproduced only on certain species. The quicker the aphid colony reach its population peak, the smaller the colony size they could build (Table I), indicated that the aphid failed to reproduce or reproduced only at the beginning of colonization. The such situation was found on *C. zerumbet, Z. officinale*, *C. zanthorrhiza* and *A. sagittifolium* which can be considered as the unsuitable hosts for the aphid.

The colony longevity showed the ability of the aphid to live on each plant, even if the plant was not the suitable one. Colonies of *P. nigronervosa* could live more than two weeks on all plant cuttings except those of *C. domestica* and *C. zanthorrhiza*, but only on two species the aphid colony could reach 25 days of age, *A. galanga* and *C. bicolor*. The longevity of banana aphid colony on each plant cutting depended on how the aphid could suit the nutritional value [38] and tolerate the deterioration process occurred in the cuttings. On the cuttings of plant containing appropriate nutritional value, banana aphids reproduced more prolifically and developed longer life colony as shown by *A. galanga* and *C. bicolor*. The *P. nigronervosa* colonies built on *C. domestica*, *K. galanga*, *C. zanthorrhiza*, *C. esculenta*, *C. bicolor*, *X. sagittifolium*, and *T. flaglliforme* showed moderate longevity, more than two weeks, bigger than those built on *Z. officinale*, *C. zanthorrhiza* and *A. macrorrhizos*.

On all plant cuttings, the development of banana aphid population was considerably slow, at no time was a cutting had more than 40 aphids, and the average colony never reached 30 aphids. However, the ability of the aphid to stay for more than a week in non-banana plants might have enough effect on the BBTV transmission in the field, since viruliverous aphids can retain the virus for the rest of their life. Furthermore, there has been no report that the viruliverous aphid would lost their transmitting efficiency after feeding on non-hosts. This could answer the question concerning the lack of aphid banana as the vector of BBTV in the field that BBTV incidence is increasing significantly [39]. The aphids may spend some time on non-banana hosts while they are transmitting the virus. Nevertheless, Niyongere et al. [31] reported that there were no correlation between *P. nigronervosa* population fluctuation and banana bunchy top disease incidence in the field.

The growth rate of *P. nigronerosa* on *Z. zerumbet*, *Z. officinale*, *C. zanthorrhiza,* and *A. macrorrhizos* were slower than those on *A. galanga, C. domestica, K. galanga, C. esculenta, C. bicolor, X. sagittifolium,* and *T. flagelliforme* (Table I). The difference of growth rate of banana aphid indicated that not all zingiberaceous and araceous plants are suitable host for the aphid. This result was quite surprising since there have been many reports stating that *P. nigronervosa* could live and breed on zingiberaceous and araceous plants, especially *Z. officinale* [9], [27], [40]. Based on the growth rate of banana aphid on the test plants, we can divide the test plants into three groups, most suitable, moderately suitable and unsuitable hosts of *P. nigronervosa*. The most suitable hosts are those on which the banana aphid could multiply more than 5 times of initial number, moderately suitable host are those on which banana aphid could multiply 2 to 5 times of initial number, while unsuitable hosts are those on which banana aphid could not multiply. This is in accordance with the report of Givovich, Weibull, and Pettersson [32] that aphids' growth rates were much slower on the unsuitable host plant than on the suitable one, and the aphids invariably settled in higher numbers on the suitable host than on unsuitable one.

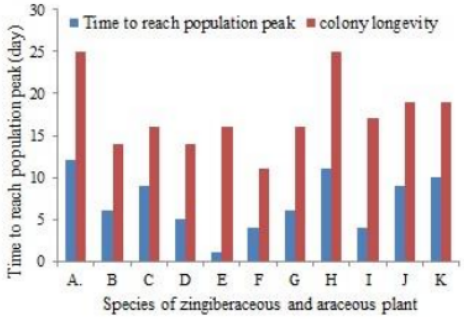

Figure 2. Time to reach population peak and the colony longevity of *Pentalonia nigronervosa* on zingiberaceous and araceous plant cutting. (A) *A. galanga*, (B) *C. domestica*, (C) *K. galanga*, (D) *C. zerumbet*, (E) *Z. officinale*, (F) *C. zanthorrhiza*, (G) *C. esculenta*, (H) *C. bicolor*, (I) *A. macrorrhizos*, (J) *X. sagittifolium*, (K) *T. flaglliforme*.

P. nigronervosa could only breed prolifically on *A. galanga*, *C. esculenta*, *C. bicolor*, and *X. sagittifolium*. These four species might have better nutritional quality, in term of quality required by the aphid. Sandström and Pettersson [41] reported that unsuitable host plants could affect aphid´s performance through the nutritional quality presents in the phloem and, according to Jansson and

Ekbom [42], nutritional quality of the plant may also alter aphids's fecundity. In Table I, it was shown that only on *A. galanga*, *K. galanga*, *C. bicolor and T. flaglliforme* the banana aphid still had positive growth rate on 10th after infestation, and only on *A. galanga* and *C. bicolor* the growth rate increased. On 15th day, the aphid growth rate on all plant cuttings were negative, except on *C. zerumbet, Z. officinale, C. zanthorrhiza, C. esculenta* and *A. macrorrhizos* where no aphid could be found alive anymore. *Z. officinale* was the only plant showed negative growth rate from the beginning of measurement, no single adult of the aphid could reproduce on the plant.

TABLE I. POPULATION PEAK AND BREEDING POTENTIAL OF *PENTALONIA NIGRONERVOSA* ON ZINGIBERACCEOUS AND ARACEOUS PLANTS

Plant species	Population peak*	Rate of *P. nigronervosa* multiplication*		
		on 5rd day	on 10th day	on 15th day
A. galanga	31.6	1.58	3.54	-0.76
C. domestica	8.4	0.66	-1.04	-2.12
K. galanga	12.3	1.38	0.08	-2.12
C. zerumbet	4.6	0.12	-0.58	-
Z. officinale	4.0	-0.32	-0.22	-
C. zanthorrhiza	4.3	0.04	-0.8	-
C. esculenta	20.2	3.04	-1.14	-
C. bicolor	28.9	2.22	2.64	-2.08
A. macrorrhizos	4.5	0.12	-0.54	-
X. sagittifolium	25.9	2.84	1.36	-3.36
T. flaglliforme	16.9	1.98	0.6	-2.62

*) average of 20 replication

Winged adults (alatae) of banana aphid play more important role in the transmission of BBTV. Even though the aphid of all phases of development are able to transmit the virus, only the winged adults are actively move from one plant to others. However, winged adults were rarely seen during the experiment. The winged aphids appeared only on two zingiberaceous plant cuttings, *C. zerumbet* and *C. zanthorrhiza*, and two araceous plant cuttings, *C. esculenta* and *A. macrorrhizos*. Winged adult of aphid develops in response to food shortage or too crowded population. In this experiment, the formation of winged adults could be induced by the ageing or deteriorating process of the given host. The populations were not crowded enough to stimulate the aphid to produce winged adult. This was in line with the finding of Waterhouse and Norris [30] that in new colonies, banana aphid needs around 7-10 generations of wingless females before winged females start to appear.

IV. CONCLUSIONS

Overall, it is concluded that the banana aphid *P. nigronervosa* can feed and live on some species of zingiberceous and araceous plant as alternative hosts with different degree of suitability. Based on behaviour and performance of the aphid on detched shoots of zingiberceous plants and detached petioles of araceous plants, the suitability of the alternative hosts is classified into three different groups. The first group is the most suitable alternative host consists of greater galangal (*A. galanga*), taro (*C. esculenta*), angle wing (*C. bicolor*) and blue taro (*X. sagittifolium*). The second groups moderately suitable alternative hosts consists of turmeric (*C. domestica*), galangal (*K. galanga*) and rodent tuber (*T. flaglliforme*). The third group is the least suitable alternative hosts including bitter ginger (*C. zerumbet*), ginger (*Z. officinale*), Javanese ginger (*C. zanthorrhiza*, and elephant ear (*A. macrorrhizos*).

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Suparman was born in Banyumas, Central Java, Indonesia, on 02 January, 1960. The author received his B.Sc. in Plant Pathology and Entomology from Bogor Agricultural University, Indonesia in 1984 and received his Ph.D. in Plant Pathology from University of Wales, Aberystwyth, The UK in 1994.
He has 32 years of working experiences as a senior LECTURER in Sriwijaya University, Indonesia. During his services as a lecturer, he has visited numbers of university in United Kingdom, Thailand, Japan, Malaysia and Vietnam as parts of his capacity improvement and career development. His subject of interests are Plant Disease Epidemiology and Biological Control. His current and previous research interests and publications are related to epidemiology of banana bunchy top disease and entomopathogenic virus as biological control agent of insect.

Yulia Pujiastuti was born in Yogyakarta Indonesia, on 18 May 1962. She got her Ph.D degree from Applied Bio-Science Department, Graduate School of Agriculture Hokkaido University, Sapporo Japan in 2000. Currently, she is senior LECTURER in Department Plant Protection, Facuty of Agriculture, Sriwijaya University.

Bambang Gunawan was born in Palembang, South Sumatra, Indonesia on 17 August 1959. He received his M.Si in Plant Sciences from Sriwijaya University, Indonesia in 2006. He is a senior LECTURER in Department of Plant Protection, Faculty of Agriculture Sriwijaya University.

Arsi was born in Ogan Komering Ilir, South Sumatra, Indonesia on 17 Oktober 1985. He received his M.Si in Plant Sciences from Sriwijaya University, Indonesia in 2014. He is a junior Lecturer in Department of Plant Protection, Faculty of Agriculture Sriwijaya University.

Rizky Randal Cameron is a graduate student of Department of Plant Protection, Faculty of Agriculture, Sriwijaya University.

Alternative Hosts of Banana Aphid Pentalonia nigronervosa Coq. (Hemiptera: Aphididae), the Vector Transmitting Banana Bunchy Top Virus

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