***BANANA BUNCHY TOP VIRUS* INFECTION TO BANANA PLANTS INDUCES THE INCREASE VOLATILES EMISSION AND THE VIRUS VECTOR ATTRACTIVENESS, *PENTALONIA NIGRONERVOSA* COQUEREL (*HEMIPTERA*: *APHIDIDAE*)**

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**Consent for publication** all coauthors consent for publication

**Abstract-**

Banana plants are affected by various viral diseases, among which the most devastating is the "bunchy top", caused by the *Banana bunchy top virus* (BBTV) and transmitted by the aphid *Pentalonia nigronervosa* Coquerel. The effect of BBTV on attraction mechanisms of dessert and plantain banana plants by the vector remains far from elucidated. For that, attractiveness tests were carried out using a two columns olfactometer for apterous aphids, and a dispositive allowing short-distance flight in a cage for alate aphids. Volatile Organic Compounds (VOCs) emitted by either healthy or BBTV-infected banana plants were identified using a dynamic extraction system and gas-chromatography mass-spectrometry (GC-MS) analysis. Behavioral results revealed a stronger attraction of aphids towards infected banana plants (independently from the variety), and towards the plantain variety (independently from the infection status). GC-MS results revealed that infected bananas produced VOCs of the same mixture as healthy bananas, but in much higher quantities. In addition, VOCs produced by dessert and plantain banana plants were different in nature, and plantains produced higher quantities than dessert bananas. This work opens interesting opportunities for biological control of *P. nigronervosa*, for example by luring away the aphid from banana plants through manipulation of olfactory cues.

**Key Words- *Musa* spp., phytovirus, behavior, Volatiles Organic Compounds, metabolites, biological control.**

**INTRODUCTION**

Banana viral diseases are responsible for major economic damages to producers (Kumar *et al*., 2015). They constitute a major constraint in banana fruit production, and threaten worldwide germplasm distribution (Thomas *et al*., 1994; Mobambo *et al*., 2010; Shiragi *et al*., 2010; Walangululu *et al*., 2010; Mukwa *et al*., 2014). Banana bunchy top disease (BBTD), caused by *Banana bunchy top virus* (BBTV), is recognized as the most devastating viral affection of banana plants in the world (Thomas and Iskra-Caruana, 2000; Chandrassekar *et al*., 2011; Qazi, 2016), sometimes resulting in 100 % yield loss (Qazi, 2016). Currently, BBTV is present in Africa, Asia and Australia, while its vectoris located in all regions of the world, even where BBTV is not yet reported, such as in South America and Europe (CABI, 2021). The diseased plant present a plant dwarfism, the narrow leafs, chlorosis of leaf margins and discontinuous dark-green streaks on leaves, petioles and pseudotem. The leaves of infected plants become progressively smaller and stand more erect giving the plant a bunchy appearance (Gatsinzi, 1987). BBTV belongs to the *Nanoviridae* family, genus *Babuvirus*, whose genome is composed of several segments of single-stranded circular DNA, encapsidateds in small isometric particles (18-20 nm), which makes it the one of the smaller viruses (Burns *et al*., 1995 ; Timchenko and Bernardi, 2007; Stainton *et al*., 2015; Mukwa *et al*., 2016).

In the plant BBTV is restricted to phloem tissues of infected plants, and the cells surrounding the phloems contain an abnormal number of chloroplasts, giving rise to the macroscopic symptoms of dark green streaks. After infection, BBTV replicates and gradually accumulates in all parts of the plant, except in leaves formed before infection where the virus is present but does not replicate. This explains the fact that the vector is not able to acquire the virus from these leaves (Hafner *et al*., 1995; Iskra-Caruana, 2003).

BBTV is transmitted by the banana aphid, *Pentalonia nigronervosa* Coquerel (*Hemiptera*: *Aphididae*) in a persistent, circulative and non-propagative manner (Iskra-Caruana, 2003; Anhalt and Almeida, 2008). This implies a lifelong retention of the virus in the vector, with a relatively long acquisition time during a meal on an infected plant (some hours to many days) (persistent transmission). After its ingestion by the vector, the virus reaches the intestine, crosses the intestinal wall then diffuses in the hemolymph to the salivary glands, and becomes transmissible on a new plant (circulative transmission), without any viral replication (multiplication) during transfer (circulative and non-propagative transmission) (Raccah and Fereres, 2009; Gray and Ghanim, 2014).

Even if in certain situations visual stimuli (color, movement, shape) seem to be involved (Döring, 2014 ; Leather, 2014), the search and selection of the host plant by aphids remain essentially facilitated by Volatile Organic Compounds chemicals (VOCs) emitted by plants (Bernasconi *et al*., 1998; Pickett *et al*., 2013; Piesik *et al*., 2013). These molecules are volatile secondary metabolites of plants (Bernasconi *et al*., 1998; Piesik *et al*., 2013) which are detected by insect olfactory systems located on their antennae (Storer *et al*., 1996). In aphids, VOCs influence insect behaviors, in particular regarding the search for food resources, leading to potential virus transmission (Whittaker, 1971; Herrbach, 1985; Berenbaum, 1995). Herbivorous insects such as aphids have developed a strong co-evolution with their host plants, so that variations in the composition (quality and quantity) of VOCs emitted by their host plants always trigger changes in their behavior (van Emden, 1973; Peccoud *et al*., 2010).

Qualities and quantities of VOCs produced by plants vary depending on their infection status by phytoviruses (Chaudhry *et al*., 1999 ; Bosque-Pérez and Eigenbrode, 2011). This is the case, for example, of the study of Eigenbrode *et al*. (2002), in which the profile of VOCs emitted by potato plants (*Solanum tuberosum*) infected with Potato leafroll virus (PLRV) was of different mixtures and quantity (concentration) than that of the profile of VOCs emitted by healthy plants. Also, the study by Jiménez-Martínez *et al*. (2004) found that wheat plants infected with barley yellow dwarf virus (BYDV) emitted VOCs in total concentrations greater than the VOCs emitted by the healthy wheat plants. It has been shown that virus vectors, such as aphids, respond changes in the quality of the host plant as a consequence of viral infection. Thus, studies on the host - parasite - vector relationship indicate that some plants infected with the virus are higher quality hosts for the vectors compared to the plants free from the virus, with regard to the attractiveness, the growth rate of the vector, fecundity and survival of the latter. This is the case with potato plants (*S. tuberosum*) PLRV, vis-à-vis the *Myzus persicae* aphid, which is preferentially attracted to plants affected PLRV in comparison to plants free or affected by other viruses (Eigenbrode *et al*., 2002; Bosque-Perez and Eigenbrode, 2011). The same situation occurs with *Rhopalosiphum padi*, an aphid which infects *Barley yellow darf virus* (BYDV) on wheat (Jimenez-Martinez *et al*., 2004b; Srinivasan *et al*., 2006). On the other hand, some other aphids avoid infected plants which are inferior hosts. This is the case for example in *Cucurbita pepo*, where plants infected with *Cucumber mosaic virus* (CMV) are relatively poor and of low quality for aphid vectors, especially *My. persicae* and *Aphis gossypii*, compared to healthy plants. In this case, it is believed that CMV manipulates the plant by causing it to produce the volatile compounds that falsely attract vectors to infected plants and then quickly disperses (Mauck *et al*., 2010).

The topic of ​​chemical interaction between insects and their biotic environment is currently the subject of increasing research for biocontrol of phytophagous insects (Bosque-Pérez & Eigenbrode, 2011; Eigenbrode *et al*., 2018 ; Xu *et al*., 2018; Chesnais *et al*., 2019a, 2019b ; Mauck *et al*., 2010, 2020). It is especially relevant in the current context of increasing resistance to synthetic insecticides and issues regarding their negative impact on the environment and human health (Penrose *et al.*, 1994; Edwards and Tchounwou, 2005). Recently, Berhal *et al*. (2017) characterized the VOCs released by the aerial part of healthy banana seedlings. However, the impact of BBTV on the recognition mecanisms of banana plants (*Musa* spp.) by *P. nigronervosa* remains unknown.

Our study aimed at better understanding the interaction between banana plants of dessert and plantain varieties, infected or healthy by the BBTV, and the aphid vector, potentially mediated by VOCs. The question is whether BBTV is capable of modifying the phenotypic characteristics of bananas to influence the attractiveness of *P. nigronervosa*, through a qualitative and quantitative variation in the profile of the VOCs emitted. This is in accordance with the conclusion of Eigenbrode *et al*., 2002; 2018 ; Mauck *et al*., 2020, tending to reinforce the “vector manipulation hypothesis-VMH”, better applied in aphids, predicting that a virus will promote its propagation from plant to plant by influencing the selection behavior of the host plant, through its effects on the moving vector (Ingwell *et al*., 2012; Roosien *et al*., 2013). As a hypothesis, it is thinks in this work that the mixtures and quantities of volatile organic compounds (VOCs) emitted by bananas vary with infection with BBTV, and that infected bananas emit a range of VOCs more attractive to *P. nigronervosa* than healthy bananas. Thus, the healthy and infected young plants of two most representative varieties of bananas in the world (dessert bananas/ banana fruit, and plantain/ cooking bananas), through their most popular and cultivated cultivars, respectively: Cavendish (AAA genome) and Plantain Pacific (AAB genome) (Simmonds 1962; Lorenzen *et al*., 2010; Ploetz 2015) were used in this work as sources of VOCs to assess the influence of the virus on the attraction behavior of *P. nigronervosa*. At the same time, we found necessary to collect and to identify the VOCs emitted by these bananas, to determine the effect of BBTV on the qualities and quantities of their emissions. From a more applied point of view, our goal is to identify how, and which VOCs could be used to repel or falsely attract banana aphids.

**MATERIAL AND METHODS**

***Insects and plants***

*Pentalonia nigronervosa* colony was obtained from a parthenogenetic female collected from a healthy banana in the province of South Kivu (Democratic Republic of the Congo), and continuously reared on live bananas, disease-free, and planted in pots (Thermoformed red MCI 17:2L pot) on a potting soil substrate. Aphids on banana plants were kept in cages (200 x 100 x 100 cm) of small mesh nets, placed in air-conditioned chambers at 25 ± 2 °C, a relative humidity of 30 ± 5%, and an artificial 12h/12h photoperiod. Experiments were also carried out under these conditions. Prior to their use in each attractiveness test, adult aphids were taken from banana plants and placed in glass Petri dishes for at least 8 hours to starve them. Alate aphids were obtained when the density of the aphid population increased sharply, or when the quality of bananas decreased significantly (Braendle *et al.,* 2006; Williams and Dixon 2007).

The plant material was constituted of dessert bananas of cultivar Cavendish (strict triploid *M. acuminata* - AAA), and plantains of the Pacific cultivar (hybrids and triploids *M. balbisiana* - AAB), either symptomatic (with symptoms of BBTV) or asymptomatic (without disease symptoms). This resulted in four plant treatments that were used for both the behavioral experiment with aphids and for VOC extraction and identification: Healthy Dessert Bananas (HDB), Healthy Plantain Bananas (HPB), Infected Dessert Bananas (IDB), Infected Plantain Bananas (IPB). Plants were identified and collected in peasant plantations in South Kivu in the Democratic Republic of the Congo (Dowiya *et al.*, 2009), with the support of the International Institute of Tropical Agriculture-IITA / Kalambo (Bukavu, RD Congo). All these plants were maintained and multiplicated in the tropical greenhouse (greenhouse n°13; G2) of the Université catholique de Louvain (Louvain-la-Neuve, Belgium) by the PIF technique (Plantes Issues de Fragments de tiges) (Kwa, 2003, 2009; Meutchieye, 2009; Sadom *et al.*, 2010; Mbunzu *et al.*, 2019), and were irrigated daily, until they reached 40 to 60 days of age (4 to 6 leaf stage), for their uses in attractiveness (apterous and alates) and extraction tests VOC. Since BBTV accumulates in the pseudostem, in the basal meristem of the bulb, and in the leaf (Hafner *et al*., 1995); suckers from an infected strain are automatically infected and show BBTV severe symptoms (van Regenmortel *et al*., 2000; Thomas and Iskra-Caruana, 1999). For this, the infected plants were obtained directly by PIF technique, from the bulbs of infected banana plants. Transmission of the virus by mechanical inoculation has never been successful (Thomas *et al*., 1994; Lepoivre, 2003). Plants had been tested twice by PCR (Table S1) in order to confirm the genotype (Supplementary Figure S1) and the infection status (Supplementary Figure S2) of each of them.

**Attractivenessof *P. nigronervosa***

***Apterous aphids.*** The attractiveness of the different types of banana plants on apterous *P. nigronervosa* aphids was determined using a two-column glass olfactometer (Y olfactometer), associated with an air flow, set at a pressure of 2.5 to 3 bars, and placed on a light table.

Banana seedlings used were in the 4th to 6th leaves stage and content in glass bells designed for this purpose. Four choice tests were carried out: HDB - HPB, HDB - IDB, HPB - IPB, and IDB – IPB (i.e., one for each plant genotype and infection status comparison). In addition, three control choice tests were done: HDB - Soil (pot containing the soil alone), HPB - Soil, and S (Soil) – E (Empty). Each test was repeated 30 times, and within each repetition, 20 apterous adult aphids, at reproduction age, were placed at the central neutral chamber of the olfactometer. The banana plants and apterous aphids used in each repetition and each treatment had never been used before. We considered that an individual aphid had made a choice between one of two sources of VOCs when they passed the middle of the tube connecting the neutral chamber to one of two olfactory sources after 30 min of observation. In order to prevent odor residue remaining from one experiment (or repetition) to the next, the pots containing the plants were inverted and the olfactometer was sterilized with 70% ethanol, then dried for five minutes before à further reuse

***Alate aphids***. The attractiveness of the different types of banana plants on alate *P. nigronervosa* aphids was determined using a dispositive allowing short-distance aphid flight in a wooden (200 x 100 x 100 cm) cage, the front face of which was covered with a fine mesh fabric to facilitate experimental manipulation.

Each test was repeated 20 times, and within each repetition, two types of banana plants at the 4th to 6th leaves stage were placed in the cage in front of 20 alates aphids, put in an open Petri dish, on the other side of the cage and at an equal distance from both plants (≈15 cm), in order to test their choice between two olfactory sources.

Four tests were performed: HDB - HPB, HDB - IDB, HPB - IPB, and IDB - IPB. As for apterous aphids, two other comparisons serving as controls were done: HDB - Soil (pot containing the soil alone), and HPB – Soil.

Aphid choices were assessed by counting the number of aphids found on each of two types of banana plants 24 h after they were deposited in the cage, and each choice experiment was repeated 20 times. The banana plants and alate aphids used in each repetition and each treatment had never been used before.

***VOC extraction and analysis***

***VOC extraction***. The VOCs were collected using a dynamic extraction system made up of a glass enclosure (40L), hermetically sealed, and connected to a vacuum pump (Rocker 300, Vacuum Pressure Pump, 80 mBar, -HG 680 mm, 20 L.min-1, NSE, GA, USA). In the glass enclosure, a live banana plant (never used before) ≈50 cm high (4 to 6 leaves) from each genotype (plantain and dessert) and from each BBTV status (infected and healthy) was placed. The pots in which the banana plants were planted were covered with aluminium foil to prevent the emission of VOCs from the underground part of the plant and the VOCs of the potting soil. Another pot containing only potting soil and also covered with aluminium foil served as a control in this part of the study. All the treatments (HDB, IDB, HPB, IPB) and controls (potting soil alone) were repeated five times, and a single banana plant was used only once, in a single repetition (and treatment). Each enclosure was covered with a Pyrex bell carrying two openings to which were connected two Teflon pipes (ELNEO: TFL 8x6 NATUR). One of the two pipes was connected to an activated carbon filter cartridge which purifies the air entering the system, and the other was connected to a bottle containing silica gel dehydrating the air rich in VOCs from the enclosure, before passing through the trap cartridge (TENAX TA wax trap, Gerstel, Mülheim an der Ruhr, Germany), capturing the extracted VOCs. The cartridge outlet was connected to the pump via an adjustable flow valve maintaining the airflow at 500 mL.min-1. The entire extraction device was placed in an air-conditioned room, at a temperature of 25 ± 2 °C, a relative humidity of 40 ± 5% and an artificial photoperiod of 12h/12h, and the VOC collection lasted 24 h.

***VOC analysis***. After each extraction from the four types of bananas (HDB, IDB, HPB, IPB) and of the control, the TENAX cartridge loaded with VOCs was thermally desorbed (Thermal desorption unit, Gerstel, Mülheim an der Ruhr, Deutschland) at 280°C for 10 min. The analysis of captured VOCs was made using Gas Chromatography (7890A, Agilent Technologies, Santa Clara, CA, USA) coupled with Mass Spectrometry (5973, Agilent Technologies, Santa Clara, CA, USA) (GC-MS). The identification of VOCs was carried out by comparing the retention time and the mass spectra of the data obtained with available standards. Quantification was performed by comparing the peak areas of the compounds with the standard peak areas (peak surfaces) provided by the Chemstation software (Agilent Technologies, Palo Alto, CA, USA). The retention times (in minutes) and peak areas (in millivolts) of the chromatograms obtained served so respectively as qualitative and quantitative parameters in this comparison. The validation of the VOCs extracted for each treatment was motivated by their presence in at least four replicates out five (De Backer *et al*., 2015), and by their absence in the control sample (potting soil alone). This protocol allowed comparing both the nature and the concentration of VOCs emitted by the aerial part of banana plantlets of each of the four treatments.

***Statistical analyses***

Comparisons of decisions made by both alate and apterous aphids between each pair of olfactory sources were carried out using Student's t tests (the normal distribution of our data was visually assessed).

Biplot PCAs of the 16 detected VOCs were constructed using the *factoextra* package (Le *et al.*, 2008). Two PCAs representations were done; one grouping datapoints by banana plant varieties (dessert or plantain) and the other by grouping banana plants according to their infection status (infected or healthy). Ellipses were added around the barycenter of the groups using a 95% confidence interval. After that, the Adonis function from the package *vegan* (permutational MANOVA) has been used by calculating a Jaccard distance matrix and running 999 permutations, to assess if the composition of the set of volatile compounds differed between varieties and between infection status (i.e., differences among created groups on the PCA).

Then, the effect of the variety factor (two levels) and of the infection state factor (two levels), as well as that of the interaction of both factors on the quantity of each VOC emitted were analyzed. To do so, Generalized Linear Models (GLMs) were fitted to the data, for each VOC, using a negative binomial distribution family for count data. Models were tested using the Anova function of the *car* package, and a Wald statistic. Then, in a more detailed approach, we compared the mean recorded value of each volatile among the four treatments. For each of the VOCs detected and measured, pairwise comparisons of the peak areas (quantification in mV) were performed using negative binomial GLMs, between HDB-HPB, HDB-IDB, IDB-IPB, and HPB-IPB. All analyses were carried out in R (v. 4.0).

**RESULTS**

***Attractiveness of aphids by banana plants***

In the protocol’s validation tests on the attractiveness banana aphids towards a banana plant or a control (potting soil only), the apterous and alate aphids were each time more attracted by the banana plant (whatever the genotype), than by the control (Table 1). On the other hand, for wingless aphids only, there were no significant differences between the two types of control: potting soil alone (S) and empty (E) (Table 1).

The apterous adults of *P. nigronervosa* were more attracted to IDB than to HDB (t = -5.0, p<0.001, Figure 1A), and to IPB than to HPB (t = -2.3, p = 0.02, Figure 1B). They were also more attracted to IPB than to IDB (t = -2.8, p<0.01, Figure 1C), and to HPB than to HDB (t = -2.3, p = 0.02, Figure 1D).

The alate adults of *P. nigronervosa* were more attracted to IDB than to HDB (t = -5.04, p<0.001, Figure 2A), and to IPB than to HPB (t = -6.68, p<0.001, Figure 2B). In addition, alate aphids were significantly more attracted to IPB than to IDB (t = -2.65, p = 0.01, Figure 2C), while they were not differentially attracted to HDB and HPB (t = -1.48, p = 0.15, Figure 2D). The attractiveness of both morphs of *P. nigronervosa* to banana plants therefore varies according to both the infection status and genotype of banana plants.

***Identification of VOCs emitted by healthy and infected banana plants***

A total of sixteen volatile organic compounds were detected (Figure 3), of which 12 VOCs were emitted by to dessert bananas and 13 to plantains (not exclusively, and regardless of the health status). Other compounds were identified but not retained in the final list because of their presence in the control treatment (potting soil only), and in less than four replicates out of the five (De Backer *et al*. 2015). Results revealed that these VOCs varied in quality and quantity between genotypes (dessert and plantain bananas) regardless. Results revealed that these VOCs varied in quality and quantity between genotypes (dessert and plantain bananas) regardless of the infection status (healthy bananas and infected bananas), while they varied mainly in quantity between infected status, regardless of the genotype (Figure 3, Table 2). Overall, the variety (F = 21.0, R²=0.43, p<0.001), the infection status (F=8.2, R²=0.17, p<0.01) had an effect on the set of VOCs emitted by banana plants, with a very light interaction effect between both variable (F=3.3, R²=0.07, p=0.02), meaning that for some volatile compounds, the effect of the virus differed between both varieties (permutational MANOVA, Figure 3).

Compounds such as α-pinene, β-pinene, and limonene were released only by dessert banana plants (infected and healthy), while alloocimene, (*E*)-β-farnesene, (*Z,E*)-α-farnesene, and 4,8-dimethyl-1,3,7nonatrien were specifically released by plantain banana plants (infected and healthy). Other compounds such as 6-methyl-5-hepten2-on, Myrcene, (*Z*) β-ocimene, (*E*)-β ocimene, linalool, methyl salicylate, 6-methyl-3,5-heptadien2-one, (*E*)-hex-2-enal and Nonanal were common to both genotypes. Considering only the VOCs common to the two genotypes, the plantains emitted the greatest amounts of VOCs, with the exception of three VOCs for the healthy bananas ((*Z*)-β-ocimene, 4,8-dimethyl-1,3,7nonatrien and 6-methyl-3,5-heptadien2-one) and five VOCs for infected bananas ((*Z*)-β-ocimene, 6-methyl-3,5-heptadien2-one, methyl salicylate, Myrcene and Nonanal) (Figure 4). In addition, infected plants emitted greater amounts of VOCs than healthy plants, except one VOC for dessert banana (6-methyl-5-heptene2-on) and four VOCs for plantain ((*E*)-hex-2-enal, 6-methyl-5-hepten2-on, methyl salicylate and Myrcene), for which the viral infection did not modify the amount of VOC emitted (Figure 4).

Overall, and still considering only the common VOCs, the banana plants genotype had a significant influence on the quantity of emitted VOCs, with the exception of 6-methyl-5-heptene2-on, (*Z*)-β-ocimene and Nonanal (Table 2). The infection with BBTV had a significant influence on emissions from banana plants, with the exception of 6-methyl-5-hepten2-on. The genotype \* infection interaction influenced only Nonanal, methyl salicylate and (*E*)-hex-2-enal. Our results therefore reveal that the genotype acts on both the quality and the quantity of VOCs emitted by bananas plants, while infection by BBTV mainly acts on the increase in the VOC quantities.

**DISCUSSION**

In this study, we tested the attractiveness of of two *P. nigronervosa* varieties towards healthy and infected banana plants with BBTV, and we found that the alates and apterous of *P. nigronervosa* were more attracted to infected banana than healthy banana plants (regardless of the variety), and by plantain bananas than to dessert bananas (regardless of the infection status by the BBTV). We also evaluated the effect of BBTV on the quality and quantity of VOCs released by banana plants, and we found that infected plants released VOCs of the same nature as healthy plants, but in much greater quantities. In addition, the set of VOCs released by dessert and plantain banana plants were of different mixtures (although some molecules were common), but plantains produced greater quantities of VOC. The greater attractiveness of *P. nigronervosa* to infected bananas could be due to the fact that plants infected with BBTV release VOCs in greater quantities than healthy plants. At this stage, however, it is difficult to determine whether this difference is linked to a physiological effect of the virus on the plant or to a real manipulation by the virus, aimed at making the plant more attractive for aphids, to increase virus transmission by vectors (Chesnais *et al*., 2019a). Indeed, it is argued that various phytoviruses transmitted by vectors (such as aphids) directly influence host plant phenotypic traits such as VOC emission, and indirectly the patterns of attraction, retention, performance, and dispersion of vectors (Belliure *et al*., 2005; Mauck *et al*., 2010 ; Bosque-Pérez & Eigenbrode, 2011 ; Shapiro *et al*., 2012 ; Carmo-Sousa *et al*., 2014 ; Tungadi *et al*., 2017 ; Eigenbrode *et al*., 2018). Such phenotypic changes, induced by viral infection of the plant, can manipulate the vector making it attack infected plant through the production of different kinds of VOCs (Thomas *et al*, 2005; Eigenbrode *et al*., 2018), which favors the propagation and epidemiological dynamics of the virus (Sisterson, 2008; Ingwell *et al*., 2012). It is possible that aphids chose to attack infected plants, because the emitted VOCs signal that plants are weakened by an infection. We clearly observed that infected plants were more attractive for aphids, whether apterous or alate, and could thus lead to higher viral transmission. These results are similar to those found in the study by Jimenez-Martinez *et al*. (2004b), presenting that the cereal aphid *R. padi* was more attracted by wheat plants infected with Barley yellow dwarf virus (BYDV) than by healthy plants. The attraction preference of aphids by the plantain variety could be due to the higher amounts of VOCs emitted by this genotype, regardless of the infection status of the plant. The Cavendish dessert genotype is a strict triploid (AAA) and belongs to the species *M. acuminata*, and the plantain is a triploid hybrid (AAB) belonging to the species *M. paradisiaca*, and derived from the cross *M. acuminata* x *M. balbisiana* (Simmonds, 1962). This genotypic demarcation between Cavendish and Pacific plantain underlies differences in their metabolic profiles, precisely in the secondary metabolites, such as VOCs, involved in the short distance communication between insects and their environments (Bernasconi *et al.*, 1998; Piesik *et al.*, 2013). Our results on the "infected dessert banana (IDB) - infected plantain (IPB)” test is consistent with those found by Chesnais *et al*. (2019), who observed that the aphid *My. persicae* preferred to settle and feed on the wild genotype of camelina (*Camelina microcarpa*) tolerant to *Turnip yellows virus* (TuYV) and infected by the same virus, compared to the cultivated genotype (*C. sativa*) and their F1 hybrid, less tolerant of this virus. The consequence was an increase in the number of viruliferous aphids (Chesnais *et al*., 2019). In our study, *P. nigronervosa* preferred to settle on infected plantain (apparently, less susceptible to BBTV), compared to infected dessert banana (each time, severely damaged by BBTV); the Cavendish variety (Banana dessert), used in our experiments, is also recognized as very vulnerable to BBTV (Su *et al*., 1992; Hooks *et al*., 2009). Indeed, dessert bananas are subject to many diseases because their genetic basis is narrow (Abadie *et al*., 2003).

According to Eigenbrode *et al*. (2018), persistently transmitted viruses more frequently attract their vectors and improve their performance on infected plants than viruses with non-persistent transmission. Indeed, in the mechanism of persistent transmission (case of BBTV), the viral vector is likely to develop a strong affinity with the host plant, because the acquisition of the virus requires sustained feeding in the phloem of the infected plant (4 hours minimum for BBTV), the time that the virus circulates inside the insect to the salivary glands where the virions reside, can be transferred for a long time (often even until his death: case of *P. nigronervosa*) to a new plant (Lepoivre, 2003; Fereres and Raccah, 2009; Gray and Ghanim, 2014). This is not the case in the non (or semi) persistent strategy (case of CMV), where the vector appears to be falsely attracted to the infected plant, then it disperses rapidly (Mauck *et al*., 2010; Tungadi *et al*., 2017). The vector immediately transmits the virus after a very short acquisition time (from a few seconds to a few minutes) (Harris, 1977). To optimize its transmission and propagation, the non-persistent virus induces a pull-push strategy from its vector to its host plant (Carmo-Sousa *et al*., 2014). This shows that vector manipulation traits appear to be adaptive depending on the virus transmission mechanism (Mauck *et al*., 2012, 2018, 2020).

We compared the attractiveness responses of two *P. nigronervosa* forms (apterous and alate) to banana plants, comparing the four plant treatments. We found that both aphid forms were attracted to banana plants in similar ways, even though the apterous aphids are reputed to be less sensitive to odors because they have fewer sensorial organs, such as rhinaria (Yongjun, 1995; Hullé *et al*., 1999). However, in the "healthy dessert banana (HDB) - healthy plantain banana (HPB)" test, apterous aphids were significantly more attracted to the HPB, while the alate aphids were attracted in a similar way to both HDB and HPB. The two forms of aphids were not confronted with the same signals because the apteras were tested with the olfactometer in Y and the alates in settling cages. For the latter (the alates), other signals resulting from the putative manipulation (color, size, texture, metabolites in the leaves, tusks and cuticles of the leaves) were involved in the orientation of the vectors (Mauck *et al*., 2020).

We identified 12 VOCs for dessert bananas and 13 for plantains, out of a total of 16 VOCs. Nine VOCs were common between the two genotypes (6-methyl-5-hepten2-on, Myrcene, (*Z*) -β-ocimene, (*E*)-β-ocimene, Nonanal, Linalool, Methyl salicylate, 6-methyl-3,5-heptadien2-one, (*E*)-hex-2-enal), corresponding to 75% of the total for dessert bananas, 69 % for plantain, and to 56.25% of the total of all emitted VOCs. We found that three compounds (α-pinene, β-pinene, and Limonene) were specific to dessert banana, while four compounds (4,8-dimethyl-1,3,7nonatrien, (*E*)-β-farnesene, (*Z,E*)-α-farnesene, and Alloocimene) were specific to plantains. We showed a clear discrimination between each of the two groups of treatments (infected vs. healthy, and plantain vs. dessert) based on the overall set of detected VOCs. In the genotypic comparison (Figure 3A), the discrimination of banana plants was mostly driven by the compounds that are specific to each genotype, as mentioned above, in addition to differences in emitted quantities. In the infection status comparison (Figure 3B), the discrimination was driven by the overall differences in emitted quantities of VOCs, but not by differences in the nature of emitted VOCs. Infected bananas emitted the greatest amounts of VOCs (at the exception of 6-methyl-3,5-heptadien-2-one), regardless of their genotype, which once again suggest an effect of the viral infection on the phenotype of banana plants. For example, in tobacco (*Nicotiana tabacum* Cv. Ky 57), the infection by a virus leads to increasing the synthesis of ethylene (Chaudhry *et al*., 1999), a volatile phytohormone acting in synergy with other volatile compounds emitted by plants on the increase in total volatile emissions (Ruther and Kleier, 2005). Some aphids are in fact preferentially attracted by the odors emitted by infected plants, whose infection by a virus induces an overall increase in their volatile emissions (Mauck *et al*., 2010).

In order to specify the compounds common between species of the genus *Musa* spp, likely to be used in the attraction of *P. nigronervosa*, we compared the VOCs identified in our study and those previously identified in the study by Berhal *et al*. (2017), which aimed to characterize only the VOCs emitted by healthy bananas (11 VOCs were identified on Cavendish and 13 VOCs on Pacific plantain in the study by Berhal *et al*. (2017), compared to 12 and 13 VOCs respectively in our study).We found that 6 VOCs (6-methyl-5-hepten2-on, Myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, Methyl salicylate, 6-methyl-3,5-heptadien2-one) were common between the two studies. The Nonanal compound identified in our study was not identified in the study by Berhal *et al*. 2017, while β-ionone and (*E, E*)-α-farnesene, present in the previous study were not identified in our study. Indeed, as in the work of Berhal *et al*., 2017, the plants used in our experiments were still young (40 to 60 days), at the stage of four to six leaves (50 cm in height), already knowing that under normal conditions, banana plants emit low amounts of VOCs (Berhal *et al*., 2017). The principal component analysis (Figure 3) clearly shows that Nonanal plays a role in distinguishing infected bananas from healthy bananas, and its recorded value is in fact higher in the presence of the virus for plantain and dessert bananas. This compound marks in fact the greatest difference between healthy and virus-infected plants for the two varieties. It therefore seems that the presence of the virus its emission. In the future, more attention will be paid to this compound to test its attractivity.

All of these VOCs are known, according to pherobase.com and Stenberg *et al*., (2015), to play a role in plant-insect interactions and insect control. Some of them are insect repellents (α-pinene, β-pinene, limonene, myrcene, (E)-β-farnesene, Ocimene and linalool) (Smith, 1965; Byers *et al*., 1979; Harrewijn *et al*., 1996; Arimura *et al*., 2004 ; Francis *et al*., 2005; Lípez *et al*., 2011; Xu *et al*., 2018). Others are attractive to natural enemies of aphids (Methyl salicylate) (James, 2003; James and Price 2004), and others to various types of insects (Nonanal, (E)-hex-2-enal and (E) -β- farnesene) (Syed *et al*., 2009; Francis *et al*., 2005 ; Hoballah and Turlings, 2005; Shiojiri *et al*., 2006; Halitschke *et al*., 2008; Allmann and Baldwin, 2010; Xu *et al*., 2019; Xu *et al*., 2020). In general, the same VOCs can be attractive and repellent, depending on whether their amount is low or high, respectively. This is the case, for example, of (E)-β-farnesene, which in aphids can act at very low concentrations as an aggregation pheromone, providing information on the superior quality of the host plant (Verheggen *et al*., 2009), while it is commonly known as an alarm pheromone in aphids (Francis *et al*., 2005). In addition, some studies of insect behavior suggest that the composition of the mixture of volatile plants is crucial and that specific mixtures are more attractive than individual compounds (Visser *et al*., 1996 ; Natale *et al*., 2003; Toby *et al*., 2005). It is therefore suspected that the differential effect of attractiveness of *P. nigronervosa* between the two genotypes would be due both to the variability of the proportions (quantities) and of the mixtures of the common VOCs; whereas it would be due, between the two infectious statuses, to the only variability of the proportions.

**CONCLUSION**

In this study, biological assays revealed a stronger attractiveness of *P. nigronervosa* alate and apterous for plantain than for dessert banana plants, and for infected bananas than for healthy banana plants. Identification of volatile compounds revealed a total of 16 VOCs (12 for dessert banana and 13 for plantain), with high emitted quantities in plantains and in infected banana plants. The compounds which seem potentially involved in the attractiveness of aphids to banana plants are 6-methyl-5-hepten2-on, Myrcene, (Z)-β-ocimene, (E)-β-ocimene, Methyl salicylate, 6-methyl-3,5-heptadien2-one and Nonanal. The next step of this study will be to determine, from these common compounds, the specific mixtures likely to be used in the semiochemical control of *P. nigronervosa*, in an integrated management approach. It will also be interesting to test the application of these compounds in field conditions, but also to extend the studies on several varieties of bananas.

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**Figure captions**

Figure 1: Effect of banana plant treatment on the choice of apterous aphids *P. nigronervosa*: (A) Healthy Dessert Banana and Infected Dessert Banana; (B) Healthy Plantain Banana and Infected Plantain Banana; (C) Infected Dessert Banana and Infected Plantain Banana; (D) Healthy Dessert Banana and Healthy Plantain Banana. The mean number of aphids per plant is displayed with the standard error, over 30 repetitions, carried out each time with 20 aphids per experiment. (\*\*): p-value < 0.01, (\*\*\*): p-value < 0.001.

Figure 2: Effect of banana plant treatment on the choice of alate aphids *P. nigronervosa*: (A) Healthy Dessert Banana and Infected Dessert Banana; (B) Healthy Plantain Banana and Infected Plantain Banana; (C) Infected Dessert Banana and Infected Plantain Banana; (D) Healthy Dessert Banana and Healthy Plantain Banana. The mean number of aphids per plant is displayed with the standard error, over 20 repetitions, carried out each time with 20 aphids per experiment. NS: not significant, (\*): p-value < 0.05, (\*\*\*): p-value < 0.001.

Figure 3: Principal component analysis (PCA, supported by 41% inertia on the first axis, and 13% on the second axis) of the 16 selected VOCs emitted by banana plants. (A): Between the plantain (blue) and the dessert banana genotypes (red), (B): Between the healthy banana plant (blue) and the infected banana plant (red). Ellipses represent 95% confidence intervals formed around the barycenter of each group (big dot or triangle). Each small dot or triangle represents a replicate (i.e., a banana plant).

Figure 4: Comparison of volatile organic compounds (VOCs) emitted by both genotypes (dessert: yellow, and plantain: blue), for both infection status by BBTV (healthy banana: light color, and infected banana plant: dark color). The observed mean (mV) with the standard error over five replicates are represented. Statistical results (GLMs): *ns* indicate non-significant results (p>0.05), stars indicate significant differences (p<0.05) between infection status (for each genotype), and different letters indicate significant differences (p<0.05) between genotypes (for each infection status). HDB: Healthy Dessert Banana; HPB: Healthy Plantain Banana; IDB: Infected Dessert Banana; IPB: Infected Plantain Banana.

Table 1: Mean number (± SE) of aphids (apterous and alates) attracted to a banana plant (or a control) and a control, as well as their statistical results (Student's t). HDB - S : Attractiveness of aphids between Healthy Dessert Banana and potting soil only ; HPB - S : between Healthy Plantain Banana and potting soil only ; S - E : between potting soil only and Empty. HDB: Healthy Dessert Banana; HPB: Healthy Plantain Banana; t: Student test ; p: p-value. Significant results (p <0.05) are in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HDB - S** |  | **HPB - S** |  | **S - E** |
| **Aphid morphe** | **HDB** | **S** | **t** | **p** |  | **HPB** | **S** | **t** | **p** |  | **S** |  **E** | **t** | **p** |
| Apterous | 6.70 ± 0.14 | 0,86 ± 0.13 | 30 | **<0.001** |  | 7.80 ± 0.23 | 0.86 ± 0.13 | 25.7 | **<0,001** |  | 1.53±0,31 | 0.90 ± 0,15 | 1.8 | 0.07 |
| Alates | 6.80 ± 0.52 | 0 ± 0 | 5.6 | **<0.001** |   | 8.85 ± 0.57 | 0 ± 0 | 5.8 | **<0,001** |   | - | - | - | - |

Table 2: Statistical results (GLM binomial negative) of the effect of BBTV quantities of VOCs emitted by different types of banana plants. Significant results (p <0.05) are in bold. For each comparison (i.e., between genotypes or between infection status), the relative proportion (Pr) of VOC emission increase is given as a ratio of plantain/dessert or infected/healthy plants. For example, Prhealthy indicates 5.7 for myrcene, which means that this compound was emitted in 5.7 times higher quantity in healthy plantain banana plants than in healthy dessert banana plants. Prplantain indicates 2.8 for myrcene, which means that this compound was emitted in 2.8 times higher quantities in infected plantain banana plants than in healthy plantain banana plants. Dashes indicate that the difference in VOC emission between two categories could not be calculated (e.g., if a given VOC is not emitted by one banana plant category). See Figure 4 for a comparison of VOCs emitted from each type of banana plant.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Genotype** | **Infection** | **Genotype \* Infection** |
|   | **healthy** | **infected** | χ² |  | **dessert** | **plantain** | χ² |  | χ² |  |
| **Molecule** | Prhealthy | Prinfected | p | Prdessert | Prplantain | p | p |
| 6-methyl-5-hepten2-on  | 1.3 | 1.6 | 3.3 | 0.06 | 1.4 | 1.8 | 0.5 | 0.48 | 1.3 | 0.26 |
| α-pinene | 0.0 | 0.1 | 17.6 | **<0.001** | 3.3 | - | 6.3 | **<0.05** | 0.001 | 0.99 |
| β-pinene | 0.0 | 0.0 | 226.2 | **<0.001** | 8.5 | - | 37.5 | **<0.001** | 0.001 | 0.99 |
| Myrcene | 5.7 | 3.1 | 10.5 | **<0.01** | 5.2 | 2.8 | 9.2 | **<0.01** | 0.5 | 0.48 |
| Limonene | 0.0 | 0.0 | 213.9 | **<0.001** | 5.4 | - | 11.6 | **<0.001** | 0.001 | 0.99 |
| (*Z*)-β-ocimene | 0.9 | 2.1 | 1.3 | 0.23 | 2.8 | 6.4 | 25.3 | **<0.001** | 1.9 | 0.15 |
| (*E*)-β-ocimene | 13.6 | 8.9 | 149.4 | **<0.001** | 5.5 | 3.6 | 54.3 | **<0.001** | 1.5 | 0.22 |
| 4,8-dimethyl-1,3,7nonatrien | - | - | 688.6 | **<0.001** | - | 1.7 | 3.5 | **<0.05** | 0.001 | 0.99 |
| Nonanal | 84.2 | 0.7 | 5.6 | 0.06 | 1517.0 | 12.4 | 110.7 | **<0.001** | 34.7 | **<0.001** |
| Linalool | 3.3 | 2.3 | 16.1 | **<0.001** | 5.4 | 3.8 | 36.1 | **<0.001** | 0.5 | 0.48 |
| (*E*)-β-farnesene | - | - | 0.001 | 0.99 | - | 2.4 | 9.8 | **<0.01** | 0.001 | 0.99 |
| (*Z,E*)-α-farnesene | - | - | 0.001 | 0.99 | - | 1.8 | 99 | **<0.001** | 0.001 | 0.99 |
| Methyl salicylate | 3.9 | 1.7 | 23.1 | **<0.001** | 3.5 | 1.5 | 17.4 | **<0.001** | 4.9 | **<0.05** |
| 6-methyl-3,5-heptadien2-one  | 2.3 | 1.8 | 7.1 | **<0.01** | 2.8 | 2.3 | 12.1 | **<0.001** | 0.25 | 0.69 |
| (*E*)-hex-2-enal | 72.2 | 3.0 | 55.5 | **<0.001** | 45.9 | 1.9 | 354 | **<0.001** | 25.3 | **<0.001** |
| Alloocimene | - | - | 0.001 | 0.99 | - | 4.3 | 10.9 | **<0.001** | 0.001 | 0.99 |