Comparative Susceptibility of Two Banana Cultivars to Banana Bunchy Top Virus Under Laboratory and Field Environments

C.R.R. HOOKS,[1,2] R. MANANDHAR,[3] E. P. PEREZ,[3] K.-H. WANG,[3] AND R.P.P. ALMEIDA[4]

J. Econ. Entomol. 102(3): 897–904 (2009)

ABSTRACT Field and laboratory experiments were carried out on the island of Oahu, HI, to compare the susceptibility of the two most commonly grown banana (*Musa* sp.) cultivars in the state ('Dwarf Brazilian' or Santa Catarina [locally known as dwarf apple] and 'Williams') to the aphid-borne Banana bunchy top virus (genus *Babuvirus*, family *Nanoviridae*, BBTV). Several morphological and physiological features of the two cultivars were monitored to determine whether the banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), transmits BBTV to both cultivars at a similar rate; and whether after successful inoculation, does each cultivar respond similarly to viral infection. Results from the laboratory experiment showed that a similar percentage of both cultivars were infected with BBTV by aphid vectors (>90% for both cultivars). However, field results showed a significantly lower percentage of dwarf apple (39%) infected with BBTV compared with Williams (79%). We also found that all physiological and morphological features measured (i.e., plant height, leaf area, canopy, chlorophyll level, and moisture content) for both cultivars were impacted similarly by BBTV. The incubation period, or the time between plant infection and initial appearance of disease symptoms, was similar for both cultivars. Results also showed that BBTV transmission efficiency was lower in the field than in the laboratory, despite that more aphids per plant were used for field than laboratory inoculation tests. The results highlight the potential use of less susceptible cultivars to help manage BBTV and the importance of screening banana varieties in the field to determine their response to vectors and associated diseases.

KEY WORDS *Musa* sp., *Pentalonia nigronervosa*, Nanoviridae, resistance

Banana (*Musa* sp.) is one of the most important cash fruit and staple crop in many tropical regions (Sharnock and Frison 1999). Hawaii ranks number one within the United States in banana production (NASS 2008). Commercial banana production occurs on all major Hawaiian Islands, with 80% of the production concentrated on the islands of Hawaii and Oahu. In addition to its economic importance, endemic cultivars introduced into the archipelago by colonizing Polynesians are of significant cultural importance to Hawaiians.

Banana bunchy top disease (BBTD), caused by Banana bunchy top virus (genus *Babuvirus*, family *Nanoviridae*, BBTV), is one of the most economically important disease of bananas in most producing regions, including Asia, Africa, and the South Pacific (Dale 1987, Dale and Harding 1998). Plants infected early with BBTV do not bear fruit, and fruit of later infected plants is typically stunted and unmarketable. Additionally, the virus spreads to suckers via the rhizome; thus, the entire banana mat eventually becomes infected (Dale and Harding 1998). Banana bunchy top virus is transmitted in a circulative, persistent nonpropagative manner by the aphid vector *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) (Magee 1927, Hafner et al. 1995, Hu et al. 1996). No additional vectors of BBTV are known. The virus was reported in Hawaii in 1989 (Conant 1992) and has since progressively spread throughout banana growing areas along the island chain. Statewide reductions in harvested acreage were reported to be 26 and 16% in 2004 and 2005, respectively (Anonymous 2005, 2006). Much of this continual decline in acreage and output has been attributed to the progressive spread of BBTV on Oahu and recently discovered infections on the eastern side of Hawaii island, the largest production area in the state.

The general belief among Hawaii banana stakeholders through casual observations is that two of the most important banana cultivars (acreage-wise) differ in their susceptibility to BBTV. 'Dwarf Brazilian' or Santa Catarina (locally known as dwarf apple; AAB genome) is assumed to be less susceptible and/or more tolerant, to BBTV infection than Williams (AAA genome, Cavendish subgroup). Thus, many growers in

[1] Department of Entomology, 4112 Plant Sciences Bldg., University of Maryland, College Park, MD 20742.
[2] Corresponding author, e-mail: crrhooks@umd.edu.
[3] Department of Plant and Environmental Protection Sciences, Gilmore Hall 310, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822.
[4] Department of Environmental Science, Policy, and Management, 137 Mulford Hall, University of California, Berkeley, Berkeley, CA 94720.

0022-0493/09/0897–0904$04.00/0

Hawaii have switched from growing Williams to dwarf apple bananas in recent years with hopes of limiting pathogen spread. However, no studies have been conducted to compare the susceptibility of these two cultivars. Recent research has quantified several morphological and physiological features of banana plants impacted by BBTV (Hooks et al. 2008). However, that work was done using only one variety; thus, there are no data on the susceptibility of banana cultivars commonly used in Hawaii to BBTV. Such information may be crucial for disease management because there are no known banana cultivars fully resistant to the virus (Magee 1948, Muharam 1984), and planting banana cultivars that are less susceptible to the virus or demonstrate some degree of resistance to BBTV could alter the rate of disease spread. Disease incidence and spread could be reduced with lower plant infection rates. However, tolerant cultivars that harbor the virus without expressing symptoms could be important sources of pathogen inoculum in plantations. Thus, determining the level of cultivar susceptibility in this system is of epidemiological importance.

Previous studies conducted to examine cultivar susceptibility to BBTV used incubation periods and/or symptom severity as evidence that cultivars were similarly affected by the virus (Magee 1948, Jose 1981). However, morphological and physiological parameters impacted by the virus were not taken into account, only presence and absence of symptoms. As such, to determine whether these two cultivars are similarly impacted by BBTV, morphological and physiological features of each known to be impacted by BBTV also were quantified for comparison. As such, the following questions were addressed: 1) Is the incubation period of BBTD similar for dwarf apple and Williams? 2) Are their growth, morphology, and physiology similarly impacted by BBTV infection? and 3) Will viruliferous aphids transmit BBTV to a similar percentage of plants for each cultivar?

Materials and Methods

Aphids, Plants, and BBTV Sources. A *P. nigronervosa* colony was started from a single apterae collected in June 2004 from a healthy dwarf apple (AAB genome) banana plant from a population in the Kahuku district, Oahu, and reared on Williams (AAA genome) banana. An isolate of BBTV was collected from an infected banana sucker in the Hawi area of the Big Island of Hawaii (North region of the island) in July 2005. The infected sucker displayed the characteristic symptoms of BBTV, and infection status was confirmed with a polymerase chain reaction (PCR)-based detection protocol (Hooks et al. 2008). Foliage from the infected sucker was used later as virus-infected source material, and aphids placed on this source material were used to inoculate BBTV to additional banana plants, which served as sources of the virus for field and laboratory trials. All experimental plants used in this study were obtained by tissue culturing following protocols described previously (Robson et al. 2007). Two cultivars were used in this study, 'Williams' (AAA genome, derived exclusively from *Musa acuminata* Colla) and 'Dwarf Brazilian' or Santa Catarina [locally known as dwarf apple] (AAB genome, derived from the intra- and interspecific hybridization of two wild diploid species, *Musa acuminata* Colla and *Musa balbisiana* Colla). Planting media used to grow the plants in the greenhouse and for the laboratory study consisted of a mixture of soil-less potting mix (Sunshine Mix; Sun Gro Horticulture Distribution, Vancouver, BC, Canada), vermiculite, and perlite at a ratio of 2:3:1, respectively. Approximately, 5 cc of slow release fertilizer (Osmocote 14:14:14 [N–P—K], The Scott's Company, Marysville, OH) was added to the planting media in each ≈14-cm-diameter pot.

Laboratory Study. In total, 50 banana plants (six- to eight-leaf stage) of each cultivar were infested with five viruliferous apterous adult aphids. The 50 test plants were inoculated following methods detailed by Su et al. (2003). As such, adult *P. nigronervosa* were collected from the laboratory colony and placed on leaf cuttings collected from diseased source plants kept in an enclosed room kept at 25 ± 5°. Test aphids and cuttings then were placed in 15-cm-diameter petri dishes, sealed with Parafilm, and put in a growth chamber set at 25°C, allowing aphids a 48-h acquisition access period (AAP). Aphids have been shown to transmit BBTV with similar efficiency if acquisition occurs on leaf cuttings or whole plants (Anhalt and Almeida 2008). After the 48-h AAP, groups of five aphids were collected with a no. 2 fine-haired paint brush and placed near the "throat" of the pseudostem at the second youngest fully expanded banana leaf of each test plant. Test plants were immediately covered by a transparent fabric with a mesh size of 36 cm^{-1} (SuperPoly Organza, Hyman Hendler and Sons, Los Angeles, CA) and secured at the bottom with a draw string and rubber band. Test plants then were placed in an isolated, insect-free, windowless room with artificial fluorescent growth lights (photoperiod of 12:12 [L:D] h) at a set temperature of ≈30°C ± 5, where aphids were allowed a 5-d inoculation access period (IAP). After the 5-d IAP, aphids were killed by spraying test plants with Provado 1.6F (1%, imidacloprid, Bayer CropScience Inc., Research Triangle Park, NC). After the treatment, plants remained in the room and were monitored to determine the percentage of plants from each cultivar that became infected with BBTV. A PCR protocol, as described in Hooks et al. (2008), was used to determine the BBTV infection status of each test plant by taking samples from the newly formed leaves irrespective of obvious symptom presence.

Field Experiment Layout and Planting Time. Two field experiments were conducted from September to December 2006 (trial 1) and from May to August 2007 (trial 2) at the University of Hawaii Poamoho Research Station (elevation, 265 m) on Oahu, HI. This area was chosen because there were no known banana plants in the vicinity, reducing the likelihood of BBTV infections from external sources. All banana plantlets used for the field experiments were micropropagated from pathogen-free banana plants as described previously (Robson et al. 2007). For trials 1 and 2, banana

plants of both cultivars were transplanted in 24- by 34- and 22- by 26-m^2 plots, respectively. For trials 1 and 2, 160 and 108 healthy transplants were planted on 29 August 2006 and 27 April 2007, respectively, and spacing between rows and plants were 2.4 and 2.1 m for each plot. The cultivar and virus treatments were arranged in a completely randomized design.

Field Inoculation. Test plants were inoculated with 12 aphids on 19 September 2006 and 12 May 2007 for trials 1 and 2, respectively. For trials 1 and 2, 21 and 12 plants of each cultivar types were randomly selected and inoculated with viruliferous adult aphids (virus treatment), and a similar number of plants from each cultivar was chosen randomly and used as nontreated controls (noninfected). Colored wire stake flags were used to mark plant cultivar and virus treatments. For trials 1 and 2, two groups of six adult aphids were removed from a single BBTV infected source leaf on which they were allowed a 48-h AAP under conditions described under the laboratory study section, by using a no. 2 fine-haired brush and placed in the throat of the pseudostem at the third and fourth leaf position. Twelve aphids were used to increase the likelihood of successful virus transmission (Magee 1948). A sleeve cage constructed of a 36 mesh cm^{-1} transparent fabric described earlier then was placed carefully over all test plants. Sleeve cages were used to protect aphids from natural enemies. After 5 days, sleeve cages were removed, and aphids were killed by spraying test plants with imidacloprid (Provado 1.6F, Bayer CropScience Inc.) at a rate of 0.7 ml/liter H_2O by using a hand-pumped backpack sprayer.

Determination of Plant Growth, Physiological Parameters, and Virus Infection. Plant growth features (i.e., height, canopy, and leaf area), leaf moisture, and chlorophyll measurements for each trial were initiated 5 d after termination of the aphid inoculation and conducted every 10 d thereafter until experiment completion, 90 d after aphid inoculation (DAI). We determined from previous field studies that the incubation period of BBTV in Hawaii is 25–85 DAI (Hooks et al. 2008). Test plants were inspected at 5-d intervals for another 20 d after plant measurement tasks were terminated to ensure no other plants were infected. Test plots for both trials were destroyed a minimum of 4 mo after study initiation, and at time of destruction no additional plants showed BBTV symptoms. Morphological and physiological features monitored during this study were selected because these characteristics are known to be significantly affected by BBTV infection (Hooks et al. 2008).

Plant height and leaf area were recorded on each sampling occasion. Plant height was measured as the distance from the ground to the fork created by the petioles of the upper most fully emerged leaf (Smith et al. 2000). The leaf area of banana leaves was estimated as described by Robinson and Neil (1985). As such, the length and maximum width of the youngest fully unfurled leaf were measured and results were multiplied by a conversion factor of 0.83 (length by width by 0.83) to obtain a better estimate of leaf area. Additionally, after aphid inoculation, the most recently open banana leaf was marked with a permanent marker (Uni Paint, Mitsubishi Pencil for Sanford Corp., Oak Brook, IL) so that the total number of leaves per test plant produced (i.e., leaf production) before the appearance of BBTV could be recorded. Leaf production rates were determined as described by Turner (1971). As such, a leaf was regarded as fully emerged when the ventral surface of the midrib was exposed fully and the entire leaf was unfurled. Otherwise, a score for the final leaf was based on the percentage of its unfurled surface. Canopy data were collected by measuring the distance end to end between the two most distant leaves of each test plant.

To estimate the percentage of leaf moisture, a cork borer was used to remove a 3.14-cm^2 circular disc sample from the most recently mature, fully expanded banana leaves of each test plant. Disc samples were collected from the area halfway between the leaf tip and petiole, and halfway between the leaf margin and mid vein. Leaf discs were placed in a plastic bag and transported to the laboratory in a chilled cooler. Afterward, leaf discs were weighed, placed into a paper bag and oven-dried at 70°C for ≈7 d. Leaf discs then were reweighed, and percentage of leaf moisture content was determined.

The relative chlorophyll content of banana leaves was determined with a Minolta SPAD-502 Chlorophyll Meter (Minolta Corporation, Ramsey, NJ). The SPAD meter determines the greenness of the leaf and the interaction of thylakoid chlorophyll with incident light (Jifon et al. 2005). Six readings representative of the entire leaf length were taken from the edge of the most recently matured fully unfolded leaf during each sample date. The mean average of the six readings was recorded from each test plant.

All field plants including border and nontest plants were checked visually for the presence of BBTV symptoms. Test plants were inspected for disease (i.e., symptoms) at 5-d intervals, commencing 10 DAI until 90 d after planting. However, if a plant inoculated with viruliferous aphids (virus treatment) did not become infected it was not included in the final statistical analysis with respect to morphological and physiological growth parameters. The virus infection statuses of noninoculated plants were monitored to make certain that BBTV infections did not occur from external sources. Initial dates that banana plants were observed displaying symptoms were recorded.

Statistics. To determine whether the morphological and physiological parameters differed among treatments, data were subjected to a repeated-measure analysis of variance (ANOVA) (SAS 9.1, SAS Institute 2002) with trial designated as a random factor (PROC Mixed). The data were initially analyzed by trial but after determining there was no significant trial × cultivar effect ($P > 0.05$) for the morphological features measured, results from both trials were pooled for analysis. The analysis was performed on measurements taken at 10-d intervals, and percentage of infected plants was analyzed using chi-square analysis, and number of leaves produced and the time that

Table 1. Laboratory response of dwarf apple (AAB genome) and Williams (AAA genome) banana to aphid inoculation of BBTV

Treatment[a]	No. leaves at inoculation	Infection rate[b] (% positive plants)
Dwarf apple	6.88 ± 0.82	90
Williams	6.80 ± 0.83	94

Means were not significantly different ($P > 0.05$) among treatment categories.

[a] In total, 50 plants of each cultivar were inoculated with groups of five aphids that fed on BBTV-infected source leaves.

[b] Percentage of plants that *P. nigronervosa* infected with BBTV.

Table 2. Field responses of dwarf apple (AAB genome) and Williams (AAA genome) banana to aphid inoculation of BBTV

Treatment[a]	No. leaves at inoculation	Incubation period		Infection rate (% positive plants)
		DAI	Leaf production[b]	
Dwarf apple	8.75 ± 0.31a	39.2 ± 2.8	10.76 ± 0.15a	39.3a
Williams	8.09 ± 0.26b	43.1 ± 4.0	9.71 ± 0.16b	78.8b

Means ± SE within a column followed by different letters were significantly different among treatments ($P < 0.05$).

[a] In total, 33 plants of each cultivar were inoculated with groups of 12 aphids that fed on BBTV-infected source leaves.

[b] Leaf production is the mean number of leaves produced before the appearance of symptoms.

passed before the appearance of symptoms were analyzed using ANOVA (PROC GLM).

Results

Incubation Period and Percentage of Infected Plants. In the laboratory experiment, the percentage of banana plants that became infected after aphid inoculation was similar at 90 and 94% for dwarf apple and Williams, respectively ($P > 0.05$; Table 1). BBTD incubation period ranged from 30 to 65 DAI and from 20 to 85 DAI for dwarf apple and Williams, respectively, for field-grown plants. Most infected plants for both cultivars expressed symptoms by 50 DAI (Fig. 1). However, significant differences existed between the percentage of infected plants (Table 2; $P < 0.005$). During the two field trials, 39% (13/33) and 79% (26/33), in total, of the test plants became infected for dwarf apple and Williams, respectively. This is based on an infection efficiency of 47.6 and 25.0% (dwarf apple) and 85.7 and 66.7% (Williams) during trials 1 and 2, respectively.

Morphological and Physiological Responses. For each plant growth parameter measured (i.e., plant height, canopy, and leaf area), there was no significant cultivar or treatment × cultivar interaction, indicating that both cultivars grew similarly, and their growth features measured were equally impacted by BBTV infection ($P > 0.05$). Despite test plants being randomly selected before planting, infected plants were visually larger than healthy plants during the initial measurement dates. Despite these earlier parameter differences, during the latter dates noninfected plants grew more rapidly than infected plants. Plant height and canopy were not significantly different between infected and noninfected plants during most of the evaluation period (Figs. 2 and 3; $P > 0.05$). However, leaf area was significantly different between infected and noninfected plants ($P < 0.0001$). Leaf growth of infected plants was significantly less than noninfected plants and differences in growth became readily observable beyond 50 DAI (Fig. 4). Chlorophyll content measured with the chlorophyll meter also differed significantly between infected and noninfected plants, and similarly to leaf area, these distinctions became more observable at 50 DAI (Fig. 5). Leaf moisture content was the only parameter measured that was significantly greater in infected than noninfected plants ($P < 0.0001$). Leaf moisture levels in noninfected plants decreased over time especially beyond 60 DAI. However, the level remained mostly constant throughout the sampling period for infected plants of both cultivars (Fig. 6).

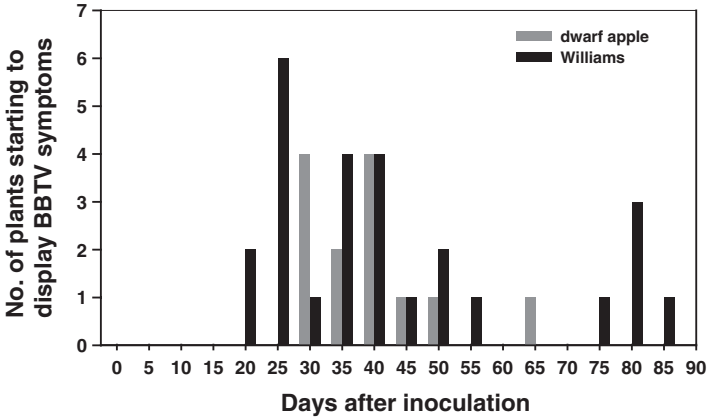

Fig. 1. Banana bunchy top disease incubation period for dwarf apple and Williams banana after inoculation of plants with infected aphids in a field environment.

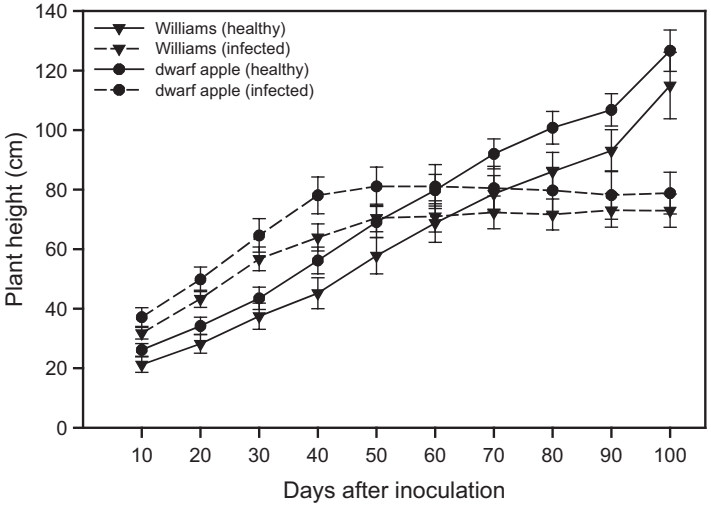

Fig. 2. Mean ± SEM plant height of Banana bunchy top virus-infected and healthy dwarf apple and Williams banana at different times after vector inoculation in a field environment.

Discussion

Results obtained from this study support earlier findings in Hawaii that various morphological and physiological characteristics of banana plants that are negatively impacted by BBTV become obvious ≈50 DAI (Hooks et al. 2008). Similar to that study, chlorophyll content, which was significantly lower in BBTV-infected plants, was the most consistent parameter for early differentiation between infective and healthy plants of both cultivars. However, unlike the current study, Hooks et al. (2008) found that BBTV significantly reduced plant height and canopy and these differences were readily apparent ≈50 DAI. During, this study differences in these growth parameters were not perceptible until 80 DAI, but, overall, no significant differences were detected. Because Williams was used in that study, this variation in response to BBTV cannot be attributed to cultivar differences. Furthermore, in that study, plants were infected at several periods throughout the year and morphological parameters measured were similar for all periods, suggesting that seasonality may not be important under Hawaii conditions. Of the Williams plants inoculated, 43.2% became infective during that study, but only five or 10 aphids per plant were used for inoculation compared with 12 for this study. Wu and Su (1990) found that when a single aphid was used for inoculation, transmission efficiency of BBTV was ≈53% but reached 100% when five or more aphids were used. Banana transplants also grew faster during this study but whether this contributed to disparities in

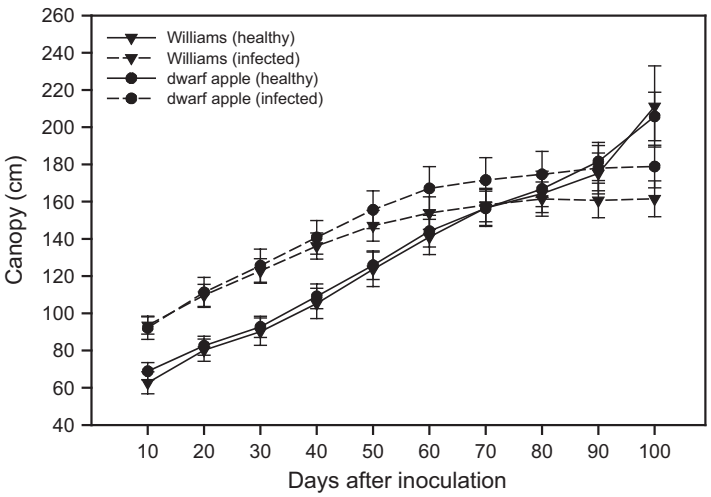

Fig. 3. Mean ± SEM plant canopy of Banana bunchy top virus-infected and healthy dwarf apple and Williams banana at different times after vector inoculation in a field environment.

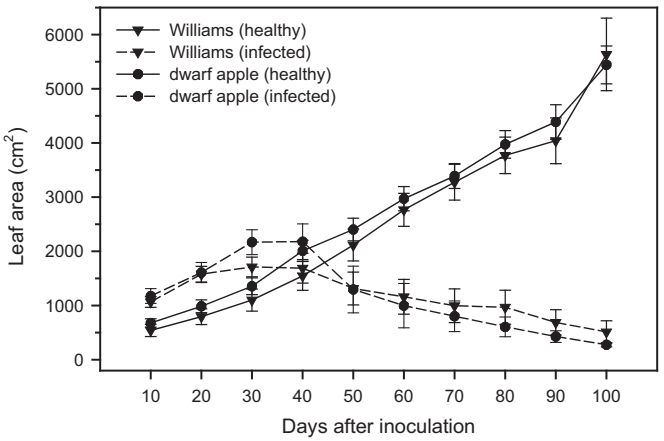

Fig. 4. Mean ± SEM leaf area of Banana bunchy top virus-infected and healthy dwarf apple and Williams banana at different times after vector inoculation in a field environment.

these two growth parameters between studies is unclear.

Despite using 12 and five aphids to inoculate each test plant in the field and laboratory experiments, respectively, a lower percentage of plants became BBTV-infected in the field than in the laboratory. In the laboratory trial, 90 and 94% of the dwarf apple and Williams became infected, respectively, compared with 39 and 79% in the field trial. Other studies showed high transmission efficiency under laboratory conditions, indicating that *P. nigronervosa* is an efficient vector of BBTV (Hu et al. 1996, Anhalt and Almeida 2008). However, plants inoculated in the field with greater number of aphids have been infected at lower rates (Hooks et al. 2008). Results obtained here support the caution proposed earlier that laboratory findings with regard to vector transmission efficiency of BBTV should not be used to predict BBTV transmission probability in the field because of its potential to significantly overestimate BBTV infection rates (Hooks et al. 2008). Further laboratory studies have shown that *P. nigronervosa* development, fecundity, and BBTV transmission efficiency are sensitive to environmental conditions (Robson et al. 2007, Anhalt and Almeida 2008). As such, we hypothesize that transmission rates are consistently lower in the field versus the laboratory and greenhouse because conditions in controlled environments are more favorable for pathogen inoculation and infection development. This observation is of importance not only to this system, because initial screening of plant genotypes in search of tolerant or resistant varieties are usually conducted under controlled conditions, potentially excluding factors that could lower disease incidence under field conditions. Furthermore, because reducing virus acquisition rates by vectors could affect secondary virus spread, any sequential sampling plan and associated threshold levels developed to help manage BBTV by controlling its vectors must

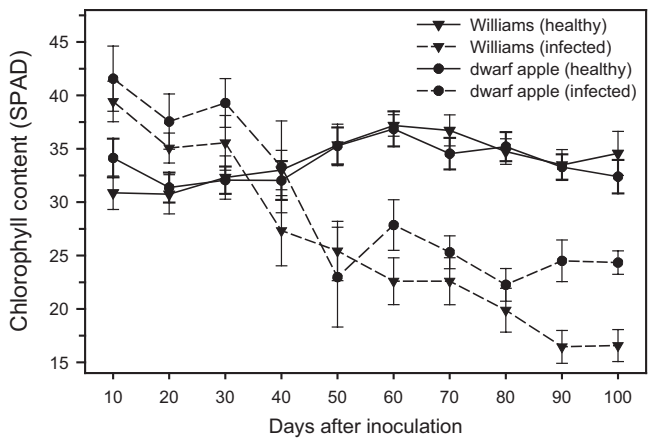

Fig. 5. Mean ± SEM chlorophyll estimate in Banana bunchy top virus-infected and healthy dwarf apple and Williams banana at different times after vector inoculation in a field environment. Chlorophyll estimate were obtained using a SPAD-502 (SPAD units) chlorophyll meter.

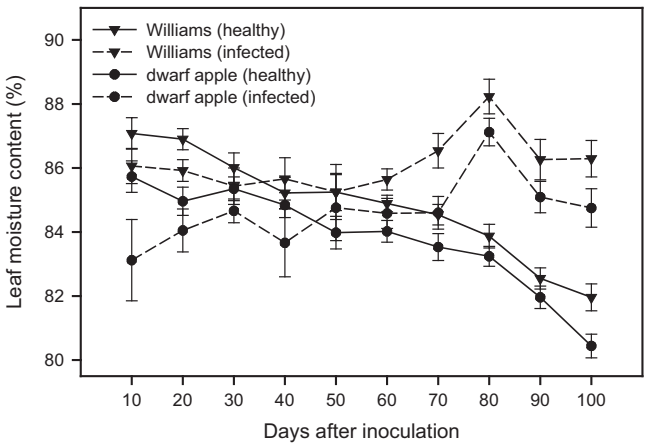

Fig. 6. Mean ± SEM leaf moisture content of Banana bunchy top virus-infected and healthy dwarf apple and Williams banana at different times after vector inoculation in a field environment.

consider *P. nigronervosa* transmission efficiency under natural field rather than laboratory conditions.

Results of the field trial also showed that BBTV infection rate was significantly lower in dwarf apple (39%) than Williams (79%). A comparison of plant infection rates between these treatments indicated that the probability of virus transmission (followed by development of symptoms) by individual aphids to Williams was 2.7 times higher than to dwarf apple (Swallow 1985). These findings support the general belief by banana growers in Hawaii that dwarf apple is less susceptible to BBTV infection than Williams. However, because the response of these cultivars to BBTV infection were similar, their common belief that dwarf apple is more tolerant to BBTV was not supported during this study. The lower field infection rates observed for dwarf apple in relation to Williams remains unexplained. We hypothesize, among many possibilities, that one or more morphological differences between the two cultivars impact *P. nigronervosa* ability to inoculate BBTV. For example, *P. nigronervosa* prefers to settle and feed in the throat of the pseudostem (Robson et al. 2006). The banana pseudostem is waxy, and differences in wax content or composition between the two cultivars may lead to disparities in virus transmission. Ashraf and Zafar (1999) found that the most distinctive characteristic to differentiate between resistant and susceptible lines of cotton cultivars to cotton leaf curl virus was epicuticular wax content. They found that the resistant lines had considerably higher wax content on their leaf surfaces than the moderately resistant or susceptible cultivars. If this is analogous with banana cultivars, it may help explain why transmission efficiency differs between laboratory and field studies. Banana plants inoculated in the field are generally larger than those tested in the laboratory, which may allow greater time for wax buildup on the pseudostem.

Espino et al. (1993) evaluated 57 banana cultivars for their susceptibility to BBTV. They determined all cultivars in the AA and AAA genomic groups were highly susceptible. However, similar to our findings cultivars containing the B genome (AAB and ABB) were less susceptible. These results provide some credence to the general supposition that cultivars within the Cavendish subgroup are generally highly susceptible to BBTV (Thomas and Iskra-Caruana 2000). However, Magee (1948) found that Gros Michel (AAA genome) was far less susceptible to BBTV than Cavendish plants (e.g., dwarf Cavendish and Williams, AAA genome). Similar to our findings, Magee concluded that, despite the cultivars differing in their susceptibility to BBTV, when they became infective, their responses to the disease (as assessed by symptom expression) were similar.

In conclusion, there are no confirmed reports of any *Musa* species or cultivars being completely resistant to BBTV. However, this and other studies have provided evidence that banana cultivars may differ in their resistant to BBTV (Magee 1948, Jose 1981, Muharam 1984, Espino et al. 1993). What is unclear is whether these differences are because of physiological or structural differences. Astier et al. (2007) discusses several resistant mechanisms that plants use to check virus infection. Two of which may be viable explanations why various banana cultivars differ in their susceptibility to BBTV. One hypothesis is that some banana cultivars although susceptible to BBTV have some resistance to virus inoculation by *P. nigronervosa.* A second hypothesis, derived from the concept of "partial" resistance, is that some banana cultivars have a lower probability of infection compared with others if inoculum level is assumed to be the same. Magee (1948) showed that the resistance of Gros Michel to BBTV compared with other banana cultivars could be overcome by increasing the number of aphid vectors. Magee (1948) findings provide credence to the second hypothesis which suggests the partial resistance to BBTV experienced by certain banana cultivars is because of their ability to escape infection under "low levels" of inoculum. It is obvious that the exact mechanisms responsible for "resistance" in some banana

cultivars are unknown and that no one mechanism may be exclusively responsible. However, we are doubtful that tolerance to BBTV is a contributor to cultivar differences. As such, future studies should investigate the mechanisms responsible for differences in virus transmission between highly susceptible and partly resistant cultivars.

Acknowledgments

We thank the crew at the University of Hawaii at Manoa Poamoho Research Station for all logistical support during the field trials. We are also grateful to Derek Kabasawa for assistance with fieldwork and detection tests. This work was supported by funding from CSREES T-STAR contract 2004-34135-14976, Western Sustainable Agriculture Research and Education program, project SW04-064, PBARC award 58-53204-534, and USDA–ARS Specific Cooperative Agreement Minor Crops Pest and Disease Control Award 58-5320-4-534.

References Cited

Anhalt, M. D., and R.P.P. Almeida. 2008. Effect of temperature, vector life stage and plant access period on transmission of *Banana bunchy top virus* to banana. Phytopathology 98: 743–748.

Anonymous. 2005. National Agricultural Statistics Service, statistics of Hawaii agriculture: Hawaii bananas. U.S. Department of Agriculture, Honolulu, HI.

Anonymous. 2006. National Agricultural Statistics Service, statistics of Hawaii agriculture: Hawaii bananas. U.S. Department of Agriculture, Honolulu, HI.

Ashraf, M., and Z. U. Zafar. 1999. Some physiological characteristics in resistant and susceptible cotton cultivars infected with cotton leaf curl virus. Biol. Plantatarium 42: 615–620.

Astier, S., J. Albouy, Y. Maury, C. Robaglia, and H. Lecoq. 2007. Principles of plant virology; genome, pathogenicity, virus ecology. Science Publishers, Enfield, NH.

Conant, P. 1992. Banana bunchy top disease, a new threat to banana cultivation in Hawaii. Proc. Hawaiian Entomol. Soc. 31: 91–95.

Dale, J. L. 1987. Banana bunchy top: an economically important tropical plant virus disease. Adv. Virol. Res. 33: 301–325.

Dale, J. L., and R. M. Harding. 1998. Banana bunchy top disease: current and future strategies for control, pp. 659–669. *In* A. Hadidi, R. K. Khetarpal, and H. Koganezawa [eds.], Plant virus disease control. APS Press, St. Paul, MN.

Espino, R. C., L. V. Magnaye, A. P. Johns, and C. Juanillo. 1993. Evaluation of Philippine banana cultivars for resistance to bunchy-top and fusarium wilt, pp. 89–102. *In* R. V. Valmayor, S. C. Hwang, R. Ploetz, S. C. Lee, and N. V. Roa [eds.], Proceedings: International Symposium on Recent Developments in Banana Cultivation Technology, 14–18 December 1992, Chiuju, Pingtung, Taiwan. Taiwan Banana Research Institute, INIBAP/ASPNET, Los Banos, Philippines.

Hafner, G. J., R. M. Harding, and J. L. Dale. 1995. Movement and transmission of banana bunchy top virus DNA component one in bananas. J. Gen. Virol. 76: 2279–2285.

Hooks, C.R.R., M. G. Wright, D. S. Kabasawa, R. Manandhar, and R.P.P. Almeida. 2008. Effect of *Banana bunchy top virus* infection on morphology and growth characteristics of banana. Ann. App. Biol. 153: 1–9.

Hu, J. S., M. Wang, D. Sether, W. Xie, and K. W. Leonhardt. 1996. Use of polymerase chain reaction (PCR) to study transmission of banana bunchy top virus by the banana aphid (*Pentalonia nigronervosa*). Ann. Appl. Biol. 128: 55–64.

Jifon, J. L., J. P. Syvertsen, and E. Whaley. 2005. Growth environment and leaf anatomy affect nondestructive estimates of chlorophyll and nitrogen in citrus sp. leaves. J. Am. Soc. Hortic. Sci. 130: 152–158.

Jose, P. C. 1981. Reaction of different varieties of banana against bunchy top disease. Agric. Res. J. Kerala 19: 108–110.

Magee, C.J.P. 1927. Investigation on the bunchy top disease of the banana. Council for Scientific and Industrial Research, Bull. 30.

Magee, C.J.P. 1948. Transmission of bunchy top to banana varieties. J. Aust. Inst. Agric. Sci. 14: 18–24.

Muharam, A. 1984. Test for resistance of some banana cultivars to banana bunchy-top disease. Bull. Penel. Hortic. 11: 16–19.

[NASS] National Agricultural Statistics Service. 2008. Hawaii bananas annual summary. (http://www.nass.usda.gov/).

Robinson, J. C., and D. J. Neil. 1985. Comparative morphology, phenology and production potential of banana cultivars 'Dwarf Cavendish' and 'Williams' in the Eastern Transvaal Lowveld. Sci. Hortic. 25: 149–161.

Robson, J. D., M. G. Wright, and R.P.P. Almeida. 2006. Within-plant distribution and binomial sampling of *Pentalonia nigronervosa* (Hemiptera: Aphididae) on banana. J. Econ. Entomol. 99: 2185–2190.

Robson, J. D., M. G. Wright, and R.P.P. Almeida. 2007. Biology of *Pentalonia nigronervosa* (Hemiptera: Aphididae) on banana using different rearing methods. Environ. Entomol. 36: 46–52.

SAS Institute. 2002. Statistics, version 9.1 SAS Institute, Cary, NC.

Sharrnock, S., and E. Frison. 1999. *Musa* production around the world- trends, varieties and regional importance, pp. 42–47. *In* Networking banana and plantain: INIBAP annual report 1998. International Network for the Improvement of Banana and Plantain, Montpellier, France.

Smith, M. K., C. Searle, P. W. Langdon, B. Schaffer, and A. W. Whiley. 2000. Comparison between micropropagated banana (*Musa* AAA; 'Williams') and conventional planting material during the first 12 months of development. J. Hortic. Sci. Biotechnol. 76: 83–87.

Su, H.-J., L.-Y. Tsao, M.-L. Wu, and T.-H. Hung. 2003. Biological and molecular categorization of strains of Banana bunchy top virus. J. Phytopathol. 151: 290–296.

Swallow, W. H. 1985. Group testing for estimating infection rates and probabilities of disease transmission. Phytopathology 75: 882–889.

Thomas, J. E., and M. L. Iskra-Caruana. 2000. Diseases caused by viruses: bunchy top, pp. 241–253. *In* D. R. Jones [ed.], Diseases of banana, abacá and enset. CABI Publishing, CAB International, Wallingford, United Kingdom.

Turner, D. W. 1971. Effects of climate on rate of banana leaf production, J. Trop. Agric. 48: 283–287.

Wu, R.-Y., and H.-J. Su. 1990. Transmission of banana bunchy top virus by aphids to banana plantlets from tissue culture. Bot. Bull. Acad. Sin. 31: 7–10.

Received 3 August 2008; accepted 6 February 2009.