

Aphid Transmission of Banana Bunchy Top Virus to Bananas After Treatment With a Bananacide

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ABSTRACT Field and laboratory studies were conducted to determine the impact of using a herbicide as a bananacide on aphid transmission of Banana bunchy top virus (family *Nanoviridae*, genus *Babuvirus*, BBTV) to healthy banana (*Musa* spp.) plants. BBTV-infected banana plants in a commercial orchard were treated with Roundup Weathermax herbicide. Using polymerase chain reaction, the time after herbicide treatment that BBTV could no longer be detected in the infected plants was determined. The impact of the herbicide treatment on *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) virus acquisition and ability to inoculate healthy banana plants with BBTV also were determined. Generally, banana plants were dead beyond 42 d after herbicide injection (DAI), and BBTV was detected in a similar high percentage of treated plants from 0 up to 21 DAI. During two field trials, 0 and 32% of *P. nigronervosa* acquired the virus from treated plants at 42 DAI, respectively, but none successfully inoculated a healthy banana plant beyond 35 DAI. Finally, 22% of *P. nigronervosa* colonies collected directly from the pseudostem of injected plants at the final sample date (42 DAI) tested positive for BBTV and infected 9.5% of the healthy banana plants. The findings indicate that banana plants may remain a potential source of virus inoculum 6 wk after injection with a bananacide. The implications of these findings with respect to BBTV management are discussed.

KEY WORDS herbicide, *Nanoviridae*, persistent virus, *Pentalonia nigronervosa*

Plant disease management strategies are developed considering many epidemiological factors, but they are often based on the concept that limiting pathogen spread within fields will result in fewer diseased plants and greater yields. Management decisions may vary for annual and perennial crops, because losses in annual crops are typically seasonal, whereas losses in perennial plant systems accumulate over multiple years. One important tactic in the management of insect-transmitted viruses in perennial crops, which is usually economically unfeasible in annual systems, is roguing or destruction of symptomatic plants. Roguing infected plants that serve as pathogen inoculum may reduce secondary disease spread within a field (e.g., Allen 1987). However, definitive plant removal may not be practical in all perennial systems. Furthermore, roguing must be carefully considered in vector-borne disease systems because physically removing plants may disturb resident vector colonies, resulting in increased pathogen spread.

Banana (*Musa* spp.) is among the most important fruit crops in Hawaii in terms of production and eco-

nomical importance, and Hawaii ranks number one in the United States in banana production. Banana bunchy top disease (BBTD), caused by Banana bunchy top virus (family *Nanoviridae*, genus *Babuvirus*, BBTV), is one of the most economically important diseases of bananas in many production regions, including Asia, Africa, and the South Pacific (Dale and Harding 1998). The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is the only known vector of BBTV, transmitting the virus in a circulative, nonpropagative manner (Magee 1927, Hafner et al. 1995, Hu et al. 1996). Since BBTV was first documented in Hawaii in 1989 (Conant 1992), concerns regarding its impact on the banana industry have mounted. Mitigating its economic impact has become a top priority of Hawaii banana stakeholders (Constantinides and McHugh 2003).

Currently, the recommended strategies for controlling BBTV in different regions of the world include 1) identifying and destroying virus-infected plants as early as possible, 2) replanting with virus-free tissue-cultured plants, and 3) controlling aphid-vector populations (Robson et al. 2006). In Hawaii, commercial banana farmers generally do not remove BBTV-infected plants from their orchards but instead use bananacides to kill them. Bananacides are herbicides that are registered to kill banana plants. The rationale of growers using a bananacide instead of physically roguing diseased plants is that it reduces the amount

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of labor and cost associated with removing infected plants from a field. It also ensures that the entire banana mat (i.e., mature plant and connected suckers) is killed reducing the likelihood that BBTv-infected suckers will emerge later and become sources for secondary virus spread.

The current working hypothesis among Hawaii growers and some agricultural personnel is that the virus is no longer viable in banana plants within days after injection with a bananacide; therefore, treated plants will not likely serve as potential sources of virus inoculum. This assumption is important with respect to BBTv management because *P. nigronervosa* is present on banana plants year-round in Hawaii (Young and Wright 2005) and is an efficient vector of BBTv (Hu et al. 1996, Anhalt and Almeida 2008). However, there have been no studies conducted to determine the length of time banana plants remain sources of BBTv inoculum after a bananacide treatment. Therefore, the purpose of this study was to address three questions surrounding the use of herbicides to destroy plants in a perennial system impacted by a persistently transmitted virus. How long after treatment with a herbicide/bananacide 1) does a banana plant remain positive for BBTv, 2) can aphids acquire BBTv from treated plants, and 3) successfully inoculate healthy banana plants?

Materials and Methods

Aphid Maintenance. A *P. nigronervosa* colony was started from a single apterae aphid collected from a healthy banana plant from a population in the Kahuku district of Oahu and reared on either 'Santa Catarina' (=dwarf Brazilian or locally known in Hawaii as dwarf apple; *Musa* spp. AAB genome) or 'Williams' (*Musa acuminata*, AAA genome, Cavendish subgroup). The aphid colony was maintained in a laboratory at $30 \pm 5^\circ\text{C}$ under a photoperiod of 12:12 (L:D) h. When aphid populations or honeydew accumulation on plants approached high levels, aphids were transferred to a new plant using a no. 2 fine hair paint brush, or plant material was cut from the old plant and placed on soil next to the new plant. All plants used to rear insects and for transmission experiments were tissue-cultured following similar protocols described by Robson et al. (2007). Planting media used to grow banana plants in the greenhouse for aphid rearing and transmission experiments 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, The Scott's Company, Marysville, OH) was added to the planting media in each ≈ 14 -cm-diameter pots.

Field Sample and Collection Procedures. Two field trials were conducted to determine how long banana plants remained as sources of BBTv inoculum after treatment with a bananacide. Trials 1 and 2 were conducted from 11 June to 23 July 2006 and 28 February to 11 April 2007, respectively. The trials were conducted at a commercial banana farm consisting of

the dwarf Brazilian cultivar in Kunia on the island of Oahu, HI. For trials 1 and 2, 15 and 10 banana plants, respectively, displaying BBTv symptoms (pale chlorotic thinning and curving leaf margins, upright bunchy leaf appearance) were randomly selected and marked numerically for purpose of identification. Afterward, a caliper was used to measure the pseudostem (trunk) diameter of each test plant. A 2.5- by 2.5-cm solid steel square rod was inserted at an angle of 45° into the pseudostem of each test plant at a height and depth of 30.5 and 10 cm, respectively. The opening was then injected with Roundup Weathermax (glyphosate, Monsanto Corp., St. Louis, MO) at the highest label recommended rate for its use as a bananacide (1 ml of concentrate per 5 cm of pseudostem diameter). If fruit were present, they were removed before injection as required by the product's label. Additionally, all banana plants within a mat were treated, but only the largest plant within the mat was used as the test plant. Trunk diameter of test plants ranged from 10.5 to 28 cm with an average diameter of 20 cm. Before injecting each test plant, an ≈ 13 - by 6.5-cm leaf sample was cut from the vicinity of and parallel to the midvein of the most recently matured viable leaf (i.e., chlorophyll present, succulent), placed in a 15-cm-diameter petri dish lined with moistened filter paper, transported to the laboratory, and used to conduct transmission assays as described below. Tissue samples were continually collected from field test plants every 7 d for 6 wk. At ≈ 35 d after bananacide injection (DAI), leaves of test plants were no longer viable (i.e., dried tissue); so, at 35 and 42 DAI, samples were collected by cutting a similar size section from the pseudostem.

Transmission Assays. In the laboratory, a 6-mm-diameter cork borer was used to extract a subsample from each leaf or pseudostem cutting. The 6-mm extract (disc sample) was then placed into a microcentrifuge tube and later tested for the presence of BBTv by using a polymerase chain reaction (PCR)-based detection protocol described in Hooks et al. (2008). Transmission assays were performed similarly as described by Su et al. (2003). Thus, the remaining leaf or pseudostem cutting was placed back inside the petri dish and groups of 20 healthy (virus-free) adult *P. nigronervosa* were collected from the laboratory colony and placed on each cutting. The petri dish was then sealed with Parafilm and placed in a growth chamber set at 25°C , allowing aphids a 48-h acquisition access period (AAP). Laboratory experiments have demonstrated that aphids can readily acquire the virus at this temperature by using this leaf cutting technique with a 48-h AAP (Anhalt and Almeida 2008). After the 48-h AAP, 10 aphids were collected from each petri dish using a no. 2 fine-haired paint brush. Five aphids were then placed in a microcentrifuge tube and individually tested for BBTv by using the PCR protocol indicated above, and the remaining group of five aphids was transferred to a four- to five-leaf stage tissue-cultured plantlet (dwarf Brazilian). The plantlets 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 (i.e., 15 and 10 per test for trials 1 and 2, respectively) were then placed in an isolated, insect-free windowless room with artificial fluorescent growth lights (12-h photoperiod) at a set temperature of $30 \pm 5^\circ\text{C}$ 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 1.6 F (1%, imidacloprid, Bayer CropScience Inc, Research Triangle Park, NC). After which plants remained in the same room and were monitored to determine the percentage of plants that became infected with BBTV. All test plants were tested for BBTV by using PCR irrespective of symptom presence.

At 42 DAI, each test plant in the field was inspected for the presence of *P. nigronevosa*, and when found, a minimum of 10 aphids were collected from each plant, five of which were individually tested for BBTV and the remaining five were placed on a healthy banana plantlet, later treated with Provado 1.6F and plants monitored and tested for BBTV as described above. The percentage of viruliferous aphids and plantlets that became infected with BBTV was recorded. Beyond the sixth week (42 DAI) of each field trials, test plants were no longer viable (i.e., leaves and petiole were completely dry and pseudostem decayed), so the experiment was terminated.

Statistical Analysis. Data on the percentage of aphids acquiring BBTV were analyzed using a one-way analysis of variance (ANOVA) PROC GLM procedure of SAS, and mean differences between comparisons were separated using protected least significant differences LSDs. Because of a significant trial \times treatment interaction ($P = 0.0002$) with respect to data on the percentage of aphids acquiring BBTV, these data sets were analyzed by trials. The percentages of field plants that remained infected after bananacide injection and tissue cultured plants that aphids inoculated with the virus were measured as a binary response and analyzed using logistic regression PROC LOGISTIC (SAS 9.1, SAS Institute 2002), respectively. Effect of number of infective aphids on transmission efficiency was tested using a logistic regression (Crawley 2007).

Results

Virus Detection in Aphids. There was a significant effect of time (days after bananacide injection [DAI]) on the percentage of *P. nigronevosa* that tested positive for BBTV after feeding on leaf and pseudostem samples collected from treated plants (trial 1: $F_{6,97} = 45.2$, $P < 0.0001$; trial 2: $F_{6,49} = 3.39$, $P < 0.007$). During trial 1, percentages of infected *P. nigronevosa* were similar on 0 DAI (day of bananacide injection) through 21 DAI (Fig. 1A). However, by 42 DAI, BBTV was not detected in any *P. nigronevosa* allowed to feed on treated plant samples. During trial 2, percentages of infected *P. nigronevosa* were similar at 0, 14, and 21 DAI, but dropped notably by 35 DAI (Fig. 1B). However, unlike trial 1, BBTV was detected in some *P.*

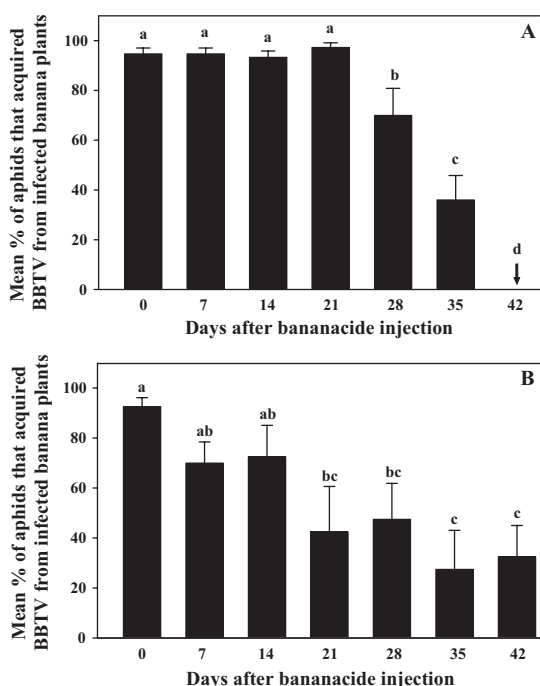


Fig. 1. Mean percentage of *P. nigronevosa* (\pm SE) that acquired BBTV from banana plants treated with a bananacide (Roundup Weathermax) during trial 1 (A) and trial 2 (B). Bar means with the same letters are not significantly different ($P > 0.05$; protected LSD).

nigronevosa (32.5%) that fed on treated plant samples at 42 DAI.

At 42 DAI, aphids were found on and collected from the pseudostem of 21 bananacide-treated plants (14 and seven during trials 1 and 2, respectively); of which 25.7 and 20% tested positive for BBTV during trials 1 and 2, respectively. In addition, individual aphid groups collected directly from the treated plants successfully transmitted BBTV to two of the 21 (9.5%) banana plants on which they were allowed to feed.

Virus Transmission to Plants. There was a significant effect of DAI on BBTV transmission rate by *P. nigronevosa* after a 48-h AAP on bananacide-treated plant samples ($F = 42.9$, $P < 0.0001$). *P. nigronevosa* was less likely to infect plants during the latter weeks after bananacide treatment (Table 1). Transmission efficiency decreased to 54% by 28 DAI; and the odds of aphids not transmitting BBTV to healthy banana plants at 35 DAI than during time of bananacide injection (0 DAI) was estimated to be ≈ 83 times greater and by 42 DAI aphids did not successfully infect healthy plants. In addition, we found that most aphid groups tested had zero, four, or five PCR-positive individuals, and the groups consisting of four or five positive individuals usually transmitted BBTV to plants, whereas the negative groups did not (Table 2, $P < 0.0001$). Few groups had an intermediate number of infected aphids and these had intermediate transmission rates. However the number of groups falling within this category was relatively small.

Table 1. Odds that *P. nigronervosa* will not transmit BBTv to healthy banana plants after feeding on an infected plant treated with a bananacide (Roundup)

DAI ^a	Transmission ^b (%)	Effect (DAI)	Point estimate	Confidence limits (95%)		Odds ratio ^c
0	84					
7	92	7 vs. 0	1.568	0.239	10.300	0.638
14	80	14 vs. 0	0.545	0.115	2.581	1.835
21	72	21 vs. 0	0.351	0.079	1.554	2.315
28	54	28 vs. 0	0.161	0.038	0.686	6.211
35	8	35 vs. 0	0.012	0.002	0.078	83.333
42	0	42 vs. 0	<0.001	<0.001	>999.999	>1,000

^a DAI represents days after bananacide injection.
^b Percentage of plants successfully infected with BBTv.
^c Odds ratio is estimated by taking the reciprocal value of the point estimate.

BBTV Detection in Bananacide-Treated Plants. There was a significant DAI effect on the presence of BBTv in banana plants after bananacide treatment ($F = 27.4, P < 0.0001$). At 35 and 42 DAI, BBTv was detected in 76 and 32% of treated plants, respectively. The odds of BBTv infected plants testing negative for BBTv after treatment with a bananacide increased significantly over time and were 50 times greater at 42 DAI compared with 0 DAI (Table 3).

Discussion

Although roguing of infected plants is an important component of disease management, in some commercial cropping systems, it may be economically and tactically unfeasible to physically remove infected plants from the field in certain situations. This includes cropping systems composed of large plants such as banana, for which elimination of infected plants involves removing the entire banana mat which typically consist of one large “parent plant” and two or more banana suckers or vertical shoots, some of which may still be below the soil surface. In those cases, killing infected plants in situ is sometimes the most practical management option. However, one disadvantage of this management approach is that infected plants may still serve as sources of pathogen inoculum before their complete destruction. Field and laboratory studies were carried out to determine the length of time that BBTv-infected banana plants remain virus reservoirs after treatment with a bananacide. Generally, banana plants were dead (e.g., foliage and petiole completely senesced and pseudostem decayed) be-

yond 6 wk after treatment with a bananacide (42 DAI). BBTv was detected in a similar high percentage of treated plants from 0 to 21 DAI. More important, some *P. nigronervosa* were able to acquire the virus from treated plant material up to 42 DAI, but no aphid groups infected a healthy banana plant beyond 35 DAI.

We anticipated the ability of *P. nigronervosa* to acquire BBTv would decline weeks after bananacide treatment and that the degeneration of test plants overtime would make them less suitable as sources of BBTv inoculum. However, the period required for this reduction to occur and the direct and/or indirect impact of the bananacide on the ability of *P. nigronervosa* to acquire BBTv was unknown. It has been suggested that the quantity and quality of phloem sap may be the main cause for aphids to abandon a feeding site for a new site (Gould et al. 2007). Roundup (glyphosate) enters the phloem flow after being applied and is transported with photosynthetic product (Sprinkle et al. 1975, Gougler and Geiger 1981). Aphids feed on phloem sap and thus could conceivably ingest or encounter small quantities of glyphosate that may cause an antifeedant effect. Davidson (1925) reported that *Aphis rumiscus* removed its stylet and moved when synthetic chemicals were present in plant sap and further suggested that aphids react to changes in the sap of the host plant. The effect of glyphosate on aphid feeding was not quantified in this study, but it was likely negligible. Although the highest label recommended rate was used, aphids were found feeding on test plants in the field at study completion (42 DAI) and if the bananacide had caused an anti-feedant behavior, it is expected aphids would have decamped treated plants before that time. The fact that our ability to detect BBTv in field plants also declined weeks after bananacide application implies that degenerative changes in the plant were likely the most important contributor to reduction in virus detection frequency. However, ≈35 DAI, the foliage of test plants were dry and no longer viable (completely senesced). As such, samples were collected from the banana pseudostem, which was a feasible approach because *P. nigronervosa* also feeds on the pseudostem (Magee 1927, Robson et al. 2006) and can readily

Table 2. Transmission efficiency of BBTv to banana plantlets by different numbers of *P. nigronervosa*

No infective aphids ^a	No. plants tested (n)	No. plants infected	Mean % transmission
0	52	7	13.5
1	9	3	33.3
2	11	4	36.4
3	6	4	66.7
4	31	23	74.2
5	72	60	83.3

^a At the end of the IAP, five aphids per group were tested for BBTv. This represents the number of aphids that tested positive for BBTv.

Table 3. Odds that a BBTV infected banana plant will no longer be an inoculum source after treatment with a bananacide (Roundup)

DAI ^a	BBTV (+) ^b	Effect (DAI)	Point estimate	Confidence limits (95%)		Odds ratio ^c
0	96					
7	100	7 vs. 0	>999.999	<0.001	>999.999	<0.001
14	96	14 vs. 0	1.000	0.059	16.928	1.000
21	88	21 vs. 0	0.306	0.030	3.159	3.268
28	89	28 vs. 0	0.208	0.022	2.017	4.808
35	76	35 vs. 0	0.107	0.012	0.950	9.346
42	32	42 vs. 0	0.020	0.002	0.172	50.000

^a DAI represents days after bananacide injection.

^b Percentage of plants that remained virulent after a bananacide injection.

^c Odds ratio is estimated by taking the reciprocal value of the point estimate.

acquire and transmit BBTV by feeding on the pseudostem (Magee 1927, Anhalt and Almeida 2008).

Generally, mean numbers of *P. nigronervosa* acquiring BBTV after bananacide treatments were higher during trial 1 than trial 2. This difference between trials may be attributed to *P. nigronervosa* not readily settling on some foliage samples during trial 2. On occasions during trial 2, aphids were found walking within the petri dishes as they were being removed from the growth chamber; and if aphids spent more time wandering than feeding, they would have had less time to acquire the virus. Reducing the AAP may correlatively reduce the level of virus in aphid vectors and thus the probability that aphids will transmit virus to other host plants (Gray et al. 1991). Anhalt and Almeida (2008) found that longer plant access periods increased virus acquisition and inoculation efficiencies in a range of 1–24 h. Because five aphids were placed on each laboratory test plant, if one fed on the leaf or pseudostem sample for a protracted period, this could result in successful virus transmission. However, our results showed that inoculation was only successful when most aphids in a group tested positive for BBTV. It is possible that the virus titer within aphids was lower in groups with fewer than four or five BBTV-positive individuals, consequently reducing overall transmission rates as BBTV is transmitted in a circulative, nonpropagative manner (Hafner et al. 1995). However, fewer aphids within a group generally tested positive for BBTV during the latter weeks after bananacide injection. As such, reductions in BBTV titer levels in individual aphids may have occurred because titer levels within infected test plants decreased over time after bananacide injections resulting in a lower number of aphids becoming infected. An additional difference between trials is that during trial 1, *P. nigronervosa* were not detected as having acquired the virus from plant samples collected at 42 DAI. However, during trial 2, 32% of the aphids were diagnosed as acquiring BBTV at 42 DAI. Trial 2 was conducted during the winter, and from casual observation, we noted that plants senesced at a slightly slower pace compared with the earlier trial. This may have meant that BBTV titer levels in test plants were higher at 42 DAI during trial 2 compared with trial 1, allowing the virus to be acquired during this period. Virus titer within plants can have a strong influence on the acquisition and inoculation efficiencies of aphid vectors (Gray et al. 1991).

The standard recommendations for managing BBTV include frequently scouting for and destroying infected plants immediately. The longer infected plants remain in a field, increases the opportunity for secondary virus spread (Allen 1978a,b). Generally, growers in Hawaii use a bananacide such as Roundup or other brands containing glyphosate as their active ingredient. During this trial, the highest label recommended rate was used, and it was found that banana plants can potentially serve as a source of virus inoculum for 6 wk after bananacide injection. This time period may vary somewhat if bananacides with a lower active ingredient are used or a lower rate is applied. Aphids have the adaptive ability to anticipate the onset of adverse conditions within their host plant, and when the quality of their host plant declines this signals them to produce wing morphs (Dixon 1998). It is uncertain at what period after application can aphids recognize a decline in plant quality. Assuming that aphids detected an adverse change in plant quality when visual symptoms of the bananacide application were initially apparent (≈ 21 DAI) that would not allow aphids enough time to produce winged morphs. Aphids generally require a minimum of one generation from the time the reduction in plant quality is noticed by parent aphids to produce wing morphs. Additionally, alatae generally take more time to reach maturity than apterae developing under similar conditions (Dixon 1998). By this time, treated plants may have degenerated to a stage where aphids cannot acquire enough nutrients needed for full development. However, if winged morphs already inhabit banana plants before treatment, these individuals may decamp to neighboring plants causing secondary virus spread. Hill (1983) suggested that chemical treatments are generally only effective if accompanied by eradication of the infected plants. We agree with Hill's supposition. As such, in addition to recommending that BBTV infected plants are destroyed, crop advisors in Hawaii may further suggest that they be treated with an insecticide just before destruction. Many growers in areas where BBTV is prevalent use preemptive sprays throughout their entire orchard to reduce the spread of BBTV by *P. nigronervosa*. Spraying and destroying only those plants infected with the virus, offers an alternative BBTV management plan to indiscriminate preemptive sprays throughout banana fields. This management practice also further reduces

the probability that aphids inhabiting infected plants will transmit virus to neighboring host plants.

It has been suggested that if a symptomatic plant is found, growers should consider roguing apparently healthy neighboring plants (Allen 1978). However, this may result in the unwarranted destruction of several banana plants and mats that were virus-free (Allen 1987). Thus, this strategy of spraying only infected plants may be extended to neighboring banana mats and in the likelihood that a neighboring plant is infected, this will reduce the risk of secondary virus spread without the potential wasteful destruction of healthy banana plants or preemptive sprays directed at an entire banana planting. Spraying infected and neighboring banana plants may be used to help improve roguing efficiency. However, the potential gains of this strategy would need to be investigated.

The experiment described in this article was conducted to determine the probability that *P. nigronevosa* would acquire and transmit BBTV after application of a bananacide. The major conclusion of this study is that banana plants may remain a potential source of virus inoculum several weeks after bananacide treatment. These findings are of potential importance in any cropping system where herbicides are used to eradicate plants infected with a persistently-transmitted virus. Determining the impact of the bananacide on aphid movement was not an objective of this study. However, Bailey et al. (1995) found that several perturbations, including herbicide applications to oat, *Avena sativa* L., plants resulted in greater incidence of Barley yellow dwarf virus and movement of apterous *Rhopalosiphum padi* L. (Homoptera: Aphididae) compared with control treatments. Herbicides used for treating diseased banana plants are injected in a small opening in the pseudostem of the infected plant; as such, sprays are not applied throughout the entire orchard or on the foliage and thus will not come in direct contact with other banana plants or aphids. Therefore, the risk of a bananacide treatment resulting in mass movement of aphids throughout the orchard is mitigated. Additionally, unlike oats and similar plant systems in which virus spread may occur relatively fast (Bailey et al. 1995), BBTV seems to be a slow moving virus in Hawaiian plantations (C.R.R.H. et al., unpublished data). Still, future studies aimed at determining the survival of *P. nigronevosa* on dying banana plants may help provide further insight on the worth of accompanying a bananacide with an insecticide application to further mitigate the potential of secondary virus spread. However, monitoring the movement and/or survival of *P. nigronevosa* on banana plants within a banana orchard is not as definite as for other virus aphid systems. The epidemiology of BBTV, large size of banana plants, structural complexity of banana orchards, and the small size and cryptic behavior of banana aphids would make surveying aphid movement from plants and associating their movement with virus incidence an ambitious undertaking for this system.

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