Within-Plant Distribution and Binomial Sampling of *Pentalonia nigronervosa* (Hemiptera: Aphididae) on Banana

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ABSTRACT The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), infests banana (*Musa* spp.) worldwide. *Pentalonia nigronervosa* is the vector of Banana bunchy top virus (family *Nanoviridae*, genus *Babuvirus*) the etiological agent of Banana bunchy top disease (BBTD). BBTD is currently the most serious problem affecting banana in Hawaii. Despite the importance of this vector species, little is known about its biology or ecology. There are also no sampling plans available for *P. nigronervosa*. We conducted field surveys to develop a sampling plan for this pest. Ten plots were surveyed on seven commercial banana farms on the island of Oahu, HI, for the presence of *P. nigronervosa* on banana plantlets. We found aphids more frequently near the base of plants, followed by the newest unfurled leaf at the top of the plant. Aphids were least likely to be located on leaves in between the top and bottom of the plant. Aphid infestation on surveyed plots ranged from 8 to 95%. We developed a sequential binomial sampling plan based on our surveys. We also discovered that the within-plant distribution of *P. nigronervosa* is an important factor to consider when sampling for this pest. Our sampling plan will assist in the development of sustainable management practices for banana production.

KEY WORDS aphid, Banana bunchy top virus, circulative virus, vector

Banana (*Musa* spp.) is an important staple crop in many tropical areas of the world. In Hawaii, banana is a significant contributor to the agricultural economy; in 2004 the state produced 16.5 million pounds of banana worth $8.1 million (National Agricultural Statistics Service 2005). Approximately one-half of the commercially grown banana in Hawaii is of the Dwarf Brazilian variety, which is classified as *Musa* AAB and is part of the plantain subgroup (Robinson 1996). *Musa* AAA Cavendish subgroup banana and other minor varieties are also grown in Hawaii. The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is the only known vector of Banana bunchy top virus (family *Nanoviridae*, genus *Babuvirus*, BBTV) (Magee 1927, Hu et al. 1996). This virus causes Banana bunchy top disease (BBTD), which can have serious effects on crop yield. In Hawaii, banana showed a 27% reduction in yield and a 26% reduction in harvested acreage from 2003 to 2004 (National Agricultural Statistics Service 2005). Much of this loss has been attributed to BBTD. *P. nigronervosa* was first recorded in Hawaii in 1924 (Zimmerman 1948) and BBTD has been present since 1989 (Conant 1992), when it was first detected on the island of Oahu. Despite various control efforts, the virus has now spread to all of the main Hawaiian Islands except for Lanai.

BBTV can be disseminated through vegetative propagation practices, common with seedless crops such as banana. This multicomponent single-stranded DNA virus is efficiently transmitted by *P. nigronervosa* in a circulative, persistent, and likely nonpropagative manner (Hu et al. 1996, Franz et al. 1998). In Australia, successful control of BBTV was possible after stringent plant quarantines, destruction of infected plants, and legally enforced establishment of new plantings with clean material (Magee 1967). The use of tissue culture plantlets to establish new plantations, although common in some areas with BBTV, such as Taiwan (Robinson 1996), is not widely used in Hawaii.

Population numbers of *P. nigronervosa* in Hawaii remain constant throughout the year (Young and Wright 2005). Aphids most often display an aggregated distribution within plantations; rainfall and aphid numbers showed no significant correlation (Young and Wright 2005). However, flight frequency of *P. nigronervosa* has been shown to have a positive correlation with rainfall in Surinam (van Hoof 1962). In India, the percentage of alate forms in field populations of *P. nigronervosa* ranged from 0.56 to 2.04% in a 1-yr survey, indicating that alates are also relatively unaffected by seasonality and remain a comparatively small percentage of the population throughout the year (Rajan 1981). This is important because it is generally thought that alate forms of *P. nigronervosa* are primarily responsible for the spread of BBTV. A

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Table 1. Incidence of *P. nigronervosa* in Oahu banana plantations

Sampling date	Sampling location[a]	No. plants sampled	Proportion positive ± SD
22 Mar. 2005	Waialua[1]	671	0.52 ± 0.02
23 Mar. 2005	Ewa west[2]	668	0.82 ± 0.02
20 April 2005	Poamoho[3]	97	0.95 ± 0.02
23 May 2005	Waimanalo[4]	156	0.76 ± 0.03
13 July 2005	Kahuku[5]	202	0.42 ± 0.04
20 July 2005	Waianae[6]	175	0.82 ± 0.03
25 July 2005	Ewa east[7]	195	0.08 ± 0.02
27 July 2005	Waialua[1]	154	0.31 ± 0.04
24 Aug. 2005	Waimanalo[4]	138	0.16 ± 0.03
1 Sept. 2005	Ewa west[2]	157	0.54 ± 0.04

[a] Superscript number corresponds to locations indicated on map in Fig. 1.

quantitative epidemiological study of BBTD showed an exponential increase in disease incidence over time (Smith et al. 1998).

Despite BBTD's economic importance, little is known about the biology of *P. nigronervosa* and the epidemiology of BBTV in Hawaii. To our knowledge, there is currently no sampling plan or action threshold available for *P. nigronervosa* on banana. The goal of this work was to determine the within-plant distribution of *P. nigronervosa* and to use aphid incidence surveys to develop a sampling plan for this pest.

Materials and Methods

Sampling Locations. We sampled for *P. nigronervosa* on commercial banana plantations on the island of Oahu, HI (Table 1). We chose sampling locations that allowed for representation of the different climatic conditions of the island, most notably the typical difference between the wet, eastern side and the dry, western side of the island (Fig. 1). For example, the average daily temperature at Waianae (Fig. 1, location 6) is 22.8°C, and the average annual precipitation is 77.2 cm versus an average daily temperature of 20.6°C and an average annual precipitation of 145.5 cm at Kahuku (Fig. 1, location 5) (National Weather Service 2006). These climatic regions also match banana-growing areas on other Hawaiian islands. Young and Wright (2005) showed that populations of *P. nigronervosa* do not fluctuate significantly throughout the year; thus, we sampled over a period of 7 mo (March–September 2005). Only plants of the Dwarf Brazilian variety (*Musa* AAB) were sampled. Cultural practices were similar on all plantations; banana mat spacing of ≈2 m between plants and between rows. Every other row was wider (≈4 m) to allow farm vehicles to pass. Plants were maintained on a drip irrigation system. Most growers did not use any chemical control for *P. nigronervosa.* Only one of the plantations sampled, Ewa west (Table 1), had been sprayed for insect pests before sampling (≈3 mo before our 25 March 2005 sampling date).

Within-Plant Distribution of *P. nigronervosa.* *P. nigronervosa* is found in greater numbers on suckers rather than on mature banana plants (Young and Wright 2005), although we also have observed aphids on plants bearing fruit (J.D.R., unpublished data). In terms of development of a practical sampling procedure, suckers are more useful than mature plants for reasons of accessibility for samplers. Thus, our decision to sample only smaller plants was made based on the distribution of aphids on banana and the goal of developing a user-friendly plan for growers. We quan-

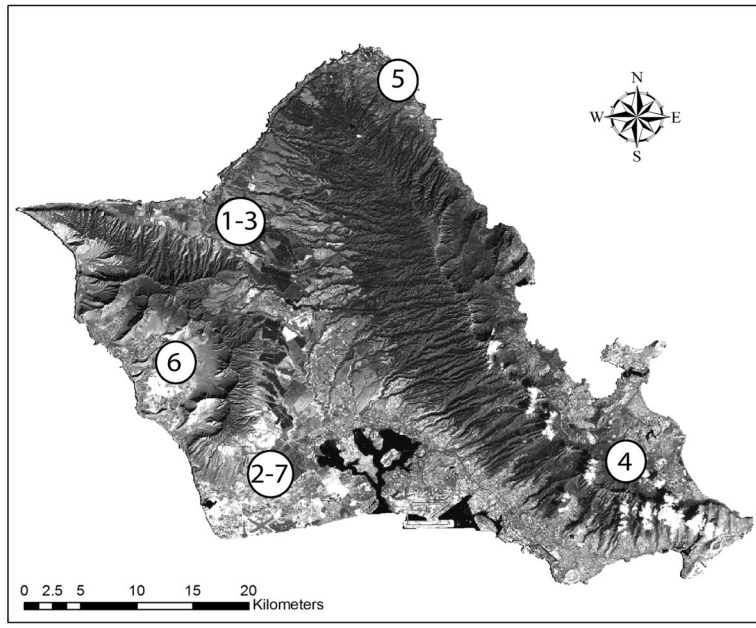

Fig. 1. Locations of banana plantations surveyed on the island of Oahu. Numbers match locations listed in Table 1.

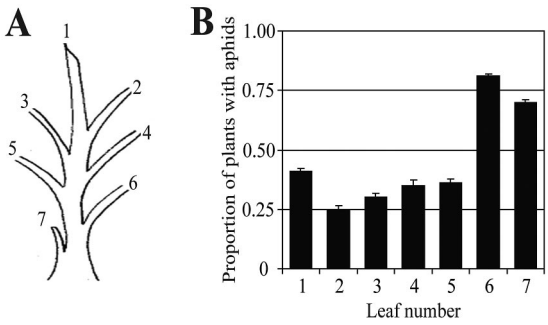

Fig. 2. (A) Diagram of banana plant used to determine spatial distribution of *P. nigronervosa* by leaf position or "number." Leaf 1 is the newest unfurled leaf, leaves 2–5 are the next fully expanded leaves, leaf 6 represents all blade-like leaves, and leaf 7 represents the dry bract around the base of the pseudostem. (B) Leaf number and incidence of *P. nigronervosa* on banana plantlets.

tified within-plant distribution of aphids by using a diagram representative of a small banana plant (Fig. 2A). On this diagram, leaf 1 represents the newest, unfurled, uppermost leaf (commonly referred to as the "cigar leaf"); leaves 2 to 5 are the next fully expanded leaves; leaf 6 represents all thin, blade-like leaves below the fully formed leaves; and leaf 7 represents the dry bract around the base of the pseudostem near soil level. The tip of dry bracts is composed primarily of dry tissue; however, the base of the leaf connected to the pseudostem remains hydrated. In addition, aphids are often found under this bract feeding on the pseudostem rather than on the bract itself. Banana morphology and terminology followed Robinson (1996). Because plants varied in size and leaf number, we sampled plants with variable numbers of leaves. All plants had a top and bottom leaf (positions 1 and 7) but the number of leaves in between varied. We randomly chose rows within a field and treated them as transects through the plantation. The number of rows sampled ranged from 10 to 30 depending on the size of the plantation. Along a row, all plants that were 1.5 m and less in height were sampled. When sampling a plant, the cigar leaf was first examined, then we moved down the plant, examining the spaces between the petioles and the pseudostem where aphids are located (van Hoof 1962). The cigar leaf was designated as leaf 1; the next oldest leaf down was designated leaf 2, and so on. If the plant was very small (i.e., >30 cm in height), we omitted leaf positions between leaves 1 and 7. For example, many of the smallest plantlets had only leaves 1, 6, and 7; unfurled leaves were absent. Leaves were usually pulled gently away from the pseudostem to determine the presence of *P. nigronervosa.* We peeled leaves completely away from the pseudostem in some instances when the grower involved gave permission for this more destructive sampling method. Aphids could be found equally as well using either technique, but we found the latter to be less time consuming. Proportion of leaf-sheaths harboring aphids at different positions was compared using a 2×7 contingency table analysis with leaf position as the categories and frequency of encounter of aphids or absence as the contingencies.

Binomial Sequential Sampling. Because we were sampling for an efficient aphid vector (Hu et al. 1996) and not a direct pest, we used a binomial sampling approach, with aphids being designated as present or absent from a plant. We used data obtained on surveys for within-plant distribution of the aphid to test our sampling plan. Enumerative field sampling for *P. nigronervosa* has been done (Young and Wright 2005), but it is likely to be difficult and time-consuming for producers because of the pest's small size, potential for large infestations, and cryptic nature. Sequential sampling is one of the most efficient sampling schemes for making control decisions in an integrated pest management program (Romoser and Stoffolano 1998).

Statistical Analysis and Validation of Sampling Plan. Decision stop lines for the binomial sequential sampling plan were calculated using the binomial distribution and Iwao's sequential procedure (Binns et al. 2000). Upper (equation 1) and lower (equation 2) stop lines were respectively determined by the following equations:

$$U_n = n\,(cp + z_{\alpha/2}\sqrt{V/n}) \quad [1]$$

$$L_n = n\,(cp - z_{\alpha/2}\sqrt{V/n}) \quad [2]$$

where n is sample size, cp is critical proportion, and V is binomial variance = cp (1 − cp). Sampling plans were calculated for a critical proportion of 0.10 and a power level of 80%.

Testing of sampling plans was done using simulations for Iwao's procedure in MathCad (Binns et al. 2002). Random variables were generated to construct data sets based on a positive binomial distribution. Simulation inputs included a minimum mean density of 0.08, a maximum mean density of 0.95, a critical density of 0.10, an α error rate of 0.2, and a maximum sample size of 125. One thousand simulation repetitions were performed. We also computed the operating characteristic (OC) and average sample number (ASN) functions from the simulations.

Results

Within-Plant Distribution of *P. nigronervosa*. Our survey of Oahu plantations for *P. nigronervosa* showed that this pest is not evenly distributed within banana plantlets. Frequency of encountering *P. nigronervosa* was highest on the lowest parts of the plant (percentage of infestation ± SD, 81 ± 1.1 and 70 ± 1.2% for leaves 6 and 7, respectively) followed by the newest leaf (41 ± 1.3%). Insects are least likely to be located on the leaves in the central height region of the plant (25 ± 1.5, 30 ± 1.9, 35 ± 2.2, and 36 ± 1.6 for leaves 2, 3, 4, and 5, respectively (Fig. 2B). Contingency table analysis showed a significant difference in aphid presence associated with leaf position ($\chi^2 = 378.04$, df = 6, $P \ll 0.0001$). Calculated cumulative probabilities across the entire plant for the detection of *P. nigron-*

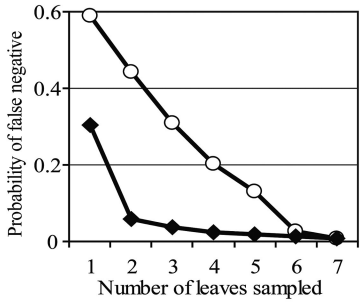

Fig. 3. Decreasing probability of obtaining a false negative for the absence of *P. nigronervosa* with location (plant top or bottom) and number of leaves sampled on a plantlet. Empty circles indicate sampling starting at the top of the plant, filled diamonds represent sampling starting at the base of the plant.

ervosa also showed that infested plants are more likely to be discovered if sampling is started at the bottom rather than at the top of the plant (Fig. 3).

Binomial Sequential Sampling and Sampling Plan Formation. Sampling for *P. nigronervosa* on Oahu showed that this insect is common in commercial banana plantations. Binomial sampling revealed infestation rates of 8 to 95% on banana plantlets <1.5m in height (Table 1). The binomial sequential sampling plan developed using Iwao's procedure (Fig. 4) indicates that at a critical density threshold of 0.10 and 80% power, a minimum sample size of ≈10 plants is appropriate.

Testing the Sampling Plan by Using Simulation. At our designated critical density of 10% infestation, the OC curve (Fig. 5) shows that the probability of choosing not to treat for banana aphid is 50% and then very quickly drops to close to zero for higher aphid population densities. The ASN curve (Fig. 5) at this critical density shows a minimum sample size of ≈45 plants, with the ASN dropping rapidly with an increase in aphid density.

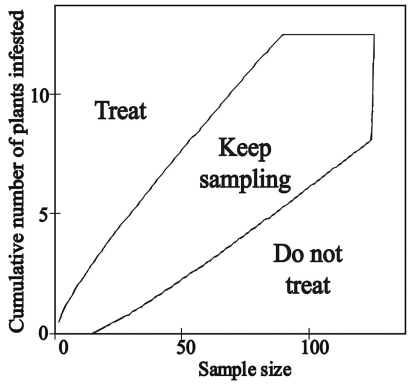

Fig. 4. Binomial sequential sampling plan for *P. nigronervosa* on banana.

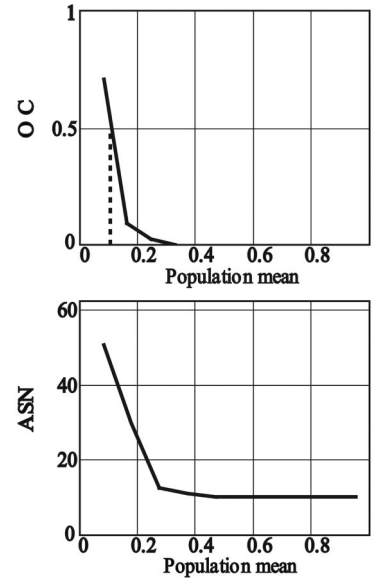

Fig. 5. Attributes of the OC and ASN for the sampling plan for *P. nigronervosa* on banana. Dashed line represents the arbitrary 10% critical density.

Discussion

We surveyed commercial banana plantations for the presence of *P. nigronervosa.* Our study showed that aphids were found more often at the base of plants instead of on the new, upper unfurled leaf. This significant finding has implications in the management of *P. nigronervosa*, because surveys must be performed in plant sections previously considered not preferred by aphids. We also developed a binomial sampling plan for *P. nigronervosa*, which could be used by growers to detect and manage aphid populations.

Sampling for *P. nigronervosa* on Oahu showed that this pest is widespread on banana plantations. We observed BBTD on every plantation surveyed, but we did not quantify disease level. BBTD is, however, widespread on Oahu (R.P.P.A., unpublished data). The exponential increase in BBTD incidence in banana fields observed in other studies (Smith et al. 1998), in addition to *P. nigronervosa*'s high transmission efficiency (Hu et al. 1996), suggest that disease incidence can increase rapidly without control of aphid populations. Low rates of observable BBTD in a plantation (i.e., symptomatic plants), along with inadequate surveys for vectors (i.e., sampling newest leaf), could result in high levels of BBTD in areas previously assumed to be disease free or to have low disease incidence. The importance of BBTV-infected but asymptomatic banana plants on the spread of BBTD is unknown. Thus, managing *P. nigronervosa* populations may be important even if disease incidence is unknown.

We found *P. nigronervosa* to prefer the base of plantlets instead of new leaves. When sampling only one location (i.e., one leaf) on a plant, we found on average a 59% chance of missing an infestation if only

the newest leaf on a plant is sampled. If sampling was instead done only at the lower bract at the base of the plant, the probability of missing an infestation drops to 30%. Sampling the two uppermost leaves still leaves a 44% chance of making a false conclusion regarding the presence of *P. nigronervosa*, whereas sampling the bottom two leaves will result in a 6% chance of a false negative. Hummel et al. (2004) showed that binomial sampling for potato aphid, *Macrosiphum euphorbiae* (Thomas), on only upper leaves of tomato, *Lycopersicum esculatum* Mill., was effective and more efficient than sampling both upper and interior leaves. Nibouche et al. (2004) developed a sampling plan for lepidopteran pests in cotton that reduced sampling time by up to 60% by sampling only upper fruiting branches, although power was decreased when mean infestations were lower than the critical density. These studies illustrate how understanding within-plant distribution of a pest can have a great effect on sampling procedures and treatment decisions.

To develop a practical sampling plan for *P. nigronervosa*, we set a critical proportion of 10% infestation, which serves as an action threshold. Because no formal thresholds for *P. nigronervosa* currently exist, we derived this from a nominal threshold, a commonly used method that is usually based on a manager's experience (Pedigo 2002). A critical proportion of 1% (i.e., treat on detection) might be more effective for disease control because *P. nigronervosa* is a viral vector [as suggested by Mowry 2001 with green peach aphid, *Myzus persicae* (Sulzer) and Potato leafroll virus (family *Luteoviridae*, genus *Polerovirus*, PLRV)]. However, we were unable to perform simulations with this level because it was out of range of the actual data we used to construct our sampling plan (samples ranged from 8 to 95% infestation). In addition, this level of 1% would provide sampling plans with prohibitively large sample sizes unlikely to be adopted by growers.

The OC and ASN (Fig. 5) functions derived from simulations using our sampling data indicate that at a population density close to the critical density of 0.10, the probability of making a "no treat" decision is ≈50%, and the average sample number is 45 plants. The OC curve drops off very quickly after exceeding the critical density and the average sample number decreases in a similar manner. Our calculated mean proportion of infested plantlets across our sampling areas was 0.58, showing that the incidence of *P. nigronervosa* on banana on the island of Oahu is typically much higher than 0.10. At this mean infestation rate of 0.58, the OC function shows a probability of making a no treat decision of zero, and the ASN function shows an average sample size of ≈10 plants. These results clearly show that our sampling plan is effective across a range of population densities, as supported by the steep OC curve and low ASN.

Mowry (2001), working with PLRV, which, like BBTV, is transmitted by aphids in a circulative, persistent manner, recommended insecticide application upon detection of aphid vectors and advised against the use of a nonzero action threshold. This recommendation was based partially on the rapidity with which aphids are able to increase their populations. In addition, the tendency of aphids to remain hidden under leaf sheaths on banana plants (van Hoof 1962, this study) may limit the efficiency of insecticide applications to reduce populations. Currently, there are no systemic insecticides registered to control aphids on banana in Hawaii. Thus, our sampling plan should be envisaged as an approach to quickly lower aphid numbers and reduce chances of aphid–virus encounters. A sustainable control strategy also must incorporate roguing and use of healthy tissue culture plantlets, similar to other strategies to control the spread of aphid-borne plant viruses; for example, roguing of infected plants is accepted as the most effective way to control BBTD (Magee 1927, Dale 1987, Conant 1992, Smith et al. 1998). Moreover, roguing has proven to be very successful in controlling BBTD, being the main focus of a control strategy used in an outbreak in Australia in the 1920s (Magee 1927). Once the disease has been introduced into an area, however, eradication is not considered to be a viable option.

Monitoring pest densities allows for informed decision making regarding application of pesticides. Using pesticides to control insect vectors of viral plant diseases can be useful in some instances (Perring et al. 1999). However, vector thresholds may need to be low to control disease spread. Mowry (2001) showed that insecticidal control of *M. persicae* reduced within-field incidence of PLRV when monitoring was frequent and when a zero pest threshold was used. Many local banana growers tend not to monitor for banana aphid and therefore do not detect infestations until they are at a very high level (J.D.R., unpublished data). The availability of a sampling plan will assist banana growers to reduce aphid populations. However, as discussed by Smith et al. (1998) for BBTD and Perring et al. (1999) and Mowry (2001) for other systems, lowering vector numbers alone may not result in reduced disease spread. Thus, this sampling plan should take into consideration disease incidence and be tested together with other cultural practices such as rouging for the development of improved disease controls strategies.

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