Dispersion and Sequential Sampling Plan for *Xylosandrus compactus* (Coleoptera: Curculionidae) Infesting Hawaii Coffee Plantations

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ABSTRACT The black twig borer, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae), is a serious pest of coffee (*Coffea arabica* L.) in the Kona region of the island of Hawaii, the center of the largest area of coffee production within the state of Hawaii. This study indirectly characterizes the spatial distribution of *X. compactus* in coffee plantations through assessment of twig borer damage, and presents a sequential sampling plan for monitoring *X. compactus* population densities. Taylor's Power Law (TPL) and Iwao's mean crowding index showed that *X. compactus* infestations were highly aggregated within plantations, with *b* and ß values significantly larger than 1. The TPL linear regression of log variance against log mean ($R^2 = 0.92$) provided a better fit to the data than the linear regression of mean crowding on the mean ($R^2 = 0.68$). Subsequently, Taylor's power law parameters were used to develop the Green's sequential plan to estimate densities of *X. compactus* at the 90 and 75% precision levels.

KEY WORDS Taylor's power law, sampling plan, black twig borer, *Coffea arabica*

The black twig borer, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae), first was found in Hawaii in 1960, and has been a sporadic pest in many crops since then. In recent years, it appears to have become a significant and predictable pest in coffee, particularly in the Kona area (Bittenbender and Easton Smith 1999). In January of 2008, coffee growers in the Kona coffee growing region, Hawaii, identified the black twig borer as the primary obstacle to coffee production in the Kona District (coffee growers, personal communication). Coffee is grown not only in the Kona area of the Big Island of Hawaii, but also on large plantations on Oahu, Molokai, Maui, and Kauai (Bittenbender and Easton Smith 1999). However, Kona coffee is the best-recognized Hawaiian coffee, and it obtains the highest value per acre. Kona produces ≈1.59 million kg of green coffee valued at US$35–42 million annually (Hawaii Farm Facts 2012).

The black twig borer female bores into the living tissues of a plant and creates a tunnel, which she then inoculates with an ambrosia fungus (Ngoan et al. 1976, Hara and Beardsley 1979). It is primarily the tunneling action of the adult beetle that results in economic damage to crops, rather than the resulting injury from the larvae or the fungus (Daehler and Dudley 2002).

In an integrated pest management (IPM) program, a pest's density must be estimated during sampling so that treatment thresholds can be established and timely management actions may be implemented (Davis 1994). The spatial distribution (dispersion) of the target pest is the basis for the development of a sampling plan and an indispensable tool for work on pest population ecology (Pedigo 1994). Insect populations may have aggregated, random, or uniform distributions, and dispersion characteristics are typically consistent for a species. Dispersion of an insect population within a habitat can significantly impact the outcomes of sampling applied to those populations (Pedigo 1994). Characterizing the dispersion of the black twig borer and development of a sequential sampling protocol will contribute to accurately quantifying infestation levels, monitoring beetle populations, and correct timing for management actions. Implementation of accurate sampling requires a monitoring system that is straightforward and easy to implement for coffee growers. Sampling protocols for estimating the density of the black twig borer in coffee have not been developed previously.

An enumerative sequential sampling plan requires counting all the individuals present in each sample unit, and using estimates of variance to mean relationships, development of a sampling plan that estimates optimal sample size based on a set precision level and insect density (Wilson 1994). Sequential sampling thus entails sampling a batch of samples, and then deciding whether to sample further units, or stop sampling and estimate pest density. The time and cost

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involved in sampling, if based on counting every insect in a defined population, is impractical for most pest management purposes or for data collection for research (Pedigo and Rice 2006). Enumerative sampling plans have been considered to be excessively time-consuming for practical decision making by growers, but efficient and accurate set precision plans can be devised depending on aspects of pest biology. However, binomial sequential sampling plans, offer a viable labor-reducing alternative to enumerative sampling in many situations (Wilson 1994). With binomial sampling, a sampling unit is classified as either infested or uninfested and the sampling unit infestation is used as a surrogate for the insect counts. Batch-sampling is used, with decisions whether to stop and estimate pest density, or continue sampling, based on proportion of sample units infested, rather than counting all individuals (Binns et al. 2000). Information on the number of insects within each sampling unit is often not needed for purposes of pest scouting (Binns et al. 2000). The advantage of using binomial sampling plans is that they are frequently much more time efficient when determining whether an action threshold has been reached, compared with enumerative counts of insects (Naranjo et al. 1996).

The objective of this study was to characterize the dispersion of black twig borers in coffee plantations and to develop sequential enumerative sampling and binomial sampling plans to classify infestation levels of the black twig borer, through quantifying infestation levels of coffee branches. Developing a sampling method for the black twig borer will provide a means to determine the number of sample units required before a set-precision estimate of damage density is made, which will facilitate pest management decisions.

Materials and Methods

Study Area. This study was conducted in 2007 and 2008 in the Kona area of the Big Island of Hawaii. Eight commercial coffee farms were selected at different elevations (140-, 246-, 344-, 368-, 504-, 535-, 674-, and 756-m elevation). Coffee plants (*Coffea arabica* L., variety Kona typica), were spaced 1.2 m apart in rows that were 3.5 m apart. Coffee plants were approximately 8 yr old and the average plant height was 1.5 m. The farms had a range of landscape characteristics. At some farms, coffee was shaded with mango (*Mangifera indica* L.), litchi (*Litchi chinensis* Sonn), avocado (*Persea americana* Mill), macadamia (*Macadamia integrifolia* F. Muell), or monkey pod (*Samanea saman* Jacquin Merrill). Other farms had no shading overstory trees present, but bordered uncultivated areas with ohia (*Metrosideros polymorpha* Gaud), *Eucalyptus* spp., christmas berry (*Schinus terebinthifolius* Raddi), and coral trees (*Erythrina* spp.). Except for pruning systems, cultural practices were similar in all coffee plantations sampled. Coffee was hand-harvested and the farms were not irrigated. The pruning systems used were the Kona style, in which one or two vertical branches is pruned in successive years, or the Beaumont–Fukunaga style, in which all the verticals on the tree are pruned in the same year, once every 3–5 yr (Bittenbender and Easton Smith 1999).

Meteorological Conditions. The Kona coffee belt has an annual mean temperature of 22°C and average annual rainfall of 1,245 mm (Bittenbender and Easton Smith 1999). Spring and summer are the rainy season in the Kona area (April to September). A low rainfall period occurs during the coldest months of the year, which are December through February (Bittenbender and Easton Smith 1999).

Data Collection. Black twig borer infestations were monitored every month for 23 mo, by randomly selecting and sampling 55 coffee trees from the entire field in each farm. Sample size (number of coffee trees examined for black twig borer infestation) was dictated by time available to sample the full complement of farms monthly (from January of 2007 to December of 2008). Each tree was classified as infested (at least one branch attacked), or uninfested by black twig borer, and the number of infested branches and uninfested branches was recorded for each plant. Fresh damage caused by black twig borer is readily distinguished on newly developed branches, and old branches are pruned, so historic damage attributable to this insect is unlikely to be included in estimates of current damage levels. Number of infested branches per tree, rather than insect density, was considered to be the variable of interest in terms of quantifying extent of damage to coffee trees. Infestation was classified as the presence of wilted leaves and dieback of twigs and tunnels in the bark area, which are symptoms of black twig borer infestation.

Spatial Distribution. Dispersion was characterized by analyzing the mean-to-variance relationship (Taylor's power law [TPL]) (Taylor 1961), and Iwao's patchiness or mean crowding regression (Iwao 1968). These two regression techniques have been widely used to measure dispersion and develop sampling protocols (Davis 1994). Taylor's power law, relates variance to mean as

$$s^2 = am^b \qquad [1]$$

where s^2 is the sample variance, m is the sample mean, a and b are the power-equation parameters, which provide an index of dispersion, measuring the rate of increase in the variance to mean ratio (Taylor 1961). The Taylor's power law model is typically expressed in linear form as

$$\log s^2 = \log a + b \log (m), \qquad [2]$$

for simplified estimation of parameters. The index of aggregation (regression slope, b) characterizes the dispersion of the species such that if $b > 1$, the distribution is aggregated, if $b < 1$, insects are distributed uniformly in the field and $b = 1$, the distribution is random (Taylor 1984).

Another regression technique for measuring dispersion is the Iwao's patchiness- or mean crowding regression (Davis 1994). The mean crowding regression or Lloyd's mean crowding index indicates the level of competition among individuals (Lloyd 1967). This method has been used to examine the relationship

Table 1. Estimates of dispersion parameters for *X. compactus* in coffee

	Parameter estimates					
Model	Intercept ± SE	Slope ± SE	R^2	*F* value	df	*P*
Taylor's power law	0.55 ± 0.01	1.33 ± 0.02	0.94	170.75	1, 188	0.0001
Iwao's mean crowding index	0.80 ± 0.11	2.87 ± 0.14	0.68	407.76	1, 188	0.0001

between mean crowding index (Lloyd 1967) (m*) and mean density *m*. Mean crowding is estimated as:

$$m^* = m + [(s^2/m) - 1] \qquad [3]$$

Therefore, the linear relationship between mean crowding index (m*) on the mean density (*m*) was derived from equation 3 by Iwao (1968) as:

$$m^* = \alpha + ßm \qquad [4]$$

The parameter α indicates the tendency to crowding greater than zero and ß indicates the dispersion pattern of the clumps of individuals of the species, in a similar manner as *b* is interpreted in TPL.

Enumerative Sampling Plan. The objective of this model was to develop a means to estimate the infestation density of branches by *X. compactus* by sequential batch sampling, estimating the cumulative number of infested branches per production unit sampled. The Green's sequential sampling method (Green 1970) requires that trees be sampled sequentially until the cumulative number of infested branches exceeds the stop-line value for the number of trees sampled. The mean density of infested branches per tree for a particular production unit can then be estimated by dividing the cumulative number of infested branches by the number of trees sampled.

After parameter estimation (see results section), values of a and b based on the variance: mean relationship described by TPL were used (see results section for fit of models) in Green's formula (Green 1970) to calculate stop lines for the sequential sampling plan. The parameters *a* and *b* were used directly from the TPL regression. The stop lines represent the relationship between T*n* (cumulative number of damaged branches) and *n* (number of trees sampled) required to achieve a desired precision level estimate of damage density, although accounting for dispersion of the target damage on the coffee plants. The sequential sampling stop line (T*n*) is calculated as:

$$ln\ Tn = (ln(D^2/a)/b - 2) + b - 1/b - 2\ ln\ n \qquad [5]$$

where T*n* is the cumulative number of infested branches, *n* is the total number of sample units, *D* is the desired level of precision, expressed in terms coefficient of variation, and *a* and *b* are TPL parameters. The anti-ln T*n* was plotted against *n* to generate the stop lines. The resulting sequential sampling plan may be used to estimate the population density of the pest damage, by collecting a series of sample units and recording the number of damaged branches accumulated. Sampling is halted when the cumulative number of individuals exceeds the stop line for that sample size; sampling is continued as long as the cumulative number sampled remains below the stop line, or when the number of samples reaches the predetermined maximum sample size, and mean population (damage) density is the estimated.

Binomial Sampling Plan. The objective of this plan was to provide a means to estimate the infestation density of *X. compactus* damaged branches by quantifying the proportion of infested coffee trees per production unit. Counting the number of infested branches is potentially time consuming, especially during summer seasons where higher black twig borer populations occur and damage levels increase. A binomial sampling plan is recommended by pest management specialists as an option for classifying damage levels based on pest or damage presence or absence data. In this study, each coffee tree sampled per plantation each month was scored as uninfested (0) or infested (1). In an attempt to develop a binomial sampling option, we plotted proportion of trees infested per plantation against mean number of branches infested per tree, in a manner similar to that of Wilson and Room (1983), who characterized mean insect density in relation to proportion of sampling units infested. A close relationship between proportion of trees infested and mean number of branches infested per tree would provide a sound basis for a binomial sampling plan with any level of infestation. The best-fitting curve was estimated and plotted on the scatterplot of proportion of trees infested using SigmaStat (2004).

Statistical Analysis. In this study, the mean and variance of infested coffee branches were calculated for each farm and sampling date. Values of the TPL coefficients a and b were estimated using linear least squares regression (PROC REG, SAS Institute 2002). The Iwao's patchiness regression was calculated with the mean of infested branches and the mean crowding index (m*) by using linear least squares regression (PROC REG, SAS Institute 2002). The stop lines for the sequential sampling plan were calculated only with TPL parameters (see results section for analyses of TPL and Iwao's crowding). After parameter estimation, values of a and b were used in Green's formula (Green 1970) to calculate stop lines for the sequential sampling plan. The precision levels used were 90% (D = 0.10) and 75% (D = 0.25). These two levels of precision were suggested by Southwood (1984) as appropriate for research purposes and for monitoring programs in an IPM, respectively.

Results

Spatial Distribution. TPL regression showed a highly significant positive relationship between variance and mean (Table 1; Fig. 1A). The TPL slope was

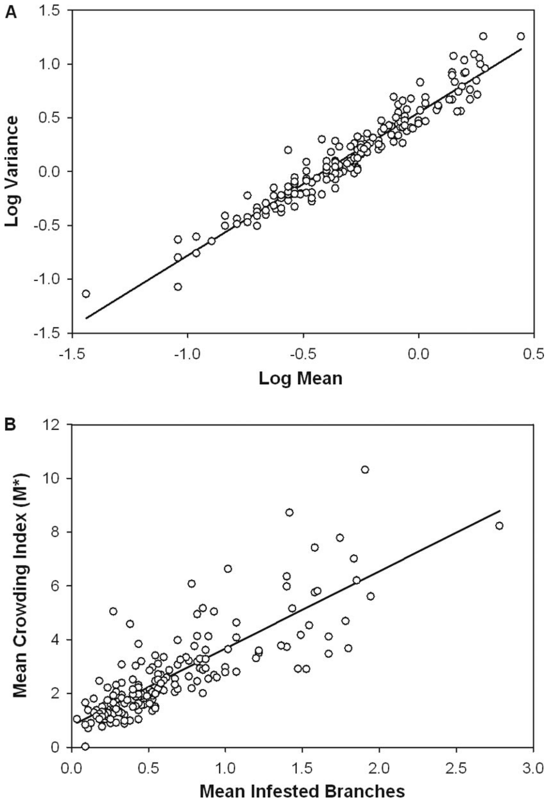

Fig. 1. Taylor's power law (log mean damaged branch density, log variance damage density) (A) and Iwao's mean crowding index (B) for *X. compactus*.

significantly greater than one ($b = 1.33$) ($t = 53.09$, $P < 0.001$), indicating an aggregated dispersion of the black twig borer damage in coffee plantations. Iwao's regression showed a significant relationship between mean crowding and the density of *X. compactus* damaged branches (Fig. 1. B; Table 1). The slope ß was significantly greater than one (ß = 2.87), ($t = 2.160$, $P < 0.005$), consistent with and further confirming an aggregated dispersion of *X. compactus.* The TPL model provided a superior fit to the data, providing a better characterization of the relationship between mean and variance. Stop lines with precision level of D = 0.10 and 0.25 were calculated with Green's formula by using the estimated TPL parameters (Fig. 2A and B).

Enumerative Sampling Plan. To estimate the mean density of infested branches with a precision level of D = 0.10, sampling stops when, after 30 trees have been sampled, the cumulative number of infested branches exceeds the value of 1,042, or if after 40 trees have been sampled, the cumulative number of infested branches exceeds the value of 915 (Fig. 2A). The estimated mean infestation density in the first case is 34 infested branches per tree, and in the second example the mean estimated infestation density is 22 infested branches per tree. With a set precision level of D = 0.25, sampling is terminated, after 20 trees have been sampled, the cumulative number of infested branches exceeds the value of 87, and in this case the mean density estimate is four branches damaged per tree.

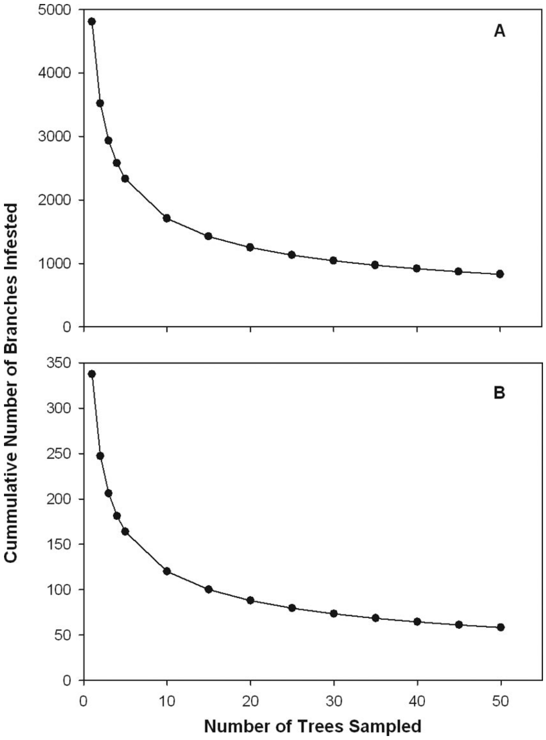

Fig. 2. Enumerative sequential sampling chart for *X. compactus* on coffee plants. Green's stop lines were calculated at two different precision levels D = 90% (A) and D = 75% (B).

Binomial Sampling. There was a significant relationship between the proportion of infested trees and the mean number of infested branches ($F = 1184.19$; df = 1,190; $P < 0.0001$; $R^2 = 0.86$) (Fig. 3).

Initially, as the mean number of infested branches increased, the proportion of infested trees increased rapidly up to ≈30% of trees infested. The proportion

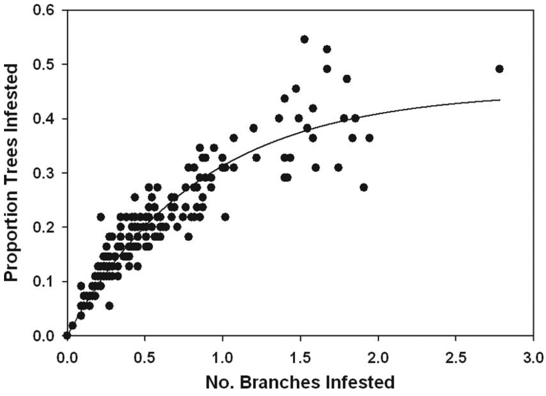

Fig. 3. Relationship of proportion of branches infested to proportion of trees infested by *X. compactus* on coffee plants.

of branches infested then became saturated, so that for further increases in the density of infested branches, there was only a small corresponding increase in infested trees. Mean number of branches infested per tree never exceeded three in this study. Counting presence or absence of infested branches, rather than all branches infested, may be used as a time saving sampling procedure, but in this case, in spite of the proportion of plants infested (presence or absence data) showing a good fit as a function of number of branches infested, very few branches per plant ever were infested.

Discussion

The aggregation parameters in TPL and mean crowding regression were greater than one, indicating that the spatial distribution of *X. compactus* was aggregated or clumped. Host odors (semiochemicals) play an important role in determining insect aggregation, and their role in attracting insects has been well studied for the development of pest management practices (Dufour and Frérot 2008). Coffee plantations in Kona usually are characterized by the presence of alternate hosts, either within or bordering the farms, for black twig borer. These include koa (*Acacia koa* A.Gray), eucalyptus, ohia, mango, and avocado. The presence of these alternate hosts might influence the aggregation pattern of black twig borer by the release of semiochemicals. Indeed, the presence of alternate hosts has been reported to enhance other ambrosia beetle populations. For instance, Weber and McPherson (1991) reported that populations of the ambrosia beetle, *Xylosandrus germanus* Blandford, a pest of walnut trees (*Juglans nigra* L.), were higher in areas where the walnut trees were near larger stands of trees containing a wide variety of alternate hosts. Contagious dispersion has been observed in other borer beetles such as the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytidae) (Ruiz et al. 2001). The coffee berry borer has the capacity to fly for periods of up to 3 hr; dispersal is aided by wind and apparently influenced by semiochemicals released by damaged coffee berries (Baker 1984, Dufour and Frérot 2008). The infested berries increase the liberation of kairomones that attract females, which release pheromones that elicit the attraction of more beetles (Baker 1984, Ruiz et al. 2001).

In some beetle species it has been reported that suitable hosts alone do not produce adequate attraction for beetles, and host selection by later colonizers depends more on the attractiveness of the first attacking beetles, also called pioneer beetles, than on the attractiveness of the plant per se (Rudinsky 1962). In the case of black twig borer, the female disperses and causes the damage to plants, whereas the males are flightless (Hara and Beardsley 1979). The goal of scolytine beetles, indeed any gravid female insect, is to locate a suitable host tree of a preferred quality for oviposition (Rudinsky 1962). It may be possible to manipulate the primary behavior of an insect to reduce its impact as a pest in a cropping system. Cultural techniques such as manipulating the landscape surrounding coffee might contribute to the management of the black twig borer.

Ruiz et al. (2001) reported that the aggregation pattern of the coffee berry borer may be influenced by differences in microclimate provided by shade. In the current study, the presence of alternate hosts of the black twig borer provided shade that reduced temperatures in some areas of the farms (Elevitch et al. 2009). As the ambrosia fungus is the only resource of food for black twig borer, local temperature and moisture content of the wood might be a limiting factor for development, survival, and dispersion. Accordingly, it is possible that the aggregation pattern of black twig borer females is mediated by access to areas where temperatures are optimum for ambrosia fungus growth and larval development.

In the case of species with a highly aggregated spatial distribution, a large number of samples are required to achieve a high level of precision in estimating population densities (Pedigo and Rice 2006). The higher precision level sampling plan (90%) developed during this study requires very large sample sizes and is impractical for field implementation, but may be useful for research requiring a high level of accuracy. A lower level of precision (75%) was more appropriate to produce a sampling plan practical for coffee growers' use. Regular sampling during summer, when black twig borer populations are higher and most damaging (Burbano 2010), using this sampling plan, should provide adequate estimates of infestation levels of black twig borer. A minimum of at least 15 trees per plantation should be sampled; farmers are unlikely to continue sampling >40 trees per plantation. Examining the relationship of the proportion of trees infested in relation to mean density of beetle damaged branches showed potential for the development of a binomial sampling plan. Beetle infestations were low during this study, not exceeding three branches infested per tree, which renders binomial sampling not very useful. Changes in climate conditions, or any factors that result in increased *X. compactus* infestations in Hawaii in the long-term, would justify the development of a robust binomial sampling plan for the insect in coffee.

Although an economic threshold for black twig borer in coffee has not been established, it is recommended that growers use a nominal threshold based on their tolerance for damage and an understanding of economic implications of different damage levels, which might be based on unpublished data and the individual farmer's acceptance of damage. The density of infested branches per sampled unit can be estimated using the sampling plan and compared with the nominal action threshold level to make a management decision. The adoption of sequential sampling plans for use in decision-making can contribute to improved, data-based management of the pest. The application of sequential sampling plans should optimize the number of samples required to make a decision. This also allows the estimation of the abundance of *X.*

compactus with specified precision, providing researchers with a valid tool for the study of the black twig borer in coffee.

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