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Binomial sampling of *Paraleyrodes* Quaintance pos. *bondari* (Hemiptera: Aleyrodidae) in *Persea americana* Mill.

Muestreo binomial de *Paraleyrodes* Quaintance pos. *bondari* (Hemiptera: Aleyrodidae) en *Persea americana* Mill.

Luis Sigifredo Caicedo R.¹, Edgar Herney Varón D.², and Helena Luisa Brochero¹

ABSTRACT

Fresno (Tolima), in Colombia, is a notable avocado producer, with 36% of the national production. In this paper, two sampling methods are presented to assess natural populations of *Paraleyrodes* Quaintance pos. *bondari* attacking avocado trees of Hass and Lorena cultivars under field conditions. The presence/absence of whitefly nymph colonies on 30 leaves located at the high, medium and low strata per host plant from both cultivars was evaluated. Visual estimations were performed to count the number of whitefly nymphs on 1.25 cm² of five leaves/bud in low and medium strata per tree to evaluate the spatial distribution of whitefly population in accordance to Poisson distribution, Negative Binomial distribution and b parameter of Law of Taylor. Significant differences in percentages of infestation ($P \leq 0.03$) from leaves that belonged to the low avocado tree strata were found between the Lorena ($31.88 \pm 1.2\%$) and Hass ($15.64 \pm 1.8\%$) cultivars. Natural populations of *P. pos. bondari* were located on the abaxial leaf side, showing an aggregate distribution in avocado tree from orchards located at different altitudes. Our findings recommend entomological surveillance for *Paraleyrodes* sp. pos. *bondari* in Fresno (Tolima), sampling four branches from the medium and low avocado tree strata through inspection of five buds/branches/tree throughout each branch with the presence/absence method to count whitefly nymph colonies on the abaxial side of pre-basal leaves. In total, the sampling involved five leaves/branch (20 leaves/strata or 40 leaves/tree) on 13 avocado trees per hectare.

Key words: whitefly, pest sampling, Hass cultivar, Lorena cultivar, avocado.

RESUMEN

El municipio de Fresno (Tolima) en Colombia, es productor destacado de aguacate con una participación del 36%. Se presentan en este documento, dos métodos de muestreo para evaluar poblaciones naturales de *Paraleyrodes* Quaintance pos. *bondari*, atacando árboles de aguacate de los cultivares Hass y Lorena en condiciones de campo. Se evaluó la presencia/ausencia de ninfas de esta mosca blanca en el envés de 30 hojas en los estratos inferior, medio y superior de cada planta. Se realizó la estimación visual de infestación de ninfas en un área foliar de 1,25 cm² sobre 5 hojas/brote de los estratos inferior y medio para evaluar la distribución espacial poblacional de la especie asumiendo una distribución Poisson, Binomial Negativa y parámetro *b* de Taylor. Se encontraron diferencias significativas para porcentajes de infestación ($P \leq 0,03$) entre cultivares Lorena ($31,88 \pm 1,2\%$) y Hass ($15,64 \pm 1,8\%$), siendo los estratos inferiores los más infestados. Las poblaciones naturales de *P. pos. bondari* mostraron una distribución agregada en árboles de aguacate localizados a diferentes altitudes. De acuerdo con nuestros resultados, para la vigilancia entomológica de *Paraleyrodes* sp. pos. *bondari* en Fresno (Tolima) deben tomarse cuatro ramas de los estratos medio y bajo de los árboles de aguacate, inspeccionando 5 brotes/rama/árbol a través de cada rama con el método de presencia/ausencia de colonias de ninfas en el lado abaxial de la hoja pre-basal. En total, el muestreo corresponde a 5 hojas/rama (20 hojas/estrato o 40 hojas/árbol) en 13 árboles de aguacate por cultivar.

Palabras clave: mosca blanca, muestreo de plagas, cultivar Hass, cultivar Lorena, aguacate.

Introduction

The Tolima province is the largest producer of avocados in Colombia, 6,810 ha, an average yield of 8.6 t ha⁻¹ and annual production of 58,317 t ha⁻¹ (MADR, 2012). The municipalities with highest production are Fresno (36%), Mariquita (28.1%), Falan (8.0%), Alvarado (7.9%) and Palocabildo (2.6%) (MADR, 2012). Since 2009, a decrease in the average yield associated with abiotic and biotic factors was observed

(MADR, 2012; ICA, 2012). Fresno recorded *Paraleyrodes* sp. (Hemiptera: Aleyrodidae) (Martin, 2004) as phytosanitary limiting (Segura *et al.*, 2012) because nymphs suck the phloem, causing weakening, wilting and chlorosis in avocado trees; adverse effects on the photosynthetic rate and secondary colonization by pathogens associated with Sooty mold (Sánchez, 2005; Martin, 2006; Martin and Mound, 2007).

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Sampling estimates the relative abundance of insect species population in a crop (Silva *et al.*, 2014). For insects exploring leaves for food, sampling techniques include: a) beating of leaves on a white background tray and direct counting of insects; b) visual estimate of the number of nymphs in a predetermined standard area, in cm²; and c) collecting leaves in a plastic tray to count injury or damage associated to herbivory (Gusmão, 2004; Bueno *et al.*, 2005; Pinto-Zevallos and Vanninen, 2013; Barbedo, 2014). In this way, it is possible to find patterns of distribution of insect pest populations. If their relationships with the host plant is known, it is possible to define a specific sample unit representing its current infestation (Basso *et al.*, 2001; Moura, 2001; Bueno *et al.*, 2005; Bernal *et al.*, 2008).

So far, the efficiency of a sampling technique to estimate population densities, infestation levels and spatial dispersion of white fly in Hass and Lorena avocado cultivars in Fresno, Tolima is unknown. It represents a baseline for the establishment of strategies of entomological surveillance and phytosanitary control. This study aimed to determine a sampling method to define levels of infestation and spatial dispersion of *Paraleyrodes Quaintance pos. bondari* whitefly nymphs in order to determine an appropriate sampling strategy in accordance with characteristics of Fresno (Tolima).

Materials and methods

Study site

Fresno is located north of the department of Tolima (05°09'20"N and 075°02'25"W). Nine productive orchards of avocado (*Persea americana* Mill.) cv. Hass and Lorena with whiteflies as the main phytosanitary problem and covering an altitude range between 1,095 and 1,361 m a.s.l. were selected.

The sampling was carried out between August and November 2012. At an altitude of 1,360 m, an average temperature of 21.94°C, relative humidity of 81.37% and average monthly rainfall of 77.18 mm were recorded; while at an altitude of 1,146 m, we registered 22.14°C average temperature, 78.36% relative humidity and an average monthly rainfall of 60.21 mm. The data reported was taken from weather stations WatchDog® Series 2000 (Spectrum Technologies, Aurora, IL).

Levels of whitefly infestation

To establish differences of infestation of whitefly nymphs, we considered a biweekly sampling on the lower, medium and upper strata, taking 10 avocado trees per cultivar and

following NOM-066-FITO-2002 (Mexican Official Standard for specifications on phytosanitary management and mobilization of avocado) as the standard method (SAGARPA, 2002). In each orchard, a systematic sampling along a path following an X transect was conducted with inspection of 30 leaves per tree in each stratum checking presence/absence of the whiteflies colonies (Larral and Ripa, 2009). We evaluated the percentage of infested leaves per tree stratum, per cultivar, for each productive orchard according to its altitude. A Wilcoxon test (Wilcoxon, 1945) was run to compare whitefly infestation levels, while a Kruskal and Wallis test compared the whitefly infestation levels on tree strata (Kruskal and Wallis, 1952) using program SAS v. 9.2 (SAS Institute, 2009).

To determine the relationship between the percentage of infestation of whitefly (Y) and altitudinal distribution of productive orchard of avocado (X), PROC REG procedure was performed in statistical program SAS v. 9.2 (SAS Institute, 2009).

Spatial dispersion of whiteflies

To determinate the spatial dispersion of whitefly, biweekly sampling per three months was conducted based on the visual estimation of percentage of infestation colonies nymphs over a standard prescribed area of 1.25 cm² per leaf (Bueno *et al.*, 2005). Simultaneously, the presence or absence of colonies of whitefly nymphs on the abaxial/adaxial leaf side were considered as infestation evidence in each stratum per tree (Naranjo and Ellsworth, 2005; Larral and Ripa, 2009; Nachman, 1981). Per strata in each tree, three branches, a meter in length, were taken at 120°, 240° and 360°, with five buds of about 60 cm per sprout, five leaves of apical, intermediate, prebasal and basal disposition were inspected for the presence/absence of the whiteflies colonies.

Correlations between the percentage of visual estimation defined by presence/absence and number of *P. pos. bondari* colonies nymphs occupying an area of 1.25 cm² were determined, recording data for each productive orchard, tree stratum, number of buds and number of leaves. Pearson's correlation was established between percentage of infestation by visual estimation (1.25 cm²) compared to the number of nymphs (SAS Institute, 2009).

Once the correlation between the percentage of visual estimation and number of nymphs was established, we estimated the pattern of spatial distribution of whitefly nymphs in the sampling universe, assuming binomial and Poisson models of distribution (Basso *et al.*, 2001). In order

to establish the theoretical probability of negative binomial and Poisson distribution to define the spatial distribution of whitefly, we followed:

$$\eta = \beta_0 + \alpha_i + g_j + \delta_k + (\alpha g \delta)_{ijk} \quad (1)$$

where:

η = number of counts by percentage of visual estimation of whitefly nymphs in 1.25 cm²,

$\beta_0 = 1, 2, 3, \dots, 9$ (orchards sampled),

$i = 1, 2$ and 3 (strata tree),

$j = 1, 2$, and 3 (120°, 140° and 360° sprouts per tree),

$k = 1, 2, 3, 4$ and 5 (leaves of each sprout),

$(\alpha g \delta)_{ijk}$ = total error.

If the K parameter has values close to zero when variance is larger than average, it is considered as an aggregate distribution; while, if the K parameter has very large values when the variance (S^2) and average (X) tend to be the same, it is assumed a randomized distribution. Moments estimators for K: $K' = X^2 / (S^2 - X)$, where α and β are empirical relationship $S^2 = \alpha + X\beta$ (Taylor, 1961).

To define a spatial dispersion, values of slope (b) of the Taylor's power Law was determined by linear regression between log variance Vs log average counts (Bueno *et al.*, 2005). Because the b value can be considered a measure of aggregation on linear regression (Pedigo and Zeiss, 1996), b values less than 1.0 indicated a uniform insect pest distribution; if equal to 1.0, they indicate a random dispersion, and if the slope is greater than 1.0, an aggregation distribution is assumed. To determinate sampling accuracy, the coefficient

of variation (CV) and relative variance (VR) were obtained (Moura, 2001).

Sample size

An optimal sample size (n) was determined by sample estimate of the variance (S^2) of the normal population (Vergara, 1996), by presence or absence percentages of whitefly colonies on the abaxial leaf side. The trees included in the sampling were defined by the negative binomial mathematical model

$$n = (1/\mu + 1/k)/CV^2 \quad (2)$$

where:

n is number of trees to be sampled; k is related with negative binomial distribution dispersion rate; μ is the average of insect population estimated in sampling and; CV is the coefficient of variation found in a real sampling area (Aranda, 2004). Coefficient of variation (CV) and relative variance were determined to define the precision of sampling (Moura, 2001).

Entomological samples

The taxonomic identification of whiteflies was based on morphological characteristics (Caballero, 1994).

Results and discussion

Taxonomic identification

Whiteflies from each orchard belonged to *Paraleyrodes* Quaintance pos. *bondari* (Hemiptera: Aleyrodidae) (Caballero, 1994). Doctor John Albeiro Quiroz, entomologist expert on whiteflies at Universidad Nacional de Colombia,

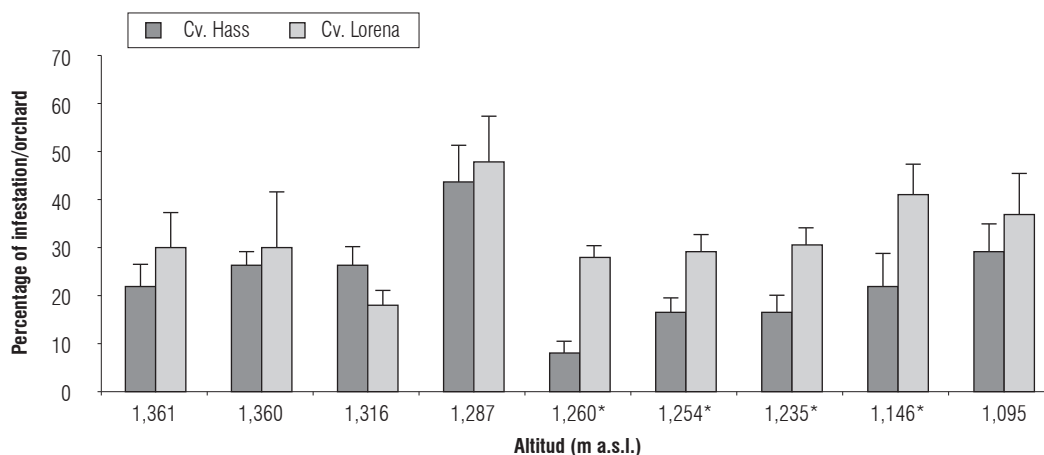


FIGURE 1. Vertical distribution of *Paraleyrodes* sp. pos. *bondari* in productive orchards of avocado, 2012. Fresno (Tolima). * Significance < 0.05 - $P > |t|$ Wilcoxon.

and Maria Del Pilar Hernandez, a former CIAT taxonomist, whitefly expert (Segura *et al.*, 2012), confirmed the taxonomic identification.

Levels of *P. pos. bondari* infestation

Of the evaluated orchards, four revealed significant differences ($P \leq 0.03$) for the *P. pos. bondari* infestation between the cultivars; 'Lorena' showed higher than average infestation ($31.88 \pm 1.2\%$) than 'Hass' ($15.64 \pm 1.8\%$) (Fig. 1).

The whitefly infestation percentage, as a dependent variable (y), was not adjusted to a linear regression model ($P > F = 0.366$; $r^2 = 0.11$), regarding independent variable (x) represented by altitudinal distribution of farms assessed. Therefore, it was not possible to determine if altitudinal difference (216 m of difference between places assessed at 1,361 m and 1,095 m) had some influence on whitefly infestation in avocado crops in Fresno, Tolima.

In this context, percentages of *Paraleyrodes pos. bondari* infestation were attributed to variations in local production conditions, i.e., pest management, fertilization, pruning, weeding, as well (Moura, 2001; Rodríguez *et al.*, 2012). For example, whitefly control activities in orchards located at 1,360 m a.s.l. had infestations below 30.55% monthly, including biological control; while orchards located at 1,146 m a.s.l. based on weekly/biweekly chemical control of whitefly, had infestation levels of 40.74%. Altitude was associated with differences in landscape composition contributing to different abiotic factors that influenced plant nutrition (wind, drainage, amount of light received by avocado trees) and insect infestation in field.

Behavior of phytophagous insects is determined by visual and chemical stimuli from host plants. In Fresno, Tolima,

mature leaves of Hass cultivar had more terpenes and steroids as secondary metabolites than 'Lorena', which was characterized by higher content of total carbohydrates and non-reducing sugars that can act as insect attractant (Sierra *et al.*, 2014). These differences, combined with differences in plant architecture of each avocado variety could explain preferences of *P. pos. bondari* by Lorena cultivar in Fresno (Tolima) in accordance with infestation percentages found there.

No statistically significant differences were found in percentage of infestation of *P. pos. bondari* nymphs on low, medium and high strata leaves on 'Hass' avocado trees between orchards evaluated. Conversely, 'Lorena' crops showed significant differences of whitefly infestation levels between strata ($P \leq 0.05$) from different orchards (Fig. 2). Nevertheless, in general, we found more whitefly infestation (8/11) on the low strata per avocado crops in Fresno, Tolima.

Clearly, herbivores prefer host plant structures to get better source of food, oviposition site and appropriate conditions to complete their lifecycle in accordance with the relative abundance and physiological stages (Naranjo and Ellsworth, 2005). *Trialeurodes vaporariorum* prefers the lower strata in *Phaseolus vulgaris* and *Phaseolus coccineus*, while *Bemisia tabaci* prefers middle leaf strata in *Sesamum indicum* crops (Bueno *et al.*, 2005).

Avocado crops are perennial, with determinate growth. These characteristics avoid abrupt changes in spatial and temporal composition of crops and allow more stable plant-insect relationships (Ríos *et al.*, 2005). Highest infestation percentages of immature stages of *P. pos. bondari* on low strata of avocado trees could be explained as response to get

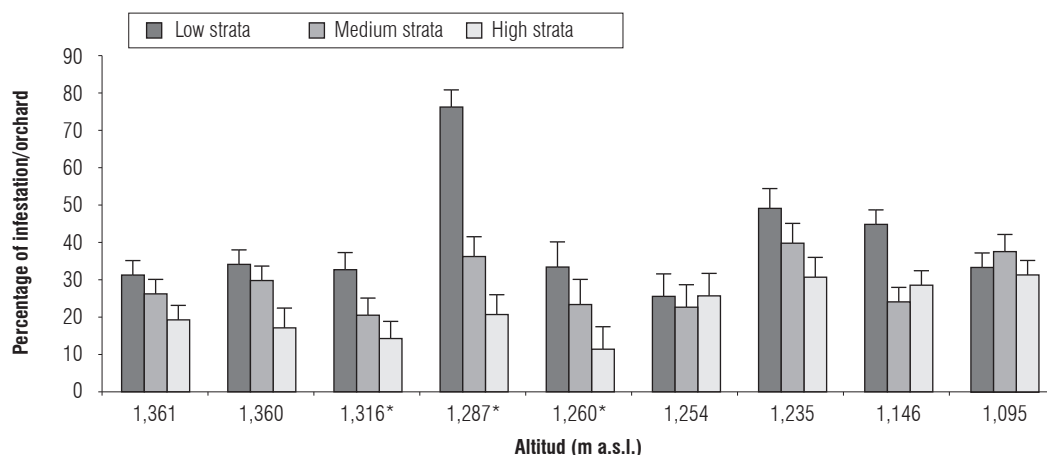


FIGURE 2. Percentage of infestation level of *Paraleyrodes sp. pos. bondari* in different strata of Lorena cultivar trees in Fresno (Tolima), 2012. * Significant level 5% - χ^2 Kruskal-Wallis.

a microhabitat as refuge of predators and stable secondary metabolites in mature leaves (Bernal *et al.*, 2008).

Spatial dispersion of *P. pos. bondari*

Equivalence correlation between percentage of whitefly nymphs found in 1.25 cm² per leaf was significant for both cultivars; 'Hass' ($P = 0.0001$; $R^2 = 92$) and 'Lorena' ($P = 0.0001$, $R^2 = 84$), this allowed for field nymph counts based on area occupied by whitefly colonies (Tab. 1).

TABLE 1. Correlation between visual estimation of infestation whitefly level and number of nymphs found in a standard area of 1.25 cm² in leaves of two cultivars of avocado in Tolima, Colombia.

Variety	Visual estimated of area occupied by whiteflies (%)		Number of nymphs on 1.25 cm ² per leaf
	LL	UL	CM
Hass	4.00	12.11	9.22
	12.11	20.22	21.33
	20.22	28.33	32.78
Lorena	4.67	11.67	9.83
	11.67	18.67	18.33
	18.67	25.67	27.83

LL, lower limit of area occupied by whitefly infestation; UL, upper limit of area occupied by whitefly infestation; MC, average between LL and UL.

During visual estimation, we did not discriminate between whitefly nymphs instars. However, Basso *et al.* (2001) found it was possible to count and sort out nymphal instars through visual estimation. These authors found a mean number of 3.01 of *T. vaporariorum* nymphs on apical, post-apical and intermediate leaves of tomato crops.

Because in this present study, variance of counts data of whitefly nymphs was greater than mean, the best-fitting model was Negative Binomial distribution (Tab. 2). In avocado crops of Fresno, Tolima, the spatial distribution of *P. pos. bondari* nymphs was "highly aggregated" with preference to buds located at 120° north ($K=0.09$) followed by prebasal leaves ($K=0.11$) (Tab. 2).

Independent of orchard and each evaluated avocado cultivar, regressions between the mean and the variance logarithmic relations of Taylor Power Law showed values of b greater than 1.0, related with aggregated distribution of insect population on lower strata ($b = 1.8$) and ($b = 1.9$) for Hass and Lorena cultivars, respectively (Fig. 3). Meristems located at 120° outline from lower strata of avocado trees showed values of $b = 2.0$ in 'Hass' and $b = 1.7$ in Lorena cultivar, while in pre basales leaves and buds located on lower strata recorded aggregation indexes were $b = 2.1$ and $b = 1.69$ for Hass and Lorena cultivars, respectively.

Similar dispersion behavior of *Paraleyrodes* sp. pos. *bondari* infesting avocado trees in Fresno - Tolima has been found in *Trialeurodes vaporariorum* attacking *Solanum lycopersicum*. In this study, authors found dispersion rates of third instar nymphs were highly aggregated on each plant strata over time, showing an average of K aggregation index of 0.17 (high strata), 0.26 (medium strata) and 0.15 (low strata) (Basso *et al.*, 2001). In cucumber (*Cucumis sativus*), regression between the variance and natural logarithm of number of nymph average were significant for adults of *Bemisia tabaci* ($F = 7.93$, $n = 8$; $p = 0.02$, $b = 1.09$); nymphs

TABLE 2. Dispersion indexes obtained through tests to adjust Poisson distribution and Negative Binomial distribution for populations of *Paraleyrodes* sp. pos. *bondari* nymphs infested 'Hass' and 'Lorena' trees in Fresno (Tolima), 2012.

Whitefly nymph colonies on 1.25 cm ² / leaf									
Variable	Level	Mean	Variance	X ² Poisson	Pr > ChiSq	X ² Negative Binomial	Pr > ChiSq		Interpretation
Orchard				954	<0.0001	103.12	0.09	<0.0001	
Strata	Low	2.19	45.56	3.36	0.056	1.59	0.11	0.207	Aggregate
	Medium								
Buds	120°	2.36	39.66	25.49	<0.0001	1.57	0.09	0.210	Aggregate
	240°	2.06	61.62	1.43	0.2325	0.05	0.11	0.830	Aggregate
	360°								
Leaves	Apical	1.45	32.58	121.54	<0.0001	13.27	0.07	0.003	
	Post.apical	1.74	38.12	26.05	<0.0001	5.01	0.08	0.025	
	Medium	1.85	30.16	26.53	<0.0001	4.04	0.12	0.044	
	Pre-basal	3.01	30.21	1.43	0.0343	0.01	0.11	0.963	Aggregate
	Basal								

X² Poisson <0.0001 was not adjusted to Poisson distribution; X² negative binomial distribution; K, aggregation index.

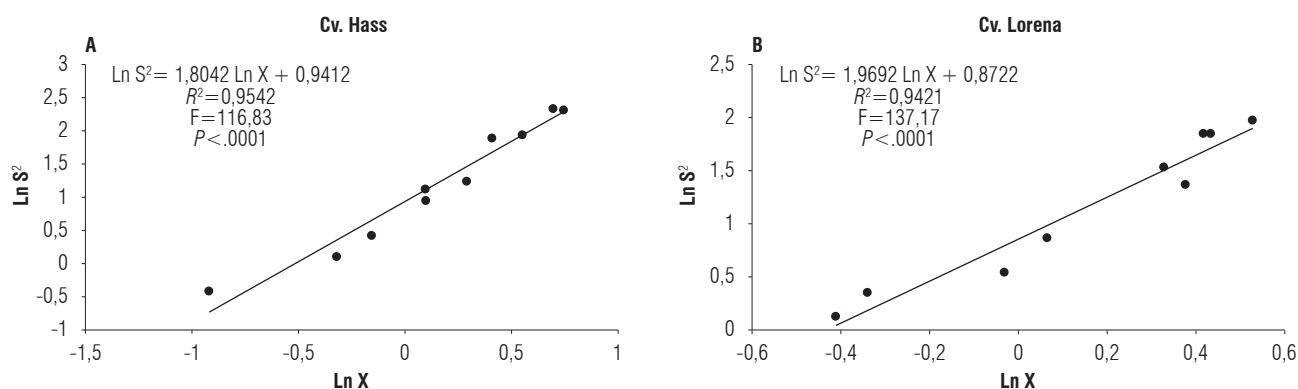


FIGURE 3. Relationship between natural logarithm of the variance ($\text{Ln } S^2$) and natural logarithm of number of nymph average ($\text{Ln } X$) on whitefly *Paraleyrodes* sp. pos. *bondari* in lower strata of Hass and Lorena avocado cultivars trees. Fresno (Tolima), 2012. A, Hass cultivar; B, Lorena cultivar.

($F = 65.46$, $n = 8$; $P < 0.0001$; $b = 1.47$) and, adults and nymphs combined ($F = 14.55$; $n = 8$; $P = 0.0051$, $b = 1.72$). Because cucumber b values were closer to 1.0, the spatial distribution of this whitefly species was considered aggregated (Moura, 2001).

Spatial distribution of natural populations of *Paraleyrodes* sp. pos. *bondari* defined a specific sampling unit to represent current whitefly infestation on avocado trees in Fresno (Tolima). This corresponded to pre-basal leaf from a bud of 60 cm located at low strata of the host plant.

Nonetheless, some authors have considered variances less than 25% improve accuracy of sampling (Moura, 2001; Laurentin and Pereira, 2002; Gusmão, 2004; Bueno *et al.*, 2005); we found that increasing number of buds per branch per tree for sampling, low relative variances (VR) can be obtained. Taking five buds/branch/tree to estimate the number of nymph colonies per $1.25 \text{ cm}^2/\text{leaf}$, we were able to have variances of 16.42 and 13.55 for Hass and Lorena cultivars, respectively. In the same way, based on presence/absence of nymph colonies per leaf, we registered values of variance of 7.49 for Hass cultivar and 6.45 for the Lorena cultivar when five buds/branch per tree were taken for the sampling. In this context, farmers and researchers should take 5 buds/branch per tree to have an accurate value of *Paraleyrodes* sp. pos. *bondari* infesting avocado trees in orchards located in Fresno (Tolima).

For *Bemisia tabaci* in sesame (*Sesamum indicum*), the most appropriate sample size for dynamics population studies is take four to five leaves in basal and medium strata of plants for four repetitions sampling (Laurentin and Pereira, 2002). These authors found relative variances of 28.20 in the medium strata and 18.17% in the basal strata by direct counts of whitefly colonies on leaves.

Likewise, low values of relative variances can be obtained by direct counts of whitefly on the medium and low strata in cucumber plants (Moura, 2001). In this study, presence/absence of nymph colonies was the accepted method by farmers due to its easy application. Because in Fresno (Tolima) this method obtained low relative variances and minor sampling times to define *P. pos. bondari* infestations in avocado trees in orchards located at different altitudes and it is possible to be adopted easily by farmers, presence/absence of whitefly on basal strata leaves is the best entomological surveillance tool to take pest control decisions.

Sample size

Sample size was defined based on the visual estimation of the number of whitefly nymph colonies in 1.25 cm^2 on five leaves/bud in low strata.

To find at least 60% of *P. pos. bondari* infestation per leaf/branch, it is necessary to have five to eight observations per avocado tree. If the number of branches/tree sampled is reduced, the number of sample sizes (No. leaves/branch) is increased and accuracy of sampling can be decreased until 75%; hence, it is necessary to spend more time and money by farmers. Consequently, we suggest taking four branches/tree, inspecting ten leaves on each branch or take eight branches per tree, inspecting four branches from the low tree strata or four branches from the medium avocado tree strata, and inspecting five pre-basal leaves on five buds of 60 cm length.

In accordance to statistical parameters of negative binomial distribution of *P. pos. bondari* whitefly: dispersion index ($K = 0.11$); mean of insect population sampled ($X = 3.01$) and its variation coefficient ($VC = 0.3$) found on pre-basal leaf on low strata of avocado trees; we found a sample size of 13 trees/ha.

Conclusions

Paraleyrodes Quaintance pos. *bondari* (Hemiptera: Aleyrodidae), whitefly found infesting avocado trees located at different altitudes from Fresno, Tolima showed an aggregated distribution as expected for pest insects on agriculture systems.

In Fresno (Tolima), sampling of *Paraleyrodes* sp. pos. *bondari* should be conducted, taking four branches from the medium and low avocado tree strata through inspection of five buds/branch per tree throughout each branch with the presence/absence method to count whitefly nymph colonies on the abaxial side of pre-basal leaves. In total, the sampling involved five leaves/branch (20 leaves/strata or 40 leaves/tree) on 13 avocado trees/ha.

The *Paraleyrodes* sp. pos. *bondari* behavior was similar to other whiteflies reported as pests in commercial crops.

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