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Short Communication

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Within-Canopy Distribution of *Stenoma catenifer* (Lepidoptera: Elachistidae) Infestation in Avocado Orchards

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Abstract

Native to the neotropics, the avocado seed moth *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae) is a specialist pest of the family Lauraceae and considered one of the most important pests of avocados worldwide. However, little is known regarding its spatial distribution within a single tree. Therefore, we designed a study to evaluate the effects of canopy height and aspect (i.e., side of the tree) on fruit infestation by *S. catenifer* larvae in avocados. The study was conducted in three commercial organic avocado orchards located in São Paulo, Brazil. At each orchard, 40 fruit from 30 random trees were sampled weekly from October 2017 through February 2018, evaluating the number of fruits infested by *S. catenifer* larvae at three tree heights (bottom, middle, and top). In addition, fruits on the ground were also sampled. We also evaluated the effect of the side of the tree where the fruits were collected, i.e., whether they were on the side facing the east (sunrise) or the west (sunset). Within the avocado canopy, the level of fruit infestation by *S. catenifer* larvae was significantly higher at the top of the trees than in the middle and bottom. Fruit on the ground had lower levels of infestation than those on the tree canopy. The level of fruit infestation was also higher on the side of avocado trees facing the east (sunrise). Understanding the within-tree distribution of *S. catenifer* will help to better target monitoring and control activities against this pest in avocados.

Key words: avocado seed moth, canopy height, canopy aspect, sampling, integrated pest management

Native to the western hemisphere, avocado [*Persea americana* Mill. (Laurales: Lauraceae)] is an important crop in many countries worldwide including Brazil, where the national production of avocado has grown by around 20% since 2006 (ABPA 2020), and is currently estimated at 195,492 tons, with a planted area of 10,868 hectares (IBRAF 2020). In Brazil, avocado fruit is produced for local consumption but also for exportation to foreign markets such as Europe and Asia. However, countries like the United States prohibit avocado imports from producing regions where the avocado seed moth, *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae), occurs (Hoddle and Hoddle 2008), which causes economic losses to exporting countries like Brazil.

Stenoma catenifer is a specialist herbivorous insect of the Lauraceae family (Cervantes Peredo et al. 1999). This insect is native to Neotropical areas and is considered one of the most important avocado pests in Mexico and Central and South America (Wysoki et al. 2002, Castillo et al. 2012, Cruz-López et al. 2020). In Brazil, *S. catenifer* is the main pest limiting commercial avocado production (Hohmann et al. 2003). In fact, crop losses of 60% have been recorded in Brazil (Hohmann and Meneguim 1993, Nava et al. 2004, Nava et al. 2005a, b). Thus, *S. catenifer* is considered a major impediment to commercial avocado production in the country (Nava et al. 2004, 2005a, b).

Stenoma catenifer females lay eggs on rough surfaces and in crevices such as the fruit pedicel or necrotic spots on fruit (Hohmann et al. 2003, Hoddle and Hoddle 2008). After hatching, first instar larvae pierce the shell (exocarp) and initially feed on the pulp (mesocarp), reaching the endocarp and then the seed, causing serious damage to the fruits, and in most cases causes the fruit to fall (Nava and Parra 2005, Hohmann and Meneguim 2006); thus, damage by this pest results in fruit that cannot be sold. *Stenoma catenifer* pupates in the soil after leaving the fruit on which they fed (Manrique et al. 2014).

In Central America, *S. catenifer* monitoring is mainly done using pheromone traps (Castillo et al. 2012, Cruz-Lopez et al. 2020); however, in Brazil, sex pheromones for this pest are not commercially available and growers rely on visual inspection of fruit infestation. Control of *S. catenifer* is predominantly carried out using synthetic chemical insecticides (Hohmann et al. 2000, Andaló et al. 2019); yet, their efficiency is limited due to the habit of the pest—it enters fruits at the larval stage, which can shelter them from the insecticides. Understanding the spatial distribution of *S. catenifer* within orchards will help to develop target-specific integrated pest management (IPM) measures to monitor and control this pest. For example, monitoring and control measures can be directed to specific sections of the canopy, which could mitigate any imbalances and contamination of the environment and allow the use of alternative pest control tactics, such as biological control (Nava et al. 2007, Parra and Coelho 2019), that can be more expensive at larger scales. However, little is known regarding the factors that influence the spatial distribution of *S. catenifer* within a single tree.

Although previous studies have investigated the effects of height on *S. caternifer* fruit infestation (e.g., Hohmann et al. 2003, Nava et al. 2006), they have shown mixed results. Also, these studies did not evaluate the effects of aspect (side of the tree) on fruit infestation. Thus, this study aimed at better characterizing the within-canopy distribution of *S. catenifer* by investigating the effects of both canopy height and aspect on fruit infestation by *S. catenifer* larvae in avocados. Because *S. catenifer* females prefer to lay eggs on the upper half of avocado trees (Hohmann et al. 2003), we hypothesized higher fruit infestation at the top of trees. To achieve this characterization, we assessed many avocado fruits from three commercial organic orchards in Brazil for *S. catenifer* larval infestation at three tree heights, namely, bottom, middle, and top, as well as on the ground. Because we were interested in the effect of the sun's position with respect to the avocado canopy on fruit infestation, we also evaluated the effects of the side of the tree where the fruits were collected, i.e., whether they were on the side facing the east (sunrise) or the west (sunset).

Materials and Methods

Study Sites

The experiment was carried out at three commercial avocado orchards (*P. americana*) located in Cristais Paulista, São Paulo, Brazil (Site 1: latitude 20°27′37″S and longitude 47°25′22″W; Site 2: latitude 20°27′34″S and longitude 47°25′15″W; Site 3: latitude 20°27′18″S and longitude 47°24′60″W; all at an altitude of 1,050 m) (Fig. 1).

All sites followed pest management practices normally adopted by organic producers (i.e., without the use of synthetic pesticides). For example, during the period of fruiting and fruit development, *Bacillus thuringiensis* Berliner and releases of the parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) were used to manage *S. catenifer*. Because these control strategies were used at all sites, we assume that they did not influence our conclusions on the pest's within-tree distribution; at least under organically managed orchards.

Assessment of Fruit Infestation

Fruits were sampled from October 2017 through February 2018, a period corresponding to the beginning of fruit development until harvest and that coincides with *S. catenifer* activity. Thirty random trees were sampled weekly at each site. For each infested avocado, we recorded its position in the tree as: 1) bottom = within 3 m from ground level, 2) middle = between 3 and 6 m above ground level, or 3) top = above 6 m from ground level, or as being on the ground. We also recorded the canopy aspect, i.e., whether the fruit was facing the west (sunset) or facing the east (sunrise). To determine the three canopy heights, a marked bamboo pole was used as a reference. At each tree height, as well as on the ground, 10 fruits were sampled, with 5 fruits on each aspect of the tree (n = 40 fruit per tree, for a total of 1,200 fruit per week per site). All sampled fruits were removed and opened in situ to confirm the presence of *S. catenifer* larvae. To minimize temporal autocorrelation, different trees were used at each sampling date. The number of infested fruits by *S. catenifer* at each tree height and canopy aspect was recorded.

Statistical Analysis

The study used a randomized complete block design with each site considered as a replicate (block). Prior to analysis, data on weekly fruit infestation by *S. catenifer* larvae were averaged to obtain a mean across all sampling dates. We thus obtained a single mean percentage for all (30) trees per site for each of the four tree heights (i.e., ground, bottom, middle, and top) and canopy aspect (i.e., fruit facing the west [sunset] or facing the east [sunrise]), and checked for normality using the Anderson-Darling test and for homogeneity of variance using the

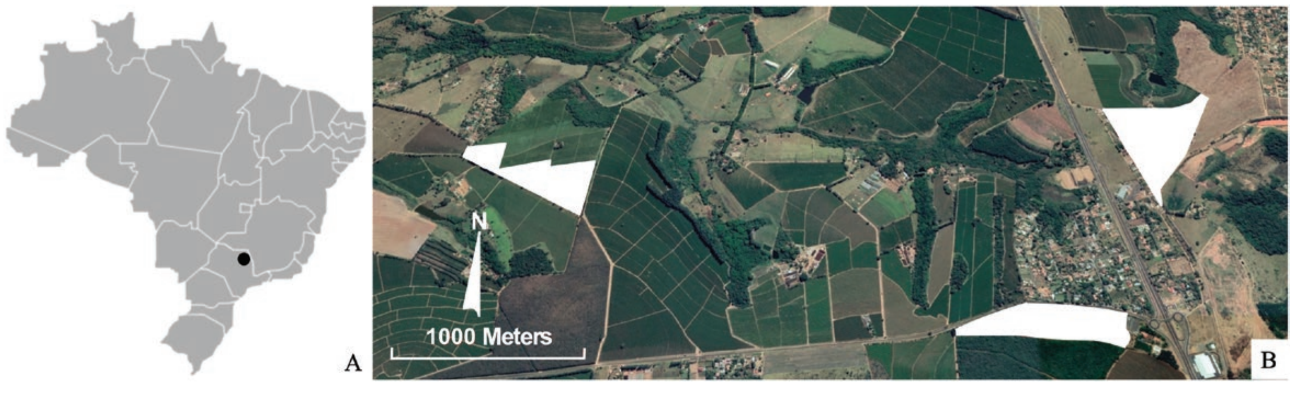

Fig. 1. (A) Map of Brazil showing the county Cristais Paulista in the state of São Paulo. (B) Sites used in field experiments. Site 1 had 7 hectares of the cultivars Breda and Margarida intercropped with coffee, with 1,300 avocado plants per hectare and an average tree age of 12 yr. Site 2 had 5 hectares of the cultivar Margarida intercropped with coffee, with 1,300 avocado plants per hectare and an average tree age of 18 yr. Site 3 had 14 hectares of the cultivar Hass intercropped with coffee, with 3,000 avocado plants per hectare and an average tree age of 15 yr.

Levene's test (Minitab 2013). Next, we tested the two main factors, tree height and the canopy aspect, and their interaction on *S. catenifer* fruit infestation by using analysis of variance (ANOVA) (fixed effects: aspect, height, and site), with means separated using Tukey's HSD test at a 5% probability (General Linear Model; Minitab 2013). Percent data were arcsine square-root-transformed before analysis. All analyses were conducted using Minitab version 17 (Minitab 2013).

Results

In total, ~72,000 avocado fruits were surveyed for *S. catenifer* larval infestation. ANOVA showed a significant effect of height ($F_{3,14} = 157.37, P < 0.001$), aspect ($F_{1,14} = 32.71, P < 0.001$), and their interaction ($F_{3,14} = 7.55, P = 0.003$) on fruit infestation by *S. catenifer* larvae. However, there was no effect of site on fruit infestation ($F_{2,14} = 0.11, P = 0.895$).

Fruit infestation by *S. catenifer* was the highest at the top of trees and lowest on the ground (Fig. 2). Fruit infestation by *S. catenifer* was also higher on the side of the plant facing the east (sunrise) (Fig. 2). However, the effects of canopy aspect interacted with tree height (significant height × aspect interaction), such that higher fruit infestation by *S. catenifer* was obtained at the sides facing the sunrise but only at the top of the canopy (Fig. 2).

Discussion

This study examined the within-tree distribution of *S. catenifer* larval infestation in avocado orchards to draw conclusions on its fine-scale distribution in the orchards. We showed that the distribution of *S. catenifer* larval infestation within trees varies significantly among canopy height and aspect. Fruit at the top (>6 m) of the canopy and facing the east (sunrise) had the highest level of infestation. The lowest level of *S. catenifer* larval infestation was found in fruit collected on the ground, which could be due to reduced oviposition on the ground or to fruit dropping prior to when the moths were active. Our study was done in organically managed orchards, that used *B. thuringiensis* and *T. pretiosum*; thus, the results need to be confirmed under other (i.e., conventional) management programs.

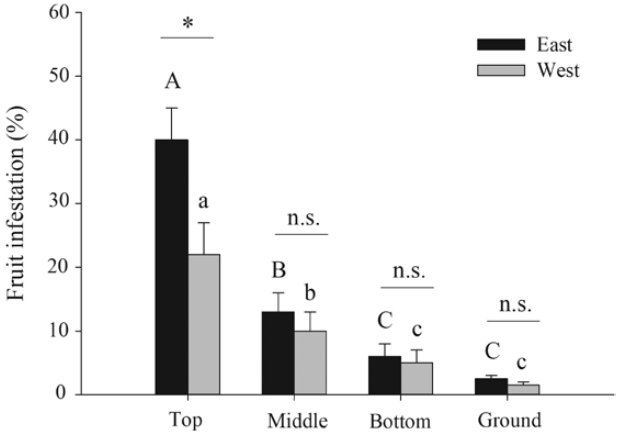

Fig. 2. Effects of canopy height and canopy aspect on avocado fruit infestation by *Stenoma catenifer*. Tree height included top, middle, and bottom, as well as on the ground. Canopy aspect refers to whether fruits were facing the east (sunrise) or west (sunset). Data are means ± SE. Different uppercase letters indicate significant differences among heights for fruit facing the east while different lowercase letters indicate significant differences among heights for fruit facing the west ($P \leq 0.05$). An asterisk indicates significant differences between east and west for each height ($P \leq 0.05$); n.s. = not significant ($P > 0.05$).

Our findings of a heterogeneous distribution of *S. catenifer* larval infestation within avocado tree canopies suggest that females might be choosing different sections of trees for oviposition, provided that egg mortality is comparable among canopy sectors. In fact, a previous study showed high correlation between oviposition site selection and larval infestation by *S. catenifer* in avocado (Hohmann et al. 2003). However, other studies showed a preference for *S. catenifer* for the middle of the avocado tree (Nava et al. 2006). Another study showed that pheromone traps capture a greater number of *S. catenifer* males in avocado orchards when placed at 6 m above ground level than at 2 m, whereas no differences in captures were observed in traps placed between 4 and 6 m (Cruz-López et al. 2020). In our study, we divided the tree canopy into more sections than in previous studies, including heights higher than 6 m. Thus, our study overcame the difficulties of collecting fruits at the highest parts of avocado trees and explains some of the discrepancies in findings between our study and previous research.

In addition, to our knowledge, this is the first study showing that *S. catenifer* larval infestation is higher in fruit facing the east (sunrise) than the west (sunset). This finding suggests a possible preference of females to lay eggs or higher egg/larval survival on the side of avocado trees exposed to the sun early in the day, which provides evidence that microenvironmental conditions with the canopy may determine maternal oviposition selection and immature performance. For example, in codling moth (*Cydia pomonella* (L.); Lepidoptera: Tortricidae), females are known to avoid cool microhabitats for oviposition (Kührt et al. 2006; Stoeckli et al. 2008). Interestingly, the effect of aspect (i.e., side of the canopy) on fruit infestation was influenced by the position within the canopy, such that higher levels of infestation were found at the top of canopies facing the east (sunrise). This further suggests that, early in the day, *S. catenifer* females might seek areas of the avocado canopy most exposed to the sun for oviposition and avoid shaded areas of the canopy, and/or immature performance is higher in this canopy section.

Stenoma catenifer females show a strong oviposition preference for Hass avocados over non-Hass avocados (Hoddle and Hoddle 2008). In our study, infestation levels did not differ by site; however, because not all orchards had the same cultivars, we could not directly conclude similar susceptibility of cultivar to *S. catenifer* larvae. Future studies should confirm whether Hass avocados are more susceptible to larval infestation than other cultivars, given that this is the most requested cultivar for foreign trade.

In conclusion, avocado fruits infested by *S. catenifer* larvae occur mainly at the top of trees and on the side of the tree facing the east (sunrise). These findings can help guide control strategies for *S. catenifer* in avocados. For instance, knowledge on the fine-scale distribution of infestation is highly relevant for accurate surveillance of *S. catenifer* in avocado orchards in regions where this pest is established as well as in newly invaded regions. To efficiently and reliably monitor *S. catenifer*, we recommend focusing on the top of trees (>6 m) and on the east-facing direction of trees when positioning pheromone traps and for visual inspection of fruit infestation. Also, this information will be useful when implementing control strategies that target adult behaviors such as mating disruption. Future studies are, however, needed to confirm that more moths are captured from traps placed on the side of trees facing the east (sunrise) to match our results on larval infestation.

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Author Contributions

Conceived and designed experiments: A.M.V. Performed the experiments: F.D., B.G.D., M.L.F.L., L.S.M.U., G.P.F., and E.O.C. Data analyses: A.M.V. and C.R.S. Manuscript preparation and editing: A.M.V. and C.R.-S. All authors have read and agreed to the published version of the manuscript.

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