Research

Captures of *Stenoma catenifer* (Lepidoptera: Depressariidae) are influenced by pheromone trap density in Hass avocado orchards

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Abstract

Stenoma catenifer Walsingham is a quarantine pest of avocado. Therefore, its detection in avocado-growing regions of Mexico that had been designated pest-free can halt national and international trade. One option for monitoring *S. catenifer* is the use of synthetic sex pheromone traps. In this study we determined the optimal density of pheromone-baited traps for monitoring *S. catenifer*. The experiment was conducted in 4 commercial Hass avocado orchards in Colima, Mexico, each with different pest infestation levels. Trap densities were established at 0.5, 1, 2, or 3 traps per ha. The traps were checked every 15 d and the number of moths recorded. The density of 3 traps per ha captured the most moths per trap followed by 1 trap per ha; similar small numbers of moths were captured at densities of 0.5 and 2 traps per ha. In general, there was a linear relationship between trap catch and trap density per ha. The overall default error rate, i.e., the proportion of traps did not detect moths when they were present, was 5.5% at a density of 3 traps per ha and 2.7% at trap densities of 0.5 and 2 traps per ha. Furthermore, at a density of 1 trap per ha all traps captured moths and so there was no default error. Since *S. catenifer* is a quarantine pest that must be detected promptly to reduce the risk of dispersal and establishment in moth-free areas, it is suggested that a density of 1 trap per ha should be used in avocado orchards to effectively monitor for the presence of this pest. However, this estimate was obtained through the bootstrapping technique, which involves the creation of pseudoreplicate datasets by resampling. Randomized field experiments with true replicates are needed to corroborate this result.

Key Words: *Persea americana*; avocado seed moth; sex pheromone trap; monitoring

Resumen

La presencia de *Stenoma catenifer* Walsingham, una plaga cuarentenaria, puede cancelar la comercialización nacional e internacional de aguacate Hass de zonas libres de plagas reglamentadas del aguacatero. Una opción para su oportuna detección es el uso de trampas con feromona sexual sintética. El objetivo del estudio fue determinar la densidad de estas de trampas para el monitoreo de *S. catenifer*. El experimento se estableció en cuatro huertos comerciales de aguacate Hass, con diferentes niveles de infestación de la plaga, en Colima, México. En los huertos, se establecieron las densidades siguientes: 0.5, 1, 2, y 3 trampas por ha. Las trampas se revisaron cada 15 días para registrar el número de palomillas capturadas. La densidad de 3 trampas por ha capturó el mayor número de palomillas, seguida de la densidad de 1 trampa por ha, mientras que las capturas con 0.5 y 2 trampas por ha fueron las menores, pero muy similares entre sí. En general, las densidades de 0.5, 1, y 2 trampas por ha en relación con la densidad de 3 trampas por ha estuvieron linealmente relacionadas en la mayoría de los casos. La tasa de error global por omisión, i.e., no detectar la palomilla cuando está presente, con referencia a la densidad de 3 trampas por ha fue de 5.5%, y de 2.7% para las densidades de 0.5 y 2 trampas por ha, mientras que en la densidad de 1 trampa por ha siempre se detectaron palomillas y no tuvo omisión de error. Por tratarse de una plaga de importancia cuarentenaria y por el interés de detectarla oportunamente y así reducir el riesgo de dispersión y establecimiento a las áreas libres, se sugiere usar la densidad de una trampa por hectárea de aguacate. Sin embargo, este estimado se obtuvo mediate la técnica de bootstrapping que utiliza pseudor-replicas. Para validar este resultado, se requieren experimentos de campo con repeticiones verdaderas.

Palabras Clave: *Persea americana*; palomilla barrenadora de la semilla del aguacate; trampas con feromona sexual; monitoreo

Stenoma catenifer Walsingham (Lepidoptera: Depressariidae), the avocado seed moth, is native to neotropical areas of the Americas (Nava et al. 2006), including several Central and South American countries (Hohmann et al. 2001; Hoddle & Hoddle 2012; Manrique 2014).

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In Mexico, *S. catenifer* is found in Chiapas, Colima, Guanajuato, Guerrero, Hidalgo, Jalisco, Nuevo León, Oaxaca, Querétaro, Tamaulipas, and Veracruz states (SENASICA 2016). When management is absent, this insect can cause losses of 45 to 80% in commercial Hass avocado (*Persea americana* Mill.; Lauraceae) (Nava et al. 2005; Hoddle & Hoddle 2008). The larvae pierce the fruit pulp and feed mainly on the seed, although they also can attack shoots (Wolfenbarger & Colburn 1979). Trapping programs are used to monitor pest dispersal into new regions, and trapping results support decision-making (Buntin 1994). Pest detection, monitoring, and control systems based on attractant-baited traps are a valuable everyday tool in integrated pest management programs, providing timely information that facilitates decisions as to whether control actions are required (Barrera et al. 2006). Optimal trap densities must be determined before pheromone-based monitoring systems can be used effectively. With respect to *S. catenifer*, trap density and the corresponding magnitude of catches must be standardized if a monitoring system for this pest is to be effective in Mexico. In this investigation we sought to determine the appropriate number of sex pheromone traps per ha for reliable detection of *S. catenifer* in commercial Hass avocado orchards in Mexico.

Materials and Methods

TRAPPING SYSTEM

Stenoma catenifer populations were monitored between Jan and May 2018 in 4 commercial Hass avocado orchards in Colima, Mexico. The orchards varied in *S. catenifer* infestation level, altitude, and tree age (Table 1). Experimental trees were in the phenological stage of flowering-fruiting (3 Jan to 9 May 2018) when the pest is most abundant. Four trap densities (treatments) were evaluated: 0.5 trap per ha (1T2h); 1 trap per ha (1Th), 2 traps per ha (2Th), and 3 traps per ha (3Th). The trap type used was a cardboard wing Pherocon® 1C (Trécé Inc., Adair, Oklahoma, USA) trap baited with a commercially available pheromone released from a rubber septum (Pherocon® Septa lures, Trécé Inc., Adair, Oklahoma, USA) (Fig. 1). Each of the 4 orchards was divided into 4 sections of 1 ha each. Treatments were placed evenly and in the center of the corresponding area inside the canopy of the avocado trees, at a height of approximately 1.6 m aboveground. Each orchard was considered 1 replicate. The experiment had a randomized complete block design, considering blocks as replicates and traps nested within the orchards. Unlike the treatments, which can be manipulated at the experimenter's convenience, orchards were considered the random component because they were selected from a pool of orchard populations (Yang 2010). The distance between traps was at least 50 m. Previous trapping studies on *S. catenifer* used 20 to 50 m between traps (Castillo et al. 2012; Cruz-López et al. 2020). The traps were checked every 2 wk from 3 Jan to 9 May 2018. Captured *S. catenifer* were removed from traps with a plastic stick, the trap bases and pheromone septa were replaced every mo. On each occasion the number of *S. catenifer* moths (all males) caught in each trap was recorded. *Stenoma catenifer* forewings have (in the apical angle) 25 small black spots that form an S-shape; this was used to distinguish *S. catenifer* from *Antaeotricha nictitans* (Zeller) (Lepidoptera: Depressariidae), which also are attracted to the *S. catenifer* sex pheromone (Castillo et al. 2012).

DATA ANALYSIS

Several analyses were conducted to evaluate the effect of trap density on moth catches. The first analysis was based on a generalized linear mixed model (GLMM) (Faraway 2006) to test the effect of sampling dates and the trap density (treatment) on the mean number of *S. catenifer* moths caught. GLMM analysis is well suited to deal with counts and heterogeneous variances because it relies on a probabilistic distribution that represents such features better than the normal distribution. Also, mixed models take into account the temporal correlation across sampling dates for designs with replications in time as in this case (Crawley 2007). Preliminary analyses were done to select among normal, Poisson, and negative binomial distribution using a canonical link function, which transforms the data based on the underlying distribution (Crawley 2007). Models were fitted using restricted maximum likelihood, and comparisons were based on examining the residuals, deviance, and the Akaike Information Criterion to choose among the distributions. We also found that the interaction between treatments and dates was non-significant. The final model was, in the notation of the R language: moths ~ treatment + date + (1|orchard per trap). This model represents the mean capture of moths as a linear function of the factors to the right of the tilde mark (~), that is, depending on the fixed effects of treatments and sampling dates. Random components were enclosed within parentheses and traps nested within the orchards. Once this model was selected, the effect of treatments was determined by applying an *F* test, and means were compared using the Tukey test, both with a significance level of 0.05. Parameters of the final model were estimated using the glmer.nb function (this function is to fit a negative binomial model with an overdispersion parameter, meaning that the data are either equal dispersed (variance = mean) or under dispersed (variance < mean), so that the estimate of the dispersion parameter becomes very large) and linked to the negative binomial distribution; means were compared using the Tukey test and 95% confidence intervals constructed with the emmeans library. All GLMM analyses were performed with the link function, but results are presented in the original units by applying the corresponding inverse link function; Gills (2001) describe common link functions and their inverse.

A second analysis was used to compare the total number of catches. While standard procedures exist to compare means, in the case of totals, which are the sum of all the captures for each treatment, there is no established procedure because replication values collapse to 1 for each treatment at each sampling date. Therefore, we generated the sampling distribution of totals using the bootstrap resampling procedure; this was used to generate variability, obtain estimates of the parameters, and construct robust confidence intervals (Thai et al. 2013).

Table 1. Hass avocado orchards selected for evaluation of the effect of sex pheromone trap density on catches of *Stenoma catenifer*, Jan to May 2018. masl= meters above sea level.

Municipality	Orchard number	Georaphical coordinates	Altitude (masl)	Area (ha)	Age of trees (yr)
Comala	(1) Agosto	19.2347°N, 103.4355°W	1,102	4.1	13
	(3) Piedra Rajada	19.2450°N, 103.4480°W	1,199	7.1	48
	(4) Rancho Alto	19.2580°N, 103.4219°W	1,338	9.9	48
Cuauhtémoc	(2) La Calma	19.2528°N, 103.3542°W	1,523	6.9	5

Fig. 1. Pherocon 1C wing trap with a commercial pheromone.

For each orchard, 1,000 non-parametric bootstrap runs were made. Every run consisted of randomly selecting sampling dates and summing the captures of all traps of each treatment. Mean and confidence intervals (95%) of total captures were calculated with the quartiles (0.025, 0.975) from the sampling distribution of each treatment. By comparing the totals, we could evaluate the relative efficiency of each trap density, that is, we expected that few traps could catch a number of moths similar to that of the highest trap density. In addition, we applied a linear regression analysis to compare the total catches from treatments 1T2h, 1Th, and 2Th against 3Th for each orchard. From this analysis, a positive relationship would indicate consistency of captures between the treatments across all the population densities, otherwise it would indicate that low trap density treatments failed to capture moths in proportion similar to the 3Th treatment.

Finally, an omission error rate was estimated for treatments 1T2h, 1Th, and 2Th relative to the treatment 3Th. The omission error represents a failure to capture moths when they are present. In this case, we assumed that the 3Th treatment had the capacity to capture moths when they were present because it had the highest density. The error rate was calculated as nfj/nt across all the orchards, where nf is the number of dates all traps of the j-treatment captured no moths, and nt is the number of dates all traps in the 3Th treatment captured moths ($x > 0$). This error rate is critical because many management decisions and free-pest area categorization rely on detecting the pest and not estimating the population densities. All statistical analyses were done using the R v. 3.4.0 (R CoreTeam 2015).

Results

In total, 2,321 *S. catenifer* moths were captured during the experimental period. The mean number of adults captured increased as the phenological stage of avocado progressed from flowering to fruiting. The largest catch of moths per trap occurred in orchard 3, with a total of 1,004 male adults (Table 1). The next largest number of moths caught per trap was in orchard 4, with 769 males; followed by 276 males caught in orchard 1, and 272 males in orchard 2. Peak captures per trap occurred on Julian day 102 (12 Apr) in orchard 4 (48 moths, 3Th); on Julian day 74 (15 Mar) in orchard 3 (37 moths, 1Th); on Julian day 129 (9 May) in orchard 2 (29 moths, 3Th); and on Julian day 18 (18 Jan) in orchard 1 (12 moths, 2Th) (Fig. 2).

Trap density affected the number of *S. catenifer* moths captured per trap ($F = 16.31$; GL = 3; $P < 0.001$). Catches of *S. catenifer* moths per trap were significantly larger in the 3Th treatment than the 1T2h and 2Th treatments in all 4 orchards and at all sampling dates, with a mean of 0.84 moths per trap per night. Catches per trap in the 1Th treatment were intermediate and not significantly different from 1T2h and 2Th, with a mean of 0.75 male moths per trap per night. Catch size in the 1T2h and 2Th treatments were very similar, with means of 0.43 and 0.44 moths per trap per night, respectively.

The comparison of mean cumulative numbers of male moth captures per trap resulted in non-significant differences between treatments 3Th and 1Th, which has the higher average of captured moths

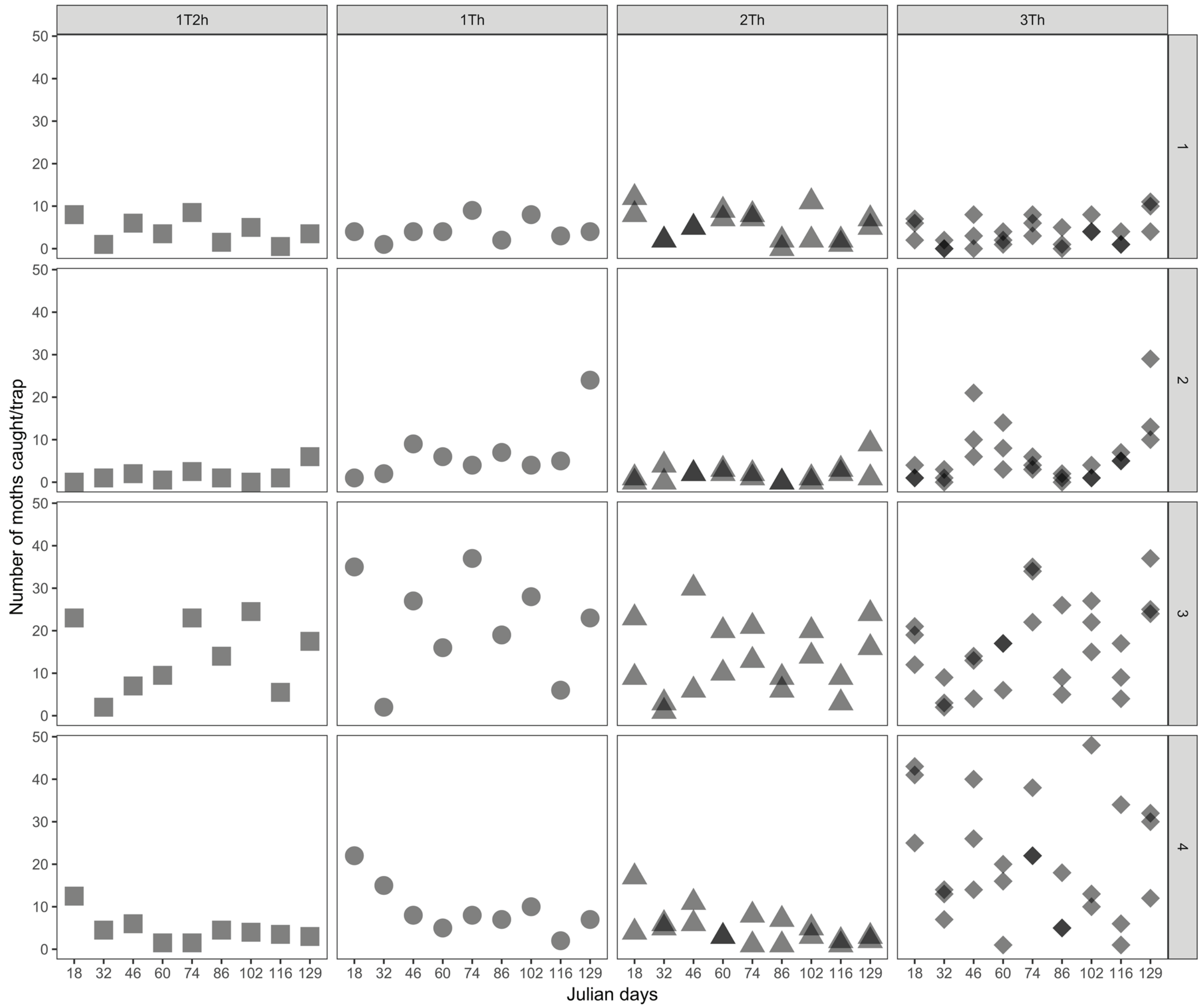

Fig. 2. Number of *Stenoma catenifer* caught in synthetic sex pheromone traps placed at different densities (1 T2h, 1Th, 2Th, and 3Th: treatments, number of traps per area) and in different Hass avocado orchards (1–4 of the Y right axis) in the municipalities of Comala and Cuauhtémoc, Colima, Mexico, 2018. The columns correspond to treatments and the rows to experimental orchards. 1T2h = 0.5 traps per ha; 1Th = 1 trap per ha; 2Th = 2 traps per ha; 3Th = 3 traps per ha.

per trap. The 1T2h and 2Th treatments had significantly lower trap captures (Fig. 3).

Using the nonparametric bootstrap analysis treatments, 1T2h, 1Th, and 2Th resulted in similar moth captures per trap in orchards 4, 2, and 3. The treatment 3Th captured significantly more moths per trap (Fig. 4).

We compared the total number of moths caught per trap in the treatment 3Th with the total moths caught per trap in treatments 1T2h, 1Th, and 2Th, in each orchard over the entire experimental period (Fig. 5). The number of moths caught in treatments 1T2h, 1Th, and 2Th correlated positively with the number of moths caught with treatment 3Th in each of the orchards (Table 2; Fig. 5). It is clear that moth populations responded to the number of traps in each treatment, reflecting the high values of moths captured. This indicates that captures made in treatments 1T2h, 1Th, and 2Th are similar to those in treatment 3Th.

The default error rate for captures was obtained only for treatment 1T2h with 5.5% and treatment 2Th with 2.7%, because these treatments did not have catches on some of the sampling dates. There was no error in treatment 1Th due to omission, indicating that the capacity to detect adults is similar to that of treatment 3Th in the observed range of trap densities.

Discussion

In this study, we found that trap density significantly affects the capture of *S. catenifer* males. Captures at densities of 1Th and 3Th were similar and larger than with the other density treatments. Hoddle et al. (2011) suggested that trap density should vary according to the level of infestation by *S. catenifer* and the size of avocado orchards. For example, the authors recommended that in orchards of up to 76 ha a total of 10 to 13 traps should be randomly installed for a period of 7 d to be able to detect at least 1 male moth with 90% confidence. They also recommended that, for detection of *S. catenifer* in export orchards, in areas of up to 2 ha, a total of 2 to 5 traps should be placed with a

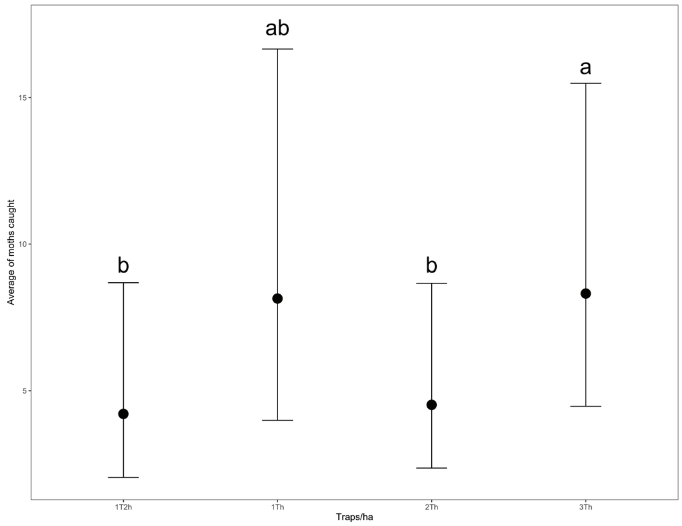

Fig. 3. Mean cumulative number by treatment of *Stenoma catenifer* (IC95) caught in traps baited with synthetic sex pheromones at different trap densities in Hass avocado orchards, Colima, Mexico, during the experiment in 2018. Means with the same lowercase letter are not significantly different from each other according to Tukey's test (X0.05). 1T2h = 0.5 traps per ha; 1Th = 1 trap per ha; 2Th = 2 traps per ha; 3Th = 3 traps per ha.

random distribution. Differences in the number of moths caught over time could be due to various factors including pheromone release rates (Anderbrant et al. 1992), trap position (Simandl & Anderbrant 1995), climate (Walton et al. 2004), wind conditions and distance between traps (Wedding et al. 1995), and the percentage of trees infested with moths in the orchards (Riedl 1980; Hoddle et al. 2011). Our estimation that 1 trap per ha as the appropriate density for detecting *S. catenifer* is of great importance because this can help avocado producers to monitor this pest effectively. However, this estimate was obtained through the bootstrapping technique, which involves the creation of pseudoreplicate datasets by resampling. Randomized field experiments with true replicates are needed to corroborate this result.

In SENASICA publications (2019, 2021) that provide guidelines for Mexican farmers, information about appropriate trap density in commercial orchards of Hass avocado orchards is unclear, although it is suggested that, in municipalities where the moth is present, traps should be placed every 550 m. In moth-free areas traps should be deployed as soon as an infestation focus is detected; traps should be placed every 2 ha, separated by approximately 130 m (17 traps within a radius of 520 m), and remain deployed for 6 mo until the infestation focus is controlled. SENASICA also suggests that, in infested municipalities adjacent to non-infested areas, a phytosanitary belt of traps should be placed 130 to 150 m apart between the 2 zones. These recommendations are very similar to those of Hoddle et al. (2011) for effective detection of at least 1 male *S. catenifer*.

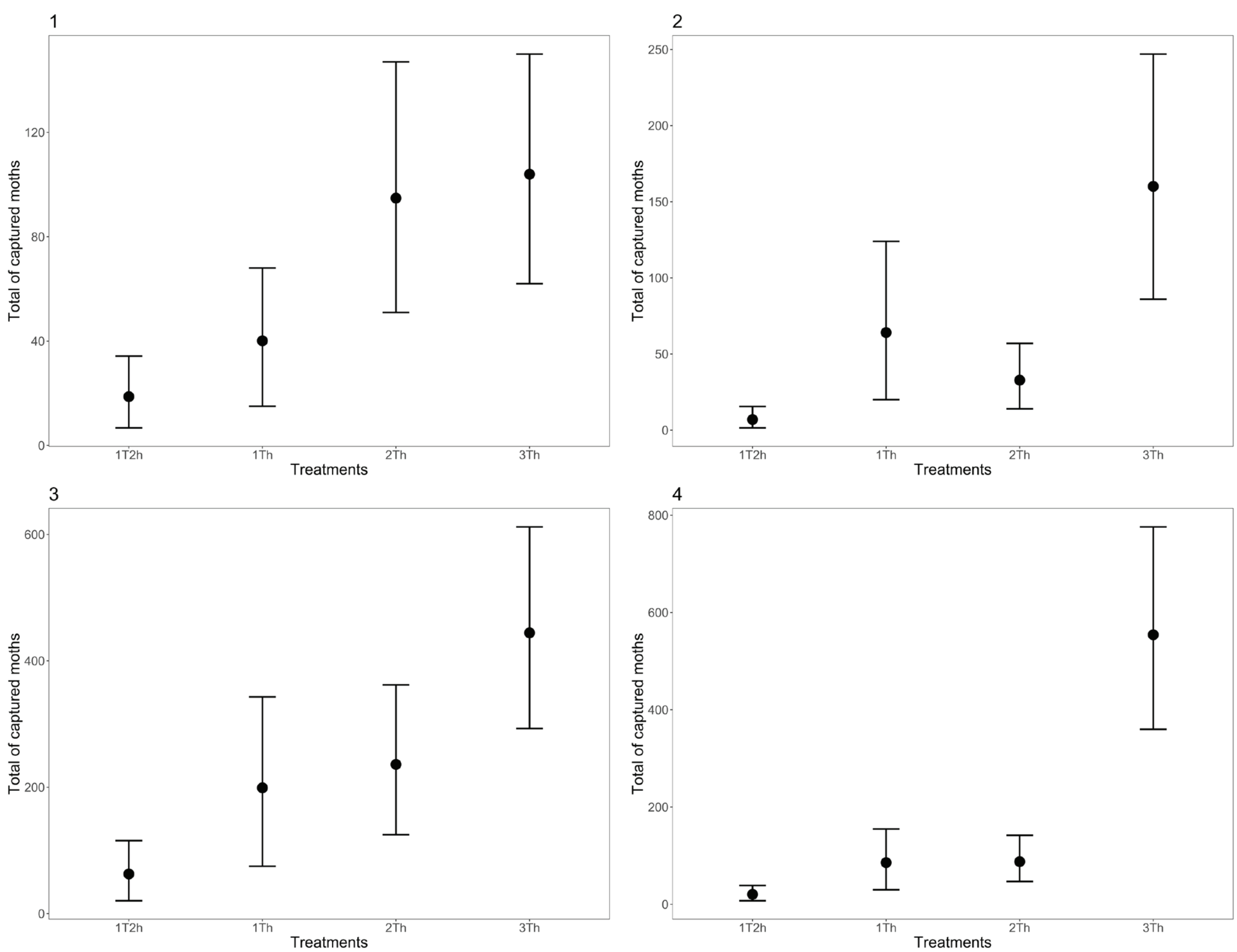

Fig. 4. Nonparametric bootstrap sampling distribution of the total numbers of *Stenoma catenifer* caught in experimental plots (CI95%) (1–4) in the linear model of the different orchards. The black dot on each line indicates the mean value of the total for each of the treatments. 1T2h = 0.5 traps per ha; 1Th = 1 trap per ha; 2Th = 2 traps per ha; 3Th = 3 traps per ha.

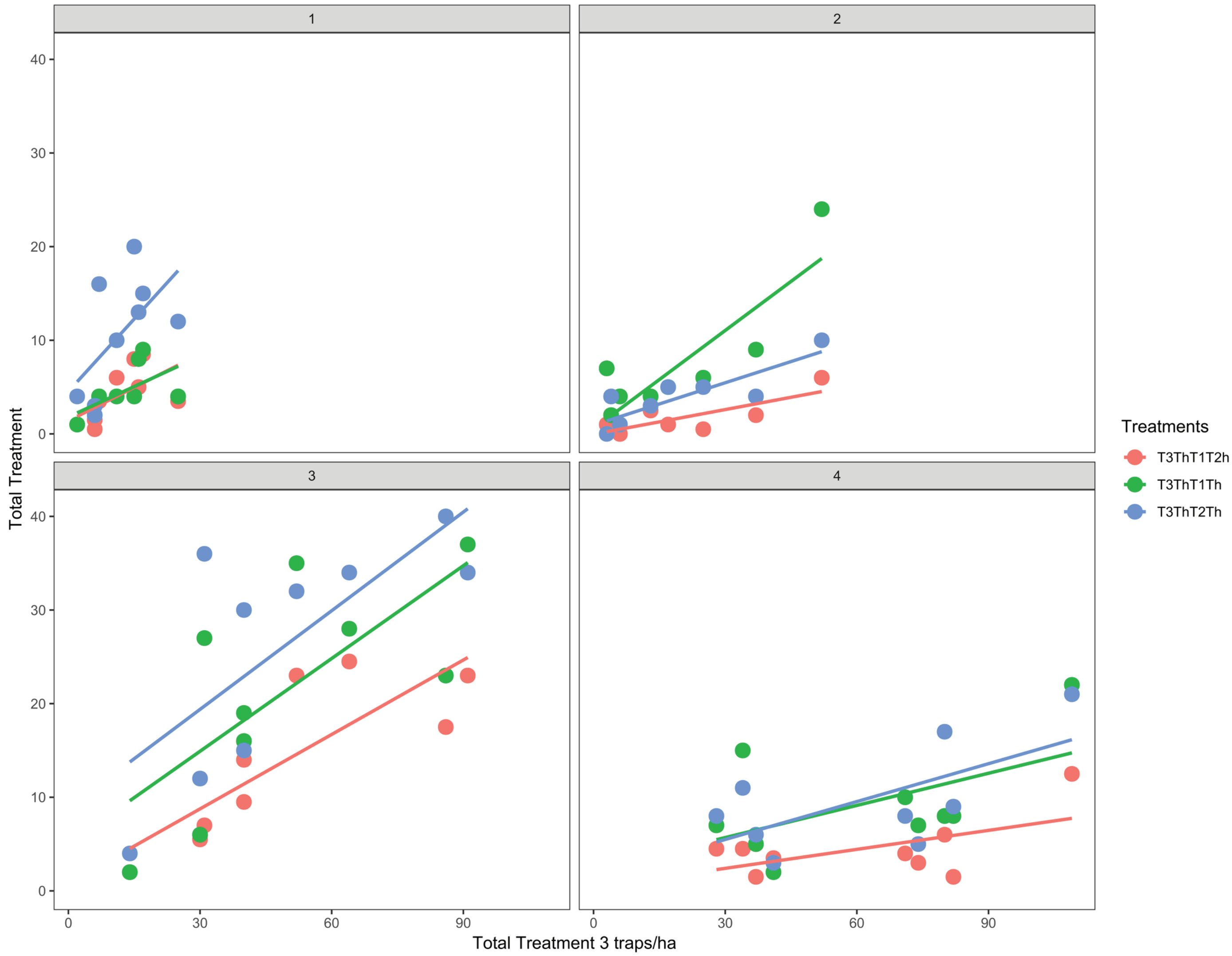

Fig. 5. Relationship between total number of *Stenoma catenifer* caught in different treatments in 4 Hass avocado orchards in Colima, Mexico, 2018. 1T2h = 0.5 traps per ha; 1Th = 1 trap per ha; 2Th = 2 traps per ha; 3Th = 3 traps per ha.

We observed that 15 d after replacing the trap bases and pheromone septa, there were increases in the number of moths caught. In other studies, such as the use of pheromone traps for the detection of *Copitarsia discolora* Guenée (Lepidoptera: Noctuidae) in broccoli, temperature and relative humidity affected pheromone emission and stability (Barrientos et al. 2011). They also noted that, during the first d after setting up the traps, there was a high emission rate of the pheromone, which continued until the dispenser release rate reached a balance with the environment.

Agronomic management practices in orchards also can influence trap catches significantly. For example, we observed that phytosanitary management was inadequate at one farm; the producer did not collect and dispose of *S. catenifer*-infested fruits that remained on trees or that had fallen to the ground, either before or after harvest. As a result, moth infestation hotspots were maintained. Furthermore, in this orchard and in another one, where the largest numbers of moths were caught, the trees were tall (> 10 m), which meant that it was not possible to cut or fully harvest all the fruits; thus potentially infested fruits were not removed. In contrast, the trees in the 2 other orchards were 2 to 3 m high, and management and harvesting were done properly, leaving fewer fruit on the ground; consequently, the numbers of moths caught in traps were smaller.

Regarding trapping systems for monitoring other pests of agricultural importance, Figueroa-Castro et al. (2016) reported that agave weevils (*Scyphophorus acupunctatus* Gyllenhal; Coleoptera: Dryophthoridae) were attracted to traps baited with synthetic aggregation pheromone and agave tissue from as far away as 120 m. They also found that traps placed furthest apart from each other captured more agave weevils than those placed closely together; they recommend a density of 1 trap every 6 ha in blue agave tequilero for monitoring *S. acupunctatus*. Vanaclocha et al. (2016) found that the size of adult catches of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was related to trap density; in 2 yr of monitoring, they demonstrated that densities of 1 trap per ha achieved results like those obtained at the recommended density of 1 trap every 0.4 to 1.6 ha. Bacca et al. (2006) found that using a density of 1 trap every 3.5 to 4 ha was the most effective for monitoring the coffee miner, *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae).

Orchard managers and producers are risk-averse, and although a single trap is sufficient to detect moths, they may consider placing extra traps out in the mistaken belief that 1 trap is insufficient to detect the moths. Risk aversion is difficult to address even when experimental results demonstrate that only 1 trap per ha is recommended because

Table 2. Results of linear regression analysis of data on trap catches of *Stenoma catenifer* when traps were placed at different densities in 4 experimental orchards in Colima, Mexico, 2018; model coefficients b0 and b1, Fc, P, r. 1T2h = 0.5 traps per ha; 1Th = 1 trap per ha; 2Th = 2 traps per ha; 3Th = 3 traps per ha.

Orchard	Treatments	b0	b1	Fc	P	r
Agosto	1T2h	1.3902	0.2380	3.60	0.098	34.1%
	1Th	1.8093	0.2163	3.90	0.087	36 %
	2Th	4.535	0.516	3.70	0.095	34.5%
La Calma	1T2h	−0.03008	0.08755	12.20	0.01	63%
	1Th	0.5594	0.3495	20.50	0.002	74.5%
	2Th	0.9327	0.1510	18.75	0.034	72.8%
Piedra Rajada	1T2h	0.8079	0.2650	14.10	0.01	66.9%
	1Th	5.0011	0.3303	7.30	0.029	51.3%
	2Th	8.8477	0.3513	7.75	0.027	52.5%
Rancho Alto	1T2h	0.37983	0.06759	3.30	0.11	32.1%
	1Th	2.2604	0.1145	2.83	0.135	28.8%
	2Th	1.4430	0.1349	4.99	0.060	41.6%

it is the most effective (and cost efficient). It is important to emphasize that, in our study, there was no error by omission in the detection of moths at 1 trap per ha.

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