Colegio de Postgraduados

Productive Characteristics, Nesting Substrates, and Colonies of the *Escamolera* Ant (*Liometopum apiculatum* M.) in Zacatecas, Mexico

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ABSTRACT
Objective: The objective of this study was to connect measurements, weights, and production of *escamoles* with nesting substrates, foraging paths, nest types, and colony sizes of the *escamolera* ant.
Design/Methodology/Approach: The data about nests, colonies, and larvae were gathered during morning and evening field walks, with the support of escamoles harvesters. The basic statistics of the data were estimated (N=59 nests/colonies) and analyzed with the Kruskal-Wallis H test. In addition, the Mann-Whitney U test was used to determine the differences per nest type.
Results: The highest production of escamoles was recorded in the *Prosopis laevigata* substrate (x=551.08 g/N=1), while the lowest production was recorded in the *Echinocereus stramineus* substrate (x=228.31 g/N=4). The length and width of the larvae (N=1,100 larvae) were similar in all the substrates. The weight of the larvae varied from 0.09 g, in the *Prosopis levigata* substrate, to 0.16 g, in the dry palm (*Yucca* spp.) substrate; therefore, 11,111 and 6,250 larvae are required, respectively, to obtain 1 kg of escamoles.
Study Limitations/Implications: The information of this study is limited to a single harvesting region.
Findings/Conclusions: The low escamoles production indicates that its harvesting must comply with a regulatory framework and a better organization, in order to guarantee the continuous presence of *Liometopum apiculatum* colonies.

Keywords: Conservation, edible insects, habitat, invertebrates, arid zones.

Citation: Romero-Jiménez, H., Tarango-Arámbula, L. A., Peredo-Rivera, E., Del Rosario-Arellano, J., Olmos-Oropeza, G., & Hernández-Roldán, E. (2024). Productive Characteristics, Nesting Substrates, and Colonies of the *Escamolera* Ant (*Liometopum apiculatum* M.) in Zacatecas, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i6.2933

Academic Editor: Jorge Cadena Iñiguez
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 11, 2024.
Accepted: May 29, 2024.
Published on-line: July 02, 2024.

Agro Productividad, 17(6). June. 2024. pp: 185-195.

INTRODUCTION

Mexico has an exceptional natural wealth; however, only a fraction of all its native species has been studied. Biological diversity diminishes constantly throughout time (Martínez-Meyer *et al.*, 2014), despite the governmental policies aimed to stop environmental and wildlife degradation (Rosas-Rosas *et al.*, 2018).

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The threats posed to biodiversity directly cause the extinction of species and change based on their taxonomic group. The major threats and causes include habitat destruction, pollution, overexploitation, introduction of exotic species, and recently climate change (Martínez-Meyer *et al.*, 2014).

The *escamolera* ant (*Liometopum apiculatum* Mayr.) can be found in a wide variety of weathers and soils of 24 Mexican states. *L. apiculatum* is a thermophilic, monogynous, polyandrous, and omnivorous ant, with a low biological vulnerability (Berumen-Jiménez *et al.*, 2021). The escamolera ant is ecologically and socioeconomically important for Zacatecas, Mexico (Rafael- Valdez *et al.*, 2014; Briones-Santoyo *et al.*, 2022). One kilogram of its larvae (escamoles) costs up to USD $30; consequently, they are harvested and sold without an appropriate control throughout the region.

As a result of its economic importance, the colonies of *escamolera* ants are overexploited (Briones-Santoyo *et al.*, 2022) and their habitats are overgrazed. In addition, there are few studies about this species. Nevertheless, their nesting substrate (Rafael-Valdez *et al.*, 2014), the use of their habitat (Cruz-Labana *et al.*, 2014), their long-range foraging (Rafael-Valdez *et al.*, 2014), the density of the nests (Cruz-Labana *et al.*, 2019; Hernández-Roldan *et al.*, 2017), the nutritional content (Cruz-Labana *et al.*, 2018), and their conservation state (Berumen-Jiménez *et al.*, 2021) have been studied.

Entomophagy is widely known and has substantially increased in Mexico and all over the world. Insects are sold in different portions and shapes in markets, street markets, and restaurants, as well as by domestic and international companies (Ramos-Elourdy *et al.*, 2006).

The larvae and pupae of *Liometopum apiculatum* are appreciated for their taste and nutritional value. Currently, the demand from restaurants, high prices, and other local factors endanger the escamolera ant populations (Berumen-Jiménez *et al.*, 2021). Although *L. apiculatum* is an important economic resource for rural communities in the arid and semi-arid regions of Mexico, this species faces problems such as overgrazed pasturelands, a lack of regulations for the management of its habitats (Hernández-Roldan *et al.*, 2017), and the harvesting of its larvae.

Therefore, the objective of this study was to connect the sizes, weights, and production of *escamoles* with the nesting substrate, the foraging path, and the type and size of the *escamolera* ant (*Liometopum apiculatum* Mayr.) nests in Pánfilo Natera, Zacatecas.

MATERIALS AND METHODS

Study Area

The study was carried out in the General Pánfilo Natera municipality (Pámanes colony), Zacatecas, Mexico, during the 2022 *escamoles* harvesting season (March and April). Pánfilo Natera is located in the southeast of the state and borders with the municipalities of Trancoso and Guadalupe (north), Villa González Ortega (south), Ojocaliente (west), and Salinas and Villas de Ramos in San Luis Potosí (east). The study area is located 48 km to the southeast of the city of Zacatecas, at 22° 40’ 00” N, 102° 07’ 00” W, and 2,100 m.a.s.l. (INEGI, 2010).

Data Gathering

The data (8 variables) were obtained during morning (7 am to 1 pm) and evening (4 pm to 7 pm) field walks, with the help of escamoles harvesters. During these hours, soil temperature does not damage the ants.

The nesting substrate and number of foraging paths per colony were visually determined when the nest was found. In addition, the nests were classified as "new nest" or "disturbed nest (previously harvested)" during the larvae collection. The location of the nests was registered using a GARMIN ETREX GPS.

To determine the *escamoles* production per nest, each nest was dug, the plant material was removed from the nest, and the larvae were collected. Although sieves were used to remove plant material, soil, rocks, and other organic waste from the larvae, some impurities were still present. The larvae harvested from the nests in the field were weighted with a Silverline digital scale; afterwards, they were washed and the rest of the impurities were removed. The impurity percentage (60%) per nest was determined with the weight recorded in the field (larvae and impurities) and the weight recorded from clean *escamoles*. This value was used to determine the actual production of escamoles in subsequent nests. The size of the colony (small, medium, and large) was visually determined once the nests were opened and the quantity of ants was observed. Once the harvesting was completed, the nests were covered and protected with plant material (dry leaves, prickly pear, and palm).

The production of *escamoles* was estimated for each nest. Twenty larvae were collected per nest and were placed in Petri dishes; subsequently, they were frozen until they were analyzed. The weight (g), length (mm), and width (mm) of the samples were recorded. Weight was determined using an ADAM Nimbus® analytical balance, while the size of the samples was determined with a TRUPER® Vernier digital caliper. The data gathered in the field included: colony number, substrate number, nest size, nest type, number of foraging paths, nesting substrate, and production per nest. Microsoft® Excel was used to develop a database in the lab.

Statistical Analysis

The database consisted of dependent and independent variables. The dependent variables were escamoles production per nest and per substrate (g), as well as length (mm), width (mm), and weight (g) of the *escamoles*. Meanwhile, the independent variables were nesting substrate, colony size, number of foraging paths, and nest type (new nest or disturbed nest).

The data were verified with the Kolmogorov-Smirnov H test (normality) and Levene's test (homogeneity of variances).

The data analysis determined the basic statistics and contrasts of the variables; in addition, a multivariate clusting was conducted. The tables include the calculation of the mean of the data and the standard deviation $(\bar{x} \pm \mathrm{SD})$. The contrasts were used to compare the nesting substrate, the colony size, and the number of foraging paths. The Kruskal-Wallis H test was carried out to establish possible differences. Afterwards, the post hoc test of the Tukey method was used to specify the differences; values $<\alpha(\alpha 0.05)$ indicate significant statistical differences.

The *escamoles* production and larvae size per nest type (new or disturbed nest) were analyzed with a Mann-Whitney U test. The comparisons were explained by the mean and its standard error $(\bar{x} \pm \mathrm{SE})$.

The optimal number of clusters was calculated using the fviz_nbclust function of the factoextra package of RStudio. Subsequently, the k-means clustering was used for the multivariate analysis of four centroids. The data were standardized, using the Ward's method and the Euclidian distance. The contrasts were based on a significance level of $p<0.05$. All the data were analyzed using the Statistica 7.0 and Project Management Framework 6.3.1 (R Project) statistical packages and the graphs were developed using the SigmaPlot 10.0 software.

RESULTS AND DISCUSSION

This research analyzed data from 59 nests: 6 new nests and 53 disturbed nests. In addition, 8 nesting substrates were analyzed. The nesting substrates chosen by the ant colonies were prickly pear (*Opuntia ficus-indica*; 10 colonies), maguey (*Agave salmiana*; 16 colonies), palm (*Yucca filifera*; 15 colonies), strawberry cactus (*Echinocereus stramineus*; 4 colonies), mezote (dry prickly pear; 3 colonies) dry palm (2 colonies), mesquite (*Prosopis laevigata*; 1 colony), and bare soil (8 colonies). Out of the 59 ant nests, 29 were large colonies, 22 were medium colonies, and 8 were small colonies.

Regarding the foraging paths, 3 colonies had 2 paths, 23 colonies had 3 paths, 24 colonies had 4 paths, and 8 colonies had 6 paths.

Nesting Substrate

Maguey was the chosen substrate of *L. apiculatum*, followed by palm. This choice can change depending on the harvesting area. In Villa González Ortega, the escamolera ant nests are mainly located in maguey and palm also (Rafael-Valdez *et al.*, 2017).

The highest production of escamoles was recorded in the mesquite, prickly pear, and maguey substrates, while the lowest production was recorded in the strawberry cactus substrate (Table 1). According to Figueroa-Sandoval *et al.* (2018), the prickly pear substrate yielded a high volume of escamoles, while the maguey substrate recorded a low production. The nesting substrates are fundamental in the production of escamoles: they provide potential foraging areas and thermal protection (Juárez-Sandoval *et al.*, 2010). However, longevity, size, and nest type are also important factors that can change production and, therefore, must be taken into account.

The width and length of the escamoles were very similar in each substrate; nevertheless, the heaviest escamoles were found in the dry palm substrate and in bare soil (Table 1).

The Kruskal-Wallis H test established differences between the 8 nesting substrates. The following results were recorded: production ($KW7, 1180=40.36$, $p<0.01$; Figure 1A); length ($KW7, 1180=40.36$, $p<0.01$; Figure 1B); width ($KW7, 1180=43.07661$, $p<0.01$; Figure 1C), and weight ($KW7, 1180=62.47$, $p<0.01$; Figure 1D).

Table 1. Production, size, and weight of escamoles per nesting substrate, based on the mean and its standard deviation $(\bar{x} \pm SD)$, in Pánfilo Natera, Zacatecas.

Nesting substrate	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
Strawberry cactus (*Echinocereus stramineus*)	282.31	186.96	8.56	0.92	4.72	0.43	0.12	0.03
Maguey (*Agave salmiana*)	431.04	280.29	8.87	1.22	4.64	0.57	0.13	0.04
Prickly pear (*Opuntia ficus indica*)	451.37	245.50	8.89	1.23	4.75	0.53	0.14	0.03
Mezote (Dry prickly pear)	380.02	330.66	8.57	1.32	4.60	0.50	0.14	0.04
Mesquite (*Prosopis laevigata*)	551.08	0.00	8.37	0.70	4.24	0.29	0.09	0.01
Palm (*Yucca* spp.)	350.32	298.05	8.46	1.23	4.59	0.56	0.14	0.04
Dry palm	351.02	263.62	8.57	1.45	4.39	0.48	0.16	0.07
Bare soil	334.34	239.66	8.80	0.99	4.70	0.44	0.15	0.04

±D.S.=standard deviation.

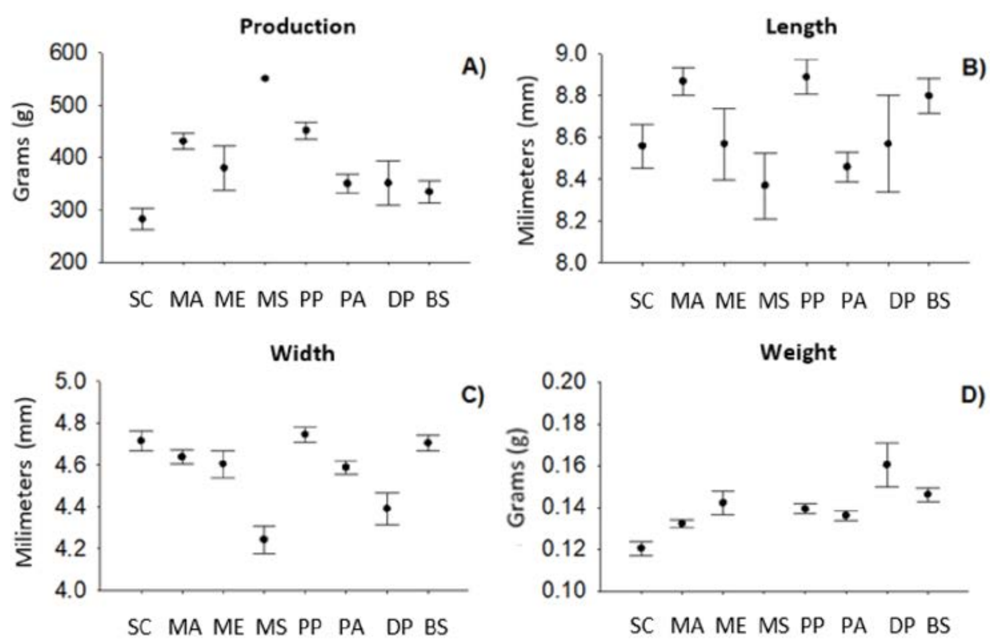

Figure 1. Comparison of eight nesting substrates based on production (A), length (B), width (C), and weight (D) of escamoles, as well as the mean ± standard error $(\bar{x} \pm SE)$. SC: strawberry cactus, MA: maguey, ME: mezote, MS: mesquite, PP: prickly pear, PA: palm, DP: dry palm, and BS: bare soil.

Colony Size

Understanding the variables that better explain the presence of the escamolera ant is fundamental to improve the management of the colonies and the habitats; consequently, the size of the nests and the colonies must be determined (Jofré and Medina, 2012; Figueroa-Sandoval *et al*., 2018). The *escamoles* production and weight are directly proportional to the size of the colony, because larger colonies have a higher average production (Table 2); however, the colonies had very similar sizes. According to Hoey-Chamberlain *et al.* (2013), a colony can house up to 250,000 ants and, consequently, an excessive population growth can impact the quality of the nest, reducing its productivity (Figueroa-Sandoval *et al*, 2018).

The Kruskal-Wallis H test established differences between the size of the colonies, regarding production (KW2, 1180=279.17, p<0.01; Figure 2A), length (KW2, 1180=63.82, p<0.01; Figure 2B), width (KW2, 1180=41.17, p<0.01; Figure 2C) and weight (KW2, 1180=78.32, p<0.01; Figure 2D).

Table 2. Production, size, and weight of the escamoles, per colony size, based on the mean and its standard deviation $(\bar{x} \pm \text{SD})$, in Pánfilo Natera, Zacatecas.

Colony size	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
Small	119.83	76.44	8.09	1.33	4.45	0.54	0.12	0.05
Medium	361.06	242.25	8.63	1.10	4.58	0.47	0.13	0.03
Large	485.43	277.21	8.93	1.16	4.74	0.55	0.15	0.04

±D.S.=standard deviation.

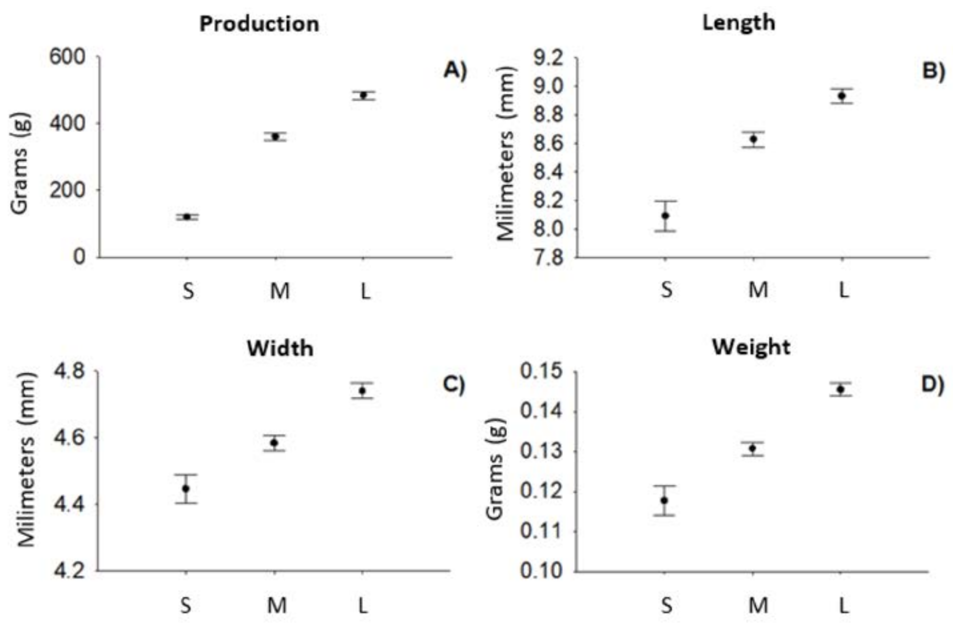

Figure 2. Contrast of the colony size of the *escamolera* ant: small (S), medium (M), and large (L), based on the mean ± standard error $(\bar{x} \pm \text{SE})$.

Foraging Paths

The colonies with four and five paths recorded the highest production, while the colony with six foraging paths registered the lowest production (Table 3). Usually, *escamolera* ant colonies have three, four, or five paths (Rafael-Valdez *et al*., 2017). The colony with six foraging paths recorded the highest *escamol* weight, followed by the colony with five paths (Table 3). Based on the method described by Rafael-Valdez *et al*. (2019), the following results were obtained: a foraging area of 607.25-2,467.2 m^2 and an average straight distance from the nest to the foraging substrate of 24.3±11.36 m.

The Kruskal-Wallis H test established differences between the number of paths per colony, regarding escamoles production (KW4, 1180=76.75, $p<0.01$; Figure 3A), length (KW4, 1180=38.94, $p<0.01$; Figure 3B), width (KW4, 1180=17.24, $p<0.01$; Figure 3C), and weight (KW4, 1180=56.97, $p<0.01$; Figure 3D).

Table 3. Production, size, and weight of the escamoles per number of paths, based on the mean and its standard deviation $(\bar{x} \pm SD)$, in Pánfilo Natera, Zacatecas.

Number of paths	Production (g)		Length (mm)		Width (mm)		Weight (gr)	
	Mean	**± D.S.**	**Mean**	**± D.S.**	**Mean**	**± D.S.**	**Mean**	**± D.S.**
2	352.49	332.32	7.77	1.38	4.34	0.64	0.10	0.04
3	328.40	235.95	8.75	1.13	4.64	0.52	0.14	0.04
4	455.06	294.38	8.74	1.12	4.66	0.54	0.13	0.04
5	412.04	248.95	8.88	1.37	4.70	0.49	0.15	0.04
6	150.97	0.00	8.10	1.14	4.58	0.36	0.16	0.06

± D.S.=standard deviation.

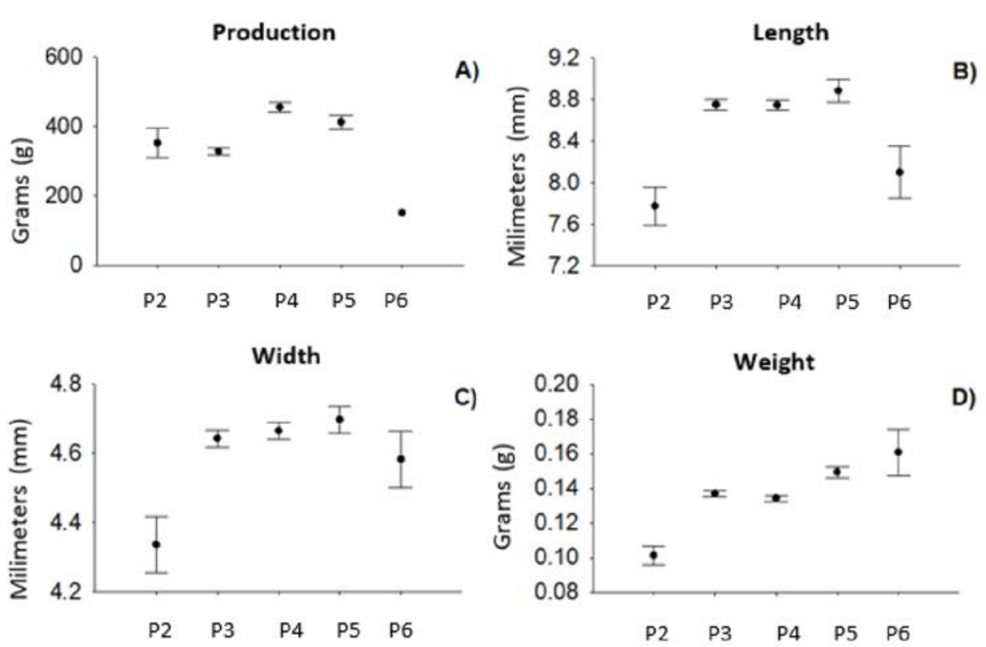

Figure 3. Contrast of the foraging paths of the *escamolera* ant colonies, based on the mean ± standard error $(\bar{x} \pm SE)$. P2: 2 foraging paths, P3: 3 foraging paths, P4: 4 foraging paths, P5: 5 foraging paths, and P6: 6 foraging paths. The *post hoc* test of the Tukey method was used for the comparison and values $<\alpha (\alpha=0.05)$ were considered significant.

Nest Type

New nests recorded the highest *escamoles* production. The escamol sizes and weights were very similar between new and disturbed nests (Table 4). Figueroa-Sandoval *et al*. (2018) evaluated 77 nests in four localities of the Potosino-Zacatecano plateau: 61 nests were disturbed, while 16 were conserved. The conserved colonies recorded a higher escamoles production (543.1 g) than the disturbed colonies (169.2 g). The study concluded that the new nests —*i.e.*, nests that had never been dug— had a higher *escamoles* production.

The comparison between types of nest established differences in *escamoles* production (U1, 1180=52 000.00, p<0.01; Figure 4A). However, there were no differences regarding escamol length (U1, 1180=63 059.00, p<0.88; Figure 4B), width (U1, 1180=62 304, p<0.71; Figure 4C), and weight (U1, 1180=61 416.50, p<0.54; Figure 4D).

Table 4. Production, size, and weight of the escamoles per nest type, based on the mean and its standard deviation $(\bar{x} \pm \text{SD})$, in Pánfilo Natera, Zacatecas.

Nest type	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
New nest	459.01	268.62	8.74	1.02	4.62	0.48	0.14	0.03
Disturbed nest	381.61	273.62	8.70	1.22	4.64	0.54	0.14	0.04

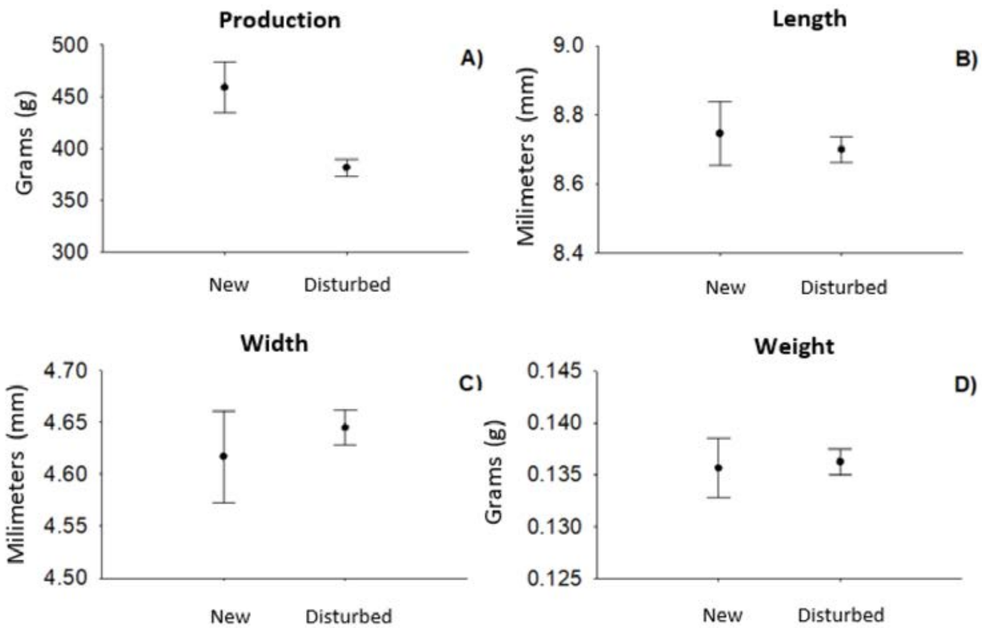

Figure 4. Nest type contrast (new and disturbed nests). The *post hoc* test of the Tukey method was used for the comparison and values $<\alpha (\alpha=0.05)$ were considered significant.

Clustering

Cluster 1 (Figure 5) (n=316 *escamoles*). This group included 8 nesting substrates; however, most of the samples (53.8%) were collected from the maguey substrate (MA) and

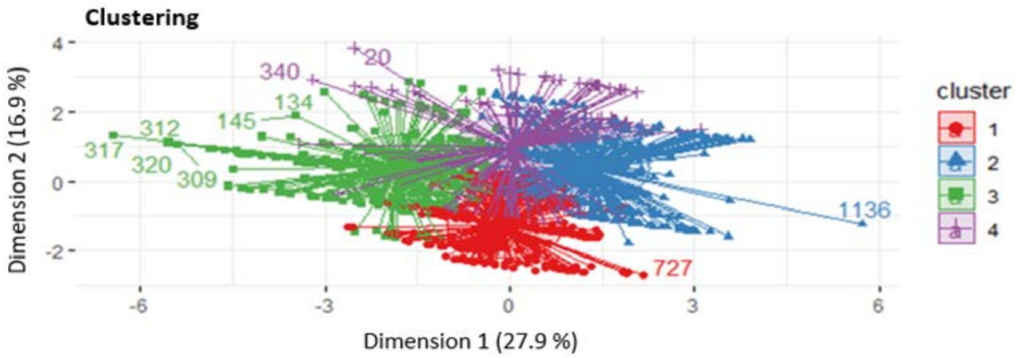

Figure 5. K-means clustering of the 4 groups. Cluster 1 (red, n=316), cluster 2 (blue, n=438), cluster 3 (green, n=306), cluster 4 (purple, n=120).

the bare soil (BS), while the lowest number of samples (0.3%) was found in the mezote (ME) substrate. Colonies with 2 foraging paths are not included in this group. Eighty-eight percent of the samples were taken from colonies with 3 and 4 foraging paths. All the nests in this group had been disturbed nest. Likewise, 77.5% of the subjects were located in medium-sized colonies. This group recorded the lowest average production of the 4 groups (244.37 g) and the minimum production (31.14 g) (Table 5).

Cluster 2 (Figure 5) (n=438 *escamoles*). This group included 7 nesting substrates; mesquite (MS) was not included in this group. The Maguey (MA) and prickly pear (PP) substrates accounted for 54.1% of the total. Only colonies with 2, 3, 4, and 5 foraging paths were found in this area. The colonies with 3 and 4 foraging paths recorded the highest number of individuals (76.6%). This group only included disturbed nests and excluded small colonies. The colonies were divided as follows: large (94%) and medium (6%). This group recorded the highest escamoles production among the 4 groups (1,271.4 g) (Table 5).

Cluster 3 (n=306 escamoles). This group recorded the lowest length (7.38 mm), width (4.10 mm), and weight (0.09 g) mean. In addition, it recorded the second maximum production (975.5 g). The highest incidence was obtained by the larger colonies (40%), all of which were disturbed nests. The colonies in this group included all the types of foraging paths (2, 3, 4, 5, and 6 paths) (Table 5). All (8) substrates were available in this area; however, the palm (PA) substrate was significantly different, because it reached 35.29% of the total, followed by prickly pear (PP) with 16.66% and maguey (MA) with 13.72%.

Cluster 4 (n=120 escamoles). This group recorded the lowest number of individuals. All the colonies included in this group were new colonies. Medium-sized colonies accounted for 50% of the total. In addition, the colonies of this group had the lowest number of foraging paths (3, 4, and 5). Nevertheless, there is an important contrast between the foraging paths, because 66.66% of the individuals were found in colonies with 4 foraging paths (Table 5). The nesting substrate of this group recorded the lowest incidence: 60.0, 16.7, and 33.3% of maguey (MA), mezote (ME), and palm (PA), respectively.

Table 5. Comparison of the groups determined using the K-means clustering, based on the dependent *escamoles* variables (production, length, width, and weight), and the group size, the mean, the standard error, and the maximum and minimum values.

Clustering	Dependent variables	Group size (n)	Mean	Standard error	Minimum	Maximum
Cluster 1	Production (g)	316	244.37	±9.47	31.14	746.73
	Length (mm)	316	9.00	±0.04	5.84	12.13
	Width (mm)	316	4.75	±0.02	3.57	5.77
	Weight (g)	316	0.14	±0.00	0.07	0.24
Cluster 2	Production (g)	438	541.73	±13.81	131.34	1271.41
	Length (mm)	438	9.40	±0.04	6.79	11.93
	Width (mm)	438	4.95	±0.02	3.82	9.70
	Weight (g)	438	0.16	±0.00	0.10	0.26
Cluster 3	Production (g)	306	294.14	±12.38	41.30	975.56
	Length (mm)	306	7.39	±0.06	1.54	9.84
	Width (mm)	306	4.11	±0.02	2.53	5.13
	Weight (g)	306	0.10	±0.00	0.03	0.19
Cluster 4	Production (g)	120	459.01	±24.52	67.02	843.54
	Length (mm)	120	8.74	±0.09	5.83	12.12
	Width (mm)	120	4.62	±0.04	3.09	5.60
	Weight (g)	120	0.14	±0.00	0.05	0.19

CONCLUSIONS

The highest *escamoles* production was recorded by the larger nests and in the mesquite substrate. Compared with colonies with other number of foraging paths, the colonies with four foraging paths recorded the best production. In addition, new nests produced more *escamoles* than disturbed nests.

The highest *escamol* individual weight (0.16 g) was recorded in the dry palm substrate —*i.e.*, 1 kg of escamoles is equal to 6,250 larvae (princesses and drones). The lowest escamol weight was recorded in the mezquite substrate (0.09 g); based on that result, 1 kg is equal to 11,111 larvae.

ACKNOWLEDGEMENTS

The authors would like to thank the Colegio de Postgraduados - Campus San Luis Potosí for the opportunity and the support in the development of this research. They would also like to thank Mr. Margarito Alfaro Briones and his family, for their support during the field research stage, at the Pámanes colony, in the municipality of Pánfilo Natera, Zacatecas.

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