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Biology of *Stigmacoccus asper* (Hemiptera: Stigmacoccidae) in Colombian High-Andean Oak Forests

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ABSTRACT

The scale insect *Stigmacoccus asper* (Hemiptera: Stigmacoccidae) is known for its interaction with oak forests and represents a sustainable alternative for honeydew honey production. However, a lack of information complicates the understanding of this interaction. Therefore, this research aimed to document aspects of the biology of *S. asper* in the oak forests of the Department of Boyacá. The study involved monitoring the life cycle and estimating population parameters under laboratory conditions at the Pedagogical and Technological University of Colombia (UPTC) in Tunja, Boyacá, Colombia. Under conditions of 18 °C and 54% relative humidity, four stages were identified (egg, nymph, cyst, and adult); the life cycle duration was approximately 39 days, with the cyst stage being the most vulnerable and the nymph stage showing the highest survival rate. The biological and ecological information obtained provides an essential tool for understanding the species and aims to incorporate its developmental cycle into the establishment of environmentally friendly practices such as apiculture. This involves the responsible use of this non-timber forest resource, with a focus on conserving High-Andean forest ecosystems.

Keywords: Life cycle, Coccoidea, Scale insect, Population parameters.

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INTRODUCTION

Records of entomofauna associated with oak forests (*Quercus humboldtii* Bonpl. 1809) are scarce [1, 2]. However, scale insects (Hemiptera: Coccoidea) have been reported in these ecosystems. These insects typically feed on angiosperms (flowering plants) by attaching themselves to various parts of the host plant (leaves, branches, roots, and twigs) and extracting nutrients from the phloem or directly from parenchymal cells [3]. The interactions between these insects and their hosts are among the most fundamental biological associations and are crucial for understanding the evolution of biodiversity, as there is often a high degree of specialization between the plants and the insects [4].

Various species of scale insects are considered important pests in agriculture; however, this group also includes beneficial species [5]. For example, the genus *Stigmacoccus* Hempe, 1900, which is primarily distributed in the Neotropics, with significant concentrations in Panama, Colombia, Venezuela, and Brazil, is mainly hosted by trees of the genera *Inga* Mill. 1754, *Cassia* L. 1753, *Quercus* L. 1753, *Schizolobium* Vogel 1837, *Bursera* Jacq. ex L. 1762, and *Psidium* L. 1753 [6]. The energy acquisition process of these scale insects involves piercing the tree and sucking sap

to utilize amino acid compounds for protein synthesis while excreting sugary compounds through an anal filament [7]. This excess of water and sugars, such as raffinose and melezitose, is commonly known as honeydew [3-8]. Honeydew provides a significant source of sugars for birds and especially for other insects like bees (Apidae), which collect it, transport it to the hive, and process it into honeydew honey [9, 10].

This substance has been exploited globally. For example, in Greece, more than 65% of honey produced by the margarodid *Marchalina hellenica* (Gennadius, 1883) comes from the Aleppo pine, *Pinus halepensis* Mill. 1768 [11]. Another successful case is from mountainous areas in California and Oregon, USA, where "white cedar honey" production has been recorded. Additionally, in New Zealand, the presence of margarodids *Ultracoelostoma assimile* (Maskell, 1890) and *Ultracoelostoma brittini* (Morales, 1991) associated with *Nothofagus* sp. Blume, 1851 (Fagaceae) forests have facilitated the establishment of beekeeping practices [12]. As cited, there is a growing body of literature documenting the importance of utilizing this natural resource worldwide.

The genus *Stigmacoccus* (Hemiptera: Stigmacoccidae) has been investigated due to its interactions with forest species and its proven ability to produce honeydew. In Brazil, Bogo *et al*. [13] detailed the relationship between *S. asper* and *Schizolobium excelsum* Vogel 1837. Similarly, Wolff *et al*. [14] studied scale insects associated with honeydew production in Rio Grande do Sul, Brazil, providing information on the distribution and host vegetation of *Stigmacoccus paranaenses* Foldi, 2006. In Colombia, Chamorro *et al*. [15] studied honeydew production in oak forests, revealing that documentation on the genus *Stigmacoccus* has been limited. Much of the research has focused on economically important scale insects, with relatively little attention to those associated with wild plants [5].

Currently, there is no specific information on the biological and ecological characteristics of the scale insect *S. asper*, including demographic parameters such as life tables, biological cycles, population dynamics, and environmental interactions. Documenting this information would provide valuable tools for understanding the species and its potential impacts [16]. This knowledge could promote the responsible use of honeydew as a natural resource, supporting ecosystem-friendly practices.

Beekeeping represents a non-timber alternative use for oak. If the primary source of honey is oak sap, oak honeydew honey could be classified as a non-timber forest product, incentivizing conservation. Therefore, studies like this are crucial for developing initiatives that promote the conservation of vulnerable oak forests [17] and the sustainable use of natural resources in these ecosystems.

This research aims to contribute to the understanding of the biological aspects of *S. asper* (Hemiptera: Stigmacoccidae) associated with the oak forest corridor, *Q. humboldtii*, in the department of Boyacá, Colombia. By studying an experimental population under controlled conditions, this research will document population parameters and describe the life cycle of this scale insect, identifying stages of higher susceptibility and proliferation. The goal is to support responsible honeydew utilization and promote effective beekeeping practices that foster the conservation of High-Andean forest ecosystems.

MATERIALS AND METHODS

Area of study

The study was conducted at the facilities of the Pedagogical and Technological University of Colombia, located in Tunja, Boyacá (lat 05°33'05'' N, long 73°21'30'' W, at 2,710 meters above sea level), in the laboratories of the Biological Crop Management Research Group (GMBC) and Bioplasma. The preparation and mounting of the collected material followed the modified protocol of Williams & Granara de Willink [18] for Pseudococcidae. Taxonomic determination of the organisms was carried out using the key by Hodgson *et al*. [6] and was confirmed by the specialist Dr. Takumasa Kondo (AGROSAVIA). For photographing and measuring the morphological description, the Leica DM750 microscope and LAS EZ version 3.0.0 software were used.

The life cycle of S. asper

Monitoring began with 11 ovisacs randomly collected from adult female *S. asper* on *Quercus humboldtii* hosts located in the North-Eastern Andes of the Department of Boyacá, specifically in the El Carmen area, Duitama-Boyacá (latitude

05°56'12.084" N, longitude 073°8'5.964" W). The samples were transported to the Biological Control of Crops - GMBC laboratory at UPTC, where a rearing batch of 1,650 eggs was established.

The eggs were placed in 60 mm Petri dishes, with 150 eggs per dish, and covered with cotton to simulate the insect's natural conditions. The eggs were maintained under stable temperature conditions of 18°C and relative humidity of 54%, monitored with a digital thermo-hygrometer (Ker Germany HTC-2). Monitoring was conducted four days a week to assess egg viability (viable/non-viable) and record hatchings. Measurements of width, length, and other morphological characteristics were documented using a Leica DM500 microscope. The life cycle of the scale insect was observed over 45 days, with daily counts (except on holidays) and using observational techniques.

First-instar nymphs (crawlers) were extracted with a brush and placed into experimental setups. These setups were located in the greenhouse of the Bioplasma Laboratory at UPTC and consisted of four *Q. humboldtii* seedlings, each approximately 75 cm in height, planted in 40 x 18 cm bags filled with a substrate composed of 15% humus, 50% black soil, 25% sand, and 10% peat. The seedlings were hydrated by spraying the substrate every other day between 07:00 and 09:00 hours with tap water, avoiding water dispersion on the foliage. Nymphs were placed on the leaves, shoots, and branches of the oak seedlings with the help of a brush. External morphological changes and behavioral data were assessed, and daily documentation of the number of survivors was maintained. Measurements were taken based on the availability of organisms at each stage, so there was no fixed number of organisms for these measurements.

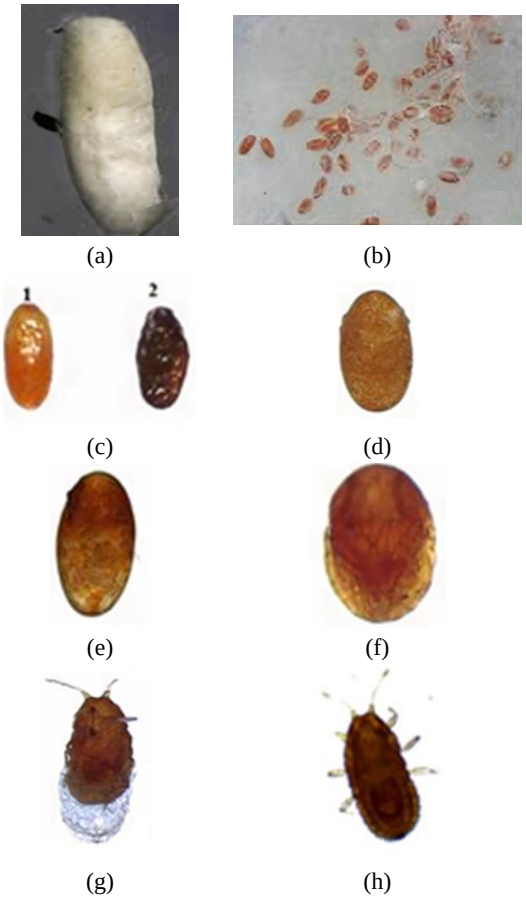
Population parameters of S. asper

Based on the survival and oviposition data for each observed female, the number of eggs per female (mx) and oviposition (x) were calculated [19]. The following parameters were considered: n_x as the number of survivors in the age interval x; d_x as the number of individuals dying between ages x and x+1; q_x as the mortality rate between ages x and x+1; l_x as the survival rate at age x; and m_x, with monitoring conducted four days a week until the emergence of adult individuals [13, 20]. These parameters were derived from a population of N = 827 eggs. Descriptive statistics were used for data exploration and analysis with R version 4.2.3.

RESULTS AND DISCUSSION

The life cycle of S. asper

Ovisacs were collected, which are characterized by more or less compact cottony secretions **(Figure 1)**. These structures are white and typically found in the cracks of oak bark. According to Hodgson *et al*. [6], the ovisac serves as an adaptation that protects the female and her offspring from adverse conditions such as desiccation and predation. It provides both protection **(Figures 1a and 1b)** and optimal conditions for the female's establishment, egg deposition, and development. Viable eggs were oval-shaped and orange in color **(Figure 1c1)**, whereas non-viable eggs were brown **(Figure 1c2)**. The eggs measured 0.43 ± 0.018 µm in width and 0.79 ± 0.016 µm in length, with developmental stages including gastrulation and structural differentiation observed **(Figures 1d-1f)** [21].



(a) (b) (c) (d) (e) (f) (g) (h)

(i) (j)

Figure 1. Life Cycle of *S. asper*. The figure illustrates different stages in the life cycle of *S. asper*. Panel (a) shows the ovisac. Panel (b) depicts the arrangement of eggs within the ovisac. Panel (c.1) presents a viable egg, while Panel (c.2) shows a non-viable egg. Panels (d), (e), and (f) illustrate eggs at 2, 6, and 14 days old, respectively. Panel (g) depicts the hatching of a nymph. Panel (h) shows a dorsal view of the nymph, Panel (i) shows a dorsal view of the cyst under a 10x microscope, and Panel (j) shows a ventral view of the adult female under a 10x microscope.

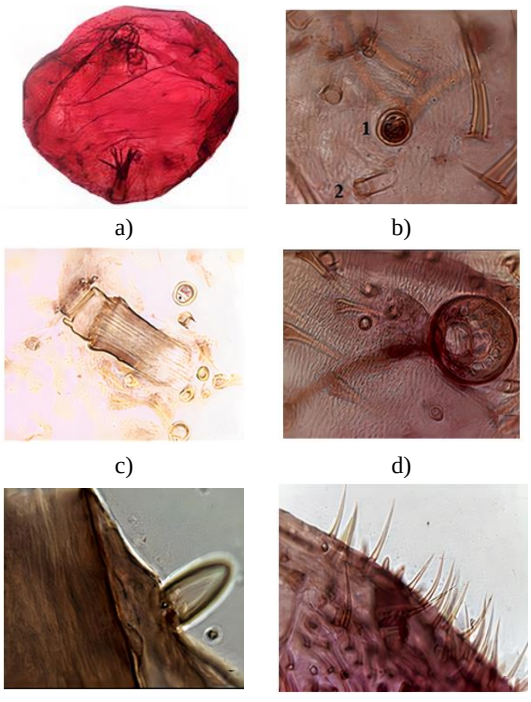
For the first instar nymphs, commonly known as crawlers **(Figure 1g)**, measurements from 14 individuals showed an average width of 464 ± 0.03 µm and a length of 833 ± 0.04 µm. These measurements are consistent with those reported by Hodgson *et al.* [6] (width 430–480 µm and length 700–770 µm), although our study recorded a 63 µm increase in length. The nymphs had well-developed locomotor appendages, with filiform antennae composed of four segments, two fewer than those reported by Hodgson *et al.* [6]. The apical segment was larger, and setae were present throughout the antennal region, resulting in a total antennal length of 377 µm ± 0.025, which is 67 µm longer than previously recorded [6].

Biological notes

Initially, nymphs exhibited restricted movement but later showed high mobility, relocating to young branches, the base of the seedling's main stem, the undersides of leaves, and among the shoots. This behavior aligns with Igua *et al.* [16], who noted that such mobility is characteristic of the dispersal stage in Coccoidea superfamily species.

Cyst stage

The cyst is slightly oval to circular **(Figure 2a)** and is marked by the presence of abdominal spiracles **(Figure 2c)**, the anus, and the mouthparts. It has a slightly sclerotized dermis covering the entire body, with spines, setae, and pores distributed across it **(Figure 2)**. The body measures 2,300 µm in length (from the mouthparts to the anus) and 2,040 µm in width. On the dorsal side, the dermis is covered with numerous dermal spines **(Figure 2f)**, measuring 46.95 µm in length. These spines are distributed from spiracle I to V and around the anal pore, with fewer spines toward the anterior area. Notably, these spines are longer than those reported by Hodgson *et al.* [6]. Additionally, conical or cone-shaped spines **(Figure 2e)** with a length of 19.34 µm were observed on various areas of the dorsal side of *S. asper*.



a) b)

c) d)

e) f)

Figure 2. Cyst State of *Stigmacoccus asper*. The figure illustrates various aspects of the cyst state of *S. asper*. Panel (a) shows the cyst mount. Panel (b.1) depicts bilocular pores, while Panel (b.2) shows tubular pores. Panel (c) displays the abdominal spiracles, and Panel (d) presents the thoracic spiracles. Panel (e) features conical or bollard-like spines, and Panel (f) shows dermal spines.

The cyst exhibited two types of pores **(Figure 2b)**. The first type, bilocular pores **(Figure 2b1)**, has a width of 14.16 µm and a length of 17.49 µm. Approximately 6 to 8 of these structures were found around the thoracic spiracles **(Figure 2d)** and were distributed in smaller numbers throughout the organism. The second type, tubular pores **(Figure 2b2)**, has a width of 9.06 µm and a depth of 19.18 µm. These tubular pores were distributed throughout the organism, with a higher concentration towards the anterior dorsal part, near the mouthparts. Between 15

and 25 tubular pores were observed around the thoracic spiracles **(Figure 2d)**.

In the anal area, three distinctive structures were present: an anal opening with a wide margin, measuring 123.12 µm in diameter, which was sclerotized. Surrounding the anal opening was a band comprising approximately eight rows of pores that increased in diameter as they moved away from the opening. While conical or bollard-like spines were not observed around the anal opening, dermal spines were found adjacent to the pore area. A sclerotized area was noted from spiracle 7 around the anal zone, which also exhibited dermal spines, and to a lesser extent, tubular and bilocular pores, with features similar to those described for *Stigmacoccus garmilleri* Foldi, 1995 [6]. The anal tube measured 101.02 µm in width and 231 µm in length, with the basal region lacking pore differentiation. The middle region featured a marked ring and slightly oval pores arranged horizontally. The anal tube also had 8 cylindrical extensions, averaging 321 µm in length, which exhibited oval pores.

The first type is the abdominal spiracles (eight pairs), which are structured in three segments **(Figure 2c)**: a basal segment with a width of 34.70 µm and a length of 54.92 µm; a middle segment with a width of 32.50 µm and a length of 11.22 µm; and a distal segment with a width of 24.19 µm and a length of 13.45 µm. The second type is the thoracic spiracles **(Figure 2d)**, located in the ventral region, with a width of 48.42 µm. There are two thoracic spiracles on the left side and two on the right side of the organism.

Biological notes

These individuals were observed on oaks, inhabiting structures such as trunks, shoots, and leaves, on both the upper and lower surfaces. It was noted that at the initial cyst stage, their coloration adapts according to their location; thus, individuals on leaves tend to have a lighter color compared to those on the trunk. Additionally, more than one honeydew-excreting filament was prominent at this early cyst stage. This observation corroborates previous documentation by Bogo *et al*. [13], who noted multiple tubes emerging from the cyst. However, only one of these tubes appears to be the primary honeydew eliminator, possibly due to damage to the other excretory tubes near the base.

Adult stage

The head, thorax, and abdomen are fused, making differentiation impossible **(Figures 1i and 1j)**. The legs are reduced **(Figure 1j)**. The adult female is wingless and sedentary, establishing itself on its host, *Q. humboldtii* **(Figure 1h)**.

Biological notes

A total of three adults were recorded, all of which settled on the main trunk, shoots, and branches of the host, with restricted movement within these areas. Based on the observed characteristics and previous discussions, including the taxonomic key by Hodgson *et al*. [6] ("A Taxonomic Review of the Margarodoid Genus *Stigmacoccus* Hempel (Hemiptera: Sternorrhyncha: Coccoidea: Stigmacoccidae)") and the expert opinion of Takumasa Kondo (AGROSAVIA), the specimens studied have been designated as *Stigmacoccus* sp. nr. *asper*.

Population parameters of S. asper

The ovisacs contained an average of 24–137 ± 34.21 eggs per clutch. These findings are consistent with those reported by Hodgson *et al*. [6], who found that females of this genus lay approximately 70 eggs. In contrast, Henderson [22] reports clutches of 60 to 80 eggs for Hemiptera: Diaspididae. Arévalo-Maldonado *et al*. [19] report clutches of 140 to 200 eggs per female in *Eurhizococcus colombianus* Jakubski, 1965 (Hemiptera: Margarodidae). Similarly, Igua *et al*. [16] documented clutches of 150 to 250 eggs per female in *Protortonia ecuadorensis* Foldi, 2006 (Hemiptera: Monophlebidae). Van Duyn and Murphey [23] reported an average of 125 eggs in *Pseudaulacaspis pentagona* (Hemiptera: Diaspididae). The number of eggs per female varies with species, environmental factors, host type, and temporal conditions [18, 24, 25].

In a cohort of 1,650 eggs, 267 progressed to the nymph stage, 61 to the cyst stage, and 3 to the adult stage, illustrating a hypothetical Type III survival curve **(Figure 3)**. For comparison, *Phenacoccus solenopsis* Tinsley, 1898 (Pseudococcidae: Hemiptera) also exhibits a Type III survival curve, with populations transitioning from nymph I = 300, nymph II = 291, nymph III = 66, to adult females = 19 [25]. For *S. asper*, a higher survival rate is observed at the nymph stage (l_x = 0.445), though this rate decreases as the life cycle progresses, similar to the early high survival rates of *P. solenopsis*,

which exceed 80% between 27 and 32°C. Conversely, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae) [24], from a population of N = 125 eggs, exhibited survival rates of nymph I = 97, nymph V = 76, and adults = 45 [26]. For *E. colombianus*, an initial population of N = 100 neonate nymphs from a single female showed a hypothetical Type II survival curve [19], a pattern also seen in *P. ecuadorensis* [16].

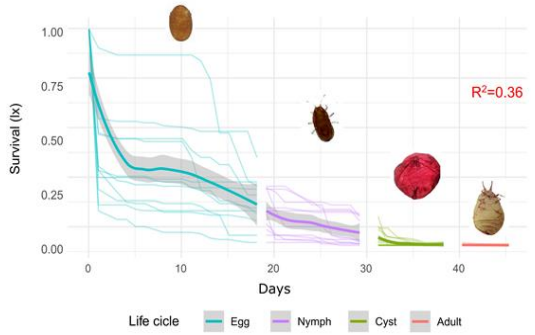


Figure 3. Survival index (l_x) of *S. asper*. The x-axis represents the stages: 1 = egg stage, 2 = nymph stage, 3 = cyst stage, and 4 = adult stage

The mortality index (q_x) **(Table 1)** shows a high mortality rate in the cyst stage (q_x = 0.951), with only 3.26% of organisms at this stage transitioning to adulthood, compared to 16.18% at the egg stage, which subsequently leads to the establishment of nymphs **(Table 1)**. This mortality and development pattern resembles that observed in *Gargaphia torresi* Costa Lima 1922 (Hemiptera: Tingidae), which showed low initial mortality rates in eggs but an increase in mortality during the nymph stage [27].

Table 1. Life Table of a Cohort (n = 1,650) of *S. asper* Under Laboratory Conditions in Tunja, Boyacá. The table presents the following parameters: n_x, the number of survivors in the age interval x; d_x, the number of individuals dying between ages x and x+1; l_x, the survival index at age x; and q_x, the mortality rate between ages x and x+1.

Stage	nx	dx	lx	qx
Egg	1650	1332	0.193	0.807
Nymph	267	148	0.445	0.555
Cyst	92	85	0.076	0.924
Adult	3	2	0.333	0.667

The embryonic phase of *S. asper* has an average duration of 8.06 ± 4.15 days **(Table 2)**. This incubation period can vary widely among different species, ranging from 3.21 days in *D. citri* Kuwayama [24] to 60.8 ± 4.4 days in *P. ecuadorensis* [16]. For *S. asper*, the duration of the life cycle from the start of observations to the appearance of the first adult individual averages 42.33 ± 6.64 days.

Table 2. Average Duration (± Standard Deviation) in Days of the Life Cycle of *S. asper* on *Q. humboldtii* Under Laboratory Conditions in Tunja, Boyacá. The table presents the average durations of different life stages of *S. asper* with a mean temperature of 18°C and an average relative humidity of 54%.

Stage	N	Mean ± S.D. (Days)	Range (Min-Max)
Egg	1650	8.06 ± 4.15	0 - 18
Nymph	267	16.27 ± 3.91	19 - 29
Cyst	92	9.24 ± 2.30	31 - 38
Total, Nymph Stage		33.57 ± 6.15	
Adul	3	8.76 ± 2.52	40 - 45
Total		42.33±6.64	

The results are consistent with those observed for *P. solenopsis*, which has a life cycle of approximately 39.5 days [25]. This duration aligns with data from tropical regions, where the life cycle of scale insects can be as short as less than a month [28]. The number of annual generations can vary widely among and within species, ranging from one to seven or eight generations per year. In contrast, species such as *P. ecuadorensis,* with a life cycle of 301.8 ± 40.5 days [16], and *E. colombianus,* with a duration of 218 ± 9.89 days [19], are characterized as univoltine, producing only one generation per year. Annual life cycles are also more common in regions with lower temperatures [29].

CONCLUSION

This exploratory and descriptive study establishes a baseline for understanding the life cycle and population parameters of *S. asper*. By collecting and analyzing ex-situ data, the study has detailed the various developmental stages of this insect, including the ovisac, egg, nymph, cyst, and adult, each characterized by distinct morphological and biological features. The average life cycle of *S. asper*, spanning approximately 42 days, along with observed mortality rates—particularly during the cyst stage—provides valuable insights into the species' reproductive strategies and developmental challenges.

The high mortality rate observed at the cyst stage and the egg clutch size suggest that honey producers relying on oak honey should develop strategies to mitigate the developmental pressures faced by *S. asper*. Understanding these aspects will help in devising effective management practices to support the sustainable use of this natural resource.

Moreover, this information supports decision-making aimed at promoting efficient apicultural practices, including the use of honeydew by bees, its collection, transport to the hive, and processing. This would ultimately facilitate the production of honeydew honey. As the primary source of this honey would be oak sap, oak honeydew honey could be classified as a non-timber forest product. Such an approach represents a valuable initiative for the conservation of high Andean forest ecosystems, given the current vulnerability of oak forests in Colombia.

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