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Biological Study of Stigmacoccus asper within the Unique Ecosystem of Colombian High-Andean Oak Forests

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ABSTRACT

The scale insect *Stigmacoccus asper* (Hemiptera: Stigmacoccidae) plays an important role in oak forest ecosystems and has potential applications in honeydew honey production. Despite its importance, knowledge gaps limit a full understanding of its ecological interactions. This study investigates the biological traits of *S. asper* within oak forests in the Department of Boyacá. Research efforts focused on tracking its development and evaluating population dynamics under controlled laboratory settings at the Pedagogical and Technological University of Colombia (UPTC) in Tunja, Boyacá, Colombia. Observations conducted at 18 °C with 54% relative humidity identified four developmental stages: egg, nymph, cyst, and adult. The complete cycle lasted approximately 39 days, with the cyst phase showing the highest vulnerability, whereas the nymph stage showed optimal survival rates. Insights gained from this study increase understanding of *S. asper* and support its potential incorporation into sustainable beekeeping initiatives. Promoting responsible use of this non-timber forest resource aligns with conservation efforts for high-Andean forest habitats.

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

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Introduction

Documentation on insect communities linked to oak forests (*Quercus humboldtii* Bonpl. 1809) remains limited [1, 2]. Among the species recorded in these ecosystems, scale insects (Hemiptera: Coccoidea) are notable. These insects are specialized in feeding on angiosperms by attaching to different parts of their host, such as leaves, branches, twigs, and roots, extracting nutrients either directly from parenchymal cells or the phloem [3]. The ecological relationship between those insects and their host plants plays a fundamental role in biodiversity, as high degrees of specialization often exist between specific insect species and their plant hosts [4].

While some scale insect species are recognized as agricultural pests, others provide ecological or economic benefits [5]. The genus *Stigmacoccus* Hempe, 1900, predominantly distributed in the Neotropics, is particularly found in Colombia, Panama, Venezuela, and Brazil. Its primary host trees belong to the genera *Inga* Mill. 1754, *Cassia* L. 1753, *Quercus* L. 1753, *Schizolobium* Vogel 1837, *Bursera* Jacq. ex L. 1762, and *Psidium* L. 1753 [6]. These insects sustain themselves by piercing the bark of trees and extracting sap, which is utilized for protein synthesis. The excess sugars and water they excrete through an anal filament form a substance known as honeydew, which consists of sugars like raffinose and melezitose [3-8]. This honeydew is an essential food source for various organisms, particularly bees, which collect it, transport it to their hives, and convert it into honeydew honey [9, 10].

The exploitation of honeydew as a resource has been well-documented worldwide. For instance, in Greece, over 65% of the honey derived from *Marchalina hellenica* (Gennadius, 1883) originates from *Pinus halepensis* Mill. 1768 [11]. Similarly, in California and Oregon, USA, the production of "white cedar honey" has been reported. In New Zealand, beekeeping activities have flourished due to the presence of margarodid species such as *Ultracoelostoma assimile* (Maskell, 1890) and *Ultracoelostoma brittini* (Morales, 1991), which are associated with Nothofagus sp. Blume, 1851 (Fagaceae) forests [12]. These cases highlight the increasing global recognition of honeydew as a valuable natural resource.

The genus *Stigmacoccus* (Hemiptera: Stigmacoccidae) has drawn interest due to its ecological relationships with forest vegetation and its potential for honeydew production. In Brazil, Bogo *et al*. [13] examined the interaction between *Stigmacoccus asper* and *Schizolobium excelsum* Vogel 1837. Similarly, Wolff *et al*. [14] investigated scale insects linked to honeydew production in Rio Grande do Sul, Brazil, detailing the distribution and host plants of *Stigmacoccus paranaenses* Foldi, 2006. In Colombia, Chamorro *et al*. [15] explored honeydew production in oak forests, emphasizing the lack of studies on *Stigmacoccus*. Most existing research has prioritized economically significant scale insects, while those associated with wild plant species remain underexplored [5].

Currently, little is known about the biological and ecological traits of the scale insect *S. asper*, including key demographic factors such as life cycle progression, population trends, and interactions with its environment. Filling these knowledge gaps would provide essential insights into the species and its ecological role [16]. A deeper understanding of these aspects could facilitate the sustainable use of honeydew as a resource, encouraging environmentally responsible practices.

Beekeeping presents a sustainable alternative for utilizing oak forests without harvesting timber. When honeydew derived from oak trees serves as the primary nectar source, the resulting honey can be classified as a non-timber forest product, thereby reinforcing conservation efforts. For this reason, research of this nature is essential to advancing strategies aimed at protecting threatened oak forests [17] while promoting the sustainable management of their natural resources.

This study seeks to enhance scientific knowledge of *S. asper* (Hemiptera: Stigmacoccidae) and its association with the *Q. humboldtii* oak forest corridor in Boyacá, Colombia. By observing an experimental population in controlled conditions, this study will analyze population parameters and map out the insect's developmental stages, identifying phases of increased vulnerability and peak proliferation. The findings will contribute to responsible honeydew exploitation and encourage beekeeping approaches that support the preservation of High-Andean forest ecosystems.

Materials and Methods

Study location

This research was carried out at the Pedagogical and Technological University of Colombia (UPTC) in Tunja, Boyacá. The study site is situated at a latitude of 05°33'05'' N and a longitude of 73°21'30'' W, at an elevation of 2,710 meters above sea level. Laboratory work was conducted within the *Bioplasma facilities* and Biological Crop Management Research Group (GMBC). The collected specimens were prepared and mounted following a modified version of the protocol by Williams and Granara de Willink [18] for Pseudococcidae. Identification of the species was performed using the taxonomic key developed by Hodgson *et al*. [6] and was further verified by Dr. Takumasa Kondo (AGROSAVIA). Morphological analyses, including imaging and measurements, were conducted with a Leica DM750 microscope and LAS EZ version 3.0.0 software.

Life cycle observation of S. asper

The investigation began by collecting 11 ovisacs from adult *S. asper* females residing on *Q. humboldtii* trees in the North-Eastern Andes of the Department of Boyacá, specifically in the El Carmen sector of Duitama-Boyacá (latitude 05°56'12.084" N, longitude 073°8'5.964" W). These samples were then transferred to the GMBC laboratory at UPTC, where a controlled breeding population of 1,650 eggs was established.

Eggs were distributed into 60-millimeter Petri dishes, with each dish holding 150 eggs, and put with cotton to mimic natural conditions. The experimental environment was maintained at a constant temperature of 18 °C and 54% relative humidity and monitored using a digital thermo-hygrometer (Ker Germany HTC-2). Observations took place four times a week to evaluate egg viability and record hatching events. Morphological measurements,

including length and width, were recorded using a Leica DM500 microscope. The full life cycle of the species was monitored over 45 days, with daily data collection except on holidays.

Newly hatched first-instar nymphs (crawlers) were carefully transferred using a fine brush into a controlled experimental setup. This setup was housed in the greenhouse at the Bioplasma Laboratory (UPTC) and included four *Q. humboldtii* seedlings, each approximately 75 cm tall, planted in 40 × 18 cm bags containing a substrate mix of 50% black soil, 25% sand, 15% humus, and 10% peat. The seedlings were watered every other morning between 07:00 and 09:00 using tap water, ensuring that only the substrate received moisture to prevent foliage wetting. Nymphs were manually placed on the young shoots, leaves, and branches of the seedlings using a brush. Morphological changes, survival rates, and behavioral patterns were systematically recorded, with daily observations continuing throughout the study. Since the number of available specimens varied at different developmental stages, measurements were taken opportunistically based on organism availability rather than a fixed sample size.

Population parameters of S. asper

To assess population dynamics, survival rates, and reproductive output, data on oviposition and the number of eggs per female (m_x) were recorded for each observed female [19]. Several key demographic parameters were analyzed: n_x (number of individuals surviving at a given age x), d_x (individuals that perished between age intervals x and x+1), q_x (mortality rate between x and x+1), l_x (survival probability at age x), and m_x, with data collection taking place four times a week until adult emergence [13, 20].

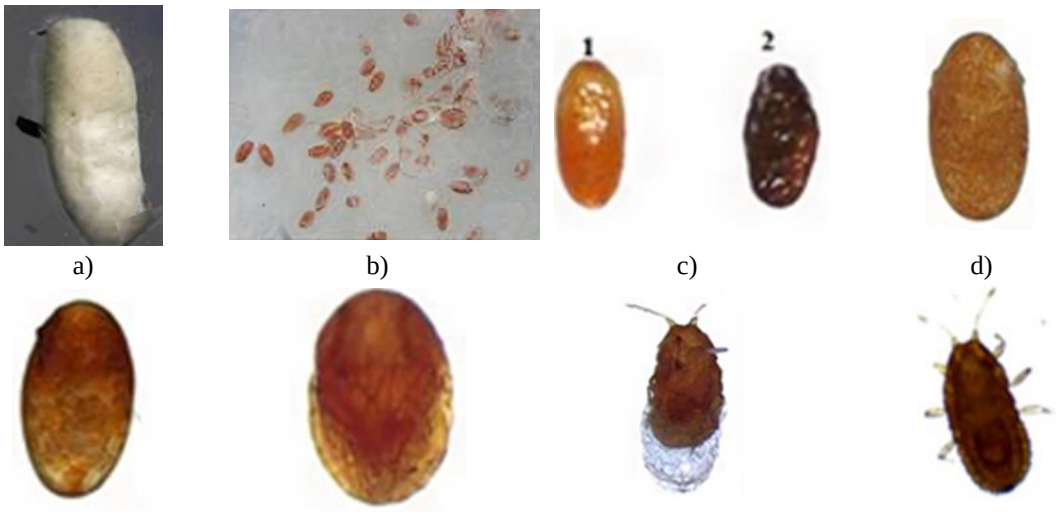
These calculations were performed using a dataset of n = 827 eggs. Descriptive statistical methods were applied to explore and interpret the data, and all analyses were conducted using R software (version 4.2.3).

Results and Discussion

Life cycle of S. asper

The collected ovisacs exhibited dense, cotton-like secretions **(Figure 1)**, forming compact white masses typically found within the crevices of oak bark. As noted by Hodgson *et al*. [6], these structures function as a protective adaptation, shielding both the female and her offspring from environmental stressors such as dehydration and predation. The ovisac not only ensures safety **(Figures 1a and 1b)** but also creates a suitable environment for egg-laying and early development.

Viable eggs displayed an orange hue and an elongated oval form **(Figure 1c1)**, whereas non-viable ones appeared brown **(Figure 1c2)**. The eggs measured approximately 0.43 ± 0.018 µm in width and 0.79 ± 0.016 µm in length. Throughout development, key embryonic stages such as gastrulation and structural differentiation were identified **(Figures 1d-1f)** [21].



a) b) c) d)

e) f) g) h)


i) j)

Figure 1. Developmental stages of *S. asper*; this figure visually outlines the various stages in the life cycle of *S. asper*; panel. a) highlights the ovisac, while panel, b) provides a view of the eggs arranged within it; a viable egg is depicted in Panel, c.1), whereas Panel, c.2) shows a non-viable egg; embryonic development is illustrated at different time points: 2 days in panel, d), 6 days in panel, e), and 14 days in panel, f); the moment of nymph emergence is captured in panel, g); a dorsal perspective of the nymph is displayed in panel, h), while panel, i) presents a dorsal view of the cyst under 10x magnification; lastly, panel, j) shows the ventral side of an adult female at the same magnification level.

First-instar nymphs (Crawlers)

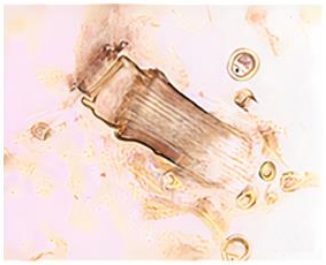
The initial developmental stage, commonly referred to as crawlers **(Figure 1g)**, was analyzed based on measurements from fourteen individuals. The average width was recorded at 464 ± 0.03 µm, while the length averaged 833 ± 0.04 µm. These values align with the range provided by Hodgson *et al*. [6] (430–480 µm in width and 700–770 µm in length), though this study observed an increase of 63 µm in length. The crawlers exhibited well-developed locomotor appendages, and their antennae were filiform, consisting of four segments—two fewer than previously documented by Hodgson *et al*. [6]. The apical segment appeared more elongated, and setae were distributed along the antennal region, bringing the total antennal length to 377 ± 0.025 µm, which is 67 µm greater than the values previously reported [6].

Biological observations

At first, the nymphs exhibited minimal movement, but as they matured, they became highly mobile, dispersing to young twigs, the lower portions of the undersides of leaves, the main stem, and emerging shoots. This pattern of movement aligns with observations by Igua *et al*. [16], who described similar dispersal behavior in species within the Coccoidea superfamily.

Cyst stage

The cyst stage appears as a slightly circular to oval structure **(Figure 2a)** and is characterized by the presence of abdominal spiracles, the anal opening, and mouthparts. The body is encased in a lightly sclerotized dermis with setae, spines, and pores distributed across its surface **(Figure 2)**. The cyst's dimensions were measured at 2,300 µm in length (from the mouthparts to the anal region) and 2,040 µm in width. The dorsal surface contained numerous dermal spines **(Figure 2)**, averaging 46.95 µm in length. These structures were concentrated around spiracles I to V and near the anal pore, with fewer spines in the anterior region. Notably, the spines in this study were longer than those recorded by Hodgson *et al*. [6]. Additionally, conical spines **(Figure 2)**, measuring 19.34 µm in length, were distributed across the dorsal region of *S. asper*.


a) b) c)

Figure 2. Cyst stage of *S. asper*; this figure presents different morphological features of the cyst stage in *S. asper*; panel (a) provides an overview of the cyst mount; panel (b) focuses on the abdominal spiracles; panel (c) depicts conical or bollard-like spines.

Pore characteristics and distribution in the cyst

The cyst structure exhibited two distinct categories of pores **(Figure 2)**. The 1st type, known as bilocular pores **(Figure 2)**, measured 14.16 µm across and 17.49 µm in length. These pores were concentrated around the thoracic spiracles **(Figure 2)**, typically appearing in small clusters of six to eight, with fewer distributed across other regions. The second category, referred to as tubular pores **(Figure 2)**, was measured at 9.06 µm in width and extended to a depth of 19.18 µm. These pores were widespread over the cyst's surface but were particularly dense in the anterior dorsal region, notably in proximity to the mouthparts. A significant concentration of tubular pores, ranging between 15 and 25, was noticed around the thoracic spiracles **(Figure 2)**.

Morphological features of the anal region

The posterior section of the cyst displayed three distinctive structures. The primary feature was a sclerotized anal opening with a broad margin, measuring 123.12 µm in diameter. Surrounding this opening was a sequence of approximately eight concentric rows of pores, which increased in size as they extended outward. While dermal spines were visible near this region, there were no conical or bollard-like spines present. A rigid, sclerotized section was evident from spiracle 7 to the anal area, exhibiting dermal spines along with a reduced presence of bilocular and tubular pores. These traits are closely aligned with those documented in *Stigmacoccus garmilleri* Foldi, 1995 [6]. The anal tube had a width of 101.02 µm and a length of 231 µm, with the basal section showing no visible pore structures. Its middle region contained a well-defined ring formation and horizontally arranged oval pores. At the distal end, the anal tube featured eight cylindrical projections, averaging 321 µm in length, each containing oval pores.

Spiracle morphology and arrangement

Two distinct types of spiracles were identified in the cyst. The first type, the abdominal spiracles **(Figure 2)**, was arranged in eight pairs and exhibited a three-part segmentation. The basal section measured 34.70 µm in width and 54.92 µm in length, followed by a middle segment measuring 32.50 µm in width and 11.22 µm in length. The final distal segment was recorded at 24.19 µm in width and 13.45 µm in length. The second type, the thoracic spiracles **(Figure 2)**, was located on the ventral side of the organism and had a width of 48.42 µm. These spiracles were symmetrically positioned, with two found on each side.

Ecological and developmental observations

Individuals were identified on oak trees, inhabiting multiple plant structures, including trunks, shoots, and the lower and upper leaf surfaces. During the early cyst phase, their coloration varied depending on their specific habitat. Those residing on leaves exhibited a noticeably lighter shade than those found on the trunk. Moreover, more than 1 honeydew-excreting filament was visible in this phase. These results are consistent with the observations of Bogo *et al*. [13], who described multiple excretory tubes emerging from the cyst. However, it appears that only one tube functions as the main channel for honeydew elimination, possibly because of damage sustained by the others near their base.

Morphological features of the adult stage

Upon reaching adulthood, the distinction between the thorax, head, and abdomen becomes imperceptible due to the fusion of these segments **(Figures 1i and 1j)**. The legs are significantly reduced **(Figure 1j)**. The adult female is apterous and remains sessile on its host, *Q. humboldtii* **(Figure 1h)**, where it establishes a fixed position.

Additional observations on adult specimens

Three adult individuals were recorded, all of which were confined to the trunk, branches, and shoots of the host plant, with minimal movement beyond these regions. Through a combination of morphological analysis, the taxonomic key outlined by Hodgson *et al*. [6] in A Taxonomic Review of the Margarodoid Genus *Stigmacoccus* Hempel (Hemiptera: Sternorrhyncha: Coccoidea: Stigmacoccidae), and expert evaluation by Takumasa Kondo (AGROSAVIA), these specimens have been identified as *S.* sp. nr. *asper*.

Reproductive parameters of S. asper

The ovisacs contained between 24 and 137 eggs, with an average clutch size of 34.21 eggs. These numbers are in line with Hodgson *et al*. [6], who reported that members of this genus generally lay around 70 eggs. Clutch sizes can vary among different Hemipteran families. For instance, Henderson [22] found that Diaspididae species

typically lay 60 to 80 eggs per clutch. In contrast, Arévalo-Maldonado *et al*. [19] documented that *Eurhizococcus colombianus* Jakubski, 1965 (Hemiptera: Margarodidae) produces between 140 and 200 eggs per female. Similarly, *Protortonia ecuadorensis* Foldi, 2006 (Hemiptera: Monophlebidae) was reported by Igua *et al*. [16] to lay between 150 and 250 eggs. Additionally, research by Van Duyn and Murphey [23] on *Pseudaulacaspis pentagona* (Hemiptera: Diaspididae) indicated a mean of 125 eggs per female. Variability in reproductive output is influenced by species-specific traits, environmental conditions, host plant characteristics, and seasonal changes [18, 24, 25].

Survival trends in S. asper

From an initial population of 1,650 eggs, 267 reached the nymph stage, 61 progressed to the cyst stage, and only 3 developed into adults. This trend follows a hypothetical Type III survival curve **(Figure 3)**. A similar pattern has been observed in *Phenacoccus solenopsis* Tinsley, 1898 (Pseudococcidae: Hemiptera), where the population declines from 300 first-instar nymphs to 291 in the second instar, 66 in the third instar, and 19 adult females [25]. For *S. asper*, survival rates peaked during the nymph stage (l_x = 0.445) before declining in later stages. This trend resembles *P. solenopsis*, where survival rates exceed 80% in temperatures ranging from 27 to 32 °C. In contrast, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae) exhibited a different survival trajectory: out of 125 eggs, 97 reached the first nymphal stage, 76 survived to the fifth stage, and 45 matured into adults [26]. Meanwhile, E. *colombianus* displayed a type II survival curve, where an initial cohort of 100 neonate nymphs from a single female followed a different survival pattern [19], a trend similarly observed in *P. ecuadorensis* [16].

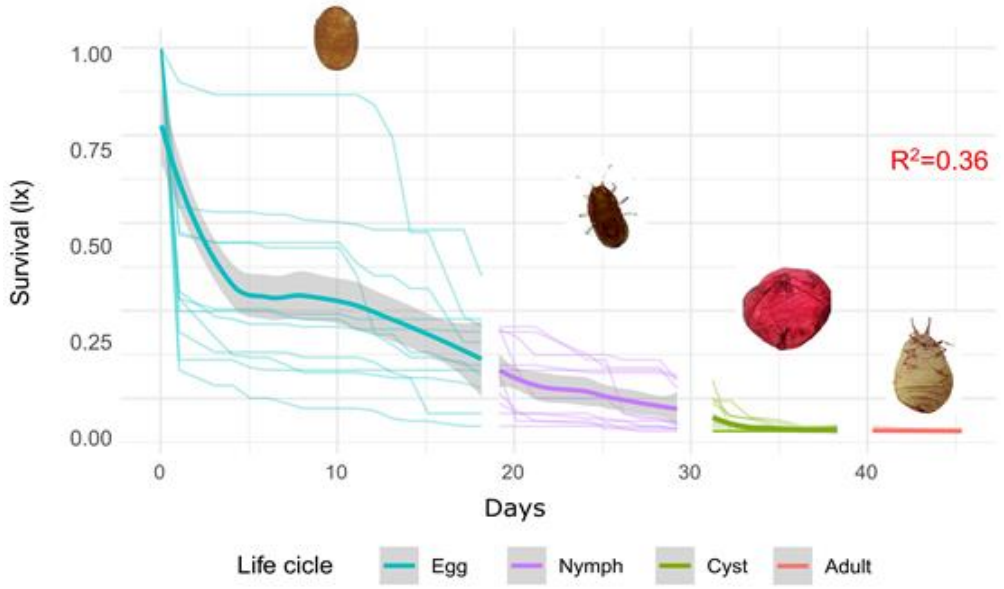


Figure 3. Survival index (l_x) of *S. asper*; the x-axis represents the developmental stages: (1) egg stage, (2) nymph stage, (3) cyst stage, and (4) adult stage.

The mortality index (q_x) **(Table 1)** indicates that the highest mortality occurs during the cyst stage (q_x = 0.951), with only 3.26 percent of individuals successfully reaching adulthood. In contrast, at the egg stage, mortality is lower (16.18%), allowing for the formation of nymphs **(Table 1)**. This pattern of survival and mortality is comparable to what has been documented in *Gargaphia torresi* Costa Lima, 1922 (Hemiptera: Tingidae), where egg mortality remains relatively low, but a significant increase in mortality is observed during the nymphal stage [27].

Table 1. Life table for an *S. asper* Cohort (n = 1,650) maintained under laboratory conditions in Tunja, Boyacá.

Stage	*n_x*	*d_x*	*l_x*	*q_x*
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

This table provides key demographic parameters: *n_x* represents the number of surviving individuals at a given stage; *d_x* denotes the number of individuals that perish between stages *x* and *x+1*; *l_x* indicates the survival index at each stage; and *q_x* reflects the mortality rate from one stage to the next.

The embryonic stage of *S. asper* lasts an average of 8.06 ± 4.15 days **(Table 2)**. The incubation period differs significantly across species, with durations as short as 3.21 days in *D. citri* Kuwayama [24] and extending up to 60.8 ± 4.4 days in *P. ecuadorensis* [16]. For *S. asper*, the complete cycle of life, from the initial observations to the emergence of the 1st adult, spans an average of 42.33 ± 6.64 days.

Table 2. Mean duration (± standard deviation) of the life cycle of *S. asper* on *Q. humboldtii* under controlled conditions in Tunja, Boyacá.

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	
Adult	3	8.76 ± 2.52	40 - 45
Total		**42.33 ± 6.64**	

This table outlines the average time for each developmental stage of *S. asper*, recorded at a mean temperature of 18 °C and an average relative humidity of 54%.

The findings align with observations made for *P. solenopsis*, which completes its life cycle in roughly 39.5 days [25]. This timeframe is consistent with studies conducted in tropical environments, where some scale insect species complete their development in less than one month [28]. The number of generations per year varies significantly across species, with some exhibiting as few as one and others producing up to 7 or 8 annual generations. In contrast, *P. ecuadorensis* follows a much longer life cycle, averaging 301.8 ± 40.5 days [16], while *E. colombianus* takes approximately 218 ± 9.89 days to reach maturity [19]. These species are considered univoltine, meaning they generate only a single generation per year. A similar trend is often observed in colder climates, where insect life cycles tend to extend over longer periods [29].

Conclusion

This descriptive and exploratory research provides foundational knowledge on the cycle of life and population dynamics of *S. asper*. Through ex-situ observations, the study has documented the insect's developmental phases, from the ovisac and egg stage to the cyst, nymph, and adult stages, each marked by distinct biological and morphological traits. With a mean life cycle of roughly 42 days and significant mortality, particularly during the cyst stage, the findings offer valuable insights into the species' reproductive strategies and survival challenges.

The elevated mortality rate during the cyst stage, coupled with the observed clutch size, highlights the need for honey producers utilizing oak honeydew to develop strategies that minimize developmental constraints faced by *S. asper*. A deeper understanding of these dynamics can contribute to the development of targeted management approaches that enhance the sustainability of this natural resource.

Furthermore, the findings support informed decision-making to improve apicultural efficiency, particularly regarding honeydew collection by bees, its transport, and subsequent honey production. Since the primary origin of this honey is oak sap, oak honeydew honey can be categorized as a non-timber forest product. This perspective presents an opportunity to reinforce conservation initiatives for high Andean forest ecosystems, especially in light of the ongoing threats to Colombia's oak forests.

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Ethics Statement: None

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