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Strategies for detection and monitoring of the small hive beetle (*Aethina tumida*) in Africanized honeybee colonies in Costa Rica

Estrategias para la detección y monitoreo del Pequeño Escarabajo de la Colmena (*Aethina tumida*) en colonias de abejas africanizadas en Costa Rica

Estratégias para a detecção e monitoramento do Pequeno Besouro da Colmeia (Aethina tumida) em colônias de abelhas africanizadas na Costa Rica"

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Abstract

The small hive beetle (SHB), *Aethina tumida*, is a pest of honeybee (*Apis mellifera*) colonies. Endemic to sub-Saharan Africa, this pest has been reported in Africanized honeybee (AHB) colonies in North, Central, and South America. Specifically in Central America, it was first found in El Salvador in 2013, in Nicaragua in March 2014, and in Guatemala in August 2020. In Nicaragua, SHB was confirmed in AHB colonies in San Juan del Sur, Department of Rivas, approximately eight kilometers north of the Costa Rican border, which increased the risk of entry to this country. After SHB was confirmed in Nicaragua, a sentinel apiary with four beehives was established close to the border in Santa Cecilia, La Cruz, province of Guanacaste, Costa Rica. In addition, epidemiological surveillance was conducted in the main beekeeping areas in the country (2015-2022) to determine SHB's possible distribution. Hives were monitored visually by examining all individual honeycombs, hive covers, and bottom boards. Furthermore, training was offered to beekeepers such as workshops and fieldwork, and training materials were distributed such as brochures focused on SHB recognition and identification and methods for colony inspection. SHB was detected and confirmed in the sentinel apiary in August 2015 in La Cruz, Guanacaste, Costa Rica, where only adult beetles were detected inside AHB colonies. To date, in collaboration with trained beekeepers, SHB has been found in different commercial apiaries, mainly in the province of Guanacaste. In conclusion, implementing strategies to detect and monitor SHB, as it spreads to new countries or beekeeping areas, requires implementing sentinel apiaries, developing epidemiological surveillance, and providing training activities for beekeepers, as demonstrated in the case in Costa Rica.

Keywords: small hive beetle, *Aethina tumida*, sentinel apiaries, Africanized honeybee

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Resumen

El Pequeño Escarabajo de la Colmena (PEC), *Aethina tumida*, es una plaga de las abejas *Apis mellifera*. Este escarabajo es endémico de África sub-Sahariana y ha sido reportado en colmenas de abejas africanizadas en Norte, Centro y Suramérica. Específicamente en Centroamérica, fue detectado en El Salvador en el 2013, en Nicaragua en marzo 2014 y en Guatemala en agosto 2020. En Nicaragua, fue confirmada su presencia en abejas africanizadas en San Juan del Sur, departamento de Rivas, aproximadamente a ocho kilómetros al norte de la frontera con Costa Rica, lo cual incrementó el riesgo de ingreso a este país. Luego de la confirmación del Pequeño Escarabajo en Nicaragua, se estableció en Santa Cecilia, La Cruz, provincia de Guanacaste, Costa Rica (cerca de la frontera), un apiario sentinela constituido por cuatro colmenas. Además, para determinar su posible distribución, se realizó vigilancia epidemiológica (2015-2022) en las principales zonas apícolas del país. Las colmenas se monitorearon visualmente, examinando los panales, la tapa y el fondo. Asimismo, se ejecutaron actividades de capacitación, como talleres y días de campo, dirigidos a los apicultores. Además, se entregó material educativo, como manuales, orientados al reconocimiento del escarabajo, así como a métodos para la inspección de las colmenas. El Pequeño Escarabajo de la Colmena fue detectado y confirmado en La Cruz, Guanacaste, Costa Rica, en el apiario sentinela, en agosto 2015, encontrando en el interior de las colmenas, únicamente escarabajos adultos. A la fecha, este escarabajo ha sido detectado en diferentes apiarios, especialmente en la provincia de Guanacaste, con la colaboración de los apicultores que se capacitaron. En conclusión, la implementación de estrategias para la detección y monitoreo del Pequeño Escarabajo, a medida que se desplaza hacia nuevos países o zonas apícolas, requiere como se ilustra en el caso de Costa Rica, la implementación de apiarios sentinela, así como el desarrollo de vigilancia epidemiológica y la ejecución de actividades de capacitación dirigidas a los apicultores.

Palabras clave: Pequeño Escarabajo de la Colmena, *Aethina tumida*, apiarios sentinela, abejas africanizadas

Resumo

O Pequeno Besouro da Colmeia (PBC), Aethina tumida, é uma praga das abelhas Apis mellifera. Este besouro é endêmico da África sub-Saariana e tem sido relatado em colônias de abelhas africanizadas em diferentes países da América do Norte e do Sul. Especificamente na América Central, foi detectado em El Salvador em 2013, na Nicarágua em março de 2014 e na Guatemala em agosto de 2020. Na Nicarágua, sua presença foi confirmada em abelhas africanizadas em San Juan del Sur, departamento de Rivas, aproximadamente oito quilômetros ao norte da fronteira com a Costa Rica, o que aumentou o risco de entrada neste país. Após a confirmação do Pequeno Besouro na Nicarágua, foi estabelecido em La Cruz, Guanacaste, Costa Rica (perto da fronteira), um apiário sentinela composto por quatro colmeias. Além disso, para determinar sua possível distribuição, foi realizada vigilância epidemiológica (2015-2022) nas principais zonas apícolas do país. As colmeias foram monitoradas visualmente, examinando os favos, a tampa e o fundo. Da mesma forma, foram realizadas atividades de capacitação, como oficinas e dias de campo, direcionadas aos apicultores. Além disso, foi entregue material educativo, como manuais, voltados para o reconhecimento do besouro, bem como métodos para a inspeção das colmeias. O Pequeno Besouro da Colmeia foi detectado e confirmado em La Cruz, Guanacaste, Costa Rica, no apiário sentinela, em agosto de 2015, encontrando apenas besouros adultos no interior das colmeias. Até o momento, este besouro tem sido detectado em diferentes apiários, especialmente na província de Guanacaste, com a colaboração dos apicultores que foram capacitados. Em conclusão, a implementação de estratégias para a detecção e monitoramento do Pequeno Besouro, à medida que se desloca para novos países ou zonas apícolas, requer, como ilustrado no caso da Costa Rica, a implementação de apiários sentinelas, bem como o desenvolvimento de vigilância epidemiológica e a realização de atividades de capacitação direcionadas aos apicultores.

Palavras-chave: Pequeno Besouro da Colmeia, Aethina tumida, apiários sentinelas, abelhas africanizadas

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Introduction

The small hive beetle (SHB), *Aethina tumida* Murray (Insecta: Coleoptera: Nitidulidae), is a honeybee pest native to sub-Saharan Africa (Hepburn & Radloff, 1998) and was first described by Murray in 1867.

In 1996, small hive beetles were detected outside their native range in colonies of European honeybee subspecies in the southeastern USA (Elzen et al., 1999), causing considerable economic damage to apiculture (Hood, 2004; Neumann & Elzen, 2004). During the last few years, SHB has been found in Africanized honeybee (AHB) colonies in several countries in the Americas.

SHB in Central America: the presence of SHB in Central America was first reported in December 2013 in 11 out of 47 AHB colonies in an apiary in Sonsonate, **El Salvador** (OIE, 2013). During the following weeks, SHB was detected in other locations across the country, such as Las Victorias, Las Delicias, La Ceiba, and El Botoncillal (OIE, 2014a). Positive colonies were not eliminated; instead, containment measures, such as quarantine and movement restrictions were applied (Bulacio-Cagnolo et al., 2023; OIE, 2014a).

Months later, in March 2014, SHB was reported in AHBs in **Nicaragua** (Calderón et al., 2015), in San Juan del Sur, Department of Rivas (southern part of the country), about eight kilometers north of the border with Costa Rica, which increased the risk of entry of this pest into this country's beehives. During an inspection conducted by researchers from the Tropical Beekeeping Research Center (CINAT) for instructional purposes, adult beetles were found in a commercial apiary and were later officially reported as SHBs by the Official Veterinary Service, with a total of seventeen out of eighteen positive colonies (OIE, 2014b). Inspections conducted in perifocal areas found two more outbreaks in neighboring communities, where adult beetles and larvae were detected (OIE, 2014c). Epidemiological control measures included disinfection of equipment, monitoring of affected colonies, and training of beekeepers; however, the affected colonies were not eliminated (Bulacio-Cagnolo et al., 2023). In 2015, samples were taken in the affected municipalities as well as in the other municipalities of the department of Rivas, and no newly infested apiaries were found; therefore, the outbreak was considered resolved (OIE, 2014d). Simultaneously, lime (calcium oxide) treatments and contact traps for SHB were used. Despite these efforts, new outbreaks were later reported in Managua, Estelí, and Madriz (OIE, 2014e). Although no other outbreaks have been reported to OIE since then, the beetle most certainly is still present in the country.

The first report of *A. tumida* in AHB in **Guatemala** was published in August 2020, when adult specimens were detected feeding on honey and pollen in the department of Petén (García-Ochaeta, 2020; OIE, 2021). Authorities hypothesized that the entrance was linked to the mobilization of colonies in border areas. Petén borders Belize to the east and Mexico to the west and north. A monitoring program conducted in different areas of Petén confirmed the presence of adult beetles in six municipalities and larvae in only one (García-Ochaeta, 2020). Containment measures included internal traps to eliminate adult beetles, prohibition of hive mobilization within the department of Petén, and beekeeper training on trapping methods (OIE, 2021).

Some countries have implemented different strategies for SHB detection, such as involving national sanitary agencies, authorities, and researchers to design joint actions, as well as establishing networks of sentinel apiaries with periodical inspections and training programs focused on SHB identification (Bulacio-Cagnolo et al., 2023).

This paper discusses the economic and biological importance of SHB, as well as the strategies for its detection and monitoring in AHB colonies in Costa Rica.

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Materials and methods

Three strategies were mainly implemented for SHB detection and monitoring in AHB colonies in Costa Rica: sentinel apiaries, epidemiological surveillance, and training programs.

Sentinel apiaries: After the Bee Pathology Program of the Tropical Beekeeping Research Center from the National University (CINAT-UNA), Calderón et al. (2015) confirmed the presence of SHB in Nicaragua and informed the local sanitary authorities of Costa Rica. The Costa Rican National Animal Health Service (SENASA-CR) placed a sentinel apiary with four AHB colonies in Santa Cecilia, La Cruz, Guanacaste, close to the border (about 20 kilometers straight line distance from the Nicaraguan border). These colonies were periodically monitored by SENASA extensionists, who visually examined individual frames, hive covers, and bottom boards for each colony.

Sentinel apiaries are established in different contexts as early warnings of sanitary problems, in this case, to perform periodical SHB surveillance and maximize the likelihood of detection in an area nearby to a potential entry point, mainly close to international borders. Sentinel apiaries are recommended in risk areas since early detection is crucial for successful eradication or control efforts (Schafer et al., 2019). Furthermore, the only known efficient attractants for adult SHBs are functional honeybee colonies. To identify the pest early, investigate new outbreaks in detail, and attract and trap beetles in areas at risk of new introductions, sentinel apiaries consisting of fully functional queenright honeybee colonies are recommended (Mutinelli, 2016; Schafer et al., 2019).

Apiary surveillance in temperate and subtropical zones should be reinforced from spring to autumn and throughout the year in tropical zones (Neumann et al., 2016; Schafer et al., 2019).

Epidemiological surveillance: After the presence of SHB was confirmed in La Cruz, Guanacaste (northern part of the country), sampling was conducted in the main beekeeping areas during the last few years (2015 - 2022) to monitor the distribution and report the situation of SHB in AHB colonies in Costa Rica (Arguedas et al., 2020; Calderón & Ramírez, 2018; Calderón & Ramírez, 2019). Such information is needed to determine the best time to act and keep beekeepers informed. A nationwide monitoring campaign was conducted between 2015 and 2018 as part of the Bee Pathology Program of CINAT-UNA, which included the inspection of 476 colonies from 77 apiaries in the provinces of Guanacaste, Puntarenas, Alajuela, San José, and Heredia (five of the seven provinces of the country) (Calderón & Ramírez, 2018). Colonies were visually monitored and all individual frames, hive covers, and bottom boards were examined (Cornelissen & Neumann, 2018; Neumann et al., 2013). In addition, 305 colonies belonging to 61 apiaries located in San Ramon, Alajuela, were sampled from May 2019 to March 2020. Furthermore, adult beetle samples from Ulloa, Heredia and Potrerillos, Liberia, Guanacaste were also analyzed.

According to Cepero et al. (2014), early detection of exotic pests and diseases is necessary for effective biosecurity control through surveillance and reporting. In beekeeping, these activities involve adequate collection, analysis, and interpretation of information on the presence or absence of diseases and their adequate reporting to competent authorities. Sentinel apiaries provide one of the tools for epidemiological surveillance.

Furthermore, two Cutts Beetle Blaster® traps with 25 ml of vegetable oil (as a killing agent) were placed in each colony for 8 to 15 days. These traps were placed inside the bee hives between two frame top bars in the bottom chambers. The content of the traps was examined for beetles (adults or larvae) at the Bee Pathology Lab (CINAT-UNA). According to Neumann et al. (2016), specialized laboratories are required to confirm or reject the presence of SHBs. This holds especially true for eggs and larvae, which cannot be assigned to SHB

based on morphometrics alone. In addition, 10 wild honeybee swarms located in trees or houses in urban areas in the provinces of Heredia and Cartago (Central Valley) captured by beekeepers and transferred to Langstroth hives were also monitored (Calderón & Ramírez, 2019).

Training programs: Training activities were directed to technicians and beekeepers and focused on SHB recognition and identification, the importance of early detection, the potential impact on beekeeping, and methods for colony inspection. Training activities have been conducted at the main beekeeping areas of the country and include workshops, fieldwork, and training materials such as brochures with images illustrating various stages of the SHB life cycle (adult and larvae) to facilitate recognition and identification. Beekeepers should be able to recognize SHB infestations via adult and larval morphology as well as clinical signs at the colony level (Neumann et al., 2016). All stakeholders, in particular beekeepers, should be provided with information on how to access tools and guidelines that enable the recognition of new infestations (Schafer et al., 2019).

Results

SHB detection in Costa Rica

SHBs were detected and confirmed in the sentinel apiary in August 2015 in Costa Rica, specifically in La Cruz, province of Guanacaste (11° 6' 0" N, 85° 25' 0" W). Only adult beetles were confirmed inside AHB colonies (Calderón & Ramirez, 2018), which were immediately eliminated, and movement was restricted to and from this region. SHB most likely entered Costa Rica through natural dispersal from Nicaragua.

SHB monitoring in AHB colonies

In September 2018, adult specimens were found through epidemiological surveillance in a wild honeybee swarm located in Heredia, Central Valley, in the middle of the country (about 265 km south of the first detection site) (Calderón & Ramírez, 2019). Calderón & Ramírez (2019) hypothesized that the beetles found in the Central Valley arrived in fruit transported into the facility from Guanacaste because of the following: 1) before this event there were no previous reports in nearby locations, 2) no intensive beekeeping is practiced at the focal point of detection in the Central Valley, and 3) this point is located close to an important distribution center that receives fruit from all over Costa Rica, including Guanacaste. SHBs can reproduce on fruits and other types of food in the laboratory and semi-field assays (Buchholz et al., 2008; Ellis et al., 2002). Nevertheless, field surveys were unable to confirm any association of SHB with alternative food sources outside social bee colonies (Mutinelli et al., 2015).

A year later, in October 2019, *A. tumida* was confirmed in a commercial apiary in Potrerillos, Liberia, Guanacaste, approximately 25 km south of the initial point of detection (Arguedas et al., 2020). In addition, the inside of 10 colonies of the stingless bee *Melipona beecheii* located near the first point of detection in La Cruz, Guanacaste was visually inspected, and no SHBs or signs of infestation were found (Calderón & Sánchez, 2021).

In collaboration with trained beekeepers, SHB has presently been found in different commercial apiaries, particularly in the province of Guanacaste, where SHB is established, approximately 100 km southwest of the initial point of detection in the country. Even though some larvae have been observed in weak colonies, adult

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beetles are the most detected in AHB colonies (Calderón, Pers. Comm.). To detect and monitor the spread of SHB, continuous epidemiological surveillance and training activities are necessary.

Discussion

As described above, SHB detection in Nicaragua and later in Costa Rica allowed us to consider two significant aspects. First, once SHB spreads within a country (as in the case of Nicaragua), invasion of border countries becomes a recurring threat. In this case, carefully positioned sentinel apiaries are useful for early detection of the invader. Second, the adoption of timely confinement measures, such as restricting colony movement in areas with low colony densities, as was the case in northwestern Costa Rica (specifically La Cruz, province of Guanacaste) has proven effective in preventing or slowing SHB dispersal (Bulacio-Cagnolo et al., 2023; Calderón & Ramírez, 2018). To limit further spread (at least within a 10 km radius), a protection zone should be established immediately, including a strict ban on the movement of bees and beekeeping equipment since migratory beekeeping poses the highest risk of SHB spreading (Neumann & Elzen, 2004).

The case in Costa Rica strongly supports the notion that by implementing sentinel apiaries a degree of the invasion's containment and deceleration could be attained. Most importantly, SHB detection at the sentinel apiary established near the Nicaraguan border most likely provided a better understanding of the dynamics at the early stages of the invasion process. In addition, measures restricting the movement of colonies in and out of the region may slow down SHB spread and allow us to track the invasion by anticipating SHB movement. Nevertheless, finding SHBs in a wild honeybee swarm in the Central Valley three years after the first report illustrates that this approach is not devoid of gaps and that we may be underestimating the potential of SHBs to be transported by alternative means, such as other bee species, or be associated with other types of incoming freight, originating in or near the areas of early detection. Apicultural trade and migratory beekeeping activities in abandoned or registered apiaries, as well as possible infestations of wild host populations, should also be considered (Neumann et al., 2016).

In conclusion, having strategies for SHB early detection as it spreads to new countries or areas requires, as illustrated by the case in Costa Rica, implementing sentinel apiaries, developing epidemiological surveillance, and providing training activities for technicians and beekeepers to aid in SHB recognition.

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