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ECOLOGY

Active phloem sap collection by *Trigona spinipes* (Fabricius) (Hymenoptera, Apidae) in *Artemisia annua* Linn (Asteraceae)

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ABSTRACT. *Trigona spinipes* Fabricius (Apidae: Meliponini), is a common stingless bee widely seen in urban and rural areas in Brazil, popularly known as irapuá, arapuá or bee-dog. Although these bees are considered pollinators of some cultivated plants, they are better known for the damage they cause in different crops. During experimental agroecological cultivation of Artemisia (*Artemisia annua* Linn, *Asteraceae*), in Jaguariúna (SP, Brazil), stingless bees *Trigona spinipes* (Fabricius) were observed sucking phloem sap directly from the plant, a phenomenon not yet described in scientific literature. This study aimed to register and describe the phloem sap-sucking behavior performed by *T. spinipes* for the first time, as well as to assess the potential impact of this behavior on *A. annua* cultivation. The behavior and the material collected by bees and the severity of attack were also analyzed. The aging and premature death of observed *A. annua* specimens occurred because of extensive lesions caused by *T. spinipes*, confirming the negative consequence of sap-sucking attacks of *T. spinipes* bees on the plants. Factors that could induce this unusual behavior were presented, pointing out the need for future studies on the development of strategies to protect plants, without causing damage to the *T. spinipes* bee populations, which are elements of Brazilian bee fauna and, therefore, protected by law.

Keywords: Medicinal plant; Insect behavior; Plant injury; Stingless bee, Farming.

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Introduction

Artemisia annua Linn (Asteraceae), popularly known as Artemisia, is a medicinal plant, originated from the Asian continent. Leaves and flowers of these species are the source of essential oils and artemisinin, a molecule with anti-malarial and therapeutic potential for other diseases. This shrub is usually single-stemmed reaching about 2 m in height with alternate branches, extremely vigorous, and usually free of disease and pest attacks (Jelodar, Bhatt, Mohamed, & Chan, 2014). It has a strong aroma and bitter taste resulting from terpenoids and sesquiterpene lactones discouraging herbivores predation (Smitha, Varghese, & Manivel, 2014).

Trigona spinipes (Fabricius, 1793) (Apidae: Meliponini), is a common widely distributed stingless bee in urban and rural areas in Brazil, commonly known as irapuá, arapuá or bee-dog. Although these bees are considered pollinators of some cultivated plants (Freitas et al., 2014, Giannini et al., 2015), they are better known for the damage they cause in different crops (Giannini et al., 2015). The aim of this work was to register, for the first time, the phenomenon of the active phloem sap-sucking behavior performed by *T. spinipes*, as well as raise the potential impacts of this behavior on *A. annua* cultivation.

Material and methods

Description of Area and Agroecological Cultivation System

The experimental plot with Artemisia (22°43'31.1"S 47°00'56.3"W) was 4 km away from the center of Jaguariúna (SP) and inserted, within a radius of 1 km, in a landscape dominated by sugarcane monoculture, pasture and a fragment of secondary forest (18 ha) 200 m far from the planted area. Four active nests of *T. spinipes* were located in this landscape; however, the existence of other active nests cannot be ruled out as this area had not been completely inspected. In the 300 m surroundings, there was a predominance of green manure plots (*Cajanus cajan* (L.) Huth *Crotalaria ochroleuca* G. Don and *Crotalaria juncea* L. - Fabaceae) and agroforestry systems in the early stages of development, as well as buildings and gardens with fruit and ornamental trees. The Artemisia planting was carried out on a plot at the Agroecological Farm of the *Empresa Brasileira de Pesquisa Agropecuária* - Embrapa Environment - located in Jaguariúna, São Paulo, Brazil. In the land preparation, there was no soil stirring and the liming was done with dolomitic limestone (PRNT 90% - 2 t ha^{-1}). The spontaneous vegetation *Urochloa decumbens* (Stapf) R.D.Webster (Poaceae), *Canavalia ensiformes* (L.) DC. (Fabaceae) and *Cajanus cajan* (L.) Huth (Fabaceae) was cut down and left on the ground as cover. For planting, the soil was grooved (20 cm deep x 20 cm wide) in double rows, spaced one meter apart, with two meters between the doubles. In these grooves 11.25 kg of thermophosphate (375 kg ha^{-1}) containing micronutrients (Yorim Master) was applied. On October 26, 2011, four hundred seedlings of *A. annua* (CPQBA-1 genotype) were transplanted on a plot of 300 m^2 (12 m x 25 m) using 8 lines with 50 plants of 50 cm spacing. For the protection against the attack of leaf-cutting ants, *Crotalaria ochroleuca* G. Don and *Crotalaria juncea* L. (Fabaceae) was planted around the whole plot. Drip irrigation was used and the control of spontaneous plants was carried out through selective weeding, only on the lines, on November 24th and December 26th, 2011.

Behavior evaluation and follow-up

From January to March 2012, after observing the presence of injuries by *T. spinipes* bees in Artemisia, all 400 cultivated plants were monthly inspected regarding the occurrence of lesions. On February 15, 2012, 400 Artemisia specimens (CPQBA-1 genotype) from another experimental plot 12.6 km far from the study area were inspected in an experimental area at the Center for Chemical, Biological and Agricultural Research (CPQBA) of the *Universidade Estadual de Campinas* (UNICAMP) in Paulinia (22°47'51.0"S 47°06'41.0"W), São Paulo, Brazil. In the two cultivated areas, *T. spinipes* bee nests were surveyed nearby and the distance between the nest and the Artemisia plot was determined by the GPS Garmin (GPSmap 60CSx).

The presence of healed lesions (dry wounds) and unhealed lesions (with the presence of exudates or bees) and their length were verified. As the lesion width did not vary, this parameter was not used. The degree of attack severity was classified as low (wounds up to 1.5 cm in length and without bees), medium (lesions between 1.5 cm and 5.0 cm in length with presence of bees or exudates) or high (lesions more than 5 cm in length). During the monthly assessments and six other monitoring visits to Jaguariúna, visual observations on the behavior of bees, the presence of other insects and assessments of lesion size and their effects on the life cycle of the plants were performed.

The average number of bees per severely attacked plant was estimated, by counting bees in samples of up to 11 plants on February 28, 29 and March 1, 2012 from 9:00 am to 12:30 pm. On January 25 and March 1 and 2, 2012 the volume of sap ingested by the bees and their sugar concentration were estimated between 10 and 11:30 am. The bees with distended abdomen were captured and the sucked sap was regurgitated by the pressure applied on their abdomen with fingers. This material was immediately aspirated into a graduated microcapillary (Hamilton, 50 microliters) and the sugar concentration (solute concentration) was estimated in a hand held refractometer (Atago, Brix, 0 to 32%) soon afterwards.

Results and discussion

For the first time *T. spinipes* bees sucking phloem sap were observed (Figure 1a). Plant phloem sap is a rich source in nutrients (Dinant, Bonnemain, Girousse, & Kehr, 2010) with protein/carbohydrate ratio of approximately 0.1 (Nowak & Komor, 2010).

This bee species scraped the Artemisia stem with their jaws to suck the phloem sap producing lesions about 0.5 cm wide and 0.5 to 10.0 cm long that at times displayed a translucent yellow exudate (Figure 1b). They continuously exploited the injuries: they landed on the branches with the abdomen empty (not distended), inserted the tongue into the lesion and distended the abdomen (Figure 1c), indicating the sucking activity.

An amorphous material in the corbicula of *T. spinipes*, which could have derived from the stem scraping, was also observed. However, this collection behavior was not verified. Farmers recognize *T. spinipes* bees because they present undesirable behavior in several crops, causing damages mainly on flowers and fruits (Peruquetti, Costa, Silva, & Drumond, 2010; Santos, Broglio, Dias-Pini, Souza, & Barbosa, 2012; Silveira, Raseira, Nava, & Couto, 2010).

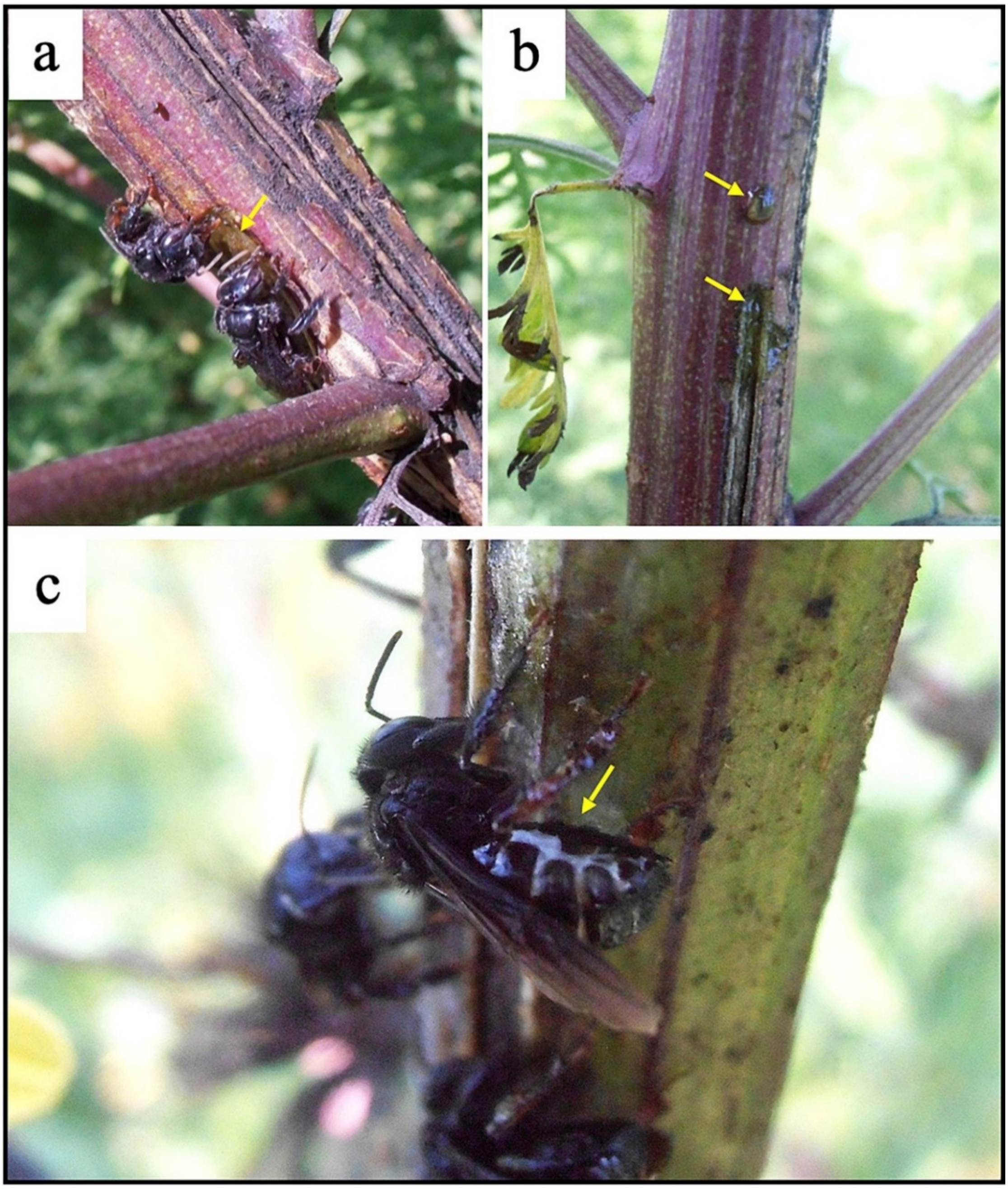

Figure 1. Stem of *Artemisia annua* Linn with: (a) lesion explored by bees *Trigona spinipes* (Fabricius, 1793), (b) unhealed and exuding lesions, (c) *T. spinipes* with abdomen distended after sap-sucking.

This undesirable behavior of *T. spinipes* is performed in order to obtain materials for the nest construction, such as resins, waxes and fibers, and food resources such as nectar and fruit pulp. These bees cut petals and anthers and scrape the epidermis from shoots, stems, leaves and fruits. However, the stem scraping activity of *T. spinipes* had never been related to the phloem sap sucking. Stingless bees are known to bite plant parts to stimulate resin production and appear to repeatedly visit and actively maintain the same resin-secreting areas by chewing for days or weeks (Howard, 1985). In this study *T. spinipes* actively maintained the exploration of the phloem sap for months, preventing the healing and closure of the lesions through an unknown mechanism. In most plants, the phloem seals itself upon wounding, and no exudate is secreted (Guelette, Benning, & Hoffmann-Benning, 2012).

The average regurgitated sap volume from bee stomach was 5.6 µl (SE = 0.56, n = 19), ranging from 3.0µl to 13.0µl. The difficulty in capturing bees with a sap-filled stomach probably produced this data variation. The average concentration of the regurgitated sap (obtained from 1 to 3 bees) was 25.5% (SE = 0.66, n=9). Data obtained in more than 50 experiments (41 plant species) show that the average concentration in the phloem sap varies from 18.2 to 21.1% (Jensen, Savage, & Holbrook, 2013). The slightly higher concentration of regurgitated sap than phloem sap was due to the dehydration process carried out by *T. spinipes*. During field observations the bees dehydrated the sucked solution, regurgitating and reabsorbing it several times on the plant branches, before going back to the nest. Observation of this behavior and the increased abdominal volume of the bees after landing and handling the lesions confirm the active collection of phloem sap by this stingless bee species. It is amazing that *T. spinipes* had exhibited this behavior even without having mouth parts adapted for phloem sap-sucking like sap-sucking insects and mites.

The average number of *T. spinipes* per plant severely attacked was 11.4 (SE= 1.52, n=29), ranging from 2 to 30 bees. The most extensive lesions occurred in the lower half part of the plant and in all those lesions the fungus *Capnodium* sp. (Capnodiaceae), popularly known as "fumagina", had developed.

In the lesions caused by *T. spinipes* on *Mimosa scabrella* Benth (Fabaceae), the establishment of *Capnodium* sp. fungus was also observed, once the removal of the stem bark by *T. spinipes* caused the release of photoassimilates favoring its development in *M. scabrella* (Caron et al., 2013).

The continuous exploitation of the lesions by the bees for phloem sap sucking as observed in our experiment, attracted other insects (Figure 2a) and also resulted in the rotting of the branch next to the injured areas (Figure 2b). Wasps, flies and beetles were frequently observed on the injuries and were not observed causing the reported damage.

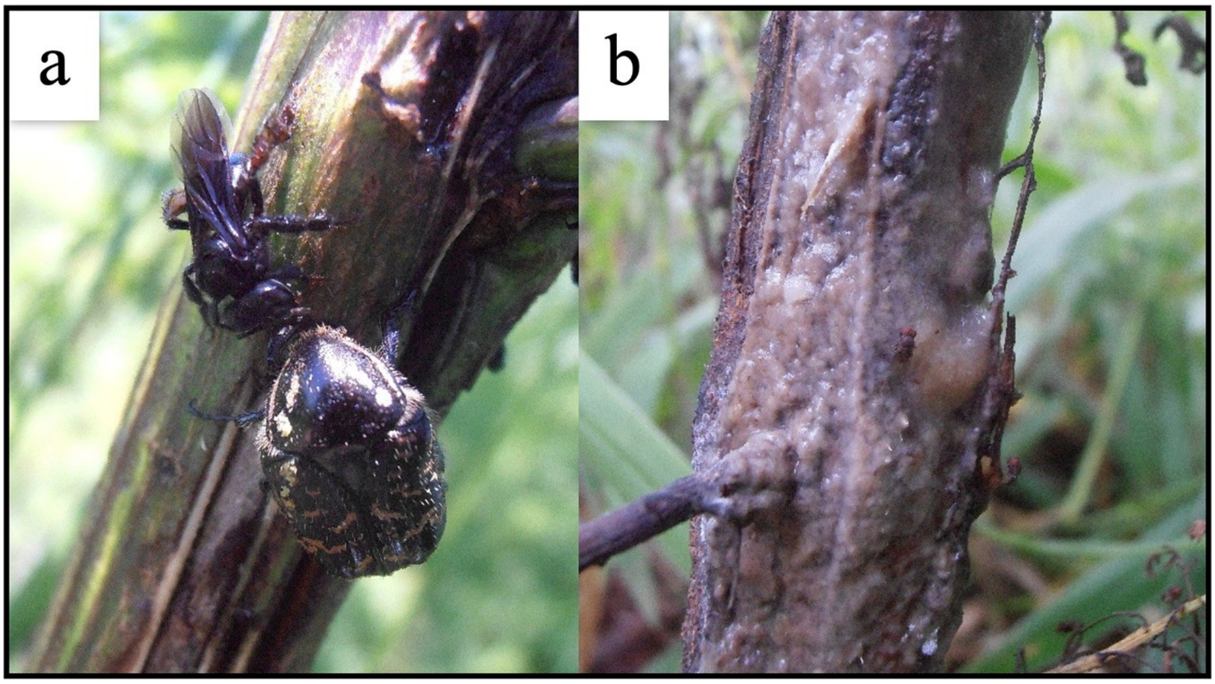

Figure 2. Stem of *Artemisia annua* Linn with: (a) Bee *Trigona spinipes* (Fabricius, 1793) and beetle *Euphoria lurida* (Fabricius, 1775) exploiting resources of the same injury, (b) advanced rot that will result in the death of the plant before flowering.

There was a 15.3% increase of severely attacked plants throughout the time (Table 1), totaling 81 plants with this level of damage in the month of March 2012 (3rd evaluation). At the end of February 2012, the senescence of severely attacked plants was observed, with 5.5% of them dying in April, before completing their life cycle. This unusual behavior could have been stimulated by the proximity and the presence of some attractive substance in Artemisia sap for this bee species once no important climate and vegetation change was noticed in the foraging area of *T. spinipes*. Moreover, the development of Artemisia occurred during the warm and humid season (from October to March), which offers more bloomy resources than the cold and dry season (April to September).

Table 1. Percentage of *Artemisia annua* Linn plants under different levels of damage caused by *Trigonas spinipes* (Fabricius, 1793) in the evaluations carried out in the year 2012 (from January to March), in Jaguariúna, SP, Brazil.

Severity level (%)	Absent	Low	Medium	High
1st evaluation (January 18)	69.8	19.0	6.3	4.9
2nd evaluation (February 16)	26.8	43.7	14.8	14.7
3rd evaluation (March 15)	14.0	44.1	21.7	20.2

During the evaluations, four nests of *T. spinipes* were located near the planted area in Jaguariúna: one at 83 m and the others between 400 and 650 m away from the plot with Artemisia. All the *T. spinipes* nests were within the expected maximum flight distance defined for this species (Araújo, Costa, Chaud-Neto, & Fowler, 2004). However, it is likely that the undesired behavior was only manifested by bees whose nests were near Artemisia plants, since the greater proximity to the source represents an advantage for bees by a reduction in the cost/benefit relation to obtain the supply they need. This could be the case of the nest 83 m away but unfortunately, the bees were not marked. In *Khaya ivorensis* A. Chev. (Meliaceae), the proximity among trees and nests of *T. spinipes* contributed with the occurrence of attacks to these plants (Moura et al., 2017). In Paulinia (SP), no similar damage was found in Artemisia plants and unfortunately, no nest of *T. spinipes* was located around this crop, despite the presence of these bees in flowers of other medicinal plant species cultivated in the place.

The purpose of bee foragers is to maximize the collection of the resource in relation to collection costs, and the strategies used for this purpose include the costs of discovery, collection and, occasionally, resource defense (Roubik, 1981). Along the distance, the absence or scarcity of food sources may be conditioning this behavior (Santos et al., 2012). Colonies of *T. spinipes* reach up to 180,000 individuals and the presence of several nests overlaps the exploitation of resources in the same area, suggesting that the environmental capacity to sustain these large populations may be exceeded leading to the emergence of alternative behaviors to ensure colony survival. The presence of these four nests of *T. spinipes* in the study area could result in a shortage of nectar sources for the bee population, sources that are also shared with bees of other species and other insects. However, the area's supply of resources was not evaluated in this study.

Conclusion

These results revealed that *T. spinipes* is capable of sucking phloem sap using it as a resource, and that this behavior may impair the yield of this crop. However, the degree of impact and the factors that determine this unusual behavior of *T. spinipes* still need to be studied in order to develop strategies to protect crop plants, without causing damage to the *T. spinipes* bee populations, which are elements of Brazilian bee fauna and, therefore, protected by law.

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