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Production of Honeydew by Scale Insects Associated with Bracatinga (*Mimosa scabrella* Benth) in Serra Catarinense, Southern Brazil

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ABSTRACT

Scale insects *Stigmacoccus paranaensis* (Foldi, 2006) associated with bracatinga trees (*Mimosa scabrella*) in Santa Catarina, Brazil, can play an important economic role and guarantee the production of "bracatinga honeydew honey". This scale insect has the ability to excrete large amounts of honeydew, of economic importance for beekeepers, especially due to the natural occurrence of host plants at high density, known as "bracatingais". In this sense, the objective of this study was to evaluate the honeydew produced by scale insects associated with *M. scabrella* in the Bom Retiro, Santa Catarina, Brazil. We determined the density of the host plant in the studied area, the intensity of infestation by scale insects, and the volume and sugar concentration of excreted honeydew. The average density of bracatinga was 437.5 individuals ha^{-1}, and the average density of scale insects per plant was 8,287. During 10 minutes, the average rate of honeydew excretion by scale insects was 0.45 µl and 0.34 µl, respectively, in 2002 and 2004. The estimated volume of honeydew production was 0.54 L in 2002 and 0.41 L in 2004 tree^{-1} day^{-1}. The highest sugar concentration was observed in 2004 (28.13%). The excretion rate was affected by climatological factors and the size of the insects. This is the first study presenting a systematic evaluation of honeydew production by scale insects in Southern Brazil. The results of the present study support production of honeydew honey in the Serra Catarinense region as an important sustainable economic and traditional activity.

Introduction

Scale insects are small phytophagous sucking insects of the superfamily Coccoidea (Hemiptera). More than eight thousand scale insect species have been described worldwide (Wolff, 2012; García Morales et al., 2016). Females feed on phloem sap from different plant species, and they are considered as pests when associated with plants of economic interest (Gullan and Kosztarab, 1997; Hodgson and Hardy, 2013). From the digestion of the sap, scale insects excrete a sugary solution, called melato in Portuguese and honeydew in English, serving as an energy source for various trophic levels and ensuring the maintenance of positive ecological interactions (Beggs, 2001). Honeydew is also economically important since honeybees (*Apis mellifera* L.) produce a high-quality honey using the scale insects' honeydew (De-Miguel et al., 2014).

Plant hosts targeted by scale insects are quite diverse. For example, honeydew-producing insects interact with *Abies* spp. and *Prunus* spp. (Santas, 1983), *Nothophagus* spp., (Nothofagaceae) (Kelly et al., 1992), *Pinus* spp. (Pinaceae) (Hodgson and Gounari, 2006; Kondo et al., 2008; Chamorro et al., 2013), *Calocedrus decurrens* (Torr.), Florin (Cupressaceae) (Chamorro et al., 2013), and *Quercus* spp. (Fagaceae) (Santas, 1983; Gamper et al., 2011; Lara et al., 2011), among other species distributed around the world. In Brazil, scale insects of the genus *Stimacoccus* are associated with *Mimosa scabrella* Benth. (Fabaceae), *Schizolobium parahyba* (Vell. 1825) Blake (Fabaceae, Caesalpinioideae) and *Inga* sp. (Fabaceae, Mimosoideae) (Bogo et al., 1999; Hodgson et al., 2007; Wolff et al., 2015), suggesting that these scale insects are oligophagous on Fabaceae.

In southern Brazil, the scale insects of the species *Stigmacoccus paranaensis* Foldi 2006 (Hemiptera; Coccoidea) stand out for honey production, specifically when they occur associated with *M. scabrella*, commonly called bracatinga. Honeydew honey produced in this interaction is known as "bracatinga honeydew honey". This honey is economically important from both domestic and foreign consumers, as the food industries in Central and Eastern European countries, which import great part of the Brazilian production. The growing market for bracatinga honeydew honey in many European countries requires its differentiation from other honey types as a response to consumer

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demands (Simova et al., 2012). As such, an initiative to establish an appellation of origin has been ongoing and could be issued soon by the National Institute of Industrial Property, a state agency that grants registration.

Honeydew honey of bracatinga is darker when compared to floral honeys, in addition to presenting flavors and odors that vary according to maturation and origin of the honeydew. More than 95% of honeydew dry weight is formed by amino acids and secondary compounds from plants, differing from the phloem sap and floral nectar in its composition (Crane and Walker, 1985; Völkl et al., 1999). In the honeydew excreted by *Stigmacoccus* sp. and *Coccus hesperidum* L. scale insects, oligosaccharides (tri-, tetra- and pentasaccharides) were identified, showing a significant metabolism of sugar transformation by the insect and / or by specific microbial symbionts that occur widely within the Coccoid body surface (Bogo and Mantle, 2000; Bogo, 2003).The honeydew remains attached to the anal waxy filament of scale insects, forming droplets (Fig. 1) which are collected by bees and subjected to a second set of digestive processes. Because it undergoes two enzymatic processes, honeydew honeys have higher pH, ash content, nitrogen content (Simova et al., 2012), and mineral compounds, but lower monosaccharide content (Azevedo et al., 2017), compared to floral honeys.

The bracatinga tree (*M. scabrella*) is considered an important bee plant in southern Brazil. It offers floral trophic resources from July to September and honeydew from March to April (Barth, 1989). In the environment, forests, in which bracatinga is the predominant species, are known as “bracatingal” or “bracatingais” (Burkart, 1979; Bartoszeck et al., 2004). The molecular identification of scale insects (Hemiptera) associated with bracatinga has still not been performed. Morphologically, the precise identification of the insect species associated with bracatinga is complicated by the similarity of phenotypic patterns exhibited by distinct insects (Gullan and Kosztarab, 1997). However, Wolff et al. (2015) recorded the occurrence of *Stigmacoccus paranaensis* associated with this plant in Cambará do Sul (RS), Brazil, with evidence that bees use the honeydew of these insects as a source for producing dark honeydew honey. Owing to its unique nutritional and therapeutic characteristics, honeydew honey produced from scale insect excretion on bracatinga trees in the Brazilian southen Plateau has been highly valued abroad (Mariano-da-Silva et al., 2011; Mazuchowski et al., 2014). In some regions of Europe, honeydew honey is more valued in relation to floral honey based on its benefits associated with antioxidant and antimicrobial activity, as well as the presence of highly bioaccessible minerals (Simova et al., 2012; Azevedo et al., 2017; Seraglio et al., 2017).

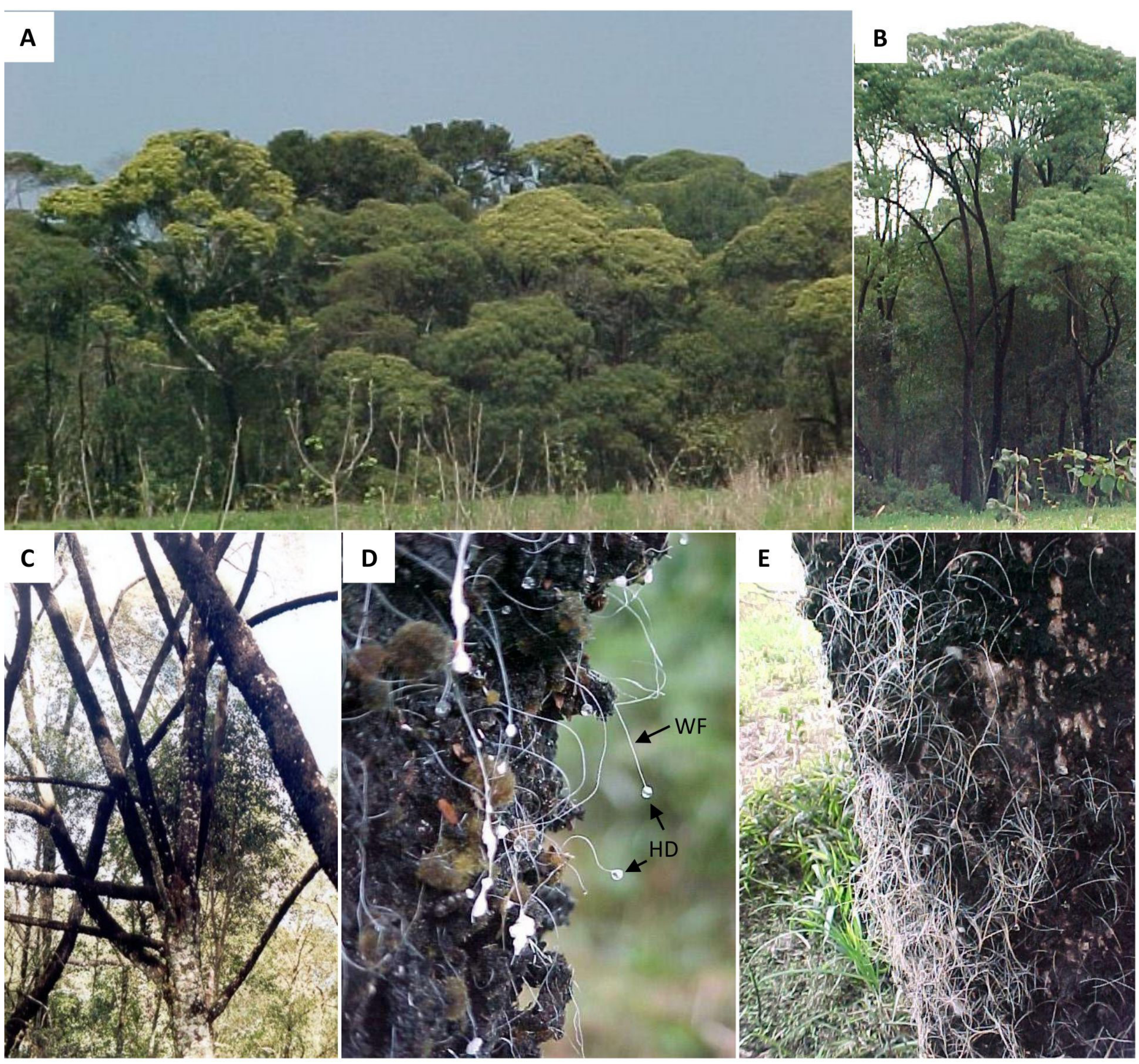

Figure 1 Studied Bracatingal, Bom Retiro, SC, Brazil (A), bracatinga plant (*Mimosa scabrella* Benth.) (B), trunk of bracatinga infested by scale insects (C), and detail of trunk showing anal filaments of excretion of the scale insect (D and E). WF: wax filament; HD: honeydew drop. Photo: Maritza Martins.

The ability of scale insects to excrete large amounts of honeydew has significant ecological and economic, being important for beekeepers in Santa Catarina. In this region, the scale insect has a biannual life cycle, with greater body size and honeydew excretion during pair years, when honeydew honey is also produced. In this sense, the objective of the present study was to determine the production of honeydew by *S. paranaensis* associated with *M. scabrella* in bracatingais areas, over the months of three year, in the municipality of Bom Retiro, Santa Catarina, Brazil. To achieve this objective, we determined the density of the host plant species in the bracatingais at the studied area, the intensity of infestation by scale insects, and the volume and sugar concentration of excreted honeydew. Abiotic data were recorded to verify their influence on the amount of excreted honeydew.

Material and Methods

Study area - The study was carried in the locality of Caneleira, municipality of Bom Retiro, State of Santa Catarina, Brazil (27°51'33" S and 49°35'24" W, 890 m a.s.l.), whose land falls within the domain of Ombrophilous Mixed Forest (Reitz and Klein, 1966). The bracatingal used in this study had an area of 10,000 m^2 (Fig. 1A), was about nine years old, and had an average height of approximately 12 m (Fig. 1B).

Density of plants and external surface of the trunk and branches of bracatinga – To perform the population density of *M. scabrella* infested by scale insects, four parcels of 400 m^2 (20 m x 20 m) each divided again into four subplots of 100 m^2 (10 m x 10 m) were established in the study area. A demographic density survey within the plots included all pre-reproductive stage plants and reproductive stage plants that had a breast height diameter greater than 10 cm. Data were collected from height of the trunk (TrH; height between the soil surface to the first bifurcation of the plant), total height (ToH), diameter at breast height (DBH) (1.30 m from the ground) and the number of plants of the species (NuP). Descriptive statistics of plant density and external surface area of the trunk were estimated. The outer surface area of the bracatinga trunk was calculated from the following formula drawn from Machado et al. (2008): Aset= C*ToH*Fc, where C is the mean circumference, ToH is the mean total height, and Fc is the correction factor of 0.389.

Evaluation of the instantaneous production of honeydew in bracatingas trunks – The instantaneous availability of honeydew, that is, the volume of honeydew from the scale insects associated with the bracatinga trunk, was monitored monthly from February 2002 to October 2004. In the months of November and December 2002, January, February, April and November of 2003 and in March 2004, no collections were carried out because of the low availability of honeydew. To reduce the effect of the developmental stage of the scale insects on honeydew production, evaluations were carried out monthly, for three years. In the study area and throughout the southern region of Brazil, the scale insects of the species *S. paranaensis* are uniform in the stages of development among individuals, during their life cycle, which takes two years to complete. Thus, in each month of evaluation, the insects were at the same stage of development, with the youngest phases occurring in the first months of 2003 and the most advanced stages, observed in the months of 2002 and 2004 (Martins-Mansani et al. unpubl. data). Within the forest fragment, all bracatinga trees infested by scale insects were labeled, i.e., five samplings collected randomly from six plants (30 samples). Each replicate consisted of 10 drops of honeydew excretion collected in the morning and afternoon periods at the apex of the waxy filaments emitted by 10 distinct scale insects. These drops were collected with the help of a stylet and packed into microtubes containing 20 ul of distilled water. Total sugar concentration was measured in a portable refractometer (Bellingham and Stanley), with scales of 0 to 50% degrees Brix and corrected owing to the dilution of honeydew in the distilled water. The data were submitted to ANOVA to test the statistical significance of different periods, months of the years, and different trees used for the collections.

Evaluation of the potential production of honeydew on the bracatinga trunk - To estimate the rate of excretion, five plants infested by scale insects were randomly selected. Four replicates (honeydew content produced by two scale insects) were sampled in each plant twice a day (morning and evening). The volume of the microcapillary (capacity of 2 and 5 μl) filled with honeydew for ten minutes was measured with the aid of a pachymeter, and the sugar content was determined with the aid of a portable refractometer (Bellingham and Stanley) with 0 to 50% Brix scales. Sampling sufficiency tests were performed to determine the number of replicates of each experiment. In order to satisfy the parametric tests, the volume (μl) and sugar concentration (%) data were transformed into log10 (X + 1) (Ordano and Ornelas, 2004) and analyzed by one-way analysis of variance (ANOVA). When differences were detected, the Newman-Keuls mean separation test (α=0.05) was applied. Pearson's simple correlation (α=0.05) was estimated from the monthly average of the real data (Sokal and Rohlf, 1981).

Evaluation of the density of scale insects Stigmacoccus paranaensis on bracatinga plants – The density of scale insects infesting the bracatinga plants was evaluated in five randomly chosen trees with a diameter at breast height (DBH) of more than 10 cm. All the scale insects present within the area of a Petri dish (70.88 cm^2) were counted. This count was performed in five distinct points on the trunk of the plant, being one at 1 m above the soil surface, and the other four, successively every 0.5 to 0.5 m (that is, 1.5 m, 2 m, 2.5 m and 3 m above the soil). At each point, counts were made in the four cardinal orientations (north, south, east and west), totaling 20 sampling areas per tree. To calculate the density of the scale insects per tree, the rule of three was used, based on the average data of the number of scale insects per 70.88 cm^2 and the external surface area of the trunk and branches of the bracatinga.

Weather data - Meteorological data were collected every day within the experimental units of the study area. These data were obtained by means of a dry bulb and a wet bulb thermometer placed at about 1.30 m from the soil, always in the same place and in the shade. The relative air humidity was obtained through the difference in the data between the two thermometers and using the conversion table presented in Laroca (1995). The temperature was obtained from the dry bulb thermometer (°C) in the same way (Fig. 1S). The meteorological information was correlated with the flow of the honeydew trophic resource. These correlations were evaluated through Pearson's simple correlation (α=0.05).

Results

Density of bracatinga plants

The density of bracatinga plants was highly variable across forest fragments from 0 to 600 trees per hectare (Table 1). Thus, only the number of plants in plots 1 and 2 was considered in order to infer the number of individuals per hectare, since the other two plots did not present a sufficient number of plants for this assessment.

The surveyed area exhibited an average of 225 individuals per ha; however, the mean density of plots 1 and 2 was 437.5 individuals per ha. The diameter at breast height, total height and height of the trunk of bracatinga plants in plots 1 and 2 were 13.8 cm (± 5.9), 12.3 m (± 1.0) and 8.9 m (± 1.2), respectively (Table 1).

Instantaneous and potential production of honeydew

The volume of honeydew excreted by scale insects differed significantly among years, both for instantaneous production and

Table 1
Number of Plants, diameter at the breast height (DBH, taken at 1.30 m from soil surface), the total height of the plant (ToH), height of the trunk (TrH), and respective coefficient of variation (CV) of the demographic survey of bracatinga (*M. scabrella* Benth.) in Bom Retiro - SC, 2002.

Plot	Number of plants		DBH (cm)		ToH (m)		TrH (m)	
	Total	Per ha	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
1	24	600	12.4	49.5	12.1	29.7	9.0	35.4
2	11	275	15.4	42.1	12.4	23.6	8.8	25.4
3	1	25	23.7		14.0		11.0	
4	0	0						
Mean	9	225	171		12.8		9.6	
Mean*	17.5	437.5	13.8		12.3		8.9	
SD*	11.2	279.1	5.9	5.3	1.0	4.3	1.2	7.1

Plots 1 and 2; SD – Standard Deviation

Table 2
Average and amplitude of the instantaneous and potential production of honeydew (A) and sugar concentration (B) produced by *Stigmacoccus paranaensis* associated with *Mimosa scabrella* in Bom Retiro - SC, evaluated in the years 2002, 2003 and 2004.

(A)	Honeydew instantaneous			Honeydew potential (10 min)	
Volume (µl / drop^{-1})	2002	2003	2004	2002	2004
N	1268	365	270	292	140
Average	1.519	0.105	0.678	0.45	0.34
Amplitude	0.01 – 6.8	0.001-0.85	0.2 – 6.0	0 – 1.3	0 – 6.0
Standard deviation	1.08	0.123	0.789	0.41	0.52
Probability of F-test among years		<0.001		<0.001	
(B)	Honeydew instantaneous			Honeydew potential	
Sugars (%)	2002	2003	2004	2002	2004
N	1244	283	235	28	57
Average	30.47	45.07	52.67	22.39	28.13
Amplitude	4.86 – 91.0	5.5 – 98.8	12.98 - 99.67	17.5 – 28.0	23.5 – 36.0
Standard deviation	16.7	21.77	20.78	4.9	2.99
Probability of F-test among years		<0.001		<0.01	

N - sample size

potential production (Table 2A). Sugar concentration also showed significant differences between years, and concentrations were higher in 2004, both for instantaneous and potential honeydew (Table 2B). In addition, significant differences between the averages for all years assessed for instantaneous honeydew availability, in both volume and concentration of sugars, were revealed by the Newman-Keuls separation test (SNK) (Fig. 2).

The instantaneous volume available in a drop of honeydew excreted in the year 2002 was on average 1.519 µl (± 1.08). In 2003, the volume available in one drop of honeydew was lower than in the previous year, with a mean of 0.105 µl (± 0.123). The honeydew volume per drop in the second year was 14.4 times lower than that measured in 2002. Intermediate values were measured in 2004, since the volume available in one drop of honeydew was 0.678 µl (± 0.789). Differences were also verified in the average volume of potential honeydew between 2002 and 2004 (0.45 µl and 0.34 µl, respectively) (Table 2).

Average total sugar concentration in instantaneous honeydew (Fig. 2A) in 2002 was 30.47 (± 16,7), significantly lower than that in 2003 and 2004. In 2003, the concentration of sugars in honeydew showed an average of 45.07%, and in 2004, it was even higher at 52.67%. The concentration of sugars in potential honeydew was also significantly different between 2002 and 2004; it was lower in 2002 (22.39%) compared to 2003 (28.13%) (Table 2B).

The months in the Southern Hemisphere with the highest available volume of instantaneous honeydew in 2002 were late autumn (May and June) to winter months (July), in which the mean volume availability was 2.206, 2.149 and 2.040 µl per drop of honeydew, respectively. In addition, available volume occurred in decreasing order during the months of April, September, August and March, which presented an average of 1.365; 1.319; 1.292 and 1.053 µl per drop of honeydew, respectively. The month of lowest instantaneous availability of honeydew was February, providing only 0.598 µl per drop (Fig. 2B). The concentration of sugars in the honeydew volume that accumulated in the filaments of the scale insects during the months of 2002 was, on average, 30.47%. Similarly, the monthly average sugar concentration ranged across months, from 17.7% in June to 54.3% in August. Thus, an inverse relationship between available volume and sugar concentration in honeydew is evident (e.g. Fig. 2B). In other words, the larger the available volume, the lower its total sugar concentration, as revealed by the Pearson correlation performed for the years of higher excretion, 2002 and 2004 ($r = -0.51$, $p<0.05$, $N = 17$).

Interestingly, the available volume of instantaneous honeydew during 2003 was much lower than that in 2002 and 2004 (Fig. 2A). In December of 2003, we saw the highest available volume of honeydew compared to the other months of the same year (Fig. 2C). However, the highest value in 2003 (0.461 µl per drop of honeydew) was lower than the lowest volume of excretion in 2002 (0.598 µl per drop of honeydew in February) (Figs. 2B and 2C). The lowest instantaneous availability of honeydew in 2003 occurred in March, providing 0.047 µl per drop of honeydew (Fig. 2C). The concentration of sugar in the honeydew during the months of 2003 showed, on average, 44.07% of total sugars, ranging from 23.4% in March to 73.5% in September (Fig. 2C).

In 2004, the highest volume available of instantaneous honeydew occurred in August with a mean of 2.16 µl per drop of honeydew, followed in September with 1.10 µl per drop of honeydew. These two values differ statistically from each other and from the values of other months of the year. The average monthly sugar concentration in 2004 ranged from 29.1% in January to 84.5% in July (Fig. 2D).

We found a significant and positive coefficient of correlation between the available volume of potential honeydew and the relative humidity of the air in 2002 and 2004 such that the higher the relative humidity of the air, the larger the volume secreted by the scale insects ($r = 0.66$, $p<0.05$, $N = 17$). In contrast, the volume of honeydew was negatively correlated with temperature, i.e., the higher the temperature, the lower the available drop of honeydew volume ($r = -0.62$; $p<0.05$; $N = 17$). A significant and negative correlation was also observed between the available honeydew volume and the concentration of sugars contained in the honeydew ($r = -0.51$, $p<0.05$, $N = 17$).

Evaluation of scale insects on bracatinga plants

In 2002, bracatinga plants were infested with a mean of 28.55 scale insects per 70.88 cm^2, while the amplitude ranged from two to 83 (Table 3). That mean value of 28.55 scale insects, represents an average of four insects per 10 cm^2. In addition, a significant difference was noted in the infestation intensity among analyzed plants (Table 3). Overall, we found an average abundance of 8,287 scale insects per individual bracatinga plant (Fig. 1D).

The result for the average volume of potential honeydew also showed significant differences among bracatinga trees (Table 3). However, Pearson's correlation coefficients for the volume of potential honeydew per plant between 2002 and 2004 were not significant ($r = 0.25$, $p> 0.05$, $N = 17$).

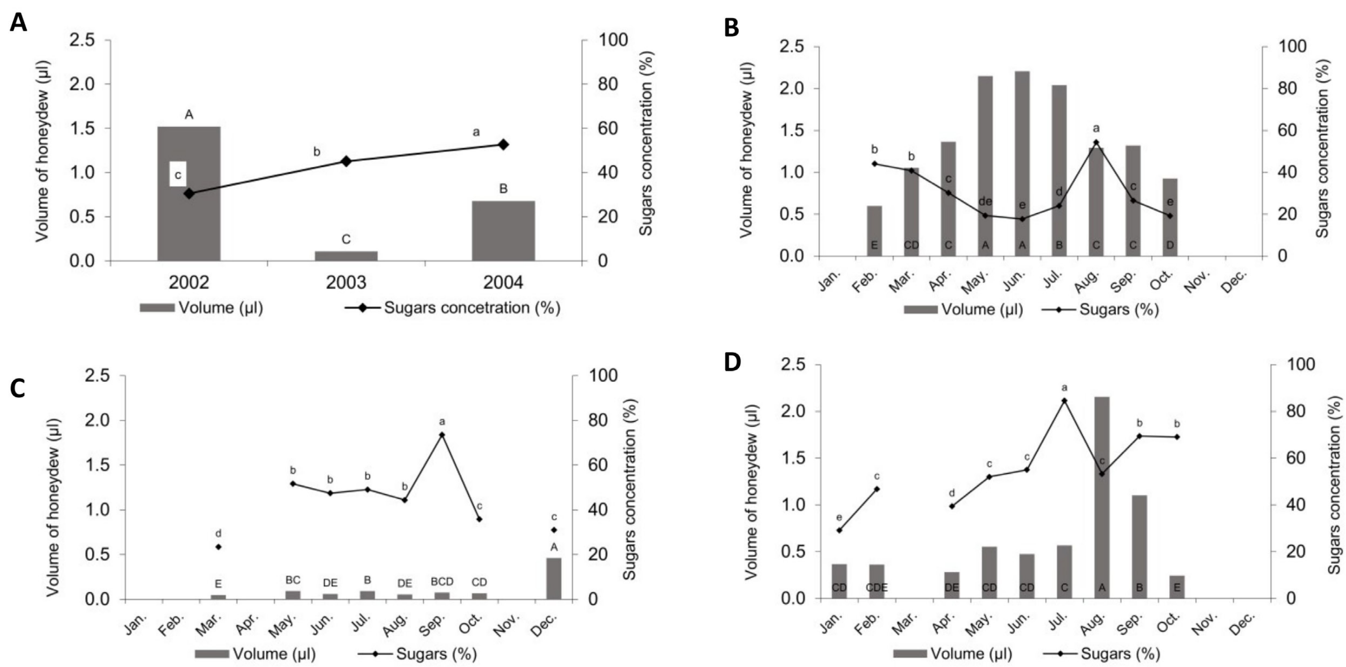

Figure 2 Instantaneous volume available per drop of honeydew (µl) (bars) and sugar concentration (%) (line). Average values of the volume and concentration of sugars during the three evaluation years (Fig. A). Average values of honeydew volume and sugar concentration over the months of 2002 (Fig. B), 2003 (Fig. C) and 2004 (Fig. D). No evaluations were carried out in January, November and December 2002; January, February, April and November 2003; or March, November and December of 2004. Capital letters in bars and lower letters in lines refer to the mean test for the volume and concentration of honeydew sugars, respectively. Means followed by the same letter (uppercase or lowercase) indicate that the values do not differ significantly (SNK = 0.05).

Table 3
Volume of potential, production per scale insect, per tree and per evaluated area of honeydew produced by *Stigmacoccus paranaensis* associated with *Mimosa scabrella* in Bom Retiro - SC, during the years 2002 and 2004.

Parameter	Years	
	2002	2004
Volume (µl scale insect $^{-1}$ h^{-1})	2.70	2.04
Volume (µl scale insect $^{-1}$ day^{-1})	64.8	49.0
Volume (L tree^{-1} day^{-1})	0.54	0.41
Volume (L ha^{-1} day^{-1})	235	177
Volume (L ha^{-1} 10 months^{-1})*	70,478	53,250
Density		
Plants of *M. scabrella* ha^{-1}	437.5	
Scale insect (tree^{-1} and ha^{-1})	8.3 x 10^3 e 3.6 x 10^6	

(*) Assuming 10 months of production (referring to the months in which the scale insect is feeding on the sap of the bracatinga) and assuming a constant volume production of honeydew.

Discussion

Density of bracatinga plants

Bracatingais show variations in plant density in naturally occurring areas. In the present study, our results revealed the occurrence of 0 to 600 plants ha^{-1}. This variation in population density is mainly related to the high correlation of regeneration from anthropic activity. Human activities, such as cattle raising, establishment of crops, extraction for use of wood or management to generate income for farmers, can decrease bracatinga plant density (Steenbock et al., 2011).

Although the evaluations of honeydew excreted by scale insects were performed in four plots, only two (plots 1 and 2) were used for the other analyses. Plots 1 and 2 showed the formation of isolated patches "capão", a characteristic of *M. scabrella*, with a resultant density of 437.5 plants ha^{-1}. In research carried out by Fonseca (1982) in a region of naturally growing bracatingais of different ages, the density of plants per area was also quite variable. In the municipality of Lages, SC, the density found was 590 plants ha^{-1} (10 years old), in Curitibanos, SC, 660 plants ha^{-1} (13 years old), in Campo do Tenente, PR, 810 plants ha^{-1} (11 years old), in Curitiba, PR, 900 plants ha^{-1} (7 years old), in Guarapuava, PR, 830 plants ha^{-1} (13 years old) and in Jaguariaiva, PR, 465 plants ha^{-1} (13 years old) (Fonseca, 1982).

It is still important to take into account the phenological characteristics of *M. scabrella* to understand the differences in plant density in bracatingais. As an exclusive species of secondary vegetation (Bartoszeck et al., 2004), bracatinga has low longevity, reaching up to 25 years of age, with the senile phase beginning at 17 years (Weber, 2007). Bracatingais at 20 years of age showed a survival of 25% of the plants, indicating that bracatingais present a reduction in population density inversely proportional to their age (Carpanezzi et al., 1988).

Evaluation of scale insects on bracatinga plants

The density of scale insects that adhered to bracatinga tree trunks showed a significant difference among the evaluated plants. The number of scale insects ranged from two to 83 individuals in the sampled units, which represented four scale insects every 10 cm^2. This difference is associated with their limited dispersion capacity restricted to a short period of its first nymph stage which is considered the main moment for scale insects to settle in host trunks. As they are apterous and have functional legs only in the first stage of the nymph, scale insects possibly undergo passive dispersion by the wind (Gullan and Kosztarab, 1997). However, Nestel et al. (1995) stated that the dispersion is not carried out over long distances; thus, scale insects remain in the host plant, resulting in an aggregate distribution. This cluster pattern was observed in the present study where plants were seen with trunks covered with scale insects, while in others, the occurrence of insects was not observed.

Difference in the density of scale insects among bracatinga trees was also observed by Reichholf and Reichholf (1973). They registered from 8 to 15 individuals / 500 cm² up to clusters containing an average of 20 scale insects per 100 cm². In New Zealand, the density of scale insects *Ultracoelostoma* spp in *Nothofagus* spp. (ha $^{-1}$) varied from 1.0 x 10^5 (Kelly, 1990) and 3.0 x 10^5 (Beggs et al., 2005) to 2.0 x 10^7 (Crozier, 1978). In this sense, it is possible to consider that the density of scale insects infesting *M. scabrella* plants is comparable to the average observed in other studies.

Honeydew production

The production instantaneous and potential honeydew in the municipality of Bom Retiro (SC) is associated with climatic conditions and the developmental stage of insects. This means that production is greater when the scale insects are at a more advanced stage. In regions with a greater production of honeydew honey, scale insects associated with *M. scabrella* have a biennial cycle, which explains the greater availability in alternate years. Thus, the greatest availability of honeydew to visitors will occur in the second year of the scale insect's life cycle, which occurs in even years, as represented in the present study during 2002 and 2004 when the insects were larger and at a later stage of development.

The difference in the amount of honeydew excreted by aphids as a function of their stage of development and body mass was also verified in other studies. According to Fischer et al. (2002), nymphs of *Metopeurum fuscoviride* (Hemiptera, Aphididae) in the first and second instars produce only half the amount of honeydew as that produced by the subsequent instars of the insect. The concentration of total sugars in the excretions of *M. fuscoviride* did not differ significantly among individuals of different ages, presenting an average of 80.2 µg of sugar / µl of honeydew^{-1} (Fischer et al., 2002). Golan (2008), studying *Coccus hesperidum* L. (Hemiptera, Coccidae) in Poland, observed that a reduction in volume and an increase in the size of the drops excreted by the insects take place in successive stages of development. Moir et al. (2018) noted that, in addition to the stage of development (body mass), the rate of excretion may also vary according to the insect's family. In this sense, these authors established and validated calculations capable of predicting the rate of excretion according to the body mass and / or family to which the aphids belong, concluding that the first parameter has a greater influence on the amount of honeydew produced (Moir et al., 2018). However, the scale insects that evaluated in our study cease the production of honeydew when they reach their highest body mass, as the reproductive stage begins.

Regarding the availability of instantaneous volume of honeydew between 2002 and 2004, which is greater than that in uneven years, the average availability per drop of honeydew in 2004 was 2.2 times lower than that in 2002. Since the life cycle of scale insects in the region where the present study was carried out is biannual, it was expected that these two years should present similar volumes of availability. Therefore, other factors are affecting production, such as the climatic conditions, which differ from year to year (Fig. S1), and the intensity of insect visitors on the honeydew drops. Therefore, the climatic conditions must be taken into account when interpreting the results of the present study.

Although no other studies have reported the influence of climatic conditions on the excretion of honeydew by scale insects in the municipality of Bom Retiro (SC), our results are corroborated by the study provided by Beggs et al. (2005). These authors suggested that climatic variation between years can affect the excretion of honeydew, both in the amount and in the concentration of sugars, owing to effects on the population dynamics of scale insects or honeydew collectors, changes in the physiology of the host plant or a combination of these factors. Additionally, the availability of honeydew can be compared to the nectar produced by flowers, which is affected by the intensity of sunlight, humidity (Dadant and Sons, 1979) and the foraging behavior of insects that feed on this nectar, i.e., "nectar standing crop" (Zimmerman and Pyke, 1988). In this sense, both the volume and concentration of sugars contained in the accumulated droplets are vulnerable to climatic conditions of the environment.

After excretion by the scale insect, we noticed that the honeydew droplets remained suspended on its anal filament and suffered from environmental activity all sort of other organisms. This could affect, for example, the concentration of total sugars in the honeydew by evaporation. In the present study, it was possible to verify the influence of climatic conditions on the available volume of honeydew, showing a higher volume and lower concentration of sugars when the air humidity was higher, but a reduction in volume and an increase in sugar concentration at higher temperatures. Because of evaporation, honeydew can become more viscous and obstruct the scale insect's anal filament (Beggs, 2001). However, the scale insect again excretes fresh honeydew if the end of the filament is cut by the action of rain (Moller et al., 1991) or insects (Barth, 1989), indicating that the collection of this resource by visitors would promote an increase in the rate of production of honeydew.

We demonstrated that the availability of honeydew excreted by scale insects was every other year (even) and seasonal; finishing in autumn-winter 2002 and at the end of winter in 2004. During summer time and every other year (odd) we noticed a marked reduction in insects collecting honeydew, particularly the activity of *A. mellifera*. It was also observed that these bees collected only the less viscous drops of honeydew, unlike other visitors, such as wasps, which did not have this preference. Moller et al. (1991) observed a reduction of up to 99% in the availability of excreted honeydew by *Ultracoelostoma assimile* after wasps visit. Thus, it is evident that the availability of honeydew at any time depends on the intensity of visits by the collectors and thus a determining factor in the amount of honeydew that remains available.

According to the results presented in this study, it is evident that honeydew excreted by scale insects associated with bracatinga presents itself as an important source of soluble carbon for different trophic levels, even with variations in the quantity and concentration of sugars within and between years. Bracatinga is a naturally occurring species of the mixed rainforest ecosystem, associated with the Atlantic Forest biome (Steenbock et al., 2011), as observed in different Brazilian states (Rotta and Oliveira, 1981), with greater concentration in the southern Brazilian Plateau (Carpanezzi et al., 1988). Thus, it is possible that the singularity of the scale insect and bracatinga interaction can be compared to that observed between *Nothofagus* trees x *Ultracoelostoma* scale insect in New Zealand beech forests in the amount of carbon per unit of forest area (Beggs et al., 2005).

In addition, the bracatingais can be conserved or managed to optimize the production of honeydew honey, an important nontimber forest product, as a source of income for beekeepers. According to Kunkel (1997), for bees to take advantage of the honeydew, it must be available in large uniform forests with hives close to the collection sites so that the density of insects can be high and constant. The formations of bracatingais with high density is a characteristic of a uniform forest, as described by Kunkel (1997), and additionally presents natural infestation by scale insects producing honeydew. In the forests of *Pinus brutia*, located in the Eastern Mediterranean, management is carried out to optimize the joint production of honeydew honey and pine wood, maximizing the expected value per area in pine plantations. Economically important for the production of wood, *P. brutia* forests can also supply pine honeydew honey produced by bees that collect honeydew from *Marchalina hellenica* (Hemiptera: Margarodidae), a scale insect that infests pines and feeds on its sap (De-Miguel et al., 2014).

Initiatives like this one described in the present study and others should be encouraged, as it is a sustainable alternative capable of promoting the maintenance of natural ecosystems and guaranteeing income for beekeepers in the southern region of Brazil and abroad.

Previous studies demonstrated that honey from melato, the honeydew excreted by scale insect in bracatinga trees, presents distinct physicochemical characteristics from those of blossom honeys (Bergamo et al., 2019). In addition to be unique (Hickenbick and Figueiredo, 2017) and its quality is influenced exclusively by the natural and human factors, the bracatinga honeydew honey can be identified and measured. Our study provides additional scientific data regarding honeydew production from this system. In this context, this contribution can support the claim of an appellation of origin of *mel de melato de bracatinga* (bracatinga honeydew honey).

Conclusion

In this study, the bracatinga (*M. scabrella*) presented a grouped distribution, equivalent to the pattern of bracatingais observed in regions of natural occurrence of the species in the states of southern Brazil. The intensity of bracatinga infestation by honeydew excretory scale insects differed from tree to tree. Both the rate of excretion and the available volume of honeydew depend on the stage of development of scale insect, increasing as the insect develops. In the municipality of Bom Retiro (SC), the highest availability of honeydew occurs in alternate years.

Climatic factors were also correlated with the rate and availability of honeydew excretion. For both available volume and secreted volume of honeydew, higher relative humidity increases volume of honeydew produced, and higher temperatures reduced volume of honeydew. However, the greater available volume correlates with lower content of sugars.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Martins-Mansani M and Orth A I performed the study conception and design. Martins-Mansani M collected the data in the field. Martins-Mansani M and Faita M R performed the data analysis. The first draft of the manuscript was written by Mansani-Martins M, Orth A I, Nodari R O and Faita M R. All authors commented on previous versions of the present manuscript. All authors read and approved the final manuscript.

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Supplementary material

The following online material is available for this article:

Figura S1 - Average rainfall (mm) and temperature (° C) data recorded during the years 2002 (A), 2003 (B) and 2004 (C) and climatological normals for the Serra Catarinense, Brazil (Data from Lages Meteorological Station - EPAGRI - CLIMERH / SC).