

Protocol & Techniques

Advances in the use of attractive traps in collecting Neotropical Social Wasp

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Abstract. It is possible to elaborate adequate methodologies for collecting social wasps from the knowledge of their behavior. Thus, the objective of the present study was to compare the attractiveness of different sized PET bottles (plastic soda bottles) (by volume), types of juice and variety of attractive flavors of juice in elaborating attractive traps to optimize the method for diverse works involving social wasps. The work was elaborated in the Botanical Garden of the Federal University of Juiz de Fora, between the years of 2015 and 2016, and was divided into three experiments. The three experiments did not present a significant difference in relation to the richness or abundance of social wasps species, therefore the bottle volume choice used as traps, the type of juice (natural or artificial) and the variety of attractive substance should be selected according to practicality and low economic value. As such, the use of 0.5 L PET bottles as attractive traps filled with 150mL of mango, guava or passion fruit artificial juice is suggested.

Keywords: Methods, Sampling, Protocol, PET bottle, Vespidae.

Social wasps actively participate in the trophic balance of ecosystems as pollinators and predators of herbivores during foraging (Hunt 2007; Prezoto et al. 2019), being a fundamental activity for the survival of colonies (Richter 2000).

The location of foraging resources (carbohydrates and prey) is guided by their perception of chemical signals, responsible for communication between insects and the environment around them (Lewis & Norlund 1984; Saraiva et al. 2017). Based on this principle, one of the most used methodologies for capturing social wasps are traps with attractive baits, which attract the insects due to the volatility of the substance used (usually fruit juice), which fall into the liquid and drown (Souza et al. 2015; Barbosa et al. 2016; Maciel et al. 2016).

Due to its practicality, this method is more used than Malaise trapping (Barbosa et al. 2016) because it uses widely available polyethylene terephthalate bottles (PET), like plastic soda bottles and baited with fruit juice (Souza et al. 2015; Maciel et al. 2016). However, the increasing use of this methodology has been generating changes over the years such as in using different substances as attractive food (Elpino-Campos et al. 2007; Jacques et al. 2015) making it difficult to compare the results of these works. Another variation is the volume of the used PET bottles. Although the majority of the studies use two-liter bottles, it is possible to find researchers who use smaller containers (Sorvari 2013; Demichelis et al. 2014; Porporato et al. 2014).

In order to understand the possible effects of variations in this methodology on the results of diversity studies, our objective was to test the performance of different models of attractive traps for standardization and optimization of the sampling of social wasps and, with that, to suggest a collection protocol that optimizes the size of the trap, as well as the substance used as a food attractant.

The work was carried out at the Botanical Garden of the Federal University of Juiz de Fora, Brazil (JB-UFJF) (21°43'28" S; 43°16'47" W), which comprises a Montana Semi-deciduous Forest fragment (Veloso et al. 1991) with an area of 84 hectares and whose fauna of social wasps is well known. The Botanical Garden is located in the urban perimeter of Juiz de Fora, southeast in the state of Minas Gerais, Brazil, at 750 m altitude and having a warm subtropical climate with dry winter and rainy summer (Cwa), according to the Köppen-Geiger classification (Sá-Júnior et al. 2012). The area was classified by Santiago et al. (2014) as a complex landscape of expressive richness and diversity, with floristic

heterogeneity of arboreal growth and predominance of pioneer plants, in addition to endangered species and considerable presence of exotic species.

The Botanical Garden is formed by distinct phytophysiognomies, and a transect of 450 m was established on a pre-existing trail in the area called "Palmital", in which there is an abundance of palmito-juçara [*Euterpe edulis* Mart. (Aracaceae)], where the traps were installed.

The work was developed between November 2015 and May 2016 and divided into three experiments: I - the performance of different bottle volumes was evaluated. Bottles of 0.5 L, 1.5 L and 2 L baited with the same attractant food, artificial guava juice, and installed in the order "0.5 L, 1.5 L and 2 L" were used. The attractive substance was chosen based on the works of Brügger et al. (2019) and Souza et al. (2015). Fifteen traps of each liter volume were used, which totaled 7,200 hours of sampling effort per volume size at the end of 20 days of collection. II - The attractiveness of natural and artificial juices was evaluated based on the results of Experiment I. To do so, natural guava juice was used made with 1 kg of fruit, 250 g of refined sugar and 2 L of water blended and then sifted, and also the artificial juice of the same flavor. Forty (40) traps of 0.5 L were alternately installed, 20 of which had natural juice and 20 had artificial juice. The sample effort for each type of juice was 9,600 hours. And III - This experiment evaluated the attractiveness of different flavors of juice: artificial juices (according to the results of Experiment II) of mango, guava, passion fruit, orange and banana, as well as light red wine (10.6% alcohol) and pilsen beer (4.6% alcohol). The installation sequence of the baits was randomly defined by lot, so that they were not repeated. Still taking into account the result of Experiment I, six traps of 0.5L of each bait were used, with a sampling effort of 2,880 hours per bait.

Each of the three experiments consisted of four trials lasting five days each, totaling 20 days of sampling effort. The traps were inspected at the end of each trial, and the social wasps were stored in 70% alcohol for later identification in the laboratory. The experiments were performed consecutively, that is, experiment one first, followed by experiments two and three.

The attractive traps were made from transparent PET bottles, with three triangular side openings of 2 x 2 x 2cm. In addition to being an easily found material, the shape of the bottles allows rainwater to drain and not enter through the openings, thus enabling use in different

seasons of the year. Based on the literature (Souza & Prezoto 2006; Jacques et al. 2015; Klein et al. 2015), all the traps were baited only on the first day with 150 mL of attractive food, determined in previous tests. They were installed at a distance of 10m from each other, always at a height of 1.5 m from the ground and remained in the field for five consecutive days as proposed by Souza et al. (2015). All the artificial juices used as an attractive substance were from the Tial® brand.

Specimens were identified to species using the keys of Richards (1978). Pinned specimens were used to create a reference collection in the Laboratory of Behavioral Ecology and Bioacoustics (LABEC) of the Federal University of Juiz de Fora.

The normality of the samples was verified by the Shapiro-Wilk test. Therefore, the Kruskal-Wallis test was applied in order to evaluate there was a statistical difference of richness and abundance of social wasps recorded by different volume traps (Experiment I) and different food attractants (Experiment III); as for the different types of juice (Experiment II), the Mann-Whitney test was applied. All the tests as generated by the BioEstat 5.0 (Ayres et al 2007) program, with was assessed at the significance level of 0.05.

The Efficiency Index (EI) was also generated, as given by the adapted formula (Giannotti et al. 1995): $EI = S * 100/SM$. For Experiment I, EI_{vol} (Efficiency Index for trap volume) S represents the number of species captured by each trap volume; for Experiment II, the index EI_{juice} (Efficiency Index for type of juice) S is the number of species registered by each type of juice; lastly, in Experiment III, S is the number of species registered by each food attractant in $EI_{attract}$. SM represents the total number of species registered for each experiments.

We recorded a total of 387 social wasps from all traps representing eight species belonging to the *Agelaia* Lepeletier, 1836, *Polistes* Latreille, 1802 and *Polybia* Lepeletier, 1836 genera (Tab. 1), which are expected for the area (Alvarenga et al. 2010; Barbosa et al. 2016).

Eight species were recorded from Experiment I (Tab. 1) and there was no significant difference regarding the performance of the three bottle trap sizes evaluated in relation to abundance ($H=0.6644$; $p=0.71$) or in relation to species richness ($H=4.8356$; $p=0.08$). Thus, the choice of the bottle size to trap social wasps be used can be made based on practicality for the researcher. We consider the 0.5 L bottles to be the best option, since they occupy less space than the commonly used 2 L bottles (which are usually used in the literature), thus facilitating the logistics of field work. Furthermore, 0.5 L bottles were the only bottles that trapped all eight species ($EI_{vol} = 100\%$) (Tab. 1). For this reason, we used this bottle trap size for Experiments II and III.

Seven species were recorded (Tab. 1) in evaluating the performance of natural and artificial juices in Experiment II. Two species were captured exclusively by the natural juice and three by the artificial juice. However, there was no significant difference in relation to abundance ($U=0.8305$; $p=0.40$) or in relation to richness ($U=0.4472$; $p=0.65$). Thus, the substance must be selected according to practicality and lowest

Table 1. List of registered social wasp species and efficiency index of each variable in each experiment.

Species	Experiment I			Experiment II				Experiment III				
	0.5 L	1.5 L	2 L	Natural	Artificial	Mg	Gu	Pa	Or	Ba	Wi	B
<i>Agelaia multipicta</i> (Haliday, 1836)	49	53	50	36	75	9	12	9	14	4	2	2
<i>Agelaia vicina</i> (Saussure, 1854)	2	6	2	1	-	1	-	-	-	-	-	-
<i>Polybia fastidiosuscula</i> Saussure, 1854	5	5	4	-	2	5	3	5	2	-	-	-
<i>Polybia jurinei</i> Saussure, 1854	3	1	10	-	1	-	-	1	-	-	-	-
<i>Polybia lugubris</i> Ducke, 1905	1	-	-	-	-	-	1	-	-	1	-	-
<i>Polybia platycephala</i> Richards, 1951	1	1	-	-	2	-	-	-	-	-	-	-
<i>Polistes pacificus pacificus</i> Fabricius, 1804	1	-	-	1	1	-	-	-	-	-	-	-
<i>Polistes versicolor</i> (Olivier, 1791)	1	-	-	1	-	1	-	-	-	-	-	-
Total richness	8	5	4	4	5	4	3	3	2	2	1	1
Total abundance	63	66	66	39	81	16	16	15	16	5	2	2
Efficiency index (EI)	100%	62.5%	50%	57.1%	71.4%	66.7%	50%	50%	33.3%	33.3%	16.7%	16.7%

Legend: Mg – Mango; Gu – Guava; Pa – Passion fruit; Or – Orange; Ba – Banana; Wi – Wine; B – Beer.

cost.

When comparing the advantages of using each type of juice, it was observed that the artificial juices had a higher efficiency index ($EI_{juice} = 71.4\%$) (Tab. 1) in relation to the performance in the field. We found the artificial juices in the field trap were more specific capturing only social wasps which facilitates material sorting in the field and exerts less impact on the local fauna. Natural juices are less practical, having to be prepared a day in advance, whereas artificial juices can be purchased and stored for the entire study period and even stored at the collection site, thus optimizing the bait transport logistics. Another advantage of artificial juices is the homogeneity of the baits throughout the study. Whereas fruit quality and storage mode vary according to the season, processed or artificial juices remain the same until the day of purchase and allows for much needed standardization during the study duration. Artificial juices are also less expensive and the cost varies less throughout the season than natural juices can facilitate better financial planning for a project. Despite these advantages, few diversity studies have used artificial juices (Henrique-Simões et al. 2012; Brügger et al. 2019).

We recorded six species of social wasps in Experiment III from traps containing one of five artificial juices, beer or wine (Tab. 1). Wine and beer only recorded one species each, banana and orange two species each, passion fruit and guava three species, and mango four species (Tab. 1). However, there was no significant difference between means in relation to abundance ($H=3.7643$; $p=0.70$) or in relation to richness ($H=3.8395$; $p=0.69$).

Thus, the bait selection criterion to be used must also be related to practicality and low cost; however, the highest efficiency index was taken into account since it is an artificial product with an approximate value, thereby corresponding to mango juice according to the results ($EI_{attract} = 66.70\%$). The juices most used in Brazil in the literature are those of passion fruit and guava (Maciel et al. 2016), which are also indicated for carrying out diversity studies according to the efficiency index ($EI_{attract} = 50\%$).

In Europe, beer-based baits are successfully used for monitoring social wasp pest species (Vespidae: Vespinae) (Dvořák 2007; Dvořák et al. 2010). We found the alcoholic substances in our study were not attractive to neotropical wasps.

Standardizing a method is the first step towards creating a standard protocol that will help new studies. From the results obtained in the experiments, using 0.5 L polyethylene terephthalate bottles (PET) with three triangular lateral openings of 2 x 2 x 2 cm to 10 cm from the base of the bottle (Fig. 1) are suggested for traps, as well as filling the trap with 150 mL of artificial mango, guava or passion fruit juice (Fig. 1), with installation of the traps at 1.5 m from the soil and having them remain in the field for five consecutive days, as already standardized in the literature.



Figure 1. The triangular side opening of 2 cm x 2 cm x 2 cm located 10 cm from the base of the 0.5 L bottle is highlighted in the white circle; the trap filled with 150 mL of artificial juice is highlighted in the white bracket.

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Authors' Contributions

Data collection and first version of the manuscript was written were performed by TTM and BCB. All authors wrote the manuscript, discussed the results, and contributed to its final version.

Conflict of Interest Statement

We have no conflict of interest to declare.

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