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First report of colored pan traps to capture Drosophilidae (Diptera)

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ABSTRACT

The use of robust sample methodologies to estimate the highest number of species with different ecological requirements and traits is essential to the knowledge construction of the biodiversity and to establish wildlife assessment and monitoring programs. Our aims were to study the performance of colored *pan traps* in the capture of Drosophilidae (Diptera), a method never used for sampling this taxon. During six months, colored pan traps (blue, yellow and white) were tested in three areas in Southern Brazil. We captured 375 individuals of 30 species belonging to four genera of Drosophilidae. The most abundant species were *Drosophila lutzii* (n=215) *p*=0.58, *Scaptomyza* sp. (n=55) *p*=0.15 and *D. bromelioides* (n=17) *p*=0.04, all of them, anthophilous species. All colored pan traps captured a high quantify species of Drosophilidae, mainly anthophilous species.

Introduction

Drosophilidae (Insecta, Diptera) is considered the ideal model for studying different ecological issues, such as edge effect and human disturbance, for example (Mata et al., 2008, 2010; Penariol and Madi-Ravazzi, 2013). One of the main reasons behind this practice is its ability to use a wide variety of resources as substrate (mainly fruits, flowers and macroscopic fungi) (Carson, 1971; Grimaldi, 1987; Markow and O'Grady, 2008; Valer et al., 2016; Mendes et al., 2017; Schmitz and Valente, 2019).

Some different methods are common to insect surveys, such as the banana attractive trap (Gottschalk et al., 2007; Bizzo et al., 2010; Hochmüller et al., 2010; Emerich et al., 2012; Garcia et al., 2012; Poppe et al., 2012; Duarte et al., 2018; Mateus et al., 2018), use of entomological nets, collection in flowers (Schmitz and Valente, 2019; Cordeiro et al., 2020), collection in macroscopic fungus resources (Gottschalk et al., 2009; Valer et al., 2016) and pan traps (Westphal et al., 2008; Halinski et al., 2018). However, although historically the use of different methodologies for capturing individuals with different ecological and evolutionary behaviors seems to be clear to measure the fauna of the natural environments, analyzing articles that present local researches of Drosophilidae reveals that the way the individuals are captured is little variable from one study to another.

In this context, there are several techniques to capture Drosophilidae in the wild, all of which with their positives and negatives aspects. For instance, banana attractive trap has been widely used to capture Drosophilidae, but is biased due to a significant collection of species of the genus *Drosophila*, while sub-sampling other groups (Penariol et al., 2008; Mendes et al., 2017). Besides that, some methods, such as active resource collection or banana/orange attractive traps (Valer et al., 2016; Cordeiro et al., 2020), are excellent for capturing a large number of dominant species, and others are less efficient in sampling species richness but are excellent for their ability to capture underrepresented species (rare species), such as malaise traps for example (Frankie et al., 2002; Agosti and Alonso, 2003). In general, the most used approaches for collecting adults are based on capturing individuals attracted by bait (Medeiros and Klaczko, 2004; Penariol et al., 2008). Thus, the use of traps with banana baits, for example, is one of the most used techniques in Drosophilidae fauna surveys.

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The use of the colored pan traps has intensified considerably since the last decade, mostly associated with floral resources, due to its efficiency in capturing a wide range of floral visitors (Westphal et al., 2008; Wilson et al., 2008; Tuell et al., 2009; Vrdoljak and Samways, 2012; Halinski et al., 2015, 2018). According to the literature, this technique also has no collector bias of insects (Westphal et al., 2008). It is a good method to capture flower dwelling Drosophilidae, but it is biased in this direction (it favors flower dwelling Drosophilidae). Moreover, different color pan traps seem to attract different frequencies of species, another bias. It is a potential method of monitoring diversity of capturing insects, since this technique does not depend on odors' attraction or eliminates the collector (Almeida et al., 1998; Campbell and Hanula, 2007; Wilson et al., 2008).

The colored traps have been used to capture many different types of insects (Campbell and Hanula, 2007). For example, some yellow traps have been used to catch a wide variety of phytophagous insects (Kirk, 1984), predators (Leksono et al., 2005), and pollinators (Halinski et al., 2015); blue pan traps catch predominantly Hymenoptera (Aguiar and Sharkov, 1997; Halinski et al., 2018), and white or yellow traps catch mostly Diptera (Disney et al., 1982; Halinski et al., 2018).

In this sense, to know the environments' biodiversity, it is always advisable to use different collection methods, estimating the largest possible number of groups with different ecological and behavioral characteristics (Schauff, 1986). Here, we tested the efficiency of pan traps methodology for the Drosophilidae and identified the species composition in different agricultural habitats in Southern Brazil.

Material and methods

Drosophilidae sampling

The study using colored pan traps was conducted in three environments in an ecoclimatic region in the state of Rio Grande do Sul, municipality of Estrela, Brazil (29°30'07"S, 51°57'57"W). The region is characterized by pasture areas, fragments of forest, and annual crops (canola, soy, corn, and wheat) (Maluf and Westphalen, 1994). According to the Köppen classification, the study area is considered cfa with humid subtropical climate, after a prior classification of Köppen-geiger (Alvares et al., 2013). The municipality of Estrela is in the Rio Grande do Sul Central-oriental mesoregion and belongs to the Northeast Lower Hillside. The average temperature is 19.3°C, the average relative humidity is 75%, annual precipitation is 1.547mm, an altitude of 52m, and predominance of subtropical forest (IBDF, 1983; IBGE, 1992; Maluf and Westphalen, 1994).

To test colored pan traps in Drosophilidae, with one collection each month, the individuals were sampled with blue, yellow, and white pan traps exposed for 24 hours in each environment (adapted from Westphal et al., 2008), from August to December 2010 and July 2011. These traps were placed in three areas associated with oilseed crops (*Brassica napus* – Hyola 61- canola crops). In the period of blooming of the crop (August to October), three plots were collect inside the crop, inside the forest fragments and near the plantation.

Description of the colored traps

The traps consist of colored pots (white, yellow, and blue) of 11cm diameter and 4mm high painted with ultraviolet spray to maximize insects' attraction. The pots were filled with water and neutral liquid detergent to break the surface tension, thus making the captured material sink (Almeida et al., 1998; Williams et al., 2001; Krug and Alves-dos-Santos, 2008; Teixeira, 2012) (Fig. 1). The plot configuration used 15 traps divided into three equilateral triangles (with three colors traps) with sides measuring three meters totalizing 270 traps (45 for each color; to 270: 15 x 3 = 45; 45 x 6 = 270). The triangles were distributed in a way that they were 15m apart (adapted from FAO, 2010). The traps were adjustable in the supports matching the height of the vegetation.

Species identification

The individuals removed from the traps were fixed in absolute alcohol and identified based on external morphology, using taxonomic keys and species descriptions according to specialized literature (Burla, 1956; Grimaldi, 1987; Vilela and Bächli, 1990; Grimaldi, 2016; Schmitz and Valente, 2019). Individuals of cryptic species were prepared and dissected for later identification of terminalia (females species were identified to external morphology and associated with males, respectively) (Bächli et al., 2004). Voucher specimens of all the recorded species were deposited in the Entomological Collection of the Museum of Science and Technology, at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Statistical analysis

The absolute and relative abundances of each species (n and p, respectively) and the species richness (number of species in the sample, S) were used to characterize the assembly captured. Sampling effort was estimated by coverage of Drosophilidae species represented in

Figure 1 Representation of colored pan traps model used in study areas, the municipality of Estrela, Rio Grande do Sul State, Southern Brazil.

these three areas associated with oilseed crops along the year with colored pan traps. Thus, the number of individuals was used and an accumulation curve was constructed in the R environment, using the "iNEXT" package (Hsieh et al., 2016).

In the iNEXT analysis, individual-based abundance data were used to select a diversity order of q, based on species richness (q = 0) (Chao et al., 2016). Significance level was calculated with 999 permutations with Chao1, an estimator based on abundance that underestimate true richness at low sample size based on the number of singletons (species captured once) and doubletons (species captured twice). Besides that, iNEXT can interpolate and extrapolate species richness by taking into account a measure of sample coverage (Chao and Jost, 2012; Chao et al., 2016).

Results

A total of 375 individuals were captured, belonging to four genera and 30 taxa (Table 1). Among the sampled genera, *Drosophila* presented the highest species richness (S=13), followed by the *Scaptomyza* (S=7), which was recorded for the first time in the Pampa Biome, in Brazil. *Drosophila lutzii* was the dominant species in the two areas evaluated (n = 215; p = 57.9%), followed by *Scaptomyza* sp. (n = 55; p = 14.8%) and *D. bromelioides* (n = 17; p = 4.6%).

The individual rarefaction curve shows the taxonomic differences between the sampled areas. However, the sample coverage curves do not indicate a trend towards asymptote (Fig. 2), suggesting that a greater number of species could be sampled; however, indicating the need for increasing sampling occasions in these sites. Furthermore, Chao1 estimator predicted 71 and 43 species to canola crops and forest fragments. Results suggest that we can collect more species in

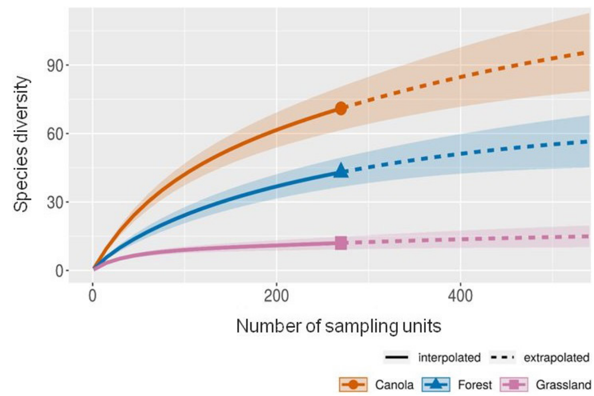

Figure 2 Drosophilidae richness (q = 0) estimated by rarefaction (solid curves) and extrapolation (dashed curves) based on sample size, with corresponding 95% of confidence intervals (shaded areas).

Table 1
Absolute abundance of Drosophilidae species collected from August to December 2010 and July 2011 in three areas of the municipality of Estrela, Rio Grande do Sul State, Southern Brazil, with pan traps with three colors (yellow - Y, blue - B, and white - W), the relative abundance of species in all sample, and the months in that each species was collected (p, relative abundances, a, p <0.01). Abbreviations: July: S, August: A, September: S, October: O, November: N, December: D.

Species	Canola crops			Forest fragments			Grassland vegetation			n	p	Sampling month
	Y	B	W	Y	B	W	Y	B	W			
Drosophila bandeirantorum Dobzhansky and Pavan, 1943		1								1	a	A
Drosophila bromeliae Sturtevant, 1921	3				2	1			1	7	0.02	J, A, S
Drosophila bromelioides Pavan and Cunha, 1947	1	8	1		1		1	1	4	17	0.04	J, S
Drosophila buscki Coquillett, 1901	1	2					1			4	a	A, S
Drosophila denieri Blanchard, 1916					4	1			1	6	0.01	J, A
Drosophila flexa Loew, 1866									1	1	a	J
Drosophila griseolineata Duda, 1927						1				1	a	S
Drosophila lutzii Sturtevant, 1916	30	74	7		32	3	8	14	47	215	0.58	J, A, S, O
Drosophila maculifrons Duda, 1927				2						2	a	S
Drosophila mediopicta Frota-Pessoa, 1954									1	1	a	J
Drosophila mediopunctata Dobzhansky and Pavan, 1943	3									3	a	S
Drosophila melanogaster Meigen, 1830				1						1	a	S
Drosophila pallidipennis Dobzhansky and Pavan, 1943	2	4	1				1			8	0.01	J, A, S
Drosophila polymorpha Dobzhansky and Pavan, 1943		1								1	a	A
Drosophila sp.1		1								1	a	S
Drosophila sp.2							1			1	a	S
Drosophila TIPO III (Schmitz and Valente, 2019)		1								1	a	S
Drosophila willistoni Sturtevant, 1916	4									4	0.01	A, S
Leucophenga sp.5 (Mendes et al., 2017)						1				1	a	S
Zygothrica sp.1		1	1							2	a	N
**Zygothrica venustipoeyi* Burla, 1956									1	1	a	O
Zygothrica prodispar Duda, 1925		2		3	6	1			1	13	0.03	S, D
Zygothrica zygopoeyi cf.					1					1	a	S
Scaptomyza sp.1	1									1	a	S
Scaptomyza sp.2	2		1							3	a	S
**Scaptomyza adusta* Loew, 1852	4	2	2							8	0.02	S
**Scaptomyza pipinna* Goñi and Vilela, 2016	3	2	1							6	0.01	S
Scaptomyza sp.5	3	3	1							7	0.02	S
Scaptomyza sp.6	1		1							2	a	S
Scaptomyza sp.7	10	3	2	28	9	3				55	0.15	S
Total	68	105	18	34	55	11	12	15	57	375	1	

*p, relative abundances, a, p <0.01; *new record Pampa Biome*

Table 2
Indices of diversity and richness estimators for Drosophilidae in three habitats located in Estrela, Rio Grande do Sul State, Southern Brazil.

	Canola crops	Forest fragments	Grassland vegetation
Richness obs (S)	20	11	11
Absolute abundance (n)	191	100	84
Dominance	0.826	0.829	0.951
Singletons	5	5	9
Doubletons	3	1	0
Chao 1	71	43	12

this location with this type of baited trap. In grassland vegetation the curves indicated the inflection point, with Chao1 estimator predicting 12 species, close to the species richness values observed (Table 2).

Absolute abundance and species richness observed of Drosophilidae regarding the trap colors and sampled areas, showed higher abundance and species richness in the Canola crops area for almost all the trap colors (except the white trap). This results could be observed for abundance and species richness by trap color between the sampled areas (except for white color in Grassland) (Table 1).

Discussion

We know that Drosophilidae species can use a wide variety of resources as a substrate (Carson, 1971; Markow and O'Grady, 2008), which are used for feeding and/or as courting sites for reproduction. However, although the family occupies several niches, fauna surveys focus on a few techniques for capturing species, privileging certain taxonomic or ecological groups (Carson, 1971; De Toni et al., 2007; Gottschalk et al., 2009; Hochmüller et al., 2010; Mitsui et al., 2010; Garcia et al., 2012; Poppe et al., 2012; Valer et al., 2016).

The sample coverage values in the collected traps have shown superiority in the abundance and species richness of Drosophilidae captured in Canola crops, a pattern also observed for other groups of insects (Halinski et al., 2015; Le Feón et al., 2016; Halinski et al., 2018), and similar to that pointed out by Prado et al. (2017). The use of pan traps is recommended for insect inventories in open landscapes with flowers, since only a few taxonomical groups have distribution restricted to closed landscapes (Prado et al., 2017). In massive flowering, the methodology of pan traps supports the collection of almost all groups of insects, especially those that use floral resources like pollen and nectar. Because of canola's open flower, all insects, including Drosophilidae, are potential pollinators and this could explain why we found such flies in our samples, but further studies are needed.

Accumulation curves indicate a higher coverage with pan traps in the Canola crops and grassland vegetation areas. But the values are also high in the forest area, revealing the importance of micro-habitats and their influence on communities' structuring. The different efficiency profile of the trap in the open Canola crops suggests that these changes can be attributed to the floral biology of the extant plants in the sampled environment, as well as the higher incidence of light on the trap, that could attract more individuals (Campbell and Hanula, 2007; Gollan et al., 2011; Vrdoljak and Samways, 2012; Azevedo et al., 2015).

In addition, the color of the traps influenced the efficiency of capturing Drosophilidae, as it is possible to observe by the higher species-richness and absolute abundance in the blue and yellow pan traps. Different results were obtained from other groups of insects, where only the yellow pan trap was identified as better for capturing individuals (Leong and Thorp, 1999; Ramírez-Freire et al., 2014; Azevedo et al., 2015). However, blue pan traps can also catch different species or, as in our study, more individuals of *D. lutzii.* Here, yellow pan traps attract more flies in the *Brassica napus* (canola crops) period, where the canola flowers are yellow. Such as the genus *Scaptomyza*, collected only in the canola environment in September, which corresponds to the peak of the canola blooming period. Thus we hypothesized that the different colors in the area (such as yellow pan traps similar to the color of canola crops) could be the reason to attract different fauna, and very useful to know the environment's diversity. However, these results showed possible consequences on the estimated diversity of the canola environment capturing uncommon or rare species, not collected for other techniques and/or sub sampled other species groups.

Drosophila proved to be the most representative genus of fauna associated with colored pan traps, with the dominance of the species of the *Phloridosa* subgenus and *bromeliae* group in all the sampled months. Among them are *D. lutzii*, *D. bromelioides*, *D. bromeliae*, and *D. denieri*, which have a wide distribution in the Neotropical region and are found in a wide variety of plants (Frota-Pessoa, 1952; Petersen, 1960; Silva and Martins, 2004; Blauth and Gottschalk, 2007; De Toni et al., 2007; Gottschalk et al., 2007; Grimaldi, 2016; Schmitz and Valente, 2019; Cordeiro et al., 2020). In recent studies, Cordeiro et al. (2020) and Schmitz and Valente (2019) investigated the Drosophilidae fauna associated with flowers in Brazil and demonstrated that these species are the most representative, corroborating the efficiency of pan traps for capturing anthophilous Drosophilidae.

Concerning the knowledge of *Scaptomyza*, the second most abundant genus present in this study, the literature mentions that its species are mainly saprophagous (Hackman, 1959; Brncic, 1983; Markow and O'Grady, 2008). In addition to this habit, some species of *Scaptomyza* are randomly sampled using other breeding sites, such as spider egg bags, mustard leaf-miners (Brassicaceae), and some are predators (Montague and Kaneshiro, 1982; Magnacca et al., 2008; Markow and O'Grady, 2008; Lapoint et al., 2013; Schmitz and Valente, 2019).

In Brazil, the genus is virtually ignored with just seven described species recorded, five of them from Pampa Biome (Santos and Vilela, 2005; Gottschalk et al., 2008; Poppe et al., 2016; Schmitz and Valente, 2019), and many waiting to be described, as those sampled in this study. The results show significant taxonomic gaps in the knowledge of the genus for the Neotropics, which can be attributed to the sampling difficulty. For these reasons, we consider that the pan traps are efficient and recommend them for future studies of *Scaptomyza*. Other benefits of this method are the absence of resource bias and the low cost, since human resources for collections can be reduced.

Considering the diversity of species and knowing part of their hosts, both in the larval and adult stages, it is assumed that *Zygothrica* is predominantly mycophagous (Burla, 1956; Grimaldi, 1987). Even so, some species also use flowers as a trophic resource, such as *Zygothrica dispar* and *Z. prodispar* sampled here, once again reinforcing the use of this technique for sampling anthophilous species. Besides, we captured *Z. venustipoeyi*, which represents a new record of occurrence and expands its distribution to the south (29° 30' 07"S, 51° 57' 57"W) (Gottschalk et al., 2008; Poppe et al., 2016; Bächli, 2019).

Fauna surveys in natural environments based on banana, flower, and fungi baits in Pampa biome, for example, have sampled between 9 to 46 species in temporal studies (Hochmüller et al., 2010; Poppe et al., 2012; Valer et al., 2016; Mendes et al., 2017; Schmitz and Valente, 2019; Cordeiro et al., 2020). The species richness obtained in this study, compared to those studies, praises colored pan traps as a good alternative to assess sample coverage in different environments, regardless of abundance since this technique has found species that are not usually detected by traditional surveys.

This colored traps demonstrated a marked efficiency in sampling Drosophilidae species (375 individuals), mainly anthophilous and some are bycatch in flowers in the Neotropical region, such *D. melanogaster* and

D. willistoni (Frota-Pessoa, 1952; Pipkin, 1964; Brncic, 1983; Grimaldi, 1987; Schmitz and Hofmann, 2005; Schmitz and Valente, 2019). Besides that, others groups of insects were sampled on this study with colored traps such as wasps (814 individuals), bees (350 individuals), Diptera taxa (1.110 individuals), coleopterans (116 individuals) and butterflies (58 individuals), corroborating its efficiency in capturing a wide range of floral visitors (Halinski et al., 2015, 2018).

The positive results obtained with this technique bring new implications for biodiversity conservation for this group of entomofauna, in addition to proposing the method as a new alternative for capturing adult anthophilous species that can yield a sufficiently representative portion of species richness in different habitats (Hagler and Jackson, 2001; Wilson et al., 2008; Grootaert et al., 2010). Robust methodologies help to sample the largest possible number of species with different ecological and behavioral characteristics. This methodology, for example, may prove useful in the future as a good alternative to collect bioindicator species monitoring the effects of anthropogenic activities. Furthermore, knowledge of new methodologies is essential to help establish faunal surveys, for example, in order to monitor programs in environments.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

MFM and RH contributed with the experimental design and collected field; MFM, MSG, RH and VLSV contributed with manuscript writing; MFM, MSG, HRM and CD contributed with species identification and data analysis. All authors read, revised and approved the manuscript.

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