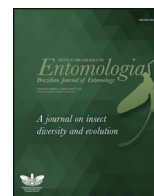





SOCIEDADE BRASILEIRA
DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution



Diversity of sarcosaprophagous Diptera (Calliphoridae, Sarcophagidae) in organic and conventional mango plantations in the Brazilian semi-arid region

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ARTICLE INFO

Article history:

Received 28 October 2020

Accepted 18 February 2021

Available online 22 March 2021

Associate Editor: Rodrigo Kruger

Keywords:

Caatinga

Agroecosystem

Forensic Entomology

Chrysomya

Oxysarcodexia

ABSTRACT

Increase on intensive monocultures is an important threat to Caatinga biodiversity decrease. The use of chemical insecticides directly affects insect natural enemies of crops, but also non-target species. We aimed to test whether the diversity of sarcosaprophagous flies would be affected by agricultural management (conventional and organic). Adult dipterans were collected monthly, from July 2015 to January 2016, using a grid composed of 12 suspended traps distant 20 m from each other, baited with 150 g of sardine or bovine spleen, in each type of management. We first calculated α -diversity by Hill numbers, then evaluated the difference of diversity metrics by ANOVA. We also graphically visualise the species composition distribution by NMDS. We recorded a total of 4,651 specimens of 15 Calliphoridae and Sarcophagidae species. *Chrysomya* spp. were the dominant species (93.7% of all blowflies). Sarcophagidae species were sampled in low abundance, but with exclusive presence of some species in each treatment (e.g. *Blaesoxipha stallengi* and *Ravinia belforti* in organic management). Although there was no difference on sarcosaprophagous fly diversity between treatments, we demonstrate empirically that mango plantations in the Caatinga harbour diverse and abundant assemblages of sarcosaprophagous dipterans, whose richness of species is comparable to other ecosystems such as rainforests and urban areas. Our results expand the known geographical range for forensically relevant species by providing a checklist of sarcosaprophagous dipterans that can thrive in agroecosystems in the Caatinga and, potentially, colonize cadavers disposed in that type of environment.

Replacement of native forests by intensive monoculture is one of the major causes for biodiversity loss in the Neotropical Region (Laurance et al., 2014). This is the case of the Caatinga, the largest contiguous area of seasonally dry tropical forest (SDTF) in South America (Silva et al., 2017). The domain is characterized by high temperature, low relative humidity, and low and irregular rainfall (< 700 mm/yr) (Silva et al., 2017), even so, it has become the largest productive area of exotic crops such as grape and mango in Brazil, particularly in the municipality of Petrolina, State of Pernambuco (IBGE, 2017). However, intensive use of machinery, irrigation and synthetic agrochemicals has raised concerns about changes in the assemblage of native insect species.

While the impact of agricultural practices has been thoroughly studied for insect natural enemies (see Garratt et al., 2011 for a review), their effect on other insect guilds has been largely ignored.

Sarcosaprophagous dipteran species, for example, have been more frequently associated with carrion, but several species exhibit a secondary nectarivore behaviour and can act as pollinators of crops, including mangoes (Larson et al., 2001). In addition to their potential role as pollinators (Huda et al., 2015), blowfly (Diptera: Calliphoridae) and flesh fly (Diptera: Sarcophagidae) are the most important taxa in Forensic Entomology, due to their ability to use carcasses as food and as sites for mating and oviposition.

Data on the geographical distribution, seasonal occurrence and response to environmental factors are essential to subsidize protocols for the estimation of the minimum post-mortem interval and provide inferences about the site of death based on entomological evidence. Recent studies confirm the establishment of exotic Calliphoridae species of the genus *Chrysomya* in the Caatinga (Oliveira and Vasconcelos, 2020), and the ability of Sarcophagidae species (e.g., *Blaesoxipha (Gigantotheca) stallengi* (Lahille, 1907)) to outdo competitors in the

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colonization of animal baits (Barbosa et al., 2019). The extent to which these ecological features affect their likelihood as cadaver colonizers in rural areas in the semi-arid region is unknown.

Motivated by the lack of empirical studies on sarcosaprophagous insects in an increasingly human-modified Caatinga, we investigated the diversity of Calliphoridae and Sarcophagidae species in a typical agroecosystem in the Caatinga, that is, mango plantations. We aimed to test whether the diversity of sarcosaprophagous flies would be affected by agricultural management (hereafter, conventional and organic). We expected that the diversity of blowflies and flesh flies would be lower in conventional plantations, due to the potential deleterious effect caused by the application of synthetic insecticides on non-target organisms.

The experiments took place in farms of fresh mangoes (*Mangifera indica* L, cultivar Tommy Atkins) intended to export, inserted in SDTF areas in Petrolina, Brazil (09°09'S, 40°22'W). Climate is semiarid (Köppen) with irregular rainfall distribution (average 500 mm/year), concentrated between November and April. Intense solar radiation, low relative humidity, and high temperature (mean 26 °C) throughout the year makes it an excellent site for large scale fruit cultivation (Siqueira et al., 2008).

To test our hypothesis, we used two treatments: conventional mangoes management (CM - with use of chemical insecticides, based on Espinetoram, Lambda-cialotrina, Indoxacarb and Lufenuron + Profenofós), with 8.0 ha; and organic management (OM - free of synthetic insecticides and fungicides) reaching 5.6 ha. For each treatment, two plantation areas were selected (CM-1: 9°21'40.20"S, 40°41'12.75"W; CM-2: 9°21'5.91"S, 40°33'2.00"W; OM-1: 9°20'41.38"S, 40°42'50.01"W; OM-2: 9°22'58.72"S, 40°38'53.06"W), at similar phenological stages (full flowering: more than 50% of panicle flowers opened), and presented the same micro-sprinkler irrigation systems. Also, all four plantations are surrounded by Caatinga fragments. The sampling sites were separated from each other from at least 1 km. Agricultural practices in all areas are similar (except for chemical products) and follow the guidelines for Integrated Pest Management (IPM) from the local government institution for research and extension activities (*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Semi-Arid*).

Adult dipterans were collected using suspended traps (120 cm above ground) baited with 150 g of sardine or bovine spleen, previously exposed in environmental temperature for 24 h without access of insects (Oliveira and Vasconcelos, 2020). In each experimental area, we established one grid composed of 12 traps distant 20 m from each other, which were exposed in the field for 48 hours, following previous studies that evidenced the independence of samples (Vasconcelos et al., 2015). We performed monthly sampling expeditions from July 2015 to January 2016, which encompassed both dry (three samplings) and rainy seasons (four samplings), totalling 336 independent samples. We identified the insects using the taxonomical keys of Carvalho and Mello-Patiu (2008) and Vairo et al. (2011).

We first calculated α -diversity by Hill numbers. Hill diversity (known as *true diversity*) is composed by a family of mathematical indices, which differ from each other by exponent "q". For convenience, we used $q = 0$ (species richness); $q = 1$ (common species, equivalent to Shannon index) and $q = 2$ (diversity of dominant species) (Hill, 1973). Then, we estimated the overall rarefaction and extrapolation of species diversity using "iNEXT" package (Chao et al., 2014). Finally, we used *one-way* ANOVA to evaluate the difference of alpha diversity ($q=0$, $q=1$ and $q=2$) between the treatments. We used a PERMANOVA analysis associated with Betadisp to evaluate species composition difference within and between treatments. Also, a NMDS was built to demonstrate to graphically visualise the species composition distribution along the treatments.

When all samples were combined, 4,651 specimens of 15 species of Calliphoridae and Sarcophagidae were recorded. Calliphoridae was the

most abundant family, with 88.5% of all specimens and was represented by four genera and six species (Table 1). The combined abundance of *Chrysomya albiceps* (Wiedmann, 1819), *Chrysomya megacephala* (Fabricius, 1974) and *Chrysomya putoria* (Wiedmann, 1819) represented 93.7% of all blowflies. Three native blowflies species were registered, of which *Lucilia eximia* (Wiedemann, 1819) was the most abundant (Table 1). Sarcophagidae adults were collected in low abundance (11.5% of total), represented by six genera and nine species, of which *Peckia (Sarcodexia) lambens* (Wiedemann, 1830) and *Oxysarcodexia thornax* (Walker, 1849) were the most abundant (Table 1). However, more than 88.3% of the specimens in this family were unidentified females at the species level.

After seven months we were able to capture virtually the totality of the diversity of common ($q = 1$) and dominant species ($q = 2$) for both, conventional and organic system (Fig. 1a, b, c). All species of Calliphoridae were registered in both management types. The sarcophagids *B. stallengi* and *Ravinia belforti* (Prado & Fonseca, 1932) occurred exclusively in organic management and *Microcerella analis* (Townsend, 1927) and *Oxysarcodexia fringidea* (Curran & Walley, 1934) were restricted to conventional mango plantations. Despite that, there is no species variability within ($F_{1,25} = 0.602$; $P = 0.443$) and between the treatments (PERMANOVA- $F_{1,25} = 1.745$; $R^2 = 0.058$; $P = 0.154$) (Fig 1d). Overall, the richness ($q = 0$) of species did not differ between the treatments ($F_{1,292} = 0.072$; $P = 0.787$). The same pattern was observed to common ($q = 1$: $F_{1,292} = 0.045$; $P = 0.832$) and dominant species ($q = 2$: $F_{1,292} = 3.096$; $P = 0.079$). To Calliphoridae, *C. albiceps* was the dominant species in both management types (Figs. 2a and 2b), but to the Sarcophagidae there was no dominant species (Figs. 2c and 2d).

We demonstrate empirically that mango plantations in the Caatinga harbour diverse and abundant assemblages of sarcosaprophagous dipterans, whose richness of species is comparable to other ecosystems such as rainforest fragments and urban areas (Vasconcelos et al., 2015). The relatively low species richness found in our study can be explained by the environmental monotonicity of the agro-ecosystem, associated with the urban pressure of the surroundings which promotes, along-time, biological homogenization. The type of management did not affect the species diversity, as all conventional and organic plantations shared the same Calliphoridae and most of Sarcophagidae species (Fig. 1a). Thus, we refuse the initial hypothesis that the organic mango plantations would harbour higher diversity when compared to the conventional system, due to potential negative effects of wide spectrum insecticides, which can negatively affect non-target insect species.

The diversity overlap between the sarcosaprophagous flies in conventional and organic management suggests that IPM guidelines towards rational use of chemical insecticides (Barbosa et al., 2000) are strictly followed by mango producers in Petrolina – if we use those species as indicators of side effects of wide spectrum agrochemicals. However, Sarcophagidae species, such as *B. stallengi* and *R. belforti* occurred exclusively on organic management, being reasonable to think that such species can be a secondary target on chemical compounds used on conventional plantation. Pollinators are by far the most affected ecological niche among insects in agroecosystems exposed to conventional insecticides (Brittain et al., 2010). Nonetheless, the high frequency of Calliphoridae and Sarcophagidae species along our experiment, denotes the possible use of mangoes orchard as feed resource by these species.

Huda et al. (2015) demonstrated this pollination potential in a field experiment, recording a great visitation rate and high number of pollen grains attached to the body surface of blowflies and flesh flies. In support of this, Rajan and Reddy (2019) demonstrated experimentally that *Chrysomya* species can be as efficient as *Apis* species on pollination success and consequently on the mango fruit quality. Despite the low

Table 1

Richness, abundance and relative frequency of Calliphoridae and Sarcophagidae species sampled in organic and conventional mango plantations in Petrolina.

Family/Species	Conventional		Organic	
	N	%	N	%
Calliphoridae				
<i>Chrysomya albiceps</i> (Wiedmann, 1819)	2,019	82.31	1,450	87.09
<i>Chrysomya megacephala</i> (Fabricius, 1794)	318	12.96	39	2.34
<i>Chrysomya putoria</i> (Wiedmann, 1819)	20	0.82	11	0.66
<i>Chloroprocta idioides</i> (Robineau-Desvoidy, 1830)	2	0.08	3	0.18
<i>Cochliomyia macellaria</i> (Fabricius, 1775)	20	0.82	39	2.34
<i>Lucilia eximia</i> (Wiedemann, 1819)	74	3.02	123	7.39
Total Calliphoridae	2,453	100	1,665	100
Sarcophagidae				
<i>Blaesoxipha (Gigantotheca) stallengi</i> (Lahille 1907)	-	-	1	2.50
<i>Microcerella analis</i> (Townsend, 1927)	2	9.09	-	-
<i>Microcerella halli</i> (Engel, 1931)	3	13.64	7	17.50
<i>Oxysarcodexia fringidea</i> (Curran & Walley, 1934)	2	9.09	-	-
<i>Oxysarcodexia thomax</i> (Walker, 1849)	7	31.82	11	27.50
<i>Peckia (Sarcodexia) lambens</i> (Wiedemann, 1830)	6	27.27	11	27.50
<i>Ravinia aureopyga</i> (Hall, 1928)	1	4.55	2	5.00
<i>Ravinia belforti</i> (Prado & Fonseca, 1932)	-	-	6	15.00
<i>Tricharaea (Sarcophagula) canuta</i> (Wulp, 1896)	1	4.55	2	5.00
Sub Total	22	100	40	100
Sarcophagidae spp. (female)	256		215	
Total Sarcophagidae	278		255	
TOTAL	2,731		1,920	

abundance, Sarcophagidae cannot be neglected as potential pollinators, because some species (e.g. *Sarcophaga*) have already been registered visiting mango flowers (Sung et al., 2006; Huda et al., 2015). These results reinforce the importance of expanding studies involving sarcosaprophagous flies in different phytophysiognomies.

Numerical dominance of calliphorids over sarcophagids was registered in all sampling months, which was greatly explained by the dominance of *C. albiceps* in both management types, a pattern that was observed for other environments such as rainforest, Caatinga, islands, urban area and sugarcane plantations (Vasconcelos et al., 2015; Carmo and Vasconcelos, 2016). Habitat simplification, as exemplified by the establishment of large monocultures, is prone to favour the replacement of a diverse group of disturbance-sensitive species by a less diverse group of disturbance-adapted species (generalist species). The major subjacent process to land use transformation is the reduction of realized niche, which is less harmful to species with a great niche range, an assumption that is corroborated by the dominance of *C. species* (> 90% of all Calliphoridae specimens).

It is worth noticing that the arrival of *Chrysomya* species in the Brazilian territory (Guimarães et al., 1978) is contemporary to the instalment of irrigation projects that led up to the development of Petrolina as a major agricultural site in Brazil.

Successful establishment of *C. albiceps* can be also explained by its high biotic potential (r-strategist), predatory behaviour in immature stage, and pioneering on resource detection (Faria et al., 1999), which explain the non-differences on diversity metrics between treatments (high abundance and dominance). Worryingly, these biological and ecological traits raise concern about the community dynamics in environments under strong anthropogenic impact in a scenario of

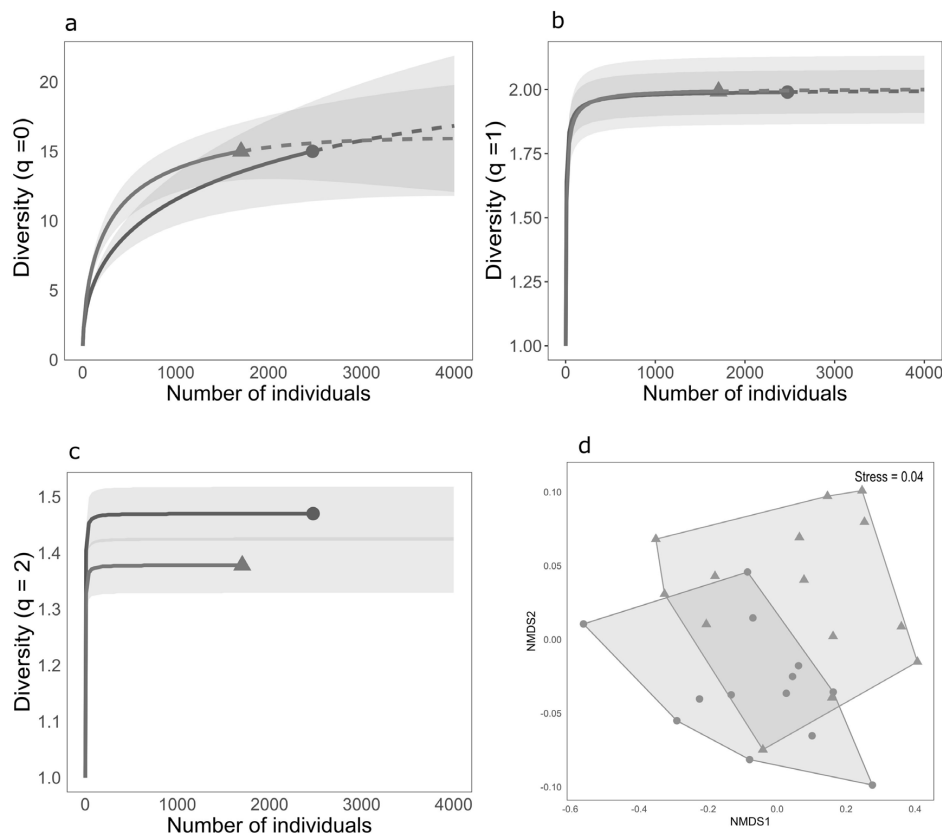


Figure 1 Rarefaction (solid lines) and extrapolation (dashed lines) curves for species richness (a), diversity of common (b) and dominant species (c) of Sarcosaprophagous in both, conventional (circle) organic (triangle) managements. Shadings refer to confidence interval after 999 simulations; d) non-metric multidimensional scaling (NMDS) showing species composition distribution in both conventional (circle) organic (triangle) managements.

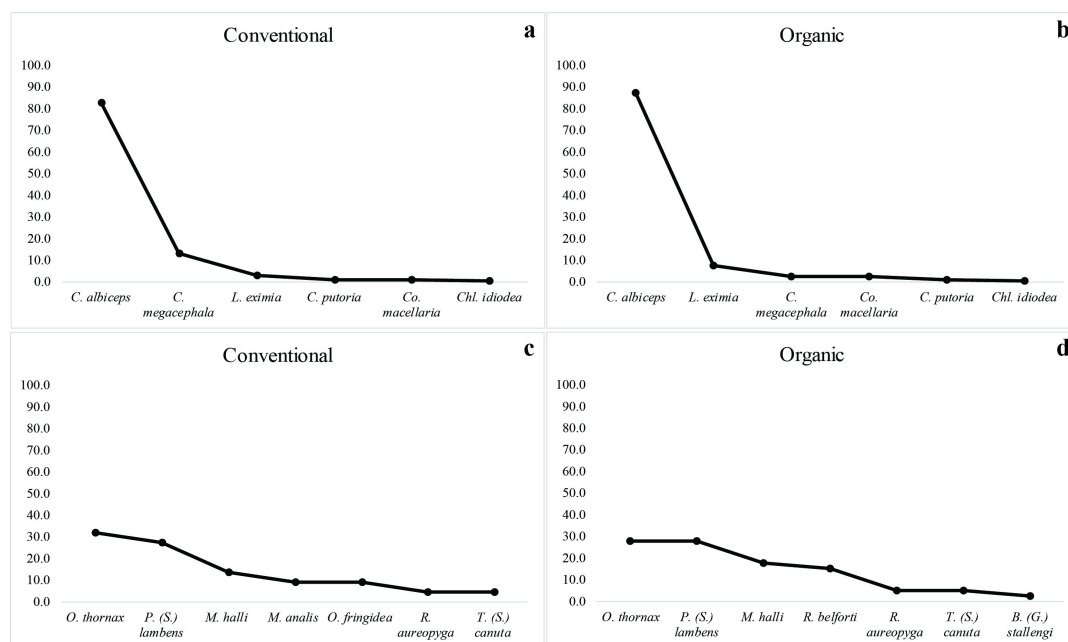


Figure 2 Diptera species dominance ranking by management types. (a and b) Calliphoridae; (c and d) Sarcophagidae.

global changes, because this exotic species may displace or outcompete native species (Faria et al., 2004).

Under a forensic approach, data on the occurrence and dominance of sarcosaprophagous species have tremendous relevance because Calliphoridae and Sarcophagidae are the most frequently used taxa as entomological evidences in criminal investigations. All Calliphoridae species registered here (except *C. idiodea*) and the sarcophagid *Microcerella halli* (Engel, 1931), *R. belforti*, *P. (S.) lambens* e *O. thornax* have been reported as colonizers of human cadavers (Oliveira-Costa et al., 2001; Oliveira and Vasconcelos, 2010; Vairo et al., 2017). *Blaesoxipha stallengi*, on the other hand, is a promising species for biotomical and ecological studies due to its apparent endemism to the Caatinga and unusual reproductive strategies (oviparity) (Barbosa et al., 2019).

Recent reports of Intentional Lethal Violent Crimes (ILVC) indicate changes in the geographical distribution of homicides in Northeastern Brazil – with increasing rates of homicide in the countryside (Pernambuco, 2020). In view of that, the Institute of Scientific Police of Pernambuco have opened working stations in several municipalities inserted into the Caatinga domain, such as Salgueiro, Arcoverde, Ouricuri, Afogados da Ingazeira and, the case of our study, Petrolina. Data on the distribution and seasonal occurrence of necrophagous insect species may help in strengthening scientific procedures associated with the routine of criminal experts. This is interesting in the case of the little-studied *L. eximia*, a species that has been found colonizing human cadavers in the states of Pernambuco and Paraíba (Guimarães, 2019).

Our results expand the known geographical range for forensically relevant species by providing a checklist of sarcosaprophagous dipterans that can thrive in agroecosystems in the Caatinga and, potentially, colonize cadavers disposed of in that type of environment. This type of entomological evidence is needed to document the likelihood of post-mortem corpse movement and to strengthen database aimed at estimating minimum post-mortem intervals.

Acknowledgments

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico

e Tecnológico (CNPq) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for providing PhD scholarship and productivity grants to the authors, Dr. Diego Oliveira and the staff at the Instituto Federal do Sertão and Universidade do Vale do São Francisco for logistical support, Dr. José Siqueira Alves for suggestions, and the owners of the mango farms in which the experiments were performed. This work is the result of integrated cooperation between public universities responsible for ca. 90% of the Brazilian scientific production, which has been suffering severe lack of investment from the current government.

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