

Figure 6. A possible cannibalistic aggregation observed in nature- growing on a banana in the wild.

Alternately, depriving both the species from normal diet and environment, as well as space limitation for considerable period (*D. virilis* has also been cultured in the laboratory for some decades from now) might compel them to adapt cannibalistic approach, which thus might have originated as the product of parallel evolution in recent time after speciation. The alternative approach logically demands absence or lower rate of conspecific

consumption in larvae living in the wild or introduced very recently in the laboratory. Interestingly, larval cannibalism has been noticed in all culture vials where flies collected from the wild- from three distinct geographical regions were introduced in the lab just a couple of weeks prior to the experimental observations. Moreover, aggregation behavior has also been observed in larvae growing in bananas in nature as well (Figure 6).

Thus it will be wise to speculate that artificial food and environment are not the exclusive cause of predatory cannibalism and such behavior is genetically predisposed in the genome of most, if not all, species of *Drosophila* and might have possibly evolved to compensate inadequate protein required for their metabolism allowing us to conclude on the facultative nature of the cannibalistic behavior.

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Drosophila suzukii (Matsumura) found in Uruguay.

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Abstract

Drosophila species collected from two southern localities in Uruguay are reported. The spotted wing *Drosophila suzukii* was the most abundant species (96%) of the total *Drosophila* flies (n = 46) emerged from ripened, decayed, or damaged blueberries collected at rural Canelones Department, while it represented 0.50% among the *Drosophila* samples (n = 5007) collected from banana-baited traps in urban Montevideo city. Data suggest that *D. suzukii* has successfully invaded anthropic environments in urban and agrosystem ecosystems in southern localities in Uruguay.

Introduction

In February 2013, upon returning from an academic exchange program at the *Universidade Federal de Pelotas* (UFPel) in Brazil, Lic. MSc Maria Victoria Calvo, PhD student at the *Facultad de Agronomia*, UdelaR, alerted that the invasive *Drosophila suzukii* has had already been found in the states of Santa Catarina and Rio Grande do Sul, southern Brazil. A few months later it was confirmed by Prof. Vera L.S. Valente during a visit of one of us (B.G.) to her lab at the *Universidade Federal do Rio Grande do Sul* (UFRGS), whose findings were later reported (Deprá *et al.*, 2014). Soon after, in August 2013, we applied for an undergraduate research project to study the occurrence of *D. suzukii* and its relation to host fruits in Uruguay. Here we report an advance of relevant findings on the occurrence and abundance of *D. suzukii* and other drosophilid species in two southern localities of Uruguay.

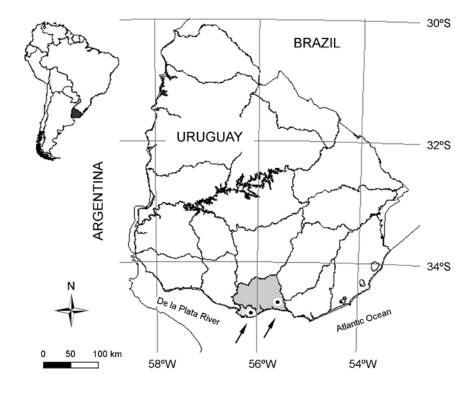


Figure 1. Map showing two Uruguayan localities surveyed: Montevideo city (left arrow) and rural area of Department of Canelones (right arrow).

Materials and Methods

Uruguayan climate is temperate, with no dry season, and with hot summers ("Cfa", Köppen, 1931). At the southern region, the annual mean temperatures ranges from 16 to 17°C, and in the warmest month the means are between 21 and 23°C: the mean monthly precipitation is between 80 and 110 mm/month, though rainfall variability is the most crucial aspect of the Uruguayan climate

(Caffera, 2010). Our study was carried out in two localities in southern Uruguay during summer 2014 (Figure 1). At Montevideo city, Department of Montevideo (34°52'57.09"S, 56°7'5,35"W, alt. 41 m, Figure 1, left arrow) flies were collected by using banana-baited traps set at the Native Flora Garden of the Facultad de Ciencias (Figure 2 a) [i.e., Butia odorata (Barb. Rodr.) Noblick, 1891 (Arecaceae) ("butiá"), Ficus luschnathiana (Miq.) Miq. 1868 (Moraceae), Opuntia arechavaletae Speg. (Cactaceae), Phytolacca dioica L. 1762 (Phytolacaceae) ("ombú"), Syagrus romanzoffiana (Cham.) Glassman 1968 (Arecaceae) ("pindó), Tipuana tipu (Benth.) Kuntze, 1868 (Fabaceae) ("tipa")]. Traps were regularly examined twice a day (mornings and afternoon) during the collecting period. The second locality was a rural area of Canelones Department, known as Empalme Maldonado (34°40'16,.69"S, 55°35'57,.88"W, alt. 83 m, Figure 1, right arrow), characterized by small to medium-sized productive forestry units, fruit plantations, or livestock farming. A total of 3.5 kg of over-ripened, decayed or damaged blueberries, Vaccinium ashei (Ericaceae) of the "Ochlockonee" cultivar were collected from the ground, at end of the harvest (Figure 2b). "Ochlockonee" cultivar is a late season rabbiteye blueberry, released in 2002 (NeSmith, 2012), that produces medium-largesized fruits and is planted together with other rabbiteye blueberry varieties for cross pollination. The blueberry farm has an average volume of harvested berries per year of about 1.75 tons per hectare (grown on 4 hectares). Collected berries were taken to the laboratory, weighted and placed in a plastic container with sand at the bottom. Emerged flies were collected at regular intervals (every 4 to 6 days) for one month. Collected



Figures 2. (a) View of the Native Flora Garden of the *Facultad de Ciencias* at Montevideo and banana-baited trap used for collecting flies (left corner). (b) Blueberries of the "Ochlockonee" cultivar at *Empalme Maldonado* farm, Department of Canelones.

drosophilids from both localities were placed under uncrowded conditions in vials with minimal fly medium (agar-sugar-nipagin solution) and kept at 22°C in laboratory conditions until being analyzed. Adults were anesthetized with vapors of triethylamine, counted, classified by sex, and identified to species level using external morphology, and in some cases, by the inspection of male terminalia and preserved in ethanol 70%. The keys and/or illustrations of Freire-Maia and Pavan (1949), Brncic and Santibañez (1957), Spassky (1957), Heed and Russel (1971), Val (1982), Vilela (1983), Vilela and Bächli (1990), Moreteau *et al.* (1995) were used. Samples of male and female specimens used for species identification were labeled and deposited at the collection of Sección Entomología, Departamento de Biología Animal of the Facultad de Ciencias, Universidad de la República, Montevideo (Uruguay). Adult samples of *D. suzukii* from Uruguay were identified by the characteristic sexually dimorphic wing pattern, male foreleg sex combs, male and female terminalia (Bock and Wheeler 1972; Vilela and Mori 2014).

Table 1. Relative abundance of *Drosophila* species emerged from blueberries collected at *Empalme Maldonado*, Department of Canelones, Uruguay, January 11th and 14th, 2014.

Male	Species	Male	Female	Total	%
melanogaster	D. suzukii (Matsumura, 1931)	20	24	44	95.65
willistoni	D. nebulosa Sturtevant, 1916	2	0	2	4.35
Total		22	24	46	100

Results and Discussion

Table 1 shows the abundance of *Drosophila* species emerged from blackberries (Figure 2b) collected at fruit plantation located 3 km far from the coastal line in the Canelones Department. The thermal amplitude in this region is slighter lower than rest of the Uruguayan territory (Caffera, 2010). The relative low number of *D. suzukii* (n = 44) out of a total of 46 *Drosophila* adults that emerged from 3.5 kg of fruits collected at the end of the harvest suggests a low infestation of the spotted-wing fly, not noticed by the farm grower. Most of the blueberries produced in southern region of Uruguay are commercialized in local markets to satisfy the summer demand; however, large commercial producers of blueberries, located in northern regions of

Uruguay, grow early-season berries that are exported to countries of the northern hemisphere. Furthermore, while examining 80 McPhail traps set for monitoring and mass trapping tephritid species [i.e., Ceratitis capitata (Wiedemann 1824) and Anastrepha fraterculus (Wiedemann 1830)] in several fruit production farms located in southern Uruguay (13 March 2014), Iris Beatriz Scatoni and collaborators (pers. comm.) detected small flies in the attractant liquid that resembled the *Drosophila melanogaster* species but showed a distinct spot at the wing tips, later identified as *Drosophila suzukii* males.

The invasive *Drosophila suzukii* (Matsumura, 1931) (Diptera, Drosophilidae) known as the cherry fly or spotted-wing *Drosophila* (SWD) is a fruit pest that it is expanding rapidly in the Americas and Europe (see Department of Agriculture, Fisheries and Forestry Biosecurity, 2013). This polyphagous species seriously damages commercial and several backyard soft skin fruits including table and wine grapes, loquats, peaches, pears, and plums (Kanzawa 1939), that have been reported to host the new invasive species. The female has a strongly sclerotinized oviscapt (Vilela and Mori, 2014) that allows the penetration of the skin of healthy ripening fruits, and larvae cause their collapse in a few days (Sasaki and Sato, 1995). In August 2008, D. suzukii was first detected in the continental US, California (Lee et al., 2011), in 2009 in British Columbia, Canada (BCMA, 2014), and in December 2011 in the municipality of Los Reves, Michoacan, Mexico (SENASICA, 2013). Deprá et al. (2014) reported the presence of D. suzukii for the first time in Brazil, stating it was first collected in the Biological State Reserve Aguaí, Nova Veneza, State of Santa Catarina, on 27.II.2013. They also recorded the relative abundance of this species, ranging from 0.67 to 7.97% in collections made in the states of Rio Grande do Sul and Santa Catarina, in March and April of that year. Two other reports indicate the invasion of D. suzukii in natural areas of Brazil. Paula et al. (2001) reported a few adults of this species collected April 2014 in gallery forests and savannas in the IBGE Ecological Reserve, Distrito Federal, Brazil, located in the Brazilian Savanna, a tropical biome in the center of South America, locally known as Cerrado biome. Using also banana-baited traps as collecting method, Bitner-Mathé et al. (2014) found a few adults of D. suzukii on traps set in November 2014 in the Parque Nacional da Serra dos Órgãos (PARNASO), Petrópolis, state of Rio de Janeiro, southeastern Brazil, a conserved area of the tropical Atlantic Rainforest.

Table 2. Drosophila species, and its relative abundance, attracted to banana-baited traps at the Native Flora Garden of the Facultad de Ciencias Faculty of Science, Montevideo city, Uruguay, February 14th to 20th, 2014.

Species group	Species	Male	Female	Total	%
cardini	D. cardini Sturtevant, 1916	1	1	2	0.04
immigrans	D. immigrans Sturtevant, 1921	32	46	78	1.56
mesophragmatica	D. gaucha Jaeger & Salzano, 1953	4	4	8	0.16
repleta	D. hydei Sturtevant, 1921	15	17	32	0.64
	D. mercatorum Patterson & Wheeler, 1942	1	0	1	0.02
melanogaster	D. melanogaster Meigen, 1830	355	308	663	13.24
	D. simulans Sturtevant, 1919	2330	1838	4168	83.24
	D. suzukii (Matsumura, 1931)	21	4	25	0.50
willistoni	D. nebulosa Sturtevant, 1916	6	12	18	0.36
	D. willistoni Sturtevant, 1916	5	5	10	0.20
ungrouped	D. busckii Coquillett, 1901	2	0	2	0.04
Total		2772	2235	5007	100

Concerned with the possible invasion of D. suzukii through the national and international fruit trade in the southeastern states of Brazil, Carlos R Vilela and Lyria Mori at the University of São Paulo analyzed commercialized fruits bought in a local market in the city of São Paulo (February 2014). They reported the emergence D. suzukii in the blueberries harvested in São Joaquim, state of Santa Catarina (Vilela and Mori, 2014). Santos (2014) recorded the first attack of D. suzukii in a strawberry cultivar (variety of San Andreas) in the municipality of Vacaria, Rio Grande do Sul, Brazil (January 2014). The local grower estimated that about 30% of its production was infested. Recently, Geisler et al. (2015) reported new host fruits of D. suzukii in Brazil: loquats and peaches, collected at the municipalities of Porto Victoria, and União da Victoria, state of Paraná, respectively, from February to December 2014.

Table 2 shows the Drosophila species collected at the new campus of Facultad de Ciencias (Malvin Norte campus, Figure 2a), Montevideo city. Adults of both sexes of D. suzukii were attracted to the banana traps at low, though comparable, frequencies to those calculated for the other *Drosophila* species. Excepting for *D. suzukii* all other *Drosophila* species were previously reported in urban and suburban Montevideo city (Goñi *et al.*, 1997, 1998).

The new data discussed above expand the geographic/climatic range of the invasive *D. suzukii* in the Americas, and place an alert to the Uruguayan and regional agricultural sanitary control authorities about the potential economic effects this propagule on fruit production.

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"Pack Hunting" or "Social Digestion" as a possible cause of larval clustering associated with the evolution of cannibalistic behavior in *Drosophila* species larvae.

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The seminal observation of cannibalism in *Drosophila melanogaster* cultured in laboratory culture media for more than nine decades (Lindsley and Grell, 1968) has led us to a few of the major questions - as to