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Foreign Invasive Pests *Drosophila Suzukii* (Matsamura) and *Zaprionusindianus* Gupta (Diptera: *Drosophilidae*) Threaten Fruit Production in Sub-Tropical Argentina

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Abstract

The sub-tropical region of north western Argentina (Tucuman province) shelters a major soft fruit production and exporting industry. *Drosophilasuzukii* (spotted-wing *Drosophila*; SWD) is a major global pest of soft fruits because females can lay eggs under the epidermis of healthy, ripening fruit. Recently, Argentina was invaded by the SWD, which has rapidly spread towards all the cardinal points, showing a great ability of adaptation to different climates and fruit crops. We report for the first time the presence of two invasive *drosophilid* species, SWD and *Zaprionusindianus* (African fig fly) in the sub-tropical rainforest of the Yungas (Köppen-Geiger climate classification CWa), adjacent to a high-value fruit production region, in the province of Tucumán (northwestern Argentina). Both species were recovered from wild guava fruit (*P.sidiumguajava)*. The SWD was found in healthy, ripe fruit attached to the trees (65%) and in damaged fruit lifted from the ground (35%); while *Z. indianus* was only recovered from damaged fruit collected from the ground (100%). *Zaprionusindianus*, SWD and other drosophilid accounted for 86.6%, 7.1% and 6.3%, respectively, of the total of *drosopholids* found. The presence of both invasive insects in the region, especially SWD, is a threat for the local berry industry. Since SWD can complete its life cycle in guavas, these fruits would allow the sustainability of SWD populations during the seasons in which commercial berry crops are not in production. Berry growers and government plant protection agencies should promptly take measures to limit these pests dispersion to commercial fruit fields.

Keywords: African Fig Fly; Invasive Species; *Psidiumguajaba*;Spotted-Wing*Drosophila*

Introduction

Argentina currently exports over 1.9 million tonnes of fruits and vegetables each year, generating revenues of around 1.7 billion dollars. This condition makes Argentina one of the largest produce exporting countries of the southern hemisphere [1], being citrus, berries, pome fruits and stone fruits the most exported.The subtropical region of northwestern Argentina (Tucuman province), where the rainforest is part of the landscape, is a major soft fruit producer and exporter[2].The Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Matsumura) (*Diptera*: *Drosophilidae*), is a highly polyphagous invasive pest from South East Asia [3], detected for the first time in Europe [4] and North America in 2008 [5], and in South America (Brazil) in 2013 [6]. From then on, this species hascolonized Europe and America affecting a wide range of host plants.

The SWD is considered an important global pest of soft fruits because females are capable of laying eggs under the epidermis of healthy, ripening fruit, using their powerful, sclerosed and serrated ovipositor. In the last 3 years, Argentina has been literally invaded by the SWD, which has rapidly spread towards all the cardinal points, showing a great ability of adaptation to contrasting climates

[7] and different fruit crops [8-10]; however, there are no reports on the presence of SWD in sub-tropical regions of northwestern Argentina. *Zaprionusindianus* Gupta (*Diptera*: *Drosophilidae*) or African fig fly is native to sub-Saharan Africa and like SWD, has also rapidly spread throughout tropical and subtropical regions [11]. This drosophilid infests mainly damaged fruit of about 80 species from over 31 plant families [12,13]. However, valuable crops, such as*Ficuscarica*L and *Dimocarpuslongan*Lour. (longan) have been severely affected by this fly from Brazil to Florida (USA) [12,14]. In Argentina, *Z. indianus* was first reported in 2006 from decaying fruits of a wide range of native and cultivated host plants surveyed in the northern provinces of Corrientes, Misiones, Formosa, Chaco and Tucumán [15], but this finding has received little attention.

Wild and cultivated guava specieshave been reported as hosts for *Z. indianus* and *D. suzukii* in Brazil [14], México [16] and the USA [12]. Even though in Argentina guavas are not grown with economic purposes, guava trees are very common in the backyard of rural homes for family consumption [17] additionally, many local or native people harvest guava and other native fruits from the subtropical rainforests to make juice and jam as a way of life [18]. In Tucumán, feral guava (*Psidiumguajava* L.) fruit are found in the foothills of the mountain cloud forest, locally known as Yungas [19], bordering the humid piedmont, where most of the soft fruit (e.g. strawberry, blueberry, blackberry and raspberry) orchards are located [20].Since there is no information about both invasive drosophilid species in the subtropical region of northwestern Argentina, and considering the great economic losses that potentially these pests can cause to the fruit industry, the objectives of this study were to determine the species composition of drosophilids infesting "Feral" guavas in the Yungas, the relative abundance of SWD and *Z.indianus*, and the prevalence of drosophilid species in damaged, fallen fruit, and in healthy, mature fruit attached to the tree.

Material and Methods

During a routine tephritid fruit fly monitoring in guava fruit, a large number of unusual drosophilid specimens were observedin the collected samples. The studiedarea is in HorcoMolle (26˚45′00" S, 65˚20′00" W, 500-600 m elevation; Tucumán province, Argentina), within the "Sierra de San Javier" park, in the southernmost end of the sub-tropical Yungas forest. The site is characterized by disturbed secondary vegetation (exotic and native plant species combined) surrounded mainly by large citrus orchards [17] and soft fruitcrops. HorcoMolle´sclimate is classified as "humid warm-temperate" with a rainy-warm season from October through April, and a dry-cold season from May through September. Mean annual rainfall ranges from 1300 to 1600 mm, with an average annual temperature of 18˚C.

From a group of 30 wild guava trees (*Psidiumguajava* L., Myrtaceae) selected in the sampling site, six trees were randomly chosen for the study. Knowing that in this location ripe guava fruit are more abundant in late summer/early autumn [17], allfruit samples were collected on March 25th, 2016.Five undamaged early maturing fruit (partially yellow guava, with mottled green spots and soft texture) were harvested from the selected trees, and five damaged, ripe fruit were collected from the ground below the canopy. In both cases, fruit were chosen randomly. Each fruit sample was placed individually into a cloth bag (20 cm diameter and 30 cm depth) and transported in a plastic tray to the lab (Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y susEnemigosNaturales, LIEMEN, San Miguel de Tucumán, Argentina).

Guava fruitswererinsed with a 30% sodium benzoate solution, and weighed. Each fruit sample was placed in a plastic tray (48x28x15 cm) with a slotted bottom, which wasplacedover another plastic tray of the same size but without perforations. A 5-cm sand layer was usedas pupation substrate in the second tray. Both trays were tightly covered with organdy cloth. The double tray method was used to prevent the contact between fruit and sand, in order to minimize fungal growth and bacterial contamination. Samples were kept in a dark room with no climate control, with temperatures ranging from 22˚ to 27˚C. Sand was sifted once a week to recover drosophilid pupae for a 1-month period, after which all fruit were dissected to search for remaining drosophilid larvae or pupae inside each fruit. Drosophilid pupae were transferred to glass cups (21 cm diameter, 9 cm depth) filled with sterilized moist vermiculite. Cups were covered with a piece of organdy cloth and held until adult emergence. Adult drosophilid specimens were identified to species using taxonomic keys [20]. Species identification was based on external morphology and on the terminalia of both sexes. Voucher specimens were placed in the entomological collection of Fundación Miguel Lillo (FML) in Tucumán, Argentina.

Results and Discussion

Total sampled fruit weight was 3.25 kg, with an individual mean fruit weight of 54.2 ± 9.9 g (SD). This quantity of fruit yielded 387 *drosophilidpuparia*, from which a total of 239 resulted in emerged adults (Table 1), that were identified as *Z. indianus* (207 individuals; 86.6%), SWD (17 individuals; 7.1%) and *Drosophila* spp. (15 individuals; 6.3%; probably *D. melanogaster* and *D. simulans* among other).

Approximately, 65% SWD adults were recovered from guavas collected from the tree canopy, while the remaining 35% was recovered from fruit collected from the ground. Regarding to *Z. indianus* and *Drosophila* spp., 100% of the adults were recovered only from fruit lifted from the ground (Table 1).

***Drosophilidae* species**								
Sampled Tree	Fruit origin	Nº of Fruit	Nº of puparia	***Z. indianus***		***D. suzukii***	***Drosophila spp.***	
				Nº of adults	Sex ratio (%)[a]	Sex ratio (%)	Nº of adults	Sex ratio (%)
1		5	9			75	0	
		5	85			-	7	
	Canopy			0	-			-
	Ground			50	58			
2		5	1	0	-	100	0	
	Canopy	5	66	34	44	50	3	-
	Ground							
3		5	11	0	-	50	0	-
	Canopy	5	41	25	44	0	0	-
	Ground							
4		5	3	0	-	0	0	
	Canopy	5	82	40	40	50	3	-
	Ground							
5		5	2	0	-	100	0	
		5	59	34	32	100	0	
	Canopy							-
	Ground							
6		5	15	0	-5		0	
		5	13	24	4		2	
	Canopy					-		
	Ground							-
[a]Sex ratio = proportion of females on the total number of emerged adults.								

Table 1: Total and relative abundance and sex ratio of *Drosophilidae* species, recovered from guava fruits collected from the tree canopy and from the ground in HorcoMolle, Tucumán, Argentina.

SWD is the first drosophilid species found in the subtropical region of northwestern Argentina with capability of laying eggs below the epidermis of healthy, ripe fruit, and of developing in the fruit. In Argentina, this frugivorous fruit fly has recently been recorded in very contrasting environments at different latitudes, fruit species and climates [8-10], (Table 2). In fact, the ability of *D. suzukii* to adapt to different environments and hosts has enabled this species to establish in tropical and subtropical regions in both hemispheres [21].

Province	**Location name**	**Location latitude**	**Associated fruit species**	**Climate type (Köppen-Geiger)[4]**	**Climate description**	**Annual rain (mm)**	**Annual average temperature (ºC)**
Río Negro[1]	ChoeleChoel	39˚17′09″S, 65˚39′15″W	Raspberry	BSk	local steppe	268	15.4
Buenos Aires[2]	Lobos	35˚11′11″S, 59˚05′46″W	Blueberry	Cfa	warm and temperate	1008	16.1
La Rioja[3]	Anillaco	28˚48’23’’S,66˚56’27’’W	Pear	BWh	desert	330	20
Tucumán	HorcoMolle	26˚45′00”S, 65˚20′00”W	Guava	Cwa	warm and temperate	949	19.4

Table 2: Name, geographicallocation, climate type and description, anual precipitation and anual average temperature of sites where the SWD was reported in argentina, including the present study [8-10,22].

In the present study, *D. suzukii* was the only drosophilid species recovered from undamaged guava fruit harvested from the plant, which is consistent with the literature. SWD has been reported in several countries infesting a great variety of fresh commercial fruits, such as blueberry, blackberry, raspberry, strawberry, cherry, plum, peach, pear, grape, fig, kiwi and guava [23,24,16], as well a wide range of non-crop fruits, including guavas [25,26].

On the other hand, *Z. Indianus*was the dominant drosophilid species found in damaged, fallen fruit. As reported previously, *Z. indianus* had been recovered from cultivated peaches (*Prunuspersica*(L.) Stokes) in Vipos (Lavagnino*et al*., 2008), located in the semi-arid region of the Tapia-Trancas basin (Tucumán,Argentina). This site is located in the northeastern part of the Tucumán province, where climate is warm semi-arid, with precipitations around 450 mm and permanent water deficit [27]. Nevertheless, we found *Z. indianus* in a completely different environment. Our sampling site is located ≈60 km south of Vipos, in a very contrasting environment: The Yungas rainforest (humid and perhumid piedmont region), with annual rains ≈1000 mm and positive water balance [27]. Köppen-Geiger climate classification for Vipos is BSh while for HorcoMolle is CWa, which reflects the plasticity of *Z. indianus,* issue previously discussed by other researchers [28,29]. The sex ratio, defined as the proportion of adult females on the total number of adults (Table 1), varied from 32.4% to 58.0% for *Z. indianus* in fruit lifted from the ground, which is consistent with previous reports in guava [16].

Regarding to SWD, the little number of specimens found is not enough to make any discussion about sex ratio. In more integral studies, SWD sex ratios were 58% in guavas collected from the tree and 66% in damaged guavas lifted from the ground [16]. An important issue to address in future research is the interaction between both drosophilid species. Strawberry fruit injured by SWD adults facilitated the infestation by *Z. indianus*, showing the opportunistic ability of African fruit fly adults to infest damaged fruit [30]. This probably occurred in our study but it was not evaluated. In hosts as guavas, SWD can complete its life cycle in 15 d under lab conditions, indicating that guavas allow the sustainability of SWD populations during the seasons in which commercial berry crops are not in production [31]. As pointed out before, in the sub-tropical region of northwestern Argentina, guavas share the same geographical space with commercial berry productions. Therefore, our results should be taken as a warning signal for growers and government plant protection agencies. Our findings reveal the need of increasing the studies about the drosophilid community in this region, including studies on population dynamics, interactions between species, potential natural enemies, geographical distribution, host range within non-crop plants and potential dispersion of both pest species to neighboring orchards. Given that SWD is considered a key pest of berry crops worldwide [25,24,32], the presence of this invasive fruit fly in the subtropical region of northwestern Argentina is a threat for the local fruit industry, and for native non-crop fruit species. Prompt measures should be taken in order to limit this pest dispersion to orchards and natural plant sanctuaries. The availability of effective insecticides for SWD control is limited, and its use can harm natural enemies. Although SWD biological control (BC) programs have not been successful yet, BC is essential in IPM programs. In this sense, the perspectives for SWD BC in invaded habitats, with emphasis on Hymenoptera parasitoids, are very optimistic.

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