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# Relative abundance and use of resources by *Drosophila melanogaster* Meigen, *D. simulans* Sturtevant and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in localities of northern Argentina

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**Abundancia relativa y uso de los recursos por parte de *Drosophila melanogaster* Meigen, *D. simulans* Sturtevant and *Zaprionus indianus* Gupta (Diptera, Drosophilidae en localidades del norte de Argentina**

**RESUMEN.** *Zaprionus indianus* Gupta es una especie plaga invasora de mosca, que ha colonizado el continente americano en los últimos 20 años. Se trata de una especie polífaga que con *Drosophila melanogaster* Meigen y *D. simulans* Sturtevant utilizan una amplia variedad de frutas como recursos de cría y alimentación. En el presente estudio cuantificamos la abundancia relativa de estas especies en individuos adultos y moscas emergidas colectados de diversos hospedadores (frutas comerciales) en diferentes localidades del norte argentino. Detectamos superposición de recursos de cría y alimentación entre las especies analizadas, aunque nuestros resultados revelaron que la abundancia relativa entre especies de *Drosophila* y *Z. indianus* varió entre las muestras de adultos y emergidos. Estos resultados sugieren un uso diferencial de los recursos y/o efecto de la competencia interespecífica.

**PALABRAS CLAVE.** Abundancia relativa. Bioinvasión. Hospedadores. *Zaprionus indianus*.

**ABSTRACT.** *Zaprionus indianus* Gupta is an invasive pest species that has colonized the American continent in the last 20 years. This species is a polyphagous species that with *Drosophila melanogaster* Meigen and *D. simulans* Sturtevant utilized a wide variety of fruits as breeding and feeding resources. In the present study we quantified the relative abundance of these species in adult and emerged samples collected from various hosts (commercial fruits) in different localities in northern Argentina. We detected overlapping of rearing and feeding resources between the analyzed species although, our results revealed that the relative abundance between *Drosophila* and *Z. indianus* species varied between both adult and emerged samples. This result revealed a differential use of resources and/or interspecific competition.

**KEYWORDS.** Bioinvasion. Host. Relative abundance. *Zaprionus indianus.*

The proliferation of alien invasive species that rapidly colonize large areas provides an amazing opportunity to investigate the causes that allow their successful colonization (biological invasion), as well as the change they cause in already established communities. *Zaprionus indianus* Gupta (Diptera, Drosophilidae), also known as the African fig fly in South America and the striped vinegar fly in the USA, is a species native to the Afrotropical biogeographic region (Yassin et al., 2008). Aided by international trade and commerce (Hulme, 2009), about 30 years ago this species begun to expand its geographical distribution from its native range in Africa to other areas in the world (Vilela, 1999; van der Linde, 2006; Lavagnino et al., 2008; Commar et al., 2012; Kremmer et al., 2017). *Z. indianus* can be classified as a category E invasive species according to Blackburn *et al*. (2011), since it is fully invasive, with individuals dispersing, surviving and reproducing at multiple sites in many habitats. This species is considered a generalist and polyphagous species (Aluja & Mangan, 2007), since it can use a wide variety of decaying fruits as breeding and feeding resources (van der Linde et al., 2006; Lavagnino et al., 2008). This characteristic is perhaps one of the principal factors contributing to its rapid geographical expansion and successful colonization history. Thus, the spread of this generalist species through different habitats in North, Central and South American continents most likely involve different developmental environments, that is, different host fruits (van der Linde et al., 2006; Lavagnino et al., 2008). *Zaprionus indianus* is typically regarded as a secondary pest, since adult females normally oviposit in fruits that have been mechanically injured by other insects (Lasa & Tadeo, 2015; Bernardi et al., 2017) although, the presence of this species along with other insects is still concerning to soft-skinned fruits farmers. Thus, the presence of *Z. indianus* as well as other *Drosophila* species that were found in fruits injured in the first place by pest insect species as *Anastrepha fraterculus* Wiedemann (Diptera, Tephritidae), *Ceratitis capitata* Wiedemann (Diptera, Tephritidae) or *Drosophila suzukii* Matsumura (Diptera, Drosophilidae), can be associated with a greater release of volatiles that function as stimuli for orientation, attraction and oviposition (Bernardi et al., 2017). However, during the process of colonization to new areas *Z. indianus* became the north-eastern Argentina would be first pest in cultivars of strawberries (*Fragaria × ananassa*, Bernardi et al., 2017) and figs (*Ficus carica L.*, Lasa et al., 2020) during geographic expansion in America. Certainly, adult females can oviposit on the fruit surface of intact ripe strawberry fruits, then larvae emerge, penetrate the epidermis and feed on the fruit pulp and yeasts (Bernardi et al., 2017). In the case of figs, females lay eggs on the bracts of the ostiole in intact fruits at the beginning of maturation and larvae enter the fruit through small natural wounds of the fruit (Lasa et al., 2020). Either as a primary or secondary pest, oviposition and subsequent larval feeding of *Z. indianus* on agricultural crops can contribute to decreased yields and rejected product. After the first record of this species in Argentina (Soto et al., 2006), it was determined that *Z. indianus* colonized different areas of Argentina (Lavagnino et al., 2008) and that the geographical expansion to north-eastern Argentina would be the consequence from a single introduction wave from Brazil (Fernandez Goya et al., 2020). On the other hand, *D. melanogaster* and *D. simulans* are cosmopolitan sibling species that exploit several fermenting fruits as feeding and *breeding* sites. Considering that the worldwide expansion of *D. simulans* is more recent than its sibling species) and that *D. melanogaster* has a stronger association of with human activity (Keller 2007, Capy & Gilbert 2004), it would be more likely for this species to be sampled in the area evaluated. However, in a previous study, Vilela et al. (1980) detected that *D. simulans* was more abundant than *D. melanogaster*.

In this study, we report the distribution, relative abundance and use of feeding and breeding resources by *Z. indianus, D. melanogaster* and *D. simulans* in different localities of Argentina (Table I). We analyzed only males, since females of *D. simulans* and *D. melanogaster* are indistinguishable (Markow & O'Grady, 2006). *D. simulans* and *D. melanogaster* were classified to species by the inspection of their genitalia (Markow & O'Grady, 2006) whereas we identified *Z. indianus* by its phenotype (van der Linde, 2006; Commar et al., 2012). Two different collection techniques were performed to quantify numbers of individuals and the relative abundance of *D. melanogaster, D. simulans* and *Z. indianus*: adult collections in the wild and emerged flies in the laboratory. We collected adults in the wild by net sweeping over: a) baits wherein flies were collected by means of 10 bucket (20 cm of diameter) with fermenting banana baits randomly distributed throughout each collecting sites and b) rotten fruits that were found on the ground (the number of resources utilized as natural attractant is indicated in Table I). In the case of emerged flies in the laboratory, the collections consisted in gathering rotten fruits from the same locations, of adult collection. Fruits were isolated in closed containers with two pieces (10 cm x 10 cm, each) of paper towel and taken to the laboratory. Emerged adult flies from each container were identified by species for 15 days to ensure that all emerged flies were offspring of flies that laid eggs in those fruits in their respective natural environments. Adult collection using fermented banana baits was ineffective to attract *Z. indianus* (see also Castrezana, 2011) although this specie has emerged from banana (Table II and Willbrand et al., 2018). Taking into account this result, we decided to exclude the adult sample collected on banana bait of the evaluation and use only the flies collected on fruits laying on the ground. We only analyzed *Z. indianus*, *D. melanogaster* and *D. simulans* even though other species as *A. fraterculus, C. capitata* and *Drosophila mercatorum* Patterson & Wheeler (Diptera, Drosophilidae; data not shown) were sampled. Taking both adult and emerged flies sampled together, a total of 4.412 flies were collected from 11 localities (Table II). More than half of the flies collected (51,4%, 2266 flies) were identified as *D. melanogaster*, whereas 1.917 (43,4%) and 229 (5,2%) of the total flies collected were *Z. indianus* and *D. simulans*, respectively. We observed that *Z. indianus* was absent in the locality of Ing. Juarez population (Table II) as was observed previously (Lavagnino et al., 2008). It is important to note that the resource available in Ing. Juarez is guava, which has been reported as host of *Z. indianus,* in this study (Table II, Fig.1) and others (Lavagnino et al., 2008; Lasa et al., 2017). Besides, Ing. Juarez exhibits similar climatic parameters than the other localities where *Z. indianus* is present. Thus, neither the lack of breeding and feeding sites nor climatic factors would be the cause of the absence of *Z. indianus* in Ing. Juarez. This gap in the distribution of *Z. indianus* in northern Argentina is strange considering that Ing. Juarez is 160 km away from Las Lomitas, where this species is found. Our results revealed (Table II) that *D. melanogaster* (59,0%, 2009 flies) was the most abundant species in adult collections in the wild (3407 flies), while the relative abundance of the other two species were 36,5% for *Z. indianus* (1243 flies) and 4,5% for *D. simulans* (155 flies). On the other hand, for emerged flies in the laboratory, our records showed (Table II) a change in the relative abundance pattern compared to adult collections in the wild, since *Z. indianus* represented 67,0% (674 flies) of the total emerged flies' sample while *D. melanogaster* constituted the 25,6% with 257 flies and *D. simulans* has the 7,4% (74 flies). The emergence record in the laboratory was significantly different respect to adult collection in the wild ($X^2$= 349,9, d.f.= 2, p < 0,0001), since we detected an excess of emerged flies corresponding to *Z. indianus* respect to *Drosophila* species, suggesting a decoupling between the proportions of the species analyzed that were attracted to and emerged from the feeding and breeding sites evaluated. There is niche overlap between the fly species studied, since flies of *Drosophila* species and *Z. indianus* emerged from most of the resources evaluated (Fig. 1, Table II). However, emergence data indicate (Fig. 1) that the percentage of *Drosophila* species respect to *Z. indianus* varied from 100% (orange) to 6% (mango). The result on orange should be explained: on the one hand, orange resource was sampled in Ituzaingo, wherein *Z. indianus* was found using other resources (Mango and Guava) as breeding sites (Table II). On the other hand, *Z. indianus* exhibited a good performance when it developed in orange as breeding resource in laboratory experiments (Lavagnino et al., 2020). Thus, the absence of *Z. indianus* in orange could be the consequence of chance since there is no biological explanation to the absence of this species from both orange samples (adult and emerged collections). Finally, the proportions of *Drosophila* species and *Z. indianus* collected and emerged were significantly different (p<0.05) for all fruit resources evaluated (excluding orange). The fact that the percentages of emerged flies is different from the ones collected around fruit resources in the wild could be accounted by differences in oviposition preferences between species, and/or by interspecific competition (Fanara et al., 1999; Rodrigues et al., 2016). In this sense, Galego & Carareto (2005) demonstrated that *Z. indianus* residues significantly reduced the viability of *D. simulans* reared under lab conditions. In summary, our study indicates that the pattern of the relative abundance of *Drosophila* species and *Z. indianus* is variable depending on the locality, the fruit resource and the sample evaluated.

**Table I. Geographical coordinates and selected climatological data for the 11 localities (in or close to small villages) analyzed in this study.**

| Locality (Province) | Coordinate | Altitude (m) | Annual Precipitation (mm) | Temperature (°C) | | |
|---|---|---|---|---|---|---|
| | | | | Mean Annual | Min. monthly low mean | Max. monthly high mean |
| Oran (Salta) | 23° 08´S, 64° 19´W | 336 | 945 | 22.3 | 9.1 | 33.1 |
| Yuto (Jujuy) | 23° 35´S, 64° 30´W | 340 | 806 | 21.9 | 8.3 | 32.9 |
| Ing. Juarez (Chaco) | 23° 54´S, 61° 49´W | 155 | 639 | 23.1 | 9.7 | 35 |
| Las Lomitas (Formosa) | 24° 42´S, 60° 35´W | 128 | 893 | 22.5 | 9.6 | 34.3 |
| Palo Santo (Formosa) | 25° 34´S, 59° 20´W | 115 | 1024 | 22.3 | 9.8 | 34.6 |
| El Colorado (Formosa) | 26° 18´S, 59° 22´W | 65 | 1102 | 21.7 | 9.1 | 34.1 |
| Montecarlo (Misiones) | 26° 33´S, 54° 45´W | 175 | 1585 | 20.1 | 8.2 | 31.6 |
| Itati (Corrientes) | 27° 16´S, 58° 15´W | 60 | 1293 | 21.9 | 11.3 | 33.4 |
| Corrientes (Corrientes) | 27° 27´ S, 58° 45´W | 52 | 1289 | 21.7 | 11 | 33.5 |
| Ituzaingó (Corrientes) | 27° 36´S, 56° 40´W | 62 | 1642 | 22.1 | 11.6 | 33.8 |

Maximum/minimum monthly high/low mean' refers to an average highest/lowest temperature across all months. For each locality we obtained the temperature data from WorldClim (www.worldclim. org).

**Table II. Absolute and relative abundance (between brackets) of flies sampled of *Drosophila melanogaster*, *D. simulans* and *Zaprionus indianus* collected in each locality.**

| Locality | Date | Resources | | Collected in the wild | | | Emerged in the lab | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fruit | # | *Dm* | *Ds* | *Zi* | *Dm* | *Ds* | *Zi* |
| Bella Vista | March 2014 | Guava | 10 | 421 (67) | 0 | 207 (33) | 0 | 0 | 57 (100) |
| Corrientes | March 2014 | Guava | 9 | 200 (52.1) | 0 | 184 (47.9) | 0 | 0 | 32 (100) |
| El Colorado | March 2014 | Guava | 10 | 64 (20.3) | 0 | 252 (79.7) | 1 (7.1) | 0 | 13 (92.9) |
| Ing. Juárez | March 2014 | Guava | 9 | 30 (100) | 0 | 0 | 1 (100) | 0 | 0 |
| Itatí | March 2014 | Guava | 8 | 60 (27.1) | 0 | 161 (72.9) | 1 (3.4) | 0 | 28 (96.6) |
| | Dec. 2013 | Mango | 8 | 65 (94.2) | 0 | 4 (5.8) | 2 (4) | 0 | 48 (96) |
| Ituzaingó | March 2014 | Orange | 7 | 38 (33.9) | 74 (66.1) | 0 | 33 (34) | 64 (66) | 0 |
| | Dec. 2013 | Guava | 6 | 115 (63.2) | 0 | 67 (36.8) | 0 | 0 | 108 (100) |
| Las Lomitas | March 2014 | Guava | 5 | 154 (55.8) | 0 | 122 (44.2) | 1 (1.6) | 0 | 60 (98.4) |
| Montecarlo | March 2014 | Caqui | 14 | 126 (56) | 21 (9.3) | 78 (34.7) | 91 (48.4) | 0 | 97 (51.6) |
| | Dec. 2013 | Guava | 24 | 274 (73.3) | 60 (16) | 40 (10.7) | 84 (31.8) | 10 (3.8) | 170 (64.4) |
| Oran | March 2014 | Banana | 7 | 330 (93.7) | 0 | 22 (6.3) | 38 (88.4) | 0 | 7 (15.6) |
| Palo Santo | March 2014 | Guava | 2 | N/A | N/A | N/A | 0 | 0 | 15 (100) |
| Yuto | March 2014 | Guava | 5 | 128 (73.6) | 0 | 46 (26.4) | 3 (11.1) | 0 | 24 (88.9) |
| | | Mango | 5 | 4 (8.9) | 0 | 41 (91.1) | 2 (11.8) | 0 | 15 (88.2) |
| | | Papaya | 2 | 0 | 0 | 19 (100) | N/A | N/A | N/A |
| | | TOTAL | | **2009 (59)** | **155 (4.5)** | **1243 (36.5)** | **257 (25.6)** | **74 (7.4)** | **674 (67)** |

The fruit and number (#) of resources utilized as natural attractant to collect adult flies and then used to evaluated flies emerged in the lab. The breeding/feeding sites (fruit resources) analyzed were: *Psidium guajava* Linnaei (Myrtales, Myrtaceae, «guava»), *Carica papaya* Linnaei (Brassicales, Caricaceae, «papaya»), *Mangifera indica* Linnaei (Anacardiaceae, «mango»), *Diospyros* sp. (Ebenaceae, «caqui»), *Musa paradisiaca* Linnaei (Zingiberales, Musaceae, «banana»), *Citrus x sinensis* («orange»). N/A not available.

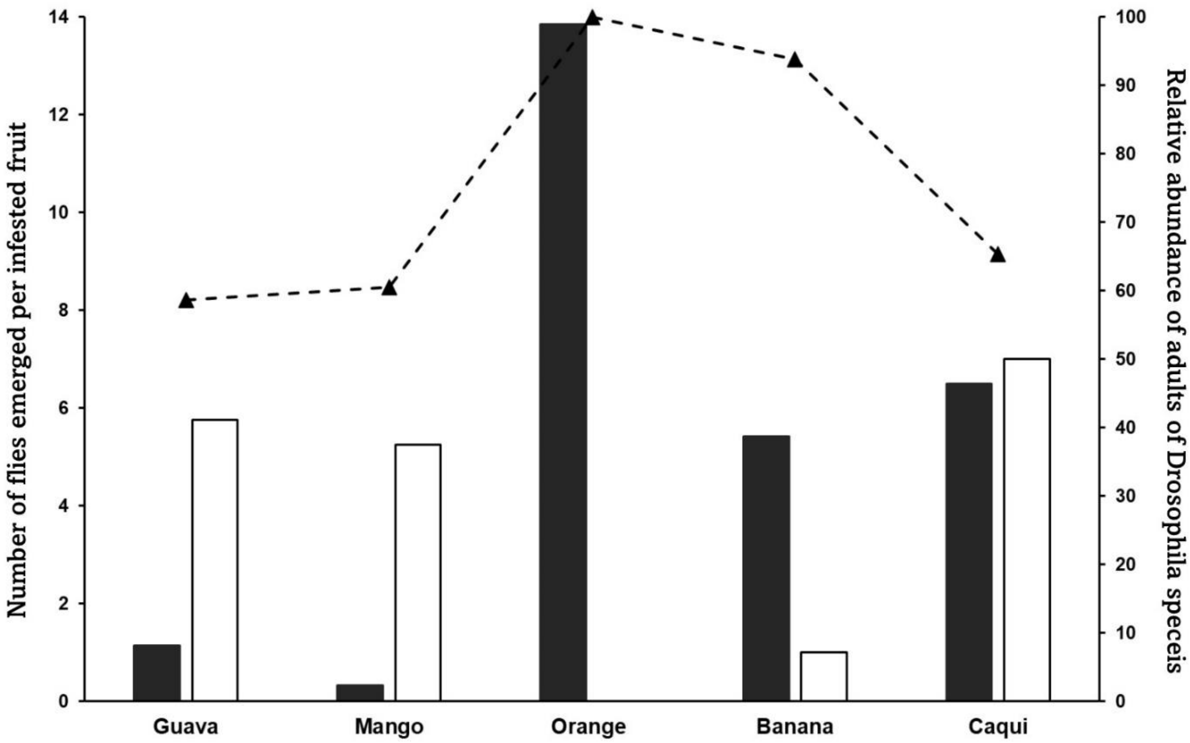


**Fig. 1. Mean numbers of both *Drosophila* species (*D. melanogaster* and *D. simulans*, filled bars) and *Z. indianus* (open bars) of emerged flies per infested fruit resources evaluated.** *Psidium guajava* Linnaei («guava», 88 resources), *Mangifera indica* Linnaei («mango», 12 resources), *Diospyros* sp. («caqui», 14 resources), *Musa paradisiaca* Linnaei («banana», 7 resources), *Citrus x sinensis* («orange», 7 resources). The relative abundance (in percentage) of adult collected sample of both *Drosophila* species respect to *Z. indianus* using as attractant guava, mango, orange, banana and caqui is indicated by triangle.


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