

Interspecific competition between the invasive species *Zaprionus indianus* and two *Drosophila* species (Diptera: Drosophilidae) in their natural hosts

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Competencia interespecífica entre la especie invasora *Zaprionus indianus* y dos especies de *Drosophila* (Diptera:Drosophilidae) en sus hospedadores naturales

RESUMEN. La historia de la biodiversidad está estrechamente relacionada con la historia de la evolución de las interacciones entre especies. La diversidad puede reducirse por exclusión competitiva en especies con ecología similar que viven en la misma región espacial. *Zaprionus indianus* Gupta (Diptera: Drosophilidae) es una especie plaga que ha colonizado el continente americano en los últimos 20 años. La expansión de esta especie en Argentina puede generar competencia, un evento importante de la ecología de comunidades de insectos que explotan recursos efímeros y fragmentados. *Zaprionus indianus* muestra un solapamiento tanto en los recursos de cría y alimentación como en la distribución con *Drosophila melanogaster* Meigen y *D. simulans* Sturtevant (Diptera: Drosophilidae) en el noreste de Argentina. En este estudio, analizamos el efecto de la competencia interespecífica entre larvas de estas especies criadas en medios de cultivo preparados a partir de tres frutos naturales. Nuestros resultados revelaron que *D. simulans* tiene una menor capacidad competitiva respecto a las otras especies. Por otro lado, tanto la colonización exitosa de *Z. indianus* como las diferencias observadas en la naturaleza en la abundancia relativa de estas tres especies entre las muestras de adultos colectados y adultos no pueden atribuirse únicamente a los efectos de la competencia interespecífica.

PALABRAS CLAVE. Bioinvasión Competencia. Índice relativo de performance. *Zaprionus indianus*.

ABSTRACT. The history of biodiversity is closely related to the history of the evolution of species interactions. Diversity can be reduced by competitive exclusion in species with similar ecology that live in the same spatial region. *Zaprionus indianus* Gupta (Diptera: Drosophilidae) is a pest species that has colonized the American continent in the last 20 years. The expansion of this species in Argentina can generate competition, a major event of the ecology of insect communities exploiting ephemeral and fragmented resources. *Zaprionus indianus* has shown an overlapping in the breeding and feeding resources and in the distribution with *Drosophila melanogaster* Meigen and *D. simulans* Sturtevant (Diptera: Drosophilidae) in north-eastern Argentina. In this study, we analyzed the effect of interspecific competition between larvae of these species reared in cultured media prepared from three natural fruits. Our results revealed that *D. simulans* has a lower competitive ability with respect to the other species. On the other hand, both the successful colonization of *Z. indianus* and the differences observed in nature in the relative abundance of these three species between samples of collected and emerged adults cannot be attributed only to effects of interspecific competition.

KEYWORDS. Bioinvasion. Competition. Relative performance index. *Zaprionus indianus*.

The proliferation of invasive alien species that colonize large areas provides an amazing opportunity to investigate the causes that allow their successes, as well as the change they bring about in already established communities (Maynard Smith & Szathmary, 1995; Thompson & Cunningham, 2002; Davis, 2009). New interactions between species are generated as a consequence of biological invasions with very different outcomes depending on the landscape wherein the species interact.

Competition plays a major role in the ecology of insect communities exploiting ephemeral and fragmented resources (Rodrigues et al., 2016). As a consequence of competition, individuals of the same species (intraspecific competition) or different species (interspecific competition) exhibit reductions in the survivorship, growth and/or reproductive success (Joshi & Muller, 1996; James & Partridge, 1998; Werenkraut et al., 2008; Gandini et al., 2024). Thus, an invasive alien species may generate interspecific competition and, therefore, shape different ecological-evolutionary scenarios depending on the environmental condition. The analysis of these scenarios will allow us to elucidate the adaptive strategy that may determine both the possible success of the invasion as well as the response exhibited by the native species.

Zaprionus indianus Gupta (Diptera, Drosophilidae) is an invasive pest species (Pfeiffer et al., 2019; Bragard et al., 2022) native to the Afrotropical biogeographical region (Yassin et al., 2008) that has colonized the American continent in the last 20 years (Vilela, 1999; Fernandez Goya et al., 2020). *Zaprionus indianus* was collected for the first time in Argentina in 2006 (Soto et al., 2006). This species is a polyphagous species that with *Drosophila melanogaster* Meigen (Diptera, Drosophilidae) and *D. simulans* Sturtevant (Diptera, Drosophilidae) utilized a wide variety of fruits as breeding and feeding resources (Lavagnino et al., 2008; Markow & O'Grady, 2008; Fanara et al., 2022). *Zaprionus indianus* colonized different areas of Argentina (Lavagnino et al., 2008; Fanara et al., 2022) from a single introduction wave from Brazil (Fernandez Goya et al., 2020). *Drosophila melanogaster* and *D. simulans* are cosmopolitan sibling species although the worldwide expansion of *D. simulans* is more recent than its sibling species (Capy & Gilbert, 2004).

Drosophila melanogaster, *D. simulans* and *Z. indianus* exhibit niche overlaps that are characterized by limited and ephemeral resources in nature (Tidon et al., 2003; da Silva et al., 2005; Galego & Carareto, 2005; Lavagnino et al., 2008; Fanara et al., 2022). In a recent study, Fanara et al (2022) analyzing natural populations from Argentina have shown differences between the proportions of these species that were attracted to and emerged from the resources evaluated. Different explanations are proposed to elucidate this decoupling detected: differential fecundity, oviposition preferences and/or interspecific competition. Here, we studied the effects of interspecific

competition on the general performance of *D. melanogaster*, *D. simulans* and *Z. indianus* in vials prepared with fermenting tissues of three alternative resources. The general performance was evaluated through the Relative Performance Index (RPI) that is estimated as viability x developmental time / body size (Ruiz & Heed, 1988; Fanara et al., 1999, 2006; Dettler et al., 2024).

We collected flies in different localities: *D. melanogaster* from General Lavalle (Mendoza, Argentina), *D. simulans* from Valle Fértil (San Juan, Argentina) and *Z. indianus* from Montecarlo (Misiones, Argentina). We selected these populations of *D. melanogaster* and *D. simulans* because there are no records of *Z. indianus* in these localities. Therefore, this will be a "first encounter" situation for both *Drosophila* populations with *Z. indianus*. Stocks of each species were generated using flies collected from each population and were maintained for five generations in lab medium, at temperature, humidity and photoperiod controlled (25 °C, 60 % and 12:12 light : dark).

Large quantities of first instar larvae of each species were obtained by placing batches of 100 pairs of sexually mature flies into egg-collecting chambers (Fanara et al., 1999). Eight chambers were set up for each species evaluated. Egg-laying medium was poured into Petri dishes (8 cm of diameter) and commercial yeast was spread onto the agar surface to stimulate oviposition. Chambers were prepared in the morning and 8 h later all flies were removed. Batches of 32 first instar larvae were collected from the dishes and seeded in vials containing one of the three different reared medium: *Mangifera indica* L. ('mango'), *Diospyros kaki* L. ('caqui') and *Pyrus communis* L. ('pear'). Two different types of vials were set up: single species culture (control) and mixed (interspecific competition) species cultures. In single species culture all 32 larvae were from the same species while interspecific competition (mixed species cultures) was evaluated by means of substitution experiments. Briefly, in each vial larvae of two species were initially seeded in the same proportions. As an example, 16 larvae of *D. melanogaster* (the species evaluated) were seeded with 16 larvae of *Z. indianus* whereas in other vial 16 larvae of *D. melanogaster* were seeded with 16 larvae of *D. simulans*. We set up five replicates for each combination single species culture and resources and 10 replicates for each combination mixed species culture and resources evaluated. It is important to note that in the case of mixed species cultures, the number of replicates was doubled in order to evaluate each species combination independently. The vials used to evaluate interspecific competition respect to one species were randomly chosen. All experiments were conducted under the same conditions of stocks maintenance. Emerging adults were collected twice daily at 8 (am and pm) and classified by species when applicable. *Drosophila melanogaster* and *D. simulans* were identified by the inspection of the male genitalia

(Markow & O'Grady, 2006) whereas *Z. indianus* was identified by its phenotype (Lavagnino et al., 2008). All three RPI traits components were scored only in males since females of both *Drosophila* species are morphologically indistinguishable. We determined that this procedure was valid as the proportion of males and females emerged from single species cultures in all reared medium did not depart from the expected 1:1 (data not shown), indicating that survival was independent of sex. Viability was measured in each vial, as the percentage of males of the species evaluated emerged relative to the

number of first instar larvae of this species initially seeded in the vials divided by two. Developmental time was estimated as the time elapsed since first instar larvae were transferred to the vials until adult emergence. We also quantified wing length as an estimation of body size (Flaibani et al., 2024) in all males emerged from single and mixed culture vial. RPI was analyzed by means of ANOVA with species (for single species culture) or treatment (for mixed species culture) and resources as fixed factors using STATISTICA 9.1 software package (StatSoft, 2010).

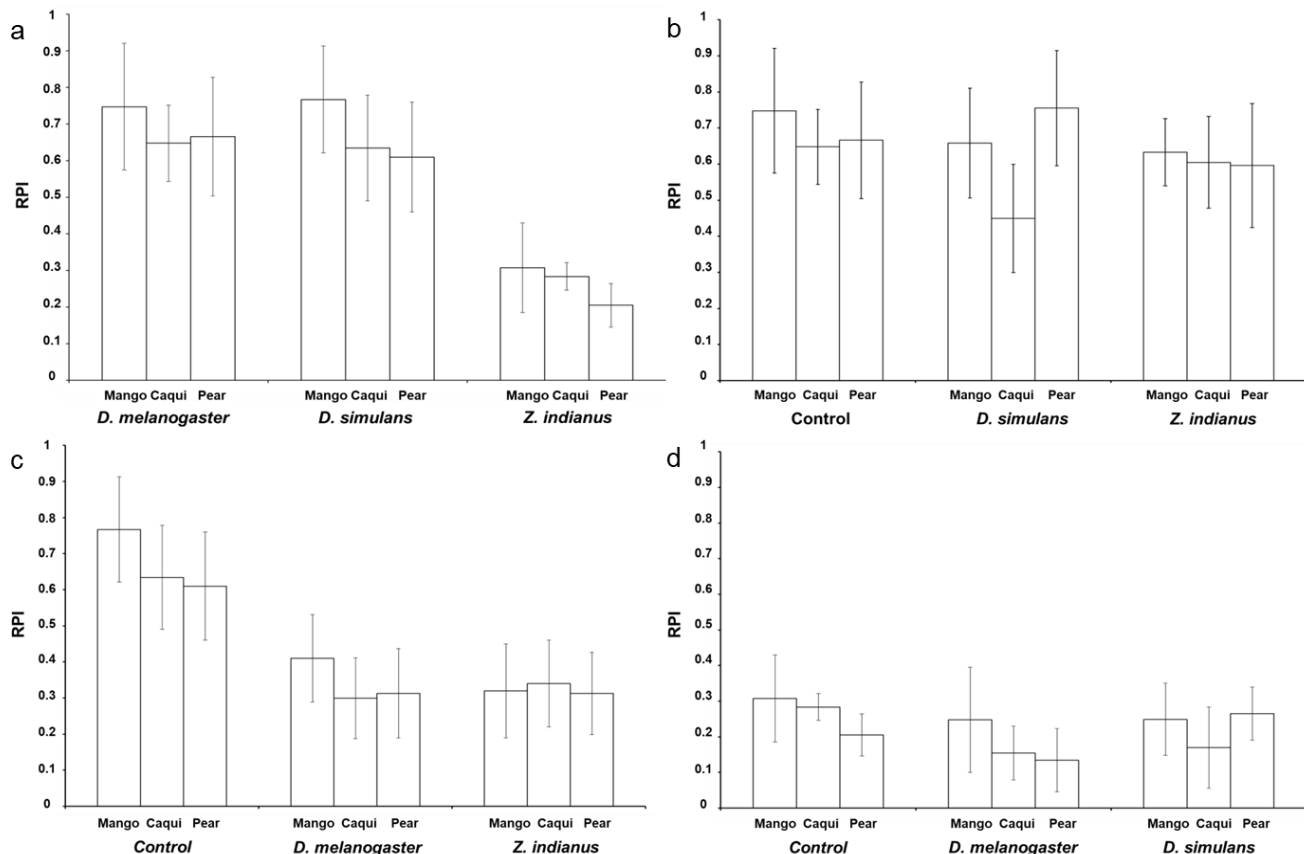


Fig 1. Relative performance index (RPI) estimated in single species culture. a. and for the two interspecific competition (mixed species culture) evaluated for *Drosophila melanogaster*. b. *D. simulans*. c. and *Zapionus indianus*. d. reared in *Mangifera indica* (mango), *Diospyros kaki* (caqui) and *Pyrus communis* (pear). Control indicates the RPI value obtained for each species reared under single species culture. Error bars corresponds to standard deviations.

To evaluate the effect of interspecific competition in each species, we compared the RPI value obtained in the control (single species culture) and both interspecific competition possibilities (mixed species culture). In all cases each vial (replicate) was considered as the experimental unit. The analysis revealed that *Z. indianus* exhibited a significant lower RPI relative to both *Drosophila* species when single species culture was evaluated ($F = 44.46$; $df = 2,36$; $p < 0.001$, Fig. 1a). Our results also determined that the resources evaluated as well as the species by resources interaction did not present significant differences. Therefore, *Z. indianus* had the lower RPI independently of the resources evaluated (Fig. 1a). This result is a consequence of the decrease of viability and a slower developmental time ($F = 23.09$ and

$F = 22.37$, respectively; $df = 2,36$; $p < 0.001$ in both cases) exhibited by *Z. indianus* respect to *D. simulans* and *D. melanogaster* (Table I) whereas, wing length showed similar size in all species studied ($F = 0.34$; $df = 2,36$; $p = 0.71$). When we evaluated mixed species culture (Fig 1b-d) only *D. simulans* was negatively affected by interspecific competition ($F = 13.76$; $df = 2,36$; $p < 0.001$) since the presence of any of the other two species reduces its RPI (Fig. 1c). Furthermore, under mixed species culture, the treatment factor showed significant differences for all three traits used to calculate RPI, ($F = 23.18$, $F = 23.58$ and $F = 13.28$, for viability, developmental time and wing length, respectively; $df = 2,36$; $p < 0.001$ in all cases), being the viability the trait most affected (Table II).

Table I. Mean values of viability (V), developmental time (DT) and wing length (WL) for *Drosophila melanogaster*, *D. simulans* and *Zaprionus indianus* reared in single species cultures.

	V (%)	DT (h)	WL (mm)
<i>D. melanogaster</i>	86.22a (0.20)	243.72a (21.75)	1.98a (0.31)
<i>D. simulans</i>	83.55a (0.15)	248.57a (28.78)	1.96a (0.26)
<i>Z. indianus</i>	56.79b (0.17)	429.29b (48.34)	2.01a (0.25)

Standard deviation is indicated between brackets. Means sharing different letters in the same column indicate significant differences between species ($p < 0.05$).

Table II. Mean values of viability (V), developmental time (DT) and wing length (WL) for *Drosophila simulans* reared in single specie culture (control) and under interspecific competition (mixed species culture) with *D. melanogaster* and *Z. aprionus indianus*.

	V (%)	DT (h)	WL (mm)
Control	83.55a (0.15)	248.57a (28.78)	1.96a (0.26)
<i>D. melanogaster</i>	46.90b (0.22)	207.48b (11.51)	1.78b (0.18)
<i>Z. indianus</i>	42.29b (0.17)	216.11b (16.66)	1.89a (0.11)

Standard deviation is indicated between brackets. Means sharing different letters in the same column indicate significant differences between treatments ($p < 0.05$).

All in all, our results suggest that interspecific competition could be one of the causes about the absence of *D. simulans*, among the emerged species when this species shares the resource with *D. melanogaster* and *Z. indianus* (Fanara et al., 2022). On the other hand, the successful colonization of *Z. indianus* cannot be attributed to interspecific competition. Certainly, the fitness of *D. melanogaster*, measured through RPI, was not affected when this species reared with *Z. indianus*. Thus, future studies should address the reason that allowed *Z. indianus* to successfully expand its distribution in Argentina.

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