

**INTRASPECIFIC AND INTERSPECIFIC PRE-ADULT COMPETITION
ON THE NEOTROPICAL REGION COLONIZER *ZAPRIONUS
INDIANUS* (DIPTERA: DROSOPHILIDAE) UNDER
LABORATORY CONDITIONS ⁽¹⁾**

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ABSTRACT

This study analyzes the pre-adult interactions of *Zaprionus indianus*, a recently-introduced species in Brazil, with two others Drosophilidae under laboratory conditions. The effects of larval residues on the viability and on the developmental time of *Z. indianus*, *Drosophila simulans* and *D. sturtevantii* were used to evaluate pre-adult competitive interactions, conditioning the culture medium with larval residues. Pre-adult interactions between *Z. indianus*, *D. sturtevantii* and *D. simulans* may affect their relative abundance over time, since the viability of *Z. indianus* was negatively affected by residues of *D. sturtevantii*, and its residues reduced the viability of *D. simulans* and the developmental time of both *D. simulans* and *D. sturtevantii*.

Key words: *Zaprionus indianus*; fitness components; competitive interactions; viability; developmental time.

RESUMO

**COMPETIÇÃO PRÉ-ADULTA INTRA E INTERESPECÍFICA, EM *ZAPRIONUS INDIANUS*
(DIPTERA: DROSOPHILIDAE), ESPÉCIE COLONIZADORA DA REGIÃO NEOTROPICAL,
SOB CONDIÇÕES LABORATORIAIS**

Este estudo é uma análise das interações pré-adultas, sob condições laboratoriais, da mosca-do-figo *Zaprionus indianus*, espécie recentemente introduzida no Brasil, com dois outros drosofilídeos. A interferência de meio de cultura, acrescido de resíduos larvais, sobre a viabilidade e o tempo de desenvolvimento de *Z. indianus*, *Drosophila simulans* e *D. sturtevantii* foi utilizada para avaliar as interações competitivas pré-adultas. As interações pré-adultas entre *Z. indianus*, *D. sturtevantii* e *D. simulans* podem afetar sua abundância relativa ao longo do tempo, pois a viabilidade de *Z. indianus* foi negativamente afetada por resíduos de *D. sturtevantii*; os resíduos da mosca-do-figo reduziram a viabilidade de *D. simulans* e o tempo de desenvolvimento tanto de *D. simulans* como de *D. sturtevantii*.

Palavras-chave: *Zaprionus indianus*; valor adaptativo; interações competitivas; viabilidade; tempo de desenvolvimento.

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1. INTRODUCTION

Zaprionus indianus is a Drosophilidae species that was recently introduced into the Neotropical region. The first published record refers to individuals observed on fallen persimmon fruits (*Diospyrus kaki* L.; Ebenaceae) in the São Paulo metropolitan area, São Paulo state, Brazil (VILELA, 1999). In the same year, a large number of *Z. indianus* were found feeding on and ovipositing in figs (*Ficus carica* L.; Moraceae) making them inappropriate for human consumption and occasioning the loss of approximately 50% of that crop. So, this African insect may now be reaching pest status in the main fig growing area in the state of São Paulo (VILELA et al., 2001).

According to MACK and D'ANTONIO (1998), species removed from or added to an environment which strongly interact with native species frequently produce ecosystem structure alterations and offer model systems for understanding the mechanisms by which species alter disturbance regimes. These alterations occur in both disrupted and intact systems, resulting in profound changes in many cases, including changes in ecosystem processes that ultimately control plant and animal activities and direct species replacements (KNOPS et al., 1999).

Mixing cultures makes it possible to study different parameters as a mean of evaluating the performance of one species when in the presence of another. Of diverse possible parameters, developmental time and viability are most frequently analyzed in studies that involve competition (GONZÁLES-CANDELAS et al., 1990). OHBA (1961) noticed diverse consequences of competition in species of *Drosophila*, such as increases in the variation of the duration of the pre-adult period, decreases in larval viability, increases in variation of the flies' body size, and decreases in pupal viability.

In Drosophilidae, developmental time and egg-adult viability are modified when the development occurs in a culture medium previously used by larvae of the same or different species (WEISBROT, 1966; DAWOOD and STRICKBERGER, 1969; HUANG et al., 1971; BUDNIK and BRNCIC, 1975; 1983; HEMMAT and EGGLESTON, 1988; BUDNIK et al., 2001). BUDNIK et al. (2001) demonstrated that competitive interactions between pre-adult individuals of different species can be established, with deterioration or facilitation of the viability of one or both species.

These effects may be due to the restriction of nutrition resources or even of substratum contamination by metabolic residues during the larval development.

Larval population density is also an important competitive factor. In *Drosophila* species, population aggregation of some species generally leads to individual body size decrease, developmental time increase and fecundity decrease (MITROFANOV and BRODSKAYA, 1976; SCHEIRING et al., 1984; BRNCIC, 1987). Similar results were obtained by AMOUDI et al. (1993) in experimental populations with different initial densities of *Z. indianus* larvae. The authors observed that the bigger the *Z. indianus* population size, the greater is the larva-adult developmental time and the lesser are the survival rates and adult body sizes. These authors concluded that, under intraspecific competition, the alterations in the developmental time, survival and body size would be related to the depletion of resources and the increase in larval residues, such as uric acid and CO₂.

Z. indianus is a species that has shown a great spread in the Neotropical region (TIDON et al., 2003) and information about its interactions with native Drosophilidae is unavailable. Aiming to evaluate the nature of these interactions, we studied the impact on viability and developmental time of metabolic waste products of two species of the genus *Drosophila* that occur at high abundances in the same area as *Z. indianus* during our collections, *D. sturtevantii* and *D. simulans*.

2. MATERIAL AND METHODS

Drosophilids were collected in orchard with different fruit trees in Mirassol, São Paulo State, Brazil (49°30'W, 20°47'S). Five traps with fermented banana were used, placed 1.5 m from the ground. The collections were made in the rainy (October, 2001 and January, 2002) and dry (April and June, 2002) seasons. The most abundant species of *Drosophila* in the rainy season was *D. sturtevantii* (*saltans* group), a native Neotropical species, and in the dry season was *D. simulans* (*melanogaster* group), an introduced species. The flies of these three species obtained in the collections were separated and maintained in 250 ml bottles with banana-agar culture medium and the larvae yielded were used in pre-adult competition studies.

The method employed to investigate the effects of metabolic waste products of immature stages on development of species that share the same environment was similar to that used by BUDNIK and BRNCIC (1975). According to this method, larvae of the same (to evaluated intraspecific competition) or

different (to evaluated interspecific competition) species are transferred to vials with fresh food and maintained there during a certain period of time for releasing metabolic residues in the culture medium. Afterwards, the vials are frozen in order to kill the larvae. These vials containing culture medium with the metabolic waste products and the dead bodies of the larvae are named as "conditioned" with compounds of a particular species. The next step is the transfer of living larvae of a species to be tested into the conditioned vials. This approach can show whether the residues of a particular species can affect its own or the other species development by evaluating fitness components of such species.

In this study, three types of competition were evaluate: (1) intraspecific competition: the medium was conditioned with residues of the same species to be tested (for example, larvae of *Z. indianus* placed in vials conditioned with residues of *Z. indianus*); (2) interspecific competition: the medium was conditioned with residues of a different species (for example, larvae of *Z. indianus* placed in vials conditioned with *D. simulans*) and, (3) intra and interspecific competition: the medium was conditioned with residues of the species to be tested plus residues of a different species (for example, larvae of *Z. indianus* placed in vials conditioned with *Z. indianus* and of *D. simulans*).

The viability and developmental time of the species to be tested (ST) were studied in vials containing 5 ml of banana-agar medium, replicated 10 times: *Z. indianus* (ST1: *zp*), *D. sturtevantii* (ST2: *st*) and *D. simulans* (ST3: *sm*). Vial 1 was nonconditioned (Vnc) and the vials 2 to 4 were conditioned each one with 30 larvae one-day-old: vial 2 was conditioned with intraspecific residues (V_{zp}, V_{st} or V_{sm}), vial 3 with interspecific residues (V_{zp}, V_{st} or V_{sm}) and vial 4 was conditioned with intra and interspecific residues using 15 larvae of each species (V_{zp+st} or V_{zp+sm}). For example, ST1Vnc means that the *Z. indianus* viability or developmental time was recorded in non-conditioned medium; ST2V_{zp} means that the *D. sturtevantii* viability or developmental time was recorded in medium conditioned with residues of *Z. indianus* as well as ST3V_{zp+sm} means that these parameters of *D. simulans* was recorded in medium conditioned with larvae of *Z. indianus* plus *D. simulans*. These vials were maintained in a constant temperature chamber at 25°C during five days to allow the larvae to develop and to release the metabolic waste products. Thereafter, they were frozen at -20 °C for about 24 h in order to kill the larvae. After the

vials were thawed at 25 °C, 20 one-day-old larvae of the species to be tested were transferred to each vial and their viability and developmental time were recorded.

ANOVA and Tukey's test for pairwise comparisons (significance level at $\alpha = 0.05$) were used to compare the mean viability and developmental time of each species and between species in different conditioned mediums of culture.

3. RESULTS

The viability and the larva to adult developmental time were used to evaluate the effects of the larval competitive interactions between *Z. indianus* and the *Drosophila* species. *Z. indianus* viability ranged from 50.0 % in intra and interspecific (*D. sturtevantii*) residues to 70.0 % in intraspecific residues ($P < 0.01$) and its developmental time ranged from 19.06 to 20.24 days in interspecific residues of *D. simulans* and *D. sturtevantii* ($P > 0.05$), respectively. The variation of *D. sturtevantii* viability on the presence of *Z. indianus* residues was also not significant. However, the variation of the mean developmental time of *D. sturtevantii* on the presence of *Z. indianus* residues was highly significant ($P < 0.001$), the smallest value was observed in interspecific residues of *Z. indianus* (18.91 days) and the greatest in the non-conditioned medium (21.86 days). *D. simulans* viability ($P < 0.01$) ranged from 55.0 % in the presence of *Z. indianus* residues to 74.5 % in the presence of intraspecific and interspecific (*Z. indianus*) residues; its developmental time ($P < 0.01$) varied from 10.91 days in intra and interspecific residues (*Z. indianus*) to 11.51 days in the non-conditioned medium. Table 1 presents the averages and the standard-errors, as well as the F-values for homogeneity of means of viability and developmental time of each species in different types of conditioning.

The pairwise comparisons between the conditioning types and control inside each experimental group show that the residues of *D. sturtevantii* affect significantly the viability of *Z. indianus* as well as the residues of *Z. indianus* affect the viability of *D. simulans*. The viability of *Z. indianus* in non-conditioned vials (67%) and in vials conditioned with its own residues (70%) is significantly reduced in vials conditioned with residues of *D. sturtevantii* (57.5%) or of *D. sturtevantii* plus *Z. indianus* (50%) (Table 1). Also, the viability of *D. simulans* in non-conditioned vials (70%) is reduced by the *Z. indianus* residues (55%).

On the other hand, the developmental time of *Z. indianus* was not significantly affected by any residues, but its residues affected significantly this fitness component of *D. sturtevantii* and *D. simulans*.

The developmental time of *D. sturtevantii*, in vials non-conditioned (21.86 days) was reduced when compared to vials conditioned with larvae of *Z.*

indianus (19.97 days) or of *Z. indianus* plus *D. sturtevantii* (20.87 days) as well as was reduced in *D. simulans* developed in non-conditioned vials (11.51 days), in vials conditioned with *Z. indianus* (11.10 days) or with the *Z. indianus* plus the *D. simulans* (10.91) residues. It can be also seen that the metabolic residues of *D. sturtevantii* or *D. simulans* reduce its own developmental times (Tables 1 and 2).

Table 1. Means and standard-errors for larva-adult viability (%) and developmental time (days) of *Zaprionus indianus* (*zp*), *Drosophila sturtevantii* (*st*) and *D. simulans* (*sm*) in non-conditioned and conditioned media (ST: species to be tested; V: vial with conditioned medium).

Species tested	Conditioned vial	Viability (Mean \pm SE)	Developmental time (Mean \pm SE)
<i>Z. indianus</i>	Vnc	67.0 \pm 2.9	19.61 \pm 0.37
ST1	Vzp	70.0 \pm 3.2	19.76 \pm 0.24
	Vst	57.5 \pm 3.0	20.24 \pm 0.32
	Vsm	64.0 \pm 3.8	19.06 \pm 0.21
	Vzp+st	50.0 \pm 2.4	19.50 \pm 0.21
	Vzp+sm	59.5 \pm 2.2	19.53 \pm 0.22
	F _{6;54}	7.77**	2.04
<i>D. sturtevantii</i>	Vnc	87.5 \pm 2.4	21.86 \pm 0.32
ST2	Vst	76.0 \pm 4.5	18.91 \pm 0.34
	Vzp	73.0 \pm 4.7	19.97 \pm 0.37
	Vzp+st	84.0 \pm 3.7	20.80 \pm 0.32
	F _{3;36}	2.71	13.66***
<i>D. simulans</i>	Vnc	70.0 \pm 4.5	11.51 \pm 0.11
ST3	Vsm	67.0 \pm 3.6	11.04 \pm 0.06
	Vzp	55.0 \pm 4.1	11.10 \pm 0.10
	Vzp+sm	74.5 \pm 2.0	10.91 \pm 0.04
	F _{3;36}	5.06**	9.72***
	nc	F _{2;27} : 17.80***	F _{1;18} : 13.46***
	intra	F _{2;27} : 01.72***	F _{1;18} : 03.06***
	inter (<i>st</i> and <i>sm</i>)	F _{3;36} : 07.76***	F _{2;27} : 04.07***
	intra and inter (<i>zp</i> + <i>st</i> and <i>zp</i> + <i>sm</i>)	F _{3;36} : 15.41***	F _{2;27} : 08.46***

*p<0.05; **p<0.01; ***p<0.001

nc: non-conditioned medium.

inter: interspecific residues. i

intra: intraspecific residues.

intra + inter: intra and interspecific residues.

Table 2. Pairwise comparisons of conditioning type effects on viability and developmental time in *Zaprionus indianus* (*zp*), *Drosophila sturtevantii* (*st*) and *D. simulans* (*sm*). (nc: non-conditioned; ST: species to be tested; V: vial with conditioned medium)

Species tested/ Conditioning type	Conditioning type	Viability	Developmental time
<i>Z. indianus</i> (ST1)			
Vnc	Vzp	NS	NS
	Vst	*	NS
	Vzp+st	NS	NS
	Vsm	NS	NS
Vzp	Vzp+sm	NS	NS
	Vst	*	NS
	Vzp+st,	*	NS
	Vsm	NS	NS
Vst	Vzp+sm	NS	NS
	Vzp+st	NS	NS
	Vzp+sm	NS	NS
Vzp+st	Vsm	NS	NS
	Vzp+sm	NS	NS
Vsm	Vzp+sm	NS	NS
<i>D. sturtevantii</i> (ST2)			
Vnc	Vst	NS	*
	Vzp	NS	*
	Vzp+st	NS	*
Vst	Vzp	NS	NS
	Vzp+st	NS	*
Vzp	Vzp+st	NS	NS
<i>D. simulans</i> (ST3)			
Vnc	Vsm	NS	*
	Vzp	*	*
	Vzp+sm	NS	*
Vsm	Vzp	NS	NS
	Vzp+sm	NS	NS
Vzp	Vzp+sm	*	NS

NS: non-significant. *p < 0.05.

4. DISCUSSION

Competition can occur in Drosophilidae pre-adult stages due to high larval density and depletion of resources (AMOUDI *et al.*, 1993), or due to larval residues produced by individuals in the medium which can interact with the metabolism of larvae (BUDNIK and CIFUENTES, 1995; BUDNIK *et al.*, 2001).

These residues would intervene or promote the growth of yeast or other resources necessary for the survival and the development of these Drosophilidae, reducing or facilitating their development (WEISBROT, 1966).

We exposed larvae of *Z. indianus* to residues of *D. sturtevantii* and *D. simulans*, species that we had observed to occur at high frequencies in the same area

(Mirassol, State of São Paulo) as *Z. indianus*; the first during the rainy season and the second during the dry season. The pairwise comparison showed that the developmental time of *Z. indianus* was not affected by any type of competitor residues, however, its viability was reduced. Individuals of *Z. indianus* exposed to the non-conditioned medium and to that with intraspecific residues presented practically the same viability (67% and 70%, respectively). However, its viability was significantly reduced when developed in medium conditioned with residues of *D. sturtevantii*: a reduction of 14.9 % comparing *Z. indianus* developed in non-conditioned medium versus in medium conditioned only with residues of *D. sturtevantii*; and 17.9% and 28.6%, respectively, when comparing the viability of *Z. indianus* which was developed in medium conditioned with its own residues versus in medium conditioned only with *D. sturtevantii* or with its own residues plus those of *D. sturtevantii*.

These results indicate a possible deleterious effect of *D. sturtevantii* on *Z. indianus* survival. On the contrary, it can be seen that *Z. indianus* residues reduced significantly the viability of *D. simulans* (21.4%) as shown by the viability of *D. simulans* in non-conditioned medium versus that in environment conditioned with *Z. indianus* residues.

Neither type of conditioned medium significantly affected the viability of *D. sturtevantii*; however, the developmental time of this species was affected by residues of *Z. indianus*. A higher developmental speed is suggested by the pairwise comparisons of the developmental time of this species in non-conditioned medium and the values in medium conditioned only with *Z. indianus* (a reduction of 8.6%) or with its own residues plus those of *Z. indianus* (a reduction of 4.8%). Although some exceptions can be observed, these comparisons suggest a possible facilitator role of the *Z. indianus* larval residues on the *D. sturtevantii* development. Despite the lower degree, a similar phenomenon was observed in *D. simulans* development that was significantly reduced in the presence of *Z. indianus* (3.6%) or when exposed to the *Z. indianus* plus the *D. simulans* (5.2 %) larval residues.

BUDNIK et al. (2001) pointed out that larval residues may not only be associated with the factors that reduce viability and increase developmental time but may also act as a facilitator of development. For example, pre-adult viability is increased when *D. willistoni* and *D. simulans* grow in a culture medium with residues of their own species (BUDNIK and BRNCIC, 1976), but in *D. pavani*, viability was reduced when exposed to larval intraspecific residues (BUDNIK, 1977). BUDNIK and CIFUENTES (1995) found different viability patterns and developmental times in intraspecific competition studies involving *D. pseudoobscura* from different geographic regions. The authors concluded that each geographic population has its own genetic background as a response to the history of interactions between species that inhabit the same geographic region.

Our results suggest that the interaction between pre-adult stages of *Z. indianus*, *D. sturtevantii* and *D. simulans* in oviposition sites can affect their relative abundance over time. Possible results are the elimination of one or more of these competitor species or coexistence among them, as occurred with other invading Drosophilidae species, such as *D. malerkotliana* and *D. simulans*, which reached equilibrium with native Neotropical populations, after a population demographic explosion of these species. Monitoring the interactions among *Z. indianus*

and other Drosophilidae species over time will be important to evaluate the evolutionary dynamics and impact of this species on the Neotropical environment.

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