

Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil

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Abstract One of the most promising plant species for biofuel production in Brazil is the physic nut *Jatropha curcas*. Major phytosanitary problems include the attack of two pest mite species, the broad mite *Polyphagotarsonemus latus* and the spider mite *Tetranychus bastosi*. Owing to pesticide-related problems, there is an increasing demand for sustainable environmental-friendly control methods such as biological control. In this study we evaluated the suitability of the predatory mite species *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *P. latus* and *T. bastosi* on *J. curcas*. The number of *T. bastosi* killed by *I. zuluagai* was lower than the number of *P. latus* consumed. *Euseius concordis* preyed upon both *T. bastosi* and *P. latus* but the number of prey killed was always lower in comparison with *I. zuluagai*. However, *P. latus* and *T. bastosi* are suitable for the development of *I. zuluagai* and *E. concordis* as oviposition of both predators did not differ in relation to prey species. The preference of *I. zuluagai* for leaves of plants infested by either *P. latus* or *T. bastosi*, combined with the higher values for predation obtained by this predatory mite when fed on *P. latus*, compared to those values obtained by *E. concordis*, suggests that *I. zuluagai* can be more efficient than *E. concordis* in reducing populations of *P. latus* and *T. bastosi* under field conditions. Furthermore, we report here on the first record of predatory mites associated with *P. latus* and *T. bastosi* on native *J. curcas* plants

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in Brazil. In conclusion, we emphasize the crucial importance of predatory mites as agents of natural biological control of mite pests on *J. curcas* in small farms.

Keywords Biological control · Spider mites · Biofuel · Smallholders

Introduction

The physic nut (*Jatropha curcas*) is native to equatorial America, where it is traditionally used for hedges, green manure, land reclaim, prevention of erosion and in traditional medicine, and more recently, for biofuel (Gubitz et al. 1999; Openshaw 2000; Fairless 2007; Nature editorial 2007; Kumar and Sharma 2008). In north Brazil, *J. curcas* is becoming an increasingly popular plant due to its value in the biodiesel industry. Nowadays, 40,000 ha in Brazil are planted with *J. curcas*, and the area is still expanding. Although the plant can be cultivated on degraded land, and thus does not compete for space with crops, farmers that produce *J. curcas* predominantly use management practices that include high inputs of pesticides and chemical fertilizers. Recently, however, smallholders in Brazil have started to cultivate *J. curcas* as a cash crop, selling the seeds for biodiesel production.

The main phytosanitary problems in cultivating *J. curcas* are the attack of pest mites such as the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the red spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) (Lopes 2009; Santos et al. 2006; Kavitha et al. 2007). The broad mite is a highly polyphagous pest attacking several crops worldwide (Gerson 1992; Peña and Bullock 1994; De Moraes and Flechtmann 2008). This minute mite (0.1–0.3 mm in length) is found in the apical portion of plants, especially in shoot structures (De Moraes and Flechtmann 2008). This pest causes terminal leaves and flower buds to become malformed, and its attack may occur concomitantly with infestations of *T. bastosi*. The spider mite *T. bastosi* (0.5 mm in length) was first recorded attacking native *Jatropha gossypifolia* plants in the northeastern Brazil (Tuttle et al. 1977). The mite was also found infesting weeds, such as *Amaranthus viridis* L., *Bidens pilosa*, *Ipomoea* sp., and *Hyptis suaveolens*, as well as on crops such as *Manihot esculenta* and *Morus nigra* (Tuttle et al. 1977; de Moraes and Delalibera 1992; Bolland et al. 1998). Contrary to *P. latus*, spider mites such as *T. bastosi* have a silk spinning behavior, covering their colonies with webbing, which may difficult predatory mites to penetrate on their colonies (Saito 1985; Gerson 1985; Sabelis and Bakker 1992). They are more frequently found on underside of fully-developed leaves of *J. curcas* in comparison with younger leaves.

In Brazil, chemical control is the main method of combating pest mites (Oliveira et al. 2007). Due to excessive use of pesticides and the associated problems of pesticide resistance and environmental pollution, there is an increasing demand for sustainable environmental-friendly control methods. Biological control of pest mites is viewed as an alternative method to chemical control. Predatory mites of the family Phytoseiidae are key natural enemies of pest mites (McMurtry and Croft 1997; De Moraes et al. 2004) and the biological control provided by such indigenous species is considered an environmental service to smallholders which normally lack the resources to control pest mites in their fields. The predatory mites *Iphiseiodes zuluagai* Denmark & Muma and *Euseius concordis* Chant (Acari: Phytoseiidae) are the most common natural enemies associated with *P. latus* and *T. bastosi* on *J. curcas* plants in the region where the study was conducted (Rodrigues et al. in prep). Predatory mites forage for prey guided by volatiles that are produced by plants in response to herbivore injury (Dicke and Sabelis 1988; Dicke 1994; Turlings et al. 1990; Janssen et al. 1999;

Sabelis et al. 2001), and therefore, the ability of these predators to find prey infested plants is key in order to biological control to succeed (Oliveira et al. 2009).

Here, we evaluated the potential of biological control of the predatory mites *I. zuluagai* and *E. concordis* on the phytophagous mites *P. latus* and *T. bastosi*. We investigated the suitability of *P. latus* and *T. bastosi* as prey for *I. zuluagai* and *E. concordis* using predation bioassays and the oviposition rate experiments as a fitness measure. Another important trait to be considered when selecting a predator for biological control purposes is its ability to find prey-infested plants (Oliveira et al. 2009), therefore, we assessed the attraction of both predatory mites to leaves of plants infested with *P. latus* or *T. bastosi* through release-recapture experiments.

Materials and methods

Plant material and mite rearing

Seedlings (15 cm) of *J. curcas* were transplanted into individual plastic pots (500 ml) containing soil and manure in a 3:1 portion. Stock cultures of both *I. zuluagai* and *E. concordis* were started with mites collected on *J. curcas* plants. Stock colonies were established on arenas prepared with 6-cm diameter flexible plastic discs floating on distilled water. Castor bean pollen (*Ricinus communis* L.) was added on a daily-basis as food for the mites (Reis and Alves 1997). Stock colonies of *P. latus* and *T. bastosi* were started with mites collected from infested native *J. curcas* plants, which were subsequently transferred to uninfested plants. Both, predatory and spider mites were maintained inside a climate chamber (28 °C, 65–70% R.H. and 12 h L/12 h D photoperiod). Each experiment was carried out in separated chambers at the laboratory of Entomology of the Federal University of Tocantins, Gurupi, Tocantins, Brazil.

Predation rate relative to prey density

The predatory activity of *I. zuluagai* and *E. concordis* on *P. latus* and *T. bastosi* was evaluated on leaf discs ($\varnothing = 3$ cm) made from healthy and unsprayed *J. curcas* plants. Discs were kept in batches of 10 in plastic trays (30 × 20 cm) containing foam covered with wet cotton wool and soaked with water to prevent mites from escaping. The trays were kept inside a climate chamber at the same conditions described above. Leaf discs were infested with 2-day-old mated females of each pest mite species (*P. latus* or *T. bastosi*), according to each treatment on the following prey densities: 5, 10, 15 and 20 individuals per disc. Predators were starved in the presence of water for 2 h before used in the experiments. Subsequently, one young mated female of each predatory mite species (*I. zuluagai* or *E. concordis*) was released on each disc according to each treatment. After 8 h, the predation rate of each predatory mite was calculated by counting the number of mites preyed per disc. Each treatment consisted of 20 replicates.

Predatory mite oviposition

To evaluate the oviposition rate of *I. zuluagai* and *E. concordis* on *P. latus* or *T. bastosi* one 2-day-old female plus one male of each predatory species were confined on leaf discs ($\varnothing = 5$ cm) made from healthy and unsprayed *J. curcas* plants, infested with *P. latus* or

T. bastosi, according to each treatment. The discs with all developmental phases (eggs, larvae, nymphs and adults) ad libitum were kept in plastic trays (30 × 20 × 8 cm) containing foam covered with wet cotton wool and soaked with water to maintain the swelling of the disc and to prevent mites from escaping. The trays were kept inside a climate chamber. Eggs of each predatory mite were quantified and discarded daily for 5 days (Rosa et al. 2005).

Release-recapture experiments

Release-recapture experiments were conducted to test the attractiveness of the predatory mites *I. zuluagai* and *E. concordis* to leaves infested with *P. latus* and/or *T. bastosi* (Pallini et al. 1997). Infested leaves were obtained by placing clean (uninfested) plants in contact with mite-infested plants for 1 week. Plants were subsequently removed and the number of mites was estimated. The number of mobile stages on each leaf ranged from 50 to 100. Six young leaves of *J. curcas* plants of similar age and size (circa length = 3 cm; width = 2 cm) were removed from the plants (being three from infested plants and three from control plants) and placed in a circle (Ø = 20 cm) spiked on needles that were fixed in a plastic tray (30 × 20 × 8 cm) with silicon glue. In each experiment, uninfested and infested leaves were alternated, so that each uninfested leaf had two infested neighbors, and vice versa. To prevent the predatory mites from escaping a sticky glue (Cola Entomológica, Bio Controle, São Paulo, Brazil) was applied around the circle. Then, 25 females of each predatory mite species (*I. zuluagai* or *E. concordis*) were placed in a Petri dish (Ø = 2 cm), which was placed inside a larger Petri dish filled with wet cotton wool. Predatory mites were starved for 2 h before experiments. Subsequently, the Petri dish containing the predatory mites was positioned in the centre of the circle of leaves, and the predators were allowed to access each treatment. Starting 1 h after release, the leaves were checked for predatory mites every hour for a period of 7 h, and all predatory mites found were removed. All trays were kept in a climate chamber (27 ± 2°C, R.H. 75 ± 10%) during the 7 h of the evaluation along the day. Each treatment was replicated four times.

Statistical analysis

Predation rate of *I. zuluagai* and *E. concordis* in relation to prey density was analyzed using Generalized Linear Models (GLM) with Poisson error distribution. The difference in the number of mites preyed between predator species, prey species and prey density was compared with a full model. The contrasts between the levels within the model were assessed with Wald test (Kuhn et al. 2008). Oviposition of predatory mites was analyzed using a Generalized Linear Mixed Model (LMER) with Poisson error distribution, with time as random factor to correct for pseudoreplication due to repeated measures (Crawley 2007). Oviposition rates of predatory mites were compared with models consisting of the number of eggs laid in function of predator species and prey species as fixed effects. To analyze differences in numbers of predatory mites recaptured on each of the odour sources (pest mite infested leaves vs. uninfested leaves) we used a χ^2 test (expected probability of 0.5 for each treatment). All analyses were performed using the statistical software R (R Development Core Team 2006).

Results

Predation rate relative to prey density

The predation rate of *I. zuluagai* was higher on *P. latus* than on *T. bastosi* in all evaluated densities (Table 1; Fig. 1). There was no significant difference on the predation rate of *E. concordis* either upon *T. bastosi* or *P. latus* in any of the evaluated densities (Table 1; Fig. 2). The predation rate of *E. concordis* was almost always lower than that of *I. zuluagai* to both prey (Tables 1, 2; Figs. 1, 2).

Predatory mite oviposition

The oviposition rate of *E. concordis* did not differ between prey species ($\chi^2 = 1.0089$, $df = 1$, $P = 0.3152$). Furthermore, prey species did not affect the oviposition rate of *I. zuluagai* ($\chi^2 = 0.1766$, $df = 1$, $P = 0.6744$). The predatory mites *E. concordis* and *I. zuluagai* laid in average 0.9 ± 0.24 and 0.9 ± 0.22 eggs per day when fed on *T. bastosi* while they produced in average 0.68 ± 0.25 and 1.00 ± 0.28 eggs per day when fed on *P. latus*, respectively. Considering the same prey species there is no difference on oviposition rate between predatory mites ($\chi^2 = 4.1 \times 10^{-10}$, $df = 1$, $P = 1$ and $\chi^2 = 1.5332$, $df = 1$, $P = 0.2156$, respectively for *T. bastosi* and *P. latus*).

Release-recapture experiments

When we assessed the preference of *E. concordis* to odours of leaves infested with *P. latus* or uninfested leaves, 87% of all mites were recaptured from any of the leaves offered as choice to *E. concordis*. Infested leaves attracted on average 46% and uninfested leaves attracted 54% of the recaptured mites (Fig. 4A). When we tested the preference of *E. concordis* to odours emanating from leaves infested with *T. bastosi* or uninfested ones, 68% of the mites were recaptured from the leaves (Fig. 4B). On average, 52% of the mites were found on leaves infested with *T. bastosi* while uninfested leaves attracted 48% of the recaptured mites. These results represent no statistical preference of *E. concordis* for uninfested leaves or leaves infested with *P. latus* or *T. bastosi* ($P > 0.05$).

When the same options were given to *I. zuluagai*, the percentage of recaptured mites was also high. When it was offered a choice towards odours of leaves infested with *P. latus* versus uninfested leaves, 89% of all *I. zuluagai* released were recaptured from the leaves. From these recaptured mites, 67% were attracted to infested leaves while uninfested leaves attracted 33% mites (Fig. 4C). These difference was significant ($P < 0.05$). When offered a choice to *I. zuluagai* between odours from leaves infested with *T. bastosi* or uninfested ones, 84% were recaptured from the leaves. Leaves infested with *T. bastosi* attracted on average 71%, while uninfested leaves attracted only 29% of the recaptured mites (Fig. 4D) and this difference was statistically significantly ($P < 0.05$).

Discussion

Our results indicate that the predatory mites *I. zuluagai* and *E. concordis* may contribute to the biological control of the pest mites *P. latus* and *T. bastosi* on *J. curcas* plantations in north Brazil. The number of *P. latus* killed by *I. zuluagai* reached a maximum around 4

Table 1 Mean predation rate (\pm SE) of *Iphiseiodes zuluagai* and *Euseius concordis* at four different densities of prey (*Polyphagotarsonemus latus* and *Tetranychus bastosi*) within a period of 8 h on *Jatropha curcas*

		Densities (spider mites/disc)							
		5		10		15		20	
		<i>T. bastosi</i>	<i>P. latus</i>	<i>T. bastosi</i>	<i>P. latus</i>	<i>T. bastosi</i>	<i>P. latus</i>	<i>T. bastosi</i>	<i>P. latus</i>
<i>I. zuluagai</i>		1.53 \pm 0.29 Aa	1.89 \pm 0.17 Bb	2.06 \pm 0.41 Ba	3.47 \pm 0.40 Bb	1.76 \pm 0.39 Ba	3.94 \pm 0.31 Bb	3.28 \pm 0.54 Ba	3.95 \pm 0.56 Bb
<i>E. concordis</i>		0.60 \pm 0.16 Aa	0.86 \pm 0.29 Aa	1.10 \pm 0.37 Aa	1.33 \pm 0.35 Aa	1.00 \pm 0.30 Aa	0.45 \pm 0.16 Aa	0.79 \pm 0.24 Aa	1.38 \pm 0.29 Aa

Within the same prey density means followed by the same small letter in the same line or the same capital letter in each column, do not differ statistically (Wald test, $P > 0.05$)

Fig. 1 Mean predation (\pm SE) of *Iphiseiodes zuluagai* preying upon different densities of *Polyphagotarsonemus latus* (solid lines) and *Tetranychus bastosi* (dotted lines). Different letters denote significant differences among treatments

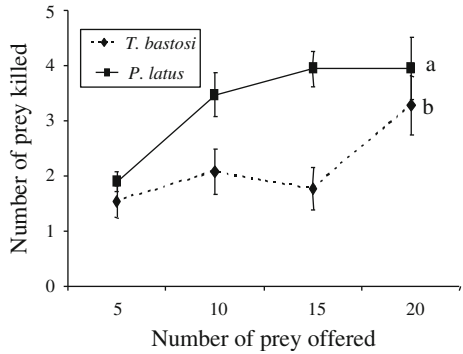


Fig. 2 Mean predation (\pm SE) of *Euseius concordis* preying upon different densities of *Polyphagotarsonemus latus* (solid lines) and *Tetranychus bastosi* (dotted lines). Same letters denote non-significant differences among treatments

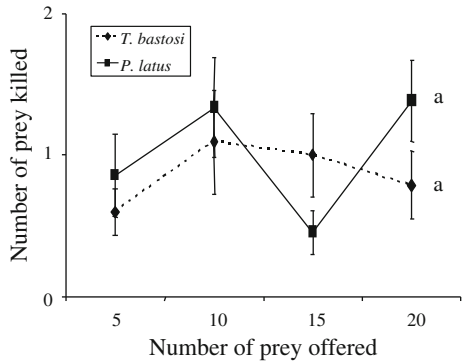


Table 2 Analysis of deviance for the best fitted model with 3 sources of variation (2 categorical: predator species and prey species plus one continuum variable: prey density)

Source of variation	df	Dev.	P
Predator	1	104.49	1.58×10^{-24}
Prey	1	14.74	1.23×10^{-4}
Density	1	20.25	6.78×10^{-6}
Predator \times Prey	1	1.02	0.31
Predator \times Density	1	2.18	0.14
Prey \times Density	1	0.04	0.84
Predator \times Prey \times Density	1	0.14	0.71

df Degrees of freedom, Dev deviance, P probability χ^2 test

mites in a period of 8 h (Fig. 1). When fed on *T. bastosi*, the number of prey killed by *I. zuluagai* was lower than that obtained by this predator when preying upon *P. latus* (Fig. 1). Unlike the spider mite *T. bastosi* the broad mite *P. latus* does not produce web making it easier for predation, although several predatory mite species (Acari: Phytoseiidae) have been reported entering dense webbing produced by spider mites (McMurtry and Croft 1997; Lemos et al. 2010). The production of a dense and sticky web on host plants is common in spider mites of the family Tetranychidae (Saito 1979, 1983; Gerson 1985). Although there are large differences in the amount of silk produced and in the structure of the web among tetranychid mite species (Saito 1979, 1983, 1985; Gerson 1985; Morimoto et al. 2006), the general consensus is that such webs have various possible functions, one of which is defense against predators (Gerson 1985; Saito 1985; Lemos et al. 2010). The web can act against predators by repelling them (McMurtry and Johnson

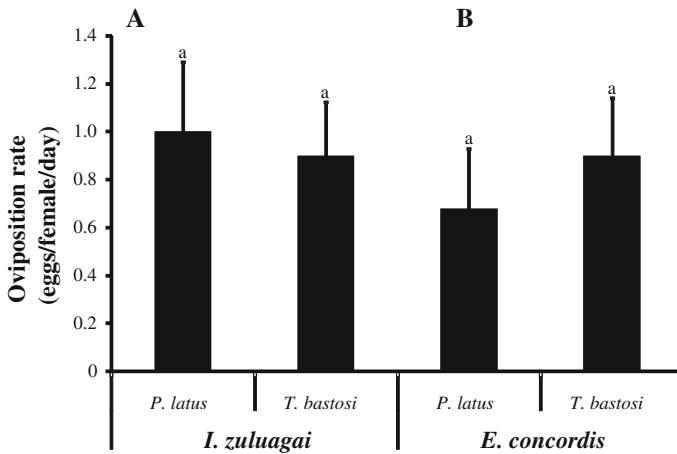


Fig. 3 Average (\pm SE) ovipositional rate of *Iphiseiodes zuluagai* (A) and *Euseius concordis* (B) fed on *Polyphagotarsonemus latus* and *Tetranychus bastosi*. Same letters denote non-significant differences among treatments

1966; Takafuji and Chant 1976), by reducing their movement on the plant (Putman 1962; McMurtry et al. 1970; Trichilo and Leigh 1986), or by sticking predators, which may result in their death (McMurtry and Scriven 1964; Trichilo and Leigh 1986; Osakabe 1988; Venzon et al. 2009). Additionally, predators experience difficulty in avoiding contact with the sticky silken threads when they enter and move inside webs (Sabelis and Bakker 1992). This would explain the higher predation rate of *I. zuluagai* on *P. latus* compared to *T. bastosi*. Furthermore, the larger size of *T. bastosi* compared to *P. latus*, could influencing less predation, thus predators would not need to consume as many individuals of *T. bastosi* as they do on the minute *P. latus*.

At the lowest density, the number of prey consumed by *I. zuluagai* was lower than two, perhaps due to the difficulty faced by the predator in finding the prey (Reis et al. 2003). Although *I. zuluagai* and *E. concordis* preyed upon *P. latus* and *T. bastosi* the predation rate of *E. concordis* was lower for both preys (Tab. 1). The number of *P. latus* and *T. bastosi* killed by *E. concordis* peaked around 1 mite in a period of 8 h (Fig. 2). When fed on *T. bastosi* the daily oviposition of *I. zuluagai* did not differ from that of *E. concordis* (Fig. 3B). Such ovipositional rates were higher than those obtained by other predatory mites fed on tetranychid mites (Ferla and Moraes 2003) indicating that both *P. latus* and *T. bastosi* are suitable for the development of *I. zuluagai* and *E. concordis*.

Release-recapture experiments showed that females of *I. zuluagai* were attracted to odours from leaves of *J. curcas* plants that were infested with *P. latus* or *T. bastosi* when offered uninfested leaves as alternative (Fig. 4C, D). These results are in line with previous studies with other predators, indicating that *I. zuluagai* uses volatile cues to localize their prey habitats (Teodoro et al. 2009). However, more research is needed to determine the nature of the volatiles used by *I. zuluagai* to perceive the presence of *P. latus* and *T. bastosi* on *J. curcas* leaves. Janssen et al. (1997) showed that the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is attracted to odours produced by plants infested with thrips and by plants infested by the two-spotted spider mite *Tetranychus urticae*. When offered a choice between these two odours, they preferred odours of plants with spider mites. Hence, predators were able to distinguish between plants with either of

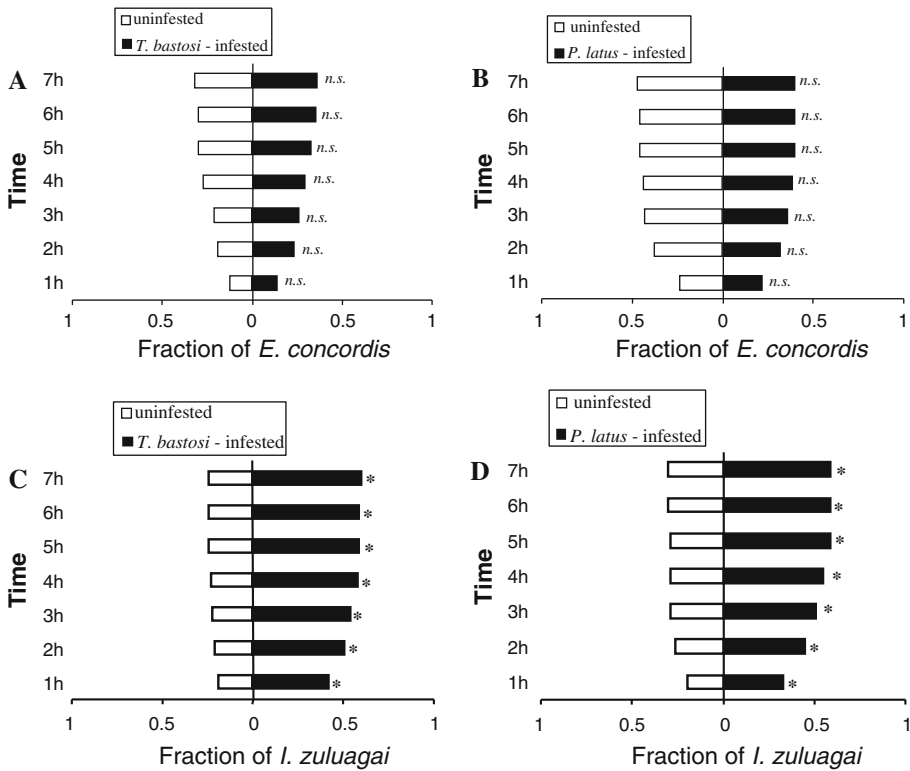


Fig. 4 Phytoseiid predators were offered a choice between uninfested *Jatropha curcas* leaves and leaves infested with *Polyphagotarsonemus latus* or *Tetranychus bastosi*. Preference of *Euseius concordis* females when offered odours coming from uninfested leaves versus **A** leaves infested with *T. bastosi* or **B** leaves infested with *P. latus*. Preference of *Iphiseiodes zuluagai* females when offered odours from uninfested leaves versus **C** leaves infested with *T. bastosi* or **D** leaves infested with *P. latus*. Within each evaluated time, asterisks denote fractions that are statistically different (χ^2 test; $P < 0.05$)

the two herbivores. It was argued that this behavior could be explained by different odour profiles being produced by the plant-herbivore complex, by the concentration of similar volatiles, or both. Therefore, it is possible that the presence of spider mites on the leaves of *J. curcas* and their feeding could have elicited the production of volatiles and *I. zuluagai* may have used such volatiles to locate leaves hosting its prey. When we assessed the preference of *E. concordis* to leaves of plants infested with *P. latus* or *T. bastosi* relative to uninfested leaves, mites did not show preference for one of the odour sources, which suggests that such odours are not attractive to or not recognizable by this predatory mite (Fig. 4A, B).

The preference of *I. zuluagai* for leaves of plants infested by either *P. latus* or *T. bastosi* combined with higher predation on *P. latus* in comparison to that values obtained by *E. concordis* suggests that *I. zuluagai* can be more efficient than *E. concordis* in reducing populations of pest mites under field conditions. Our results emphasize the key importance of predatory mites as agents of natural biological control of pest mites on *J. curcas* crops. However, more field realistic experiments are needed to elucidate tritrophic relationships among *I. zuluagai*, *E. concordis*, their prey, and the host plant.

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