Alternative food sources to predatory mites (Acari) in a *Jatropha curcas* (Euphorbiaceae) crop

Fuentes alternativas de alimentación de ácaros depredadores (Acari) en cultivos de *Jatropha curcas* (Euphorbiaceae)

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Abstract: In Brazil, smallholders produce the physic nut *Jatropha curcas* in association with crops such as pumpkin and corn, besides weeds. We evaluated the suitability of pumpkin and corn pollens, as well as pollen from a weed species (*Peltaea riedelii*), present in physic nut crop, as alternative food to predatory mites *Euseius concordis* and *Iphiseiodes zuluagai*. These species are natural enemies of *Polyphagotarsonemus latus* and *Tetranychus bastosi*, two important mite pests on *J. curcas* plants. The survival and oviposition rate of *E. concordis* and *I. zuluagai* were evaluated for five days on leaf discs supplied with pollen of pumpkin, corn or *P. riedelii*. Castor bean pollen (*Ricinus communis*) was provided to the predators as a control of suitable food. No significant difference on the oviposition rate of both *I. zuluagai* and *E. concordis* was observed when they fed with pollen from the three plants. However, the source of pollen affected the survival of the predatory mites. Pumpkin pollen was the worst food for both predatory mites while corn and *P. riedelii* was a better food. These results indicate the non-suitability of pumpkin pollen as alternative food to *I. zuluagai* and *E. concordis*. Corn and *P. riedelli* are sources of supplementary food to the predatory mites and can sustain predator populations when prey is scarce.

Key words: *Iphiseiodes zuluagai*. *Euseius concordis*. *Polyphagotarsonemus latus*. *Tetranychus bastosi*. Physic nut.

Resumen: En Brazil los pequeños agricultores producen el piñón *Jatropha curcas* L. asociado con plantaciones de zapallo, maíz, y muchas veces con malezas. Evaluamos la adecuación del polen de zapallo y maíz, así como de la maleza (*Peltaea riedelii*), frecuente en los cultivos del piñon, como fuente de alimento alternativo para los ácaros *E. concordis* y *I. zuluagai*. Estas especies son los enemigos naturales de *Polyphagotarsonemus latus* y *Tetranychus bastosi*, dos principales ácaros plagas de plantas de jatropha. La tasa de sobrevivencia y desove de los ácaros *E. concordis* y *I. zuluagai* fue evaluada por un período de cinco días en discos de hojas provistas con polen de zapallo, maíz o de *P. riedelii*. Polen de la planta *Ricinus communis* fue previsto para los depredadores, como control de alimentación adecuada. No hubo diferencia estadística en la tasa del desove de ambos *I. zuluagai* y *E. concordis* cuando alimentados con el polen de las especies evaluadas. No obstante, la fuente de polen afecta la sobrevivencia de los ácaros depredadores. El polen de zapallo fue el peor alimento para ambos ácaros depredadores, contrario al del maíz y la maleza *P. riedelii*, que fueron los mejores. Los resultados indican que el polen de zapallo es inadecuado como alimento alternativo para *I. zuluagai* y *E. concordis*. El maíz y la maleza *P. riedelli* representan una fuente alimenticia suplementaria para los ácaros depredadores y puede ser importante para sustentar poblaciones de depredadores, por lo menos algunos días, cuando la presa está ausente o escasa.

Palabras clave: *Iphiseiodes zuluagai*. *Euseius concordis*. *Polyphagotarsonemus latus*. *Tetranychus bastosi*. Jatropha.

Introduction

In diversified farming systems, the association of plant species can result in more efficient biological control of pests due to increased diversity and abundance of natural enemies (Andow 1991; Altieri 1999; Letourneau *et al.* 2011). For instance, the occurrence and abundance of predatory mites of the family Phytoseiidae can be enhanced by food sources and shelter found in natural vegetation fragments near the main crop (Addison *et al.* 2000; Zacarias and Moraes 2002; Demite and Feres 2005). Generalist predatory mites have a diverse dietary habit including pollen and nectar (Yamamoto and Gravena 1996; McMurtry and Croft 1997). Thus, some predatory mite species can benefit from plant derived food provided by crops and weeds (Moraes *et al.* 1993; van Rijn and Tanigoshi 1999a; van Rijn and Tanigoshi 1999b; Bellini *et al.* 2005).

In Brazil, physic nut (*Jatropha curcas* L.) is cultivated mainly by smallholders. The broad mite *Polyphagotarsonemus latus* Banks, 1904 (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker and Sales, 1977 (Acari: Tetranychidae) are the main damaging mite species of physic nut (Lopes 2009; Sarmento *et al.* 2011; Cruz *et al.* 2013). The broad mite is a polyphagous and cosmopolitan species, which attacks mainly the apex of the plants (Gerson 1992; Kavitha *et al.* 2007). Conversely, the spider mite *T. bastosi* is more frequently found on underside of fully-developed leaves of *J. curcas* in comparison with younger leaves (Sarmento *et al.* 2011; Pedro-Neto *et al.* 2013). Besides, tetranychid mites, such as *T. bastosi* produces a considerable amount of web, which may serve to protect their colonies from predators (Gerson 1985; Saito 1985; Sabelis and Bakker 1992; Venzon *et al.* 2009).

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The predatory mites *Iphiseiodes zuluagai* Denmark & Muma, 1972 and *Euseius concordis* Chant, 1959 (Acari: Phytoseiidae) are the most common natural enemies of *P. latus* and *T. bastosi* on *J. curcas* plants in Tocantins, Brazil (Sarmento *et al.* 2011; Cruz *et al.* 2012). Both species are considered promising in controlling *P. latus* and *T. bastosi* on *J. curcas* plants (Sarmento *et al.* 2011). *Jatropha curcas* can be cultivated associated plant species and under the natural occurrence of weeds. Smallholders and rural settlers diversified their cultivating areas of *J. curcas* with pumpkin and corn, and weeds are not controlled. Plant diversification play an important role in the maintenance of a community of predatory mites on this crop though provision of alternative plant food such as pollen and nectar (Moraes *et al.* 1993; Zannou *et al.* 2005; Cruz *et al.* 2012).

We investigated the suitability of pollen from the cultivated plants pumpkin *Curcubita pepo* L. (Cucurbitaceae) and corn (*Zea mays* L.) (Poaceae) as well as from *Peltaea riedelii* (Gürke) Standl. (Malvaceae), a weed species commonly present in *J. curcas* plantations, for survival and reproduction of the generalist predators *I. zuluagai* and *E. concordis*.

Materials and methods

The experiments were carried out at the laboratory of Entomology of the Federal University of Tocantins (UFT), Gurupi, Tocantins (11º48'29''S 48º56'39''W, 280 m), Brazil. Stock cultures of both *I. zuluagai* and *E. concordis* were started with mites collected on *J. curcas* plants cultivated at experimental field. Stock colonies were established on arenas with 6-cm diameter of flexible plastic discs floating inside a plastic box filled with distilled water. The rearing of predatory mites was maintained with castor bean pollen (*Ricinus communis* L.), which was collected from plants of natural occurrence and added on a daily-basis as food (Reis and Alves 1997). Predatory mites were maintained inside a climate chamber (28 °C, 65-70% R.H. and 12h L/12h D photoperiod). The pollens of pumpkin, corn and *P. riedelii* used in the experiments were collected from plants cultivated in association with *J. curcas*. Each pollen species was separately kept in vials (Eppendorf™) with capacity of 10 mL and stored in refrigerator (6 ± 2 °C). The stock of each pollen species was monthly renewed.

The oviposition and survival rates of *E. concordis* and *I. zuluagai* were evaluated when the mites were fed either on pollen of pumpkin, corn, *P. riedelii* and *R. communis* (control). Pollen were offered to the predators on leaf discs (Ø = 3 cm) made from healthy plants without pesticide residues. Pumpkin and corn pollen were offered to the predators on leaf discs made from pumpkin and corn plants respectively. Due to the reduced size of the leaves of *P. riedelii* plants, we offered its pollen to the predatory mites using leaf discs made from plants of *J. curcas*. Pollen of *R. communis* was offered to the predators on plastic discs as a control because it is a suitable food for both predators (Moraes & Lima 1983; Reis and Alves 1997). The discs were kept in batches of five over a piece of foam covered with wet cotton wool and placed inside plastic trays (30 x 22 x 7 cm) containing water to prevent mites from escaping. Trays were kept inside a climate chamber at the same conditions described above. Two-day-old mated females of each predator species were starved in the presence of water for 2 hours before tested. Each treatment was started with 15 predatory mite females with the exception of castor bean pollen treatment, which was started with 12 predatory females. Subsequently, one female of each predatory mite species (*I. zuluagai* or *E. concordis*) was released on each disc according to the pollen diet *ad libitum*. Leaf discs and pollen were daily replaced. Eggs of each predatory mite were quantified and discarded daily during five days.

As the species of predatory mites tested here usually lays on average less than one egg per day per female, the data on oviposition rate presents many zero which contributed to a strongly non-normal distribution data due to zero inflation. Based on this we decided to analyze the oviposition rates of predatory mites using the more conservative non-parametric Kruskal-Wallis test with multiple comparisons. This analysis was performed with the package "agricolae" (Mendiburu 2012). To analyze the effect of different foods on survival of the predators, data were subject to a Kaplan-Meier survival analysis using the package "survival" (Therneau 2013). The contrasts among the treatments levels were accessed by pairwise planned comparisons with Log-rank tests. All tests were performed using the software R2.11 (Crawley 2007, R-Development-Core-Team 2012).

Results

There was no effect of the pollen species on oviposition rates of *I. zuluagai* (Table1, Kruskal-Wallis test's, Chi^2 = 5.43; P = 0.14; d.f. = 3). However the oviposition of *E. concordis* was significantly different among the pollen species tested (Table 2, Kruskal-Wallis test's, X^2 = 9.05; P = 0.03; d.f. = 3). The higher oviposition rate of *E. concordis* was observed on pollen of *R. communis*, which was significantly different from all treatments (all P < 0.05).

However, there was an effect of pollen diets on survival of *I. zuluagai* (Fig. 1, log-rank test, X^2 = 30.20; P < 0.01; d.f. = 3) and of *E. concordis* (Fig. 2, log-rank test, X^2 = 34.10; P < 0.01; d.f. = 3). The survival of *I. zuluagai* was significantly higher when mites fed on *R. communis* pollen (log-rank test, all P < 0.05). The lowest survival was observed when *I. zuluagai* was fed on pollen of pumpkin and *P. riedelii*, which does not differed statistically (log-rank test, X^2 = 1.40; P < 0.24; d.f. = 1).

For *E. concordis*, the highest survival was obtained when the predator fed on *R. communis* pollen (log-rank test, all P <
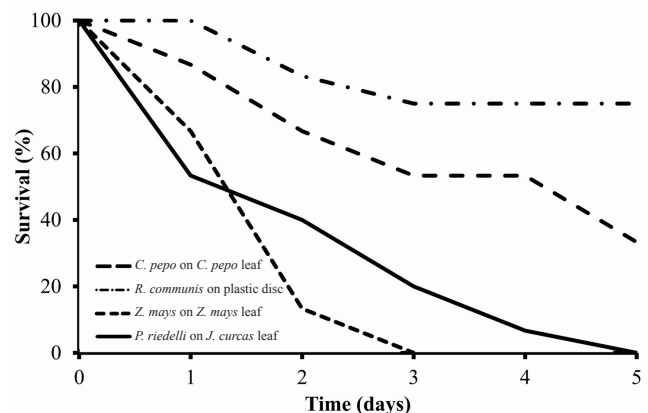

Figure 1. Survival (%) of the predatory mite *I. zuluagai* fed with pollen from four different plant species (see legend inside the figure).
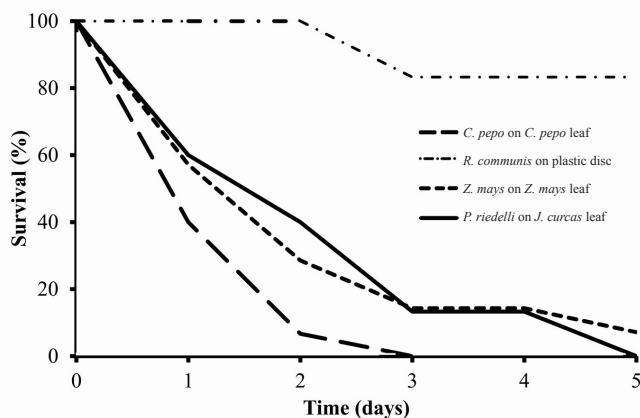
Figure 2. Survival (%) of the predatory mite *E. concordis* fed with pollen from four different plant species (see legend inside the figure).

0.05). No significant difference on survival of this predatory mite was observed when it fed on corn or on *P. riedelii* pollen (log-rank test, $X^2 = 0.00$; P = 0.99; d.f. = 1). However *P. riedelii* provided a longer survival to *E. concordis* when compared with pumpkin pollen (log-rank test, $X^2 = 4.10$; P = 0.04; d.f. = 1). The lowest survival was observed when predatory mites were fed with pollen of pumpkin and corn, which does not differed statistically from each other (Fig. 2, log-rank test, $X^2 = 2.60$; P = 0.11; d.f. = 1).

Discussion

The pollen affected survival of the predatory mites *I. zuluagai* and *E. concordis*. However, none was as suitable as *R. communis* pollen. Pollen of pumpkin was not suitable as food to the predators at all. It resulted in death of 100% of individuals of *I. zuluagai* and *E. concordis* in three days. Likewise, the pollens of corn and of *P. riedelii* suit to keep *E. concordis* alive only for five days. Instead *P. riedelii* pollen was able to promote a slight long survival on *I. zuluagai*, but this was lower than the survival on *R. cummunis* pollen. However, the between the pollen for both predatory mites.

Pollen is an important resource for generalist predatory mites such as *I. zuluagai* and *E. concordis* (McMurtry and Croft 1997). It is a source of nitrogen for many insects and mites. Pollen protein content ranges from 2.5% to 61% and this concentration may influence plant-animal interactions (Roulston *et al.* 2000; Venzon *et al.* 2006). Apart from the nutritional value of macro-nutrients found in pollen, vitamins, mineral nutrients and sterols are important for digestion and other metabolic processes (Stanley and Linskens 1974; Wald-

bauer and Friedman 1991). Thus, differences in the digestibility and assimilation of nutrients from different pollen species are expected to have influence on fecundity and survival of predators.

Pollen can be an alternative or supplementary food for predatory mites. The term alternative food is generally applied when the predator is able to survive and reproduce on this diet (Overmeer 1985). Otherwise, the food is considered only a supplementary source. Thus, the alternative food should also be able to keep the predator alive in the absence of essential food. The pollen of corn and *P. riedelii* were most promising as a supplementary food source as they were not capable of maintaining *I. zuluagai* and *E. concordis* alive for long periods.

The *I. zuluagai* oviposition rates obtained were lower than those found in the literature when the predator fed castor bean pollen (Reis *et al.* 1998). This pollen species is considered an important food to rear predatory mites, giving high oviposition rates and survival over 40 days (Reis and Haddad 1997; Reis *et al.* 1998). Regarding survival, all alternative pollen species tested were responsible for a sharp decline in survival of the predators *I. zuluagai* and *E. concordis*. However, corn pollen resulted in better survival of *I. zuluagai* compared to the other tested pollens, and it is therefore the most promising pollen species. Yamamoto and Gravena (1996) added honey solution at 10% to castor bean pollen diet for *I. zuluagai* and reported that the mean number of eggs deposited per day was twice as high as that found by Reis *et al.* (1998). This suggests that nectar may be an additional food source to the diet of this predator. Possibly, in field conditions predatory mites may complement their diet exploring extrafloral nectar or plant exudates (van Rijn and Tanigoshi 1999a; Gnanvossou *et al.* 2005).

Although the oviposition rate of *I. zuluagai* when fed on corn pollen is lower than when it fed with *T. bastosi* or *P. latus*, pollen corn may improve predatory mite performance when offered with prey items or sustain predator population when prey is absent (Sarmento *et al.* 2011). The same pattern was observed for *E. concordis*. Oviposition rate was 0.29 ± 0.11 eggs/day when predator fed of corn pollen and it was 0.90 ± 0.24 and 0.68 ± 0.25 eggs/day when it fed on *T. bastosi* or *P. latus*, respectively (Sarmento *et al.* 2011). It was also lower when this predatory mite was fed with pollen of *Typha angustifolia* L. (Ferla and Moraes 2003). Furthermore, corn pollen is able to maintain *E. concordis* ovipositing for three days in the absence of prey.

The survival rate of *E. concordis* was similar when they fed on corn pollen or *P. riedelii* pollen. However *I. zuluagai* exhibited a higher survival when fed with *P. riedelii* pollen in comparison with corn pollen. In addition,

Table 1. Oviposition rate (mean ± SE) as eggs per day of *E. concordis* females fed with pollen from different plant species. The numbers inside brackets represent the number of replicates with females alive. The minus signal in some cells denotes the absence of mean due to the absence of alive females.

Day	Pollen	*Curcubita pepo*	*Zea mays*	*Peltaea riedelli*	*Ricinus communis*
1st		0.33 ± 0.21 (6)	0.25 ± 0.16 (8)	0.11 ± 0.11 (9)	0.42 ± 0.15 (12)
2nd		0.00 ± 0.00 (1)	0.50 ± 0.29 (4)	0.50 ± 0.22 (6)	0.83 ± 0.24 (12)
3rd		-	0.50 ± 0.50 (2)	0.50 ± 0.50 (2)	0.50 ± 0.17 (10)
4th		-	0.00 ± 0.00 (2)	0.00 ± 0.00 (2)	0.60 ± 0.31 (10)
5th		-	0.00 ± 0.00 (1)	-	1.10 ± 0.10 (10)

Table 2. Oviposition rate (mean ± SE) as eggs per day of *I. zuluagai* females fed with pollen from different plant species. The numbers inside brackets represent the number of replicates with females alive. The minus signal in some cells denotes the absence of mean due to the absence of alive females.

Day	Pollen	*Curcubita pepo*	*Zea mays*	*Peltaea riedelli*	*Ricinus communis*
1st		0.30 ± 0.15 (10)	0.38 ± 0.14 (13)	0.25 ± 0.16 (8)	0.25 ± 0.13 (12)
2nd		0.00 ± 0.00 (2)	0.30 ± 0.15 (10)	0.50 ± 0.50 (6)	0.70 ± 0.30 (10)
3nd		-	0.38 ± 0.18 (8)	0.00 ± 0.00 (3)	0.78 ± 0.28 (9)
4rd		-	0.38 ± 0.18 (8)	1.00 ± 0.00 (1)	0.78 ± 0.28 (9)
5th		-	0.40 ± 0.24 (5)	-	0.67 ± 0.17 (9)

the survival of the predators on pollen of *P. riedelii* was higher than on pollen of pumpkin to both predators tested. Although the pollen of this plant species might not be ideal for development of these predators, *P. riedelii* may contribute to the maintenance of predators in the field for a few days in prey shortage. Additionally, the presence of the forage vegetation interspersed in crops is important to provide environments for refuge for beneficial organisms (Altieri 1999; Duso *et al.* 2004). *Peltaea riedelii* belongs to the Malvaceae family, has a wide distribution in Brazil and spontaneously occurs in several agroecosystems (Barth 1975). This plant could fit in this system as a spontaneous plant, which, together with *J. curcas* and corn, is able to provide food resources for maintenance of the predators *I. zuluagai* and *E. concordis*. Moreover, this plant may play a role in the dispersal of predatory mites, since these, besides dispersal with the wind and with the relationship with other forest species (Helle and Sabelis 1985), can also be dispersed by meta-population dynamics (Zemek and Nachman 1998). Thus, weeds as *P. riedelii* and intercrops such as corn may have an important role in providing alternative food and facilitating dispersion.

When fed on pollen of pumpkin, oviposition and survival of the predators *E. concordis* and *I. zuluagai* were lower than that of the other species of pollen. It may be related to the size of the pollen grain. In the laboratory it was observed that the pollen of pumpkin is considerably larger than those of corn and *P. riedelii*, which can impede handling and ingestion by predators. In addition, pollen grains usually have a tough outer wall (i.e. exine) that has protection function (Overmeer 1985). Alternatively, pumpkin pollen may not be nutritional suited to the predator due to its chemical composition. Thus, either a physical or a chemical barrier refrain predatory mite utilization of pumpkin pollen.

The presence of plant resources (e.g. pollen and nectar) in the field is responsible for maintenance of predatory mite populations when prey is scarce or absent (Aguilar-Fenollosa *et al.* 2011). Furthermore, it has been shown for other systems that biological control of phytophagous mites may be improved in the presence of plant derived food (Smith and Papacek 1991; McMurtry 1992; Liang and Huang 1994; González-Fernández *et al.* 2009).

Field studies are necessary to determine how the predators can access these pollens and to elucidate mechanisms on how the dispersion of these species occurs. Moreover, it is necessary to evaluate the net effect of such crop diversification in the control of pest mites of *J. curcas*.

Conclusion

Pollen of corn and of *P. riedelii* has the potential to be used as food source to supplement the diet of the predators *I. zuluagai* and *E. concordis* in a *J. curcas* crop system.

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