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Functional and numerical responses of the predatory mite *Amblyseius aerialis* (Acari: Phytoseiidae) to *Aceria guerreronis* (Acari: Eriophyidae)

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Original research

ABSTRACT

The coconut mite, *Aceria guerreronis* Keifer, is one of the main pests of the coconut tree (*Cocos nucifera* L.) worldwide. Its control is mainly based on the use of acaricides. However, due to the habitat of this pest mite, which is protected by fruit bracts that act as a physical barrier, chemical control is often inefficient. Thus, natural enemies have been studied as an alternative to acaricides. We evaluated the functional and numerical responses of *Amblyseius aerialis* (Muma) to increasing densities of *A. guerreronis* (40, 80, 160, 240 and 360) and additionally the oviposition rates of the phytoseiid *A. aerialis* over 11 days on different food sources: coconut pollen, *A. guerreronis* and a mix of pollen and *A. guerreronis*. Our results showed that *Amblyseius aerialis* showed a type III functional response, where prey consumption increasing as the density of *A. guerreronis* increased, with a maximum consumption of up to 175 prey per predator. All the prey densities allowed female oviposition. The number of eggs laid by *A. aerialis* increased with increasing prey density and stabilized between 240 and 360 *A. guerreronis* individuals, with an average oviposition of 1.7 eggs/female. Furthermore, our results showed that all the food sources (coconut pollen, *A. guerreronis* and a mix of pollen and *A. guerreronis*) supported oviposition. The mean oviposition was approximately 1.5 eggs/female on all the food sources. Our study suggests that *A. aerialis* can be effective in the biological control of *A. guerreronis*. However, further studies are needed to investigate whether *A. aerialis* can explore the habitat of *A. guerreronis* and if that prey can support the development of immature stages and the reproduction of *A. aerialis*.

Keywords coconut mite; biological control; *Cocos nucifera*; predation; oviposition

Introduction

The coconut mite (*Aceria guerreronis* Keifer) is a major pest of coconut (*Cocos nucifera* L.) fruits. It occurs in several countries worldwide (Haq *et al.* 2002; Lawson-Balagbo *et al.* 2008a; Fernando and Aratchige 2010; Negloh *et al.* 2011). In Brazil, it is found in all coconut plantations but with a lower incidence than in Africa and Asia (Lawson-Balagbo *et al.* 2008a). It develops in the perianth of the fruits below the bracts, initially causing a yellowish-white spot in a triangular shape close to the margin of the perianth. As the fruits grow, the damaged

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tissue becomes necrotic (Haq *et al.* 2002; Galvão *et al.* 2008; Navia *et al.* 2013). This damage leads to a reduction in the yield of solid and liquid albumens and even premature fruit fall (Wickramananda *et al.* 2007). A higher *A. guerreronis* incidence on fruits can decrease the mean number of fruits harvested per bunch by approximately 60% and decrease the liquid albumen volume by 28% (Rezende *et al.* 2016).

Chemical control has been applied to the coconut mite worldwide. Successive pesticide sprayings are necessary to achieve satisfactory control as this pest develops in a habitat protected by fruit bracts that act as a physical barrier. However, this practice can cause undesired effects such as elimination of natural enemies, environmental contamination and presence of residues in fruits (Lawson-Balagbo *et al.* 2007; Fernando and Aratchige 2010; Van Leeuwen *et al.* 2010; Lima *et al.* 2012). The protection provided by bracts in fruits attacked by *A. guerreronis* was confirmed by Silva *et al.* (2017), who showed that bracts prevented exposure to the pesticide and that the pest was only vulnerable while dispersing. Therefore, alternative forms of control are necessary to manage this important pest.

The biological control of the coconut mite has been extensively researched. Predatory mites belonging to the family Phytoseiidae are considered the main natural enemies of the coconut mite, including *Neoseiulus baraki* (Athias-Henriot), *Neoseiulus paspalivorus* (De Leon), *Amblyseius largoensis* (Muma), *Neoseiulus recifensis* Gondim Jr. and Moraes and *Euseius alatus* De Leon (Lawson-Balagbo *et al.* 2008a, b; Reis *et al.* 2008; Galvão *et al.* 2011; Negloh *et al.* 2011; Lima *et al.* 2012; Melo *et al.* 2015).

The predatory mite *Amblyseius aerialis* (Muma) was predominant in surveys in coconut plantations in the municipality of Moju, in the state of Pará, Brazil. It was found in almost the same proportion both on leaflets and fruits, mainly on the external surfaces and in lesser quantities under the bracts after the *A. guerreronis* attack (unpublished results). According to McMurtry *et al.* (2013), this predator is considered a type III generalist, as it feeds on a wide variety of prey and pollen of different plant species as complementary food sources, being found on more than 20 species of host plants throughout Brazil (Castillo and Noronha 2008; Demite *et al.* 2014; Amaral *et al.* 2018; Ferreira *et al.* 2018). This species is commonly found in the Amazon region in different crops, such as fruit trees (Mineiro *et al.* 2009; Ferreira *et al.* 2018), physic nut (*Jatropha curcas* L.) (Cruz *et al.* 2012), rubber tree (*Hevea brasiliensis* L.) (Nuvoloni *et al.* 2014), coconut (*C. nucifera*) (Cruz *et al.* 2015), nance (*Byrsonima crassifolia* (L.) Kunth) (Noronha *et al.* 2020) and oil palm (*Elaeis guineensis* Jacq.) (Cruz *et al.* 2019). However, there are no reports on the interaction between this species and the coconut mite in spite of its high abundance in this region.

The study of the functional and numerical responses of a predator represents an important step in determination of its ability to regulate pests (Filgueiras *et al.* 2020) providing relevant information about the predation rate and prey density (Solomon 1949), and its ability to convert food into eggs (Hassell 1978). Thus, our study evaluated the functional and numerical responses of this predator to different densities of *A. guerreronis*. Additionally, to determine the relative nutritional value of coconut pollen compared to *A. guerreronis* we assessed *A. aerialis* fecundity when offered coconut pollen, *A. guerreronis* and a mix of coconut pollen and *A. guerreronis*.

Material and methods

Rearing of the predatory mite

All bioassays were conducted in the Laboratory of Entomology of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Amazônia Oriental), Belém, state of Pará, Brazil.

Colonies of the predatory mite, *A. aerialis*, were established from individuals collected from leaflets and fruits in commercial coconut plantations owned by Sococo S. A. Agroindústria da Amazônia, in the municipality of Moju, state of Pará (02°06'38.73" S, 48°40' 45.71" W). The mites were kept in rearing units made of plastic trays (16 × 22 × 7 cm) containing a piece

of polyethylene foam (2 cm thickness) that was kept permanently moist by the daily addition of distilled water. Additionally, a 10 × 15 cm resin plate (Paviflex®) was laid on top of the foam piece, the edges of which were covered with cotton strips moistened with distilled water to prevent the mites from escaping. In the middle of the plate, pieces of cotton fibre were placed under a microscope coverslip (2 × 2 cm) to serve as a shelter, providing a place for the oviposition and development of the mites. The predators were fed daily with fresh coconut pollen, and 400 individuals of *A. guerreronis* in different stages were provided on perianth fragments (~1 cm³). The predators were transferred weekly to new rearing units kept in an incubator (25 ± 1 °C, 70 ± 10% RH, 12: 12 h L: D).

Functional and numerical responses

The functional and numerical response experiment was conducted in an experimental unit composed of a Petri dish (2 cm high, Ø 5 cm) containing a disc of filter paper and a piece of coconut leaflet with the abaxial side facing up. All the edges of the coconut leaflet were surrounded with hydrophilic cotton moistened with distilled water to prevent the mites from escaping. In each experimental unit, one of five densities of *A. guerreronis* (40, 80, 160, 240 and 360 mites/experimental unit) were offered to one female of *A. aerialis* (four days old), replicated 20 times for each prey density. During two days the number of prey consumed and the number of eggs deposited by *A. aerialis* females were counted every 24h, with daily replacement of prey to the initial amount offered. Data from the first day were excluded to avoid the influence of previous feeding experience (Sabelis 1990, Carrillo and Peña 2012). Prey consumption was assessed by subtracting the number of *A. guerreronis* that remained in the arena from the number initially provided. The experiment was in random blocks and conducted in an incubator (25 ± 1 °C, 70 ± 10% RH, 12: 12 h L: D).

Oviposition

The oviposition of the predator fed on three food sources, *A. guerreronis*, coconut pollen and a mixture of coconut pollen and *A. guerreronis* was investigated under the same environmental conditions used for the functional and numerical response. A mated female of *A. aerialis* (4 days old) was placed in each unit, and food sources (~240 *A. guerreronis* individuals, pollen (0.5 g) and mixture of ~240 *A. guerreronis* individuals + pollen (0.5 g)) was offered daily to each female. All the units were kept in an incubator (25 ± 1 °C, 70 ± 10% RH, 12:12 h L:D). The number of predator eggs laid in each replicate was recorded, and the eggs were removed daily for 11 days. Oviposition data from the first day were excluded to avoid the influence of previous feeding experience (Sabelis 1990, Carrillo and Peña 2012). The experiment was completely randomized, and for each food source, 20 females (replicates) were tested.

Data analysis

Functional and numerical responses

To determine the type of functional response (Type I, II or III), logistic regression curves were adjusted between the proportion of prey consumed and prey density, according to Juliano (1993). The type of functional response was determined by the significance and sign of the linear coefficient of the equations obtained for the proportions of prey eaten. Non-significant and linear coefficients indicate a type I response, negative and significant coefficients indicate a type II response, and positive and significant coefficients indicate a type III response (Holling 1959, 1961).

The parameters of attack rate (a') and handling time (Th) were estimated by the equation $Ne = N_0 \{1 - \exp[a'(ThNe - T)]\}$ (Rogers 1972) (for experiments without prey replacement) using nonlinear least-squares regressions. Here, Ne refers to the number of prey eaten; N_0 , the initial prey density; a' , the attack rate (the proportion of prey captured by a predator per unit of

Figure 1 Functional response of *Amblyseius aerialis* fed with different densities of *Aceria guerreronis*, expression in number of prey consumed (a) and the proportion of prey eaten (b). In panel (a), the curve was fitted by Roger's equation III to the functional response, while in panel (b) the line represents the predicted values of Holling's type III disc equation.

search time); and Th , the prey handling time (the time needed for a predator to capture, kill and digest the prey). All the statistical procedures were performed in SAS (SAS Institute 2018).

The numerical response was estimated through the polynomial regression of the number of eggs per *A. aerialis* female as a function of the prey density. The model was selected based on simplicity, parsimony and high values of F and R^2 using Table Curve 2D 5.01 software (Systat Software 2014).

Oviposition

The oviposition rate data were analysed using repeated measures ANOVA and Tukey's test (HSD) to determine differences among the food sources. SAS software (2018) was used for the analyses.

Results

Functional and numerical responses

The number of prey consumed increased as availability increased (Figure 1a). The proportion of prey consumed initially increased under lower prey densities (40 and 80 mites) and decreased afterwards (Figure 1b), suggesting a type III functional response.

A type III functional response was confirmed by the significance and sign of the linear coefficient of Hollings's equation, which showed a significant and positive linear coefficient (Table 1). The attack rate (a') and the handling time (Th) of *A. aerialis* fed with *A. guerreronis* were 0.0011 h^{-1} and 0.11 h , respectively (Table 2).

All prey densities offered allowed oviposition by *A. aerialis* females. The number of eggs laid increased with increasing prey density and stabilized at high densities (240 and 360), with an average oviposition rate of 1.7 ± 0.09 eggs/female (Figure 2). Therefore, these findings supported the following bioassays using only coconut mite as prey.

Figure 2 Mean number of eggs laid by *Amblyseius aerialis* females provided with different densities of *Aceria guerreronis*.

Oviposition

The mean number of eggs laid by *Amblyseius aerialis* females was not affected by food sources ($F_{2,53} = 1.97, P = 0.15$). The females fed on *Aceria guerreronis*, coconut pollen and a mixture of coconut pollen and *A. guerreronis* laid an average of 1.4, 1.4 and 1.6 eggs/female/day (respectively) during the 11-day oviposition period (data from the first day were excluded) (Figure 3).

Discussion

The functional response of *A. aerialis* showed a sigmoid curve, indicating a type III response, i.e. an increase in prey consumption with prey density. In contrast, the proportion of prey eaten decreased with increasing density. However, according to Holling (1965), type III predators show a reduced response at low prey densities, unlike type II predators. Since the prey in this experiment was an eriophyoid and was smaller than other mites (approximately 200 μm in length), the consumption by the predators increased, as they need to ingest a greater quantity

Table 1 Holling’s equation and type of functional response of *Amblyseius aerialis* fed on different densities of *Aceria guerreronis*.

Species	Holling Equation	χ^2	DF	P	Logistic regression coefficient				Type ⁵
					I ¹ (P)	L ² (P)	Q ³ (P)	C ⁴ (P)	
<i>A. aerialis</i>	$y = \frac{\exp(0.00000056x^3) - (0.00035x^2) + (0.053x) - 0.2015}{1 + \exp [(0.00000056x^3) - (0.00035x^2) + (0.053x) - 0.2015]}$	223.91	96	<.001	-0.2015 (0.27)	0.053 (<.001)	-0.00035 (<.001)	5.692e ⁷ (<.001)	III

¹Intercept; ²Linear; ³Quadratic; ⁴Cubic; ⁵Type of functional response.

Table 2 Parameters ($\pm$ SE) of functional responses of *Amblyseius aerialis* fed on increase densities of *Aceria guerreronis*.

Parameter	Estimate ($\pm$ SE)	CI (95%)
a'	$0.00119 \pm 0.000250 \text{ h}^{-1}$	(0.000694 – 0.00168)
Th	$0.1171 \pm 0.00563 \text{ h}$	(0.1060 – 0.1283)

of *A. guerreronis* to meet their nutritional requirements; this phenomenon characterizes the potential regulation of the prey population. However, it is difficult for predators to reach *A. guerreronis* under bracts due to the tight adherence of the bracts to the surface of the fruit; this prevents larger mites, such as most Phytoseiids, from penetrating this space at the same time as eriophyoids (Silva *et al.* 2016). The predation of *A. guerreronis* by other generalist predators, such as *A. largoensis* and *E. alatus*, may occur on the exposed surface of fruits, on spikelets when mites leave older fruits for younger ones, when mites move from one plant to another by aerial dispersion, and rarely, under the bracts of a fruit (Melo *et al.* 2015).

The phytoseiid *N. baraki* showed a type III functional response when fed with different densities of *A. guerreronis* (Lima *et al.* 2012; Teodoro *et al.* 2020). Lima *et al.* (2012) reported a maximum mean consumption of ~ 100 mites at a density of 240 *A. guerreronis*, and Teodoro *et al.* (2020) reported a mean consumption of ~ 150 mites at the same density. In our study, we observed a mean consumption by *A. aerialis* of ~ 140 *A. guerreronis* at a density of 240 mites. Despite being different species of predators, our results showed a predation rate similar to those found by other authors and suggest that *A. aerialis* may be effective in controlling *A. guerreronis*.

The handling time and attack rate shown by *A. aerialis* feeding on *A. guerreronis* (0.0011 and 0.11, respectively) were close to the values reported by Teodoro *et al.* (2020) for *N. baraki*

Figure 3 Average oviposition rates (eggs/female $\pm$ SE) by *Amblyseius aerialis* females given different food resources per 10 days.

(0.004 and 0.13) feeding on the same prey species. The time required by *A. aerialis* to identify, attack, subdue, and consume a unit of prey (handling time) was lower. This indicates that this predator needed less time to consume a single prey, which increased its prey consumption over a 24 h period. The attack rate determines the ability of a predator to capture its prey within a specific area (Holling 1959), and our findings demonstrate that *A. aerialis* was effective in capturing more prey per area than *N. baraki*.

The predatory mite *Proctolaelaps bickleyi* (Bram) (Acari: Melicharidae) had a higher attack rate (0.1907) and a shorter handling time (0.0292) when provided with different densities of *A. guerreronis* (Lima *et al.* 2012). These values are better than those observed in our study, but the attack rate and handling time can change according to the predatory species and kind of prey.

The numerical response showed that even at lower prey densities (i.e., 40 and 80), *A. aerialis* could oviposit and that the number of eggs laid/day increased at high densities, indicating that the increment of individuals in the population will be greater as prey density increases. It is expected that to control pest mites, a good predatory mite should have, among other characteristics, the capacity to convert their prey into egg biomass and increase their population given abundant prey (Sabelis 1990). Galvão *et al.* (2007) reported a daily oviposition of 1.2 eggs per female for *A. largoensis* fed on *A. guerreronis*. *Amblyseius largoensis* is a natural enemy frequently found on coconuts and may also play a key role in the natural control of this pest. Lima *et al.* (2012) observed similar results for *N. baraki* fed on *A. guerreronis*. The mean number of eggs laid per day by *N. baraki* females at the highest density (140) was ~1.2. In our study, at a comparable density (160 *A. guerreronis*), we observed a mean of 1.5 eggs laid per day. Thus, these results reinforce that *A. aerialis* can be effective in controlling *A. guerreronis*.

The possible benefit in the predator's fitness was verified by the daily oviposition of the predator *A. aerialis* (in the oviposition test when fed with *A. guerreronis* and coconut pollen). According to the classification by McMurtry *et al.* (2013), mites belonging to the genus *Amblyseius* genus have type III feeding habits, which are characterized by successful reproduction when feeding on a wide variety of prey, including mites of the Eriophyidae family. However, some species also successfully reproduce when feeding on pollen. The results obtained in this experiment are important, as they show that there was no difference in the predator's oviposition rate when fed coconut mites, coconut pollen or both. This generalist feeding habit may support the conservation of this predator in the field when prey is scarce feeding on coconut pollen.

This study represents the first effort to investigate the predatory and reproductive behaviours of *A. aerialis* on *A. guerreronis* through oviposition and predation activity. Our results suggest that this predator has the potential to control *A. guerreronis*. Moreover, the reproduction of *A. aerialis* is supported by either *A. guerreronis* or coconut pollen, suggesting that *A. aerialis* could be sustained under field conditions on coconut pollen as alternative food. Additional studies are necessary to evaluate life table parameters and to investigate under which circumstances *A. aerialis* can enter the microhabitat of *A. guerreronis*.

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