

## Life history of *Proctolaelaps bulbosus* feeding on the coconut mite *Aceria guerreronis* and other possible food types occurring on coconut fruits

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**Abstract** *Aceria guerreronis* Keifer (Acari: Eriophyidae) is a major pest of coconut fruits (*Cocos nucifera* L.) in many countries of the Americas, Africa, and parts of Asia. Considerable attention has been given to studies of biological control agents of *A. guerreronis*. *Proctolaelaps bulbosus* Moraes, Reis and Gondim Jr. is a predator recently discovered in association with *A. guerreronis*. Nothing is known about its biology. The aim of this study was to determine suitable food sources for *P. bulbosus*, among items commonly found on coconut fruits, including *A. guerreronis*. Food sources evaluated included the mites *A. guerreronis*, *Steneotarsonemus concavuscutum* Lofego and Gondim Jr., and *Tyrophagus putrescentiae* (Schrank), the fungus *Rhizopus* aff. *stolonifer* (Ehrenb.) Vuill and coconut pollen; the mite *Tetranychus urticae* Koch was also included in the assessments, for being a commonly used prey for mass production and laboratory rearing of predatory mites. *Proctolaelaps bulbosus* was able to develop up to adulthood when fed *A. guerreronis*, *R. aff. stolonifer* and *T. putrescentiae*. It had the highest population growth rates when feeding on the former ( $R_0 = 17.5$ ;  $r_m = 0.392$ ). These results indicate that *A. guerreronis* is the most suitable food for *P. bulbosus* among the possible food sources found on coconut fruits and that *P. bulbosus* can survive in the absence of eriophyid using *R. aff. stolonifer* as a food source.

**Keywords** *Cocos nucifera* · Biology · Ascidae · Eriophyidae · Biological control · Alternative food

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## Introduction

The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is a major mite pest of coconut (*Cocos nucifera* L.) in many countries of the Americas, Africa and parts of Asia (Moore and Howard 1996; Haq et al. 2002; Seguni 2002; Lawson-Balagbo et al. 2008a). It lives underneath the perianth causing necrosis, distortion, reduced growth or even abortion of attacked fruits (Haq et al. 2002; Nair 2002). Moore et al. (1989) reported yield losses of up to 31.5%; however, in our own (unpublished) observations, losses are frequently much higher, especially in crops for coconut drinking water.

Control of coconut mite is difficult because of the tall nature of the palm and because the pest is hidden under the perianth which is not reachable by many acaricides and many potential biological control agents (Mariau and Tchiboza 1973; Moore et al. 1989; Ramaraju et al. 2002). However, use of suitable predatory mites as biological control agents has a potential (Moraes and Zacarias 2002; Lawson-Balagbo et al. 2007a, b, 2008a, b; Domingos et al. 2010). In Brazil, predatory mites most commonly found in association with *A. guerreronis* are Phytoseiidae, especially *Neoseiulus baraki* (Athias-Henriot) and *N. paspalivorus* (De Leon), and Ascidae (*sensu* Lindquist and Evans 1965), especially *Proctolaelaps bickleyi* Bram (Moraes and Zacarias 2002; Lawson-Balagbo et al. 2007a). The suitability of *A. guerreronis* as prey to those predators has been investigated under laboratory condition (Lawson-Balagbo et al. 2007a; Domingos et al. 2009). *Proctolaelaps bulbosus* Moraes, Reis & Gondim Jr. was recently described from northeast Brazil, where it has been found in association with *A. guerreronis* (Lawson-Balagbo et al. 2008a; Moraes et al. 2008). Information on the biology of *P. bulbosus* is not available.

This study constitutes a first step in the evaluation the potential role of *P. bulbosus* in the control of *A. guerreronis*. Its specific objective was to assess the suitability of various potential food sources present on coconut fruits for development, reproduction and population growth rates of *P. bulbosus*.

## Materials and methods

### Source and maintenance of the predator stock colony

Specimens of *P. bulbosus* used in this study were obtained from a colony established with about 100 mites collected from fruits of *C. nucifera* in Juazeiro (9°25'S, 40°30'W), State of Bahia, northeastern Brazil, about 2 months before starting the study. The stock colony was maintained in a unit consisting of a black PVC disk placed on a filter paper disk resting on a foam mat in a tray (13, 13 and 16 cm in diameter, respectively). The mat and the filter paper were maintained permanently wet by daily addition of distilled water to the tray. A mixture of all developmental stages of *A. guerreronis* on pieces of meristematic tissue of coconut fruits was provided every other day as food for *P. bulbosus*. The stock colony was kept at  $25.0 \pm 1.0^\circ\text{C}$ ,  $75 \pm 10\%$  RH and 12 h photophase.

### Experimental procedure

A group of about 50 adult female *P. bulbosus* was transferred from the stock colony to each of 7 units similar to it, in which they were also fed with a mixture of the different developmental stages of *A. guerreronis*. After 12 h, the females were removed, retaining in each unit only 50 of the eggs they laid in that period.

The units were examined every 12 h to determine the incubation period. After hatching, each larva was transferred to an experimental unit consisting of a  $2 \times 2$  cm piece of jack-bean, *Canavalia ensiformis* L., leaf (upper side down) laying on a piece of filter paper ( $2 \times 2$  cm) resting on a mat ( $2 \times 2$  cm) in a tray ( $6 \times 7$  cm). The margins of the bean leaf were covered with a band of wet cotton wool to prevent mites from escaping and to provide drinking water to them. The foam mat, the filter paper and the cotton band were maintained permanently wet by the daily addition of distilled water to the tray.

The following food sources were tested for their suitability for *P. bulbosus*: all developmental stages of the mites *A. guerreronis*, *Steneotarsonemus concavuscutum* Lofego and Gondim Jr. (Tarsonemidae) and *Tyrophagus putrescentiae* (Schrank) (Acaridae); eggs of the mites *T. putrescentiae* and *Tetranychus urticae* Koch; hyphae of the saprophytic fungus *Rhizopus* aff. *stolonifer* (Ehrenb.) Vuill; coconut pollen. *Aceria guerreronis*, *S. concavuscutum*, *T. putrescentiae* and *R. aff. stolonifer* are commonly found under the bracts of coconuts, whereas coconut pollen is often found on the surface of coconut fruits. *Tetranychus urticae* is not found on coconut; it was included in the experiment for being a commonly used prey for mass production and laboratory rearing of predatory mites. The origin of the food items was as follows: *A. guerreronis* and *S. concavuscutum*, from fruits periodically collected in Itamaracá, State of Pernambuco; *T. putrescentiae*, from a laboratory colony maintained on dog food (Pedigree adult for small breeds®); *R. aff. stolonifer*, from a laboratory culture maintained on the same substrate used for *T. putrescentiae*, as described by Lawson-Balagbo et al. (2007a); coconut pollen, from flowers collected periodically on the campus of “Universidade Federal Rural de Pernambuco” (UFRPE); eggs of *T. urticae*, from a colony maintained on jack-bean plants, from which they were extracted using the method described by Bakker et al. (1992). The amount and form of presentation of food in the different treatments were: approximately 100, 75 or 80 specimens of all developmental stages of *A. guerreronis*, *S. concavuscutum* or *T. putrescentiae*, respectively; the first 2 species were offered on pieces (about  $1.0 \times 1.5$  cm) of infested coconut fruits, while the latter was offered on approximately 10 mg of the substrate on which it was produced; approximately 10 mg of the substrate of cultivation of *R. aff. stolonifer*, containing approximately 2,300 sporangia of the fungus; about 10 mg of coconut pollen, 80 eggs of *T. putrescentiae* or 10 mg of eggs of *T. urticae*, each on a glass coverslip ( $18 \times 18$  mm). All food items were replenished daily.

Each experimental unit was examined every 12 h to determine the duration of each immature stage. Soon after emergence, each adult female was mated with a male taken at random from the stock colony. Thereafter, each unit was examined every 24 h to determine the oviposition. Dead males were replaced by new males until the death of the female. All immatures obtained were reared up to adulthood to determine the offspring sex ratio. The experimental units were kept at  $25.0 \pm 1.0^\circ\text{C}$ ,  $75 \pm 10\%$  RH and 12 h photophase.

### Data analysis

Data were analyzed as a completely randomized design, considering each mite as a replicate. The developmental time of each immature stage and in total (egg—adult) were compared among food sources by analysis of variance and subsequent pairwise Tukey’s test ( $P = 0.05$ ), considering only the treatments in which the predator was able to complete the life cycle. When food was either *A. guerreronis* or *R. aff. stolonifer*, periods of pre-oviposition, oviposition, post-oviposition, female longevity and total daily oviposition rate per female were subjected to *T* tests ( $P = 0.05$ ). Sex-ratios were compared by chi-square test. Population growth parameters ( $R_0$ ,  $r_m$ ) were estimated using the program developed

by Maia et al. (2000), which use the Jackknife method to estimate the confidence intervals of treatment means and allows comparisons between pairs of treatments using *T* tests. All analyzes were performed using SAS Institute (1999–2001).

## Results

*Proctolaelaps bulbosus* developed from egg to adult only when fed *A. guerreronis*, *T. putrescentiae* (eggs and all stages) or *R. aff. stolonifer*; all predators died in the larval stage when offered other food sources. Thus, the subsequently reported comparisons refer only to these food sources.

Duration of the larval stage was shorter ( $F_{3,199} = 12.87$ ,  $P = 0.0001$ ) on *A. guerreronis* and *R. aff. stolonifer* than on *T. putrescentiae* (Table 1). Significant differences were not observed for other developmental stages between food sources ( $P > 0.05$ ). The duration of the combined immature stages was shorter ( $F_{3,199} = 6.95$ ,  $P = 0.0010$ ) when the predator was fed *A. guerreronis* and longer when it was fed *T. putrescentiae*. The survivorship of the combined immature stages was lower on a combination of all stages of *T. putrescentiae* and higher on *A. guerreronis* and *R. aff. stolonifer* ( $F_{3,199} = 10.65$ ,  $P = 0.0002$ ).

When fed eggs or all stages of *T. putrescentiae*, all adult females of *P. bulbosus* died within 48 h after emergence; predator females survived longer and oviposited only when fed *A. guerreronis* or *R. aff. stolonifer*. No significant differences ( $P > 0.05$ ) were observed between the periods of pre-oviposition of predators on those food sources, but periods of oviposition ( $F_{1,59} = 5.34$ ,  $P = 0.0032$ ), post-oviposition ( $F_{1,59} = 100.72$ ,  $P < 0.0001$ ) and female longevity ( $F_{1,59} = 71.10$ ,  $P < 0.0001$ ) were significantly longer and fecundity ( $F_{1,59} = 28.46$ ,  $P < 0.0001$ ) and daily oviposition rate ( $F_{1,59} = 7.53$ ,  $P = 0.0133$ ) were significantly higher on *A. guerreronis* (Table 2). Intrinsic rate of increase ( $r_m$ ), and net reproductive rate ( $R_0$ ) were also higher when the predator was fed on *A. guerreronis*. Predator sex ratios were not statistically different from each other on those food sources ( $\chi^2 = 0.06$ ;  $P = 0.7945$ ). The period of oviposition of *P. bulbosus* was slightly but significantly higher on *A. guerreronis*, while peak daily oviposition rate was about twice higher and happened 2 days earlier on the same prey (Fig. 1).

**Table 1** Mean duration (in days  $\pm$  SE) and survivorship of the immature stages of *Proctolaelaps bulbosus* on different food sources at  $25.0 \pm 1.0^\circ\text{C}$ ,  $75 \pm 10\%$  RH and L12:D12 h.  $N = 50$

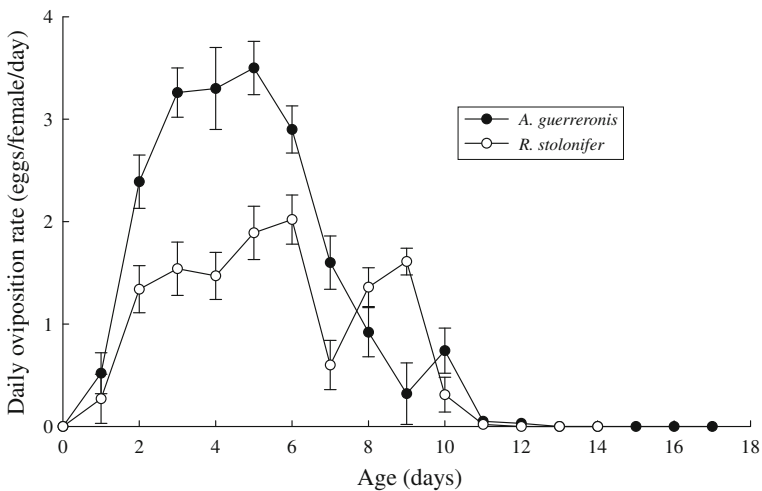
Developmental stage/ survivorship	Food source <sup>a</sup>			
	<i>A. guerreronis</i> (combined stages)	<i>R. aff.</i> <i>stolonifer</i>	<i>T. putrescentiae</i> (eggs)	<i>T. putrescentiae</i> (combined stages)
Egg	1.1 $\pm$ 0.03 a	1.2 $\pm$ 0.03 a	1.1 $\pm$ 0.07 a	1.3 $\pm$ 0.09 a
Larva	1.0 $\pm$ 0.01b	1.0 $\pm$ 0.02 b	1.4 $\pm$ 0.06 a	1.4 $\pm$ 0.07 a
Protonymph	1.0 $\pm$ 0.01a	1.0 $\pm$ 0.01 a	1.2 $\pm$ 0.08 a	1.1 $\pm$ 0.09 a
Deutonymph	1.1 $\pm$ 0.02 a	1.1 $\pm$ 0.07 a	1.2 $\pm$ 0.05 a	1.1 $\pm$ 0.09 a
Egg-adult	4.2 $\pm$ 0.09 c	4.4 $\pm$ 0.08 bc	4.8 $\pm$ 0.15 ab	5.0 $\pm$ 0.19 a
Survivorship (%)	85.5 $\pm$ 3.8 a	88.0 $\pm$ 3.0 a	75.0 $\pm$ 3.7 a	50.0 $\pm$ 6.4 b

<sup>a</sup> Means followed by the *same letter* within each row are not significantly different (*T* test;  $P > 0.05$ )

**Table 2** Mean duration (in days  $\pm$  SE) of different adult phases, oviposition rate, fecundity, sex ratio and population growth parameters of *Proctolaelaps bulbosus* on two food sources at  $25.0 \pm 1.0^\circ\text{C}$ ,  $75 \pm 10\%$  RH and L12:D12 h.  $N = 30$ 

Parameters	Food source <sup>a</sup>	
	<i>A. guerreronis</i>	<i>R. aff. stolonifer</i>
Pre-oviposition	$0.6 \pm 0.05$ a	$0.7 \pm 0.08$ a
Oviposition	$9.2 \pm 0.20$ a	$8.5 \pm 0.14$ b
Post-oviposition	$4.7 \pm 0.23$ a	$2.7 \pm 0.13$ b
Female longevity	$14.5 \pm 0.33$ a	$11.9 \pm 0.13$ b
Eggs/female/day	$1.8 \pm 0.05$ a	$1.4 \pm 0.04$ b
Total fecundity	$25.7 \pm 0.53$ a	$16.6 \pm 0.48$ b
$R_0$	$17.5$ (16.39–18.63) a	$11.3$ (10.34–12.30) b
$r_m$	$0.392$ (0.384–0.403) a	$0.307$ (0.295–0.319) b
Sex ratio	$0.78$ (0.68–0.88) a	$0.77 \pm (0.68–0.85)$ a

<sup>a</sup> Means followed by the *same letter* within each row are not significantly different ( $T$  test;  $P > 0.05$ )

**Fig. 1** Daily oviposition rate of *Proctolaelaps bulbosus* on two food sources at  $25.0 \pm 1.0^\circ\text{C}$ ,  $75 \pm 10\%$  RH and L12:D12 h

## Discussion

Although *P. bulbosus* had a better performance on *A. guerreronis*, it was able to complete its development and reproduce on *R. aff. stolonifer*, a fungus commonly found in the microhabitat occupied by *A. guerreronis*. This saprophytic fungus develops on damaged tissues on the surface of the fruit covered by the bracts, or on the underside of the bracts themselves. Thus, even when the population of *A. guerreronis* is low, the predator is able to maintain itself in the environment, developing and reproducing on that fungus.

Other fungi are occasionally found in the same microenvironment. Species of *Penicillium* and *Aspergillus* were reported by Chuku et al. (2007) under the bracts of coconut in

Nigeria, but they have not been identified in Brazil. Fungivory has also been reported for other species in this same genus, namely *Proctolaelaps pygmaeus* (Muller) (Shereef et al. 1980), *P. striatus* Westerboer (Afifi et al. 1984), *P. bickleyi* (Lawson-Balagbo et al. 2008b) and *P. deleoni* Nawar, Childer and Abou-Setta (Nawar 1992).

The determined rates of population growth of *P. bulbosus* were slightly lower than that determined for *P. bickleyi* by Lawson-Balagbo et al. (2007a), on both *A. guerreronis* and *R. aff. stolonifer*. *Proctolaelaps bickleyi* is often found on aborted coconut fruits (Lawson-Balagbo et al. 2008a). Despite the lower fecundity of *P. bulbosus* than reported by those authors for *P. bickleyi*, duration of the immature phase (egg-adult) was slightly shorter for the former species than determined by those authors for *P. bickleyi*. The Ascidae, especially *Proctolaelaps*, are generally known for their ability to increase their population in a short period of time (Nawar 1992; Abou-Awad et al. 2001), as also observed for *P. bulbosus* in this work.

Ascid mites are commonly found in soil and in moist environments (Gerson et al. 2003). The number of *A. guerreronis* on aborted fruits on the ground is usually reduced, but *R. aff. stolonifer* is commonly found on those fruits, and could probably facilitate the persistence of *P. bulbosus* under those circumstances.

The unsuitability of *S. concavuscutum* and coconut pollen observed in this study suggests that they are not important food items for *P. bulbosus* under natural conditions, despite their common occurrence in areas where *P. bulbosus* is known to be found (Reis et al. 2008). The unsuitability of *T. urticae* for *P. bulbosus* is not surprising, given that the former is never found in association with that predator. Lawson-Balagbo et al. (2007a) also observed *P. bickleyi* to be unable to reach adulthood when offered *T. urticae* as prey. Congruently, Abou-Awad et al. (2001) also reported the inadequacy of *T. urticae* as prey for another ascid species, *Lasioseius athiasae* Nawar and Nast.

Although not sufficiently suitable to allow the oviposition of *P. bulbosus*, *T. putrescentiae* could still allow its survivorship in the absence of more favorable food sources, as suggested by the fact that the predator could at least develop up to adulthood feeding on that mite. There is no other published evaluation on the suitability of *T. putrescentiae* as prey for other ascid species.

The phytoseiids *N. baraki* and *N. paspalivorus* have been determined to be able to go underneath the perianth at an early stage of development of the fruit (Lawson-Balagbo et al. 2007a, 2008a), because of their small size (Moraes et al. 2004). Given the reduced dimension of the space underneath the perianth, it seems that the larger *P. bulbosus* (Moraes et al. 2008) would only be able to reach that microhabitat on older fruits, when the space becomes larger. Lawson-Balagbo et al. (2008a) found this predator, reported as *Proctolaelaps* n.sp., only on fruits attached to the plants, whereas they found the still larger *P. bickleyi* (Lawson-Balagbo et al. 2008a) on attached coconut fruits and also on aborted coconut fruits on the ground.

This study provides basic information on the biology of *P. bulbosus* on some of its potential food sources. Complementary studies should be conducted, to evaluate its possible role as a control agent of *A. guerreronis*. It seems that its relatively large size could restrict the association with *A. guerreronis* to aborted fruits. Additionally, it is possible that its preferred habitat is not coconut fruits, but other habitat where other sources of food, not considered in this study, might be available.

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