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Rearing of coconut mite *Aceria guerreronis* and the predatory mite *Neoseiulus baraki* in the laboratory

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Abstract A method was developed for the rearing of coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), and its predatory mite *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) on embryo culture seedlings of coconut (*Cocos nucifera*) in the laboratory. Seedlings in the ages of <2, 2–4 and 4–6 months were infested with 75 field-collected coconut mites and the population growth was determined up to six weeks after introduction. The populations of coconut mites increased exponentially up to five weeks after introduction and declined thereafter on seedlings of all ages with significant differences among the three groups of seedlings occurring over time. At week 5, a significantly higher mean number (±SE) of coconut mites (20,098 ± 3,465) was bred on 4–6-month-old seedlings than on smaller seedlings, and on the largest seedlings the numbers were highest at all time intervals, except at week 2. *Neoseiulus baraki* was reared on embryo culture seedlings of the three age groups infested with coconut mites, by introduction of five female deutonymphs and one male, three weeks after introducing coconut mites. Predator numbers progressed significantly over time, but the size of seedlings did not significantly influence the numbers. On all groups of seedlings, the mean number of *N. baraki* increased up to two weeks after introduction on to seedlings and then declined. Many coconut mites were successfully reared in the laboratory for a longer period by this method and it could also be used as an alternative method to rear *N. baraki.* Development of this method may contribute to the progress of studies on the biology and ecology of coconut mite and its interactions with natural enemies.

Keywords *Aceria guerreronis* · *Cocos nucifera* · Coconut · *Neoseiulus baraki* · Rearing

Introduction

Despite extensive research efforts in the past four decades, an effective and sustainable management method for the coconut mite *Aceria guerreroris* Keifer (Acari: Eriophyidae),

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one of the most intractable pests of coconut (*Cocos nucifera*) in the Americas, Africa, India and Sri Lanka has not yet been developed (Mariau 1977; Mariau and Julia 1970; Hall and Espinosa 1981; Sathiamma et al. 1998; Fernando 1998). The microscopic size of the mite, its hidden habitat underneath bracts of the fruit and the tall nature of the coconut palm have hindered progress of research and management. Further, difficulty in rearing coconut mite in the laboratory to obtain a steady supply of mites has delayed the study of biology and ecology of the mite and its interactions with other organisms, especially natural enemies.

Coconut mite appears to have a narrow host range. So far, it has been reported from the fruits of palmyra palm, *Borassus flabillifera*, in India (Ramajaru and Rabindra 2001) and Sri Lanka (Moraes et al. 2004) and from the apical meristematic tissue of cocosoid palm, *Lytocaryum weddellianum*, in Brazil (Flechtmann 1989) and young queen palm, *Syagrus romanzoffia*, in California, USA (Anasloni and Perring 2004).

Several attempts have been made to rear coconut mite in the laboratory. Young coconut bunches of 3–5 months-old showing damage symptoms of coconut mite were maintained successfully by directly dipping the peduncle of the bunches in water containing salicylic acid or 10% sucrose solution for 10–15 days without affecting the density of coconut mites (Haq 2001). Rearing on excised bud leaves of coconut by repeated transfers of coconut mites to fresh tissues at intervals of 8–10 days was successful for a short period (Wickramananda et al. 2005). Similarly, attempts in maintaining colonies on leaf tissues of queen palm in the laboratory were only briefly successful (Anasaloni and Perring 2004). With none of these methods could large numbers of *A. guerreronis* be reared over a longer period.

The present study aimed to develop a method of rearing coconut mite in the laboratory continuously, and to establish a culture of the predatory mite, *Neoseiulus baraki* Athias-Henriot (Acari: Phytoseiidae) fed with these coconut mites.

Materials and methods

Production of embryo culture seedlings

Coconut mites were being reared on coconut seedlings produced by the embryo culture technique (Weerakoon et al. 2002). Mature coconut fruits of 12–13 months after pollination were collected from harvested heaps, de-husked and split open to collect the embryos from the kernel. Excised embryos were sterilized in 3% calcium hypochlorite for 5 min, followed by rinsing in several changes of sterile distilled water. Embryos were then cultured in glass test tubes (3 × 20 cm) and sealed with cotton plugs. Standard growth medium for culturing was modified Eeuwens Y3 liquid medium. Enlarged embryos that do not germinate or grow further after sprouting were treated with 0.35 μM giberellic acid (GA_3), incorporated into the culture medium until germination and further growth occurred. Then they were transferred again to the standard medium devoid of GA_3. If spontaneous rooting did not occur in germinated embryos, the shoots were dipped in a solution of 100 μM indoleacetic acid (auxin) for 3 days, followed by culturing in an auxin-free medium to induce rooting. Cultures were incubated in the dark during the first 8–10 weeks and then transferred to light conditions (16 h photoperiod and 25–30 μM s^{-1} m^{-1} light intensity). Incubation temperature was 29 ± 1°C.

Fig. 1 About 2–4-month-old embryo culture seedlings infested with *Aceria guerreronis*

Rearing of coconut mite

Each of 30 seedlings from three age groups of <2, 2–4 and 4–6 months from the day of culturing the embryos were used for the experiment. The seedlings were <5, 5–15 and 15–30 cm long, respectively, at the time of using in the experiment. Leaves were unfolded in the <2 and 2–4 month-old seedlings, while 1–2 leaves were opened in the >4 month-old seedlings. To infest the seedlings, coconut mites were obtained from infested coconut fruits collected from the field. The bracts of the fruits were removed and 75 active coconut mites in different stages of development were transferred to each seedling. They were picked from the bracts using a single-hair brush while watching under a stereo microscope and carefully placed on the outer leaves of the seedlings where leaf bases overlap taking extreme care not to loose any mites while transferring. After transferring the mites on to seedlings of 2 and 2–4 months old, the glass tubes were closed with a piece of polyethylene secured by a rubber band (Fig. 1). The tubes containing 4–6 month-old seedlings were enclosed in 30-cm-long polyethylene sleeves with one end tied on to the test tube and the other end kept open. Seedlings were placed on tube holding racks and kept at 27 ± 1°C and 70–80% relative humidity.

From 2 to 6 weeks after the introduction of coconut mites, six seedlings of each age group were randomly selected at weekly intervals, for counting of the coconut mites. The seedlings were removed from the growth medium, leaves were removed carefully and cut into 5–10 pieces and each piece was placed on a Petri plate lined with black paper. The motile coconut mites and eggs on the leaf pieces and the black paper were counted under a stereo microscope.

Rearing of *Neoseiulus baraki*

Coconut mites reared on each of 30 embryo cultured seedlings aged <2, 2–4 or 4–6 months were used for rearing *N. baraki*. Seedlings were infested with coconut mites as described above. About 3 weeks after introduction of coconut mites five female deutonymphs and

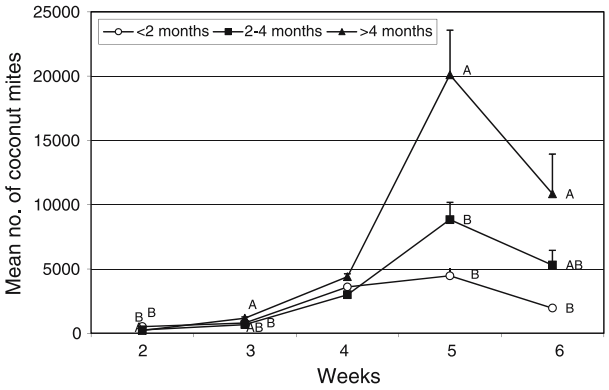

Fig. 2 Mean number (±S.E.) of coconut mites (*Aceria guerreronis*) reared on embryo culture seedlings of <2, 2–4 and >4 months-old at different intervals after introduction. Mean numbers with different letters at each time interval are significantly different. (Wilks' Lambda statistic)

one male of *N. baraki* were introduced on to each seedling by placing them at the base of the leaves using a fine brush. The predator was raised in the laboratory on flour mite *Tyrophagus putrescentiae* Shrank (Acaridae) as prey (Fernando et al. 2004). The seedlings were kept at 27 ± 1°C and at 70–80% relative humidity. About 10 seedlings of each age group were randomly selected and dissected as described above at weekly intervals up to three weeks after introduction of *N. baraki*. The motile stages and eggs of *N. baraki* on the plant parts and on the polyethylene sleeve of older seedlings were counted under a stereo microscope.

Data analysis

Numbers of motile stages and eggs at each time interval were combined for the analysis. Data analysis was carried out using repeated measures analysis of variance (SAS 1996) and Wilks' Lambda statistic was used to determine differences in mite numbers among different categories of seedlings and over time.

Results

Many coconut mites were successfully bred on all ages of embryo culture seedlings. On all seedling stages, an exponential increase occurred up to peak numbers at 5 weeks after introduction (Fig. 2). However, populations progressed differently with respect to the age of seedlings ($P = 0.0012$) and with time ($P < 0.0001$). Also, population growth of coconut mites on each group of seedlings were significantly different over time ($P = 0.0005$). Also, the temporal population growth of coconut mites varied significantly ($P = 0.0005$) among the three group of seedlings. Peak numbers (at week 5) were significantly higher on 4–6-month-old seedlings than on the younger seedlings (Fig. 2). Peak numbers of coconut mites were approximately 60, 118, and 267 times higher than the initial numbers on <2, 2–4 and 4–6-month-old seedlings, respectively.

Neoseiulus baraki bred on seedlings of all ages infested by coconut mite. Mean numbers of *N. baraki* varied significantly over time ($P = 0.0001$). Peak numbers were found at two

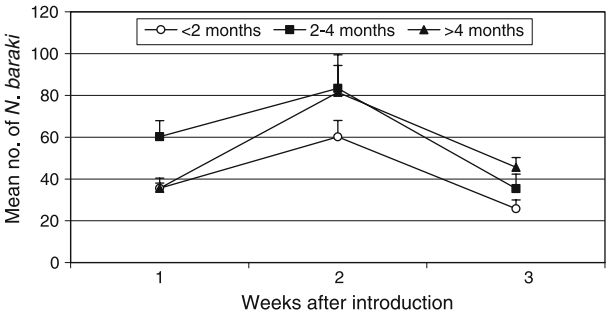

Fig. 3 Mean number (±S.E.) of *Neoseiulus baraki* developed on coconut mite reared on embryo culture seedlings of <2, 2–4 and >4-months-old, at different intervals after introduction of the predators

weeks after introduction (Fig. 3). Seedling size did not influence the development of *N. baraki* populations ($P = 0.059$). Nevertheless, populations of *N. baraki* developing on different-sized seedlings varied marginally significantly over time ($P = 0.044$), suggesting that the prey mite numbers on seedlings of all sizes were just sufficient as food source in certain time periods, but insufficient in others. A single female of *N. baraki* produced on average 12, 16.7, and 16.3 offspring at the peak population level on <2, 2–4 and 4–6-month-old seedlings, respectively.

Discussion

Coconut mites colonize the area beneath the bracts of coconut fruits. The meristematic tissue underneath the bracts provides ample food, and the narrow space between the bracts and the fruit surface creates a physical and climatic micro-environment, which is difficult to reproduce artificially. The compact apical region of embryo culture seedlings resembles the perianth region (bracts) of a coconut fruit. The study showed that the meristematic tissue of the bud region of embryo culture coconut seedlings is a suitable substrate for rearing of *A. guerreronis*. Growth rate of coconut mites was highest on the largest seedlings, with the largest meristematic regions. Perhaps the high breeding rate and as a consequence the scarcity of undamaged meristematic tissue have limited the rate of population increase beyond the five weeks after introduction, even on the larger seedlings.

Coconut mites can be reared effectively on embryo culture seedlings. Conditions can be carefully controlled, so that predatory mites or entomopathogenic fungi are kept away. This has the advantage that the coconut mite population growths fast and the mites can be used directly in studies in situ or on other substrates, without taking effort in removing any contaminants. With the method described by Haq (2001), the young nuts were maintained fresh only for 10–15 days, which is insufficient for the completion of many studies. Furthermore, these cultures could not be kept free from natural contaminants, because fruits were used directly from the field. With the method of Wickramananda et al. (2005) coconut mites could be maintained on excised bud leaf tissue for about 8–10 days, but then they needed to be transferred to fresh tissue due to contamination by saprophytic fungi. Repeated transfer of coconut mites to fresh tissues is a cumbersome procedure, often resulting in the loss of many mites. Rearing of coconut mite on embryo culture seedlings is superior to Haq's and Wickramananda et al.'s methods with respect to the mite numbers

produced and the durability of the cultures. A disadvantage is that the seedlings need to be raised in aseptic laboratory conditions, which takes several months before they can be used to breed coconut mites.

Neoseiulus baraki was also reared successfully on the coconut mites raised on embryo culture seedlings. Although, seedling size had no direct effect on the progression of *N. baraki* numbers over time, the numbers of coconut mites bred on smaller seedlings may have been limiting occasionally. On average, a single *N. baraki* female produced 16.7 offspring in two weeks on 2–4-month-old seedlings, which was comparable to the 16.6 offspring when fed on its' laboratory host *T. putrescentiae* during the same period (Fernando 2004). Therefore, breeding on coconut mites raised on embryo culture seedlings is a suitable method for mass breeding of predatory mites, at least for laboratory studies because it involves minimum culture maintenance and it is prone to less contamination. These advantages may well outweigh the longer initial production time, compared to rearing *T. putrescentiae*.

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