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A new tray-type arena to mass rear *Neoseiulus baraki*, a predatory mite of coconut mite, *Aceria guerreronis* in the laboratory

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ABSTRACT

Neoseiulus baraki Athias-Henriot (Acari: Phytoseiidae) is an important predatory mite of the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae) and attempts are now being made to evaluate the effectiveness of augmenting *N. baraki* to control coconut mite in the field. These studies require a steady supply of *N. baraki* in large numbers. One major constraint of the existing method to mass rear *N. baraki* on *Tyrophagus putrescentiae* Shrank (Acari: Acaridae) in a closed arena without a water barrier was frequent contamination of cultures by other mites. This paper describes the development of an efficient method to mass rear *N. baraki* in the laboratory with less monitoring and relatively minimal contaminants. Three box-type arenas and a tray-type arena were tested to mass rear *T. putrescentiae*. Mites were successfully developed on two box-type arenas and the tray-type arena, but the production (2197 mites per tray in 4 weeks) of *T. putrescentiae* was significantly higher on the tray-type arena. A 110-fold increase of *T. putrescentiae* was achieved in tray-type arenas in 4 weeks. None of the box-type arenas could be maintained more than 5 weeks without acarine and fungal contaminations. *N. baraki* could be successfully reared on the tray-type arena at least for 6 weeks. More than 4800 mites per tray, a 240-fold increase was achieved in 5 weeks when *T. putrescentiae* was added at 3-week intervals. Advantages and disadvantages of the new method are described.

1. Introduction

Despite various attempts to contain the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), which was first reported in Sri Lanka in 1997 (Fernando et al., 2002), it has now spread to almost all coconut growing areas in the country. Chemical control has failed as a long-term control strategy, mainly due to difficulties in application and environmental hazards. Although, biological control has been recognized as the most sustainable, economical and environment-friendly pest management strategy, evaluation of the biological control agents against coconut mites has been limited. Previous studies mainly focused on the use of the entomopathogenic fungus, *Hirsutella thompsonii* Fisher (Deuteromycetes: Monilianes) (Hall et al., 1980; Cabrera, 1982, 2002; Espinosa-Becerril and Carrillo-Sanchez, 1986; Suarez et al., 1989; Beevi et al., 1999; Rabindra and Sreerama Kumar, 2003), perhaps due to the very limited number of biological control species available for the pest. Few attempts have been made to evaluate the effectiveness of predatory mites against the coconut mite in the laboratory (Fernando et al., 2003; Lawson-Balagbo et al., 2007, 2008; Negloh et al., 2008).

Neoseiulus baraki is a predatory mite associated with the coconut mite in many parts of the world (Howard et al., 1990; de Moraes and Zacarias, 2002; de Moraes et al., 2004; Lawson-Balagbo et al., 2008; Negloh et al., 2008), including Sri Lanka (de Moraes et al., 2004; Fernando and Aratchige, 2006). It has a flattened idiosoma with a small cross-sectional diameter (de Moraes et al., 2004; Aratchige et al., 2007), which enables it to reach the area underneath the bracts (perianth) where the coconut mite feeds. Moreover, it shows a strong temporal relationship with the abundance of the coconut mite on palms (Fernando et al., 2003). Hence *N. baraki* is considered a potential biological control agent against the coconut mite. However, in nature they are unable to maintain the coconut mite populations below the expected economic levels. Therefore, addition of *N. baraki* to the environment to supplement the natural population in controlling coconut mite has a potential.

An essential pre-requisite to field augmentation is an effective mass rearing method. Use of coconut mites to mass rear *N. baraki* in the laboratory has limitations because it develops either on the fruits of coconut naturally or on embryo-cultured seedlings in the

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laboratory (De Silva and Fernando, 2008), which is expensive and time consuming. Eggs of *Tetranychus urticae* Koch (Acari: Tetranychidae), coconut pollen and maize pollen were found to be suitable alternative foods for rearing *N. baraki* (Negloh et al., 2008). But *N. baraki* can be more easily reared on an alternative prey, *Tyrophagus putrescentiae* Shrank (Acari: Acaridae), which develops on a rice bran-wheat flour mixture in a box-type arena without a water and/or insect glue barrier (Fernando et al., 2004). One major constraint of this method to mass rear *N. baraki* in the laboratory is the frequent contamination of cultures by other mites, particularly *Lasioseius* sp. (Acari: Ascidae) who could crawl into the arena and feed on *N. baraki* and *T. putrescentiae*. Additionally these arenas were excessively moulded by fungi in 4–5 weeks making them unsuitable to rear *N. baraki*. Therefore, experiments were conducted to develop an easy and efficient method (without contaminations and less intervention) to rear *N. baraki* in the laboratory that would enable a continuous supply of *N. baraki* for field releases. This paper describes such a method of mass rearing *N. baraki* in the laboratory.

2. Materials and methods

2.1. Mother cultures

Mother cultures of *T. putrescentiae* and *N. baraki* were maintained in a closed arena without a water barrier (Fernando et al., 2004). The arena consisted of a black polyvinyl plastic sheet (13 cm × 9 cm) laid on a piece of foam of 13 cm × 9 cm × 2.5 cm in a plastic box (13.5 cm × 9.5 cm × 3 cm) covered with a screened lid. Wet tissue paper strips were stretched along the periphery of the plastic sheet and folded upward to line the side of the box to discourage the escape of mites from the arena, to prevent contaminants entering the arena and to provide drinking water for the mites. They were wetted when they became dry, usually 2–3 times a week. Tufts of cotton wool placed under a microscope slide (2.2 cm × 2.2 cm) on the plastic sheet served as the oviposition sites for *N. baraki*. Each box was covered with a screened lid and three such arenas were kept in a plastic tray (44 cm × 35 cm × 3 cm) containing water and covered with another tray of the same size to minimize contamination by undesirable arthropods and to provide sufficient humidity in the rearing arenas. *T. putrescentiae* was provided as the alternative food for *N. baraki* and approximately 1 g of a mixture of rice bran and wheat flour (1:1) was sprinkled on to the arena once a week as the food source for *T. putrescentiae*.

2.2. Rearing of T. putrescentiae

Four types of arenas were compared for their suitability to rear *T. putrescentiae*. These included three box-type arenas and a tray-type arena. The first box-type arena (T1) was similar to the arena used for rearing mother cultures except that a free water barrier was maintained around the foam. Wet tissue paper strips stretched around the periphery of the black polyvinyl plastic sheet and suspended in water were used to discourage the escape of mites and to provide drinking water for them. This further discouraged external contaminants entering in to the arena. Water was added into the water barrier 2–3 times a week (*c.* 5 ml/time) depending on the requirement. Tufts of cotton wool under a microscope cover slip (2.2 cm × 2.2 cm) served as oviposition sites. A mixture of 1 g of rice bran and wheat flour (1:1) was spread on the arena to serve as the food source of *T. putrescentiae*. Each box was covered with a screened lid and two such boxes were kept in a plastic tray of 44 cm × 35 cm × 3 cm and covered with another plastic tray of the same size.

The second box-type arena (T2) was prepared and maintained as the arena that was used to maintain mother cultures of *T. putrescentiae* and *N. baraki* (Fernando et al., 2004). The difference between the box-type arenas that were used in T1 and T2 was the presence of a free water barrier around the black polyvinyl sheets in the arenas in T1. In the arenas in T2, wet tissue paper strips were folded upward along the sides of the box.

The third box-type arena (T3) was structurally similar to the second box-type arena (T2), except for the fact that the black polyvinyl plastic sheet (18 cm × 18 cm), foam piece (18 cm × 18 cm × .5 cm) and the plastic box (18 cm × 18 cm × 3.5 cm) were bigger. Wet tissue paper strips around the periphery of the polyvinyl sheet with the edges folding upward to line the side of the box effectively confined the mites to the surface of the polyvinyl plastic sheet and provided mites with drinking water. Tissue paper strips were wetted 2–3 times a week (*c.* 10 ml/time) and rice bran and flour mixture (1:1, approximately 1 g) was provided once a week. Loosely arranged cotton wool underneath a microscope cover slip (2.2 cm × 2.2 cm) was used as an oviposition site for the mites. Each box was covered with a transparent screened lid and two such boxes were placed in a plastic tray of 44 cm × 35 cm × 3 cm and covered with another plastic tray of the same size.

The tray-type arena (T4) consisted of a black plastic sheet of 30 cm × 38 cm pasted on a plastic tray (44 cm × 35 cm × 3 cm). A thin layer of insect glue of approximately 1 cm wide (Stikem Special®, Sea Bright Associates, USA) applied along the periphery of the sheet prevented the escape of mites and safeguarded the arena from contaminants. A sufficiently wetted plastic foam pad (10 cm × 10 cm × 5 cm) wrapped with a tissue paper was placed on a glass sheet (10 cm × 10 cm) to provide the mites with drinking water and to maintain high humidity in the rearing unit. A piece of fine net (4 cm × 4 cm) underneath the glass sheet served as an egg laying substrate for the mites. Water was added to the sponge 2–3 times a week depending on the requirement (*c.* 15 ml/time). Approximately, 1 g of rice bran and flour mixture (1:1) was sprinkled on to the black polyvinyl plastic sheet once a week. Each individual tray was covered with another inverted tray of the same size.

All the arenas were kept on shelves in an air-conditioned room (27 ± 1 °C, >70% RH). Each type of arena was replicated 10 times. Twenty adult females of *T. putrescentiae* were introduced into each arena and the production of mites in each arena was determined by counting the females and the other motile stages weekly up to 4 weeks after the introduction of *T. putrescentiae*. Production of *T. putrescentiae* per unit area of each type of arena was calculated by dividing the number of mites on each arena by the area available for mites on the particular arena.

Fungi growing on the rice bran and flour mixture were identified using the manual by Barnett (1962).

2.3. Rearing of N. baraki

Box-type arenas that were used to test their suitability to rear *T. putrescentiae* became unsuitable in 4 weeks after introducing *T. putrescentiae* as they were excessively contaminated by fungi (*Aspergillus* sp. and *Penicillium* sp.) and by airborne mites, particularly, *Lasioseius* sp (Acari: Ascidae). Therefore, only the tray-type arena was used for rearing of *N. baraki*. Thirty-two, tray-type arenas were prepared as the method described above. Three weeks after introducing *T. putrescentiae* in to the arenas (when *c.* 1000 *T. putrescentiae* were present on each arena), one set of arenas was maintained without introducing *N. baraki* (T5). Into the other set of arenas, adult females of *N. baraki* were introduced (20 per each arena). After introducing *N. baraki*, arenas were maintained either without adding *T. putrescentiae* (T6) or adding the same at 2-week (T7) or 3-week (T8) intervals. Each individual tray was covered with