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MASS-REARING TECHNIQUE FOR *Proprioseiopsis lenis* (Corpuz & Rimando) and *Neoseiulus calorai* (Corpuz & Rimando) (PHYTOSEIIDAE, ACARI) WITH NOTES ON THE BIOLOGY OF *P. lenis*[1]

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ABSTRACT

An easy and simple laboratory technique for mass-rearing the predatory mites, *Proprioseiopsis lenis* (Corpuz & Rimando) and *Neoseiulus calorai* (Corpuz & Rimando), using cheap, readily available materials and the flour mite *Suidasia pontifica* Oudemans as prey, is described.

The development of *P. lenis* was studied on the red spider mite, *Tetranychus piercei* McGregor and flour mite *S. pontifica*. The progenies produced were all females. Developmental stages included the egg, larva, protonymph, deutonymph and adult. The four developmental stages lasted one day each on the average, including the egg, with *T. piercei* as prey. Longevity of adults averaged 10.17 days, pre-oviposition period 2.08 days, oviposition period 7.25 days and post-oviposition period 0.83 days. Average total fecundity was 17.75 eggs or 2.35 eggs per day. Development period of *P. lenis* on *S. pontifica* was one day longer than on *T. piercei* (average 5.32 days), due to the doubling of incubation period of eggs on the former.

Key words: *Neoseiulus calorai*, phytoseiid mass rearing, *Proprioseiopsis lenis*, spider mites, flour mites

INTRODUCTION

Proprioseiopsis lenis (Corpuz & Rimando) and *Neoseiulus calorai* (Corpuz & Rimando) are among the most widespread leaf- and litter-inhabiting phytoseiid mite predators in the Philippines. They occur in diverse habitats, in woody shrubs and trees, grasses and annual crops. Among their recorded habitat

plants are economically important crops such as citrus, cucumber, eggplant, potato, rice, strawberry and ornamentals, notably chrysanthemums and roses (Corpuz-Raros, 1989; Schicha and Corpuz-Raros, 1992; Corpuz-Raros and Garcia, 1994; Corpuz-Raros *et al.,* 2004). Laboratory observations have confirmed their feeding on various spider mites, namely, *Oligonychus biharensis* (Hirst), *Tetranychus piercei* McGregor, *T. truncatus* Ehara, *T. urticae* Koch, as well as on the flour mite, *Suidasia pontifica* Oudemans. Efforts to mass-rear them at the National Crop Protection Center and the Acarology Laboratory, University of the Philippines Los Baños (UPLB) started in 2002 under a project funded by the Bureau of Agricultural Research, Department of Agriculture.

P. lenis was originally described by Corpuz and Rimando (1966) as *Amblyseius lenis* based on specimens collected from *Citrus nobilis* at the UPLB Campus. Its present distribution range within the Philippines covers many provinces in the islands of Luzon, Leyte, Camotes and Mindanao and its recorded habitat plants totalled to more than 50 species, as well as litter of these plants, in cultivated fields and natural forest stands (Corpuz-Raros 2002). It is also known to occur in Queensland, Australia where it was first reported on strawberry under its junior synonym, *Amblyseius sullivani* Schicha and Elshafie (1980). This predator is dark reddish brown (Fig. 1a), darker than most phytoseiids that are usually light brown or becoming reddish when newly fed on spider mites. Morphologically, it is recognized by the absence of dorsal seta *J2*, and in having long setae *Z5, Z4* and s4, pentagonal and reticulate ventrianal shield, disc-shaped spermathecal cervix and 3 setaceous macrosetae in leg IV, of which the middle (tibial) is shortest (Corpuz-Raros *et al.* 2004).

We have maintained mass cultures of this predator for over three years during which no males have been produced. Males are also extremely rare in the field, and only two individuals were recently collected since its discovery in 1966, despite intensive collections for over three decades. Parthenogenetic reproduction in the laboratory cultures is due to infection with *Wolbachia*, a symbiotic cytoplasmic bacterium (Corpuz-Raros, 2005). The predator has a very short developmental period of only about four days, relatively high fecundity, and feeds voraciously on spider mites.

The present stock of *N. calorai,* on the other hand, originated from colonies invading cultures of *P. lenis*, which were being reared and maintained on *S. pontifica* in the laboratory. Likewise, a rearing technique based on *S. pontifica* was devised to rear and maintain cultures of *N. calorai.*

Figure 1. Adult females of *P. lenis* (a) and *N. calorai* (b), spider mite prey *T. piercei* (c) and flour mite prey *S. pontifica* (d).

Like *P. lenis, N. calorai* was originally described by Corpuz and Rimando in 1966 under the genus *Amblyseius* but the species is now combined with *Neoseiulus* following contemporary classification of the family Phytoseiidae (*e.g.* Chant and McMurtry, 2003). It is light brown like most phytoseiids, becoming dark brown as it grows older (Fig. 1b). Morphologically, it is recognized by having 19 pairs of dorsal setae including 2 pairs of sublaterals which are borne on membrane lateral to dorsal shield; rather long and serrated setae Z4 and Z5, all others short and smooth; pentagonal and creased ventrianal shield; thick and tube-like cervix of spermatheca; and only one setaceous macroseta on basitarsus

of leg IV (Corpuz-Raros, *et al.* 2004). The spermatodactyl on movable chela of male is large and T-shaped. *N. calorai* also feeds voraciously on spider mites. The biology of this predator was recently studied by Malveda and Corpuz-Raros (In Press) using *T. urticae* as prey. Because of their desirable qualities, *P. lenis* and *N. calorai* are two of the phytoseiids whose potential as biological control agents is being investigated in our laboratory. The present paper reports on: 1) a mass-rearing technique for both *P. lenis* and *N. calorai* utilizing *S. pontifica* as prey and 2) developmental rate of *P. lenis* on two prey species, the spider mite *T. piercei* and flour mite *S. pontifica.*

MATERIALS AND METHODS

Mass-rearing of *P. lenis* and *N. calorai*

Stock culture of the flour mite. The stock of the flour mite was mass-reared on Brewer's yeast in glass bottles. Twenty grams of yeast was dispensed per bottle and infested with a scoop of the flour mite from the pure stock following the method of Navasero and Calilung (1997) and Navasero et al 2003). After at least four weeks, the substrate started to deteriorate and another substrate was prepared for infestation.

Stock culture of *P. lenis*. The initial laboratory stock of *P. lenis* originated from 2 deutonymphs collected from leaves of mayana *Plectranthus scutellarioides* growing beside the Entomology greenhouse, Biological Science Building, UP Los Baños, Laguna in May 2001. This UPLB stock serves as source for various laboratory experiments.

Stock culture of *N. calorai*. The laboratory stock of *N. calorai* was started from individuals that invaded some rearing units of *P. lenis* reared on *Suidasia.* It was successfully reared also on spider mites, initially on *T. piercei* with soybean as host plant, and later on *T. urticae* with soybean or water hyacinth as host. Like *P. lenis,* the culture of *N. calorai* was subsequently maintained on *S. pontifica* as prey. These were initially confined in trays with *T. piercei* on excised soybean leaves as prey, but as the population increased, the predator and prey were reared in plastic cages with potted soybean plant as host for the spider mite prey. After observing that *P. lenis* voraciously feeds and normally develops and reproduces on *S. pontifica,* part of the stock was conditioned to feeding on this flour mite and has been thus maintained up to the present. Another stock of *P. lenis* from a rose plantation in Bahung, La Trinidad, Benguet was similarly reared on *T. piercei,* then on *S. pontifica.*

Development of *P. lenis* on *T. piercei* and *S. pontifica*

Ten deutonymphs of *P. lenis* were confined in Munger cells fabricated out of plexiglass measuring 7.8 cm x 2.7 cm and bearing a bore with a diameter of 2.2 cm and a depth of 0.4 cm. The bore was covered above and below with pieces of ordinary glass of the same size as the middle piece plexiglass. A piece of 2-ply tissue paper of similar size as the glass pieces was lined in between the plexiglass and bottom cover. The resulting closed cell became the arena within which the predator was reared on spider mite prey on a leaf. A soybean leaf with mixed stages of spider mite prey, *T. piercei* (Fig. 1c), was sandwiched between the bottom and middle pieces. Prey on a fresh leaf was replenished as the need arose. Each culture was examined daily under a dissecting microscope to monitor egg-laying, molting into the next stage, and death of the adult. The fecundity of females, duration of various stages, adult longevity and other aspects of the predator's life were determined. From the progeny of this parental stock, 30 eggs were similarly confined separately in Munger cells to record life history data. The same set-up and procedure was used later in studying the development of *P. lenis* on its flour mite prey, *S. pontifica* (Fig 1d).

Stock culture of the spider mite. The initial colony of *T. piercei* was collected from leaves of *Moringa oleifera* from Anos, Los Baños. Four to five weeks old soybean plants were infested with adult spider mites from the purified stocks. When adults of the next generation started to eclose, usually 7-10 days after initial infestation, infested leaflets were detached and each of them was pinned on mature leaves of new soybean plants. After five days, the host started to deteriorate and another fresh substrate was prepared for infestation. The process was repeated continuously to maintain the cultures of spider mites.

Mass-rearing unit. This was a rectangular, commercially available plastic soapbox, the bottom part of which was lined with 2-ply tissue paper (Fig. 2a) to absorb excess moisture and serve as refuge for the predators. The tissue paper was also observed to be a good receptacle for *Suidasia* prey mites as it prevented them from wandering about and thus kept them within the reach of the predators. To further increase the volume of refuge material for both the prey and predator, a small amount (0.5g) of sterile coconut coir dust was scattered on the tissue paper. Each box was placed on a moat of water in a rectangular plastic vat with perforated cover and the vats were placed on wooden shelves (Fig. 2c). The water in the rectangular vat served also as moisture source for the predator. The boxes were emptied, cleaned and dried after use, ready for rearing another batch of predators.

Mass-rearing procedure. Adults of *P. lenis* from the pure stock were introduced to rearing boxes containing a scoop of *Suidasia* mite prey. A scoop of the prey contained mixed stages. When adults of the next generation started to

Figure 2. Close-up of the mass rearing unit for the predator *P. lenis* (a) and *N. calorai* (b) showing the coir dust-*Suidasia* prey mixture, and the mass rearing set-up for *P. lenis* (c) and *N. calorai* (d).

eclose, usually 4-5 days after initial inoculation and twice a week thereafter, more prey (for the predator) or yeast (for the prey) was added. The water in the moat was replaced every 7 days to provide fresh water to the predator. The cultures got dense, usually after a month of continuous rearing, and these were split up by scooping onto fresh substrates (rearing units). When some of the cultures became contaminated or invaded by another predator, these were discarded and fresh substrates were inoculated from the pure stock. The process was repeated continuously to maintain the cultures of *P. lenis*.

To maintain the cultures of *N. calorai* the rearing units were placed in a rectangular plastic vat measuring 32cm x 23m x 9 cm, lined at the bottom with an inch thick moistened coir dust (Fig. 2b). A plastic sheet was placed on top of the coir dust as additional resting place for the predators. Similarly, the rearing boxes were placed on a wooden shelf (Fig 2d). Room temperature was maintained at 27 $\pm$ 1 0C.

RESULTS AND DISCUSSION

Mass-rearing of *P. lenis* and *N. calorai*

A mature culture of both *P. lenis* and *N. calorai* had an average of 79 eggs and 106 active stages (larva, protonymph, deutonymph and adult) per scoop of about 1.55 ml after a month of inoculation. Likewise, a scoop of the prey of the same volume contained mixed stages of about 337 eggs and 16,161 active stages. This provided the predators prey of various slopes sufficient to support succeeding generations. The cultures got dense, usually after a month of continuous rearing, and had to be split up by scooping onto fresh substrates (rearing units) or releasing in the field.

The rectangular plastic soapbox as a rearing unit for both *P. lenis and N. calorai* appeared appropriate for these predators and offers the following advantages. First, it is clear and transparent enough for easy observation. The bottom part is flat and can be easily lined with 2-ply tissue paper to absorb excess moisture, and to serve as receptacle for prey *Suidasia* mites, and as refuge for the predators. Second, it is easy to clean and disinfect. Coconut coir dust, a readily available, cheap coconut by-product, is a suitable refuge material for both the predators and prey mites, and can provide additional moisture to the predators. Lastly, the container is cheap and reusable.

The method of rearing *P. lenis* and *N. calorai* using the flour mite as prey proved to be simple and convenient compared with using spider mite. The rearing unit is simple, cheap, easy to construct, and permits easy regulation of moisture

and aeration. It allows continuous reproduction of the prey mite by adding small amounts of the yeast medium, as the predator itself continues to propagate. Addition of pure stocks of *S. pontifica* becomes necessary only when the predator gets overpopulated. The rearing system can be kept under minimal supervision for several months, requiring only the changing of water and the addition of prey and yeast.

Comparative Development of *P. lenis* on the Two Prey Species

On *T. piercei*. Total developmental period was very short, with a range of 4.00 to 4.23 days or a mean of 4.08 ± 0.08 (Table 1) when reared on the spider mite, *T. piercei*. Each of the four developmental stages lasted for one day on the average, including the egg, which in prey spider mites lasts for a relatively longer period, or about half the time needed for development. Longevity of adults ranged from 4 to17 days, with a mean of 10.17 days. Pre-oviposition period averaged 2.08 days. Eggs were laid by females 1 to 4 days old. Egg-laying or oviposition period lasted for 1-14 days (average of 7.25 days) during which the female laid an average total of 17.75 eggs or 2.35 eggs per day. Adults lived for 0-2 days after laying their last egg, with this post-oviposition period averaging 0.83 day.

Table. 1. Duration (days) of the different life stages of *P. lenis* fed with *T. piercei* under laboratory conditions.

Life Stages	No. of Samples	Duration	
		Range	Mean ±SE
Developmental Period			
Egg (incubation period)	30	1.00 – 1.13	1.05 ± 0.06
Larva	8	0.92 – 1.05	0.90 ± 0.04
Protonymph	8	0.88 – 1.14	1.01 ± 0.08
Deutonymph	8	0.97 – 1.21	1.04 ± 0.08
Total Developmental period (egg to adult)		4.00 – 4.23	4.08 ± 0.08
Post-developmental Period			
Pre-oviposition	12	1.00 – 4.00	2.00 ± 0.75
Oviposition	12	1.00 – 14.00	7.25 ± 3.29
Post-oviposition	12	0.0 – 2.00	0.83 ± 0.42
Longevity	12	4.00 – 17.00	10.17 ± 3.03

Prey consumption rate of *P. lenis* on *T. piercei*, as expected, increased as the predator became bigger and older. To complete development, the average number of prey, mainly eggs, consumed by the predator at each developmental stage are as follows: larva 3.63 ± 3.53, protonymph 9.11 ± 7.15, deutonymph 15.84 ± 9.06, and to complete development 28.59 ± 9.91. The predator consumed a total of 83-639 prey eggs of *T. piercei* or an average of 336.17 during its entire lifetime. Prey consumption was greatest during the oviposition period when an average of 274.25 preys was consumed, or 81 % of the total consumed during the entire adult stage. This implies that for mass rearing purposes, there is a need for an efficient mass rearing technique for *T. piercei* to produce the very high prey requirement of *P. lenis*.

On *S. pontifica*. Total developmental period of *P. lenis* on the flour mite *S. pontifica* was slightly longer than on *T. piercei*, ranging from 4.58 to 6.17 days or a mean of 5.32 ± 0.53 (Table 2). The egg stage lasted for 2.23 days on the average, or twice as long as when reared on the spider mite. The protonymphal stage lasted for a day, deutonymphal stage for 1.46 days, but the larval stage lasted barely half a day. Longevity of adults ranged from 5 to18 days, with a mean of 10.08 days. Eggs were laid by females 2 to 8 days old. Pre-oviposition period averaged 3.37 days. Egg-laying or oviposition period lasted for 1-9 days (average of 4.74 days) during which the female laid an average total of 7.11 eggs or 1.4 eggs per day. Adults lived for 0-9 days after laying their last egg, with this

Table 2. Duration (days) of the different life stages of *P. lenis* fed with *S. pontifica* under laboratory conditions.

Life Stages	No. of Samples	Duration	
		Range	Mean ±SE
A. Developmental Stages			
Egg (incubation period)	30	1.62 – 2.75	2.23 ± 0.45
Larva	30	0.62 – 1.33	0.66 ± 0.30
Protonymph	26	0.58 – 1.58	0.96 ± 0.25
Deutonymph	25	0.58 – 2.33	1.46 ± 0.46
Total Development (egg to adult)		4.58 – 6.17	5.32 ± 0.53
B. Post-development			
Pre-oviposition	19	2.00 – 8.00	3.37 ± 1.50
Oviposition	19	1.00 – 9.00	4.74 ± 2.49
Post-oviposition	19	0.0 – 9.00	2.74 ± 2.45
Longevity	25	5.00 – 18.00	10.08 ± 3.48
Fecundity			
Daily		1.00 – 3.00	1.40 ± 0.75
Total		1.00 – 21.00	7.11 ± 5.77

post-oviposition period averaging 2.74 days. Egg hatchability was 100%. The apparently longer development of *P. lenis* on *S. pontifica* was attributed to inadequacy of free moisture in the Munger cell, which was difficult to regulate because the yeast medium is hygroscopic, and once moistened, both prey and predator could get trapped in the caked medium. Nevertheless, *S. pontifica* was conveniently used as prey in a larger rearing unit that allows access of the predator to free moisture.

All progenies of *P. lenis* developed into females on both spider mite and flour mite prey.

SUMMARY

The procedure for rearing *P. lenis* and *N. calorai* using the flour mite as prey and choir dust as medium is reported for the first time. The technique proved to be very easy, simple and convenient. The rearing unit is cheap, easy to construct, and permits easy regulation of moisture and aeration. The development of *Proprioseiopsis lenis* under laboratory conditions was longer when reared on the flour mite *Suidasia pontifica* than on the prey spider mite *Tetranychus piercie.* This was attributed to inadequacy of free moisture in the Munger cell, where the development of the mites was monitored.

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