

**FUNCTIONAL RESPONSE OF THREE PREDATORY
PHYTOSEIID MITES (PHYTOSEIIDAE, ACARI)
TO TWO-SPOTTED MITE, *Tetranychus urticae* Koch
(TETRANYCHIDAE, ACARI) ¹**

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ABSTRACT

The functional responses of three species of predatory phytoseiid mites, *Neoseiulus calorai* (Corpuz and Rimando), *Neoseiulus longispinosus* (Evans) and *Proprioseiopsis lenis* (Corpuz and Rimando), to eggs, larvae, protonymphs, deutonymphs and adult females of *Tetranychus urticae* Koch, were determined. The predation tests were conducted on rose leaf discs inside Mungger cells over a 24h period at 27 ± 1 °C. Prey densities ranged from 1 to 60 per leaf disc for each prey stage. All three species were effective predators of eggs of *T. urticae* but *N. calorai* and *P. lenis* were effective also on larvae. Based on R^2 values, both Type I and Type II curves provide good fit on the functional response data.

Key words: functional response, Phytoseiidae, Tetranychidae, Acari, *Neoseiulus calorai*, *Neoseiulus longispinosus*, *Proprioseiopsis lenis*, *Tetranychus urticae*

INTRODUCTION

The green-spotted or two-spotted spider mite, *Tetranychus urticae* Koch, was recently reported as the most prevalent spider mite pest of commercially grown roses, chrysanthemums, other ornamentals, strawberry and other important crops in Benguet and wherever these crops are commercially grown in other parts of the Philippines (Corpuz-Raros *et al.*, 2004). An earlier report by Cadatal (1998) showed that it has long been a serious pest of roses in the

Philippines. *T. urticae* is an emerging pest also of sampaguita, eggplant, pigeon pea (Navasero *et al.*, 2005). For this reason, studies have been initiated to develop an IPM program on ornamental crops with the use of native predators especially phytoseiids. The present study is part of the effort towards this objective. It reports on the functional responses of three phytoseiid predators, *Neoseiulus longispinosus* (Evans), *N. calorai* (Corpuz and Rimando), and *Proprioseiopsis lenis* (Corpuz and Rimando), that commonly inhabit roses, chrysanthemums and other high value ornamentals.

In the Philippines, *N. longispinosus* is one of the most common phytoseiids on crop plants, and is exclusively arboreal but maybe found occasionally on newly fallen leaves on the ground. It exhibits the Type II life style, with strong specificity for spider mites (McMurtry and Croft, 1997). In our laboratory, we observed this predator to feed on all laboratory reared spider mites offered, including the rose spider mites, *Oligonychus biharensis* (Hirst), *Tetranychus piercei* Mc Gregor, *T. truncatus* Ehara and *T. urticae* (Corpuz-Raros *et al.*, 2004). Towards selecting a better prey for mass rearing the predator, De Leon-Facundo and Corpuz-Raros (2004) found no difference in predator development and reproduction when reared on *T. kanzawai* and *T. truncatus* but the predator required significantly fewer eggs of the latter to produce an egg. *N. longispinosus* has been used successfully against the cassava spider mite, *T. kanzawai*, in commercial cassava plantations of the Matling Industrial Corporation at Lanao del Sur (Vasquez and Gonzales, 1994).

N. calorai and *P. lenis*, on the other hand, are widespread leaf- and litter-inhabiting phytoseiid predators in the country. They occur on diverse plant types, on woody shrubs and trees, grasses and annual crops like 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 similarly confirmed their feeding on various spider mites, namely, *O. biharensis*, *T. piercei*, *T. truncatus* and *T. urticae*, as well as on the flour and litter mite, *Suidasia pontifica* Oudemans. The biology of *N. calorai* was recently studied by Malveda and Corpuz-Raros (In Press) with *T. urticae* as prey while notes on the life history of *P. lenis* are given in Navasero and Corpuz-Raros (2005).

MATERIALS AND METHODS

Mass Culture of Spider Mite Prey, *Tetranychus urticae* Koch

T. urticae was reared under laboratory conditions following the method described by Navasero *et al.* (2005) on water hyacinth. Briefly, from the initial laboratory stock of *T. urticae* that was field collected from rose leaves and

maintained on soy bean in the laboratory, a portion was taken, maintained on 30-45 cm high water hyacinth contained in one-gallon plastic containers and allowed to reproduce. Each container was filled with tap water and placed on a moat of water on a rectangular tray or vat to ward off ants and predators, which may invade the culture. Infested leaves from the pure stock culture were cut into four pieces per leaf and each piece was pinned on an uninfested water hyacinth leaf. After two to three days when active stages of the mite had freely transferred to the new leaves, the dried pieces were removed and the culture was allowed to develop. After one week when the host started to deteriorate, another container of fresh water hyacinth was prepared for infestation. The process was repeated continuously to maintain the cultures of spider mites.

Mass Culture of Predatory Mites

Predatory mites used for experimentation were mass reared from stocks that have been maintained in the Acarology Laboratory, UPLB since 2001. Room temperature was maintained at $27 \pm 1^\circ\text{C}$. The technique for mass rearing *N. longispinosus* was adopted from De Leon-Facundo and Corpuz-Raros (2004) while those for *N. calorai* and *P. lenis* were based on Navasero and Corpuz-Raros (2005).

N. longispinosus. The parental stock of *N. longispinosus* was collected and purified from a population that naturally colonized potted cassava plants infested with *T. kanzawai* in the greenhouse. After changes in prey and host plant, from *T. kanzawai* on cassava to *T. truncatus* on soybean, it is now maintained on *T. urticae* with soybean as host plant. Four- to five-week old soybean plants were infested with *T. urticae* inside a plastic cage mounted on a steel frame zipped on front side, and provided with aeration windows covered with silkscreen cloth to prevent build-up of moisture and allow free flow of gases. When the soybean plants started to deteriorate, usually after five days, these were cut at the base and infested onto fresh substrates with *T. urticae*. When these cut plants dried up, usually after three or four days, spent substrates were discarded as the predators and remaining prey had already transferred to the new host.

P. lenis* and *N. calorai. While *P. lenis* and *N. calorai* both feed on spider mites, they can be mass reared on the storage mite *S. pontifica* which is easier to maintain than spider mites and their host plants. These predators have been maintained in our laboratory on *S. pontifica* since 2002.

The stock of *P. lenis* was originally collected in 2001 from leaves of mayana (*Plectranthus scutellarioides*) growing beside the Entomology Greenhouse. The predator was first transferred to soybean infested with *T. piercei*, but later, part of the stock was conditioned to feed on *S. pontifica* and subsequently mass reared on this latter prey. On the other hand, the stock of *N. calorai* was started from

individuals that invaded some rearing units of *P. lenis* reared on *S. pontifica*. *N. calorai* was also successfully reared on spider mites like *T. piercei* and *T. urticae* with soybean or water hyacinth as host plant. However, like *P. lenis*, the culture of *N. calorai* was subsequently maintained using the same prey and procedure.

To start a mass culture of either predator, adults from the pure stock were introduced to a rearing unit containing a scoop of *S. pontifica* reared on yeast granules. The rearing unit is a rectangular plastic soapbox, the bottom part of which was lined with 2-ply tissue paper. When predator adults of the next generation started to eclose, usually 4-5 days after initial inoculation and twice a week thereafter, additional prey (for the predator) and yeast (for the prey) were added. Water in the moat, on which the rearing unit was placed, was replaced every 7 days. After about a month, the dense cultures were split up by scooping onto fresh substrates in new rearing units. The process was repeated continuously to maintain cultures of *P. lenis*.

To maintain cultures of *N. calorai*, a slightly different procedure was followed. The rearing units were placed in rectangular plastic vats measuring 32 x 23 x 9cm and lined with an inch thick moistened coir dust. A plastic sheet was placed on top of the coir dust as additional resting place for the predators.

Functional Response Tests

In a series of tests the functional responses of *N. calorai*, *N. longispinosus*, and *P. lenis* to various densities of *T. urticae* eggs, larvae, protonymphs, deutonymphs and adult females of were determined.

The mite predation arena consisted of a rose leaf disc placed at the bottom of a Munger cell built out of a 7.8 x 2.7 cm piece of plexiglass bearing a bore with a diameter of 2.2 cm and a depth of 0.4 cm and two pieces of glass plates of the same size to cover the bore from the top and bottom. The leaf disc was laid with the nether side up where the spider mite prey normally feeds. A piece of 2-ply tissue paper of similar size as the slide was lined in between the plexiglass and the bottom cover to keep the leaf fresh and turgid. Eggs of *T. urticae* at varying densities (1, 10, 20, 40, 60) were confined in each cell with a test predatory mite. Using a fine pointed camel's hair brush, care was taken to insure that eggs were transferred to the cell, undamaged. Predation response was determined for the adult female of the three predators. For each prey density and predator, the test was replicated to have a total of 10. After 24h, the Munger cells were examined to record the number of mite prey eggs fed upon by the predator. The same set-up and procedure were used and the same data were gathered to measure the functional response of female predators to the larvae, protonymphs, deutonymphs, and adults of *T. urticae*.

Regression analysis was made for each prey stage and species of predator to compare the results and fit the data to Holling's Disc Equation:

$$N = \frac{a^1 T_1 D_i}{1 + a^1 T_h D_i}$$

where N= number of prey killed; a^1 =consumption rate per predator; T_1 = exposure time to predator; D_i =prey density; and T_h =handling time

RESULTS AND DISCUSSION

The functional responses of *N. calorai*, *N. longispinosus* and *P. lenis* to different population densities of *T. urticae* egg and active stages are shown in Tables 1 to 3 and Figure 1.

The functional response of *N. calorai* to *T. urticae* is similar for all developmental stages of the prey, that is, consumption rate tended to decrease from the egg to adult stage except for larva. At the highest prey density of 60, average consumption rates of females were: egg, 40.2; larva, 54.4; protonymph, 11.8; deutonymph, 6.1; and adult female 0.6. High consumption of *N. calorai* on eggs and larvae of *T. urticae* is an efficient response, which is about five times as much as that on the older stages, collectively. The higher consumption of prey larvae than eggs suggests that *N. calorai* prefers young mobile prey but the rate declines as the prey becomes bigger and older, from protonymph to adult. We observed that when a prey bumped into the predator while it was feeding on another prey, the predator ceased feeding, moved away immediately but subsequently attacked and captured another prey. Disturbance seemed to elicit aggressive response, stimulating the predator to make more prey captures than it normally does to reach satiation. The increased number of contacts at high prey density apparently also led to increased prey activity caused by movements of the disturbed predator. In general, therefore, the first contact of a predator with a stationary prey results in stimulated activity of several prey individuals, ultimately resulting in greater predator-prey contact. Since the area of the experimental arena approximates one-half to two-thirds the area of an average sized rose leaf, a prey density of 60 in the experimental arena would correspond to a density of 120 to 180 prey on a rose leaf, which is about the same number observed in the field under heavy infestation. This suggests that a release rate of 2 predators per leaf would be sufficient to clean up the prey at high densities, granting that the predator is hungry as it was in the laboratory tests. However, field performance of *N. calorai* remains to be evaluated and many factors are expected to affect its behavior and prey consumption in actual field situations.

Table 1. Number of eggs, larvae, protonymphs, deutonymphs and adults of *T. urticae* killed by adult females of the predatory mite, *N. calorai*, on rose leaf discs inside Munger cells over a 24h period at 27± 1°C.

Life Stage of Prey	Number of Test Predators	Density of Prey Offered	Consumption per Predator	
			Range	Mean
Egg	10	1	0-1	0.7
	10	10	3-10	9.3
	10	20	6-20	14.4
	10	40	5-40	23.4
	10	60	10-60	40.2
Larva	10	1	0-1	0.9
	10	10	5-10	8.4
	10	20	15-20	18.9
	10	40	22-40	34.7
	10	60	49-60	54.4
Protonymph	10	1	0-1	0.4
	10	10	0-6	2.4
	10	20	1-11	6.3
	10	40	0-25	10.5
	10	60	2-21	11.8
Deutonymph	10	1	0-1	0.2
	10	10	0-4	1.1
	10	20	0-4	1.9
	10	40	0-11	4.0
	10	60	1-18	6.3
Adult	10	1	0-1	0.2
	10	10	0-1	0.3
	10	20	0-2	0.7
	10	40	0-2	1.0
	10	60	0-2	0.6

Table 2. Number of eggs, larvae, protonymphs, deutonymphs and adults of *T. urticae* killed by adult females of the predatory mite, *N. longispinosus*, on rose leaf discs inside Munger cells over a 24h period at 27 ± 1 °C.

Life Stage of Prey	Number of Test Predators	Density of Prey Offered	Consumption per Predator	
			Range	Mean
Egg	10	1	1	0.8
	10	10	2-10	7.0
	10	20	6-19	13.5
	10	40	12-38	22.0
	10	60	15-37	26.7
Larva	10	1	0-1	0.8
	10	10	3-10	6.0
	10	20	3-18	9.7
	10	40	3-23	13.1
	10	60	3-26	15.5
Protonymph	10	1	0-1	0.9
	10	10	1-8	3.9
	10	20	1-10	3.5
	10	40	3-12	7.1
	10	60	1-11	6.8
Deutonymph	10	1	0-1	0.9
	10	10	1-7	4.5
	10	20	3-8	5.3
	10	40	2-7	4.5
	10	60	3-10	6.8
Adult	10	1	0-1	0.5
	10	10	0-8	3.8
	10	20	2-9	4.3
	10	40	1-7	3.4
	10	60	3-6	3.2

Table 3. Number of eggs, larvae, protonymphs, deutonymphs and adults of *T. urticae* killed by females of the predatory mite, *P. lenis*, on rose leaf discs inside Munger cells over a 24h period at $27 \pm 1^\circ\text{C}$.

Life Stage of Prey	Number of Test Predators	Density of Prey Offered	Consumption per Predator	
			Range	Mean
Egg	10	1	1	1.0
	10	10	2-10	8.1
	10	20	3-20	14.4
	10	40	28-37	30.7
	10	60	30-58	43.4
Larva	10	1	0-1	0.9
	10	10	2-9	7.1
	10	20	3-18	14.4
	10	40	13-35	28.3
	10	60	32-53	40.2
Protonymph	10	1	1	1.0
	10	10	1-7	4.3
	10	20	2-15	9.1
	10	40	2-15	9.4
	10	60	7-19	13.6
Deutonymph	10	1	0-1	0.1
	10	10	0-4	1.9
	10	20	0-9	4.2
	10	40	0-9	4.5
	10	60	0-6	3.3
Adult	10	1	0-1	0.1
	10	10	0-6	1.5
	10	20	0-7	2.5
	10	40	1-6	3.1
	10	60	0-5	2.3

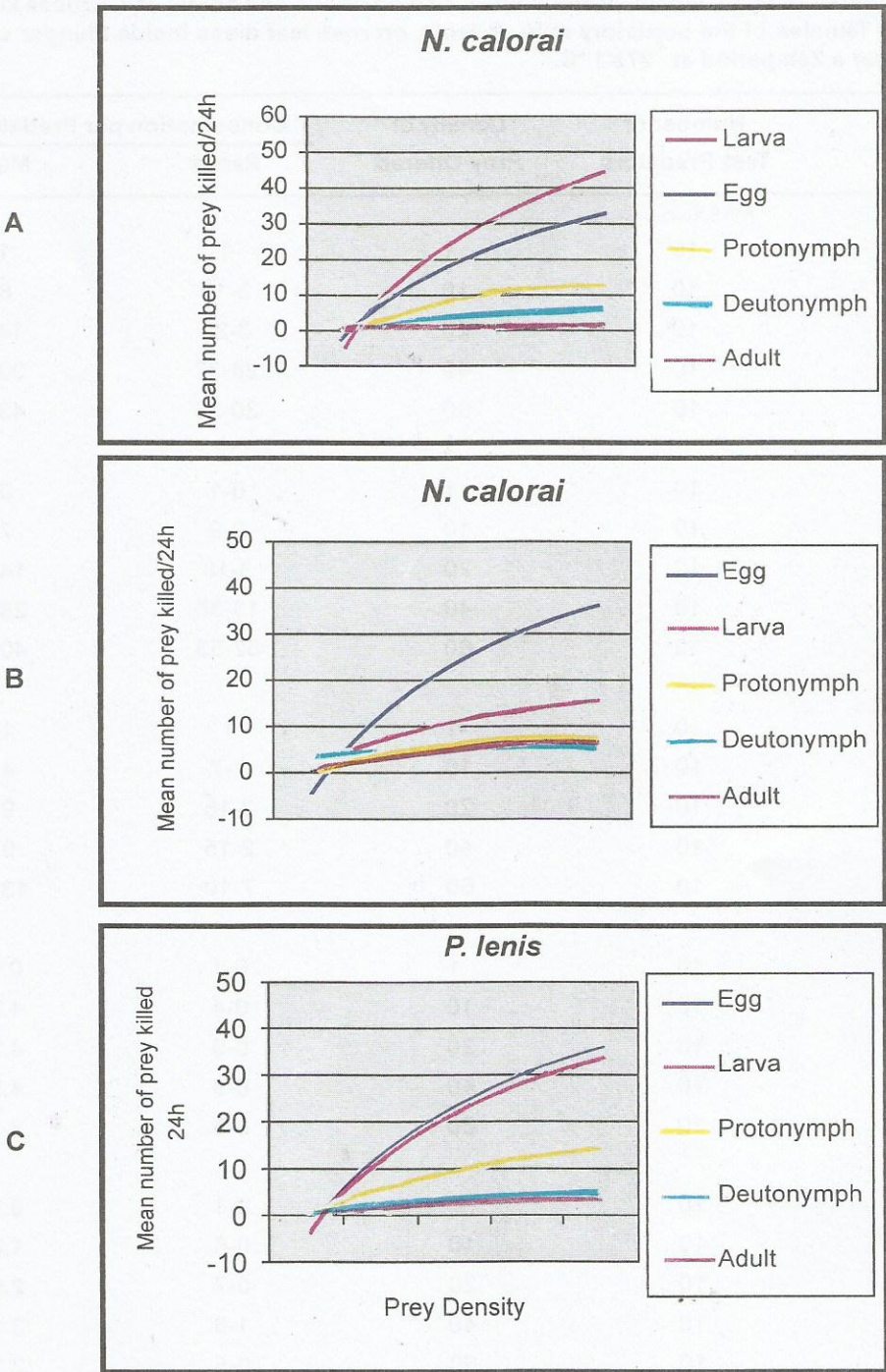


Figure 1. Functional response of *N. calorai* (A), *N. longispinosus* (B) and *P. lenis* (C) females to the spider mite prey, *T. urticae* at various life stages.

The randomly distributed prey on the flat surface within the limited confines of the Munger cell allowed the predator to discover its prey easily. The cover of the cell provided a place to rest on, and it was often observed doing so, in an inverted position like other phytoseiids used in laboratory studies. While resting, prey activity subsided correspondingly.

The difference in the predation rates affected the shape of the functional response curves of *N. calorai* (Fig. 1A). Based on R^2 values, both Type I and Type II curves (Holling 1966) provide good fit (Table 4). According to Holling, prey consumption of a predator increases at a decreasing rate until the curves level off. The satiation point or leveling off of the response curve appears to be reached at a density of 40-50 for prey larvae and 30-40 for prey eggs. The functional response curves of *N. calorai* are reported for the first time.

A similar response was observed with *N. longispinosus* using *T. urticae* as prey. The consumption rates of the predator on the following stages of the prey were: egg, 26.7; larva, 15.5; protonymph, 6.8; deutonymph, 6.8; and adult female, 3.2 at 60 prey density. Consumption of eggs increased with density but the rate leveled off at prey density of 40, approaching the level reported by Ibrahim and Abdul Rahman (1997) for the same predator and prey. The functional response to the eggs is efficient; in contrast, response to the older stages appears to be less efficient as the predator consumed only an average of 3.2 out of 60 adult prey offered. The data also show that *N. longispinosus* is primarily an egg feeder and predation efficiency progressively decreases as the prey in active stages grow and mature from the larva to adult. As in *N. calorai*, the difference in the predation rates affected the shape of the functional response curve of *N. longispinosus*. Based on R^2 values, both Type I and Type II curves (Holling 1966) provide good fit but Type II (Fig. 1B) appears to have a better fit (Table 4). This predation response was also obtained by Kim and Lee (1993) on *N. longispinosus* to the eggs of *T. urticae*, which was affected by spatial distribution and density of spider mite prey.

The functional response data for *P. lenis* follow a trend similar to that for *N. calorai* and, like the latter predator, it is also an egg and larval feeder. The consumption rates at prey density 60 were: egg, 43.4; larva, 40.2; protonymph, 13.6; deutonymph, 3.3; and adult female, 2.3. There was also an increasing trend in the functional response to the eggs and larvae which is an efficient response. Its response to older and mobile prey, from protonymph to adult, appears to be less efficient especially larger deutonymphs and females. The difference in the predation rates affected the shape of the functional response curve of *P. lenis* (Fig. 1C). Based on R^2 values, both Type I and Type II curves (Holling 1966) provide good fit (Table 4). Being a relatively smaller predator, *P. lenis* consumed fewer prey at all stages than *N. calorai* and satiation point was reached at a lower density of 30 eggs or larvae.

Table 4. Types of functional responses of adult females of three predatory phytoseiid mites to the spider mite, *Tetranychus urticae*, at different life stages.

Species of Predator	Stage of Prey	Response	Regression Equation	R ²
<i>N. calorai</i>	Egg	Type I	9.31x-10.3300	0.9530
		Type II	21.755 Ln(x)-3.2305	0.8386
	Larva	Type I	13.33x-10.5300	0.9656
		Type II	30.907Ln(x)-6.1331	0.8410
	Protonymph	Type I	3.09x-2.9900	0.9752
		Type II	7.4695Ln(x)-0.8720	0.9206
	Deutonymph	Type I	1.47x-1.75000	0.9523
		Type II	3.3908Ln(x)-0.5867	0.8185
	Adult	Type I	0.15x+0.1100	0.5461
		Type II	0.4011Ln(x)+0.1760	0.6308
<i>N. longispinosus</i>	Egg	Type I	14.8x-18.2000	0.9654
		Type II	34.348Ln(x) - 6.6885	0.8401
	Larva	Type I	3.65x-1.9300	0.9811
		Type II	9.1235 Ln(x)+0.2842	0.9903
	Protonymph	Type I	1.5x-0.0600	0.8538
		Type II	3.7628 Ln(x)+0.8371	0.8680
	Deutonymph	Type I	1.18x+0.8600	0.7391
		Type II	3.1318 Ln(x)+1.4013	0.8410
	Adult	Type I	1.9x-1.2600	0.7190
		Type II	4.4762 Ln(x)+0.1541	0.6446
<i>P. lenis</i>	Egg	Type I	10.74x-12.7000	0.9654
		Type II	25.003Ln(x)-4.4200	0.8452
	Larva	Type I	9.98x-11.7600	0.9737
		Type II	23.297Ln(x)-4.1270	0.8571
	Protonymph	Type I	3.03x-1.6100	0.9577
		Type II	7.4819Ln(x)+0.3161	0.9433
	Deutonymph	Type I	09x+0.1000	0.6136
		Type II	2.5229Ln(x)+0.3844	0.7790
	Adult	Type I	06x+0.1000	0.6716
		Type II	1.0647Ln(x)+0.3060	0.8352

The high consumption rate of both *N. calorai* and *P. lenis* on eggs and larvae of prey *T. urticae*, suggests that these predators are more effective against the prey at these stages. When adults are preferentially preyed upon, they are less effective since the pest prey may have already produced too many progenies or have already caused substantial injury to their host plant (Huffaker *et al.*, 1970). The ability of *P. lenis* and *N. calorai* to utilize all available prey at various stages, would indicate greater effectiveness of these predators than *N. longispinosus* which is primarily an egg feeder. However, because the latter feeds selectively on spider mites and resides more permanently on arboreal plant parts, it has more chances to encounter the pest mite. In contrast, *N. calorai* and *P. lenis* are generalist predators with broad prey range, including not only spider mites, but also acaroids found on both leaves and litter on the ground. Their being frequently collected in litter suggests that they are not strictly arboreal residents, limiting their chances to find spider mites. There is a need to study the prey preferences of these two voracious predators, as well as their behavior and performance, once released in the field.

SUMMARY AND RECOMMENDATION

The functional responses of *N. calorai*, *N. longispinosus* and *P. lenis* to different population densities of the egg and active stages of *T. urticae* were determined under laboratory condition. The predation tests consisted of rose leaf discs in Munger cells over a 24h period at $27 \pm 1^{\circ}$ C.

All three experimental predators are voracious predators of eggs of the foremost spider mite pest of premium crops, *T. urticae*. *N. longispinosus* is primarily an egg predator, whereas the two other phytoseiids feed almost equally on eggs and larvae of the prey. Consumption increased with increase in prey density until the response curves leveled off or the predator reached satiation point at prey density of around 40 eggs for *N. longispinosus*; between 40 and 50 larvae or between 30 and 40 eggs for *N. calorai*; about 30 eggs or larvae for *P. lenis*. Based on R^2 values both Type I and Type II curves provide good fit on the functional response data for the three predators.

Although *N. calorai* is the most voracious of the three predators, its preference for leaf-inhabiting spider mites and litter-inhabiting acaroids (like *Suidasia pontifica*) has to be evaluated vis-a-vis *N. longispinosus* which selectively preys on spider mites. The same is true for *P. lenis*, another generalist predator with wide habitat, ranging from plant foliage to ground litter, like *N. calorai*. The natural existence of generalist and specific predators in cultivated crop ecosystems points to the need for ecological approaches that can harness the complementary effects of these two types of predators rather than concentrating research efforts on just one of them. Although some progress has been achieved towards mass rearing the predators, and some biological information based on laboratory studies are now available for these predators, their field performance has yet to be evaluated on specific crop types.

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