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Prolonged laboratory rearing as it affects host preference and reproductive capacity of *Neoseiulus idaeus* Denmark and *Muma* (Acari: Phytoseiidae)

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ABSTRACT: Host preference and reproductive capacity of both feral and colonized strains of the Colombian biotype of *Neoseiulus idaeus* Denmark and Muma (Acari: Phytoseiidae) were studied in the laboratory and screehouse. Two tetranychid mites *Mononychellus tanajoa* Bondar (the cassava green mite, CGM) and *Tetranychus urticae* Koch (the two spotted mite, TSM) (Acari: Tetranychidae) were used as prey in potted plant, cassava leaf lobe and artificial arena experiments.

The numbers of feral and colonized strains of *N. idaeus* harvested from cassava leaves infested with CGM were significantly higher ($P < 0.05$) than those harvested from leaves infested with TSM. The rate of population increase of the feral strain was significantly higher ($P < 0.05$) than that of the colonized strain at all sampling times. Free-choice trials on field-infested leaves showed that CGM was preferred by both strains although it supported a higher non-significant population of the feral strain. Feral *N. idaeus* consumed more prey eggs than the colonized strain implying higher feeding capacity. A significantly higher number of feral adult females preferred washed eggs of CGM to that of TSM, while colonized *N. idaeus* females showed no preference. Reduction in prey preference and reproductive capacity of *N. idaeus* in the laboratory is suggested.

Key Words: Host preference; Reproductive capacity; Cassava green mite; Two spotted mite; *Neoseiulus idaeus*.

Introduction

Africa's attempt to control the rapid spread of the accidentally introduced cassava green mite *Mononychellus tanajoa* Bondar CGM (Acari: Tetranychidae) in the early seventies was centred on classical biological control. This involves using phytoseiid predators (1, 2, 3, 4, 5, 6, 7). The Colombian biotype of *Neoseiulus idaeus* Denmark and Muma (Acari: Phytoseiidae) was the first biological control candidate imported into Africa for the control of CGM. It came through the Africa-wide biological control programme of the International Institute of Tropical Agriculture, IITA (8, 9, 10, 11). Unfortunately, over six years after its maiden introductory release into the African cassava agroecology, no establishment of this biotype was recorded (12, 13, 14).

In establishing a laboratory population of phytoseiid predators, the founder's sample taken from a much larger field population undergo natural selection that affect the distribution of genes in the now-closed newly established laboratory colony (15). Individuals responding favourably to the new environment may be very small)pers. obs.), suggesting laboratory selection for increase in the frequency of certain genotypes while
decreasing the frequency of others (15, 16). This selection has in several cases affected host preference and reproductive capacity of colonized population (17, 18, 19).

Knipling (20) expressed concern that entomophagus arthropods reared on unnatural host change their host preference as a result of preimaginal conditioning that may reduce their effectiveness when released against the natural host. *Trichogramma semifumatum* showed a significant reversal by preferring eggs of its alternate host, *Sitotroga cereallella* over those of its natural host, *Trichoplusia ni*, when reared for > 100 generations on *S. cerealella*. The reproductive capacity which can be expressed in terms of female weight was also affected negatively in *Aphytis lingnannsis* (21) and *Encarsia formosa* (22) when reared on unnatural hosts *Aspidiotus lingnannsis* (21) and *Encarsia formasa* (22) when reared on unnatural hosts *Aspidiotus nerri* and *Aleyrodes proletella* respectively. They also had higher number of ovarioles implying higher parasitism rates. The action of such laboratory-reared organisms against their target pests when released in the field is therefore questionable especially if both the natural and unnatural hosts are present in the same agroecosystem.

Among other areas of research, it is therefore expedient that the host preference and reproductive capacity of the laboratory strain be studied and compared with the feral strain to determine whether laboratory rearing of the predator has led to its delayed establishment and yet unsuccessful control of CGM in Africa.

Materials and Methods

a. Preliminary experiments to determine the rate of CGM to TSM necessary for infestation

Twenty potted cassava (variety odongbo) plants about four weeks old were used, ten for each prey species. The experiment was carried out in the screenhouse at a fluctuating temperature of 23-35°C and relative humidity of 70-80%. The number of leaves per plant was reduced to one. Ten of the plants were infested with 50 *M. tanajoa* females and the remaining ten with 30 *T. urticae* females. This infestation ratio was determined after considering the intrinsic rate of increase (Tm) values of the prey species and by trial and error means. The numbers of different developmental stages per leaf were counted at two days intervals. Days after infestation with counts from both prey species having approximately equal tetranychid populations were used as reference infestation data.

b. Host preference and reproductive capacity of feral and colonized strains of N. idaeus

Three experiments were carried out in the laboratory to determine host preference of *N. idaeus*.

(1) On potted plants: Fourteen potted cassava (variety Odongbo) plants about four weeks old were used, seven for each strain of *N. idaeus*. The experiment was carried out in the screenhouse at a fluctuating temperature of 23-25°C and relative humidity of 70-80%. The number of leaves per plant was reduced to two each on opposite side of the main stem to which a leaf disc was glued. Individual phytophagous and predaceous mites were placed on the leaves and leaf disc respectively using a slightly moistened No. 000 camel hair brush.

Based on known differences in the intrinsic rate of natural increase (rm) among the Tetranychidae (23) and the results of preliminary experiments, ovipositing females of CGM and TSM, were released separately on each of the two leaves of the experimental plant at a ratio of 50:30, respectively. TSM with a higher rm was released 8 days after CGM. This ratio gives approximately equal numbers of different developmental stages of both prey species 8 days after the release of TSM (Table 1). To prevent mixing of prey species during the period of population increase, a barrier of wet cotton wool smeared with Tanglefoot(R) was

applied to the stream between the two leaves. When the two prey species reached approximately equal numbers (Table 1), the predator strains were released via the leaf disc glued to the main stem. Preference of each predator strain on both prey species was tested by releasing on each plant, 10 well-fed, mated females of either the colonized or feral strain. Thus seven replicates of each strain were set up. The barrier between the two leaves was removed after the predators' infestation to allow free movement of predators within the plant. Predators were prevented from escaping through the pot/soil by smearing tanglefoot close to the base of the plant. The number of adult female predators per leaf, number of predator juvenile actives per leaf and number of predator eggs per leaf was recorded every 24 hours. The experiment was terminated 96 hours after predators' introduction due to limited space for the different prey to increase and consequently shortage of food for the predators. Differences in the population of the two strains of the predator on the two prey species were used as a measure of preference.

Table 1: Number of different developmental stages of CGM and TSM (Mean ± S.E.) per cassava leaf, 16 and 8 days after release at a ratio of 50 CGM : 30 TSM ovipositing females, respectively.

	Egg	Juveniles	Adults
CGM	417.00 ± 10.50	364.90 ± 23.00	44.09 ± 2.98
TSM	546.09 ± 13.56	226.69 ± 19.60	38.94 ± 4.00

(2) **On cassava leaf lobes:** The experimental arena consisted of two leaf lobes from the first fully expanded cassava leaves of a 4 week-old plant (variety Odongbo) on wet cotton wool in a petri-dish. One of the two leaf lobes was heavily infested with 800 CGM individuals of the various developmental stages and the other with same number of TSM. The two leaf lobes were then separated by another fully expanded but clean and uninfested leaf lobe. Both prey and predators were prevented from escaping from the leaf lobes by using a pin to lift up the cotton wool along the leaf edges. The experiment was replicated ten times. In each replicate, ten young adult female *N. idaeus* of each strain were introduced onto the clean lobe midway between the infested lobes. The number of female predators per leaf lobe was recorded every two hours over a 10 hour period. This experiment was designed to determine the ability and the extent to which feral and colonized *N. idaeus* distinguish between the two prey species. In contrast to experiment 1, preference can be assessed within a shorter period.

(3) **On an artificial arena:** The experimental area consisted of an 11cm - diameter black polythylene sphere made from black trashcan liner material to ease observation. Eight 0.5cm diameter circles equidistant from one another were drawn with the aid of a compass along the circumference of the sphere. A one-centimeter circle was drawn at the center of the sphere, which served as predator's point of release. The sphere was then placed on wet cotton wool in a 29cm- diameter Petri dish. Twenty petri dishes were set up in this manner. 500 washed eggs of CGM and TSM were introduced into the small circles in an alternate fashion. Ten young well fed *N. idaeus* females were released onto the center circle of each arena, replicated 10 times for each predator strain. The experiment lasted five hours. The number of prey mite eggs consumed and the number of female *N. idaeus* per prey mite patch were recorded every 30 minutes.

Results

a. Preliminary experiments to determine the ratio of CGM to TSM necessary for infestation

The number of eggs produced by 30 TSM females every two days (136) was significantly higher ($P < 0.05$) than that produced by 50 CGM females (64) (Table 2). The development of TSM was much faster than that of CGM. Eggs of TSM had developed into adults by the 8th day while it took CGM about 11 days

to achieve this. Approximately equal numbers of the different developmental stages of the two prey species were observed on day 8 for TSM and day 16 for CGM. Webbing in the TSM-infested leaf was observed at the second day of the study and gradually become very dense as the experiment progressed. Sparse webbing in the CGM-infested leaf throughout the experimental duration shows the inability of this species to produce copious webbing.

Table 2: Population increase of CGM and TSM per cassava leaf after releases at a ratio of 50 CGM : 30 TSM ovipositing females respectively.

Day	TSM			CGM		
	Eggs	Actives	Adults	Eggs	Actives	Adults
2	136	-	30	64	-	50
4	291	-	30	124 -	-	50
6	394	110	30	189	56	50
8	**526**	**299**	**45**	257	109	48
10	685	386	57	340	167	48
12	816	451	80	385	224	46
14	959	567	97	450	287	44
16	1081	678	126	**513**	**310**	**46**
18	1224	1085	145	577	392	51
20	1367	1128	173	641	457	59

The bold underlined data (day 8 for TSM and day 16 for CGM) signified when populations of species were approximately equal.

b. Host preference and reproductive capacity of feral and colonized strains of N. idaeus

In all experiments, introduced female predators were seen moving within the different experimental area/arenas randomly. There was a time lag of 2 to 3 minutes before predators settled on a patch or leaf containing prey mites.

Experiment 1:

Table 3 shows the distribution of predator's eggs, juveniles and adults of colonized and feral strains of *N. idaeus* present in CGM- and TSM-infested leaves in the potted plant experiment. CGM supported and attracted significantly higher ($P < 0.05$) numbers of predators than TSM irrespective of strain.

At 24 hours after initial infestation (HAI) of *N. idaeus*, there were generally more predators on the leaf infested with CGM than on TSM-infested leaf regardless of strain (Table 3). The number of eggs (7.1) of the feral strain laid on CGM-infested leaf was significantly higher ($P < 0.05$) than that (1.0) on TSM-infested leaf. Similarly, significantly ($P < 0.05$) more eggs of the colonized strains (4.4) were laid on CGM-infested leaf than on the STM-infested leaf (1.3). A significantly higher number of feral *N. idaeus* adults' (6.6) moved to the CGM-infested leaf than the TSM-infested leaf (1.9). Although there was a higher adult population of the colonized strain on the CGM-infested leaf (4.3 adults) than the TSM-infested leaf (1.4 adults), this difference was not significant. Feral and colonized strains of *N. idaeus* exposed to the same prey species showed no significant differences in the number of eggs deposited and the number of adult

females (with the exception of feral females) present on these leaves. There were no juveniles of either strain during this period.

At 48 HAI, there was an increase in the number of eggs produced by both the colonized and the feral strains on the CGM- and TSM-infested leaves. Statistically the population remained the same as at 24 HAI. The number of eggs deposited by colonized *N. idaeus* on the CGM-infested leaf (8.3 eggs) was not significantly different from the number of eggs deposited on the TSM-infested leaf (3.3 eggs) by the same strain. However, the number of eggs deposited by the feral *N. idaus* on GCM-infested cassava leaves (15.4 eggs) was significantly higher ($P < 0.05$) than the number of eggs deposited by colonized *N. idaeus* (8.2 eggs) on the same prey species. There were significantly more feral adult females present on the CGM-infested leaf ($P < 0.05$) than on TSM-infested leaf. In contrast, however, colonized *N. idaeus* females present on CGM and TSM-infested leaves were not significantly different (Table 3).

Table 3: Number of (a) eggs; (b) juveniles, and (c) adults of the feral and colonized strains of *N. idaeus* recorded on TSM and CGM, 24, 48, 72 and 96 hours after introduction of each predator strain.

Predator strain	Prey species	Hours after introduction (Mean ± S.E.)			
		24	48	72	96
a) Eggs					
Feral	CGM	7.1 ± 1.2b	15.4 ± 2.3b	20.7 ± 3.2b	24.0 ± 3.2b
	TSM	1.0 ± 0.3a	3.3 ± 0.6a	5.6 ± 1.0a	7.7 ± 1.1a
Colonized	CGM	4.4 ± 1.2b	8.3 ± 2.2a	8.6 ± 2.0a	8.0 ± 2.7a
	TSM	1.3 ± 0.7a	3.3 ± 1.9a	2.6 ± 1.5a	3.6 ± 1.8a
b) Juveniles					
Feral	CGM	-	-	6.9 ± 1.2b	13.9 ± 2.7b
	TSM	-	-	0.9 ± 0.3a	2.7 ± 0.7a
Colonized	CGM	-	-	3.9 ± 1.0b	7.0 ± 2.5a
	TSM	-	-	1.1 ± 0.6a	1.3 ± 2.5a
c) Adults					
Feral	CGM	6.6 ± 0.6b	6.1 ± 0.7b	6.7 ± 0.4b	5.9 ± 0.5b
	TSM	1.9 ± 0.5a	1.9 ± 0.3a	1.7 ± 0.2a	2.0 ± 0.4a
Colonized	CGM	4.3 ± 1.2a	3.7 ± 0.9a	3.1 ± 0.7a	2.1 ± 0.6a
	TSM	1.4 ± 0.6a	1.4 ± 0.6a	2.0 ± 0.7a	2.4 ± 0.7a

Means (±,S.E.) followed by the same letter in a column are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test).

At 72 HAI, the juveniles were present for the first time. There were significantly ($P < 0.05$) more feral *N. idaeus* eggs (20.7), juveniles (6.9) and adults (6.7) on CGM-infested cassava leaves than on TSM-infested leaves (5.6 eggs, 0.9 juvenile actives and 1.7 adults). The population of colonized juveniles (3.9) on CGM-infested leaves was significantly higher ($P < 0.05$) than that on TSM-infested leaves (1.1). However, there was no significant difference in the population of feral and colonized strains of the predator when foraging on TSM-infested leaves. But the number of eggs (20.7) and adults (6.7) of the feral strains

were significantly higher ($P < 0.05$) than those of the colonized strain (8.6 eggs and 3.1 adults), respectively, when both were foraging on CGM-infested leaves (Table 3).

At 96 HAI, the total population trend of the feral strain remained the same as at 72 HAI. However, the trend in the population of the colonized strain was different. The population of the colonized strain on the CGM-infested leaf was considerably higher than that on TSM-infested leaf. The number of each stage: 8 eggs, 7 juveniles and 2.1 adults were however not significantly different from that on the TSM-infested leaf with 3.5 eggs, 1.3 juvenile actives and 2.5 adults, respectively (Table 3). In contrast, the number of each stage of the colonized strain was significantly lower than the feral strain counterparts when both foraged on CGM-infested leaves. Generally, as the experimental period increased, the number of adult females of both strains decreased.

The sum total of the various stages of each predator strain on CGM- and TSM- infested leaves showed that the population of the feral strain of *N. idaeus* was significantly higher ($P < 0.05$) than that of the colonized strain given the same prey at all times (Fig. 1). The feral population was 45, 63, 100, and 133% more in number at 24, 48, 72 and 96 hours, than that of the colonized strain when both strains fed on CGM. A corresponding but less dramatic population increase of the feral strain fed on TSM was also observed. The sum total of the feral strain of *N. idaeus* present in both CGM- and TSM-infested leaves was higher than that of the colonized strain (Fig. 2) giving an estimated growth rate index of 0.50 and 0.18 *N. idaeus*/leaf/hr. respectively from the slopes of the lines.

Experiment 2:

Table 4 shows the distribution of female *N. idaeus* of both strains foraging on leaf lobes infested with either CGM or TSM at different times. In general, the number of female predators of both strains was significantly higher on the CGM-infested than on TSM-infested leaf lobes after 8 hours. The number of predators from either strain was not significantly different on both CGM- and TSM-infested leaves during the first 8 hours of the experiment. Motile stages of TSM, particularly the adult females, were seen to be much faster and more aggressive than the corresponding CGM stages. Consequently "fights" between predators of both strains and such motile stages were observed more frequently on the TSM leaf lobes. Although the initial population of prey mites on each leaf were approximately equal, the CGM leaf lobes had to be replenished with more prey 4 - 6 hours after the start of the experiment as food became a limiting factor. Predators on these lobes were seen searching "vigorously" for food and began to disperse. A lot of CGM cadavers and empty cases were seen on the lobe.

Table 4: Numbers of feral and colonized strains of *N. idaeus* foraging on TSM and CGM infested cassava leaf lobes.

Predator strain	Prey species	Hours after introduction (Mean ± S.E.)				
		2	4	6	8	10
Feral	CGM	4.9 ± 0.74a	4.7 ± 0.54a	5.2 ± 0.49a	5.0 ± 0.54a	5.6 ± 0.53b
	TSM	3.7 ± 0.72a	4.2 ± 0.68a	4.3 ± 0.56a	4.5 ± 0.54a	4.5 ± 0.60a
Colonized	CGM	5.3 ± 0.42a	4.6 ± 0.4a	4.5 ± 0.45a	4.5 ± 0.4a	5.5 ± 0.37b
	TSM	4.1 ± 0.46a	4.7 ± 0.56a	4.6 ± 0.58a	3.5 ± 0.27a	3.1 ± 0.23a

N = 10; Means (±S.E.) followed by the same letter in a column are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test).

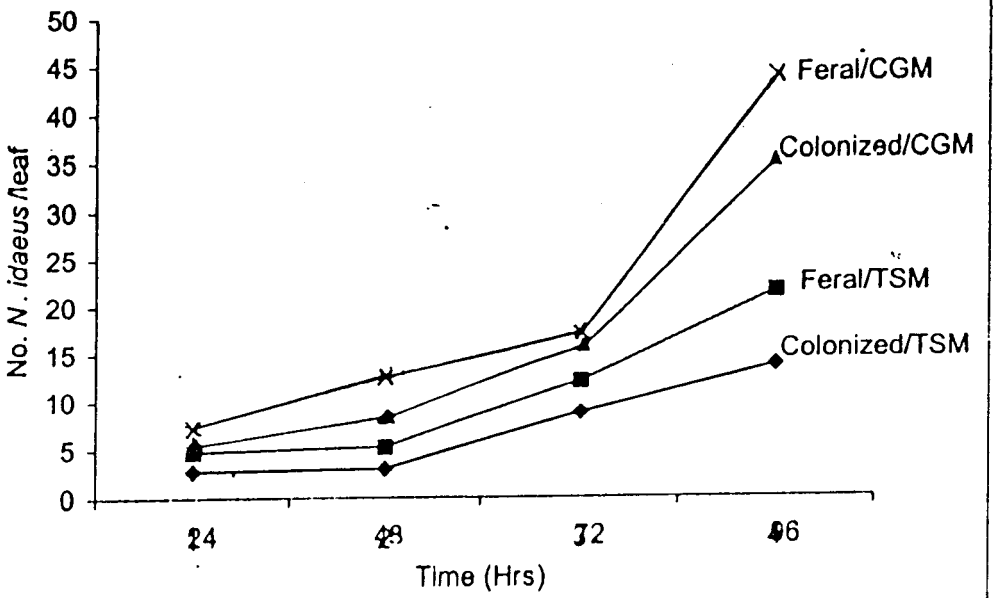

Fig. 1. Changes in the populations of feral and colonized strains of *N. idaeus* foraging freely on populations of TSM and CGM on a two-leaf potted cassava plant.

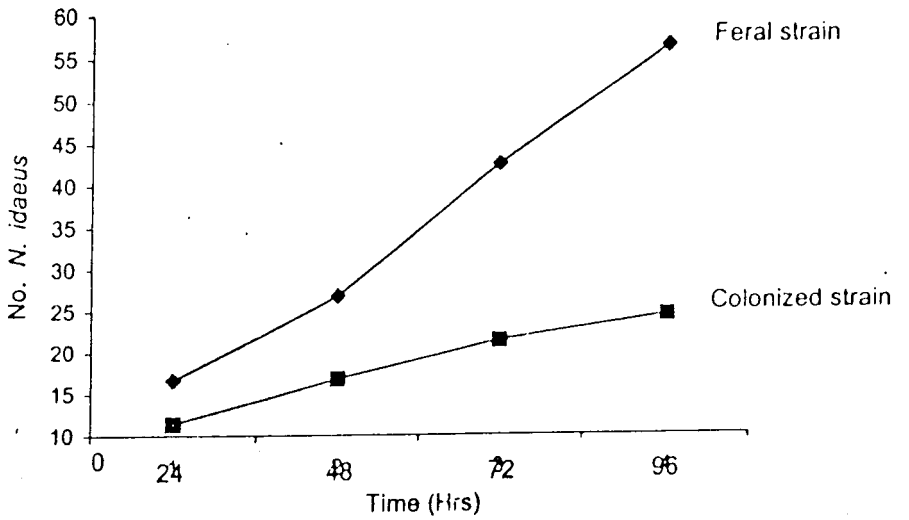

Fig. 2 Trend of population increase of feral and colonized strains of *N. idaeus*

Experiment 3:

Table 5 shows the distribution of female *N. idaeus* of both strains foraging on the artificial arena containing egg patches of both prey mites species. Significantly higher number of feral *N. idaeus* were seen on the CGM egg patch ($P < 0.05$) than on the TSM egg patch. Colonized *N. idaeus* however did not differentiate between the two prey species. Feral *N. idaeus* consumed significantly ($P < 0.05$) more CGM eggs than those of TSM while the colonized strain consumed approximately equal amount of both prey species (Table 6).

Table 5: Distribution of feral and colonized strains of *N. idaeus* foraging in the presence of CGM and TSM eggs on artificial arena.

Predator strain	No. *N. idaeus*	
	CGM	TSM
Feral	3.52 ± 0.08b	2.72 ± 0.10a
Colonized	2.96 ± 0.14a	2.86 ± 0.16a

Means (±S.E.) in a row followed by the same letter are not significantly different at $P < 0.05$.

Table 6: Number of CGM and TSM eggs consumed by feral and colonized strains of *N. idaeus* foraging on artificial arena.

Predator strain	No. of eggs consumed	
	CGM	TSM
Feral	44.0 ± 3.5b	25.0 ± 2.9a
Colonized	30.0 ± 4.0a	26.0 ± 3.7a

Means (±S.E.) in a row followed by the same letter are not significantly different at $P < 0.05$.

Discussion

The observed difference in the growth rate of CGM and TSM is similar to reports in literature (24, 25, 26, 27, 28, 29) regarding the various parameters that determine the growth rate of Tetranychidae. These include total fecundity, sex ratio and survivorship, which are higher for TSM than for CGM. TSM with higher capacity to increase should therefore be infested earlier than CGM if comparable populations within a time frame are to be achieved. Initial infestation of 30 TSM females and 50 CGM females gave approximately equal number of the various developmental stages of TSM and CGM 8 and 16 days respectively after infestation. In other words, infesting 50 CGM females 8 days before infesting 30 TSM females will produce comparable populations of both species 8 days later. The difference in timing adequately accounts for the 1.5 times difference in reproductive potential.

It was apparent from Fig. 1 that prey species is important in *N. idaeus* preference. Both strains of *N. idaeus* generally preferred CGM but to different degrees. Similar results were recorded for a laboratory strain of *N. idaeus* in a mixed culture of *M. tanajoa* and the red spider mite *Tetranychus talrius* where it was found that *N. idaeus* preyed more readily on CGM (30). The higher degree of preference for CGM exhibited by the feral strain in all experiments could be attributed to the fact that CGM is the primary food source of *N. idaeus*. The colonized strain having been reared exclusively on TSM eggs for several generations may have lost a substantial degree of host specificity for its primary prey.

The capacity for increase by the feral strain was higher than that of the colonized strain (45-133%) (Fig. 2). Colonization of *N. idaeus* seemed to have led to a decrease in its reproductive capacity which is contrary to the aim of general laboratory rearing programmes that emphasize, among other attributes, higher intrinsic rate of natural increase for the laboratory strain (31). This apparent decrease in reproductive capacity may be due to the prey species supplied for mass rearing. It appears that CGM, as a food source is better for enhanced reproductive response in *N. idaeus* than TSM. Dicke *et al.*, (19) reported that *Amblyseius potentillae* ovipositing females reared on an alternative food, *Vicia faba* pollen, had lower predation rates and hence lower reproduction than those reared on its main food. *T. urticae* eggs or other spider mite species such as *Panonychus ulmi* and *A. schiechtendali*. This change was attributed to the diet used to mass-rear the predator in the laboratory, supplied in such a manner that the encounter rate and successful catch ratio could have been affected.

Changes in the foraging ability of the colonized strain could be responsible for the smaller populations recorded. This strain had for over 70 generations in optimal environmental conditions free of competition been exposed to ample amount of prey mites where its searching capacity is seldom put to use. Selection of poor genotypic individuals with limited foraging traits such as searching capacity and handling ability could have resulted during colonization. This may have been the result of a possible decrease in genetic variability of the colonized strain (32, 33).

Another parameter that may affect *N. idaeus* preference for host prey may be prey density. Results at 96 HAI (Table 3) showed that although *N. idaeus* generally preferred CGM, at low density of this prey, experienced as the experiment progresses, the level of significance between the two predator populations decreased. The increasing predator population on TSM-infested leaf as the CGM population on the other leaf declined was probably due to predator emigration from an area where food was scarce. This was true for both feral and colonized strains and this is of interest because TSM and other tetranychids in the cassava agroecosystem could keep predator population going at periods of CGM scarcity. Similar results have been reported for the Brazilian biotype of *N. idaeus*. This biotype continued to be recovered from syrrounding weeds with high population of TSM, *Oligonychus gossypii*, plant pollen and exudates when no recoveries were made on cassava during the wet season when CGM in the agroecosystem is very scarce (IITA Biological Control Database).

Decrease in the number of adult females as the experiment progressed was due to three major factors. Dead predators were noticed on the leaves, tangle foot and stem of the cassava plant. These, however, did not account for the total number of disappearances recorded. About 3% of all disappearances of both strains of *N. idaeus* were probably due to fall off from the leaf abaxial surface onto the screenhouse floor. Increasing predator population and decreasing prey population probably caused emigration from the leaves. Kuchlein (34) reported increased emigration at high predator densities. A kairomonal cue to feed on CGM as compared to TSM is questionable since the predators' choice of washed prey mite eggs (presumably without surface kairomones as a result of washing by agitation) were significantly different.

CGM or other *Mononychellus* species appear to be the preferred prey for *N. idaeus* but this preference seemed to be affected when this predator was reared on the alternate host TSM for a prolonged period in the laboratory.

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