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Releasing of the Predatory Mite, *Neoseiulus californicus* (McGregor) for Controlling the Citrus Red Mite, *Panonychus citri* (McGregor)

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ABSTRACT

To determine the most convenient releasing level of the predatory mite, *N. californicus* for reducing numbers of the citrus mite, *P. citri*. L. an area with dimension 65.5 × 35 meter cultivated with 70 trees of Navel orange was chosen for the experiment. The treated lines were divided to three groups, each group contained two lines, the first was for low releasing of the predatory mite, *Neoseiulus californicus* (75 females and 25 males/ tree) and the second for high releasing treatment (150 females and 50 males/ tree). The first and second groups were assigned to one and twice releases, respectively; while the third group for three. One line was left without releasing, as control, located in the area left side. Two lines were left, to isolate between the three-treatment groups and one line to isolate the treatment groups from the control line. Results demonstrated the ability of *Neoseiulus californicus*, to regulate *Panonychus citri* populations. Moderate reduction of *Panonychus citri* was obtained from predator single release but didn't prevent the pest from exceeding the economic threshold level. Multiple releases with rates 100 predator females and 50 males/tree were the best for suppressing *Panonychus citri* population and preserving it from exceeding the economic threshold level.

Key words: Release; *Neoseiulus californicus*; Biological control; Citrus red mite; *Panonychus citri*.

INTRODUCTION

The tetranychid *Panonychus citri* (McGregor) is one of the most important pests that attack citrus in many parts of the world (Gotoh and Kubota, 1997; Jamieson *et al.*, 2005; Childers *et al.*, 2007; Gotoh *et al.*, 2003 and Hui De *et al.*, 2004). In addition to citrus trees, the genus *Panonychus* infests over 80 plants species, including rose, almond, pear, castor bean, and several broadleaf evergreen ornamentals (Bolland *et al.*, 1998 and Zhang, 2003). Spider mites such as the citrus red mite may be important outdoor allergens among children living in rural areas (Kim *et al.*, 2001). It usually infests upper leaf surfaces around the mid rib and when damage increases; leaves may bleach or burn at the tips and drop. On fruit, it causes stippling and later silvering on the rind of mature oranges and lemons. However, peel stippling does not hurt the quality of the fruit inside (Kranz *et al.*, 1977; Tan *et al.*, 1989; Knapp *et al.*, 1996 and Childers *et al.*, 2007).

P. citri is polyphagous; but citrus is its major host (and even there, it is not serious CABI, (2007). If populations are low, *P. citri* is mainly found in the upper parts of trees, where there is strong sunlight; but found over the entire tree at high infestation (Izquierdo *et al.*, 2002).

P. citri and associated predaceous phytoseiid mites occurring on citrus were well documented by McMurtry, 1985; but did not include species commonly found in western Oregon (Hadam *et al.*, 1986). For example, *Euseius* sp. is most common on citrus but is of semi-tropical and tropical distribution (McMurtry and Croft, 1997).

Mites of the family Phytoseiidae are widely utilized because of their potential as biological control agents of phytophagous mites and, more recently, of thrips on various crops (McMurtry, 1983; Wood *et al.*, 1994; Heikal *et al.*, 2004; Jamieson *et al.*, 2005 and Arthurs *et al.*, 2009). *N. californicus* has proven its potential as biological control agent of red spiders and eriophyid mites in Egypt (Mowafi *et al.*, 2009 and Ebrahim *et al.*, 2013). In addition, it appears to act as a generalist tetranychid predator and can initiate multiple attacking behaviors. It can survive periods of starvation in the laboratory (Xiao and Fadamiro, 2010) and tolerate high field temperatures (McMurtry and Croft, 1997). Moreover, it is highly active with high prey searching efficiency (Pratt and Croft, 2000 & Blackwood *et al.*, 2001) and adapted to disturbed habitats, such as intensively-managed orchards (McMurtry and Croft, 1997). In addition, it proved its effectiveness as an important biological control agent against *P. citri* (Ebrahim *et al.*, 2013). Also, it showed effective maintainance at low densities (< 1.5 motiles per leaf) when applied 2 releases of *N. californicus* or *P. persimilis* at a rate of 200 per tree with each release (Fadamiro *et al.*, 2013). Thus, this study aims to determine the most convenient releasing level of the predatory mite, *N. californicus* for reducing the citrus mite, *P. citri* population.

MATERIALS AND METHODS

Land area with 70 trees of Parent Washington navel orange with dimension 65.5 × 35 meter, was chosen for this experiment at Gharbia governorate. The area was divided into 10 equal lines each of 7 trees. Six lines treatments were applied for releasing the predatory mite, *N. californicus* and divided into three groups. Each group contained two lines, the first line was left for low releasing treatment (75 females and 25 males/ tree) and the second for high releasing treatment (150 females and 50 males/ tree). The first and second groups were assigned to once and twice releases, respectively; while the third group for three releases. One line was left without releasing (as control), located in the area left side. Two lines were left, to isolate between the three-treatment groups and one line to isolate the treatment groups from the control line.

The predator individuals were collected in gelatin capsules (0.5 – 1.5 cm) by using a special vacuum pump. The predator individuals were released on its own field line by opening the gelatin capsules and pasting (by stick glue) the separated capsule for each 15-20 leaflet/each branch tree. Seventy five capsules belonged to low releasing treatment, 50 capsules had 1 female in each and 25 capsules had 1 female and 1 male in each. For high releasing treatment, 150 capsules had 1 female in each and 50 capsules had 1 female and 1 male in each. The capsules were put in assigned Petri-dishes and transferred to field with Ice-box.

Randomized samples of 20 leaflets/tree were collected just before every release and then biweekly, where the first sample was considered as the pre-count and the second one as the first post-count and this was repeated with the subsequent samples.

RESULTS AND DISCUSSION

The effect of releasing the predatory mite, *N. californicus* with single, double and multiple releases at low and high release treatments are presented in table 1. The mean number of citrus red mite, *P. citri* was generally low in the pre-count (just before the predator release) on 16th of February 2015, ranged between 3.7 and 7.0 different stages/replicate. The first post-count (time of second release for double and multiple releasing treatments groups) showed increase of the mean number of *P. citri* to reach 4.4, 5.5, 5.3, 7.4, 5.6 4.8 and 14.8 different stages at, low single releasing, high single releasing, low double releasing, high double releasing, low multiple releasing, high multiple releasing and no releasing treatments groups, respectively.

The second post-counts (time of third release for multiple releasing treatments groups) and third post-counts showed increase of group A (single release treatment) of the mean number of *P. citri* to reach 7.1 and 8.2 different stages at low single release group and 8.7 and 8.0 different stages at high single release group, respectively. On the contrary, it showed decreases for groups B and C of the mean number of *P. citri* down to 4.5 and 3.5 different stages at low B group, 3.6 and 2.9 different stages at high B group, 4.0 and 1.2 at low C group and 2.7 and 0.4 different stages at high C group, respectively.

Meanwhile, the fourth post-count showed increase of the mean number of *P. citri* for low A, high A and low B groups to reach 11.3 10.9 and 4.0 different stages, respectively. This decline in high B, low C and high C groups remained steady and reached 1.7, 0.9 and 0.8 different stages, respectively.

There were no changes for nonreleasing treatment group, which showed rising increases for the number of *P. citri* different stages, which reached 64.8 different stages at the fourth post-count.

Reduction of *P. citri* population at the first post-count reached 48.4%, 50.8%; 58.5%, 54.4% and 57.6%, 65.4% at low (single release), high (single release), low (double release), high (double releases) and low (multiple releases) and high (multiple releases), respectively. These values increased gradually at next post-counts to reach 69.8%, 77.8%; 92.8%, 97.6% and 98.4%, 98.6% (at the fourth post-count) on the previous groups, respectively. Low mean numbers of the predatory mite, *N. californicus* were found in different releasing groups at the first and second post-counts. Its mean numbers /replicate at the first post-count were 0.1, 0.4; 0.0, 0.6 and 0.1, 0.5 at low and high single release, double releases and high multiple releases groups, respectively. The mean number of the predatory mite, *N. californicus* increased at the fourth post count to reach 1.3& 1.8; 3.2 & 3.5 and 3.8 & 5.2 at the aforementioned releasing groups, respectively.

Repeated measures MANOVA showed significant effects of sampling dates treatment interaction (Wilks' λ = 0.077, DF= 25, P< 0.0001), on the numbers of *P. citri*. Analyzed the data by sampling date utilizing one-way ANOVA (Table 2), showed no significant differences in the number of *P. citri* within treatments at pre-count (date of first release) and first post-count (date of second release for B and C groups). Meanwhile, significant differences (decreases) were found in the number of *P. citri* within treatments at second post-count (date of second release for groups C). Moreover, high significant differences (decreases) were recorded at third and fourth post-count. In general, *P. citri* densities were significantly higher in the control than in the releasing treatments on most of the sampling dates.

N. californicus seemed to be a successful biocontrol agent of *P. citri* and was effective in applying multiple releases as a one of the integrated pest management. Moreover, high *P. citri* reduction

Table (1): Release of *N. californicus* to control *P. citri* on citrus trees

Date	Release	Treatments	Mean no. of *P. citri*/replicate	Reduction of *P. citri*	Number of *N. californicus* MS	Eggs	Total	Mean Air temp. °C	Mean R.H. %
*16 Feb. 2015	Single release group (A)	Low	3.7	--	--	--	--	17.1	66.8
		High	4.9	--	--	--	--		
	Double release group (B)	Low	5.5	--	--	--	--		
		high	7.0	--	--	--	--		
	Multiple release group (C)	Low	5.7	--	--	--	--		
		high	6.0	--	--	--	--		
	No releasing (Control)		6.4	--	--	--	--		
**2 Mar, 2015	Single release group (A)	Low	4.4	48.4	0.1	0.0	0.1	18.5	53.1
		High	5.5	50.8	0.0	0.4	0.4		
	Double release group (B)	Low	5.3	58.5	0.0	0.0	0.0		
		high	7.4	54.4	0.5	0.1	0.6		
	Multiple release group (C)	Low	5.6	57.6	0.0	0.1	0.1		
		high	4.8	65.4	0.2	0.3	0.5		
	No releasing (Control)		14.8	--	--	--	--		
***16. Mar, 2015	Single release group (A)	Low	7.1	52.6	0.1	0.0	0.1	18.9	56.1
		High	8.7	56.0	0.2	0.1	0.3		
	Double release group (B)	Low	4.5	79.8	0.4	0.5	0.9		
		high	3.6	87.3	0.9	0.6	1.5		
	Multiple release group (C)	Low	4.0	82.8	1.1	0.9	2.0		
		high	2.7	88.9	1.9	0.4	2.3		
	No releasing (Control)		25.9	--	--	--	--		
****30 Mar, 2015	Single release group (A)	Low	8.2	57.4	0.4	0.2	0.6	20.8	51.3
		High	8.0	68.5	0.3	0.8	1.1		
	Double releases group (B)	Low	3.5	87.8	0.6	0.7	1.3		
		high	2.9	92.0	1.3	0.4	1.7		
	Multiple releases group (C)	Low	1.2	96.0	2.4	0.9	3.3		
		high	0.4	98.7	3.9	0.6	4.5		
	No releasing (Control)		33.4	--	--	--	--		
*****13 April, 2015	Single release group (A)	Low	11.3	69.8	0.9	0.4	1.3	22.3	51.6
		High	10.9	77.8	1.2	0.6	1.8		
	Double releases group (B)	Low	4.0	92.8	1.9	1.3	3.2		
		high	1.7	97.6	2.6	0.9	3.5		
	Multiple releases group (C)	Low	0.9	98.4	2.1	1.7	3.8		
		high	0.8	98.6	2.8	2.4	5.2		
	No releasing (Control)		64.8	--	--	--	--		

Low= 50♀+25♂ (1♀/20leaves+ 1♂/40leaves) predators/tree, High = 100♀+50♂ (1♀/10leaves + 1♂/20leaves) predators/tree.
MS = Moving stages. *Pre-count - time of 1st release for single, double and multiple release group
**First post-count - time of 2nd release for double and multiple release group ***Second post-count - time of 3rd release for multiple release group
****Third post-count *****Fourth post-count

Table (2): One-way anova values for the number of *P. citri* on citrus trees released with single, double and mutable release of low and high numbers of the predatory mite, *N. californicus*

Dependent Variable	F	df	Sig.
Precount	1.631403	5	0.176685
first post count	1.166431	5	0.344522
second post count	4.971812	5	0.001458
third post count	16.58294	5	0.000000
fourth post count	30.11124	5	0.000000

could be obtained when *N. californicus* was released on trees with moderate initial prey densities below 7 different stages per leaf (1.4 moving stages of *P. citri*/leaf). At single release, *N. californicus* did not prevent *P. citri* from exceeding the economic threshold level (5 motiles/leaf as proposed by Childers *et al.* (2007) along 8 weeks of the entire experiment duration. This is because the intrinsic rate of natural increase of *N. californicus* is lower than that of *P. citri*. These differences may be attributed to presence of certain substances, probably hormones or with hormone like effect, which suppress fertility and laying eggs with *P. ciri* (Ebrahim *et al.*, 2013). In addition, the high single release of *N. californicus* did not provide an advantage in suppressing *P. ciri* over the low single release. The double and multiple releases of *N. californicus* provided higher reduction rate of *P. ciri* and kept its population below the economic threshold level. Although the low and high single releases of *N. californicus* provided convenient reduction rate reached 69.8% and 77.8% at the end of our experiment, respectively; however these could not prevent *P. citri* from exceeding the economic threshold. Low or high multiple release of *N. californicus* at the rate of 75 (50 females and 25 males) and 150 (100 females and 50 males) provided the highest reduction rates and kept the mean population of *P. citri* below one different stage per leaf. This disagreed with that obtained by Fadamiro *et al.* (2013) who reported that with 2 releases of rates 100 or 200 per tree for each release of *P. persimilis* or *G. occidentalis*, (which substituted with *N. californicus in* Fadamiro's experiment) on trees with ≥ 5 motiles per leaf of *P. citri*, were not promising for adequate suppression of *P. citri* below the economic threshold. This could be attributed to the different weather factors in either of this experiment or Fadamiro's experiment, which affected the rapid suppression of *P. citri*. Temperature affects prey consumption, generation time, oviposition and longevity (Pruszynski, 1976; Sabelis, 1981; Shaw, 1982 and El Taj and Jung, 2012). On the other hand, the number of deutonymphs devoured by the most voracious stage generally increased as temperature increased. In addition, the male to female ratio of *N. californicus* was highest (0.77) at 25°C (El Taj and Jung, 2012). Worthwhile, the releasing distribution methods should have supreme attention as this might be due to that *Panonychus* species produce less webbing than genus *Tetranychus*, which may have adverse effect on *N. californicus* dispersal in finding and elimination the citrus red mite population (Schmidt, 1976; Sabelis, 1981 and Sabelis and Bakker, 1992). In addition, *N. californicus* may be like *P. persimilis*, which is attracted *by* volatiles (synomones) produced by leaves infested with *T. urticae* (Bruin *et a1.*, 1992 and Dicke *et al.*, 1993). This might be the case in our study where lesser volatiles were produced by the low numbers of *P. citri* enrolled, thus affecting the number of attracted and dispersed *N. californicus*. This point could be solved by releasing predator 1 female / 20 adjacent leaves (at prey density 7 deferent stages/leaf) and in addition, conducting multiple successive predators releases on non-inoculated leaves not previously inoculated.

The dynamics of citrus red mite population seemed sensitive and correlate with weather changes, at the end of April and beginning of May as temperature raised to reach 40.5°C in some days. This led to citrus red mite population number suppression in control and treatment. This may be attributed to high population number of *P. citri* mortality, which reached 75.8% at 35°C (Ismail, 2009). In addition, it worth to mention that *N. californicus* eggs are incapable of hatching at 37.5°C, although females oviposit (Gotoh *et al.*, 2004).

In conclusion, our results demonstrated the ability of the commercially available *N. californicus*, to regulate populations of *P. citri*. Moderate reduction of *P. citri* was obtained from the single release of *N. californicus* but did not prevent *P. citri* from exceeding the economic threshold level. Multiple releases with rates 100 females and 50 males of the predatory mite, *N. californicus* was the best for suppressing *P. citri* and preserving it from exceeding the economic threshold level.

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