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Prey Suitability of *Tuta absoluta* Larvae (Lepidoptera: Gelechiidae) for Three Predatory Phytoseiid Mites (Acari: Phytoseiidae) Under Laboratory Conditions

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Tomato crop in the Mediterranean Basin has been recently affected by the exotic pest *Tuta absoluta* (Meyrick), which is difficult to control due to its high reproduction rate and potential to develop resistance to insecticides. In this paper, the suitability and effectiveness of three predatory phytoseiid mites *Cydnoseius negevi* (Swirski and Amitai), *Neoseiulus barkeri* (Hughes) and *Amblyseius largoensis* (Muma), an indigenous species, were evaluated on larvae of *T. absoluta* under laboratory conditions. First instar larvae of *T. absoluta* proved to be possible food source for tested phytoseiid mites under laboratory conditions.

Females of *C. negevi*, *A. largoensis* and *N. barkeri* were able to feed and sustain oviposition on unfed, first instar larvae of *T. absoluta.* A diet of insect larvae provided the shortest oviposition period and adult longevity of *C. negevi* and *A. largoensis,* while *N. barkeri* showed the longest corresponding periods. The total and daily number of insect larvae consumed was significantly higher in *N. barkeri* than in *A. largoensis* and *C. negevi*. Likewise, *N. barkeri* laid significantly higher number of eggs (23.6 eggs / female) than that deposited by *C. negevi* and *A. largoensis* (2.5 and 3.9 eggs / female). The sex ratio of the progeny was female biased and ranged: (females / total = 0.62–0.68%) when insect larvae were provided for females of *C. negevi*, *A. largoensis* and *N. barkeri*.

Keywords: Acari, Phytoseiidae, *Tuta absoluta*, Lepidoptera, Gelechiidae, biological control.

The use of biological control strategies against arthropod pests in solanaceous has been proven to be effective and reliable (van Lenteren, 2012). Currently, the most important challenge that biological control is facing the continuous invasion of exotic pest species into new region. In this context, indigenous natural enemies may play a key role in developing biocontrol strategies against new pests (Ehler, 1998; Messelink et al., 2012). Generalist predators are able to use various food resources (such as alternative prey or plant material), in order to establish their populations prior to pest infestation, resulting in

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crop system that are more resilient to pest invasions (Messelink et al., 2012; Pina et al., 2012). However, generalist predators are increasingly used for biocontrol.

In the Mediterranean Basin and Europe, the tomato crop has recently been affected by the new exotic pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which is able to cause serious yield losses with its larval feeding activity (Desneux et al., 2010). Through their feeding activity within the mesophyll of the leaves, the larvae produce large mines, thus affecting the plants photosynthetic capacity; furthermore, they burrow into apical buds and fruits (Ferracini et al., 2012). *Tuta absoluta* is a very challenging pest to control by chemicals because of the mine-feeding behavior of larvae, in addition to the resistance developed to many conventional insecticides and the side effects for useful organism in Integrated Pest Management (IPM) programs (Lietti et al., 2005; Cabello et al., 2009).

Research on predators and parasitoids able to control tomato borer has been carried out throughout the Mediterranean area. Several species of predators, such as, *Macrolophus pygmaeus* (Rambur), *Nesidiocoris tenuis* (Reuter) (Hemiptera: Nabidae) (Urbaneja et al., 2009), as well as parasitoids such as *Agathis fuscipennis* (Zetterstedt) and *Bracon nigricans* Szepligeti (Hymenoptera: Braconidae) (Ferracini et al., 2012; Biondi et al., 2013).

A predatory mite has received attention as biological control agent for *T. absoluta*. de Oliveira et al. (2007) indicated that the mite *Pyemotes sp.* (Acari: Pyemotidae) can be a new alternative for biological control of *T. absoluta* since the caterpillars and adults of the pest could host many *Pyemotes sp*, physogastric females, which were allowed to grow on the moth. Recently, females of *Neosiulus barkeri* (Hughes), *Amblyseius largoensis* (Muma) and *Cydnoseius negevi* (Swirski and Amitai) (Acari: Phytoseiidae) were preyed well on *T. absoluta* eggs and all were able to sustain oviposition (Momen et al., 2013).

Studies on the nutritional value of moth's eggs and 1st caterpillars have been reported on phytoseiids ex: nymph's of *A. largoensis* preyed well on the moth's eggs, *Prays citri* Milliere and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Yponoeutidae and Pyralidae) with a high percentage reaching maturity; also nymphs fed occasionally on 1st instar larvae of both insects (Kamburov, 1971).

The predatory phytoseiid mites, *N. barkeri*, *C. negevi* and *A. largoensis* are native natural enemies that spontaneously appear in various crops in Egypt (Momen, 1995; Abou-Awad et al., 1998; Momen et al., 2009; Momen et al., 2013).

Our laboratory observations designated three phytoseiid mite species among several mites associated with tomato plants, of which can feed on larvae of *T. absoluta;* since these species were seen grasping the 1st instar larvae soon after hatching eggs and before their way to penetrate the mine.

Thus, the objective of this study was to evaluate whether the observed co-occurrence of *N. barkeri*, *C. negevi* and *A. largoensis* and *T. absoluta* eggs and 1st instar larvae was just occasional or whether the latter could be important as food source for the former [especially all females of above phytoseiid mites were proved to be an efficient predators of *T. absoluta* eggs (Momen et al., 2013)], assumed by laboratory evaluation of the ability of these predatory phytoseiid mites to consume and reproduce on 1st instar larvae of *T. absoluta* under laboratory conditions. In particular, the reproductive potential and consumption rates of the phytoseiid predatory mites were evaluated and compared under laboratory conditions using 1st instar larvae of *T. absoluta*.

Materials and Methods

All species tested (*C. negevi*, *N. barkeri* and *A. largoensis*) were indigenous in the Egyptian fauna and common on leaves and in debris of tomato plants at Giza Province.

Maintenance of mite stock cultures

Adult females of *A. largoensis* and *N. barkeri* were taken from stock colonies maintained on larvae and nymphs of *Tetranychus urticae* Koch (Acari: Tetranychidae) as prey in the laboratory of (N R C) since 2011. Females of *C. negevi* were fed pollen of castor bean *Ricinus communis* L. (Euphorbiaceae) in the laboratory. Feeding experiments were piloted in the laboratory at 30 ± 2 °C and 70–75% RH.

The rearing substrate was detached leaves of copper leaf *Acalypha wilkesiana* cv. *Marginata* Muell. (Euphorbiaceae), placed with upper down on water-saturated cotton in plastic Petri dishes. Water was added daily to keep the cotton saturated. Whenever a leaf began to deteriorate (approximately every 5 days), it was replaced with a fresh one.

Insect material

A colony of *T. absoluta* was established with pupae and caterpillars collected in tomato (var. G.S.N.) commercial plantation at Giza Province, Egypt. Females and males were maintained in nylon cage (100×60×70 cm), fed with a 10% honey solution and provided with tomato terminal buds and leaves for egg laying overnight. Leaf petioles containing eggs were kept inside vials with water. The vials were kept in a tray containing a 1 cm layer of sterilized sand, as *T. absoluta* pupation may occur on different parts of the plant but also in the ground. Pupa was then transferred to the adult cage (for more details on processing tomato plants and rearing insect, see Momen et al., 2013).

Plants were removed after 24 h and eggs were collected under a stereoscopic binocular microscope with a small brush and placed on healthy tomato stem sealed with parafilm on both sided (since first larval instars is difficult to produce mines in stem) positioned at top of copper leaf acalypha, placed on water-saturated cotton in Petri dishes. Four to 5 days later, all eggs started to hatch the 1st instar larvae, so easy to be collected. All larval instars of *T. absoluta* frequently abandon the mine to penetrate new galleries (Fernandez and Montagne, 1990); therefore, larvae could be collected easily from the colonies also.

Experimental procedure

Arenas (4×4 cm) of copper leaf acalypha were placed on upper surface down on sponge pads soaked in distilled water in Petri dishes; the margin of each disc was covered with a band of cotton wool to prevent insect larvae and predatory mites from escaping. Water was added daily to the Petri dishes to maintain the sponge pad soaked.

Effects of Tuta absoluta *first instar larvae on the development and survival of three phytoseiid mites*

Eggs of each predator were transferred singly to the rearing discs, and the newly hatched phytoseiid larvae (0–24 h) were kept on the arenas till they developed to next stages (all three tested species are non-feeding larvae). Insect larvae were gently transferred under a steromicroscope with the aid of a thin brush onto copper leaf for a total of 2 larvae per leaf. Arenas were examined daily and predator development and survival were recorded. Prey larvae consumed were replenished once per day. Survival and developmental progress was assessed twice per day in 8-h and 16-h intervals until the mites reached adulthood or died. Developmental progress was determined by presence of the shed skin of the proceeding stage.

Effects of Tuta absoluta *first instar larvae on adult longevity, consumption rate and fecundity of three phytoseiid mites*

Newly-emerged mated females of each predator, starved for 24 h, were confined individually on test arenas, 1st instar larvae was exposed to predatory females of each species. The larvae were gently transferred under a steromicroscope with the aid of a thin brush onto copper leaf for a total of 5 larvae per leaf.

Every 4–5 days, a new male was introduced into each arena (for each predator) for repeated mating. Phytoseiid eggs was counted and removed daily in order to estimate fecundity. Progeny were reared to adulthood to determine the sex ratio. All larvae were replaced on a daily basis, regardless of their status. Larvae were considered consumed if they were shriveled or shrunken. Arenas were checked daily and the following recorded: number of live larvae, prey consumed, phytoseiid eggs, duration of adult longevity, non-consumed dead larvae and predator mortality. Experiments lasted until all individuals of the original cohort died. Whenever a leaf began to deteriorate (approximately every 3 days), it was replaced with a fresh one.

Statistical analysis

A total of 17 individuals of each species were tested in our experiments, but 15–16 individuals (replicates) only of *N. barkeri*, *T. negevi* and *A. largoensis* per *T. absoluta* larvae were analyzed using 1-way ANOVA; the treatment means were compared by Tukey HSD at a 5% probability level.

Result

Effect of Tuta absoluta *first instar larvae on the development and survival of three phytoseiid mites*

All phytoseiid larvae of various species developed to protonymphs without feeding but none of all individuals were able to feed and develop beyond the protonymphal stage and never reached the deutonymphal stage.

Effects of Tuta absoluta *first instar larvae on adult longevity, consumption rate and fecundity of three phytoseiid mites*

The preoviposition period was significantly shorter for *N. barkeri* than *C. negevi* and *A. largoensis* (ANOVA: $F = 52.18$, $df_{2,46}$, $p = 0.000$). Likewise *N. barkeri* had significantly longer oviposition period (20.43 ± 0.31 days) than *C. negevi* and *A. largoensis* (7.31 ± 0.72 and 9.20 ± 0.78 days), respectively (Table 1). *Amblyseius largoensis* and *C. negevi* had significantly shorter adult longevity than *N. barkeri* (ANOVA: $F = 37.52$, $df_{2,46}$, $p = 0.000$). The number of tomato borer larvae consumed was only statistically higher with *N. barkeri* than larvae eaten by *A. largoensis* and *C. negevi* (Table 1). The total eggs production and the average daily egg production per female of *N. barkeri*, *C. negevi* and *A. largoensis* showed highly significant difference (ANOVA: $F = 1090.06$, 309.63; $df_{2,46}$, $P = 0.000$) (Table 1). Sex ratio was in favor of females for phytoseiid mites tested. When considering the oviposition periods for female predators, *N. barkeri* quickly attained peak consumption rate and oviposition rate (Fig. 1).

Discussion

Mites used in lab experiments are generalist predators that feed on a variety of organisms, including mite and insect eggs (Abou-Awad et al., 1998; Momen and El- Laithy, 2007), thrips (Bonde, 1989) and whitefly (Momen et al., 2013). Under laboratory conditions, there were clear differences in the acceptability and suitability of the tomato leaf miner for the mite taxa examined. The generalist-feeders tested in this study may have been limited in their ability to consume the first instar larvae and may be more likely to penetrate the egg chorion (Momen et al., 2013).

Protonymphs and deutonymphs of predatory phytoseiid mites, *Typhlodromus athiasae* Porath and Swirski sometimes successfully attacked first instar caterpillars of *Prays citri* Milliere, while males and females attacked eggs as well as first instar larvae, but seemed to prefer the latter (Swirski et al., 1967). First instar larvae of *P. citri* were often successfully attacked by the three young stages of phytoseiid *Amblyseius limonicus* German and McGregor, while insect eggs were observed to be consumed only occasionally (Swirski and Dorzia, 1968). It was observed that in some cases, *A. largoensis* occasionally fed on 1st instar larvae of *P. citri* and *E. ceratoniae* during its deutonymphal and adult stages (Kamburov, 1971).

Table 1

Adult longevity, fecundity and consumption rates (mean ± SE) of multiple-mated females of *Neoseiulus barkeri*, *Cydnoseius negevi* and *Amblyseius largoensis* fed first instar larvae of *Tuta absoluta* and kept at 30 ± 1 °C , 70% RH and 16 L: 8 D photoperiod

Items	Phytoseiid mites			(F) Calculated
	Neoseiulus barkeri (16)[z]	*Cydnoseius negevi* (16)	*Amblyseius largoensis* (15)	
Pre-oviposition period (days)	3.37 ± 0.12 a[y]	7.12 ± 0.39 b	5.73 ± 0.18 c	52.185**
Oviposition period (days)	20.43 ± 0.31 a	7.31 ± 0.72 b	9.20 ± 0.78 b	125.920**
Post-oviposition period (days)	3.75 ± 0.28 a	6.93 ± 0.83 b	7.33 ± 0.84 b	8.034**
Adult longevity (days)	27.56 ± 0.29 a	21.37 ± 0.71 b	22.27 ± 0.57 b	37.521**
Number of eggs laid / female	23.62 ± 0.56 a	2.50 ± 0.16 b	3.87 ± 0.19 c	1090.06**
Daily number of eggs laid / female	1.16 ± 0.03 a	0.36 ± 0.02 b	0.44 ± 0.02 b	309.632**
Number of prey larvae consumed / female	26.69 ± 0.37 a	20.06 ± 0.69 b	20.93 ± 0.58 b	41.331**
Number of prey larvae consumed during oviposition period	20.31 ± 0.31 a	7.12 ± 0.68 b	9.20 ± 0.79 b	132.521**
Daily number of prey larvae consumed by female	0.96 ± 0.01 a	0.93 ± 0.01 b	0.64 ± 0.01 b	3.794*
Sex ratio % ♀ : ♂	0.68 185 : 86	0.62 23 : 14	0.65 33 : 18	

[z] Numbers between parentheses represent the number of replicates.
[y] Within rows, means followed by a common letter do not differ significantly (TukeyHSD, a = 0.05).
**Highly significant; *significant.

Neoseiulus barkeri ate the most *T. absoluta* larvae and *A. largoensis* as well as *C. negevi* consumed small number of insect larvae indicating that predation rates are likely only slightly inflated compared to the field. However, it should be stressed that under our experimental conditions, with the predators confined with their prey on small leaf discs, prey location was much easier than under field conditions. When averaged across the entire experiment, *N. barkeri* female ate 0.96 insect larvae per day, which is lower than daily predation rates on nearly similar-sized thrips *Thrips tabaci* (Lind.): 4.3 nymphs (Bonde, 1989); ate 1.6 eggs of *T. absoluta* (Momen et al., 2013). One reason for lower rates of mite predation on insect larvae could be related to prey defense mechanisms. In addition, survival of *A. largoensis* and *C. negevi* was lower in arenas provisioned with insect larvae, although this was not the case for *N. barkeri*.

Tuta absoluta larvae supported limited reproduction of *C. negevi* and *A. largoensis*, with *N. barkeri* having the highest average oviposition rate (23.62 / female and 1.16 / female / day). With thrips as food source, *N. barkeri* was produced 2.3 eggs / day (Bonde, 1989) and 1.7 eggs / day on eggs of *Ephestia kuehniella* Zeller (Momen and El-Laithy,

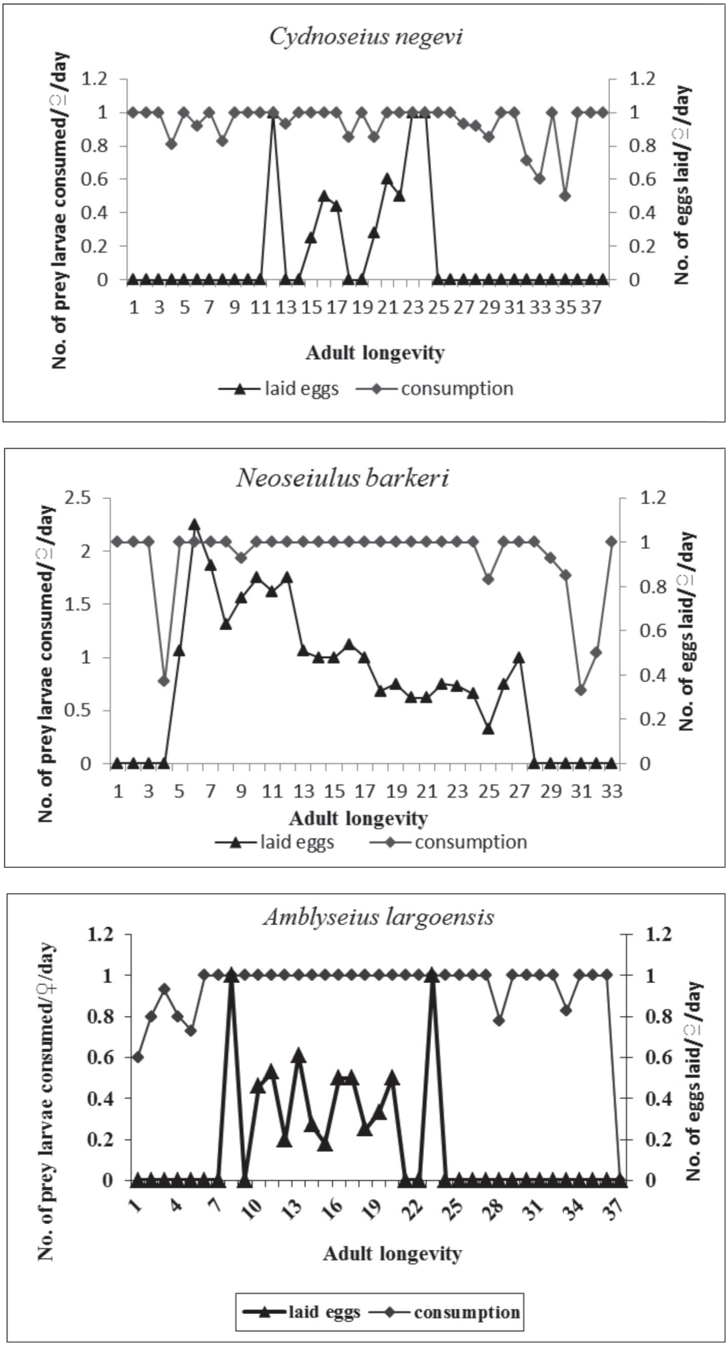

Fig. 1. Influence of *Tuta absoluta* larvae on adult longevity, survival, oviposition and consumption rate in multiple-mated females of three predatory phytoseiid species

2007). A higher fecundity (30.6 and 30.8 eggs / ♀) was reported when *N. barkeri* fed *Aleuroglyphus ovatus* Toupeau (Acari: Acaridae) at 24 ºC and 28 ºC (Xia et al., 2012). Kamburov (1971) confirmed poor development and reproduction of *A. largoensis* when fed on scale crawlers of *Aonidiella aurantii* (Maskell) and *Chrysomphalus aunidum* (L.) (Hemiptera: Diaspididae). With *Raoiella indica* hirst and eggs of *T. absoluta* as food source, *A. largoensis* was produced 1.6 and 1.1 eggs per day, which was higher than on insect larvae (0.4 eggs/ day, present study), but close to that reported on oak pollen *Quercus virginiana* Miller (0.5 egg / day) (Carrillo et al., 2010; Momen et al., 2013).

For *C. negevi*, female was laid an average of 2.5 eggs during its oviposition period, which lower than value reported on eggs of *Bemisia tabaci* Gennadius and scale insect *Phoenicoccus marlatti* Cockerell as well as pollen grains of *Ricinus communis* L.

Our study indicates that predatory mite *N. barkeri* may contribute to the biological control of the pest insect *T. absoluta* since it was proved to feed well and sustain oviposition on an egg stage (Momen et al., 2013) and first larval instar of the pest (present study). However, it is important to point out that under natural conditions, *N. barkeri* could potentially feed on a variety of prey and alternative food source (mites and insects) in absence of its primary food. In addition, results indicate that *T. absoluta* larvae appear to be a sub-optimal food source for *A. largoensis* and *C. negevi* tested here.

To the best of our knowledge, this study was the first to evaluate the effects of *T. absoluta* first larval instar on development, consumption rate and fecundity of *N. barkeri*, *C. negevi* and *A. largoensis* under laboratory conditions.

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