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## Intraguild predation between *Macrolophus pygmaeus* and *Nesidiocoris tenuis*

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**Abstract:** The omnivorous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae) are important biological control agents of pests on tomato crops. In this study the Intraguild Predation Interactions (IGP) between these two species were investigated. The experiments concerned the effect of hetero-specific treatments on: a) their within plant distribution in the field, b) the distribution on tomato caged plants, c) their behavioral interactions, d) the development of *M. pygmaeus* nymphs when together with adults of *N. tenuis* and e) the effectiveness in biological control. *N. tenuis* was most frequently recorded on the apex but also the upper 4 leaves of the plant whereas *M. pygmaeus* was recorded on the 2<sup>nd</sup> to 6<sup>th</sup> leaf from the top. The presence of *M. pygmaeus* caused a tendency of *N. tenuis* population to aggregate on the uppermost part of the plant. In hetero-specific treatments the mobility of the predators and particularly that of *N. tenuis* was increased. Without extraguild prey, all *M. pygmaeus* nymphs of 2<sup>nd</sup> and 3<sup>rd</sup> instars failed to develop to adulthood, whereas a high percentage of nymphs found dead had their body fluids totally sucked, indicating IGP by *N. tenuis*. When occurred together, a significant negative impact on the suppression rate of whitefly population was recorded, despite this negative effect was lessened in more complex environments. Thus, although there might not be severe IGP interactions, sublethal effects associated to IGP may occur.

**Key words:** *Trialeurodes vaporariorum*, whitefly, IGP, Heteroptera, Miridae, polyphagous predators, tomato.

## Introduction

Species belonging to the genera *Macrolophus* and *Nesidiocoris* (Hemiptera: Miridae) are important biological control agents of several pests such as whiteflies, aphids and mites on solanaceous crops (Albajes & Alomar 1999). *Macrolophus pygmaeus* (Rambur) can reproduce at a high rate feeding on *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae) (Perdikis & Lykouressis, 2002). *Nesidiocoris tenuis* (Reuter) is also common in tomato crops in the Mediterranean region and is considered as an important natural enemy of whiteflies (Tavella *et al.*, 1997, Camero *et al.*, 2000, Sanchez *et al.*, 2003, Arnó *et al.*, 2006, Lykouressis *et al.*, unpublished data). It exhibits diverse feeding habits being able to survive on plant sap for a short period (Urbaneja *et al.*, 2005). Both species have been reported to share the same habitats as they to

co-occur in tomato fields. Additionally, both species could naturally enter and establish in protected tomato crops, a fact that could potentially disrupt a biological control program in case that either one of these predatory mirids had been already released. Their omnivorous habits of sharing the same resources and their co-existence indicate that predatory or competitive interactions between them may occur.

Intraguild predation (IGP) occurs between consumers that belong to the same guild and are also engaged in predator-prey interactions with each other (Polis *et al.*, 1989). In fact, the literature on the impact of combinations of predatory species in biological control is controversial, since synergistic, neutral and antagonistic effects have been reported (i.e. Rosenheim *et al.*, 1993, Lucas *et al.*, 2005). Apart from predation, sublethal interactions have been also recorded in IGP studies. The sublethal effects linked to IGP risk include the behavioural responses of the intraguild prey.

The present work aimed at a comprehensive study of the IGP interactions between *M. pygmaeus* and *N. tenuis*.

## Materials and methods

### *Within plant distribution of predators in the field.*

This study was conducted in an open field tomato crop (cv. Formula) of 0.5 ha in the region of Trifylia, in western Peloponnesus. No insecticide sprayings were conducted in the crop during the growing season.

The within plant distribution of *M. pygmaeus* and *N. tenuis* was studied. A shoot that bore both predator species on each of 31 tomato plants was labeled. These shoots were sampled in the course of our experiments. The number of each predator's species nymphs and instar (first to fifth) or adults on each leaf, flower cluster and on the apex (upper 5cm part of the shoot) was recorded. In each sampling, 10 fully expanded leaves were examined starting from the apex of the plant. Samplings were conducted in the morning of 27 July, afternoon of 2 August, afternoon of 8 August, morning of 9 August, afternoon of 23 August and morning of 24 August.

### *Within plant distribution of predators in caged plants.*

The distribution of predators on tomato plants was studied in mono-specific and hetero-specific treatments in caged tomato plants.

In cages with a single tomato plant, *M. pygmaeus* and/or *N. tenuis* 5<sup>th</sup> instar nymphs were introduced. Four treatments were considered: 1) 8 nymphs of *M. pygmaeus*, 2) 8 nymphs of *N. tenuis*, 3) 8 nymphs of *M. pygmaeus* plus 8 nymphs of *N. tenuis*, and 4) 16 nymphs of *N. tenuis*. Caged tomato plants without insects were used as controls. The introduction of predators in cages took place on the 7<sup>th</sup> October in 2007. Records on the distribution of both predator species on the plants were conducted in 2-day intervals i.e. on 9, 11, 13 and 15 of October.

### *Behavioral IGP interactions between M. pygmaeus and N. tenuis.*

Possible occurrence of behavioral IGP interactions between *M. pygmaeus* and *N. tenuis* were investigated in the laboratory. In the experiments, 5<sup>th</sup> instar nymphs of both *M. pygmaeus* and *N. tenuis* were used. The behaviour of predators was recorded both in mono-specific and hetero-specific treatments in Petri dishes. In mono-specific treatments, the movements of either 1 or 2 nymphs of each species per dish were recorded. In the hetero-specific treatment,

1 nymph of each species had been enclosed together in each dish. The records were taken at a room temperature of  $22 \pm 1^\circ\text{C}$ . Each treatment was replicated 10 times.

**IGP interactions between *M. pygmaeus* and *N. tenuis*.**

The IGP interactions between *M. pygmaeus* and *N. tenuis* were further investigated studying the development and survival rates of *M. pygmaeus* nymphs in the presence of an adult of *N. tenuis*. In the treatments, development and survival rates of 1-day old 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar nymphs of *M. pygmaeus* were recorded in the presence of 2-day old *N. tenuis* adults in a Petri dish, with or without extraguild prey i.e. eggs of *E. kuehniella*, offered *ad libitum*. Experiments were replicated 10 times and were conducted in a growth chamber set at  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and 16:8h (L:D).

**IGP and predators' effectiveness in biological control.**

The predaceous efficiency of *M. pygmaeus* and *N. tenuis* was studied in mono-specific and hetero-specific treatments. Fifth instar nymphs starved for 24h were introduced in plastic Petri dishes (15cm diameter, 1.5cm height). One or two tomato leaves were placed upside down on a cotton layer in the dish. Each leaf was infested with 2<sup>nd</sup> and 3<sup>rd</sup> instar nymphs of the whitefly. In total, 70 whitefly nymphs of 2<sup>nd</sup> and 3<sup>rd</sup> nymphal instars, at almost equal proportion, were retained on each leaf.

Four treatments were considered: 1) mono-specific *N. tenuis*, where 1 nymph of *N. tenuis* was introduced in a Petri dish with 1 tomato leaf infested with 70 prey items in total, 2) mono-specific *M. pygmaeus*, where 1 nymph of *M. pygmaeus* was introduced in a Petri dish with 1 tomato leaf that bore 70 prey items in total, 3) hetero-specific - low prey density where 1 nymph of *N. tenuis* and 1 nymph of *M. pygmaeus* were introduced together in a dish with 1 tomato leaf that bore 70 prey items in total, and 4) hetero-specific - high prey density, where 1 nymph of *N. tenuis* and 1 nymph of *M. pygmaeus* were introduced together in a dish with 2 tomato leaves, that each bore 70 prey items. 24h after the introduction of predators in the dish, the number of the consumed prey on each leaf was recorded. Each experiment was replicated 15 times. Experiments were conducted in a growth chamber at  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and 16:8h (L:D).

## Results

The population of *M. pygmaeus* was mostly recorded on the middle leaves of the tomato plants. Highest numbers of the predator were found on the 6<sup>th</sup> leaf followed by those on the 5<sup>th</sup> and the 7<sup>th</sup>. On the contrary, *N. tenuis* individuals were most frequently recorded on the upper part of the plants. Its highest numbers were recorded on the apex followed by those recorded on the lower leaves up to the 5<sup>th</sup> leaf.

The population of *M. pygmaeus* in cages was mostly recorded on the middle leaves of the tomato plants. Higher numbers of the predator were found on the 6<sup>th</sup> leaf followed by those on the 5<sup>th</sup> and the 7<sup>th</sup> leaf. On the contrary, *N. tenuis* individuals were most frequently recorded on the upper part of the plants. Higher numbers were recorded on the apex followed by those recorded on the lower leaves up to the 5<sup>th</sup> leaf. These outcomes indicate the different pattern of distribution of the predator species on the plants, with *M. pygmaeus* mostly preferring the middle and *N. tenuis* the upper plant strata.

The study of the behavioral IGP interactions in dishes indicated that the mobility (walking activity) of each predator species was affected by the treatment. In the mono-specific experiments, the nymphs of *M. pygmaeus* remained mostly inactive in the dish. In the respective experiments, *N. tenuis* showed a much higher activity level than *M. pygmaeus*, and

the time spent moving was similar to that being inactive. In mono-specific experiments, when two individuals of a single species were enclosed in the dish, *M. pygmaeus* and *N. tenuis* remained mostly immovable. However, in the hetero-specific treatment, *M. pygmaeus* spent moving similar but *N. tenuis* significantly more time than in the previously mentioned treatment.

When extraguild prey was available, *M. pygmaeus* nymphs completed their development in the presence of a *N. tenuis* adult in almost all cases. However, in the absence of prey, all *M. pygmaeus* nymphs of the 2<sup>nd</sup> and 3<sup>rd</sup> instars failed to develop to adulthood. Among the nymphs found dead, some individuals had totally sucked their body fluids. Sucked individuals of the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar constituted 67±6%, 40±7% and 63±7% of the dead nymphs, respectively.

In mono-specific treatments, predation rates of *M. pygmaeus* were similar to those of *N. tenuis* (40.4±3.5 and 29.6±3.7, whitefly nymphs, respectively). In the low prey density hetero-specific combination a significant reduction in prey consumption was recorded (11.33±1.2 prey consumed). However, when the number of the available prey was doubled (140 prey items in total, shared equally on two leaves), the consumption was increased and was not different from that of the mono-specific treatments.

## Discussion

The study of the potential interactions between *M. pygmaeus* and *N. tenuis* showed evidence for intraguild predation. Experiments on tomato plants in the field and in cages showed that the two predator species prefer to occupy different sites on tomato plants. In addition, the presence of *M. pygmaeus* caused a significant change in the distribution of *N. tenuis* on the plants that showed a higher aggregation on the upper part. In hetero-specific combinations of *Dicyphus tamaninii* (Wagner) (Hemiptera: Miridae) and *Macrolophus melanotoma* (Costa) the predation rate on the greenhouse whitefly on tomato plants increased on the lower leaves compared to mono-specific treatments (Lucas & Alomar, 2002).

Intraguild relations become also evident if considering that development of young *M. pygmaeus* nymphs in the absence of extraguild prey was not completed in the presence of a *N. tenuis* adult. In contrast, when no prey was available on tomato leaves at 25°C *M. pygmaeus* nymphs could complete development in 77% of the cases with an identical methodology to that followed here (Perdikis & Lykouressis, 2000). However, the experiments on IGP are suggestive of an interference of relatively low intensity since IGP prey killing did not occur in the presence of extraguild prey (Lucas *et al.*, 1998).

IGP behavioral interactions resulted in the reduction of effectiveness in biological control but this effect was lowered when more prey was offered on a larger experimental arena. Increased complexity moderates the competition and enhances prey suppression (Finke & Denno, 2002). In artificial arenas without plants mirids were highly vulnerable to predation by syrphid larvae but on plants, this effect was highly reduced (Fréchette *et al.*, 2007).

Our results show clearly that, despite the absence of a strong predatory interaction (prey attacking and killing behaviour), sublethal effects associated to IGP risk constitute an important structuring force in the system.

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