



## Intraguild predation and sublethal interactions between two zoophytophagous mirids, *Macrolophus pygmaeus* and *Nesidiocoris tenuis*



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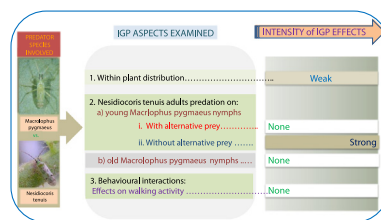
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### HIGHLIGHTS

- *Macrolophus pygmaeus* and *Nesidiocoris tenuis* had different within plant distribution.
- When co-occurred dispersed in a manner indicating non-avoidance behavior.
- *N. tenuis* adults killed young but not old *M. pygmaeus* nymphs in prey absence when alternative prey was absent.
- Their walking activity was not different in mono- and hetero-specific treatments.
- Contacts were recorded in a similar frequency and aggressive behavior was not observed.

### GRAPHICAL ABSTRACT



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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, potential intraguild predation (IGP) interactions between the two species were investigated on tomato. We examined: (a) the within plant distribution of both species in the field, (b) the within plant distribution of each predatory species when co-occurred at high densities on tomato caged plants, (c) their behavioral interactions when enclosed in experimental arenas and (d) the development young and old nymphs of *M. pygmaeus* when enclosed together with *N. tenuis* adults. Results revealed that the two predators showed a different distribution pattern on the plants, with *N. tenuis* exploiting mostly the upper part, whereas *M. pygmaeus* were mostly observed on the 5th to the 7th leaf from the top. However, when the predators co-occurred, *N. tenuis* or *M. pygmaeus* individuals were recorded with increased numbers on the lower or the higher part of the plant, respectively. In the presence of *N. tenuis* adult young nymphs of *M. pygmaeus* completed their development to the adult stage, when alternative prey (lepidopteran eggs) was present on the plant, however failed to reach adulthood in the absence of alternative prey. A high percentage of the dead nymphs found with their body fluids totally sucked indicating predation by *N. tenuis*. However, large 4th instar nymphs of *M. pygmaeus* were much less vulnerable to *N. tenuis* than younger. The behavior of *N. tenuis* was affected by the presence of *M. pygmaeus*, but at a rate similar to that when two individuals of *N. tenuis* were enclosed together. Contacts between the predators were recorded in a similar frequency in

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mono- and heterospecific treatments, whereas aggressive behavior was not observed. This study shows that intraguild interactions between *M. pygmaeus* and *N. tenuis* occur but are not intensive. The potential implications of the outcomes for biological control are discussed.

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## 1. Introduction

Omnivorous predators of the family Miridae are common in several agroecosystems (Dolling, 1991; Coll and Ruberson, 1998). In particular, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) are important biological control agents against several pests such as whiteflies, aphids, mites and the serious invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato crops (Perdikis et al., 2011; Calvo et al., 2012; Urbaneja et al., 2012). *M. pygmaeus* also exhibits phytophagous habits developing successfully and ovipositing at low rates when feeding solely on leaves of tomato or eggplant (Perdikis and Lykouressis, 2000). *N. tenuis* can also feed on plant but cannot complete development to adult hood in the absence of prey (Urbaneja et al., 2005). Both predators have commonly been reported to co-exist in tomato fields (Tavella et al., 1997). Their omnivorous habits and their co-occurrence suggest that competitive interactions between them may take place.

Intraguild predation (IGP) occurs between consumers that belong to the same guild and are engaged in predator–prey interactions (Polis et al., 1989). In these interactions three species may be involved: an IGP predator (a competitor), an IGP prey (another competitor) and an alternative (extraguild) prey that is shared by both of the competitors. The outcomes of IGP could be that the intraguild prey is killed and consumed, killed but not consumed as affected by sublethal effects due to the presence of competitor (Lucas, 2005, 2012). IGP is common in food-webs including biological control agents (Rosenheim et al., 1995; van Veen et al., 2006) such as terrestrial heteropteran species (Lucas and Rosenheim, 2011), and may influence their effectiveness (Rosenheim et al., 1995; Snyder and Wise, 1999). In fact, the literature on the combined action of co-occurring natural enemy species in biological control includes synergistic, neutral and antagonistic effects (Rosenheim et al., 1993; Chang, 1996; Ferguson and Stiling, 1996; Lucas et al., 1998; N'óia et al., 2008; Gagnon et al., 2011). The outcome varies depending on several factors such as the predator–prey relative size, mobility, population structure, environmental complexity, availability of the intermediate prey or predator aggregation (Polis et al., 1989; Lucas et al., 1998; Finke and Denno, 2002; Tommasini et al., 2002; Chacón and Heimpel, 2010; Lucas and Rosenheim, 2011).

*M. pygmaeus* has been reported to prey on other beneficials such as parasitized whitefly nymphs by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Castañé et al., 2000), and larvae of the leaf-miner parasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) (Nedstam and Johansson-Kron, 1999). It can also constitute an intraguild prey for *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Nabis* spp. (Hemiptera: Nabidae) (Lucas and Alomar, 2000) and the anthicoid species *Orius majusculus* (Reuter) (Jakobsen et al., 2004).

IGP between *M. pygmaeus* and *N. tenuis* was investigated by Lampropoulos et al. (2013) who reported that interactive effects on prey consumption of co-occurred *M. pygmaeus* and *N. tenuis* were more intense at prey densities close to predators' satiation. Moreno-Ripoll et al. (2012) have shown that *N. tenuis* females inflict a high rate of mortality on young nymphs of *M. pygmaeus* in the absence of alternative prey (i.e., the shared prey resource). Conversely, *M. pygmaeus* females caused no significant lethal effect on nymphs of *N. tenuis*. However, further investigations are required to explore whether IGP is weakened or even disappeared when larger

nymphs of *M. pygmaeus* are involved, since relative size is a determinant of predator–predator interactions (Lucas, 2005, 2012).

Apart from predation, sublethal effects have been also recorded in IGP studies, including changes in the behavior of an intraguild member. These effects have been considered as having a major impact in the structure and dynamics of the predators' guilds with important consequences in biological control (Rosenheim et al., 1995; Brodeur and Rosenheim, 2000). Among them, IGP may cause changes to spatial and temporal segregation of the IGP predators and prey in the habitat. For example, the spatial distribution of the coccinellid predator *Coleomegilla maculata* DeGeer on corn plants was changed in the presence of the predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Hoogendoorn and Heimpel, 2004). Additionally, the distribution of whitefly predation on tomato plants by *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) and *M. pygmaeus* was different between intra and interspecific treatment, due to changes in the predator's within plant distribution (Lucas and Alomar, 2002). Similarly, Martinou et al. (2010) reported that the presence of *M. pygmaeus* reduced the searching time of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae). Analogous possible effects on the distribution of *M. pygmaeus* on the plant under the presence of *N. tenuis* and vice versa were not recorded when 3 nymphs of each species co-occurred on a tomato plant (Moreno-Ripoll et al., 2012). However, *N. tenuis* and *M. pygmaeus* commonly co-occur at much higher densities than those tested in the aforementioned study and thus, further experimentation should be conducted.

The present work aimed at a comprehensive study of the IGP lethal and sublethal interactions between *M. pygmaeus* and *N. tenuis*. Specifically, the objectives were to assess whether, (i) the within plant distribution of each species is altered when they co-occur at high densities, (ii) the behavior of each predator species (i.e., walking or aggressive behavior) is different in hetero- vs. con-specific treatments in experimental arenas and (iii) *M. pygmaeus* inflicted mortality by *N. tenuis* is dependent on *M. pygmaeus* developmental instar and the presence of alternative prey.

## 2. Materials and methods

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

The within plant distribution of *M. pygmaeus* and *N. tenuis* was recorded when co-occurring on tomato plants in the field. This study was conducted in an open field of conventionally grown tomato crop (cv. Formula) of 0.5Ha in the Trifylia region at western Peloponnesus, Greece (coordinates X = 284 750; Y = 4 105 184; h = 3 m). No insecticide treatments were conducted in the crop during the growing season.

On each of 31 randomly selected tomato plants a shoot that bore both predator species was selected. Nymphs and adults of each predator's were counted on the apex (upper 4 cm part of the stem), the flower cluster and on each of 10 fully expanded leaves counting from the apex downwards, considering predators being rarely observed on lower leaves. Since *M. pygmaeus* is more active during the afternoon (Perdikis et al., 2004), sampling was accordingly conducted during the afternoon (4–7 p.m.) on 2 August 2007. On this sampling no prey was recorded on the plants.

## 2.2. Survival and within plant distribution of *M. pygmaeus* and *N. tenuis* nymphs in caged tomato plants

The survival and distribution of *M. pygmaeus* and *N. tenuis* nymphs were studied in mono- and hetero-specific treatments in caged tomato plants.

*M. pygmaeus* young nymphs were collected on tomato plants in the area of Chalkis, Evia, and *N. tenuis* in the area of Trifylia, western Peloponnesus, one week prior to the experiments. The nymphs were reared on tomatoes (cv. Primadona) in wooden-framed muslin cages (80 × 80 × 70 cm) in an experimental glasshouse of the laboratory of Agricultural Zoology and Entomology on the campus of the Agricultural University of Athens (A.U.A.). Eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Entofood™, Koppert Biological Systems) were provided *ad libitum*.

For the experiments young tomato plants (cv. Primadona), with a single shoot that bore 10 leaves and without flowers were transplanted individually in pots (32 cm in diameter) with a mixture of peat:perlite 5:1. The plants had not been treated with any chemical, were visually inspected and any pest found was removed. Each potted plant was enclosed in a fine muslin cage (32 cm diameter × 70 cm high). The cages were introduced in a non-heated plastic greenhouse on the campus of A.U.A.

In each cage, *M. pygmaeus* and/or *N. tenuis* 5th instar nymphs were introduced. Four treatments were carried out: (1) 8 nymphs of *M. pygmaeus*, (2) 8 nymphs of *N. tenuis* and (3) 8 nymphs of *M. pygmaeus* with 8 nymphs of *N. tenuis*. Since *N. tenuis* may occur at high densities on the plants an additional treatment by using 16 nymphs of *N. tenuis* was applied to investigate intraspecific effects. Each treatment was replicated 8 times.

The survival and the distribution of the nymphs on each plant in every each leaf were recorded two days later in the afternoon. The location of the predators was recorded on the upper 5 cm of the shoot (apex), on each leaf up to the 10th leaf, counting from the apex downwards, as well as on the sides of the cage and the soil of the pot. Data regarding the latter two sites were not included in the analysis since nymphs were rarely found (3.48% and 0.02% on the sides of the cage and on the soil of the pot, respectively). The survival of the predators in each cage was also recorded 4 days after the initiation of the experiment.

## 2.3. Behavioral interactions between *M. pygmaeus* and *N. tenuis* in the laboratory

In these experiments, 5th instar nymphs of both *M. pygmaeus* and *N. tenuis* were used. The nymphs of each species were reared separately in caged tomato plants with *E. kuehniella* eggs as prey. Each plant was covered with a plastic cylindrical cage (11 cm diameter, 30 cm height) with two lateral and a top openings covered with fine muslin (9 × 9 cm). The plants were kept in a growth chamber at 25 ± 1 °C, 65 ± 5% RH, and 16:8 h (L:D) photoperiod.

The behavior of the predators was observed both in mono-specific and hetero-specific treatments in petri dishes (9 cm diameter and 1.5 cm high). The top cover of each dish bore a round hole of 3 cm diameter, covered with fine muslin, to prevent excessive humidity inside the dish. On the base of the dish a layer of moistened cotton was placed on which 1 tomato leaflet without any prey was put upside down. The leaflet edges were covered with moistened cotton to prevent predators from reaching its underside.

In mono-specific treatments, the behaviors of either 1 or 2 nymphs of each species, per dish, were recorded. In the hetero-specific treatment, two nymphs (1 nymph of each species) were enclosed together in each dish. The nymphs were released with the aid of a fine brush on the leaflet in the dish. Immediately after their introduction in the dish, their behavior was recorded continuously during 30 min. These records concerned the duration of the periods

that each nymph remained immobile or walking either on the tomato leaflet, the lip or on the cotton layer in the dish. In addition, the frequency and duration of encounters was recorded. The records were taken at a room temperature of 22 ± 1 °C. Each treatment was replicated 10 times. In the cases that two con-specific or heterospecific individuals have been placed in a dish, the behavior of each individual can be dependent on the behavior of the other. For this reason, in ten replicates the behavior of the first and in additional ten replicates the behavior of the second individual was recorded.

## 2.4. IGP between *M. pygmaeus* and *N. tenuis* in the laboratory

IGP between *M. pygmaeus* nymph and *N. tenuis* adult (females and males were used at equal ratio) was further investigated. The development and survival rates of *M. pygmaeus* 1-d old 2nd, 3rd and 4th instar nymphs were recorded in the presence of 2-d old *N. tenuis* adult with or without eggs of *E. kuehniella* offered *ad libitum*. The nymphs of *M. pygmaeus* and the adults of *N. tenuis* were collected from their respective rearing units.

A single nymph of *M. pygmaeus* was introduced together with an unstarved adult of *N. tenuis* in a petri dish following identical methodology as previously described. In each petri dish, a tomato leaflet was introduced with or without the alternative prey. The leaf was daily replaced by a fresh one and the prey renewed.

At 24 h intervals, the survival of *M. pygmaeus* nymphs was recorded until adult stage. The number of the dead nymphs found sucked were also recorded, as an indicator of predation by the predator.

In 2 cases, the *N. tenuis* adult was found dead, and then replaced. Each treatment was replicated 10 times. Experiments were conducted in a growth chamber set at 25 ± 1 °C, 65 ± 5% RH, and 16:8 h (L:D).

## 2.5. Statistical analyses

The distribution of the predators (*M. pygmaeus* and *N. tenuis*) on the plants (apex, each of the upper 10 leaves and flowers) in the field was initially assessed using two-way ANOVA. Since the assumption of normality was violated a Generalized Linear Model (GLM) with binomial distribution and logit function was used. The means were compared by contrasts. Regression analysis was used aiming to relate their numbers with their respected location on the plant, starting with the apex, the first, second and in sequence the lower leaves up to the 10th leaf from the apex, excluding flower cluster.

In cage experiments the effects of the treatment (species combination) on the survival of the predators in each cage was tested using GLM with binomial distribution and logit function. The within plant distribution of the two predator species (*M. pygmaeus* and *N. tenuis*) was grouped in three classes (the upper part including the apex, 1st and 2nd leaf, the middle part with the 3rd, 4th and 5th leaf and the lower part i.e., the 6th up to the 10th leaf). The data were analyzed with a GLM with Poisson distribution and log function. As factors the “location on the plant” and the “species” were used.

In the behavioral experiment, the data of total period of walking in the dish were analyzed using a GLM with binomial distribution and logit function with factor the “species”. The frequencies of contacts were compared among the treatments with Pearson chi-square test. The Log-rank test was used to search whether the treatments had a significant effect on the time until encounter. The duration of the encounter was compared among the treatments using a one-way ANOVA.

The developmental periods of *M. pygmaeus* nymphs were compared in the presence and the absence of *N. tenuis* using one-way ANOVA. Means were separated by Tukey–Kramer HSD test. The

survival of *M. pygmaeus* nymphs were compared using a chi-square test.

Analyses were conducted with the statistical package JMP 10.0 (SAS Institute, 2012).

### 3. Results

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

A significant interaction between the factors “species” and “location” on the plant was recorded ( $\chi^2 = 46.18$ ,  $df = 11,602$ ,  $P < 0.0001$ ). *M. pygmaeus* was mostly recorded on the middle leaves of the tomato plants, with its highest numbers on the 6th leaf followed by the 5th and the 7th leaves (Fig. 1). No individual was recorded in the flower cluster. On the contrary, *N. tenuis* individuals were most frequently recorded in the upper stratum of the plants. Its highest numbers were recorded on the apex followed by significantly lower numbers recorded on the 1st, 2nd and the 3rd leaf. On the lower leaves up to the 7th leaf much reduced numbers were recorded (Fig. 1).

*N. tenuis* densities were highest at the upper part of the plant and were reduced on the lower parts following a linear negative trend ( $y = 2.95(\pm 0.36) - 0.32(\pm 0.05)x$ ,  $F = 35.42$ ,  $df = 1,9$ ,  $P < 0.001$ ,  $R^2 \text{ adj} = 0.78$ ) a relation that was not observed for *M. pygmaeus*.

#### 3.2. Survival and within plant distribution of *M. pygmaeus* and *N. tenuis* nymphs in caged plants

The survival rate of the predators was not affected by treatment (i.e., predator density or combination of species) ( $\chi^2 = 2.58$ ;  $df = 4,35$ ;  $P = 0.63$  and  $\chi^2 = 2.33$ ;  $df = 4,35$ ;  $P = 0.67$ , for records taken 2 and 4 days after the introduction of the predators in the cages, respectively). The highest survival among the treatments was recorded when 8 *N. tenuis* were placed together with 8 *M. pygmaeus* and the lowest in the treatment when 8 *M. pygmaeus* were used ( $0.90 \pm 0.03\%$  and  $0.65 \pm 0.02\%$ , respectively).

The distribution of *N. tenuis* on the plants when used alone was dependent on the density (8 or 16 individuals per cage) ( $\chi^2 = 1.42$ ;  $df = 1,42$ ;  $P = 0.028$ ), the location on the plant ( $\chi^2 = 53.68$ ;  $df = 2,42$ ;  $P < 0.001$ ), their interaction being no significant ( $\chi^2 = 4.02$ ;  $df = 2,42$ ;  $P = 0.13$ ) (Fig. 2a). Contrasts showed that the numbers of *N. tenuis* found on the lower part of the plants were significantly increased at the high population density.

The distribution of *N. tenuis* and *M. pygmaeus* on the plants when used alone or together was dependent on the treatment ( $\chi^2 = 12.98$ ;  $df = 3,84$ ;  $P < 0.005$ ), the location on the plant ( $\chi^2 = 34.38$ ;  $df = 2,84$ ;  $P < 0.001$ ) whereas a significant interaction

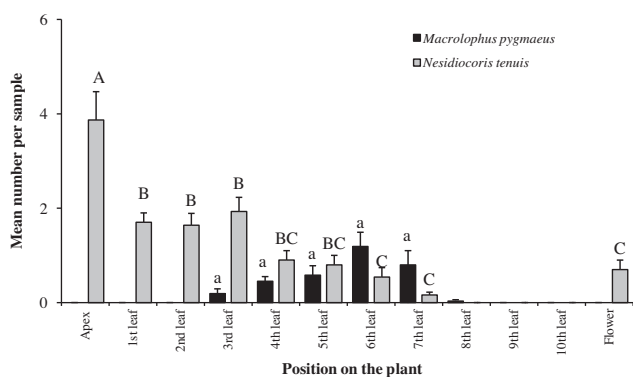


Fig. 1. Number (mean  $\pm$  SE) of *M. pygmaeus* and *N. tenuis* nymphs of all instars and adults on different parts of a stem of tomato plants in the field ( $n = 31$ ). Columns followed by a different letter differ significantly within each species.

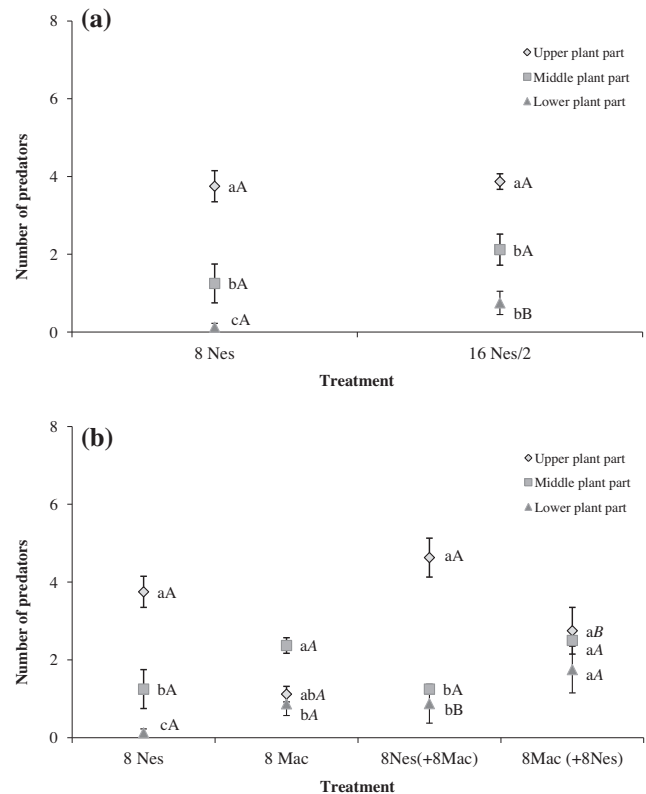
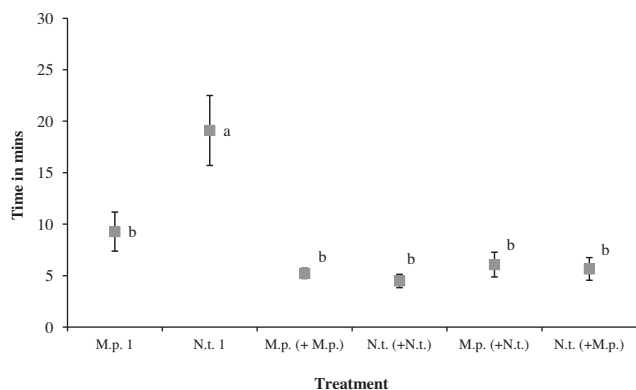


Fig. 2. (a) Distribution of *N. tenuis* 5th instar nymphs (mean  $\pm$  SE) on different parts on caged tomato plants without prey. The treatments used were 8 and 16 *N. tenuis* (N.t.) nymphs. For comparison, the numbers of *N. tenuis* in the high density were divided by two. The records were taken 2-d after the introduction of the predators in the cages. In all treatments 8 cages (replicates) were used. Means labeled with the same low case letter are not significantly different within each predator density. Different upper case letters indicate means significantly different between the two predator densities on each plant part. (b) Distribution of *N. tenuis* and *M. pygmaeus* 5th instar nymphs (mean  $\pm$  SE) on different parts on caged tomato plants without any prey. The treatments used were 8 *M. pygmaeus* (M.p.) or 8 *N. tenuis* (N.t.) nymphs, alone or together. The records were taken 2-d after the introduction of the predators in the cages. In all treatments 8 cages (replicates) were used. Means labeled with the same low case letter are not significantly different within each predator treatment. Different erect upper case letters or upper case letters in italics indicate means significantly different on each plant part between mono- and hetero-specific treatments for *N. tenuis* and *M. pygmaeus*, respectively.

was recorded ( $\chi^2 = 32.87$ ;  $df = 6,84$ ;  $P < 0.001$ ) (Fig. 2b). In the mono-specific treatment *N. tenuis* was recorded at significantly higher numbers at the upper part, followed by that at the middle, whereas at the lower part only a few individuals were recorded. *M. pygmaeus* was most commonly found at the middle part although its presence was not significantly different from that at the upper or the lower part. However, when the two species combined, significantly higher numbers of *N. tenuis* at the lower part and *M. pygmaeus* at the upper part of the plant were recorded in comparison to their respective numbers in the monospecific treatments.

#### 3.3. Behavioral interactions between *M. pygmaeus* and *N. tenuis* in the laboratory

The time walking in the dish was significantly different among the treatments ( $\chi^2 = 32.07$ ;  $df = 5,74$ ;  $P < 0.001$ ) (Fig. 3). In the mono-specific experiments with a single individual, *N. tenuis* showed a significantly longer walking activity than *M. pygmaeus*. In mono-specific experiments, when two individuals were used, *M. pygmaeus* walked for a comparable but *N. tenuis* for a



**Fig. 3.** Total time (mins  $\pm$  SE) spent walking by *M. pygmaeus* or *N. tenuis* 5th instar nymphs in petri dishes with a tomato leaflet. The treatments used were 1 nymph of *M. pygmaeus*, 1 nymph of *N. tenuis*, 2 nymphs of each species separately, and 1 nymph of each species together. Each treatment was replicated 10 times but in the cases where two individuals were placed in each dish then in ten replicates the behavior of the first and in additional ten replicates the behavior of the second individual was recorded.

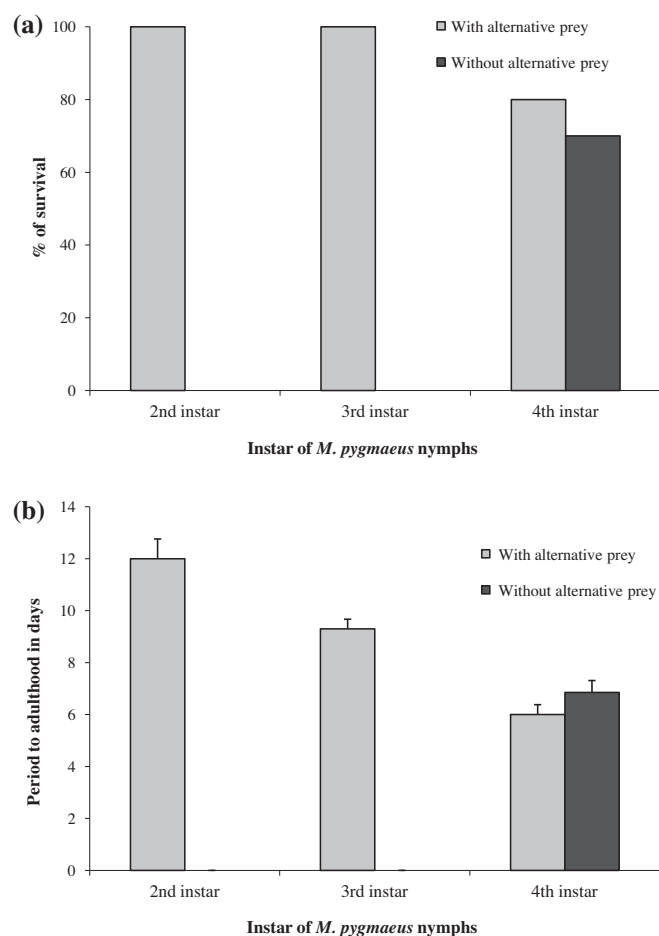
significantly shorter period than in the respective single individual, mono-specific treatment.

In treatments where two individuals were placed in a dish (mono-specific or hetero-specific) the number of encounters was counted, but in each replicate none or only a single encounter was observed. Always, after their encounter both nymphs turned to the opposite direction. The total number of encounters and the time from the start of an experiment until the contact were not significantly different among the treatments ( $\chi^2 = 1.73$ ;  $df = 2$ , Pearson  $P = 0.42$ , Log-rank test  $\chi^2 = 0.41$ ;  $df = 2$ ;  $P = 0.81$ , respectively) (Table 1). Similarly, although the duration of the encounter was shortest in the hetero-specific treatment, there was not recorded a significant difference ( $F = 1.88$ ;  $df = 2, 16$ ;  $P = 0.18$ ) (Table 1).

#### 3.4. IGP between *M. pygmaeus* and *N. tenuis* in the laboratory

All *M. pygmaeus* 2nd and 3rd instar nymphs and 80% of the 4th instar completed their development in the presence of one *N. tenuis* adult when alternative prey was available (Fig. 4a). However, in the absence of alternative prey, no nymph of the 2nd and 3rd instars developed to adulthood; however, 70% of the 4th instar nymphs were able to complete their development (Fig. 4b). The survival rate and developmental period of the 4th instar nymphs were not different in the presence or absence of alternative prey ( $\chi^2 = 0.267$ ;  $df = 1$ ; Pearson  $P = 0.60$  and  $F_{1,13} = 2.17$ ;  $P > 0.15$ , respectively).

Sucked 2nd, 3rd and 4th instar nymphs constituted  $67 \pm 6\%$ ,  $40 \pm 7\%$  and  $63 \pm 7\%$ , of the total number of dead nymphs, respectively. It was further recorded that the survival period of those nymphs was significantly shorter than that of the dead but not sucked nymphs ( $F_{1,7} = 8.69$ ;  $P < 0.02$  and  $F_{1,8} = 5.97$ ;  $P < 0.04$ , in the 2nd and the 3rd instar, respectively). In particular, the



**Fig. 4.** Percentage of *M. pygmaeus* 2nd, 3rd and 4th instar nymphs which completed their development (a) and their respective developmental period in days (mean  $\pm$  SE) (b) in the presence of a single *N. tenuis* adult, in petri dishes with or without alternative prey (eggs of *E. kuehniella*) at 25 °C. In all treatments 10 dishes (replicates) were used.

longevity of the sucked nymphs was  $2.4 \pm 0.9$  and  $3.75 \pm 1.1$  days and that of the dead (but unsucked) nymphs was  $6.0 \pm 1.41$  and  $7.83 \pm 1.44$  days of the 2nd and the 3rd instar, respectively.

#### 4. Discussion

Field and cage experiments on tomato plants showed that the survival of *M. pygmaeus* and *N. tenuis* was not affected when co-occur. In a similar study an adverse effect on *M. pygmaeus* but not on *N. tenuis* survival emerged when 6 adults of each species were caged for 4 days on tomato plants with 7 leaves in plastic cylinders, without alternative prey (Moreno-Ripoll et al., 2012). Evidence for an adverse effect on *M. pygmaeus* survival was not recorded in our study although a higher density of either conspecific or heterospecific individuals were used. Likely, this could be associated with the

**Table 1**

Total number of encounters and respective time until encounter (average  $\pm$  SE) and duration of each encounter (average  $\pm$  SE) recorded when two *M. pygmaeus* (MM), one *M. pygmaeus* and one *N. tenuis* (MN) or two *N. tenuis* (NN) 5th instar nymphs were enclosed together in a petri dish with a tomato leaflet without prey at 22 °C. The total observation period was 30 mins.

Treatment	Total number of encounters	Time until encounter (in mins)	Duration of encounter (in secs)
MM	8	7.57 $\pm$ 2.65	5.14 $\pm$ 0.91
MN	5	8.75 $\pm$ 2.93	3.12 $\pm$ 0.89
NN	8	9.25 $\pm$ 5.31	5.75 $\pm$ 1.26

higher plants used in our work since increased spatial complexity moderates the competition (Finke and Denno, 2002; Janssen et al., 2007). Fréchet et al. (2007) reported also that mirids were highly vulnerable to predation by syrphid larvae in artificial arenas but this effect was diminished when experiments performed at larger scale on plants.

Each predatory species was observed to occupy different stratum of the tomato plant. *N. tenuis* was mostly recorded on the uppermost part of the plant, whereas *M. pygmaeus* mostly found foraging on the lower leaves of this part. Arnó et al. (2010) reported also that the highest population of *N. tenuis* (88.5%) was present on the upper part of the plant.

The within plant distribution of *N. tenuis* was affected by its density, with more individuals occupying the lower plant part when occurred at high density. Similarly, in heterospecific treatments each species occupied more the part of the plant that was less preferred in the respective monospecific treatment (i.e., the lower part for *N. tenuis* and the higher part for *M. pygmaeus*). Moreno-Ripoll et al. (2012) showed that the distribution of *M. pygmaeus* and *N. tenuis* was not altered in monospecific (6 nymphs) and heterospecific treatments (3 nymphs of each species) on tomato plants. In our study more individuals of larger instar were used and this may be involved in the differences recorded between the two studies.

Our results are indicative of low intraspecific level of competition since the within-plant distribution of each predator species was affected by the presence of the other species. This segregation in within-plant distribution has been considered to be a behavioral response mediated by direct interaction or competition. For example, the distribution of the ladybird *H. axyridis* (Pallas) did not change with the co-occurrence of *C. maculata* however the distribution of the latter differed significantly between single-species and two-species treatment, being more aggregated (Hoogendoorn and Heimpel, 2004). This asymmetrical interaction was considered to be an avoidance behavior which is indicative of an IGP interaction. In our study, each species dispersed more towards the less occupied plant part in the heterospecific treatment compared to the respective monospecific treatment. This demonstrates symmetrical and less competitive interactions between the predators at least under our experimental conditions.

The results may have significant implications in biological control with the potential complementary activity of the two species. Lucas and Alomar (2002) reported when *D. tamaninii* and *M. pygmaeus* co-occurred an increased level of whitefly predation was recorded at lower leaves of the plant compared to monospecific treatments. Moreover, the predators *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) were found to disperse more when co-existed than when each species was alone on bean plants and this change enhanced the control of the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Walzer et al., 2009).

Our results provide evidence for intraguild predation in absence of alternative prey, given that development of young *M. pygmaeus* nymphs was not completed in the presence of a *N. tenuis* adult. Furthermore, Perdikis and Lykouressis (2000), reported that a percentage of 77% of *M. pygmaeus* nymphs completed their development without any prey on tomato leaves, following identical methodology. According to Moreno-Ripoll et al. (2012), when five 2nd–3rd *M. pygmaeus* nymphs were enclosed in a cage of 0.5 L with a tomato leaflet and a female of *N. tenuis* in absence of intermediate prey, only 24% of them reached adult stage. Thus, *M. pygmaeus* nymphs can be vulnerable to *N. tenuis* predation.

The current study provides evidence that the high mortality recorded in *M. pygmaeus* nymphs was accounted to *N. tenuis* adult considering that (i) a high percentage of the dead nymphs was

totally sucked and (ii) those sucked nymphs survived for a significantly shorter period than the dead but unsucked ones. *M. pygmaeus* nymphs might be a high quality food resource for *N. tenuis* as was the case with the frozen nymphs of *M. pygmaeus* that were preyed by *D. tamaninii* (Lucas and Alomar, 2001). In general, exploiting of intraguild prey is a profitable strategy to increase survival of intraguild predator, particularly in periods of prey scarcity (Polis et al., 1989; Wissinger et al., 1996; Lucas and Rosenheim, 2011).

Despite negative effects on young nymphs, our results clearly show that the larger *M. pygmaeus* nymphs developed normally to adult stage in the presence of a *N. tenuis* adult, even when intermediate prey was not available. This indicates that the relative size or the higher mobility of co-occurred individuals can affect the severity of asymmetrical IGP interactions between *M. pygmaeus* and *N. tenuis*. Therefore, although according to the results of the current study and that of Moreno Ripoll et al. (2012) the young nymphs of *M. pygmaeus* can be vulnerable to *N. tenuis* predation, the larger-older can avoid this adverse effect. This finding decreases the frequency and strength of IGP between the two predators.

Additionally, aggressive behavior between last instar nymphs of the two species was not observed. It has been demonstrated that the relative size of the opponents may mediate the significance of interactions (Rosenheim et al., 1995; Lucas, 2005, 2012; Raak-van den Berg et al., 2012). Therefore our result might be related to the comparable relative sizes of *M. pygmaeus* and *N. tenuis*. Plant-feeding habits of both predators could also be involved in this outcome. The potential of IGP predator or prey to exploit other than the shared resource food may lessen competitive interactions, promote coexistence and ultimately, alter the expectations that IGP would reduce suppression of a common prey (Daugherty et al., 2007 and references therein; Lucas and Rosenheim 2011; Lucas, 2012).

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