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Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato

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Abstract Tomato is the most important vegetable crop in Spain. The mirid bug *Nesidiocoris tenuis* (Reuter) commonly appears in large numbers in protected and open-air tomato crops where little or no broad-spectrum insecticides are used. *Nesidiocoris tenuis* is known to be a predator of whiteflies, thrips and several other pest species. However, it is also considered a pest because it can feed on tomato plants, causing necrotic rings on stems and flowers and punctures in fruits. Our objectives were to evaluate predation by *N. tenuis* on sweetpotato whitefly *Bemisia tabaci* Gennadius under greenhouse conditions and establish its relationship to *N. tenuis* feeding on tomato. Two different release rates of *N. tenuis* were compared with an untreated control (0, 1 and 4 *N. tenuis* plant^{-1}) in cages of 8 m^2. Significant reductions of greater than 90% of the whitefly population and correspondingly high numbers of *N. tenuis* were observed with both release rates. Regression analysis showed that necrotic rings on foliage caused by *N. tenuis* were best explained by the ratio of *B. tabaci* nymphs:*N. tenuis* as predicted by the equation $y = 15.086x - 0.6359$.

Keywords Miridae · Whitefly · Biological control · Zoophytophagy

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This research is part of an internal R&D project conducted at Koppert Biological Systems S.L. in Spain. The main goal was to improve control of *Bemisia tabaci* in protected crops of southeastern Spain by bringing on a new biological control agent.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the most important vegetable crop in Spain, with a production area of close to 60,000 ha in 2002 and a total production of 4 million metric tonnes. Almost 28% of this volume is produced in the Comunidad Autónoma de Murcia and in the adjacent province of Almería (INE 2002). In both regions, tomatoes are grown primarily in plastic covered greenhouses. In recent years, the most harmful pest of tomato in these regions has been the whitefly *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae). This pest causes damage by sucking the sap, thus weakening the plant, and by secreting large amounts of honeydew that favour the appearance of sooty mould. However, the greatest impact on tomato is due to its role as vector of tomato yellow leaf curl virus (TYLCV) (SIFA 2004). Serious economic losses have been caused in Spain by this geminivirus since its appearance in the 1990s (CARM 1996). Consequently, permissible population levels of *B. tabaci* are minimal, such that implementation of biocontrol based IPM programs is difficult (Stansly et al. 2004). Nevertheless, interest in biological control continues to increase in Spain (Castañé 2002; van der Blom 2002), thanks in part to adaptation of TYLCV-tolerant varieties, development of pesticide resistance (Cahill et al. 1996; Elbert and Nauen 2000) and successful use of natural enemies against this pest (Stansly et al. 2004, 2005a, b; Urbaneja et al. 2002; Calvo and Belda 2006).

The parasitoid *Eretmocerus mundus* Mercet (Hym.: Aphelinidae) and the predator *Nesidiocoris tenuis* Reuter (Hem.: Miridae) (Sánchez et al. 2003a, b; van der Blom 2002) are endemic natural enemies of *B. tabaci* that commonly appear in tomato crops in Southern Spain. The biology, behaviour and effectiveness of *E. mundus* is well documented (Stansly et al. 2004, 2005a, b; Urbaneja and Stansly 2004), and this parasitoid is mass-reared and released to control *B. tabaci* in greenhouse vegetable crops in this region (Urbaneja et al. 2003a). In contrast, only limited research has been reported on the potential of *N. tenuis* for augmentative biological control of *B. tabaci*.

Often cited with regard to its polyphagous habit (Goula 1985; Urbaneja et al. 2005) or zoophytophagous behaviour (Dolling 1991), *N. tenuis* has been observed to contribute to the control of whiteflies, thrips, leafminers, spidermites, and Lepidoptera species in greenhouses (Arzone et al. 1990; Calvo and Urbaneja 2003; Carnero et al. 2000; Marcos and Rejesus 1992; Solsoloy et al. 1994; Torreno 1994; Trottin-Caudal and Millot 1997; Vacante and Benuzzi 2002; Vacante and Grazia 1994). However, *N. tenuis* has also been classified as a pest of tomato (Malézieux et al. 1995) due to feeding damage such as necrotic rings in both leaf and flower petioles, and whitish halos on fruit (Arnó et al. 2006; El-Dessouki et al. 1976; Kajita 1978; Malausa 1989; Malausa and Henao 1988; Vacante and Grazia 1994). The necrotic rings on the flower petiole can result in under certain conditions on floral abortion (Calvo and Urbaneja 2004; Sánchez et al. 2006). Intensity of injury to tomato has been observed to decrease with increased availability of prey (Arnó et al. 2006).

The aim of the present work is to evaluate the efficacy of *N. tenuis* in controlling *B. tabaci* and to characterize damage to tomato. This information could then form a basis for management of *N. tenuis* that will optimize benefits derived from predation on *B. tabaci* while minimizing damage to the tomato crop.

Materials and methods

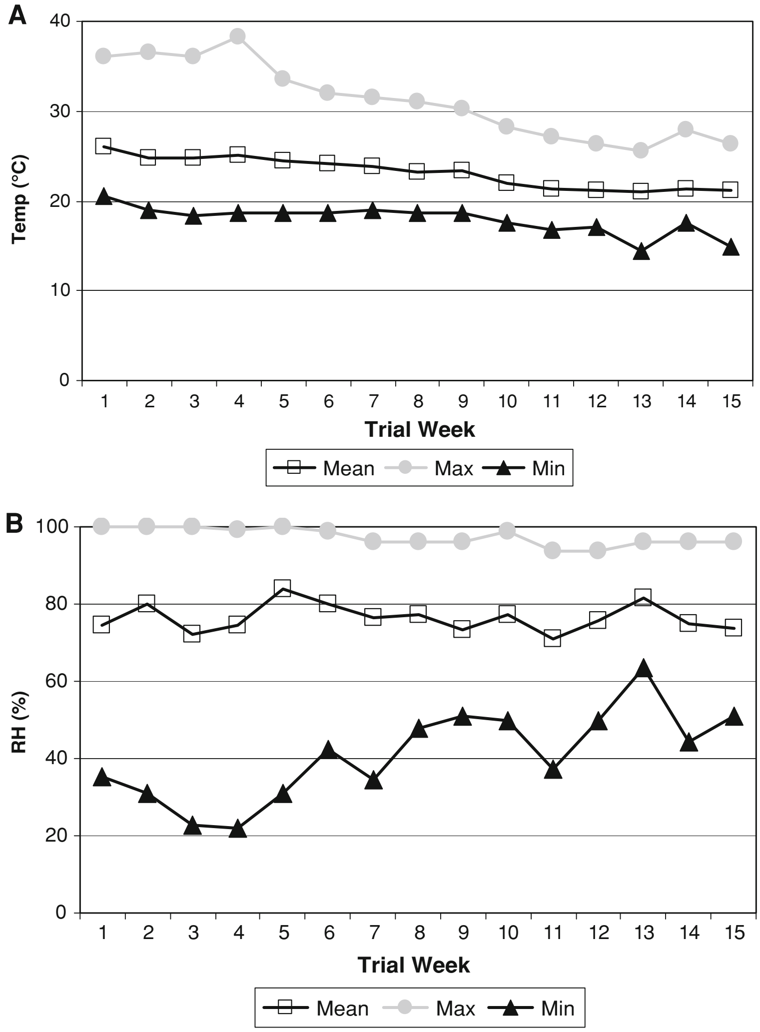
Test facilities

The experiment was conducted in a 40 × 10 m air inflated double layered polyethylene covered Quonset style greenhouse equipped with pad-and-fan cooling and diesel-fired heating, located at the Koppert Biological Systems S.A. facilities in Águilas, Murcia, Spain. The plastic tunnel was accessed through a double door and was divided into 36 experimental cages constructed of "anti-thrips" polyethylene screening with 220 × 331 μm interstices supported by heavy guy wires connected to the greenhouse superstructure. Each experimental cage was 4 × 2 × 3.5 m (l × w × h) and covered on the floor with a 2 mm-thick woven white polyethylene ground cloth. Each cage was accessed by an independent door secured with a zipper. Twelve cages were used for the present study, six on either side of the centre aisle. Dataloggers (model HOBO H8 RH/Temp, Onset Computer Company, Bourne, MA, the USA) were placed in four different cages to record temperature and relative-humidity.

Environmental conditions

The average temperature during the experiment ranged between 26°C, on 3 October 2002, to 20°C, on 16 January 2003, with an absolute minimum and maximum of 14.5 and 38.3°C respectively during the test period (Fig. 1A). Average relative humidity ranged from 84%, on 31 October 2002 to 65% on 16 January 2003 with absolute values of 100 and 22% respectively (Fig. 1B).

Fig. 1 Temperature (**A**) and relative humidity (**B**) in the greenhouse during the experiment



Plant and pest management

Tomato seeds variety 'Boludo', tolerant to TYLCV and TSWV (Seminis Vegetable Seeds Europe Enkhuizen, The Netherlands), were sown on 1 September 2002. Seeds were deposited in 5.4 cm^2 cells in expanded polystyrene trays of 11 × 19 cm filled with peat. On 27 September, seedlings were transplanted into polyethylene 6.3 l flowerpots filled with cocopeat (coir) growing medium and placed inside the experimental greenhouse in two rows of 5 plants per cage or 1.25 plants m^{-2}. Plant densities in commercial tomato crops in southern Spain vary from 1.25 to 3 plants m^{-2}. However therefore, the release rates for all the insects were calculated in individuals per plant, so results could easily be transformed to other plant, densities. Crop cultivation techniques typical of tomato greenhouse-cultivation in Spain were followed: a trellis of two wire-guides for each plant to which the main stem was trained using black polyethylene string, weekly pruning of secondary shoots, application of a standard nutrient solution for tomato by means of an automated-irrigation system with an irrigation frequency adjusted to accumulated radiation (800 W m^{-2}), and an irrigation time of 8.5 min.

Whiteflies and predators

Bemisia tabaci adults came from an experimental colony located in Águilas and identified as *B. tabaci* biotype "Q" based on DNA analysis (J.L. Cenis, IMIDA La Alberca. Murcia, España, Personal Communication). Less than 4-day-old *N. tenuis* adults were obtained from an experimental colony maintained at 25°C and 75% RH, maintained on tobacco plants and fed with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. The sex-ratio of this colony was 0.5 female/total. The colony originated from specimens collected during summer 2002 in tomato greenhouses in the province of Murcia.

Experimental design

Three release rates of *N. tenuis* were compared in the 12 cages using a randomized complete block design with three treatments replicated four times. The three treatments were a one time release of 1 or 4 *N. tenuis* plant^{-1} plus a control receiving no release. One *N. tenuis* per plant is the recommended release rate (Calvo and Urbaneja 2004), so the aim of the higher rate was to test effects of an excessive number of predators.

Each cage was infested with 55 adult *B. tabaci* on October 3rd, 2002, one week before the first evaluation. The infestation of 55 adult whiteflies per cage was chosen to simulate a strong and early whitefly attack. Two weeks later (just after the second evaluation), *N. tenuis* was released inside the cages. Special care was always taken to enter the control cages first, then the cages with the lower release rate and finally the ones with the higher release rate, to reduce risk of accidental contamination among treatments.

Evaluations

Six randomly chosen plants per cage were sampled weekly for 15 weeks, beginning 10 October, 2002. First, the number of *N. tenuis* nymphs and adults and the number of necrotic rings were counted from 7 apical leaves of the 6 plants (Castañé and Carnero 2002). Then a leaf belonging to the middle strata was selected at random from each plant and cautiously turned to count the number of *B. tabaci* adults. Finally nymphs (N_1–N_4) were counted on this same leaf using a 10× hand lens (Stansly et al. 2005a).

Statistical analysis

Values are expressed as mean ± standard error of the mean. The accumulated number of whitefly nymphs plus pupae, whitefly adults, *N. tenuis* nymphs and adults, and necrotic rings during the course of the trial were calculated (Stansly et al. 2005a, b). The resulting estimates of insect and damage accumulated × days (= area under the weekly incidence curve) was then subjected to a one-way analysis of variance joined with a Tukey's test for mean separation ($P < 0.05$).

The number of necrotic rings per leaf was fitted to the number of *B. tabaci* nymphs per leaf, the number of *N. tenuis* individuals (adults and nymphs) per leaf and the ratio of *B. tabaci* nymphs per leaf:number of *N. tenuis* adults + nymphs per leaf using linear, power, exponential, inverse and logarithmic regression analyses. All statistical analyses were carried out with SPSS v12.0 (SPSS 2004).

Results

Bemisia tabaci populations

The greatest number of whitefly adults per leaf (93.1 ± 24.7) were observed in the control treatment the last week of the test (Fig. 2A). This compared to a maximum of 17.4 ± 5.0 adults per leaf observed in week 12 in cages receiving 1 *N. tenuis* plant^{-1}, representing a relative reduction of 81%. The maximum number of *B. tabaci* adults in cages receiving 4 *N. tenuis* plant^{-1}, observed in week 9, was only 3.3 ± 0.6, representing a 96% reduction compared to the maximum in the control treatment. Accumulated *B. tabaci* adult per day reflected these relationships although differences between the 1 and 4 *N. tenuis* plant^{-1} release rates were not significantly different ($F = 43.16$; d.f. = 2, 53; $P < 0.001$) (Table 1).

Similar trends were observed in numbers of *B. tabaci* nymphs, with maxima at the end of the experiment reaching 744.5 ± 116.1, 297.0 ± 89.2 and 92.9 ± 12.6 for the control treatment, and the 1 and 4 *N. tenuis* plant^{-1} treatments respectively representing reductions of 62% and 87% respectively (Fig. 2B). Again, the number of accumulated nymph per day was significantly different between the control and the two release rates with no differences between the latter ($F = 28.94$; d.f. = 2, 53; $P < 0.001$) (Table 1).

Nesidiocoris tenuis populations

Most adult *N. tenuis* were initially observed in cages receiving the high release rate. However, their numbers

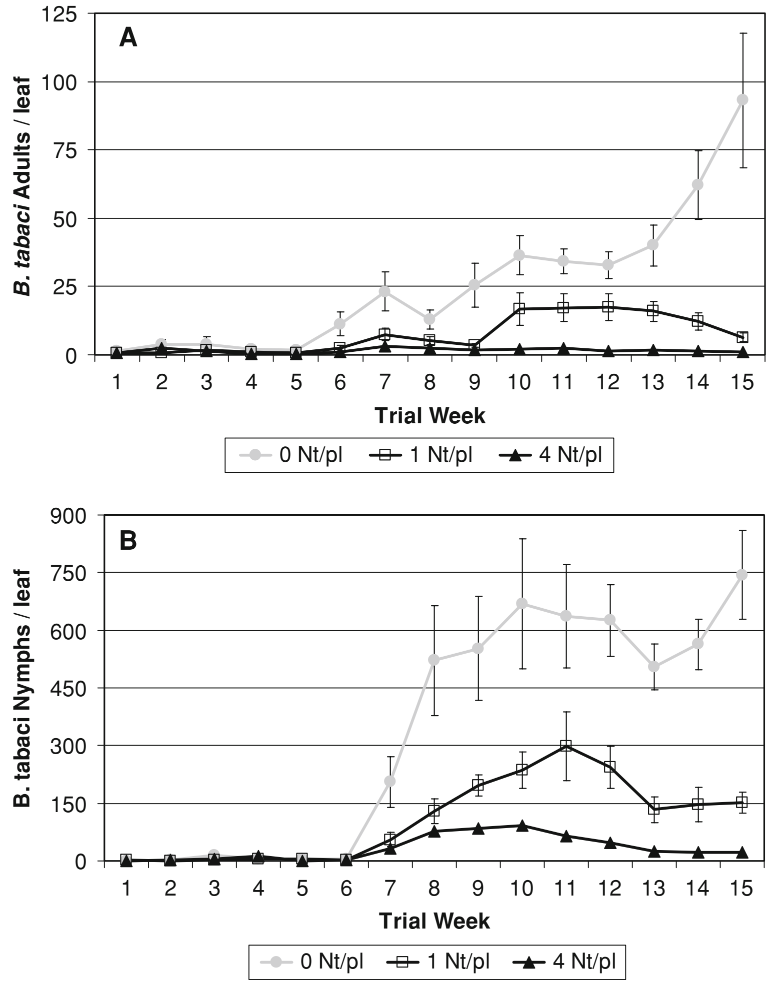


Fig. 2 Average whitefly adults (**A**) and nymphs (**B**) per leaf in each week (±SE) for treatments receiving 0, 1 and 4 *N. tenuis* plant^{-1}. Whiteflies were released in week 0 and *N. tenuis* was released just after the second evaluation in week 2

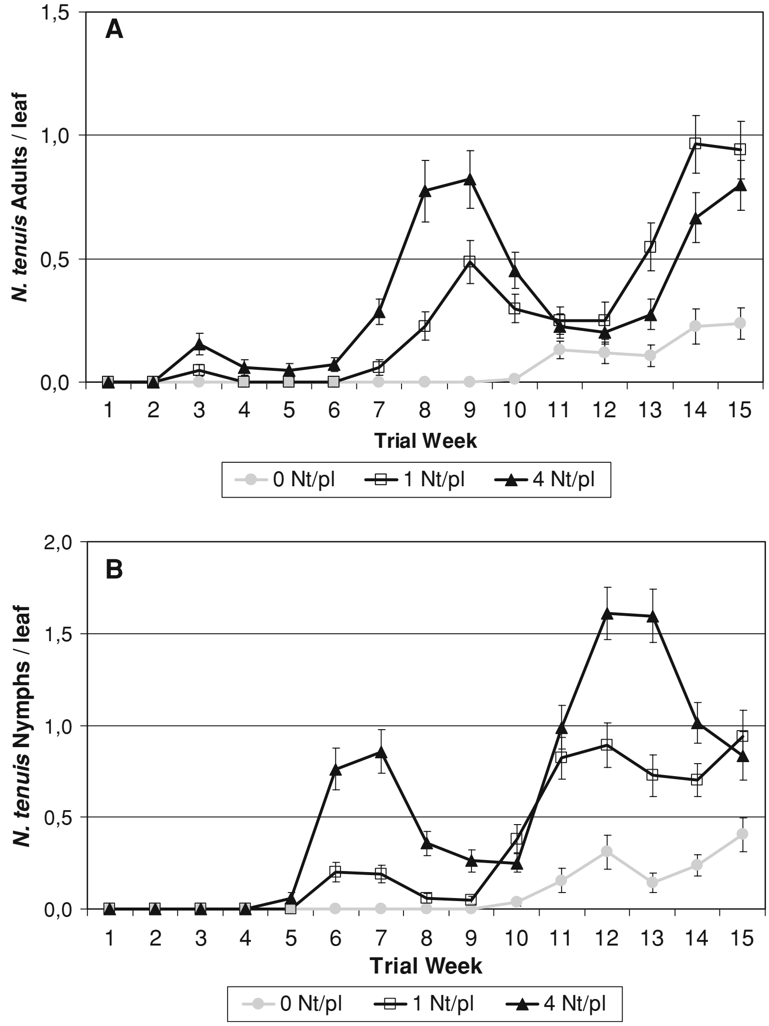
Table 1 Average nymphs and adults of *B. tabaci* (±SE), nymphs and adults of *N. tenuis* (±SE) and necrotic rings (±SE) accumulated per day for treatments receiving 0, 1 and 4 *N. tenuis* per plant

	Release rate (Ind. plant^{-1})		
	0 *N. tenuis* plant^{-1}	1 *N. tenuis* plant^{-1}	4 *N. tenuis* plant^{-1}
B. tabaci			
Adults	2360.4 ± 251.4 a	741.4 ± 164.1 b	161.0 ± 13.0 b
Nymphs	32795.2 ± 4573.5 a	10732.6 ± 1821.0 b	3339.0 ± 338.0 b
N. tenuis			
Adults	5.0 ± 1.03 b	25.2 ± 1.9 a	31.0 ± 1.8 a
Nymphs	7.5 ± 1.4 c	31.4 ± 2.0 b	57.2 ± 2.4 a
Necrotic rings	12.6 ± 2.1 c	56.7 ± 3.1 b	88.2 ± 3.1 a

Means in the same row followed by the same letter are not significant different (Tukey, $P < 0.05$)

declined after week 11 compared to low release cages (Fig. 3A) although differences in number of accumulated adults per day did not differ significantly between the two release rates ($F = 70.52$; d.f. = 2, 251; $P < 0.001$) (Table 1). Peak numbers of mirid nymphs were considerably greater in response to the 4 *N. tenuis* plant^{-1} release rate (Fig. 3B). Furthermore, more cumulative nymphs per day were observed with 4 *N. tenuis* plant^{-1} compared to 1 *N. tenius* plant^{-1} (Table 1) ($F = 157.61$; d.f. = 2, 251; $P < 0.001$). As many as 0.2 ± 0.1 adults and 0.4 ± 0.1 nymphs per terminal were seen in cages where no *N. tenuis* were

Fig. 3 Average *Nesidiocoris tenuis* adults (**A**) and nymphs (**B**) per leaf in each week (±SE) for treatments receiving 0, 1 and 4 *N. tenuis* plant^{-1}. Whiteflies were released in week 0 and *N. tenuis* was released just after the second evaluation in week 2



released due to unintentional contamination, although fewer *N. tenuis* day^{-1} were accumulated in these cages compared to release cages.

Plant injury

The first necrotic rings were observed in week 6 with the greatest incidence always in cages receiving 4 *N. tenuis* plant^{-1} (Fig. 4). Maxima observed per leaf were 3.9 ± 0.2, $1.6 \pm 0.1$and 0.5 ± 0.1 for the high, low and zero release rates respectively. Differences were significant among all treatments in the number of accumulated necrotic rings day^{-1} (Table 1) ($F = 181.67$; d.f. = 2, 251; $P < 0.001$).

Regression analysis showed best fit between number of necrotic rings and the ratio of *B. tabaci* nymphs to *N. tenuis* adults + nymphs (Table 2). R^2 and F values were highest using power regression. The regression equation was $y = 15.086x - 0.6359$, where y was the number of necrotic rings per leaf and x was the ratio of *B. tabaci* nymphs to *N. tenuis* adults + nymphs (Fig. 5).

Discussion

Nesidiocoris tenuis was highly effective in controlling *B. tabaci* on tomato under these experimental conditions, with little difference observed between the two release rates evaluated. Whitefly reductions of up 81% and 96% were recorded with only one release of 1 or 4 *N. tenuis* plant^{-1} respectively. Furthermore, *N. tenuis* established well in the tomato crop under the experimental conditions, reaching

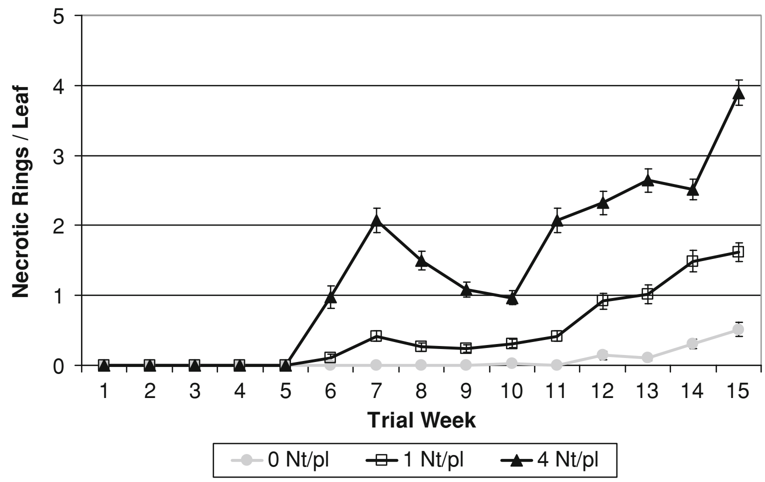


Fig. 4 Average necrotic rings per leaf in each week (±SE) for treatments receiving 0, 1 and 4 *N. tenuis* plant^{-1}. Whiteflies were released in week 0 and *N. tenuis* was released just after the second evaluation in week 2

Table 2 Lineal, power, exponential, inverse and logarithmic regression analysis of the number of necrotic rings observed with the number of *B. tabaci* nymphs per leaf, with the number of *N. tenuis* individuals (adults and pupae) per leaf and with the quotient *B. tabaci* nymphs per leaf/individuals of *N. tenuis* per leaf

Curve type	R^2	d.f.	*F*	*P*	*a*	*b*
Necrotic rings depending on the number of nymphs of *B. tabaci*/*N. tenuis*						
Lineal	0.191	53	12.288	0.001	−0.001 ± 0.000	1.647 ± 0.149
Logarithmic	0.705	53	124.270	<0.001	−0.630 ± 0.057	4.163 ± 0.259
Inverse	0.675	53	107.914	<0.001	26.867 ± 2.586	0.651 ± 0.113
Power	0.745	53	151.842	<0.001	−0.636 ± 0.052	15.086 ± 3.565
Exponential	0.351	53	28.101	<0.001	−0.001 ± 0.000	1.278 ± 0.168
Necrotic rings depending on the number of nymphs of *B. tabaci*						
Lineal	0.308	53	23.139	<0.001	−0.005 ± 0.001	1.994 ± 0.172
Logarithmic	0.477	53	47.473	<0.001	−0.761 ± 0.110	4.675 ± 0.484
Inverse	0.297	53	21.953	<0.001	26.289 ± 5.611	0.846 ± 0.176
Power	0.350	53	27.959	<0.001	−0.640 ± 0.121	14.617 ± 7.750
Exponential	0.257	53	18.021	<0.001	−0.004 ± 0.001	1.587 ± 0.278
Necrotic rings depending on the number of *N. tenuis*						
Lineal	0.543	53	61.674	<0.001	1.295 ± 0.165	−0.90 ± 0.218
Logarithmic	0.418	53	37.278	<0.001	0.849 ± 0.139	1.488 ± 0.115
Inverse	0.187	53	11.926	0.001	−0.164 ± 0.048	1.720 ± 0.160
Power	0.649	53	96.348	<0.001	1.040 ± 0.106	1.028 ± 0.090
Exponential	0.584	53	73.129	<0.001	1.320 ± 0.154	0.203 ± 0.041

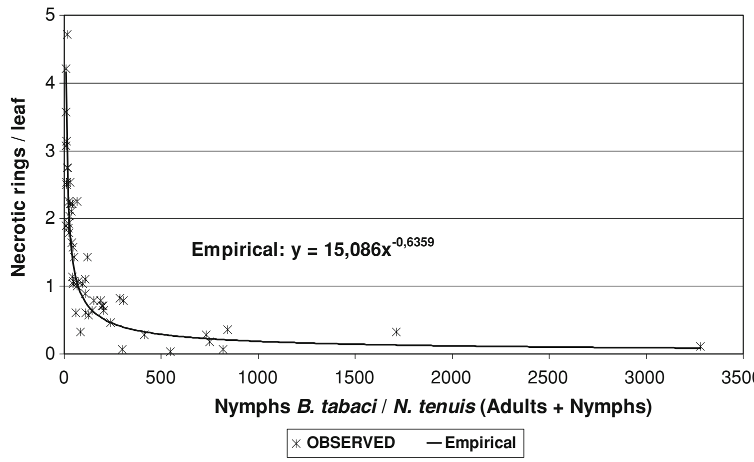
R^2 is the *r* square parameter; d.f. are the degrees freedom; *F* is the Fisher's parameter; *P* is the significant level and *a* and *b* are the constant and the dependent parameter of the curve (±SE)

high population levels with both release rates assayed. These results are consistent with studies showing that tomato plants are good host plants for *N. tenuis* (Carnero et al. 2000; El-Dessouki et al. 1976; Goula 1985; Goula and Alomar 1994; Sánchez et al. 2003a, b; Urbaneja et al. 2005).

We found in an earlier study that no *N. tenuis* developed successfully on tomato plants without prey (Urbaneja et al. 2003a, b, 2005). *Dicyphus hesperus* Knight (Het.: Miridae) was also unable to complete its development when fed exclusively on tomato leaves (Gillespie and McGregor 2000). Conversely, *Macrolophus pygmaeus* Rambur and *Dicyphus tamaninii* Wagner (Het.: Miridae), were able to reach adulthood feeding on tomato leaves and fruits, respectively (Perdikis and Lykouressis 2000; Lucas and Alomar 2001).

In the present experiment, the number of accumulated *N. tenuis* adults per day was not significantly different between both release rates, although more nymphs were observed with the high rate. Prey availability per predator must have been lower at the higher release rate, since the number of whitefly nymphs was not significantly different. This would

Fig. 5 Observed and empirical distribution based on a power model for the number of necrotic rings caused by *N. tenuis* on depend of the prey availability



result in reduced survivorship of mirid nymphs, either directly through poorer nutrition or indirectly through competitive interactions such as cannibalism, although our observations did not allow us to distinguish between these possibilities. However, cannibalism in zoophytophagous species is not common under field conditions (Castañé et al. 2002; Lucas and Alomar 2002).

The first necrotic rings surrounding the pedicel were detected on tomato leaves by week 6 of the test. The cumulative number of necrotic rings per leaf was significantly greater in the high release rate treatment. Shipp and Wang (2006) in a similar trial also obtained a strong correlation between tomato damage and prey availability when they tested different release rates of *D. hesperus* Knight (Het.: Miridae) against the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thy.: Thripidae).

Gillespie and McGregor (2000) proposed three simple models for feeding behaviour in omnivorous Heteroptera: (1) the amount of plant feeding decreases with increased prey feeding, (2) the amount of plant feeding increases with increased prey feeding and (3) the amount of plant feeding is independent of the amount of prey feeding. *Nesidiocoris tenuis* appears to follow the first model because damage to tomato decreased in response to greater availability of *B. tabaci*. These results agreed with those of Arnó et al. (2006) based on observations made under laboratory conditions. Hence, it seems that *N. tenuis* feeds on tomato plants when there is a lack of prey. This behaviour differs from *D. hesperus* for which damage to tomato increased in response to increased prey availability (model 2) due to the need to obtain water from the plant for external digestion of prey (Gillespie and McGregor 2000). In contrast to both these mirids, *D. tamaninii* seems to damage tomato fruits independently of prey availability (model 3) (Lucas and Alomar 2002).

The ratio of *B. tabaci* nymphs and *N. tenuis* individuals was the best predictor of incidence of damage by *N. tenuis*. Therefore, to avoid undue injury to a tomato crop by *N. tenuis*, special attention should be paid to this ratio. Shipp and Wang (2006) observed that damage caused by *D. hesperus* increased exponentially when a ratio of 1:10 (predator:prey) was exceeded. Alomar et al. (1991) came to a similar conclusion for *D. tamaninii* preying on greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hem: Aleyrodidae) in tomato greenhouses. They recommended close monitoring of the relative abundance of predator and prey to avoid damage to tomato fruit (Lucas and Alomar 2002). Alomar and Albajes (1996) provided a decision chart indicating that insecticidal control against *D. tamanini* was required when it exceeded 4 per plant and adult whitefly were less than 20 per plant. We focused on whitefly nymphs rather than adults in our study, but could not provide a decision chart for *N. tenuis* because no information is available to relate injury from this species to tomato yield or fruit quality. When such information becomes available, threshold ratios of *B. tabaci nymphs* to *N. tenuis* could be established.

This predator, like most mirid predators, displays a high degree of polyphagous behaviour and is able to feed on several different pest species. Urbaneja et al. (2003b, 2005) showed that on tomato leaves and under laboratory conditions at 25°C, *N. tenuis* was able to complete its life cycle in 12.8, 13.21, 20.61 and 23.44 days feeding on the eggs of *E. kuehniella*, *B. tabaci* nymphs, larvae of *F. occidentalis* and immatures and adults of *Tetranychus urticae* Koch (Acari: Tetranychidae), respectively. Thus, the presence of these other pests could potentially increase the tolerance level for *N. tenuis* per plant without significant increase in plant damage.

Mirid predators respond differently to different plant types as well. For instance, *D. tamaninii* damages tomato but not cucumber (Gabarra et al. 1995; Castañé et al. 1996). Thus, the balance between plant injury and biological control is determined by both the quantity and quality of plant and prey types (Eubanks and Denno 1997; Agrawal et al. 1999). As a consequence, the status of a mirid species such as *N. tenuis* as pest or biological control agent will depend on crop, pest complex, and possibly other circumstances. Thus, further studies may be necessary to evaluate the utility of *N. tenuis* as biological control agent in particular agroecosystems.

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