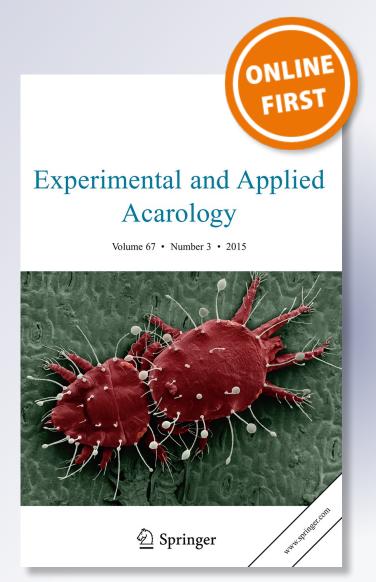
Conservation biological control in strawberry: effect of different pollen on development, survival, and reproduction of Neoseiulus californicus (Acari: Phytoseiidae)

María F. Gugole Ottaviano, Claudia V. Cédola, Norma E. Sánchez & Nancy M. Greco

Experimental and Applied Acarology

ISSN 0168-8162

Exp Appl Acarol DOI 10.1007/s10493-015-9971-7





Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This eoffprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".





Conservation biological control in strawberry: effect of different pollen on development, survival, and reproduction of *Neoseiulus californicus* (Acari: Phytoseiidae)

María F. Gugole Ottaviano¹ · Claudia V. Cédola¹ · Norma E. Sánchez¹ · Nancy M. Greco¹

Received: 10 June 2014/Accepted: 6 October 2015 © Springer International Publishing Switzerland 2015

Abstract Wild vegetation surrounding crops may provide temporary habitat and potential food sources for phytoseiids in different seasons. Monthly vegetation samples of wild plants adjacent to strawberry plants and wild plants in a vegetation strip close to the crop were taken. The frequency of *Neoseiulus californicus*, *Tetranychus urticae* and other mites and insects was recorded. In addition, in a laboratory assay, the survival, developmental time and fecundity of females fed on pollen of strawberry and pollen of wild plants where N. californicus was recorded during their flowering, were estimated. Pollen from Urtica urens, Lamium amplexicaule, Convolvulus arvensis, Sonchus oleraceous, Galega officinalis, and Fragaria x ananassa (strawberry) allowed development of N. californicus to adult, but not reproduction. Survival was 70-80 % when fed on pollen from S. oleraceus, G. officinalis and C. arvensis, 80-90 % when fed on pollen from U. urens and F. x ananassa, and more than 90 % when fed on T. urticae and on pollen from L. amplexicaule. In autumn and winter, U. urens, L. amplexicaule and S. oleraceous could promote the persistence of N. californicus when prey density in strawberry is low, offering T. urticae, thrips and pollen. In summer, pollen of C. arvensis and G. officinalis would contribute to the persistence of N. californicus when the strawberry crop is ending and offers scarce food resources. Although the pollen of these plants would not enable the predator population to increase, the presence of these plants in the vicinity of strawberry could contribute to the persistence of N. californicus population and help to limit T. urticae growth when this pest begins to colonize the crop.

Keywords Neoseiulus californicus · Conservation · Wild vegetation · Pollen · Strawberry

Published online: 12 October 2015

CEPAVE (CCT La Plata, CONICET-UNLP), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Boulevard 120 e/61 y 62, 1900 La Plata, Argentina



Nancy M. Greco ngreco@fcnym.unlp.edu.ar

Introduction

Conservation biological control involves the habitat manipulation of natural enemies to favor their survival and physiological performance and/or behavior in order to increase their effectiveness as control agents. In general, this strategy entails the removal or reduction of harmful conditions to natural enemies, or promotes favorable factors that are absent or at inadequate levels (Barbosa 1998). For example, the biological control of *Tetranychus urticae* Koch (Acari: Tetranychidae) by conservation of *N. californicus* (McGregor) from reducing acaricide applications (Greco et al. 2011) and conservation of polyphagous insect predators of the genus *Macrolophus* (Hemiptera: Miridae) on noncrop plants in the Mediterranean vegetable agroecosystems (Alomar et al. 1994; Perdikis et al. 2007).

Wild vegetation adjacent to crops encourages survival and reproduction of natural enemies, providing prey and pollen as food, microclimates favorable to their development, and shelters for extreme environmental conditions and/or for pesticides (Ferro and McNeil 1998; Penagos et al. 2003). These plants have a central role in maximizing the control of herbivores by natural enemies (Fiedler et al. 2008). Bickerton and Hamilton (2012) indicated that biocontrol of the European corn borer *Ostrinia nubilalis* (Hübner) by generalist predators in pepper can be improved by intercropping with three flowering plants.

The importance of maintaining natural vegetation surrounding crops to sustain predatory mite populations has been documented in many cropping systems and suggests that this vegetation may provide temporary habitat and potential food sources for phytoseiid mites in different seasons (De Moraes et al. 1993; Duso et al. 2003; Ferla et al. 2007). Several species of phytoseiids were found both in a strawberry crop and in the surrounding vegetation, especially on *Richardia* sp., *Agerantum conyzoides* L., *Sonchus oleraceus* L., *Bidens pilosa* L. and *Rumex* sp. in Brazil (Ferla et al. 2007).

Neoseiulus californicus has been reported as highly effective in controlling two the spotted spider mites, *T. urticae*, in several crops (Strong and Croft 1995; Sato et al. 2007; Greco et al. 2011). This predator can adapt to variation in prey populations providing stable pest suppression over time (Escudero and Ferragut 2005; Greco et al. 2005, 2011). Their ability to survive on various food sources contributes to their persistence in the agro ecosystem (Cross et al. 2001; Rhodes et al. 2006). As a type II generalist, *N. californicus* has a fairly broad diet range that includes thrips nymphs, other mites and pollen (McMurtry and Croft 1997; Makoto et al. 2005).

The persistence of *N. californicus* in horticultural farms could be enhanced by wild vegetation, since the vegetation could provide overwintering sites as well as food source. Indeed, *N. californicus* fed pollens of *Eschoschltzia californica* Cham., *Convolvulus arvensis* L. and *Helianthus annus* L. had a similar survival than when fed *T. urticae* and spite of fecundity was a little lower, the viability of eggs was a hundred percent (Sazo et al. 2006). Ragusa et al. (2009) reported that with pollens of *Carpobrotus edulis* (L.) and *Scrophularia peregrina* L. this predator had high values of survival and oviposition.

Neoseiulus californicus is the main established predator of *T. urticae* in commercial strawberry crops in La Plata, Buenos Aires, Argentina. In this region, the predator is able to colonize strawberry crops early in the season, even at low densities of its main prey, *T. urticae* (Greco et al. 1999). Moreover, it can limit the rate of prey population increase, depending on the initial prey–predator ratio (Greco et al. 2005). These characteristics suggest that this predator is a promising conservation biological control agent for *T. urticae*.



Our hypothesis is that pollen from some wild plants surrounding the strawberry crops, as well as strawberry pollen, represents alternative food to *N. californicus*. We expect to find that pollen from the plants where this predator is present allow predator survival and reproduction. Conservation of natural enemies through habitat manipulation requires a compromise in the selection of plants that provide food resources, shelter and oviposition sites, whereas at the same time are not host of other herbivores that may attack the crop (Escudero et al. 1999). Therefore, the presence of thrips, aphids and whiteflies which are secondary pests of strawberry should be considered in the selection of suitable plants for the conservation of *N. californicus*.

In view of the fact that a number of species of tydeid mites can act as alternative prey for phytoseiids (Knop and Hoy 1983) we pay attention to the mite *Tydeus kochi* Oudemans (Acari: Tydeidae), commonly recorded on strawberry, being that it could be a food resource to *N. californicus*. *Tydeus kochi* is considered an unspecialized feeder, since tydeids have been reported as fungivores, predators, scavengers or feeders on different plant exudates and pollens (Baker and Wharton 1952; Sabbatini Peverieri et al. 2009).

The specific objectives of this study were: (a) to identify wild plants surrounding the strawberry crops housing *N. californicus*, and (b) to analyze the effect of pollen of these plants as well as strawberry pollen on development, survival, and reproduction of *N. californicus*. In addition, we investigated whether the mite *T. kochi* could be a food resource to *N. californicus*. The purpose was to identify plants that may promote the presence and persistence of *N. californicus* in strawberry crops.

Materials and methods

Field sampling

Field sampling was performed in a farm located in Los Hornos, La Plata, Buenos Aires province, Argentina (38°52′S, 57°59′O). The farm was representative of horticultural farms of the region in which several seasonal crops (tomato, sweet pepper, eggplant, artichoke, leaf vegetables, and strawberry) are cultivated throughout the year under open field or greenhouse conditions. One strawberry plot of approximately 1 ha each was cultivated under low plastic tunnels and irrigation and soil management were standard for the region. Granulated fertilizer composed of total nitrogen (N) 15.0 % (8.9 % ammoniacal N, 6.1 % nitrate), assimilable phosphorus 127 (P_2O_5) 15.0 % and water-soluble potassium (K_2O) 15.0 % was applied to the soil 20 days before planting. Methyl bromide was used to disinfect the soil. The beds were covered with black polyethylene mulch and irrigated by drip.

Monthly vegetation samples were taken to characterize the community of wild plants. Samples were taken from (a) wild plants adjacent to strawberry plants (growing at the edges of beds) from April 2005 to April 2006 and from January 2008 to March 2009, and (b) wild plants in a vegetation strip close to the crop (2 m wide, 100 m long and 5 m away from the crop), from June 2006 to February 2007. In (a), 30 points were randomly selected in the crop and three leaves of each wild plant species present were taken at random. The total number of leaves collected depended on the number of plant species recorded at each point, since them were not always the same. When plants were in reproductive stage also three flowers of each were collected. In (b), 20 sample units (0.25 m² each) were delimited



at random and three leaves of each plant species present were collected. When the plants were in the reproductive stage, three flowers were also collected.

Species richness and frequency of each plant species were calculated separately from data of (a) and (b) samples. The frequency of each plant was calculated as: (number of sample units in which the plant species was present/total number of sample units) \times 100, for each season (Matteucci and Colma 1982).

Neoseiulus californicus, T. urticae and other mites and insects were counted under binocular microscope in leaves and flowers from (a) and (b), and frequency of each was calculated as: (number of times that each organism was present in the plant/total number of times that the plant was registered) \times 100.

Generalized nonlinear models were used to examine the relationship between the frequency of *N. californicus* and the frequency of each wild plant species, the frequency of *T. urticae* and the season, assuming a Poisson distribution of errors and the log link function. The statistical significance of each variable was tested in turn in the model by a forward stepwise procedure, and those that contributed to the most significant change in deviance from the null model were retained. The change in the deviance was tested by the likelihood ratio test, considering a χ^2 distribution with a significance level of 0.05. The Wald statistic was used to test the significance of the regression coefficient (Lindsey 1997).

The associations between *N. californicus*, wild plant species, and other mites and insects present during autumn–winter and spring–summer were assessed by a correspondence analysis (CA) (Salvador Figueras 2003). The distances between observed and expected frequencies were analyzed by χ^2 test according to the null hypothesis of independence.

Laboratory assays

Neoseiulus californicus and T. urticae colonies were started from individuals collected in strawberry crops and maintained separately under controlled conditions of 25 ± 2 °C, 60–70 % relative humidity and 14 L/10D photoperiod. Colonies were kept on new and completely expanded trifoliate strawberry leaves, with petiole placed in water-filled tubes (height 7 cm, diameter 2 cm) to keep the leaves turgid, inside a box (500 ml) covered with paper film to prevent individuals from escaping. Due to the morphological similarity of N. californicus with other species, phytoseiids were identified according to Schuster and Pritchard (1963) and Guanilo et al. (2008). Individuals of N. californicus were supplied, every 2 days, with eggs, nymphs and adults of T. urticae as food, using a fine brush of sable hair.

Pollens from the wild plants where *N. californicus* was recorded during flowering, and pollen of strawberry, were collected. Flowers of each species were placed individually in paper bags and kept at room temperature for 48 or 72 h to remove moisture from the anthers. Then, anthers were placed in petri dishes and preserved in refrigerator at 10 °C until they were used in the experiment.

The survival of N. californicus fed pollen of each wild plant where it was present at flowering stage, pollen of strawberry and T. urticae (10 eggs and 10 active stages) was recorded daily under binocular microscope from protonymph to adult, since larvae do not feed. Each treatment (N = 14) consisted of an initial cohort of 24 h old eggs placed individually in experimental units. The experimental unit was a petri dish (2.7 cm diameter) containing a strawberry leaf piece (1.8 cm in diameter) with the abaxial side up, on an agar disc of the same diameter and floral foam (2 mm high). The discs were surrounded by water to prevent individuals from escaping and to keep moisture in the device. To preserve strawberry discs in optimal conditions, they were replaced whenever necessary moving the



predator with a fine brush. The eggs came from females fed on eggs, larvae, nymphs and adults of T. uticae. When N. californicus eggs hatched, anthers were brushed onto the disc with a sable hair brush, dropping the pollen grains on the disc. The initial number of each cohort (n = 20–133) did not include individuals that left the experimental unit.

The survival of individuals that completed development (N = 7, n = 17–45) was estimated using the product-limit estimator of Kaplan–Meier (1958). Survival curves were compared using the multiple comparison χ^2 tests and then pairwise comparisons to identify differences between treatments were made by Gehan Wilcoxon test (Gehan and Thomas 1969). The duration of protonymph, deutonymph and total duration of nymphal stage with each food was analyzed by one-way ANOVA and Tukey tests were used to compare means. In all cases that did not meet the assumptions of normality and homoscedasticity required by ANOVA, square root transformation was applied to data and if further assumptions were not met Kruskal–Wallis test was performed.

Another assay was performed to estimate fecundity of the females fed on pollen of plants that allowed *N. californicus* development. The experimental unit was a strawberry disc of approximately 4 cm diameter placed on an agar disc inside a Petri dish (8 cm diameter). The discs were surrounded by water to prevent the escape of individuals and to maintain moisture. Fifteen *N. californicus* eggs were placed on the strawberry disc and after hatching, individuals were provided with pollen (treatments) or *T. urticae* (control treatment). Once in the adult stage, the number of eggs/female was recorded during 10 days. Each treatment was replicated six times.

To determine whether T. kochi is consumed by N. californicus, 5 eggs (n = 10), 5 nymphs (n = 14) and 5 adults (n = 14) collected from strawberry leaves were offered to newly mated females with 48 h fasting. The survival of N. californicus was recorded daily under binocular microscope until all females died.

Results

Field sampling

In total, 54 wild plant species were recorded throughout the year. Most species were present in both sites. Species richness of wild vegetation adjacent to strawberry was 32, 24, 22 and 32 in autumn, winter, spring and summer, respectively (Table 1). *Neoseiulus californicus* was recorded in 11 species of these wild plants throughout the year, exhibiting a similar pattern in all seasons. It was registered mostly (64 %) on plant species with intermediate and very low frequencies. Their main prey, *T. urticae*, was present on *U. urens*, *E. crus-galli*, *L. amplexicaule*, *D. fullonum*, *C. arvensis* and *A. viridis* (Fig. 1a).

On the strip of wild vegetation close to the crop, the plant species richness was 9, 14 and 13 in winter, spring and summer, respectively (Table 2). *Neoseiulus californicus* was found on four species, in spring and summer. It was more frequent on *C. arvensis* (very common) and *M. alba* (rare) along with its main prey, *T. urticae* (Fig. 1b).

The wild plants where N. californicus was recorded during their flowering were A. viridis, S. oleraceus, T. officinale, C. arvensis, G. officinalis, L. amplexicaule, A. cristata, E. crus-galli, E. pilosa, L. multiflorum, P. aviculare and U. urens.

The frequency of *N. californicus* was affected by the frequency of *T. urticae*, the frequency of plant species and the season (Table 3). All variables explained significantly the variation in *N. californicus* frequency. The frequency of *T. urticae* was the most



Table 1 Frequencies (%) of wild plant species adjacent to strawberry plants, and frequencies (%) of Neoseiulus californicus on them

Family	Species	Autum	n	Winter		Spring		Summer	
		Plants	N. c.						
Alliaceae	Nothoscordum inodorum (Ait) Nichols	0.99	0.00	-	-	-	-	-	_
Amaranthaceae	Amaranthus viridis L.	2.97	0.00	8.14	14.29	4.28	7.69	-	-
Asteraceae	Anthemis cotula L.	3.98	0.00	7.46	0.00	2.33	0.00	12.04	0.00
	Baccharis coridifolia DC.	3.96	0.00	_	-	-	-	11.11	0.00
	Baccharis trimera (Less) DC.	0.99	0.00	-	-	2.72	0.00	-	-
	Carduus acanthoides L.	2.17	0.00	-	-	-	-	-	-
	Coniza sp.	_	_	_	_	1.14	0.00	_	_
	Galinsoga parviflora Cav.	6.88	0.00	5.26	0.00	1.14	0.00	1.587	0.00
	Picris echioides L.	1.09	0.00	6.98	0.00	2.27	0.00	1.75	0.00
	Senecio vulgaris L.	4.35	0.00	4.93	0.00	1.69	0.00	9.17	0.00
	Sonchus oleraceus L.	22.10	1.37	24.12	1.82	23.35	0.00	42.14	0.00
	Taraxacum officinale Web.	15.82	0.00	-	-	2.38	4.17	5.00	0.00
	Vernonia platensis (Spreng) Less.	-	-	-	-	1.14	0.00	-	-
	Wedelia glauca (Oct.) Hoffmann.	-	-	_	-	1.14	0.00	-	-
Boraginaceae	Echium plantagineunm L.	-	-	1.16	0.00	-	-	-	-
Brasicaceae	Capsella bursa pastoris (L.) Medicus.	0.72	0.00	7.02	0.00	3.41	0.00	2.14	0.00
	Coronopus didymus Sm.	7.61	0.00	19.74	0.00	2.99	0.00	39.17	0.00
	Rapahnus sp.	_	_	_	_	_	_	1.59	0.00
Cariophyllaceae	Stellaria media (L.) Villars	36.84	0.00	5.70	0.00	2.26	0.00	41.43	0.00
Convolvulacae	Convolvulus arvensis L.	4.35	0.00	5.19	0.00	13.69	8.70	-	-



Table 1 continued

Family	Species	Autum	n	Winter		Spring		Summer	
		Plants	N. c.						
Cyperaceae	Cyperus eragrostis Lam.	-	-	-	-	1.18	0.00	-	-
	Cyperus rotundus L.	11.59	0.00	-	-	6.55	0.00	14.29	0.00
Dipsacaceae	Dipsacus fullonum L.	0.99	100	1.47	100	-	-	3.17	0.00
Fabaceae	Lotus sp.	0.99	0.00	_	-	-	-	1.59	0.00
	Medicago polimorpha L.	-	-	1.35	0.00	1.13	0.00	-	-
	Trifolium repens L.	8.42	0.00	10.53	0.00	1.79	0.00	4.17	0.00
Gentianaceae	Centaurum pulchellum (Sw.) Druce	-	-	1.16	0.00	-	_	-	-
Geraniaceae	Geranium molle L.	1.98	0.00	-	-	-	-	-	-
Lamiaceae	Lamium amplexicaule L.	1.32	0.00	-	-	-	-	1.75	100
	Thymus sp.	_	_	1.16	0.00	_	_	_	-
Malvaceae	Anoda cristata (L.) Schlecht.	2.97	33.33	-	-	-	-	-	-
Oxalidaceae	Oxalis sp.	0.99	0.00	_	_	4.49	0.00	_	-
Plantaginaceae	Plantago major L.	-	-	-	-	4.49	0.00	-	-
Poaceae	Bromus unioloides H.B.K.	-	-	-	-	-	-	-	-
	Cynodon dactylon (L.) Pers.	1.13	0.00	-	-	1.13	0.00	60.00	0.00
	Echinocloa crus- galli (L.) Beauv.	9.06	0.00	4.61	25.00	30.74	1.26	3.17	0.00
	Eragrostis pilosa (L.) P. Beauv.	12.43	0.00	1.16	0.00	3.39	0.00	39.29	1.82
	Eragrostis virescens Presl.	1.32	0.00	2.33	0.00	1.79	0.00	13.33	0.00
	Lolium multiflorum Lam.	-	-	-	-	1.12	0.00	-	-
	Poa sp.	_	_	12.16	0.00	_	_	_	_
	Paspalum unvillei Steud	-	-	-	-	1.27	0.00	-	-
	Paspalum dilatatum Poir	-	-	-	-	11.36	0.00	-	-
Polygonaceae	Polygonum aviculare L.	1.98	0.00	8.82	8.33	4.54	0.00	2.50	0.00
	Rumex crispus L.	0.99	0.00	-	-	-	-	3.51	0.00



Ta	L	1. 1	1 c		٠:.		
13	n	e	- C	m	m	nı	1ea

Family	Species	Autum	n	Winter		Spring		Summer	
		Plants	N. c.						
Portulacaceae	Portulaca oleracea L.	27.17	0.00	-	-	76.65	0.00	-	-
Solanaceae	Datura sp.	0.99	0.00	-	-	1.27	0.00	-	-
	Solanum sp.	_	_	_	_	_	_	_	_
Urticaceae	Urtica urens L.	29.71	0.00	42.98	3.06	16.34	0.00	34.29	4.17
Verbenaceae	Verbena sp.		-	-	-	-	-	1.75	0.00

significant variable, but the model that included all variables explained the highest proportion of the variation in *N. californicus* frequency, 45.6 % (deviance change was 1496.91 out of 2751.92) in wild plants adjacent to strawberry plants, and 82.8 % (458.85 out of 554.467) on wild vegetation close to crops.

Correspondence analysis between frequency of *N. californicus*, species of wild vegetation, and other mites and insects present in autumn–winter showed that axis 1 accounted for 76.7 % and axis 2 accounted for 14.4 % (cumulative inertia = 91.1 %) of the data variability (Fig. 2). *Neoseiulus californicus* showed no clear association with any species of wild plants, while its main prey *T. urticae* was weakly associated with *L. amplexicaule*. Moreover, whiteflies, thrips, aphids and the mite *T. kochi* showed a strong association with *S. oleraceus* and *U. urens*. From spring–summer data, the first two axes accounted for 75.2 % of the variability of the data (axis 1 = 55.6 % and axis 2 = 19.6 %). *Neoseiulus californicus* was placed next to the intersection of the axes, so that it was not associated with any plant species, *T. urticae* was associated with *C. arvensis*, thrips with *T. officinale*, the mite *T. kochi* with *D. fullonum*, and aphids with *S. oleraceus*. Whiteflies did not associate with any plant species. *Neoseiulus californicus* exhibited an association with thrips in these seasons (Fig. 2).

Laboratory assays

Neoseiulus californicus fed on pollen from A. viridis, P. aviculare, L. multiflorum, E. crusgalli, A. cristata, T. officinale and E. pilosa died at protonymph stage, whilst fed on pollen of. L. amplexicaule, S. oleraceus, U. urens, G. officinalis and C. arvensis, and F. x ananassa, and T. urticae completed development. Daily survival from protonymph to adult differed with food ($\chi^2 = 25.12$, df = 6, P < 0.001) ranging between 69 and 94 % (Fig. 3). We found a high and constant survival when the predator was fed T. urticae and pollen of L. amplexicaule, whereas when it was fed C. arvensis pollen there was a marked decrease in survival at day 2 (late protonymph or early deutonymph). With the other food, survival was intermediate and declined gradually.

Developmental time of N. californicus, from protonymph to adult, differed with food $(F_{1,6} = 7.46, P < 0.01)$. It was significantly shorter when fed on T. urticae and pollen from L. amplexicaule (Table 4). In contrast, N. californicus exhibited the longest developmental time when food was pollen from U. urens. Regarding the duration of protonymph, it was significantly lower when fed on T. urticae than when fed on pollen from G. officinalis and G. urens G is G in G in



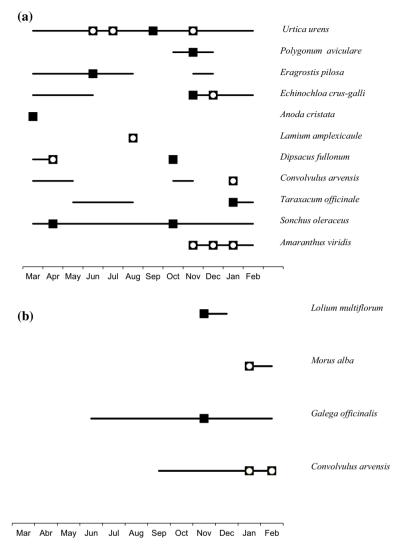


Fig. 1 Presence of *Neoseiulus californicus* on wild plants adjacent to strawberry plants (a), and wild plants in a vegetation strip close to crop (b), throughout the year in one horticultural farm in La Plata, Buenos Aires, Argentina. *Lines* represent the presence of the plant; *black squares* and *white circles* represent the presence of *N. californicus* and *Tetyranychus urticae*, respectively

significantly lower when fed on *T. urticae* than with strawberry pollen ($F_{6,17} = 2.37$, P < 0.01).

The fecundity of females of *N. californicus* fed with pollen from all species was null. Only one mating was observed when the predator was fed on pollen from *C. arvensis*. When fed on *T. urticae* the fecundity was 2.62 ± 0.22 eggs/female/day.

Neoseiulus californicus females did not consume any developmental stage of *T. kochi*. Between 40 and 60 % of females left the experimental unit during the first 24 h and survival of the remaining decreased abruptly, dying 98 % of females within 48 h.



Table 2 Frequency (%) of plant species on a strip of wild vegetation close to the crop, and frequencies (%) of *Neoseiulus californicus* on them

Family	Species	Winter		Spring		Summer	
		Plants	N. c.	Plants	N. c.	Plants	N. c.
Asteraceae	Arctium minus (Hill.) Bernh	_	_	_	_	5.00	0.00
	Baccharis coridifolia DC.	_	_	5.00	0.00	5.00	0.00
	Carduus acanthoides L.	5.71	0.00	15.00	0.00	_	_
	Carthamus lanatus L.	5.00	0.00	10.00	0.00	_	_
	Pricris echioides L.	_	_	_	_	5.00	0.00
	Sonchus oleraceus L.	_	_	10.53	0.00	_	_
Brassicaceae	Coronopus didymus Sm.	_	_	5.13	0.00	_	_
Cariophyllaceae	Stellaria media (L.) Villars	_	_	7.69	0.00	_	_
Convolvulaceae	Convolvulus arvensis L.	_	_	41.03	0.00	75.00	16.28
Dipsacaceae	Dipsacus fullonum L.	_	_	32.20	0.00	5.00	0.00
Fabaceae	Galega officinalis L.	30.91	0.00	49.15	3.39	61.67	0.00
Moraceae	Morus alba L.	_	_	_	_	5.00	50.00
Poaceae	Avena fatua L.	46.67	0.00	5.13	0.00	15.00	0.00
	Bromus unioloides H.B.K	25.71	0.00	27.12	0.00	31.67	0.00
	Cynodon dactylon (L.) Pers.	5.71	0.00	_	_	_	_
	Lolium multiflorum Lam.	_	_	70.00	21.43	20.00	0.00
	Paspalum dilatatum Poir.	25.00	0.00	_	_	5.00	0.00
	Stipa hyalina Nees.	_	_	10.00	_	_	-
Polygonaceae	Polygonum aviculare L.	_	_	_	_	5.00	0.00
-	Rumex crispus L.	5.00	0.00	_	_	_	_
Verbenaceae	Verbena sp.	15.00	0.00	12.82	0.00	5.00	0.00

Discussion

Our results agree with the findings of other authors who documented the presence of *N. californicus* on wild plants of different agroecosystems (De Moraes et al. 1993; Escudero et al. 1999; Ferla et al. 2007). The presence of *N. californicus* on *A. viridis*, *S. oleraceus*, *C. arvensis* and *U. urens*, and its absence on *P. oleracea*, in spite of the high frequency of this plant in summer and fall, is coincident with results reported by Escudero et al. (1999) in horticultural farms of Spain.

Neoseiulus californicus fed with pollen from some wild plants completed development but it was unable to reproduce. Sazo et al. (2006) found a very low rate of oviposition of *N. californicus* when fed on pollen of some species among them *C. arvensis*. Also in coincidence with Sazo et al. (2006), we found a very low survival in laboratory when *N. californicus* was fed pollen of *T. officinale*. Ragusa et al. (2009) found that *N. californicus* fed on pollen of 13 spontaneous Mediterranean plants reached adulthood and reproduced. Also, the survival of *N. californicus* fed pollen from 7 species of flora Mediterranean growing spontaneously did not differ from that of individuals who were fed their main prey. As reported by Raworth et al. (1994) and Ragusa et al. (2009), *Hordeum murinum L.* and *Stellaria media* L. seems to play an important role in the early increase of the *N.*



Table 3 Summary of the forward stepwise procedure used to build a multiple regression model for the relationship between the frequency of *Neoseiulus californicus*, the frequency of wild plant species, the frequency of *Tetranychus urticae* and seasons

Model	Deviance	Change in deviance	df	P	Wald	P
Wild plants adjacent to strawberry pl	lants					
No terms added (null model)	2751.92		195			
Int + plant freq.	2740.87	11.049	194	< 0.001	8.82	0.003
Int $+ T$. urticae freq.	1874.86	877.05	194	< 0.001	1051.49	< 0.001
Int + seasons	2627.64	124.27	192	< 0.001	75.59	< 0.001
Int + plant freq. + <i>T. urticae</i> freq. + seasons	1496.91	1255.00	190	< 0.001		
Wild vegetation on a strip close to th	e cultivation p	olots				
No terms added (null model)	554.47		62			
Int + plant freq.	457.59	96.88	61	< 0.001	23.48	0.001
Int $+ T$. urticae freq.	322.14	232.33	61	< 0.001	23.51	< 0.001
Int + seasons	461.01	93.45	60	< 0.001	23.41	< 0.001
Int + plant freq. + <i>T. urticae</i> freq. + seasons	95.61	458.85	59	<0.001		

The model assumes a Poisson distribution of errors and uses the log link function. The change in deviance after inclusion of a term in the model was tested through a likelihood ratio test (P < 0.05)

californicus population. However, in spite of *S. media* was often found in horticultural farms of this study, *N. californicus* was never registered on it.

Besides *F.* x ananassa, on *U. urens*, *L. amplexicaule* and *C. arvensis* the predator was found along with their main prey, so these plants could also be a habitat to maintain the predator–prey interaction. In addition, *U. urens* and *S. oleraceus* provide alternative preys like thrips, so it is difficult to discern in what extent predator presence in these plants can be explained by pollen and/or by prey presence.

In autumn and winter, *U. urens*, *L. amplexicaule* and *S. oleraceous* could favor the persistence of *N. californicus* in horticultural farms when prey density in strawberry is low, providing *T. urticae*, thrips and pollen as they bloom in these seasons. Chant (1959) found that phytoseiids inhabiting annual herbaceous plants disperse in the autumn to ever green plants such as *U. dioica* where they concentrate to spend winter. Nevertheless, it is important to consider that *S. oleraceous* and *U. urens* house aphids, whiteflies and thrips, so further research should address the risk that these plants could become sources of crop colonization by secondary pests.

In spring and summer, pollen of *C. arvensis* and *G. officinalis*, which flower in these seasons, would contribute to *N. californicus* persistence at the end of the strawberry growing season when it offers scarce food resources. Moreover, *G. officinalis* was the only plant on which the predator was found in absence of *T. urticae* and thrips, and its pollen allowed *N. californicus* development. It is important to consider that *S. oleraceous* and *U. urens* house aphids, whiteflies and thrips.

Pollen would represent an alternative food source allowing the survival of predators during times of shortage or absence of prey. Escudero and Ferragut (1998, 1999) suggested that absence of wild vegetation surrounding crops could delay crop colonization because predators would need more time to colonize from distant plants, increasing the risk of mortality during dispersal. The number and extension of available habitats for biological



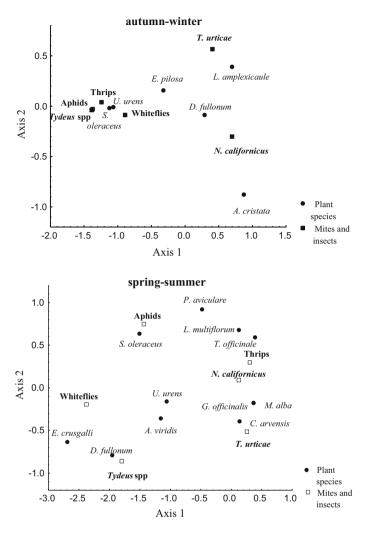


Fig. 2 Correspondence analysis of *Neoseiulus californicus* frequency, wild plant species, and other mites and insects present during autumn-winter and spring-summer in horticultural farms in La Plata, Buenos Aires, Argentina

control agents can determine the degree and duration of control in the context of conservation biological control (Barbosa and Benrey 1998).

As a type II generalist, the diet of *N. californicus* includes, in addition to *T. urticae*, thrips and other mites (McMurtry and Croft 1997). Regarding the thrips, it is known that *N. californicus* can feed on them, and indeed in this study *N. californicus* was associated with thrips in spring–summer. However, the mite *T. kochi* was not eaten by this predator in the present study, suggesting that it is very unlikely that it could affect this predator presence.



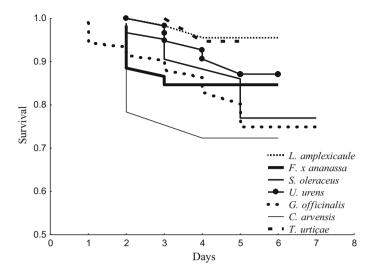


Fig. 3 Daily survival of *Neoseiulus californicus*, from protonymph to adult, fed with pollen from wild plants adjacent or close to strawberry crop, pollen from strawberry plants, and *Tetranychus urticae*

Table 4 Mean (\pm SD) developmental time (days) of immatures of *Neoseiulus californicus* fed on pollen from *F.* x ananassa and different wild plants, and the prey *Tetranychus urticae*

	Protonymph	Deutonymph	Total
Lamium amplexicaule	$1.55 \pm 0.11 \text{ ab}$	$1.57 \pm 0.09 \text{ ab}$	$3.14 \pm 0.18 \text{ ab}$
Fragaria x ananassa	1.57 ± 0.12 ab	$1.87 \pm 0.10 \text{ b}$	$3.43 \pm 0.16 \text{ bc}$
Sonchus oleraceus	1.62 ± 0.11 ab	$1.62 \pm 0.09 \text{ ab}$	$3.23 \pm 0.14 \text{ b}$
Urtica urens	$2.10 \pm 0.13 \text{ bc}$	$1.80\pm0.15~ab$	$3.90 \pm 0.15 \text{ c}$
Galega officinalis	$2.04 \pm 0.13 \text{ bc}$	$1.56 \pm 0.11 \text{ ab}$	$3.59 \pm 0.18 \text{ bc}$
Convolvulus arvensis	$2.00\pm0.31~ab$	$1.57 \pm 0.20 \text{ ab}$	$3.51 \pm 0.29 \text{ bc}$
Tetranychus urticae	$1.15\pm0.08\;a$	$1.30\pm0.15~a$	$2.45 \pm 0.11 \ a$

Different letters in the same column indicate a significant difference between means (P < 0.05 Kruskal–Wallis test)

To conclude, increasing or maintaining the wild plants *L. amplexicaule*, *C. arvensis* and *G. officinalis* close to the strawberry crop could contribute to *N. californicus* persistence in horticultural farms and improve *T. urticae* control.

Acknowledgments We thank strawberry grower H. Marengo for permission to carry out the field work at his farms and F. Ferragut (Universidad Politécnica de Valencia, España) for taxonomic determination of *Tydeus kochi*. We also thank Martha Roggiero for technical assistance. This research was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 1182/2008, PIP 2012/2014) and Universidad Nacional de La Plata (N 453 2005-2008, N 576 2009-2011).

References

Alomar O, Goula M, Albajes R (1994) Mirid bugs for biological control: identification, survey in noncultivated winter plants, and colonization of tomato fields. IOBC/WPRS Bull 17(5):217–223



- Baker EW, Wharton GH (1952) An introduction to acarology. McMillan Company, New York
- Barbosa P (1998) Agroecosystems and conservation biological control. In: Barbosa P (ed) Conservation biological control. Academic San Diego, CA, Ch 3: pp 39–54
- Barbosa P, Benrey B (1998) The influence on insect parasitoids: implications for conservation biological control. In: Barbosa P (ed) Conservation biological control. Academic San Diego, CA, Ch 4: pp 55–82
- Bickerton MW, Hamilton GC (2012) Effects of intercropping with flowering plants on predation of *Ostrinia* nubilalis (Lepidoptera: Crambidae) eggs by generalist predators in bell peppers. Environ Entomol 41:612–620
- Chant DA (1959) Phytoseiid mites (Acarina: Phytoseiidae). Part I. Bionomics of seven species in south-eastern England. Part II. A taxonomic review of the family Phytoseiidae, with descriptions of 38 new species. Can Entomol Suppl 12(91):1–119
- Cross JV, Easterbrook MA, Crook AM, Crook D, Fitzgerald JD, Innocenzi PJ, Jay CN, Solomon MG (2001) Review: natural enemies and biocontrol of pests of strawberry in North and Central Europe. Biocontrol Sci Technol 11:165–216
- De Moraes GJ, de Alencar JA, de Lima JLS, Yaninek JS, Delalibera I Jr (1993) Alternative plant habitats for common phytoseiids predator of the cassava green mite (Acari: Phytoseiidae, Tetranychidae) in Northeast Brazil. Exp Appl Acarol 17(1):77–90
- Duso C, Malgnini V, Paganelli A, Aldegheri L, Bottini M, Otto S (2003) Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. Biocontrol 49:397–415
- Escudero LA, Ferragut F (1998) Comunidades de ácaros del ecosistema hortícola mediterráneo: composición y distribución geográfica. Bol SanVeg Plagas 24:749–762
- Escudero LA, Ferragut F (1999) Abundancia y dinámica estacional de las poblaciones de fitoseidos en los cultivos hortícolas valencianos (Acari: Tetranychidae, Phytoseiidae). Bol Sanid Veg Plagas 25:347–362
- Escudero LA, Ferragut F (2005) Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari: Tetranychidae). Biol Control 32:378–384
- Escudero LA, Roselló J, Aleixandre E, Bramardi S, Ferragut F (1999) Colonización y dispersión de los ácaros en un ecosistema hortícola protegido: características y factores responsables. Bol Sanid Veg Plagas 25:143–155
- Ferla NJ, Marchetti MM, Gonçalves D (2007) Ácaros predadores (Acari) associados à cultura do morango (Fragaria sp, Rosaceae) e plantas próximas no Estado do Rio Grande do Sul. Biota Neotropica 7(2):1–8
- Ferro DN, McNeil JN (1998) Habitat enhancement and conservation of natural enemies of insects. In: Barbosa P (ed) Conservation biological control. Academic San Diego, CA, Ch 7: pp 123–132
- Fiedler AK, Landis DA, Wratten DS (2008) Maximizing ecosystem services from conservation biological control: the role of habitat management. Biol Control 45:254–271
- Gehan EA, Thomas DG (1969) The performance of some two sample tests in small samples with and without censoring. Biometrika 56:127–132
- Greco N, Liljesthröm G, Sánchez N (1999) Spatial distribution and coincidence of Neoseiulus californicus and Tetranychus urticae (Acari: Phytoseiidae, Tetranychidae) on strawberry. Exp Appl Acarol 23:567–580
- Greco NM, Sánchez NE, Liljesthröm GG (2005) *Neoseiulus californicus* (Acari: Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari: Tetranychidae): effect of pest/predator ratio on the pest abundance on strawberry. Exp Appl Acarol 37:57–66
- Greco NM, Liljesthröm GG, Gugole Ottaviano MF, Cingolani MF, Cluigt N, Sánchez NE (2011) Pest management plan for the two-spotted spider mite, *Tetranychus urticae*, based on the natural occurrence of the predator y mite *Neoseiulus californicus* in strawberries. Int J Pest Manag 57(4):299–308
- Guanilo A, De Moraes G, Toledo S, Knapp M (2008) Phytoseiid mites (Acari: Phytoseiidae) from Argentina, with description of a new species. Zootaxa 1884:1–135
- Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481
- Knop NF, Hoy MA (1983) Biology of a tydeid mite, *Homeopronematus anconai* (n. comb.) (Acari: Tydeidae), important in the San Joaquin Valley vineyards. Hilgardia 51:1–30
- Lindsey JK (1997) Applying generalized linear models. Springer, New York
- Makoto M, Tomotoshi K, Shigeki M, Masami T (2005) Predation rate of *Neoseiulus californicus* (McGregor) on three species of greenhouse thrips. Kyushu Plant Prot Res 51:73–77
- Matteucci SD, Colma A (1982) Metodología para el estudio de la vegetación. Secretaría General de la Organización de los Estados Americanos, Washington DC
- McMurtry JA, Croft BA (1997) Life styles of phytoseiid mites and their roles as biological control agents. Ann Rev Entomol 42:291–321



- Penagos DI, Magallanes R, Valle J, Cisneros J, Martinez AM, Goulson D, Chapman JW, Caballero P, Cave RD, Williams T (2003) Effect of weeds on insect pests of maize and their natural enemies in Southern Mexico. Int J Pest Manag 49(2):155–161
- Perdikis D, Favas Ch, Lykouressis D, Fantinou A (2007) Ecological relationships between non-cultivated plants and insect predators in agroecosystems: the case of *Dittrichia viscose* (Asteraceae) and *Macrolophus melanotoma* (Hemiptera: Miridae). Acta Oecol 31:299–306
- Ragusa E, Tsolakis H, Jordá Palomero R (2009) Effect of pollens and preys on various biological parameters of the generalist mite *Cydnodromus californicus*. Bull Insectol 62(2):153–158
- Raworth DA, Fauvel G, Auger P (1994) Location, reproduction and movement of *Neoseiulus californicus* (Acari: Phytoseiidae) during the autumn, winter and spring in orchards in the south of France. Exp Appl Acarol 18:593–602
- Rhodes EM, Liburd OE, Kelts C, Rondon SI, Francis RR (2006) Comparison of single and combination treatments of *Phytoseiulus persimilis*, *Neoseiulus californicus*, and Acramite (bifenazate) for control of twospotted spider mites in strawberries. Exp Appl Acarol 39:213–225
- Sabbatini Peverieri G, Simoni S, Goggioli D, Liguori M, Castagnoli M (2009) Effects of variety and management practices on mite species diversity in Italian vineyards. Bull Insectol 62:53–60
- Salvador Figueras M (2003) Análisis de Correspondencias. 5campus web. http://www.5campus.com/leccion/correspondencias. Accessed 10 Nov 2011
- Sato M, Silva M, Souza Filho M, Matioli A, Raga A (2007) Management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberry fields with *Neoseiulus californicus* (Acari: Phytoseiidae) and acaricides. Exp Appl Acarol 42:107–120
- Sazo L, Araya JE, Iturriaga P (2006) Efecto del tipo de polen sobre la supervivencia, fertilidad y viabilidad de los huevos de *Neoseiulus californicus* (Mc Gregor) (Acari: Phytoseiidae). Bol San Veg Plagas 32:619–623
- Schuster R, Pritchard A (1963) Phytoseiid mites of California. Hilgardia 34(7):191-285
- Strong WB, Croft BA (1995) Inoculative release of phytoseiid mites into the rapidly expanding canopy of hop for control of *Tetranychus urticae* Koch. Environ Entomol 24:446–453

