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Biological control of an acarine pest by single and multiple natural enemies

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\textbf{A B S T R A C T}

When multiple predator species are introduced to control a single prey species, predator species may act synergistically if the rate of mortality of the prey exceeds the rate of mortality from all single predator species combined. Alternatively, the release of multiple predator species may yield a lower than expected prey mortality due to competitive interactions (including intraguild predation) amongst predators. The question of whether and when multiple natural enemies interact to produce lower or higher prey mortality than each predator species acting alone depends on the details of predator–predator interactions. In this study, we investigated whether combined releases of \textit{Phytoseiulus persimilis} and \textit{Neoseiulus californicus} provide better biological control of \textit{Tetranychus cinnabarinus} on strawberry than releases of each predator species alone. Experiments were conducted in two commercial strawberry fields (\textit{Fragaria ananassa} Duch. cv. ‘Camarosa’). The release of \textit{P. persimilis} alone and the combined release of \textit{N. californicus} and \textit{P. persimilis} reduced the numbers of \textit{T. cinnabarinus} on strawberry equally. The densities of \textit{T. cinnabarinus} in the treatment with \textit{N. californicus} alone were significantly higher than in the other treatments, and this was the only treatment in which the economic injury level was surpassed. The densities of \textit{P. persimilis} in absence of \textit{N. californicus} were similar to those in presence of \textit{N. californicus}. Hence, there was no significant adverse effect of the presence of \textit{N. californicus} on the densities of \textit{P. persimilis}. The densities of \textit{N. californicus} in the absence of \textit{P. persimilis} were lower than the densities in the presence of \textit{P. persimilis}, but this difference was not significant. We conclude that, compared to releases with \textit{P. persimilis} alone, there is no advantage to releasing \textit{N. californicus}, either alone or in combination with \textit{P. persimilis}.

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1. Introduction

There are many studies that compare the effectiveness of multiple species of natural enemies to that of single species in order to control single or multiple prey species (Ferguson and Stiling, 1996; Schausberger and Walzer, 2001; Denoth et al., 2002; Matsumoto et al., 2003; Fitzgerald et al., 2007; Snyder et al., 2008). Some of these studies show no difference between releasing single species and adding other enemy species, mainly due to low rates of establishment of the additional enemy species (Denoth et al., 2002). Other studies show negative effects on pest control due to exploitative competition for prey or interference among enemy species, including intraguild predation (e.g., Rosenheim et al., 1995; Rosenheim and Harmon, 2006; Janssen et al., 2006, 2007). Yet, it is also possible that different enemy species facilitate each other and have a synergistic effect on pest control (e.g., Ferguson and Stiling, 1996). In this article, we investigate whether multiple species of predatory mites interact to produce lower or higher spider mite pest control than each of these species of predatory mites acting alone.

Various studies examined the effectiveness of introducing multiple predatory mite species versus single predatory mite species to control tetranychid mites (Schausberger and Walzer, 2001; Walzer et al., 2001; Barber et al., 2003; Rhodes et al., 2006; Fitzgerald et al., 2007). Phytoseiid mites have been shown to be effective in controlling tetranychids on many cultivated crops (Helle and Sabelis, 1985; van Lenteren et al., 1992). Single predatory mite species are able to suppress spider mites rapidly if they have a high numerical response (Janssen and Sabelis, 1992). However, some predatory mite species tend to disappear from the crop after reducing pest mites and thus provide only short-term control (Walzer and Schausberger, 1999a). Therefore, combined release of different predatory mite species has been investigated for long-term spider-mite control (Walzer and Schausberger, 1999a; Walzer et al., 2001; Schausberger and Walzer, 2001; Barber et al., 2003; Rhodes et al., 2006; Fitzgerald et al., 2007; Onzo et al., 2003, 2004, 2005), but the results vary from negative effects and no effects to positive effects, and the underlying causal mechanisms leading to these different outcomes are not clear in most cases.
We investigated whether the release of two species of predatory mites would yield better control of the carmine spider mite *Tetranychus cinnabarinus* on strawberry. This mite is one of the most important pest species, widely distributed across the world and attacking more than 100 types of host plant, including crops such as strawberry, cotton, deciduous fruits, vegetables and ornamental plants (Jeppson et al., 1975). It is one of the most serious pests on strawberry in Aydin, Turkey. *T. cinnabarinus* causes severe damage to both leaves and fruits, and it is controlled by acaricide sprays (Cakmak et al., 2003). In Turkey and other countries, very few new acaricides are available for use on strawberry, because pesticide companies are deterred by the costs of registration for a minor crop. As a result, excessive usage of the few registered acaricides in strawberry caused the development of resistance to pesticides, in many cases after only a few years of use (Cross et al., 2001). Owing to such problems of resistance and because of environmental pollution with pesticides, there is an increasing demand for sustainable, environment-friendly control methods. Therefore, efforts have been made to apply biological control against spider mites in this crop.

One of the most specialized biological control agents of the two-spotted spider mite, *Tetranychus urticae* is *Phytoseiulus persimilis* Athias-Henriot. Its reproduction depends on the availability of spider mites as prey, and when the number of spider mites on a crop is low scarce due to predation, it tends to disperse (Cakmak et al., 2005). Therefore, new predator releases are required to combat new spider mite infestation of a crop. Moreover, *P. persimilis* does not function well at temperatures above 30°C or humidities below 60% R.H. (Sabelis, 1981). Because of these characteristics, researchers have considered to combine releases of this species with the predatory mite *Neoseiulus californicus* McGregor (Walzer and Schausberger, 1999a,b; Schausberger and Walzer, 2001; Walzer et al., 2001; Barber et al., 2003). The advantages of *N. californicus* would be (1) that it has a broader diet, including several plant feeding mites and pollen (McMurtry and Croft, 1997); (2) it can survive at lower spider mite densities (Frieze and Gilstrap, 1982); (3) it survives at lower humidities (Bakker et al., 1993). However, the success of combining these two species also depends on the effect they have on each other via exploitative competition and interference, including intraguild predation (Walzer and Schausberger, 1999a,b; Schausberger and Walzer, 2001; Walzer et al., 2001; Barber et al., 2003; Cakmak et al., 2006; Rhodes et al., 2006). Walzer and Schausberger (1999a) showed that intraguild predation between *N. californicus* and *P. persimilis* is strongly asymmetric in favor of *N. californicus*. They showed that *N. californicus* discriminates between con- and hetero-specific eggs and immatures and prefers to prey on the latter, whereas *P. persimilis* does not discriminate between conspecific eggs and *N. californicus* eggs (Schausberger and Croft, 1999; Walzer and Schausberger, 1999b). In addition, *P. persimilis* tends to avoid plants with two-spotted spider mites and conspecifics (Janssen et al., 1997), whereas it does not avoid plants with two-spotted spider mites and *N. californicus* (Janssen et al., 1999; Cakmak et al., 2006). However, *N. californicus* does not avoid visiting bean leaves with spider mites and conspecifics, or bean leaves with two-spotted spider mites and *P. persimilis* (Cakmak et al., 2006). Both *P. persimilis* and *N. californicus* attack and feed on heterospecific eggs and larvae, but the predation risk is much lower in the presence than in the absence of the pest (Cakmak et al., 2006).

Theory predicts that intraguild predation among natural enemies would result in reduced biological control (Rosenheim and Harmon, 2006; Janssen et al., 2006), but there is not much evidence for this in the literature (Janssen et al., 2006, 2007). We therefore investigated whether combined releases of *P. persimilis* and *N. californicus* provide better biological control of *T. cinnabarinus* on strawberry than releases of each of these predator species alone.

## 2. Materials and methods

### 2.1. Mite rearing

*Tetranychus cinnabarinus* was collected from strawberry fields in Aydin, Turkey, in April 23, 1998 and reared on bean plants (*Phaseolus vulgaris* cv. Barbunia) at 25 ± 2°C and 65 ± 10% RH under a 16-h light regime. Bean plants were previously grown under the same conditions in a pest-free climate room for a period of 2 weeks.

The predatory mites *P. persimilis* and *N. californicus* were reared at 25 ± 1°C on bean plants infested with *T. cinnabarinus*. About 2–3 plants, taken from the spider mite culture, were added to the predator cultures twice a week. We used strains of the predatory mite *P. persimilis* and *N. californicus*, collected from bean and strawberry fields in Turkey, respectively (Sekeroglu and Kazak, 1993; Cakmak and Cobanoglu, 2006).

### 2.2. Strawberry fields

During the growing seasons of 2006 and 2007, experiments were conducted in two commercial strawberry fields, each with an area of approximately 1 ha, in the Sultanhisar district of Aydin, Turkey. Strawberry planting beds were covered with polyethylene (110 μm thick) and exposed to sunlight to control soil-borne diseases and weeds for 44 days (from June 14th) and 38 days (from June 30th) in the two fields, respectively. Strawberries (*Fragaria ananassa* Duch. cv. Camarosa) were planted at a density of 5500 plants per 0.1 ha in the two fields on August 10 and 15, 2006, respectively. They were arranged in four rows on elevated beds, mulched with black polyethylene, and drip-irrigated. In the beginning of January, the plants were pruned and the leaves were removed from the fields. At the end of January, strawberries were covered with high polyethylene tunnels. In both fields, the polyethylene covers were removed in early June.

Pesticides were applied in all experimental plots throughout the growing season at rates recommended by the manufacturer. Sprays of fenarimol, kresoxim-methyl and fenamiphos + quinoxyfen were used in September and October against *Sphaerotheca macularis*. Lufenuron and acetamiprid were applied in October against *Spodoptera* sp. and aphids, respectively. Sprays of fenhexamid, cyprodinil + fludioxonil and imazalil were applied in February and March to control *Botrytis cinerea*. Tebufenpyrad was applied against *T. cinnabarinus* according to grower’s practice. After predators were released, no insecticides and acaricides were applied in the predator release plots. Fertilizers were applied via drip-irrigation according to grower’s practice.

Population growth and compatibility of *P. persimilis* and *N. californicus* for control of *T. cinnabarinus* were studied in twelve ‘walk-in’ tunnels using the following treatments (a) *P. persimilis*; (b) *N. californicus*; (c) *P. persimilis* + *N. californicus*; (d) pesticides, no predators (grower’s practice). A randomized complete block design with three replicates per treatment was used in each field. Each replicate included 200 plants (50 plants × four rows), and was conducted in a separate tunnel, leaving one tunnel as a buffer between replicates. In the tunnels in-between the treatment tunnels, spider mites were controlled with pesticides according to grower’s practice.

### 2.3. Predator releases

A natural infestation of *T. cinnabarinus* occurred in both fields. When the density of spider mites reached 2–3 active stages per leaflet, predators were released at the ratio of 1 predator to 20 prey (Cakmak et al., 2005). The initial density of the two predator species together in the combined predator releases was the same as
in the single predator releases (a so-called replacement design); hence, one predator of each species was released per 40 prey. The total amount of P. persimilis and N. californicus released was determined by calculating the mean number of T. cinnabarinus motiles from sampled leaflets from each treatment. Predators were released on leaf discs of 2–3 cm² excised from bean leaves. Each disc harboured ca. 20 eggs and active stages of predators (Kazak et al., 2002; Cakmak et al., 2005).

2.4. Mite sampling

Strawberries were covered with high polyethylene tunnels at the end of January. Starting 2 weeks later, 30 leaflets per plot (90 leaflets per treatment) were randomly collected every week, and taken to the laboratory, where the number of T. cinnabarinus motiles and eggs were counted under a stereo microscope (magnification 10×). Leaflets were collected from the top, middle and lower stratum of each plant. After predators were released, the numbers of predators and their eggs were counted.

2.5. Statistical analyses

Data are repeated measures of the average number of mites per 30 leaflets and are analyzed with a linear mixed effects model (LME) (Crawley, 2007). Contrasts between treatments were assessed through model simplification (Crawley, 2007).

3. Results

Natural populations of T. cinnabarinus were found in all plots of field A from week 2 to 3, and from week 6 in all plots of field B (Fig. 1). In both fields, T. cinnabarinus populations increased gradually until around week 10. There was a significant effect of treatment on the number of active stages ($F_{3,20} = 10.9, P = 0.0002$), and no significant difference between the two fields ($F_{1,19} = 2.95, P = 0.10$). The densities of T. cinnabarinus in the treatment in which only N. californicus was released were significantly higher than in the other treatments (Fig. 1, contrasts through model simplification: $F_{1,22} = 31.3, P < 0.0001$). The differences between the other treatments were not significant (all $P > 0.4$). The economic injury level (EIL, 15 active stages per leaflet for T. cinnabarinus on strawberry, Turkish Ministry of Agriculture and Rural Affairs, 1995) was surpassed only in treatments with N. californicus alone (Fig. 1). Phytoseiulus persimilis reached its highest levels in week 9–10 in field A and in week 10–13 in field B (Fig. 2). The densities of P. persimilis were similar in the presence and absence of N. californicus (Fig. 2, $F_{1,10} = 0.30, P = 0.60$). Hence, there was no significant adverse effect of the presence of N. californicus on the densities of P. persimilis. P. persimilis populations suppressed the populations of T. cinnabarinus to an extent equivalent to chemical control (Fig. 1).

Populations of N. californicus also increased (Fig. 3), but despite the growth of the populations, it suppressed the prey populations less than the other treatments (Fig. 1). Although densities of N. californicus seemed lower in the presence of P. persimilis, this difference was not significant ($F_{1,10} = 2.05, P = 0.18$), probably due to the large variation in densities.

4. Discussion

To compare multiple and single predator releases, we assessed the effect on prey density in replacement experiments: the total number of predators released was kept constant in all treatments. This design allows for straightforward interpretation of the results only in two cases (ignoring the trivial case of all treatments yielding equal prey suppression): (1) if the prey density under multiple predator releases is suppressed more than under any of the single predator releases, then the predator species should have acted synergistically; (2) if the prey density under multiple predator releases is suppressed less than under any of the single predator releases, then the predator species should have acted antagonistically. If, however, multiple enemy releases have an effect intermediate between those of the single predator releases, this may arise from synergism, antagonism or absence of any interference between the predator species. This is because lower numbers of each predator species are released in a multiple enemy release than in the single enemy releases. Moreover, predators and prey reproduced at the time scale of the experiments, making it difficult to predict the effect of multiple predators on prey density in absence of predator interference without the aid of an explicit population dynamical model. Thus, if multiple predator releases yield results intermediate between those of single predator releases, no pertinent conclusions can be drawn. Below we will focus first on the results of single predator releases and proceed by interpreting the results from the multiple predator releases.

4.1. Single predator releases

A significantly higher population of active stages of T. cinnabarinus occurred in the N. californicus release plot in both fields (Fig. 1). In fact, the release of N. californicus alone did not result in success-
ful control of *T. cinnabarinus*. Similarly, Sato et al. (2007) found that three releases of *N. californicus* without acaricide application were not sufficient to significantly reduce *T. urticae* densities. A possible reason for the ineffectiveness of *N. californicus* is the initial predator–prey ratios. When released at higher predator–prey ratios, some studies showed that *N. californicus* was able to control spider mites (Greco et al., 2005; Fraulo and Liburd, 2007).

*Phytoseiulus persimilis* alone gave effective control of *T. cinnabarinus* after release at a predator–prey ratio of 1:20, whereas at the same predator–prey ratio *N. californicus* alone did not reduce *T. cinnabarinus* numbers. These results are in agreement with earlier findings (Cakmak et al., 2005), showing that the Turkish *P. persimilis* strain was successful in controlling *T. cinnabarinus* at the initial predator:prey ratio of 1:20.

### 4.2. Multiple predator releases

We found that not only *P. persimilis* alone, but also the combination of *P. persimilis* and *N. californicus*, was significantly more effective in suppressing pest populations than *N. californicus* alone. Yet the two treatments with *P. persimilis* were not significantly different from each other. Barber et al. (2003) reported that *P. persimilis* and *N. californicus* eliminated spider mites on dwarf hops in the UK faster than *N. californicus* alone, but slower than *P. persimilis* alone. On strawberries in Florida, Rhodes et al. (2006) also found qualitatively similar results in short term (2 weeks) studies in greenhouses, but these authors found that the combined release of *P. persimilis* and *N. californicus* and the release of *N. californicus* alone gave equally effective spider-mite control and even better control than *P. persimilis* alone after 4 weeks in the greenhouse and in two consecutive full season experiments. Thus, our experiments and the two referred to above lead to the conclusion that multiple predator releases result in prey suppression intermediate between that achieved under single predator releases. Hence, given that all three studies employed a replacement design, no definitive conclusions can be drawn as to synergy or antagonism among the predator species released.

Scrutiny of the densities of the two predator species in our experiments also did not reveal definitive evidence for facilitation or interference. On the one hand, one may argue for interspecific interference because the densities of *P. persimilis* in the presence of *N. californicus* were similar to those in the absence of *N. californicus* (despite the fact that their numbers at release were twice as low as a consequence of the replacement design) and the densities of *N. californicus* seemed lower in the presence of *P. persimilis*, albeit not significantly so. On the other hand, the same trends in the densities of the two predator species may arise from a lower predation impact of *N. californicus*, thereby first allowing the spider mite population to build up and then the *P. persimilis* population to reach a larger size. Moreover, the densities of *N. californicus* in the presence of *P. persimilis* may be lower than in its absence, simply as a result of the lower numbers released under the replacement design. Thus, we cannot infer interference from the densities of the two predator species and for much the same reasons, this also applies to other studies employing a replacement design (Rhodes et al., 2006; Barber et al., 2003; Fitzgerald et al., 2007). Absence of evidence for interference was also concluded from another study on *N. californicus* and *Stethorus punctillum* (Rott and Ponsonby, 2000).

All these arguments, however, should not be taken as ‘evidence for absence’ of interference. In laboratory experiments, bidirectional intraguild predation has been found: *N. californicus* females
feed on young stages of *P. persimilis*, and *P. persimilis* females do feed on young stages of *N. californicus* (Walzer and Schausberger, 1999a; Walzer et al., 2001; Cakmak et al., 2006). Moreover, neither of the two predator species avoided prey patches with the heterospecific competitor, both when inexperienced with the other predator and when experienced with prey patches occupied by the heterospecific predator (Cakmak et al., 2006). Nevertheless, *P. persimilis* hardly suffered from intraguild predation by *N. californicus* as long as the shared prey was present (Cakmak et al., 2006). Perhaps, this is a reason why it is hard to detect intraguild interference between these two predator species in the field, even if it would now and then occur.

Because the initial numbers of each predator species were not equal across treatments, evidence for interference or facilitation can only be obtained from the population growth rates of the predators. The weekly growth rates of both predator species became negative earlier in presence of the other species than when alone (Fig. 4, weeks 8–12 for *P. persimilis*, weeks 8–14 for *N. californicus*). As the initial densities of each species were lower in the combined releases of the predators than in the single releases, this suggests that there was interference between the two species. We found that control of *T. cinnabarinus* was not improved by releasing a combination of *P. persimilis* and *N. californicus* instead of *P. persimilis* alone. However, scrutiny of the predator growth rates per week revealed that when densities of *T. cinnabarinus* declined, *N. californicus* was able to maintain positive growth rates for a longer time than *P. persimilis* (Fig. 4). This shows that *N. californicus* has a capacity to persist in the crop for a longer time. Because these low densities arose in the end phase of the experiment, no clear advantage of *N. californicus* may have become manifest with respect to spider-mite control. In case lower densities arise earlier in the crop growing season or locally in the space occupied by a crop, *N. californicus* might add to overall spider-mite control by its ability to persist longer at lower densities. Perhaps this is the reason why Rhodes et al. (2006) found better performance of *N. californicus* with and without *P. persimilis* when considered over two crop seasons.

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**References**


Fig. 4. Weekly growth rates of *Phytoseiulus persimilis* (top) and *Neoseiulus californicus* (bottom). Growth rates were calculated as ln(x(t + dt)/x(t))/dt, with x(t) = density of eggs and motiles at time t and dt = time between two measurements. Treatments are as explained in the legend under Fig. 1.