MORGANA MARIA FONSECA PORTO

INTRAGUILD INTERACTIONS BETWEEN THE PREDATORY MITES *Neoseiulus californicus* AND *Phytoseiulus macropilis* (ACARI: PHYTOSEIIDAE)

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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Eraldo Lima
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Angelo Pallini Filho
(Coorientador)

Arnoldus Rudolf Maria Janssen
(Orientador)

“Sê todo em cada coisa.
Põe quanto és
No mínimo que fazes.”

Ricardo Reis, heterônimo de *Fernando Pessoa*.

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“Mestre, meu mestre querido!
Coração do meu corpo intelectual e inteiro!
Vida da origem da minha inspiração!
Que triste a grande hora alegre em que primeiro te ouvi!
Depois tudo é cansaço neste mundo subjetivado... ”
Fernando Pessoa

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SUMÁRIO

ABSTRACT

PORTO, Morgana Maria Fonseca, D.Sc., Universidade Federal de Viçosa, August, 2017. **Intraguild interactions between the predatory mites *Neoseiulus californicus* and *Phytoseiulus macropilis* (Acari: Phytoseiidae).** Adviser: Arnoldus Rudolf Maria Janssen. Co-adviser: Angelo Pallini Filho.

Predatory mites are commonly used as biological control agents and the species *Phytoseiulus macropilis* and *Neoseiulus californicus* have been considered to be released together to control an important pest, the two-spotted spider mite *Tetranychus urticae*. Because interacting predators may interfere with each other, an important step towards the implementation of successful release of multiple predators in biological control programs requires to resolve how predators respond to the presence of each other and whether they are involved in intraguild interactions. Given this, initially, I investigated whether these two predatory mites avoid the presence of each other. It is known that predators and parasitoids can use volatiles associated with the presence of competing species when foraging for patches with prey or hosts. I therefore investigated whether the two predator species use volatiles emanating from plants with prey and heterospecifics to avoid prey patches with the other predator species. Furthermore, I assessed whether these predators interact trough intraguild predation, in which competing predators also kill and eat each other. However, because there is no consensus regarding criteria to evaluate the occurrence of such interaction, I first explored existing criteria and suggested guidelines for the design of experiments. Based on these guidelines, I subsequently evaluated both the capacity of *P. macropilis* and *N. californicus* to kill stages of the other species and the capacity to benefit from feeding on these stages, both prerequisites for the occurrence of intraguild predation. The most important findings regarding the possible interactions among these predators are that neither of the predators used volatiles to avoid prey patches occupied by the heterospecific predators. However, they did engage in reciprocal intraguild predation. Moreover, I show that ontogeny clearly played a critical role in determining the occurrence of intraguild predation within this predator system. I discuss the possible explanations for the lack of odour-mediated avoidance and the interplay between ontogenetic stages and intraguild interactions.

RESUMO

PORTO, Morgana Maria Fonseca, D.Sc., Universidade Federal de Viçosa, agosto de 2017. **Interações intraguilda entre os ácaros predadores *Neoseiulus californicus* e *Phytoseiulus macropilis* (Acari: Phytoseiidae).** Orientador: Arnoldus Rudolf Maria Janssen. Coorientador: Angelo Pallini Filho.

Os ácaros predadores são comumente usados como agentes de controle biológico e as espécies *Phytoseiulus macropilis* e *Neoseiulus californicus* têm sido consideradas para serem liberadas em conjunto para controlar uma importante praga, o ácaro rajado *Tetranychus urticae*. Porque predadores que interagem podem interferir uns com os outros, um passo importante para a liberação bem-sucedida de múltiplos predadores em programas de controle biológico requer determinar como os predadores respondem à presença uns dos outros e se eles estão envolvidos em interações intraguilda. Diante disso, inicialmente, foi investigado se esses dois ácaros predadores evitam a presença um do outro. Sabe-se que predadores e parasitoides podem usar voláteis associados à presença de espécies competidoras quando forrageiam por áreas com presas ou hospedeiros. Portanto, foi analisado se as duas espécies de predadores usam substâncias voláteis que emanam de plantas com presas e heteroespecíficos para evitar locais de presa com a outra espécie de predador. Além disso, foi avaliado se esses predadores interagem através da predação intraguilda, em que predadores competidores matam e comem-se uns aos outros. No entanto, como não há consenso em relação aos critérios para avaliar a ocorrência de tal interação, primeiro foi explorado os critérios existentes e então foi sugerido diretrizes para o desenho de experimentos. Com base nessas diretrizes, foi investigado tanto a capacidade de *P. macropilis* e *N. californicus* para matar os estágios da outra espécie como a capacidade de se beneficiar alimentando-se destes estágios, ambos pré-requisitos para a ocorrência de predação intraguilda. As descobertas mais importantes sobre as possíveis interações entre esses predadores são que nenhum dos predadores usou voláteis para evitar locais de presas ocupadas pelos heterospecíficos. No entanto, eles se envolveram em predação intraguilda recíproca. Além disso, foi mostrado que a ontogenia claramente desempenhou um papel crítico na determinação da ocorrência de predação intraguilda dentro deste sistema de predadores. Foi discutido as possíveis explicações para não se evitar os odores produzidos e a interação entre estágios ontogênicos e interações intraguilda.

GENERAL INTRODUCTION

Before the publication of *On the Origin of Species* (Darwin 1859), it was already claimed that the fighting for survival of species was not merely driven by predator-prey interactions, but that resource limitation could also play an important role in this battle among species (see Mayr 1977). Crucially, this idea was adopted and illuminated by Darwin, who conceived that interspecific competition was the key element deciding what he calls "*the struggle for existence*" and that it was the dominant force driving evolution by natural selection (Darwin 1859; Mayr 1977; Diamond 1978; Milinski and Parker 1991). Accordingly, the recognition that competition among species is central and ubiquitous in nature has prompted modern research to investigate the mechanisms by which such interactions shapes community structure (Schoener 1974, 1983; Polis 1988). Within predator guilds, a first step to understanding the dynamics and functioning of interspecific competition requires to resolve how foraging behaviour of interacting predators affect their distribution and coexistence (Milinski and Parker 1991; Janssen et al. 1997, 1999).

When competing for resources, the success of individual predators depends more on their behavioural strategies than on their physical strength (Milinski and Parker 1991). The distribution of predators over prey patches is expected to be directly influenced by the availability of resources, however, the presence of competitors on these patches may also affect their foraging decisions (Janssen et al. 1997; Gnanvossou et al. 2003; Choh et al. 2010; Maleknia et al. 2013). It is well known that arthropod predators explore various chemical cues to locate prey patches (Vet and Dicke 1992; Dicke et al. 1998) and it has been shown that they may also use these cues to avoid competition (Janssen et al. 1997; Gnanvossou et al. 2003; Maleknia et al. 2013). If predators are not able to detect and avoid the presence of heterospecifics in prey patches, they might interact through the joint use of the resources and this may lower the foraging success of one of the competing species (Pianka 1974; Milinski and Parker 1991). The absence of avoidance of competitors and the resultant exploitation competition potentially increase the chance of other interactions such as intraguild predation, which is a combination of resource competition and predation (Polis 1988; Polis et al. 1989).

Intraguild predation (IGP) is the killing and eating among potential competitors that use similar resources, and is a widespread interaction (Polis et al. 1989; Rosenheim et al. 1995; Arim and Marquet 2004; Gagnon et al. 2011). When predators interact through competition, different sizes/stages of the involved species commonly coexist and may attack each other's vulnerable stages (Polis 1988; Choh et al. 2012; Montserrat et al. 2012). Therefore, most IGP depends on the ontogenetic stage of the individuals (Polis et al. 1989; Woodward and Hildrew 2002; Montserrat et al. 2012), however, this dependency on size/stage structure has received limited attention and the occurrence of IGP may therefore be underestimated (Polis and McCormick 1987; Polis 1988; Polis et al. 1989; Wissinger 1992). The combination of competition and predation in addition to size/stage structure features of IGP result in more complex dynamics and can lead to exclusion, coexistence or alternative states (Polis 1988; Polis and Holt 1992; Holt and Polis 1997). Despite this potential of IGP to shape population dynamics and community structure (Mylius et al. 2001; Hin et al. 2011) and its implications for species conservation and management of pest populations, there is still no consensus regarding criteria to evaluate its occurrence (Fonseca et al. 2017. Chapter 2).

The raising concern that intensive industrial agriculture did not eradicate hunger worldwide and that the excessive use of pesticides is harmful to human health and the environment has led to a strong growth in use of biological pest control approaches (Matson et al. 1997; Margni et al. 2002; Calvo et al. 2015; UN Human Rights Council 2017). Amongst the different strategies used, augmentative biological control consists of the seasonal release of large numbers of natural enemies to suppress the population of the target pest species (Hajek 2004; van Lenteren et al. 2017). This approach often involves the release of several natural enemies which may have complementary effects on the pest species (Sih et al. 1998; Cardinale et al. 2003; Straub and Snyder 2008). The presence of multiple natural enemies can give rise to several types of interactions that further increase food web complexity and may affect the efficacy of biological control (Janssen et al. 1998; Sih et al. 1998; Messelink et al. 2012). For instance, two predators sharing the same prey may interfere with each other through competition or intraguild predation and the possible effects of such interactions has been increasingly considered in the design of effective biological control programs (Rosenheim et al. 1995; van Lenteren et al. 2003; Janssen et al. 2006; Messelink et al. 2012).

Predatory mites (Acari: Phytoseiidae) have proven to be suitable biological control agents in many agricultural systems, particularly to control species of phytophagous mites and other small insects (van Lenteren et al. 2017). The two-spotted spider mite *Tetranychus urticae* (Koch (Acari: Tetranychidae)) is among the most polyphagous pests known: it attacks over 1100 plant species and is highly resistant to pesticides (Van Leeuwen et al. 2010; Migeon and Dorkeld 2015). Two predatory mite species, *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae), have been considered for combined releases to control the two spotted spider mite in the Neotropics. *Phytoseiulus macropilis* is considered a specialist predator of Tetranychus species and is mass-produced and commercially available as a biological control agent (McMurtry and Croft 1997; Gerson et al. 2003; Oliveira et al. 2007, 2009). *Neoseiulus californicus* has a broader diet, feeding on various phytophagous mite species and types of pollen (McMurtry and Croft 1997; Croft et al. 1998; Gerson et al. 2003). The use of these predators combined is because while *P. macropilis* tend to disperse when the density of spider mites is low, *N. californicus* stays because it can feed on other food sources and is more resistant to starvation (F. Colares and M. Fonseca, pers.

obs.). Besides being investigated for combined releases, both *P. macropilis* and *N. californicus* have been found to co-occur naturally in extensive regions of Brazil on various crops and on spontaneous vegetation (Ferla et al. 2007; Roggia et al. 2009; de Freitas 2014). Therefore, these two predators potentially compete for spider mites and may be involved in intraguild interactions.

In this thesis, I have investigated how these two predatory mites respond to the presence of each other, how to evaluate the occurrence of IGP and whether they interact trough IGP. In Chapter 1, I assessed whether the predators respond to odours emanating from prey patches invaded by the other species. In Chapter 2, I evaluated the existing criteria to investigate the possible occurrence of IGP in order to offer guidance on how to design appropriate experiments to determine whether or not two species may engage in IGP. In Chapter 3, I verified whether reciprocal intraguild predation occurs in this predator system and explored the interplay between ontogenetic stages and IGP interactions.

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CHAPTER 1

Absence of avoidance of the competitor in two interacting predatory mites

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Abstract

Arthropod predators are known to exploit a wide diversity of volatile cues during their foraging activities. Response to these volatiles determines habitat selection and distribution of species, as well as the occurrence and strength of interactions within food webs. For example, these chemical cues also mediate avoidance of competitors within trophic levels. It is therefore of paramount importance to explore how foraging behaviour of interacting predators affect their distribution and coexistence. Predatory mites are commonly used as biological control agents and the species *Phytoseiulus macropilis* and *Neoseiulus californicus* have been considered to be released in combination to control an important pest, the two-spotted spider mite *Tetranychus urticae*. It is not knowns how these predators respond to odours emanating from prey patches invaded by heterospecifics, which is important to determine their interactions and, ultimately, their combined effects on the pest. Therefore, we investigated whether these two predatory mites avoid the presence of the other species using the volatiles emanating from plants with prey and heterospecifics. Whereas both *P. macropilis* and *N. californicus* were significantly attracted to volatiles of plants infested with spider mites, neither of the predators avoided volatiles from prey patches occupied by the heterospecifics. We discussed the possible explanations for this lack of odour-mediated avoidance and suggest that studies on this interaction should be extended to other predator species.

Key words: interspecific competition, predator-predator interactions, biological control, volatile chemical cues, olfactometer.

Introduction

Foraging behaviour is central in animal behaviour as it is the means by which organisms obtain essential resources for their survival and persistence in ecosystems (Bell 2012). This behaviour is of particular interest in evolutionary ecology because it determines habitat selection and distribution of species over the landscape and also the occurrence and strength of interactions within food webs (Abrams 1996; Janssen et al. 1998, 1999). Optimal foraging theory assumes that natural selection will favour foraging strategies that lead to the highest possible fitness for the forager and thus, it predicts that organisms behave so as to maximize the net rate of intake of energy and nutrients when searching for food (Belovsky 1978; Tinbergen 1981; Stephens and Krebs 1986; Křivan 1996). Resource quantity and quality directly affect patch selection of foraging animals but detrimental factors such as the presence of predators and intra- and interspecific competitors may also influence their foraging decisions (Byers 1993; Janssen et al. 1995a, b, 1997, Pallini et al. 1997, 1999; Kats and Dill 1998; Gnanvossou et al. 2003).

Animals make foraging decisions based on information from their environment (Stephens and Krebs 1986; De Boer and Dicke 2004). Among the various cues used during foraging, chemical information plays an important role, especially within arthropods (Dicke and Sabelis 1988; Vet and Dicke 1992). Carnivorous and herbivorous arthropods are known to exploit a wide diversity of volatile cues to locate their resources (Dicke 2000; Dicke et al. 2003) and there is a wealth of studies demonstrating the importance of volatiles in simple tritrophic interactions consisting of a plant, an herbivore and its natural enemy (Vet and Dicke 1992; Dicke et al. 1993; Tumlinson et al. 1993). It is well known that in the first steps of the foraging process, natural enemies base their decisions on chemical cues released by herbivore-damaged plants that are associated with the presence of their hosts or prey, whereas the cues produced by these herbivores alone become more important at short distances (Vet and Dicke 1992; Dicke 1999). These chemical cues can also mediate avoidance of intra- and interspecific competition within trophic levels. For instance, avoidance of cues from competitors has been reported within the second trophic level in bark beetles (Byers 1993) and phytophagous mites (Pallini et al. 1997). Within the third trophic level, parasitoids (Price 1970; Janssen et al. 1995a, b; Geervliet et al. 1998; Tamò et al. 2006; Cancino et al. 2012), and predators use volatiles to avoid competition (Janssen et al. 1997; Gnanvossou et al. 2003; Maleknia et al. 2013).

Interspecific competition among predators is a prevalent interaction in natural and managed food webs and an important determinant of the structure and functioning of these ecosystems (Schoener 1983; Polis 1988). Therefore, a major challenge in ecology is to understand how foraging behaviour of interacting predators affect their distribution and coexistence (Milinski and Parker 1991; Janssen et al. 1997, 1999). Predators provide a key ecosystem service by directly suppressing herbivore populations that are potentially agricultural pests (Ives et al. 2005; Snyder et al. 2006) and there is a strong growth in their use as biological control agents (van Lenteren et al. 2017). Natural communities typically comprise multiple interacting predators and release strategies of biological control agents often involve several natural enemies, which may have complementary effects on the target pest species (Sih et al. 1998; Cardinale et al. 2003; Straub and Snyder 2008). However, two predator species sharing the same prey may interact through exploitative or interference competition, or by the combination of these latter two which may result in intraguild predation (Polis 1988; Polis et al. 1989; Rosenheim et al. 1995; Janssen et al. 2006, 2007). In attempting to prevent such antagonistic effects, predators could avoid the presence of each other, leading to temporal or spatial segregation of their niches (Pianka 1974), hence, affecting their efficacy of controlling the herbivore population. Therefore, exploring how interacting predators respond to the presence of each other may be an important step towards the implementation of successful release of multiple enemies in biological control programs.

Predatory mites (Acari: Phytoseiidae) are widely used as biological control agents in many agricultural systems, particularly to control species of phytophagous mites and other small insects (van Lenteren et al. 2017). *Tetranychus urticae* (Koch (Acari: Tetranychidae)), known as the two-spotted spider mite, is one of the most important species of mite pests that attacks over 1100 plant species (Migeon and Dorkeld 2015). The predatory mite *Phytoseiulus macropilis* (Banks) is commonly used for the control of the two spotted spider mite in many crops in several regions of the world (Prasad 1974; Hamlen 1980; Hamlen and Lindquist 1981; Watanabe et al. 1994; Oliveira et al. 2007, 2009; Gigon et al. 2016). This predatory mite has been considered to be released in combination with another predatory mite species, *Neoseiulus californicus* (McGregor), which has a broader diet, feeding on various phytophagous mite species and types of pollen (McMurtry and Croft 1997; Croft et al. 1998; Gerson et al. 2003). Besides being investigated for combined releases, both *P. macropilis* and *N. californicus* have been found to co-occur naturally in extensive regions of Brazil on various crops and on spontaneous vegetation (Ferla et al. 2007; Roggia et al. 2009; de Freitas 2014). Hence, these two predators potentially compete for spider mites and may be involved in various intraguild interactions.

Predatory mites are blind and chemical cues are central in their foraging behaviour (Sabelis and Baan 1983; van Wijk et al. 2008). Although it is known that both *P. macropilis* (Oliveira et al. 2009; Fadini et al. 2010) and *N. californicus* (Janssen et al. 1990) are attracted to odours from prey patches, it has not yet been reported how these predators respond to odours emanating from prey patches invaded by heterospecifics predators. Therefore, using a Y-tube olfactometer, we investigated whether these two predatory mites respond to such odours and avoid the presence of the other species.

Material and methods

Cultures

The two-spotted spider mite (*T. urticae*) was reared on jack bean plants (*Canavalia ensiformis* (L.) DC) in a climate-controlled room (25 ± 3 °C, 70 – 90 % relative humidity, with controlled photoperiod 12:12 L:D). Clean jack bean plants were grown in a greenhouse until they were 2 weeks old and were subsequently added biweekly to the spider mite culture. The predatory mites *P. macropilis* and *N. californicus* were reared under the same conditions as above on detached bean leaves infested with two-spotted spider mites. These leaves were put in a plastic tray (l × w × h = 45 × 30 × 8 cm) that was placed inside a second, water-containing tray (55 × 40 × 10 cm) to prevent the mites from escaping. New bean leaves with spider mites were added to the cultures 2–3 times per week. The cultures of spider mites and predatory mites were started with individuals obtained from cultures from Econtrole Pesquisa & Consultoria Ltda (Viçosa, MG, Brazil). Both predatory mite species had been reared for about a year on jack bean leaves with two-spotted spider mites prior to the experiments.

Olfactometer experiments

To assess the response of adult females of predatory mites in response to volatiles from the heterospecific competitor, two-choice tests were done in a Y-tube olfactometer (Sabelis and Baan 1983; Janssen 1999). The olfactometer consisted of a Y-shaped glass tube (27 cm in length × 3.5 cm in diameter), with a black Y-shaped metal wire in the middle to guide the predator, with the base of the tube connected to a pump that causes an airflow from the arms of the tube to the base (Janssen 1999). Each arm was connected to a glass container (50 x 36 x 43 cm) in which the volatile sources were kept. The wind speed in each arm of the Y tube was measured with a hot-wire anemometer and calibrated to 0.50 m/s (VelociCalc® Air Velocity Meter 9545-A). When wind speeds in both arms are equal, the air flows coming from the containers form two separate fields in the base of the Y-tube, with the interface coinciding with the metal wire (Sabelis and Baan 1983). Each volatile source consisted of two jack bean plants that were infested with spider mites (*T. urticae*). The heterospecific predator was present on one group of plants and absent on the other group. The plants were 2 weeks old with two fully developed leaves and were infested with 300 adult females of *T. urticae* per leaf and they were subsequently incubated in this way for 2 days. This resulted in some visual damage, but plants were far from being overexploited. After this, one of two different densities of the predatory mites was placed on one group of plants: 20 or 40 adult females per leaf. Subsequently, plants were incubated for 2 more days. Plants were incubated in a climate room until they were used for olfactometer experiments (conditions as above).

Prior to the experiments, mated adult females of the predatory mites were taken from the rearing and were starved for 1-3 hours, because this motivates the predators to walk upwind in the olfactometer (Sabelis and Weel 1993). The predatory mites were tested individually in the olfactometer and each trial consisted of twenty adult female predatory mites that reached the end of one of the arms of the Y-tube within five minutes. Each female was put on the metal wire inside the Y-tube and after reconnecting the pump, the female started moving upwind to the junction of the wire, where she had to choose for one of the two arms. Each female was observed for a maximum of 5 minutes and was subsequently removed, after which the next female was introduced. Females that did not make a choice within 5 minutes were scored as having made no choice and were excluded from further analysis. To correct for unforeseen asymmetry in the experimental set-up, volatile sources were switched to the opposite arm of the olfactometer after each 5 females tested.

To ensure that both predator species did respond in the olfactometer, we first did an olfactometer test with clean and spider-mite-infested jack bean plants as the volatile sources. Subsequently, we tested the response of the predators with plants harboring prey plus adults of the other species. Three replicates were done per combination of volatile sources, each with a new set of plants and a new group of predators. The choices offered were: 2 jack bean plants infested with spider mites vs. 2 jack bean plants infested with spider mites plus the heterospecific predatory mite; this was repeated for both predatory species and with both densities of predators on the plants.

The data of each two-choice olfactometer experiment were analyzed with a log-linear model for contingency tables with Generalized Linear Models (GLM) using a Poisson error distribution (Crawley 2013). We first tested whether there was a preference for one of the arms of the olfactometer, which would point at an asymmetry in the set-up. We found no evidence for this (all P > 0.418). We subsequently assessed whether the choice of the predators differed significantly among replicates, by constructing a GLM with volatile source, replicate and their interaction as factors. A significant interaction between the volatile and replicate would indicate that the preference of the predators varied significantly among replicates, and a significant effect of replicate on the preference would indicate that the overall preference was more strongly determined by some replicates. All statistical analyses was done using R (R Core Team 2016).

Results

Both predators were significantly attracted to volatiles of plants infested with spider mites. Of *P. macropilis*: 19 females chose the infested plants and 1 chose the clean plant (binomial test, P < 0.001). For *N. californicus*, these numbers were 15 vs 5 (binomial test, P = 0.021). Hence, both predator species performed well in the olfactometer.

Phytoseiulus macropilis did not show a preference when offered a choice between odours of plants with spider mites and those of plants with spider mites plus *N. californicus* both with a density of 20 (Fig. 1, GLM, Chi^2 = 1.67, d.f. = 1, P = 0.19) or 40 heterospecific predators per leaf (Fig. 2, GLM, Chi^2 = 0.60, d.f. = 1, P = 0.44). The preference of the predators did not differ significantly among replicates (interaction of replicate with volatile source: 20 *N. californicus*: Chi^2 = 0.55, d.f. = 2, P = 0.758; 40 *N. californicus*: Chi^2 = 3.70, d.f. = 2, P = 0.158). In the experiment with 20 heterospecific predators per leaf, 58% out of 60 females tested chose volatiles from plants with spider mites plus *N. californicus* and in the experiment with 40 heterospecific predators per leaf, 45% of the 60 females chose for plants with heterospecific predators. There was no significant difference between the preference with both predator densities (both predator densities: Chi^2 = 0.0, d.f. = 2, P = 1.0).

Neoseiulus californicus also did not show a preference when offered a choice between odours of plants with spider mites and those of plants with spider mites plus *P. macropilis* in any replicate of both experiments with 20 (Fig. 3, GLM, Chi^2 = 0.07, d.f. = 1, P = 0.80) or 40 (Fig. 4, GLM, Chi^2 = 2.42, d.f. = 1, P = 0.12) heterospecific predators per leaf. The preference of the predators did not differ significantly among replicates (interaction of replicate with volatile source: 20 *P. macropilis*: Chi^2 = 1.74, d.f. = 2, P = 0.418; 40 *P. macropilis*: Chi^2 = 5.69, d.f. = 2, P = 0.058). In one of the replicates, *N. californicus* showed a significant preference for the volatiles of plants with 40 heterospecifics per leaf (Fig. 4). In the experiment with 20 heterospecific predators per leaf, 48% out of 60 females chose volatiles from plants with spider mites plus *P. macropilis* and in the experiment with 40 heterospecific predators per leaf, 40% of the 60 females made that same choice.

We conclude that, despite the potential for competition and possible intraguild predation between individuals of the two predatory mite species, they do not avoid volatiles of plants on which heterospecifics are present.

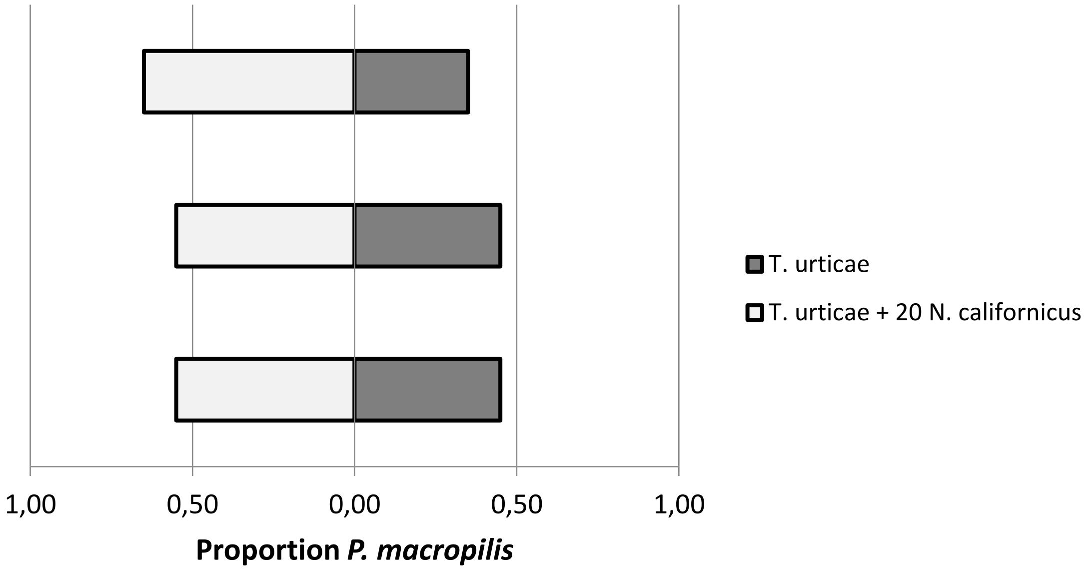

Figure 1. The response of *P. macropilis* in an olfactometer when offered the choice between odours from plants with spider mites (right-hand side of the bars) and plants with spider mites and 20 *N. californicus* per leaf (left-hand side). Shown are results of 3 independent replicates. Each bar represents the choice of twenty predatory mites.

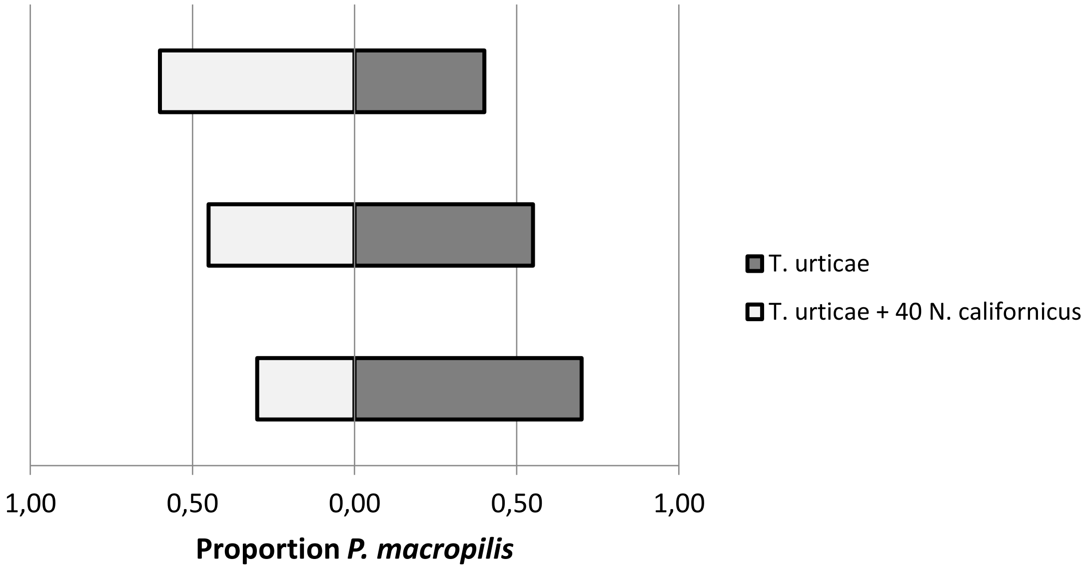

Figure 2. The response of *P. macropilis* in an olfactometer when offered the choice

between odours from plants with spider mites (right-hand side of the bars) and plants with spider mites and 40 *N. californicus* per leaf (left-hand side). Shown are results of 3 independent replicates. Each bar represents the choice of twenty predatory mites.

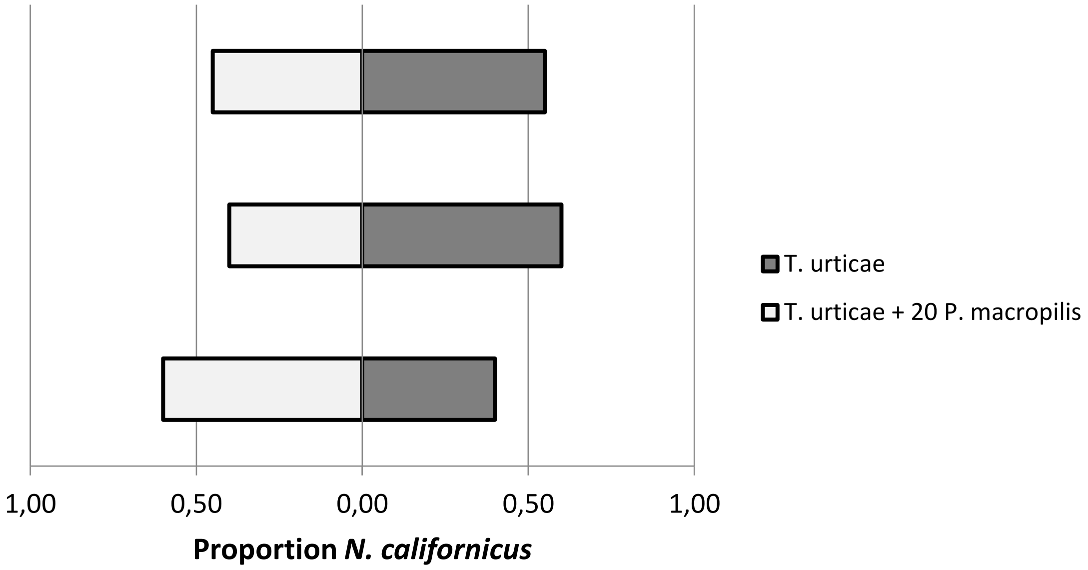

Figure 3. The response of *N. californicus* in an olfactometer when offered the choice between odours from plants with spider mites (right-hand side of the bars) and plants with spider mites and 20 *P. macropilis* per leaf (left-hand side). Shown are results of 3 independent replicates. Each bar represents the choice of twenty predatory mites.

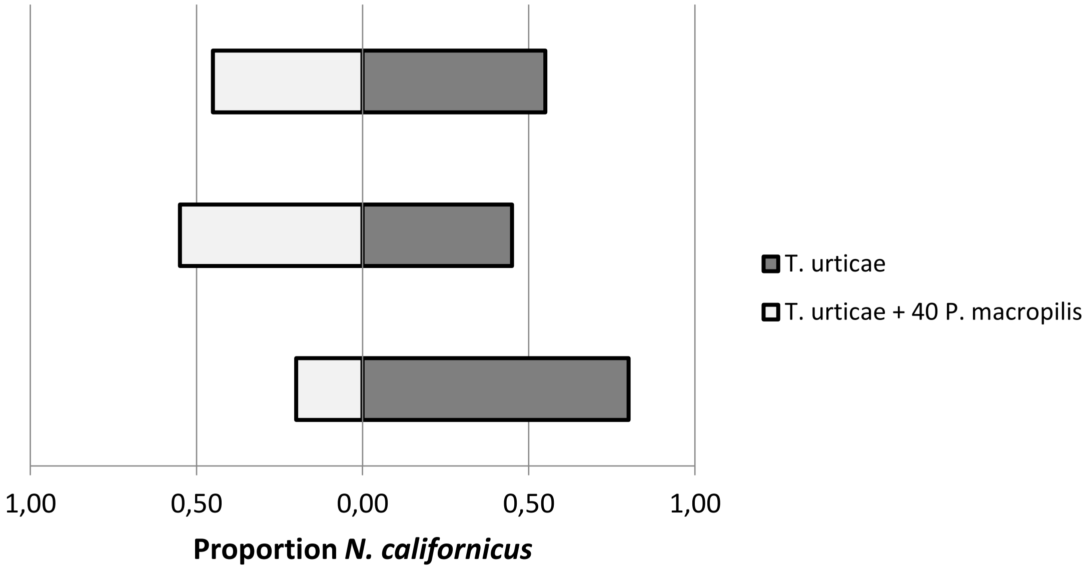

Figure 4. The response of *N. californicus* in an olfactometer when offered the choice between odours from plants with spider mites (right-hand side of the bars) and plants with spider mites and 40 *P. macropilis* per leaf (left-hand side). Shown are results of 3 independent replicates. Each bar represents the choice of twenty predatory mites.

Discussion

Whereas both *P. macropilis* and *N. californicus* were significantly attracted to volatiles of jack bean plants infested with spider mites, neither of the two predator species avoided volatiles from prey patches occupied by the heterospecifics, both with 20 or 40 predators per leaf. These results are in agreement with observations made in a very related system of predatory mites, *Phytoseiulus persimilis* (Athias-Henriot) and *N. californicus,* which also showed that these predators do not avoid volatiles of plants with the presence of the heterospecific species (Çakmak et al. 2006). In this latter system, it was also demonstrated in greenhouse release experiments that *P. persimilis* does not avoid visiting plants with spider mites and *N. californicus* (Janssen et al. 1999). Nevertheless, this absence of avoidance of competitors is not always the case: it was shown that *P. persimilis* avoided odours from prey patches occupied by conspecifics (Janssen et al. 1997; Maleknia et al. 2013). Additionally, another study has demonstrated avoidance of volatile cues from both co- and heterospecific competitors by all three predatory mites tested (Gnanvossou et al. 2003). Because of the limited attention given to such interactions, the mechanisms leading odour-mediated responses to con- or heterospecific predators are not yet well understood (but see Janssen et al. 1997).

Although *P. macropilis* and *N. californicus* have recently been found to co-occur naturally (Ferla et al. 2007; Roggia et al. 2009; de Freitas 2014), they may have not share enough evolutionary history and this could be a reason for why they do not distinguish volatiles of plants on which heterospecifics are present. Another conceivable explanation for the lack of avoidance found in our predatory system, is that the predators did not have previous experience with heterospecifics. It is known that individual foragers have the ability to learn from previous foraging experiences (Bernstein et al. 1988, 1991), and it was already shown that predatory mites can change they response to volatiles after a negative experience (Dicke et al. 1990; Takabayashi and Dicke 1992; Drukker et al. 2000; De Boer and Dicke 2004). In parasitoids, selection pressures due to competitive interactions may drive the evolution of avoidance mediated by volatiles cues, they are known to learn profitable cues that improve foraging success (Vet 1999). Predatory mites that interact through intraguild predation were shown to avoid patches with cues (Choh et al. 2010) or with the presence of the heterospecific predator (van der Hammen et al. 2010). Since our predatory system has the potential for competition and possibly intraguild predation, their response to each other could be expected to change after experiencing such antagonistic interactions, however, both assumptions need further investigation.

Besides avoidance, another mechanism to prevent adverse effects of competitive interactions, which allows for coexistence of two related predator species, is niche differentiation (May 1974). We observed invasions of *P. macropilis* in the culture of *N. californicus* and the two species coexisted for about 3 months. *Neoseiulus californicus* is much more resistant to starvation, 33% of its protonymphs died after four days without food, whereas 62% of *P. macropilis* protonymphs had died on the third day (M. Fonseca, pers. obs.). This may explain the tendency of *P. macropilis* to disperse and die in the water barrier surrounding the rearing units when prey availability is low. These different aspects of their foraging behaviour and the constant presence of resources may have cause this coexistence we observed, but the mechanisms involved remain to be experimentally verified.

In conclusion, further study is needed of the direct and indirect interactions between *P. macropilis* and *N. californicus* and their effects on biological control of the two-spotted spider mite. The increased joint use of several species of natural enemies for biological control highlights the importance of exploring the mechanisms and functions of volatiles mediating interactions within the third trophic level. Chemical cues have important implications for ecological interactions and evolutionary processes, however, studies of odour-mediated avoidance are limited to a small number of species, but deserve more attention.

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CHAPTER 2

How to evaluate the potential occurrence of intraguild predation

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Abstract

Intraguild predation is the combination of exploitative competition and predation among potential competitors that use similar resources. It has the potential to shape population dynamics and community structure. Although there is much empirical evidence for the occurrence of intraguild predation in natural ecosystems, the study of its effects is mainly limited to short-term microcosm experiments. There is, therefore, certain skepticism about its actual significance in nature. A relevant concern is that there is no consensus regarding criteria to evaluate the possible occurrence of intraguild predation in short-term experiments, and methodological differences may therefore underlie apparent inconsistencies among studies. Our purpose here was to evaluate existing criteria to offer guidance for the design of experiments to determine whether two species may potentially engage in intraguild predation. The criteria are based on the condition that intraguild predators need to experience immediate energetic gains when feeding on the intraguild prey. Thus, a relevant experimental design must quantify predation but also fitness benefits of feeding on the other species, i.e. increases in reproduction, somatic growth, or survival.

Key words: Predator–predator interactions, experimental design, population dynamics, food webs, evolution.

Introduction

Intraguild predation (IGP) consists of the combination of exploitative competition and predation among potential competitors that use similar resources (Polis et al. 1989). It has received considerable attention in the theoretical literature since the influential paper of Polis et al. (1989) pointed out its potential to shape population dynamics and community structure (Diehl and Feissel 2000; Mylius et al. 2001; van de Wolfshaar et al. 2006; Ellner and Becks 2011; Hin et al. 2011). Theoretical models predict that IGP results in more complex population dynamics than either competition or predation alone and can lead to exclusion, coexistence or alternative states (Polis and Holt 1992; Holt and Polis 1997). Despite the ubiquity and importance of IGP, empirical evidence for its effects is mainly limited to microcosm experiments, and there exists, therefore, certain skepticism about its actual occurrence and significance in nature (Kindlmann and Houdkova´ 2006; Gagnon et al. 2011). Nevertheless, there are various examples of its occurrence in a wide variety of communities (Polis et al. 1989; Polis and Holt 1992; Rosenheim et al. 1995; Arim and Marquet 2004), and new methods, using primers specific for the IG prey, are increasingly used to demonstrate the occurrence and importance of this interaction in the field (for example, Yang et al. 2017).

The simplest system with IGP involves three species: one competitor species (the intraguild predator, IG predator hereafter) that feeds on the other competitor (the intraguild prey, IG prey hereafter) and a shared prey (or shared resource), which is consumed by both competitors (Fig. 1a, Polis and Holt 1992; Holt and Polis 1997). Predictions of the dynamics and coexistence of these three species generally depend on several parameters, such as predation rates, prey preference of the IG predator, ontogenetic diet shifts, and productivity (or carrying capacity of the resource). Whereas there are no publications that systematically investigate the effects of all of these, most publications present predictions as a function of productivity. This is also the parameter that is most easily manipulated experimentally. We therefore focus primarily on this parameter in this paper. Consistent predictions of IGP theory are that coexistence of IG predators and IG prey depends on the productivity level of the resource and the competitive dominance of the IG prey compared to the IG predator (Polis et al. 1989; Diehl and Feissel 2000; Mylius et al. 2001). For coexistence of all three species, the IG prey must be superior at exploitative competition for the shared resource, and even then, coexistence is only possible at intermediate levels of productivity (Polis and Holt 1992, Table 1). If the IG predator is the superior competitor or when productivity levels are high, the IG prey is always excluded (Diehl and Feissel 2000; Mylius et al. 2001, Table 1). At low productivity levels, the best competitor dominates (Holt and Polis 1997; Diehl and Feissel 2001; Mylius et al. 2001, Table 1).

If the two competitors feed on each other, they are engaged in a more complex type of IGP termed reciprocal intraguild predation (Fig. 1b), which is also frequently observed in systems of true predators (Polis et al. 1989; Wissinger 1992; Woodward and Hildrew 2002; Montserrat et al. 2012). When such mutual predation is included in the basic model of IGP, the possibilities for coexistence of the three species are dramatically reduced compared to simple IGP (Table 1, HilleRisLambers and Dieckmann 2003; Montserrat et al. 2012; Schellekens and van Kooten 2012). The predictions are that reciprocal IGP could lead to mutual exclusion or alternative states, in which either one or the other competitor persists alone with the resource, depending on the initial densities (Table 1, HilleRisLambers and Dieckmann 2003; van der Hammen et al. 2010; Montserrat et al. 2012), and there is only limited possibility for coexistence (Schellekens and van Kooten 2012). At low productivity levels, only the best competitor can coexist with the shared resource, whereas priority effects determine which competitor will persist at intermediate to high productivity levels (Table 1, Montserrat et al. 2008; van der Hammen et al. 2010; Montserrat et al. 2012). In the latter case, the competitor that arrives first can build up a population and exclude the other competitor through intraguild predation, thus, the order of invasion is a decisive factor in systems with reciprocal IGP (HilleRisLambers and Dieckmann 2003; Montserrat et al. 2008; van der Hammen et al. 2010; Montserrat et al. 2012).

The theoretical predictions above concern the state of populations in the long-term, when the populations reach stable equilibria or persistent fluctuations. However, laboratory experiments on IGP are frequently only short-term because of experimental logistics (Briggs and Borer 2005; Janssen et al. 2006). Such short-term experiments can only provide qualitative information about whether or not two species are potentially able to engage in IGP (Guzmán et al. 2016). Nevertheless, long-term dynamical predictions are commonly inferred from these short-term experiments and consequently may lead to inaccurate interpretations (Brown et al. 2001; Hastings 2004; Briggs and Borer 2005). Recently, Guzmán et al. (2016) introduced another relevant concern about the methods used in these short-term experiments, that is, there is no consensus regarding criteria to evaluate the occurrence of IGP, and thus, methodological differences may underlie apparent inconsistencies among studies. These authors proposed criteria to evaluate IGP in predatory mites, but these criteria are easily generalized to other arthropod systems, e.g., aquatic arthropods (Wissinger and McGrady 1993; Crumrine 2005), arthropod pests (Rosenheim et al. 1995) and spiders (Polis and McCormick 1986). However, we think that the criteria deserve some further clarification given the number of publications on this topic, both within acarology and in ecology in general. Our purpose here is therefore to further explore such criteria in order to offer guidance on how to design appropriate experiments to determine whether or not two species may potentially engage in IGP.

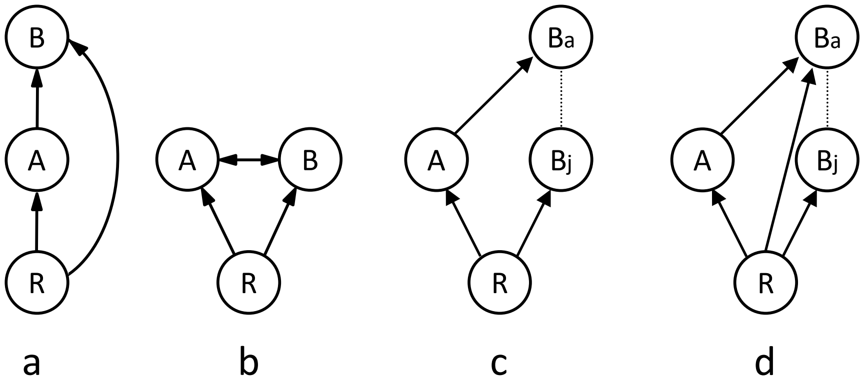

Figure 1. Different types of intraguild predation. The capitals in the circles indicate the shared resource (R), and the two species involved in intraguild predation (*A* and *B*). *Arrows* point from victim to attacker, interrupted lines indicate development. Juveniles and adults are indicated with ‘‘j’’ and ‘‘a’’, respectively. *a* Simple intraguild predation: species A feeds on the shared resource, species B feeds on the resource and on species *A*. *b* Simple reciprocal intraguild predation: species *A* and *B* feed on the shared resource and on each other. *c* Intraguild predation with complete ontogenetic diet shift: juveniles of species *B* feed on the shared resource, whereas adults feed on species *A*. *d* Intraguild predation with partial diet shift: juveniles of species *B* feed on the shared resource, adults feed on the resource and on species *A*. After Mylius et al. (2001) and van der Hammen et al. (2010).

Table 1. A comparison of the predicted consequences of competition, intraguild predation (IGP) and reciprocal intraguild predation on species persistence. Shown are the species that will persist at three different productivity levels (Low, Intermediate, High), with A being the superior competitor and B being the inferior competitor.

Interaction	Productivity			Details[2]
	Low[1]	Intermediate	High	
Competition	A	A	A	a
IGP with A as IG-predator	A	A	A	b
IGP with B as IG-predator	A	A, B or AB	B	b
Reciprocal IGP	A or None	A or B	A or B	c

These coexistence patterns are mostly based on analytical or numerical stability analyses and invasion criteria (Holt and Polis 1997; Mylius et al. 2001; HilleRisLambers and Dieckmann 2003), but also hold for unstable, paradox-of-enrichment types of dynamics. Holt and Polis state that a Lotka–Volterra model showing unstable dynamics show such high amplitude cycles that populations effectively would go extinct. Possibly, cyclic dynamics increases possibility for coexistence, as was shown for resource competition (Armstrong and McGehee 1980; Huisman and Weissing 1999). 1 Low productivity levels allow for the superior competitor to persist with the resource, but not the inferior competitor; 2 a: Tilman (1980); b: Holt and Polis (1997), Mylius et al. (2001), Diehl and Feissel (2000), van de Wolfshaar et al. 2006), Hin et al. (2011); c: Priority effects occur, except for intermediately strong trade-offs between feeding on the shared resource or the other predator (HilleRisLambers and Dieckmann 2003). Montserrat et al. (2008, 2012) present experimental evidence for bistability, and show that the inferior competitor can eliminate the superior competitor at low productivity levels, but subsequently also goes extinct.

IGP or interspecific killing

The experimental design proposed by Guzmán et al. (2016) to evaluate the occurrence of IGP consists of all combinations of two main factors resulting in four treatments: the presence or absence of the IG predator, the presence or absence of the shared resource, with the IG prey always present. Such a design would allow assessing the mortality rate of IG prey and it assumes previous knowledge that both IG prey and IG predator feed on the shared resource. However, this design does not allow assessing one further prerequisite for the occurrence of IGP, which is that the IG predator does not only kill the

IG prey, but also feeds on it. This may seem obvious, but predators may simply kill other predators without feeding on them (Palomares and Caro 1999), in which case the interaction is qualified as interspecific killing (an extreme form of interference competition). In many systems, especially those with large predators, it is possible to distinguish interspecific killing from intraguild predation through observing the IG predator consuming the IG prey (e.g. Bjorklund et al. 2016). We can safely assume that this consumption results in nutritional or energetic gains in natural systems; if not, IG predators would be selected not to feed. However, in other systems, including those with predatory mites and other small arthropods, observing consumption of prey is often difficult because of the size of the study organisms and the low frequency of predation events. Therefore, prey and predators are often confined and the mortality of the prey is assessed after some period through quantification of their shriveled remains, but it is then unclear whether the predator only killed the prey or also fed on it. It is with such a common experimental set-up in mind, that we suggest the experiments below.

Thus, predation of the IG prey by the IG predator should provide direct nutritional and energetic gains that result in increased growth, reproduction or survival of the IG predator (Polis et al. 1989). Although this is essential, many publications, including some of our own, do not present it as an important component of IGP. It is relatively easy to assess gains when IG prey are an equally good or better food source than the shared resource. For instance, the larval performance of the ladybird Harmonia axyridis was equal on intraguild prey and the shared resource (Yasuda and Ohnuma 1999; Cottrell 2004; Sato et al. 2008), and two predatory mites performed better when feeding on each other’s immature stages than on the shared resource (Buitenhuis et al. 2010). However, there is one potential complication in assessing gains when the IG prey is a trivial component in the diet of the IG predator, and removing it from the IG predator’s diet may not result in any direct effects on the latter’s fitness. However, consuming the IG prey should result in direct positive fitness effects in the absence of other food, and this absence will occur as a result of competition for the shared resource between the IG prey and IG predator. Even when it is a minor diet component when other prey are present, IG prey may contribute to the persistence of IG predators in periods of food scarcity (Janssen et al. 2006).

Experimental assessment of the occurrence of intraguild predation

Guzmán et al. (2016) suggest as main criterion that short-term predation experiments should show that IG predators attack both IG prey and shared resource when both are present. If they do not attack the IG prey or do not feed on the shared resource, the authors conclude that the two species are not engaged in IGP, but are involved in competition for resources or a predator–prey interaction, respectively. With hindsight, we think that this criterion is somewhat too strict. It is certainly true that the two predators are involved in intraguild predation when the IG predators attack both the IG prey and the shared resource in such experiments. However, IGP can still occur in nature when IG predators do not feed on both the IG prey and the shared resource within the arbitrary duration of an experiment. Actually, the strength of predation and competition will vary over time, depending on the densities of the shared resource and the IG prey. Accordingly, if IG predators forage only on the shared resource in the presence of the IG prey, this does not mean that there are no circumstances in which they will feed on the IG prey.

For example, Holt and Polis (1997) argued that if IG predators follow the rules of optimal diet theory, the IG prey would be dropped from the IG predator’s diet when the resource is abundant and a better diet than IG prey, allowing for coexistence to occur. After being dropped from the diet, IG prey populations would recover, resource levels would decrease as a result of the combined consumption by the IG prey and IG predator, and the IG prey would again be included in the IG predator’s diet (Krivan 2000). In this way, adaptive foraging of the IG predators would result in temporal changes in the diet of the IG predators, with alternating periods in which the IG predator feeds on the IG prey and the shared resource and periods in which the IG predators would only feed on the resource. Similar diet restrictions and expansions are expected when the IG prey is a better diet, with the shared resource being excluded or included from the diet depending on the densities of the IG prey (Krivan and Diehl 2005). Although the system of IG predators, IG prey and shared resource will then go through periods in which there is effectively no IGP, the long-term dynamics of the system are still determined by the occurrence of IGP (Krivan and Diehl 2005).

A second example of IGP where consumption of resource and IG prey by the same individual is separated in time is found in systems with ontogenetic diet shifts (Polis et al. 1989; Mylius et al. 2001; van de Wolfshaar et al. 2006; Hin et al. 2011). Organisms are also not necessarily restricted to one ecological role during their entire lives (Polis et al. 1989; Mylius et al. 2001). Hence, IG predators may sometimes feed on the IG prey, and feed on the common resource at other times. In its most extreme form, adult IG predators may feed on IG prey, whereas juvenile IG predators consume resource only (Fig. 1c). Although each ontogenetic stage separately is involved in one interaction only (adult IG predators are predators of the IG prey, juveniles are competitors), each individual is involved in both interactions, but not during the same life stage or age. Nevertheless, this interaction also falls under the definition of IGP (Polis et al. 1989).

Besides these two examples, there are also conditions in which the consumption of IG prey and shared resource by the IG predator within an experiment remains undetected. Consider no diet preference and a satiating functional response in the IG predator. When the density of the shared resource is high relative to that of the IG prey, attacks on the IG prey will be low, simply because the IG predators spend time handling the shared resource or because they become satiated when feeding on the abundant shared resource. As a result, the observed mortality rate of the IG prey may be low and not significantly different from its natural mortality in the absence of IG predators. Hence, a non-significant difference in the observed mortality rates of the IG prey in the presence or absence of the IG predator does not necessarily prove that IGP does not occur at lower densities of the shared resource or higher densities of the IG prey. This could perhaps be solved by increasing the number of replicates of the experiment, which might ultimately result in a significant difference in mortality of the IG prey in the presence vs the absence of the IG predator. However, there are better alternatives, for example, changing the densities of IG prey or the shared resource. The most extreme case of reducing densities is setting them to zero, which results in exactly one of the treatments suggested below: the IG predator with the IG prey in the absence of the shared resource.

Criteria for assessing the possible occurrence of intraguild predation

Based on the above arguments, we suggest that, to ascertain the potential occurrence of IGP between two competitors A and B, a relevant experimental design must quantify predation but also consumption, or otherwise increased reproduction, somatic growth or survival of one species feeding on the other (i.e. immediate fitness benefits). We assume that it is known that both species attack the shared resource and can grow, develop and reproduce on it (i.e. they are competitors). If not, extra experiments need to be done to ascertain this. We thus suggest the following design with three treatments (Fig. 2), with A being the possible IG predator and B being the possible IG prey, each of the appropriate stage: (1) species A with species B; (2) the same stage of species B alone; (3) the same stage of species A alone. When the mortality of B in the first treatment is significantly higher than in the second treatment, it can be concluded that A kills B. This interaction can then be intraguild predation or interspecific killing (Fig. 2). If the mortality of species

B is not higher in the first treatment, the species are involved in resource competition. Subsequently, if the fitness of A in the first treatment is higher than in the third treatment, it is concluded that A consumes B and benefits from it: A is the intraguild predator and B is the intraguild prey (Fig. 2). If the fitness in the first treatment is not higher than in the third treatment, the interaction can be classified as interspecific killing or interference competition.

To verify whether killing or predation occurs may often take less time than to demonstrate effects of feeding on the IG prey on reproduction, growth and survival of the IG predator. In predatory mites, for example, predation of the IG prey can be assessed within one day, but it may take several days to observe effects on IG predator oviposition, and even longer to perceive effects on survival and development. The experiments proposed here may therefore take more time than the simple predation experiments that are often done.

When reciprocal intraguild predation is suspected (Montserrat et al. 2012), these experiments should be repeated with the suspected stage of species B serving as predator and the suspected stage of species A as prey. In cases of ontogenetic diet shifts (Fig. 1c, d), the appropriate stage of the IG predator (for example the adults) should be tested, which is not the stage that also feeds on the shared resource (Fig. 1c).

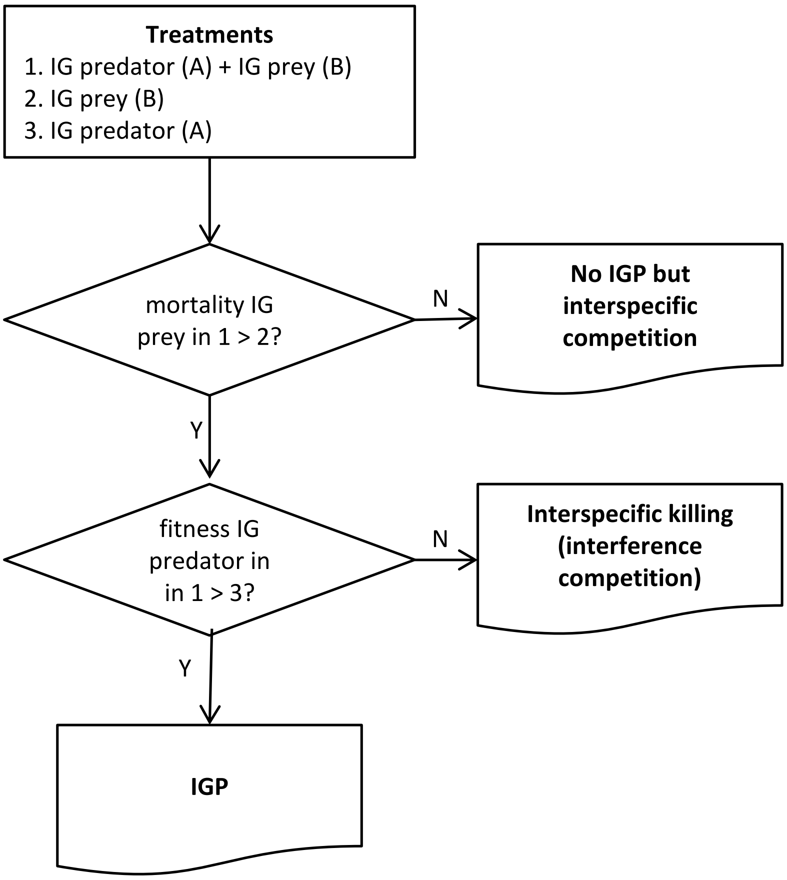

Figure 2. A flow chart of the experiments needed to assess the potential occurrence of intraguild predation in systems where direct observations of IG predators consuming IG prey are difficult to obtain. Letters A and B refer to the letters used in the text, with A being the stage of the species that is the suspected IG predator, and B being the stage of the species that is the suspected IG prey. It is assumed that some stage of both the IG prey and IG predator feed on the shared resource, but not necessarily the same stage as tested here. If this is not known, extra predation experiments with the shared resource and the IG prey or IG predator need to be done.

Re-evaluation of the reviewed literature

Guzmán et al. (2016) evaluated 18 papers on IGP between phytoseiids, and found that three of them met their criteria. Seven of these 18 papers met the criteria suggested here: they reported the predation of the IG predator on the IG prey as well as some fitness benefit due to this predation (Meszaros et al. 2007; Momen and Abdel-Khalek 2009; Momen 2010; Buitenhuis et al. 2010; Mendel and Schausberger 2011; Montserrat et al. 2012; Gotoh et al. 2014). The other papers, including some of our own (Cakmak et al.

2006; Montserrat et al. 2008), did not include the treatment with the potential IG predator alone to establish fitness benefits (i.e. the comparison of the first and the third treatment as suggested above). The experiments of Guzmán et al. (2016) do involve all necessary treatments, but there is no specific comparison of the oviposition rate of the IG predator in the presence or absence of the IG prey (as measure for fitness benefit). However, the reported significant effect of the interaction between the presence/absence of shared resource and IG prey on IG predator oviposition suggests that there is such a fitness benefit.

The evolution of intraguild predation

As explained above, besides the indirect benefit of decreasing the population of potential competitors, thus freeing resources, IGP also results in direct energetic benefits (Polis et al. 1989). If the killing of competitors is not profitable and does not provide any energetic gains to the aggressor, they are not engaged in intraguild predation but in an extreme form of interference competition. Interference is a common interaction among competitors and a frequent coevolutionary response to exploitation competition (Simberloff 1982; Connell 1983; Schoener 1983; Polis 1988). Eliminating the ability of a competitor to exploit resources through interference is one possible evolutionary response to lessen potential harmful effects of exploitation competition (Polis 1988). Intraguild predation may be the next step in the evolution of interference between predators (Polis et al. 1989). There will probably be selection for those predators that do not only kill the competitors, but also feed on them, even if this only results in small increases in fitness. Subsequently, selection can act on the IG predators to be more effective at converting IG prey. Thus, the immediate energetic gains distinguish IGP from traditional concepts of competition and its multiple fitness benefits further facilitate the evolution of this interaction (Polis 1988; Polis et al. 1989). Another advantage of being able to feed on the IG prey is that it may increase the persistence of IG predators in periods of scarcity of the shared resource (Janssen et al. 2006).

An alternative evolutionary route to IGP occurs in a tritrophic system with a predator, a prey and the food of the prey. When a predator subsequently evolves to include the food of the prey into its diet, the predator and the prey become involved in intraguild predation. Although it is difficult to know which of the two routes have historically been followed, there are perhaps some indications that this has happened, for example when the IG prey is a better food source for the IG predator than the shared resource is. For instance, in a system with reciprocal IGP between predatory mites used to control the western flower thrips, *Amblyseius swirskii* had higher oviposition rates and a faster development time when feeding on immature stages of the IG prey than on the shared resource (Buitenhuis et al. 2010). Furthermore, a mixed diet of IG prey with the resource may also be important to fulfill energy requirements or supplement nutritional deficiencies, for example, of protein for IG predators that feed primarily on plants which mostly consist of carbohydrates (Polis et al. 1989).

The importance of intraguild predation for dynamics and persistence

The criteria suggested above will reveal whether IGP can occur between species pairs, but the next question is whether this IGP is important for population dynamics and coexistence of the species involved. This can only be evaluated in replicated, long-term population experiments and invasion experiments, of which there are not many around (Morin 1999; Diehl and Feissel 2000; Montserrat et al. 2008, 2012; Hiltunen et al. 2013; Wilken et al. 2014). These experiments serve to discriminate between IGP, classical competition and predation (Table 1). With resource competition, the species that reduces the level of resources the most wins (Table 1, Tilman 1980; but see Armstrong and McGehee 1980; Huisman and Weissing 1999). In classical tritrophic predator–prey systems, predators only persist with the resource and the prey when the productivity of the system is sufficiently high (Oksanen et al. 1981). As outlined above, in systems with IGP, coexistence depends on both productivity levels and on which of the competitors is the IG predator (Holt and Polis 1997; Diehl and Feissel 2000; Mylius et al. 2001). It furthermore depends on ontogenetic diet shifts; if the intraguild predator can only survive or reproduce when feeding on the IG prey (Fig. 1c), they can only persist in the presence of IG prey and at sufficiently high productivity levels, similar to a classical predator–prey system (Mylius et al. 2001; Hin et al. 2011). More complex forms of diet shifts, where adult and juvenile IG predators differ gradually in the rate of attack of the resource (Fig. 1d), can give rise to various alternative equilibria, depending on the importance of predation relative to competition (Hin et al. 2011).

Theory predicts that the possibilities for coexistence of species involved in IGP are limited and depend on productivity levels, on which species is the superior competitor, and on the occurrence of reciprocal IGP. In contrast, IG prey and IG predators co-occur frequently enough in nature to allow the observation that IGP is a common interaction (Arim and Marquet 2004). Various causes have been suggested for this discrepancy between theory and reality, such as habitat heterogeneity or the presence of refugia for IG prey (Finke and Denno 2002; Janssen et al. 2007; Ferreira et al. 2011; Lemos et al. 2015), and flexible foraging behaviour of the IG-predator (Kondoh 2003). Furthermore, the various interactions occurring in complex food webs may decrease the strength of the interaction, resulting in increased coexistence of IG prey and IG predators (Fagan 1997). The importance and consequences of IGP for species coexistence ultimately need to be assessed in population-dynamical experiments or invasion experiments at various productivity levels (e.g. Morin 1999; Diehl and Feissel 2000, 2001; Montserrat et al. 2008; Wilken et al. 2014). Moreover, the effects of age or stage structure on the success of invasion of one species into a population of the other species should be studied experimentally (Montserrat et al. 2012).

Predator–prey theory has historically focused on interactions between individual prey and predator species and largely ignored the possible effects of predator-predator interactions (Holling 1965; Connell 1983; Kareiva 1994; Losey and Denno 1998). It has become clear in the last 3 decennia, however, that it is paramount to understand how a complex of predators can interact to affect each other's behaviour and dynamics, as well as their subsequent effects on behaviour and population dynamics of the shared prey (Polis et al. 1989; Lima 1992; Kareiva 1994), including the consequences of this for biological pest control (Rosenheim et al. 1995; Janssen et al. 2006). Although it is important to identify the occurrence of IGP, we suggest that further research should consider the magnitude (interaction strength), the population dynamics and the coexistence of the interacting species, which may differ profoundly from that of systems of competing species or of predators and prey. The challenge is to investigate the effects of IGP in natural systems more rigorously, and laboratory tests of the possible occurrence of IGP, as proposed here and elsewhere (Guzmán et al. 2016), are a necessary first step.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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CHAPTER 3

Ontogenetic stage-specific reciprocal intraguild predation

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Abstract

Intraguild predation is the killing and eating of potential competitors, and often depends on ontogenetic stage of the individuals, which generally determines whether it is an intraguild predator or an intraguild prey. Although ubiquitous, these ontogenetic stages have mostly been neglected in predator interactions which can result in underestimating the potential for intraguild predation. Here, we investigated predatory interactions among all mobile stages of the two predatory mite species *Neoseiulus californicus* and *Phytoseiulus macropilis*, which compete for two-spotted spider mites. We evaluated both the capacity to kill stages of the other species and the capacity to benefit from feeding on these stages, both prerequisites for the occurrence of intraguild predation. Ontogeny clearly played a critical role in determining the occurrence of intraguild predation within our predator system. Whereas the juveniles of *P. macropilis* developed from larva until adulthood when feeding on *N. californicus* eggs, interestingly, large adult female *P. macropilis* did not feed on the smaller stages of the other species. We furthermore show that intraguild predation was reciprocal: both juveniles and adult females of *N. californicus* preyed on *P. macropilis* smaller stages. We suggest that it is crucial to investigate intraguild predation among all ontogenetic stages of the species involved.

Key words: predator-predator interactions, stage structure, ontogenetic diet shifts, predatory mites, biological control.

Introduction

Whereas there is an intuitive appeal to view predators as large and fierce animals attacking small and helpless prey, it is less observable, however, that these dangerous predators often begin their lives small and vulnerable (Wilbur 1988; Mukherjee and Heithaus 2013). In about 90% of the feeding links among animal species, prey are smaller than predators (Cohen et al. 1993b), hence, the killing capability of the predator depends on the sizes of both predator and prey (Wilbur 1988; Claessen et al. 2002). Because body size often changes drastically during ontogeny, an individual performing as a predator when adult and large may be vulnerable to attacks and killings when small (Polis et al. 1989; Polis 1991; Elgar and Bernard 1992; Palomares et al. 1999; Janssen et al. 2002). Such killings of small predators may arise both within species through cannibalism (Elgar and Crespi 1992) and among species through hyperpredation (Rosenheim 1998), intraguild predation (Polis et al. 1989; Polis 1991; Palomares et al. 1999), by omnivory (Faraji et al. 2001; Janssen et al. 2002, 2003) and, intriguingly, even through predation by prey that are considered purely herbivorous (Aoki et al. 1984; Saitō 1986). Thus, due to ontogenetic changes, individuals of a species may start their lives at a lower trophic level and move up through trophic levels. This allows for reversals of predator and prey roles within individuals (Barkai and McQuaid 1988; Magalhaes et al. 2005; Choh et al. 2012).

There is growing awareness that these ontogenetic shifts of trophic levels may have profound effects on the structure and dynamics of food webs because they result in size- or age-structured interactions (Polis 1984; Cohen et al. 1993a, b; Woodward et al. 2005; Rudolf 2006, 2007). Yet, food web analyses often represent networks of feeding links between species (Pimm et al. 1991; De Ruiter et al. 2005; Jonsson et al. 2005), but not between stages of species (Preston et al. 2012, 2014). Large bodies of theoretical and empirical work are still framed as each species occupying one trophic level and thus, we tend to see food webs as simplistic and static representations of biological communities (Polis and Strong 1996; Woodward et al. 2005; de Ruiter 2005). Nevertheless, natural food webs contain multiple links of various type and strength of ecological interactions that frequently depends upon ontogenetic stage of the individuals (Pimm 1982; Polis 1991; Polis and Strong 1996; Persson 1999). Therefore, the traditional approach of appointing species to one trophic level can be an oversimplification of reality (Werner and Gilliam 1984; Choh et al. 2012; Rudolf et al. 2014).

Intraguild predation (IGP), the killing and eating of heterospecific competitors, is a widespread interaction in which the stage or size of the individual generally determines whether it is a predator or a prey (Polis et al. 1989; Arim and Marquet 2004; Montserrat et al. 2012). Because predators are vulnerable in the early stages of ontogeny, most IGP is asymmetric, with larger stages preying on smaller ones (Polis et al. 1989; Woodward and Hildrew 2002; Montserrat et al. 2012). The simplest conceivable case of IGP involves three species: one competitor species that feeds on the other competitor and a shared prey that is consumed by both competitors (Polis and Holt 1992; Holt and Polis 1997). However, in such size-structured systems, different sizes of interacting predatory species commonly coexist, hence, the two predator species may frequently attack each other's vulnerable stages (Polis 1984; Choh et al. 2012; Montserrat et al. 2012), thus engaging in a more complex type of IGP, termed reciprocal intraguild predation (RIGP). Reciprocal intraguild predation also occurs in natural systems but has only been given limited attention (Polis et al. 1989; Wissinger 1992; Woodward and Hildrew 2002; van der Hammen et al. 2010; Montserrat et al. 2012). Furthermore, stage structure due to ontogenetic changes has mostly been a neglected subject of research within predator interactions (but see Wissinger 1992, Woodward and Hildrew 2002, Montserrat et al. 2012, (Preston et al. 2012, 2014) and accordingly, may underestimate the potential for IGP (Polis and McCormick 1987; Polis 1988; Polis et al. 1989; Wissinger 1992).

Moreover, this obscures the potential of IGP to shape population dynamics and community structure (Polis et al. 1989), as well as its implications for species conservation and other management of animal populations.

The best studied systems of intraguild interactions consist of biological control systems, especially in greenhouse crops, because these artificial food webs (Ehler 1996) are easier to manipulate than natural systems (Messelink et al. 2012). The increased use of biological control agents in agricultural crops has led to a growing complexity of these artificial food webs, hence, the densities of the target pests species are more and more determined by the joint effects of various direct and indirect interactions among prey and predators (Janssen et al. 1998; Çakmak et al. 2006; Messelink et al. 2012; van Lenteren et al. 2017). With the presence of several natural enemies, particularly generalist predators that can interact more with other natural enemies, IGP can occur more frequently and is increasingly considered in the development of successful biological control programs (Rosenheim et al. 1993, 1995, Janssen et al. 1998, 2006; Messelink et al. 2012).

Here, we investigated the interplay between ontogenetic stages and IGP interactions in a system consisting of the two predatory mite species, *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks), (Acari: Phytoseiidae). These two species are mass-produced in several regions of the world and used as biological control agents of the two-spotted spider mite *Tetranychus urticae* (Koch (Acari: Tetranychidae)) (McMurtry and Croft 1997; Gerson et al. 2003), which is a cosmopolitan pest that attacks over 1100 plant species and is resistant to pesticides (Van Leeuwen et al. 2010; Migeon and Dorkeld 2015). In Brazil, both *P. macropilis* and *N. californicus* co-occur naturally in extensive regions on various plant species (Ferla et al. 2007; Roggia et al. 2009; de Freitas 2014). *Phytoseiulus macropilis* is considered a specialist predator of Tetranychus species and tend to disperse from crops when the densities of prey are low (McMurtry and Croft 1997; Oliveira et al. 2007) while *N.*

californicus has more generalist feeding habits and thus can adapt to oscillations of prey populations (Croft et al. 1998; Gerson et al. 2003). Therefore, these predators have been considered for combined releases to control *T. urticae*.

Our aim was to evaluate whether these predatory mites interact through intraguild predation and which stages are involved in this. Both predators have five developmental stages: egg, larva, protonymph, deutonymph (the latter three here together referred to as juvenile) and adult. The size of each stage is similar for both species, and the duration of their development from egg to adult is about 5 days for both species (Escudero and Ferragut 2005; Souza-Pimentel et al. 2016). Both protonymphs and deutonymphs need to feed to complete their development and although most predatory mite larvae do not need to feed to reach the next stage, the larvae of our system were observed feeding. We systematically explored intraguild predation by juveniles and adults of the two species and thus, we verified whether reciprocal intraguild predation occurred. We evaluated both the capacity to kill stages of the other species as well as the capacity to benefit from feeding on these stages, both prerequisites for the occurrence of IGP (Polis et al. 1989; Fonseca et al. 2017).

Material and methods

Cultures

The two-spotted spider mite (*T. urticae*) was reared on jack bean plants (*Canavalia ensiformis* (L.) DC) in a climate-controlled room (25 ± 3 °C, 70–90 % relative humidity, with controlled photoperiod 12:12 L:D). Clean jack bean plants were grown in a greenhouse until they were 2 weeks old and were subsequently added biweekly to the spider mite culture. The predatory mites *P. macropilis* and *N. californicus* were reared under same conditions as above on detached bean leaves infested with two-spotted spider mites. These leaves were put in a plastic tray (l × w × h = 45 × 30 × 8 cm) that was placed inside a second, water-containing tray (55 × 40 × 10 cm) to prevent the mites from escaping. New bean leaves with spider mites were added to the cultures 2–3 times per week. The cultures of spider mites and predatory mites were started with individuals obtained from cultures from Econtrole Pesquisa & Consultoria Ltda (Viçosa, MG, Brazil). Both predatory mite species had been reared for about a year on jack bean leaves with two-spotted spider mites prior to the experiments.

Experimental set-up

The experimental units used for all experiments consisted of plastic Petri dishes (diameter 6 cm, 1.5 cm high). Each Petri dish contained a small piece of wet cotton wool as a water source and was closed with cling film (Alpfilm®, Alpfilm Indústria e Comércio de Plásticos Ltda, São Paulo, Brazil). All adults used in the experiments were gravid females, aged between 10 and 15 days since the egg stage. These adult females were placed singly in the experimental units and were starved for 24 h prior to the experiments to prevent possible effects from the previous diet. Only females that had oviposited during this starvation period were used. Cohorts of newly laid eggs and larvae for experiments were obtained by transferring females from the cultures to separate arenas of bean leaves with spider mites and allowing them to lay eggs for 24 h. Subsequently, the eggs were separated on a new arena, which were checked for hatched larvae every 12 h. Preliminary experiments were done to check how many individuals to use as prey. All experiments were conducted in a climate-controlled room (conditions as above).

IGP of adult females on heterospecific eggs and larvae

We evaluated the predation rate of adult females of both species on heterospecific eggs and larvae and the effect of this feeding on their oviposition rate to verify whether adult females gained from predation (Polis et al. 1989, Fonseca et al. 2017). Predation experiments were started by placing a single gravid female in each experimental unit together with 8 heterospecific eggs or larvae. To measure natural mortality, only eggs or larvae were placed in another set of arenas. One day later, the deflated eggs or the shrivelled corpses of the dead larvae were counted as evidence of predation. All treatments were replicated 14 times for *N. californicus* with eggs and 18 times with larvae of the other species and for *P. macropilis* 28 times with eggs and 27 times with larvae of *N. californicus*.

The oviposition rates of adult females feeding on heterospecific eggs or larvae were measured by offering adult females 8 eggs or 8 larvae of the other species and the oviposition rate of adult females without prey was assessed on another set of arenas. Newly-laid eggs were counted every 24 h and the adult females were transferred to new experimental units with new prey, or without prey in the control treatments. Each treatment lasted 5 days and was replicated 14 times for *N. californicus* on eggs of *P. macropilis* and lasted 4 days and was replicated 10 times on larvae of *P. macropilis*. We did not measure oviposition rates for adult females of *P. macropilis* on eggs or larvae of the other species because the predation experiment showed no evidence that these females preyed on eggs or larvae of *N. californicus*.

IGP of juveniles on heterospecific eggs and juveniles

To examine whether other ontogenetic stages were potential intraguild predators, we assessed the predation rate, development and survivorship of juveniles of both species on heterospecific eggs and juveniles. The first experiment was started by placing a newly hatched larva in each experimental unit together with 6 heterospecific eggs. To measure natural mortality of eggs and as control for juvenile survival, there were two further treatments: only eggs or only larvae without eggs. Every 24 h, the state (dead or alive) and developmental stage of the juveniles were checked and preyed eggs were counted. Subsequently, the juveniles were transferred to new experimental units with new eggs or without eggs in the control treatment and the eggs of the control treatment were also renewed. The experiments lasted until the juveniles reached adulthood or died. Each treatment was replicated 12 times for *N. californicus* juveniles and 16 times for *P. macropilis* juveniles.

In another experiment, we investigated whether juveniles eat juveniles of the other species. One newly hatched larva of each species was placed in each experimental unit together with a newly hatched heterospecific larva. Individuals of both species were held separately in controls. The predation and developmental stage of juveniles were assessed every 24 h and the number of replicates was 14 per treatment. This experiment lasted for 2 days, when predators were at the protonymph stage, because the previous experiment showed that more than 60% of the *P. macropilis* juveniles without food had died on the third day and thus, we could no longer discriminate between death due to starvation or predation. Therefore, it was not possible to measure intraguild predation among the deutonymphs in this experiment of development. Moreover, we did not investigate each juvenile stage separately because their duration can vary and may not last a day.

Statistics

Because generalized linear models did not result in a satisfactory distribution of residuals, predation rates were compared with the Wilcoxon rank-sum test (Crawley 2013). To analyse the effects of feeding on heterospecific eggs on survival of juveniles, we used a time-to-event analysis (Cox proportional hazards model) using the function ‘coxph’ of the ‘survival’ package (R Core Team 2016). The Kaplan–Meier estimate, which takes censored data into account, was used to assess survival through time. Because none of the juveniles reached the deutonymph or adult stage in the controls, the proportions of juveniles reaching these stages were compared through Pearson's Chi-squared test. Oviposition rates of adult females was compared between treatments with a Wilcoxon rank-sum test. All analyses were performed with the statistical software R, version 3.3.3 (R Core Team 2016).

Results

IGP of adult females on heterospecific eggs and larvae

None of the adult females of *P. macropilis* killed any of the heterospecific eggs (Fig. 1). Mortality of *N. californicus* larvae in the presence of *P. macropilis* was very low and not significantly different from mortality without *P. macropilis* (Fig. 2; Wilcoxon rank-sum test: W = 317, P = 0.3461). We conclude that adults of *P. macropilis* are no intraguild predators of *N. californicus* eggs and larvae.

In contrast, there was no mortality of eggs of *P. macropilis* in the absence of adult females of *N. californicus* but a significantly higher mortality in their presence (Fig. 1; Wilcoxon rank-sum test: W = 140, P = 0.016). The mortality of larvae and young protonymphs of *P. macropilis* was also higher in the presence of adult females of *N. californicus* than in their absence (Fig. 2; Wilcoxon rank-sum test: W = 294.5, P < 0.001). *Neoseiulus californicus* females did not oviposit in the absence of eggs of *P. macropilis*, and half of the females oviposited in the presence of eggs. This difference was significant (Fig. 3; Wilcoxon rank-sum test: W = 147, P = 0.0058). All adult *N. californicus* oviposited in the presence of larvae of *P. macropilis* and only one female produced one egg in their absence. This difference was also significant (Fig.3; Wilcoxon rank-sum test: W = 98, P < 0.001). We conclude that adults of *N. californicus* are intraguild predators of *P. macropilis* eggs and larvae and young protonymphs.

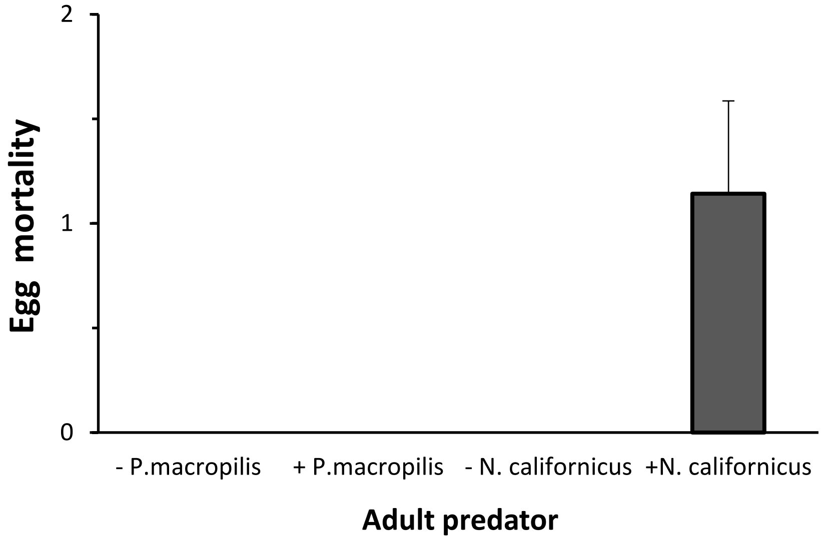

Figure 1. Average mortality (+ s.e.) of *N. californicus* (light bars) and *P. macropilis* (dark bars) eggs after 24 hours in the presence (+) or absence (-) of adult females of the other species.

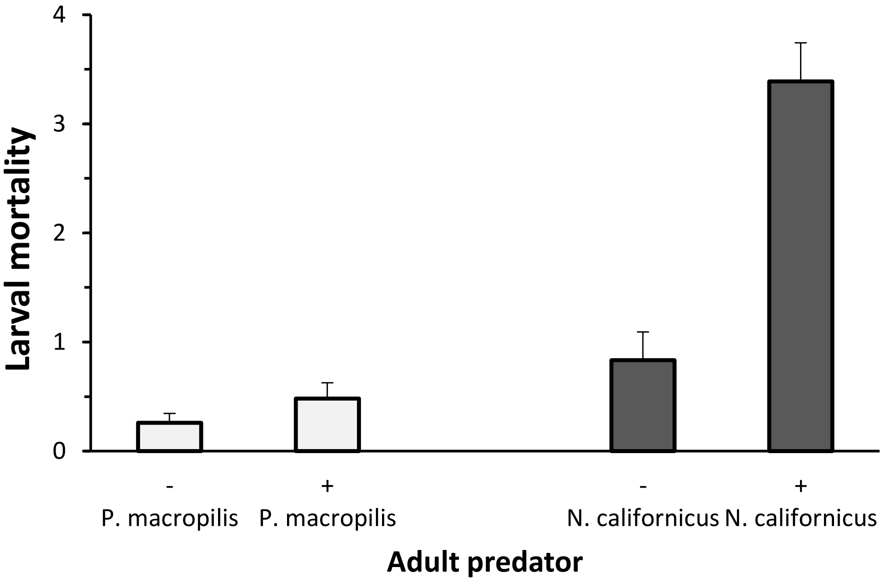

Figure 2. Average mortality (+ s.e.) of *N. californicus* (light bars) and *P. macropilis* (dark bars) larvae after 24 hours in the presence (+) or absence (-) of adult females of the other species.

Figure 3. Mean oviposition (± s.e.) of *N. californicus* fed on *P. macropilis* eggs (during 5 days, light bars) or larvae (during 4 days, dark bars) and without food (control).

IGP of juveniles on heterospecific eggs and juveniles

Juveniles of both *N. californicus* (Fig. 4, Wilcoxon rank-sum test: P = 0.0013) and *P. macropilis* (Fig. 4, Wilcoxon rank-sum test: P < 0.001) preyed on eggs of the other species until they reached adulthood. Juvenile survival of *N. californicus* significantly increased by feeding on heterospecific eggs (fig. 5, Cox proportional hazards: log rank = 6.52, d.f. = 1, P = 0.0106); juvenile survival of *P. macropilis* also increased by feeding on eggs of *N. californicus* (fig. 6, Cox proportional hazards: log rank = 13.25, d.f. = 1, P = 0.0003). In the absence of *P. macropilis* eggs, none of the *N. californicus* juveniles (n = 12) reached the deutonymph or adult stage and in their presence, 58% reached the deutonymph stage (Pearson Chi-Square: 12.3, d.f. = 3, P = 0.0063) and 33% reached adulthood (Pearson Chi-Square: 13.3, d.f. = 3, P = 0.0039). Juveniles of *P. macropilis* (n = 16), did not develop into deutonymph or adulthood in the absence of *N. californicus* eggs and in their presence 63% reached the deutonymph stage (Pearson Chi-Square: 17, d.f. = 3, P = 0.0007) and 44% adulthood (Pearson Chi-Square: 16.25, d.f. = 3, P = 0.0010). We conclude that juveniles of both species are intraguild predators of heterospecific eggs.

Larvae and protonymphs of both *N. californicus* and *P. macropilis* did not prey on the same stages of the other species in any of the 14 replicates. We conclude that juveniles (larvae and protonymphs) of both species are no intraguild predators of heterospecifics of the same stage.

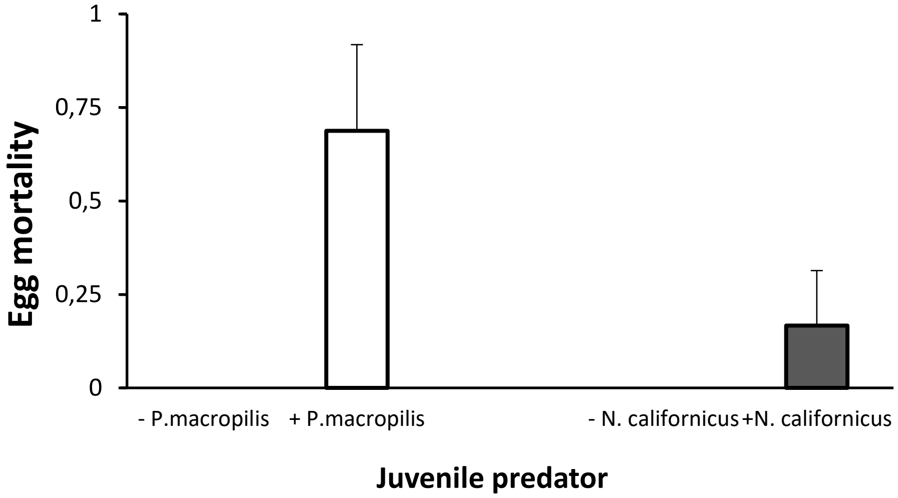

Figure 4. Average mortality (+ s.e.) of *N. californicus* (during 5 days, light bars) and *P. macropilis* (during 7, dark bars) eggs in the presence (+) or absence (-) of juveniles of the other species.

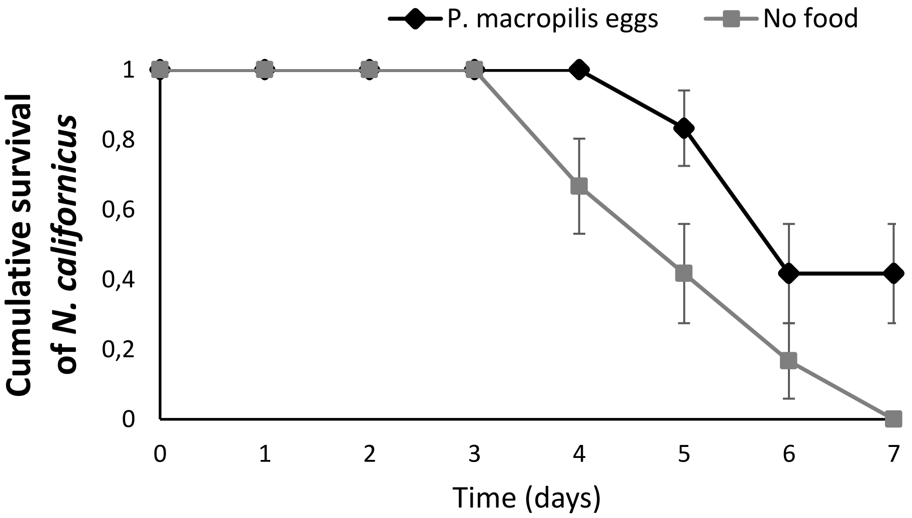

Figure 5. Cumulative survival (± s.e.) of *N. californicus* juveniles fed on *P. macropilis* eggs (black diamonds) and without food (grey squares, No food).

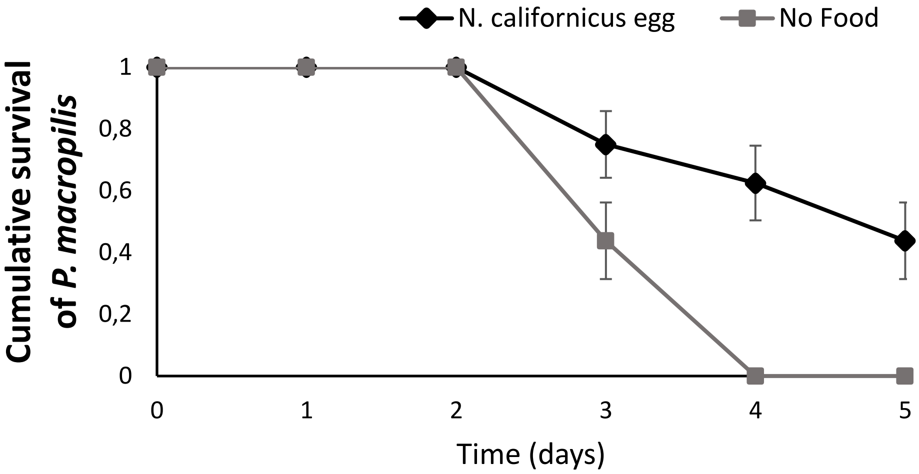

Figure 6. Cumulative survival (± s.e.) of *P. macropilis* juveniles fed on *N. californicus* eggs (black diamonds) and without food (grey squares, No food).

Discussion

Our results show that ontogeny clearly played a critical role in determining the occurrence of intraguild predation (IGP) within our predator system. Whereas the juveniles of *P. macropilis* develop from larva until adult feeding on *N. californicus* eggs, interestingly, the large adult females were no intraguild predators (IG predators) of the smaller stages (eggs and larvae, Fig. 7) of the opposite species. So far, studies on the existence or the absence of IGP have often focused on adult individuals preying on juveniles. In contrast, we show here that juvenile stages can be IG predators whereas adults are not. It is therefore crucial to investigate IGP by all ontogenetic stages of the species involved. Because IGP frequently originates from ontogenetic diet shifts (Polis et al. 1989) and because this represent the mode of life in 80% of animal taxa (Werner 1988), similar phenomena may occur in many other IGP systems. Moreover, this demonstrates the complexity of interactions among stage-structured populations and the ways in which stage-structured IG predators and IG prey can interact. We furthermore show that the IGP in our system was reciprocal (Fig. 7). Although there was no IG predation in the experiments between juveniles of the same stages of both species, and also not between adult females (M. M. Fonseca, personal observation), both juveniles and adult females of *N. californicus* were IG predators of *P. macropilis* smaller stages.

Besides stage structure, another fundamental criterion for assessing the possible occurrence of IGP is that the predation should result in direct nutritional and energetic gains, i.e. increased growth, reproduction or survival (Polis et al. 1989). We argued earlier (Fonseca et al. 2017) that, although this is essential, many publications do not present fitness gains as an important component of IGP. Here, we show that juveniles of *P. macropilis*, a species considered as a highly specialized predator of Tetranychid mites (McMurtry and Croft 1997), can complete their juvenile development feeding only on IG prey. *Neoseiulus californicus* was first classified as a selective predator of Tetranychids (McMurtry and Croft 1997; Croft et al. 1998), but its juveniles also develop until adulthood feeding only on IG prey, and 50% and 100% of the adult females reproduced on eggs or larvae of *P. macropilis* respectively. We therefore suggest that the concept of specialist predatory mites used in the literature may be too strict. Because many predator-prey systems in nature are subjected to a wide range of recurrent disturbances that affect the distribution and abundance of primary prey, organisms will commonly encounter situations without prey and therefore have to adapt to other available food sources. Thus, what defines a predator's diet, are the encounter rates with different prey types (Rosenheim et al. 2004) and its ontogenetic stage. In case of IGP, such adaptations to alternative food likely originate from interference competition because there will probably be selection for those predators that do not only kill their competitors, but also feed on them (Fonseca et al. 2017). Subsequently, selection can act on the IG predators to be more efficient at converting IG prey (Polis 1988; Polis et al. 1989; Fonseca et al. 2017). Besides providing energy and nutrition, IGP also eliminates potential competitors and this may indicate a potential for negative interactions between predatory species

(Polis et al. 1989; Rosenheim et al. 1995; Janssen et al. 2006; Montserrat et al. 2008; Buitenhuis et al. 2010).

The possibility of adverse interactions among natural enemies through IGP has been a concern for successful biological control (Rosenheim et al. 1995), however, there is not much empirical evidence for IGP limiting pest control by the predators involved (Janssen et al. 2006; Rosenheim and Harmon 2006; Messelink and Janssen 2014). Considering a three-species module (a IG predator, a IG prey and a shared prey), IGP may in theory result in the exclusion of one of the predator species. For coexistence of all three species, the IG prey must be superior at exploitative competition for the shared resource, and even then, coexistence is only possible at intermediate levels of productivity (Polis and Holt 1992; Holt and Polis 1997; Mylius et al. 2001). Nevertheless, there are many factors that may increase the possibilities for coexistence of predators in IGP systems. First of all, in nature and in most experimental systems in the field, there are more species than the three taken into consideration by theory. Moreover, most theory considers equilibrium dynamics whereas experiments mostly concern short-term, transient dynamics (Venzon et al. 2001; Briggs and Borer 2005; Janssen et al. 2007). The narrow region of coexistence of predators in IGP might also be extended by structural complexity of the habitat due to the availability of spatial refuges and niche partitioning (Finke and Denno 2006; Amarasekare 2007; Janssen et al. 2007; Ferreira et al. 2011), as well as by the presence of alternative food (Daugherty, P et al. 2007; Holt and Huxel 2007; Messelink and Janssen 2014). Furthermore, as mentioned above, the stage structure of populations is not incorporated in most theoretical models of IGP and also not fully investigated in experimental systems. More recently, however, a few theoretical and empirical studies have shown that coexistence of IGP predators and prey may sometimes be enhanced by stage structure (Mylius et al. 2001; Borer 2002; Crumrine 2005; Rudolf and Armstrong 2008; Hin et al. 2011). In contrast, Montserrat and colleagues (2008,

2012) experimentally demonstrated that stage structure of predatory mite species involved in reciprocal IGP may result in exclusion of one of the species.

Traditionally, the food web approach describes the occurrence of feeding links among species (Paine 1980), however, this becomes arbitrary when different stages of the same species have different ecological roles with respect to a particular other species. Because the large majority of animal populations are stage-, age-, or size-structured (Werner 1988), we suggest that feeding links in food webs should be represented at levels lower than the species (Preston et al. 2012, 2014). Although scientists strive for generalizations and simplifications, and with good reasons, we should always be aware of the intrinsic complexity of natural ecosystems and interactions, driven by two inherent features of nature: change and chance (Botkin 1990; Meyer 1993). Our results add to previous evidence of the importance of accounting for the stage structure of populations to reach reliable predictions of food web patterns and processes and of community structure and dynamics. There is much to understand about the possible consequences of ontogenetic changes for species interactions and we therefore suggest that theory and experiments should focus on this.

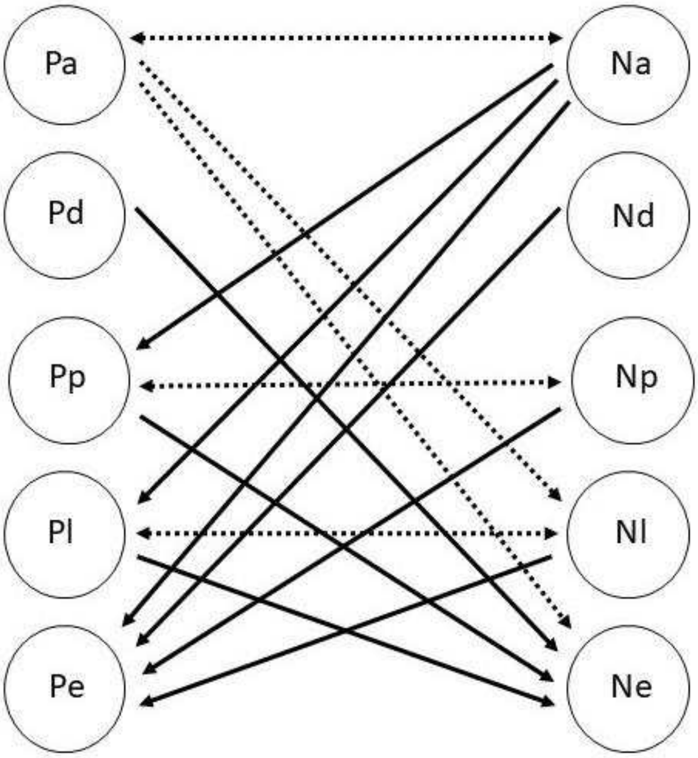

Figure 7. Intraguild predation among various stages of the predatory mites *Phytoseiulus macropilis* and *Neoseiulus californicus*. The capital letters in the circles indicate the predator species ("P" for *P. macropilis* and "N" for *N. californicus*) and the normal type signifies the ontogenetic stages ("a" for adults, "d" for deutonymphs, "p" for protonymphs, "l" for larvae and "e" for eggs). Arrows point from attacker to victim. The solid lines indicate the occurrence of intraguild predation and interrupted lines indicate its absence.

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GENERAL CONCLUSIONS

In this thesis, I took the first steps towards understanding the possible interactions between the predatory mites *P. macropilis* and *N. californicus*. The results showed that neither of the predators used volatiles to avoid prey patches occupied by the heterospecifics. Studies of odour-mediated avoidance are limited to a small number of species and thus, the mechanisms involved are not yet well understood. I proposed some possible mechanisms to explain this absence of avoidance, however, the assumptions remain to be experimentally verified (Chapter 1).

Because the two predators did not avoid patches with the other species, there is a distinct probability that they will end up on the same plant with prey, especially when used together for biological control. It is therefore important to investigate the interactions between individuals of these two species, such as resource competition and intraguild predation (IGP). Because there was no agreement on how to test for the possible occurrence of IGP, for natural enemies in general and for predatory mites in particular, I formulated guidelines on how to design appropriate experiments to determine whether or not two species may potentially engage in intraguild predation. The criteria are based on the condition that intraguild predators need to experience immediate energetic gains when killing and feeding on the intraguild prey. Thus, a relevant experimental design must quantify predation but also fitness benefits of feeding on the other species. The proposed criteria are easily generalized to other arthropod systems besides predatory mites (Chapter 2).

Based on these guidelines, I show that *P. macropilis* and *N. californicus* interact through reciprocal intraguild predation and that ontogeny of the two species clearly played a critical role in determining the occurrence of this interaction within our predator system. These results add to previous evidence of the importance of accounting for the stage structure of populations to reach reliable predictions of food web patterns and processes and of community structure and dynamics (Chapter 3).

In conclusion, to ascertain how *P. macropilis* and *N. californicus* affect each other's dynamics, as well as their subsequent effects on behaviour and population dynamics of the two-spotted spider mite, their shared prey, further study on the effects of intraguild predation between stages of these species is needed.