



**Alien predator meets a native prey: is adaption
to the opponent by experience possible?**

Master thesis

by

Jasmin Deimel B.Sc.

Supervised by Dr. Andreas Walzer and Ao.Univ.Prof. Dipl.-Ing.

Dr.nat.techn. Elisabeth Helene Koschier

Department of Plant Protection

University of Natural Resources and Life Sciences, Vienna

Vienna, 29th of May, 2020

Statutory declaration

I declare on my word of honor that I have prepared the present work independently, have used no aids other than those specified and have quoted all formulations and concepts taken from unprinted sources, printed literature or the Internet in their wording or essential content in accordance with the guidelines of scientific work and have indicated the exact source. This written work has not yet been submitted anywhere.

Date, Place

Jasmin DEIMEL

Abstract

The alien predatory mite *Amblydromaus limonicus* is used as biocontrol agent against greenhouse thrips species in Austria. It cannot be ruled out that *A. limonicus* is able to overwinter in the field in mild winter seasons because of climate change. In such cases, *A. limonicus* may invade native predatory mite communities. However, it is largely unknown how native predatory mite species respond to an unknown alien predator. Thus, the defensive behavior of the native predatory mite *Kampimodromus aberrans* and the predation behavior of the alien predatory mite *A. limonicus* was tested, when they meet each other for the first time. In a second trial, females of both species, which had experience with their opponents were used as study objects. All tested gravid females (*K. aberrans* or *A. limonicus*) had to choose between a bean leaf with or without cues of the opponent, whereby pollen and spider mite eggs were additionally available. The prey patches with predator cues contained also four predator eggs. The prey patch selection and the oviposition site selection of the predatory mite species were evaluated in regular intervals and the intraguild predation rates were recorded after 24h and 48h.

In experiment 1, the *K. aberrans* females clearly preferred to stay in prey patches without cues of the alien predatory mite species and also deposited more eggs in these prey patches. Additionally, intraguild predation on eggs of *A. limonicus* was negligible in *K. aberrans*. Contrary, the alien predator *A. limonicus* laid more eggs in prey patches with *K. aberrans* cues and consumed eggs of *K. aberrans*. In experiment 2, the behavior of the alien *A. limonicus* females was not changed by experience. However, experience strongly affected the behavior of the native females of *K. aberrans*. Experienced *K. aberrans* females showed no prey patch preference for oviposition, but strong intraguild predation on the *A. limonicus* eggs.

Our results revealed that alien predator females might associate cues from the native species with additional food independent of experience. Contrary, *K. aberrans* females associated the unknown IG predator cues with prospective predation risk and avoid oviposition in such prey patches. *Kampimodromus aberrans* females experienced with the cues of the alien predator displayed a more effective strategy: they did not leave the prey patch, but reduced the number of IG predator eggs by killing them. Thus, the behavioral responses of the native species *K. aberrans* to the alien predator *A. limonicus* may allow the co-existence of the two species in the case of the establishment of *A. limonicus* in the field. However, this assumption has to be tested at population level both under controlled and natural conditions.

Zusammenfassung

Die exotische Raubmilbe *Amblydromalus limonicus* wird in Österreich zur Thrips-Bekämpfung in Glashäusern eingesetzt. Es kann nicht ausgeschlossen werden, dass in milden Wintern, bedingt durch den Klimawandel, der exotische Räuber gelegentlich im Freiland überwintern kann. In solchen Fällen könnte *A. limonicus* in einheimische Raubmilben-Gesellschaften einwandern. Wie aber einheimische Raubmilben auf einen unbekanntem Räuber reagieren, ist unbekannt. Daher wurde das Abwehrverhalten der in Österreich beheimateten Raubmilbe *Kampimodromus aberrans* und der exotischen Raubmilbe *A. limonicus* getestet, als diese zum ersten Mal aufeinandertrafen. In einem zweiten Versuch wurde überprüft, ob sich das Verhalten der Weibchen mit Erfahrung ändert, wenn diese mit den Duftstoffen der jeweils anderen Art konfrontiert werden. Alle getesteten, fruchtbaren Weibchen mussten sich innerhalb von 48h zwischen zwei Buschbohnenblättern, eines mit und das andere ohne Duftstoffe und Eiern der anderen Art, entscheiden. Zusätzlich standen Pollen und Spinnmilbeneier als Nahrung zur Verfügung. Es wurde die Eiablage-Präferenz der Weibchen erhoben und die Anzahl der konsumierten Raubmilben-Eier nach 24 und 48 Stunden aufgezeichnet.

Im Experiment 1 bevorzugten die *K. aberrans*-Weibchen die Buschbohnenblätter ohne den Duftstoffen der unbekanntem Raubmilbenart zur Eiablage. *Kampimodromus aberrans* konsumierte auch keine Eier der exotischen Art. Die Weibchen der exotischen Art hingegen legten deutlich mehr Eier in Nahrungsplätze mit den Duftspuren und Eiern der einheimischen Art, welche auch konsumiert wurden. Im Experiment 2 zeigten die erfahrenen *A. limonicus*-Weibchen ein ähnliches Verhalten wie die unerfahrenen Weibchen im ersten Experiment. Im Gegensatz dazu zeigten die erfahrenen *K. aberrans*-Weibchen keine Eiablage-Präferenz, sondern konsumierten die Eier der exotischen Raubmilbenart.

Die Resultate zeigten, dass die exotische Raubmilbenart *A. limonicus* Duftstoffe anderer Raubmilbenarten mit Nahrung assoziierte. Im Gegensatz dazu stufte die einheimische Raubmilbe *K. aberrans* Duftstoffe unbekannter Raubmilben als Gefahr für ihre Nachkommen ein und vermeidet die Eiablage in solchen Nahrungsplätzen. *Kampimodromus aberrans* Weibchen, die mit den Duftstoffen der exotischen Raubmilbe vertraut waren, zeigten eine deutlich effizientere Verteidigungsstrategie: Sie verließen nicht die Nahrungsplätze mit den Duftspuren und Eiern der exotischen Art *A. limonicus*, sondern reduzierten das Prädationsrisiko ihrer Nachkommen durch Konsumation der exotischen Raubmilbeneier. Diese Abwehrstrategien könnten die Ko-Existenz der exotischen und der einheimischen Raubmilbenart gewährleisten. Es sind aber noch Populationsexperimente unter kontrollierten und natürlichen Bedingungen notwendig, um diese Annahme zu verifizieren.

Table of Contents

1	Introduction.....	7
1.1	Intraguild predation	7
1.2	Defense mechanisms to avoid IGP	8
1.3	Study organisms	8
1.3.1	Amblydromalus limonicus	9
1.3.2	Kampimodromus aberrans.....	11
1.4	Study objectives	13
2	Materials and methods	14
2.1	Species origin and rearing arenas	14
2.2	Experimental units and pre-experimental processes.....	15
2.3	Oviposition site selection of naïve females.....	16
2.3.1	Generating gravid <i>K. aberrans</i> and <i>A. limonicus</i> females.....	16
2.4	Oviposition site selection of experienced females.....	17
2.5	Pre-experimental procedure	17
2.5.1	Generating experienced and naïve gravid females.....	17
2.6	Statistical analysis and data presentation.....	18
3	Results	19
3.1	Experiment 1: Oviposition site selection of <i>K. aberrans</i> & <i>A. limonicus</i> females in the presence of IG predator cues.....	19
3.1.1	Prey patch selection	19
3.1.2	Total egg production and oviposition site selection	19
3.1.3	Extra- and intraguild predation	20
3.2	Experiment 2: Oviposition site selection of naïve and experienced <i>K. aberrans</i> & <i>A. limonicus</i> females in the presence of IG predator cues	22
3.2.1	Prey patch selection	22
3.2.2	Total egg production and oviposition site selection	23
3.2.3	Extra- and intraguild predation	24

4	Discussion	26
4.1	<i>Kampimodromus aberrans</i> & <i>A. limonicus</i> females in the presence of IG predator cues	26
4.2	Learning effects on the behavior of <i>K. aberrans</i> & <i>A. limonicus</i>	28
5	Conclusions and Outlook.....	30
6	References.....	31
7	List of figures	36

1 Introduction

Whenever an alien predator migrates in a native community for the first time, then it encounters an unknown prey, which is conversely confronted with an unknown predator. The long-term outcome of this interaction essentially depends on the one hand on the predator's adaptability to overwhelm, kill and consume the unknown prey and otherwise on the anti-predator behavior of its opponent. A special type of predation occurs, when predators kill each other, termed intraguild predation (Polis et al., 1989). Such interactions between alien and native predators can result in displacement of the inferior predator or to coexistence between the alien and native predator (Polis et al., 1989).

1.1 Intraguild predation

Root (1967) defined a guild as a group of species that exploit the same class of environmental resources in a similar way. In relation to a predator guild, the prey [termed as extraguild (EG) prey] is usually the shared resource of the guild members, which are consequently food competitors. Intraguild predation (IGP) means then the killing of heterospecific food competitors belonging to the same guild, whereas the young and small immature stages (eggs, larvae) are the most endangered stages being killed by intraguild (IG) predators (Polis et al., 1989). This type of predation is widespread among terrestrial and aquatic arthropod guilds including scorpions, spiders, predatory mites, beetles, dragonflies and ants (Polis et al., 1989). The killing of IG prey offers several advantages for the IG predator: (1) It will increase the survival rate of their offspring because of the killing of a potential offspring predator; (2) the elimination of native food competitor increases the availability of the shared EG prey and (3) the consumption of IG prey may provide the IG predator with high-quality food (Polis et al., 1989; Walzer and Schausberger, 2011). The direction and strength of IGP is mainly dependent on the diet specialization and IG predator size ratios. Diet-generalists are usually the more aggressive IG predators compared to diet-specialists. Larger IG predators have benefits in overwhelming smaller IG prey. Consequently, young and small juveniles are the preferred IG prey overwhelmed and killed by the larger adult IG predators (Walzer et al., 2006). In asymmetric IGP, one species is always the IG predator, whereas both predator species prey reciprocally on each other in symmetric IGP (Polis et al., 1989; Walzer and Schausberger, 2011).

There are several studies dealing with IGP among predatory mite species, which have resulted in the displacement or co-existence of the involved predatory mites. For example, the two predatory mite species *Neoseiulus neobaraki* and *Neoseiulus paspalivorus* co-occur on palm trees in Benin and Tanzania sharing the coconut mite *Aceria guerreronis*. Both predators are diet-generalists. The larger and more aggressive *N. neobaraki* could be able to suppress the smaller *N. paspalivorus*, but usually

the two species are spatially separated. Additionally, the smaller *N. paspalivorus* is numerically dominant in Benin over *N. neobaraki* and therefore runs little risk of suffering from strong IGP (Negloh et al., 2012).

In Austria, the three most important predatory mites occurring in orchards and vineyards (*Euseius finlandicus*, *Kampimodromus aberrans* and *Typhlodromus pyri*) also showed significant differences in the food competition among each other. The very fast moving predatory mite females of *E. finlandicus* strongly feed on the immature stages of the two other predatory mites, especially in periods of food shortage, whereas *K. aberrans* and *T. pyri* females are weak IG predators (Schausberger, 1997). However, *K. aberrans* prefers hairy leaves in contrast to *E. finlandicus*, which results in reduced encounters between the two species and consequently lowers the IGP events (Schausberger, 1997).

1.2 Defense mechanisms to avoid IGP

Whenever several IG predators share the same habitat, one or more of these species run risk of being displaced by the superior IG predator. The most common strategy in arthropods is to minimize the IGP risk is to avoid prey patches occupied by other IG predators. Such IG predator free patches often contain less EG prey resulting in high costs for the prey (Morosinotto, 2012; Scheuerlein et al., 2001). Therefore, it is important that the IG prey correctly assesses the danger posed by the IG predator. Only if the IG predator actually represents a danger, it makes sense for the IG prey to invest in this strategy (Venzon et al., 2000). Direct (eggs, feces and traces from the predator) and indirect cues (alarm pheromones of IG prey or EG prey) of the IG predator may inform the IG prey about the IGP risk (Morosinotto, 2012; Venzon et al., 2000).

Furthermore, it is well known in predatory mite guilds that mothers choose prey patches without potential IG predators for depositing their eggs, because small, young larvae are the most endangered IG prey stages (Walzer et al., 2006; Walzer and Schausberger, 2011; 2012). For example, the predatory mite species *Iphiseius degenerans* (Berlese) does not deposit its eggs in prey patches with cues from thrips larvae (IG predator on the eggs of *Iphiseius degenerans*), but also avoids oviposition in areas occupied by the IG predator *N. cucumeris* (IG predator on larvae of *Iphiseius degenerans*) (Choh et al., 2015). Additionally, predatory mite females can also assess the IG predation risk (high-risk or low-risk predator) and adjust their response to the level of IG predation risk. Such sophisticated strategies are often optimized by learning of the IG prey (Walzer and Schausberger, 2011). However, it is largely unknown, if a native IG prey also responds by an unknown alien IG predator.

1.3 Study organisms

Today, predaceous mites (Acari: Phytoseiidae) are well explored because of their manifold functions as important biological control agents for whiteflies, thrips and phytophagous mites for vegetable

production in greenhouse (Knapp et al., 2013). All over the world, more than 25 species of predaceous mites are commercially produced and sold as biocontrol agents for biological pest control (Chorąży et al., 2016). It is well known that alien beneficial insects often have several advantages compared to the native species: higher reproduction rates, larger body size, a more aggressive predatory behavior and a wider food range (Zenni and Nuñez, 2013). Additionally, alien pest species can be often controlled effectively only by alien species. All these advantages over native species make the use of alien beneficial insects for pest control so valuable. The prevailing climatic conditions, especially the low temperatures during winter periods, dramatically reduce the abilities of alien species to establish permanently in new habitats (Blackburn et al., 2011). However, if climatic conditions change in the course of global warming, alien beneficial insects may be able to permanently overcome this abiotic barrier of the native ecosystem, which is considered to be one of the strongest forces to prevent invasions of another ecosystem (Ward and Masters, 2007).

1.3.1 Amblydromalus limonicus

Garman and McGregor described the predatory mite *Amblydromalus limonicus* (Acari: Phytoseiidae) (Synonym: *Amblyseius limonicus*) for the first time and found some individuals on citrus- and orange-trees in California (Chant and McMurtry, 2005). In Europe, researcher started evaluations of *A. limonicus* as biocontrol agent in protected cultivation after the massive invasion of the western flower thrips *Frankliniella occidentalis* in the 1990s to control this pest in a natural way (van Houten et al., 1995). Since 2015, the alien predatory mite species *A. limonicus* is permitted (commercially available) in Austria as a biocontrol agent against thrips larvae in greenhouses (Walzer et al., 2017b). *Amblydromalus limonicus* has two advantages against other thrips predators from the family Phytoseiidae. First, *A. limonicus* is a very aggressive predator, which can also overwhelm the second instar larva of *F. occidentalis*. Second, it is a relative cold-tolerant species allowing its use as thrips predator in early spring (Knapp et al., 2013; Walzer et al., 2016). However, *A. limonicus* may survive in eastern Austria in the field because of climate warming (Walzer et al., 2016; Walzer et al., 2017b). In such a case *A. limonicus* may be able to invade native predatory mite communities and may locally displace some native species (Walzer et al., 2016). Additionally, mild winter periods may allow the occasional overwintering of *A. limonicus*, because it is a relatively cold-tolerant predatory mite species. Then *A. limonicus* has an advantage over native predatory mite species, because *A. limonicus* can begin reproduction in early spring in contrast to native species (Walzer et al., 2017a).

1.3.1.1 Natural occurrence and use as biological control agent

The food generalist *A. limonicus* naturally occurs in Australia, New Zealand, South, Central and North America and Africa. In Europe, the first specimens of *A. limonicus* (Garman and McGregor) were found

on tomato plants and apple trees in Northeastern Spain in the year 2011, where they are considered as alien species (Chorąży et al., 2016). Within Spain, *A. limonicus* was exclusively seen in Barcelona and Girona provinces in the Northeastern of Spain, where *A. limonicus* is now permanently established in native predatory mite guilds. It is still unknown, how *A. limonicus* was introduced to Spain (Chorąży et al., 2016). The natural habitats of *A. limonicus* are restricted to areas located near to the sea, to get sufficient relative humidity for egg hatching and also moderate high temperatures are necessary for juvenile development and egg production (McMurtry and Scriven, 1965). Furthermore, *A. limonicus* cannot enter diapause, which makes it impossible to establish permanently in temperate climate zones (Dittmann et al., 2016). The fact that *A. limonicus* is the only predatory mite species that can successfully kill the second very well-fortified larval stage of *F. occidentalis*, makes it particularly popular for use in greenhouses for biological thrips control (Knapp et al., 2013). Although *A. limonicus* feeds on spider mites they avoid spider mite webbings, which hinder their movement (McMurtry and Scriven, 1965).

Control of phytopagous mites other than spider mites: First, *A. limonicus* was used as biological control agent (BCA) against phytophagous mites against the avocado brown mite, *Oligonychus punicae* in California, where it successfully reduced the pest infestation (McMurtry and Scriven, 1971). Contrary, using *A. limonicus* in the field did not have a significant effect on the population of *O. punicae* (McMurtry et al., 1984).

Control of thrips: van Houten et al., (1995) firstly tested *A. limonicus* as BCA against the first instar *F. occidentalis* larvae. Results regarding to the predation rate were highest at *A. limonicus* compared to other predatory mite species. Thrips infested cucumber plants treated with *A. limonicus* were pest-free after 9 weeks of its release (van Houten et al., 1995).

Control of whiteflies: Populations of the whitefly species *Trialeurodes vaporariorum* can be successfully controlled by *A. limonicus* in greenhouse for cucumber, strawberry and rose production (Hoogerbrugge et al., 2011; Knapp et al., 2013).

1.3.1.2 Food range

Amblydromalus limonicus is known to feed on the first two larval stages of thrips (e.g *Frankliniella occidentalis*, *Thrips tabaci*), eggs of moths (*Prays citri*, *Ephesttia kuehniella*), whiteflies (*Bemisia tabaci*, *Trialeurodes vaporariorum*), spider mites (*Oligonychus punicae*, *Panonychus citri*, *Eutetranychus orientalis*, *Tetranychus cinnabarinus*, *Tetranychus urticae*), eggs of the Mediterranean fruit fly *Ceratitis capitata*, pollen and extrafloral nectar of different plant species and spores of fungi (Knapp et al., 2013; McMurtry and Scriven, 1965; Sengonca and Drescher, 2001; van Houten et al., 1995; Vangansbeke et al., 2014).

1.3.1.3 Development and reproduction

Vangansbeke et al., (2014) found out that larvae of *A. limonicus* did not develop to protonymphs without having any additional food except water. Therefore, *A. limonicus* larvae are obligatory feeding stages, because they strongly need food for their further development to protonymphs. Under laboratory conditions, the development time of *A. limonicus* from egg to total immature stage lasts on average 7.88 ± 0.16 days (females) and 6.89 ± 0.21 days (males) (23 ± 1 °C, $65 \pm 5\%$ RH and a photoperiod of 16 L:8 D), when fed with *T. latifolia* pollen (Vangansbeke et al., 2014). The highest oviposition rates of *A. limonicus* females are observed when preying on *E. kuehniella* eggs (3 ± 0.08 eggs per day), but this diet is very expensive and therefore not practicable for mass-rearing. Females fed with *T. latifolia* pollen have an oviposition rate of 1.80 ± 0.10 eggs per day (Vangansbeke et al., 2014).

1.3.2 Kampimodromus aberrans

The native predatory mite species *K. aberrans* controls phytophagous mites in Austrian vineyards and apple orchards (Kasap, 2005). In contrast to *E. finlandicus*, which is another dominant predatory mite species in Austria, *K. aberrans* is much less aggressive towards other predatory mite species (Schausberger, 1997; Schausberger and Croft, 2000). Additionally, it is a small, slowly moving predatory mite and it is suspected that *K. aberrans* is more likely to be suppressed by an alien predatory mite species as a result of IGP.

In Europe, the generalist predatory mite *K. aberrans* (Oudemans) is a very important phytoseiid species (beneficial animal) naturally occurring in apple orchards, vineyards and on hazelnut (Duso et al., 2009; Kasap, 2005; McMurtry et al., 2013). *Kampimodromus aberrans* can coexist with other phytoseiid species (e.g *Amblyseius andersoni*, *Typhlodromus pyri*, *Phytoseius finitimus*) in the same habitat, although it is confronted with IGP (Duso et al., 2009). In North-Italian vineyards, specimens of *K. aberrans* are commonly used single or in combination with *T. pyri* and *A. andersoni* to function as biocontrol agents against spider mites (Ahmad et al., 2015). In Turkey, *K. aberrans* is a very useful phytoseiid of tetranychids in apple orchards in the Van region (Kasap, 2004).

In Austria, *Kampimodromus aberrans* and *Euseius finlandicus* are the two most abundant predatory mite species found on fruit trees, which partly live in the same habitat and having overlapping food sources. Whenever there is a limitation of food they can compete for food and kill each other. In presence of *E. finlandicus*, *K. aberrans* is only able to establish on plants with strongly pubescent leaves. Therefore, the presence of hairy leaves on certain apple varieties is an important regulatory factor in limiting the consequences of IGP for *K. aberrans* (Seelmann, 2005).

1.3.2.1 Food range

The generalist predatory mite *K. aberrans* can prey on phytophagous mites like *T. urticae*, *Phytoptus avellanae* and *Cecidophyopsis vermiformis* in addition to pollen as nutritious food (Duso et al., 2009; Kasap and Atlihan, 2011; Kasap, 2005).

1.3.2.2 Development and Fertility rate

Under laboratory conditions, the development time of *K. aberrans* females from egg to total immature stage lasts on average 9.27 ± 0.11 days (22 ± 1 °C, 60-80 % RH and a photoperiod of 16 L:8 D), when fed with *T. latifolia* pollen. The development period of *K. aberrans* males is shorter (7.15 ± 0.07 days) (24 C, 70 ± 10 % R.H., 16 L: 8 D) (Broufas et al., 2007; Lorenzon et al., 2012). The fastest development of *K. aberrans* females was observed at 30 °C: 5.66 ± 0.08 days (Broufas et al., 2007). *Kampimodromus aberrans* females are able to deposit the highest egg number, when fed with pollen of *T. latifolia* with an average of 0.34 ± 0.03 eggs per female per day (24 C, 70 ± 10 % R.H., 16 L: 8 D) (Lorenzon et al., 2012).

1.4 Study objectives

In this study, the oviposition behavior and prey patch preference of the native Austrian predatory mite species *K. aberrans* and the alien predatory mite species *A. limonicus* was examined, when having the choice between prey patches with and without IG predator cues (eggs and female traces). Second, we examined, if experience with the IG predator changes the behavior of the native species *K. aberrans* and alien species *A. limonicus*.

The specific questions in detail are the following:

Experiment 1

- Do *K. aberrans* females prefer prey patches without alien IG predator cues of *A. limonicus* for oviposition to increase the survival rates of their offspring? Do *K. aberrans* females kill heterospecific *A. limonicus* eggs to minimize potential threat for their offspring?
- Do alien *A. limonicus* females behave as aggressive predators by choosing the prey patches with more potential prey including IG prey for oviposition? Do *A. limonicus* females kill IG prey to minimize the number of potential food competitors?

Experiment 2

- Does experience with the counterpart affect the oviposition site selection and IGP behavior of the native and alien species *K. aberrans* and *A. limonicus* compared to the naïve relatives?

2 Materials and methods

2.1 Species origin and rearing arenas

Specimens of the alien predatory mite *A. limonicus* were sampled 2014 in La Tallada d'Emporda (42.0541° N, 3.0614° E), near Girona, Spain from leaves of apple trees, whereas the native predatory mite species *K. aberrans* were collected 2016 from leaves of hazelnut bushes in the Türkenschanz park (48.20849° N, 16.37208° E), Vienna, Austria. The predatory mites were reared on detached bean leaves (native *K. aberrans*) or plastic tiles (alien species *A. limonicus*) placed on water-saturated foam cubes in plastic boxes (20 x 20 x 6 cm) half-filled with water. They were provided with pollen of narrow-leaved cattail *Typha angustifolia* (Nutrimite, Biobest N.V., Westerlo, Belgium) and spider mites (*A. limonicus*: alive spider mites; *K. aberrans*: frozen spider mites) in 2-3 day intervals. Water-saturated cellulose strips (5 mm height) at the margins of the leaves or tiles served as water supply for the mites and confined the arena. A barrier of fruit tree grease (Fruit Tree Grease, Vitax Ltd, UK) along the margins of the arenas hindered the mites from escaping. Cotton wool strands under a cover glass served as shelter and oviposition site for the mites. The two-spotted spider mite *Tetranychus urticae*, used as prey for the predatory mites, was reared on bean plants *Phaseolus vulgaris* ("Stromboli", Austroaat) at $23 \pm 2^\circ\text{C}$, $60 \pm 10\%$ relative humidity and 16:8 h L: D. Additionally, clean bean plants separated from infested bean plants, were reared in pots under room conditions at $22 \pm 2^\circ\text{C}$, $45 \pm 10\%$ relative humidity, and a photoperiod of 16:8 h L: D. The leaflets of these bean plants were used as prey patches in the experiments.

2.2 Experimental units and pre-experimental processes

Single, clean and detached bean leaflets (5-6 cm²), placed on water-saturated foam cubes in plastic boxes, were used as prey patches for the predatory mites. Each leaflet was populated with ten gravid spider mite females, which deposited eggs over 24h. Then two gravid *A. limonicus* or *K. aberrans* females were placed on one half of the leaflets, which were allowed to prey on the spider mites and to deposit eggs for 24h. Then all spider mite and predatory mite females were removed from all leaflets and the number of spider mite eggs was adjusted to 40 in each prey patch. Additionally, a standardized amount of pollen (*Typha angustifolia*) was added on each prey patch. In that manner we created prey patches with identical food supply (spider mite eggs, pollen), but containing IG predator cues (female traces, predatory mite eggs, killed spider mites) or not. The deposited predatory mite eggs in the prey patches with IG predator cues were adjusted to 4 and the location of the eggs was marked by blue dots to alleviate the determination of IGP. Then two leaflets (one with IG predator cues) were placed upside down on a water-saturated foam cuboid in a plastic box (20 x 10 x 6 cm) half-filled with water. They were connected by a wax-bridge.

The complete testing arena was bordered with an adhesive (Fruit Tree Grease, Vitax Ltd, UK) to prevent the mites from escaping (Fig. 1). All rearing arenas of the predatory mites and the experimental units were stored in a climate chambers at 25 ± 1 °C, 60 ± 5 % relative humidity and a photoperiod of 16:8 h L: D.

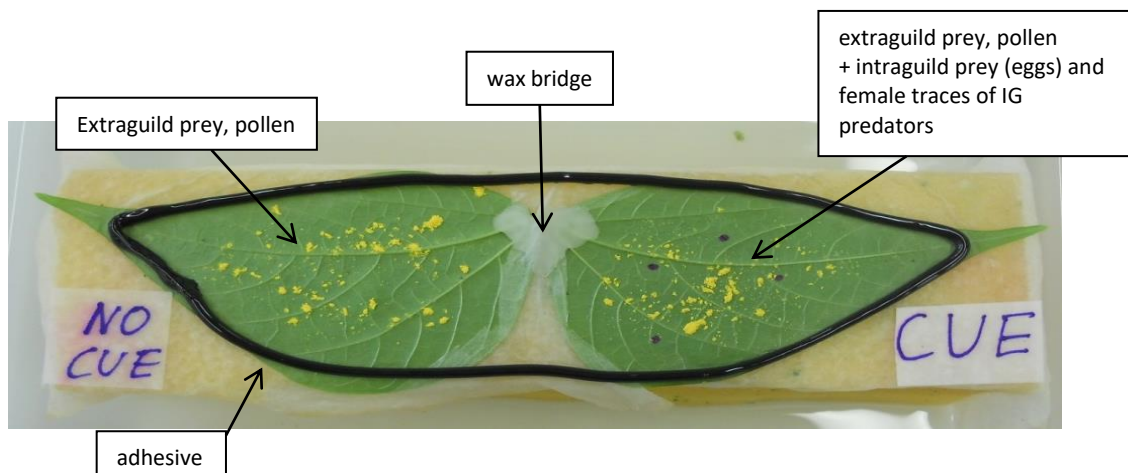


Fig. 1: Experimental unit for a single gravid female having the choice between a prey patch without carrying heterospecific predator cues (NO CUE) and a prey patch carrying heterospecific predator cues (CUE) for oviposition and prey patch selection.

To adjust the saturation level of the predators, each female was stored in an acrylic cage covered with cover glass without food for three to four hours before start of the experiment (Fig. 2). Each acrylic cage (Schausberger, 1997) consisted of an acrylic plate (8 x 3.5 x 0.3 cm) with two cylindrical circular chambers in it (\varnothing 1.5 cm, 0.3 cm high) closed by a fine nylon mesh at the bottom. To minimize the stress level, water was provided through a moist stripe of filter paper fixed on the backside of the acrylic cage, reaching into a plastic box filled with water.

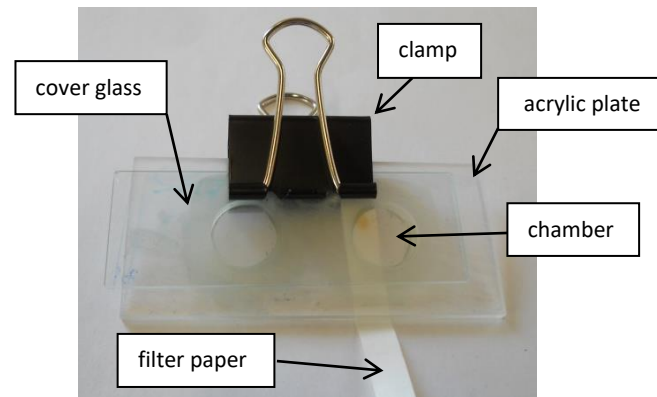


Fig. 2: Each gravid test-female was stored in one of these acrylic cages for 3-4h before the start of the experiment.

2.3 Oviposition site selection of naïve females

Single, naïve, even aged and gravid females of *K. aberrans* or *A. limonicus* were placed in the middle of the wax-bridge and the position of the females was checked nine times [immediately after release (first choice) and then after 1, 2, 3, 4, 5, 6, 24 and 48h]. After 24 and 48 hours, the amount of deposited eggs in prey patches with IG predator cues and without IG predator cues, IGP (consumption of heterospecific predatory mite eggs) and the number of consumed spider mite eggs were recorded. 38 to 40 replicates were conducted per species.

2.3.1 Generating gravid *K. aberrans* and *A. limonicus* females

Nymphs, randomly collected with a fine moistened brush from stock arena, were reared on detached bean leave arenas for seven days (*K. aberrans*) or on plastic tiles for four days (*A. limonicus*) and fed with pollen. For *K. aberrans* nymphs, detached bean leaves upside down (8-9 cm²) were placed on water-saturated foam cubes (6 x 6 x 5 cm) covered with filter paper and stored in a plastic box (10 x 10 x 6 cm) half filled with water. The margins of the leaves were surrounded by moist tissue material as water source for the mites. At the end, the arena was bordered with an adhesive (Fruit Tree Grease, Vitax Ltd, UK) to function as barrier for the mites. As shelter for the mites, cotton wool fibers was put

onto the bean leave covered with cover glass. For *A. limonicus* nymphs, arenas had same set-up as used for *K. aberrans* nymphs but acrylic plates (6 x 6 x 0.3 cm) were placed on water-saturated foam cubes (6 x 6 x 5 cm) instead of detached bean leaves. The arenas were stored in a climate chamber at 25 ± 1 °C, 60 ± 5 % relative humidity and a photoperiod of 16:8 h L: D. When nymphs reached adulthood, females of each species were isolated and provided with a single conspecific male and again reared on detached bean leave arenas for seven days (*K. aberrans*) or on plastic tiles for five days (*A. limonicus*). Pollen and spider mites serves as food resource. Only females, which deposited at least one egg during time of isolation, were used in the experiments.

2.4 Oviposition site selection of experienced females

Here we used IG-predator experienced and IG-predator naïve females as test individuals. These females of *K. aberrans* and *A. limonicus* were subjected to the identical experimental treatments as described in 1.3. 35 to 48 replicates per treatment were conducted.

2.5 Pre-experimental procedure

2.5.1 Generating experienced and naïve gravid females

For the second experiment, gravid females of *K. aberrans* and *A. limonicus* were generated in the same way as described in 1.3.1. These females of each species were split in two cohorts and confronted with IG predator cues or with clean air/draft caused by a laboratory vacuum pump (KNF LABOPORT N86 KN.18) over 24 hours before start of the experiment using the following experimental setup: Two rectangular plastic boxes (20 x 10 x 6 cm) with small holes (\varnothing : 5.2mm) on both short sides were parallel located to each other. Blue small plastic tubes were inserted in each hole with their smaller end pointing outside of the boxes. These ends outside the boxes were connected on both box sides with elastic hoses, which were in contact with the arms of y-shaped plastic connectors. The base arm of one connector was serially linked via a hose with an air-flowmeter and a vacuum pump. The base arm of the other y-shaped connector was linked with a plastic tube filled with activated charcoal. Each box was subdivided in two similarly sized compartments by a plastic plate with small holes allowing an air circulation between the two compartments. The prey patches consisted of detached bean leaves placed upside down on water saturated foam cubes (9.5 x 9.5 x 5 cm) surrounded by an adhesive (Fruit Tree Grease, Vitax Ltd, UK). One plastic box contained two serial prey patches, whereas four IG predator females were placed on the prey patch in front and provided with 20 IG prey individuals (10 eggs and 10 nymphs). The prey patch behind contained the cohorts of 8-10 test females (the prospective predator-experienced females) provided with fresh pollen and frozen spider mites. In that manner the test females were exposed to cues of the IG predators and the killed IG prey, because the

direction of the air-flow was from the prey patch with the IG predators to the prey patch with the test females. The other box contained only the prey patch with cohorts of test females (the prospective predator-naïve females), which were exposed to clean air. Then both boxes were closed and the vacuum pump created an air-flow (2.5 l min^{-1} per box) from the plastic tube filled with activated charcoal to both boxes (Fig. 3). After 24h the predator-naïve and predator-experienced females were used in the choice experiments.

2.5.1.1 Experimental setup

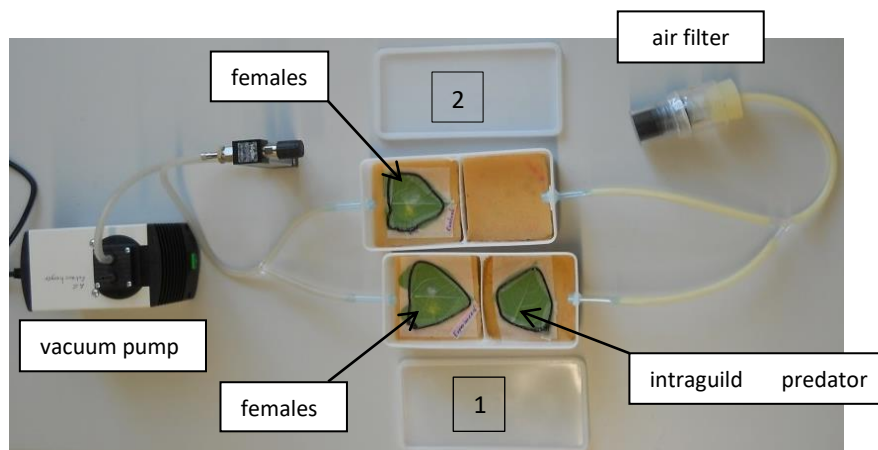


Fig. 3: Experimental construction for confronting females of *K. aberrans*/ *A. limonicus* with cues (1) from intraguild IG prey and IG predators and (2) from clean air. Females exposed to clean air were used as naïve females in the choice experiments, females exposed to IG prey and IG predator cues were used as experienced females.

2.6 Statistical analysis and data presentation

The statistic program IBM® SPSS® Statistics Version 24 (IBM Corp., USA) was used for data analyses of the two experiments. The illustrations of the results were created using the software SigmaPlot 11.0 Notebook (.JNB). Generalized Estimating Equations (GEE, autocorrelation structure among the observation points, pairwise post hoc comparisons by least significance tests) were used to analyze the effects of species and the interaction of species and time on the prey patch selection, oviposition site selection (binomial distribution, logit link function), total egg production, IG and EG predation (normal distribution, identity link function) in the first experiment. GEE's were also used in the same manner in the second experiment with a single difference. The effects of predator-experience and its interaction with time on the identical traits as in the first experiment were analyzed within each species separately. In the second experiment, all females which did not produce any eggs within the experimental period were excluded from analyses.

3 Results

3.1 Experiment 1: Oviposition site selection of *K. aberrans* & *A. limonicus* females in the presence of IG predator cues

3.1.1 Prey patch selection

The main factor species was marginally significant (GEE: Wald $X^2_1 = 2.791$, $P = 0.095$), however, the species effects were dependent on time (species*time: Wald $X^2_1 = 48.101$, $P < 0.001$). The majority of *K. aberrans* females were found in the prey patches without IG predator cues after 24h and 48h, whereas *A. limonicus* preferred the prey patches with IG predator cues (pairwise comparisons *K. aberrans* versus *A. limonicus*, 24h: $P = 0.014$, 48h: $P = 0.015$) (

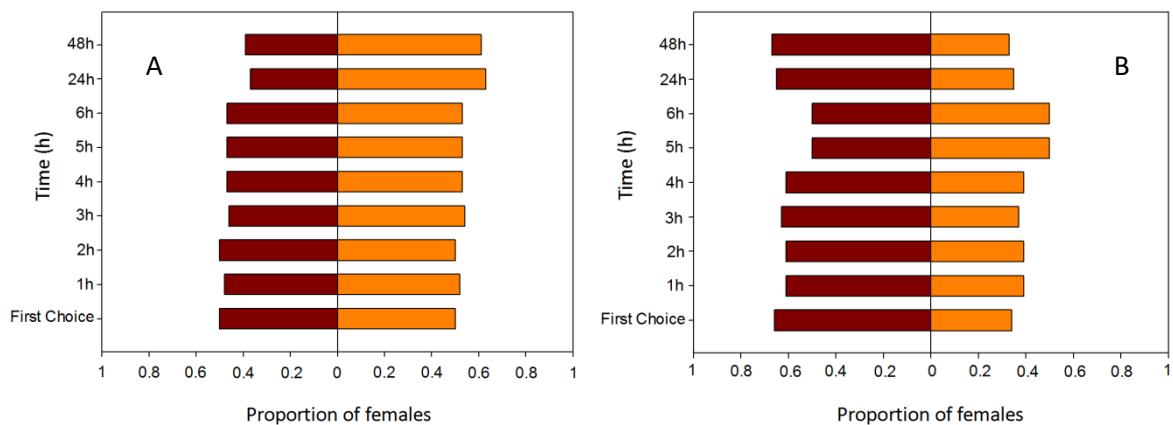


Fig. 4).

Fig. 4: Prey patch selection after first choice, and 1, 2, 3, 4, 5, 6, 24 and 48h by *K. aberrans* (A) and *A. limonicus* (B) females when having the choice between prey patches with (red bars) or without IG predator cues (orange bars)

3.1.2 Total egg production and oviposition site selection

The total egg production in both prey patches was significantly different between the species (GEE: Wald $X^2_1 = 26.795$, $P < 0.001$). Pooled over time, the oviposition rates of *A. limonicus* were higher than of *K. aberrans* (1.21 eggs/females $\pm 0.1SE$ versus 0.57 ± 0.08). The total oviposition rates, however, were also affected by the interaction of species and time (Wald $X^2_1 = 9.401$, $P = 0.009$). *Kampimodromus aberrans* females produced more eggs after 24h (0.76 ± 0.12) than after 48h (0.37 ± 0.12) (pairwise LSD test: $P = 0.011$), whereas the egg production of *A. limonicus* was constant over time (24h: 1.05 ± 0.12 , 48h: 1.37 ± 0.14 , $P = 0.084$).

The oviposition site selection of the females was marginally influenced by species (GEE: Wald $X^2_1 = 3.418$, $P = 0.064$), but significantly affected by the interaction of species and time (Wald $X^2_1 = 8.778$, $P = 0.012$). After 24h, the eggs of both species were evenly balanced between both prey patches (pairwise LSD-test: $P = 0.963$). After 48h, *K. aberrans* preferred prey patches without predator cues for egg deposition ($P = 0.007$), whereas the opposite trend in oviposition site selection was observed in *A. limonicus* ($P = 0.065$) (Fig. 5).

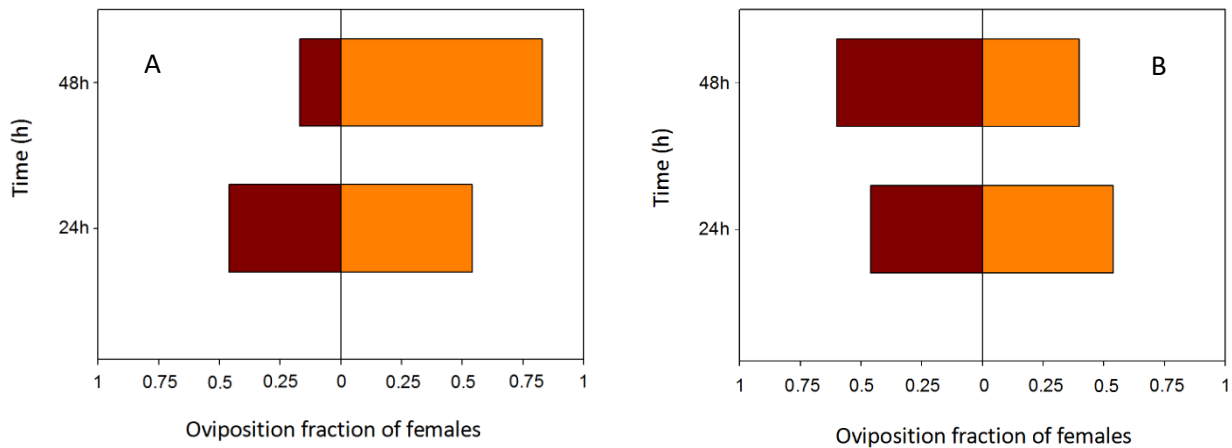


Fig. 5: Oviposition site selection of *K. aberrans* (A) and *A. limonicus* (B) females after 24h and 48h when having the choice between prey patches with (red bars) or without IG predator cues (orange bars).

3.1.3 Extra- and intraguild predation

The total extraguild predation rates (EGP) of the females on spider mite eggs differed between the species (GEE: $X^2_1 = 17.265$, $P < 0.001$) reflected in higher predation rates of *A. limonicus* compared to *K. aberrans* (17.81 eggs/females/day ± 1.09 SE versus 11.71 ± 0.97). Additionally, the interaction of species and time was also significant ($X^2_1 = 17.265$, $P < 0.001$). Both species consumed significantly more eggs on the first day (pairwise LSD-tests: $P < 0.001$) (Fig. 6).

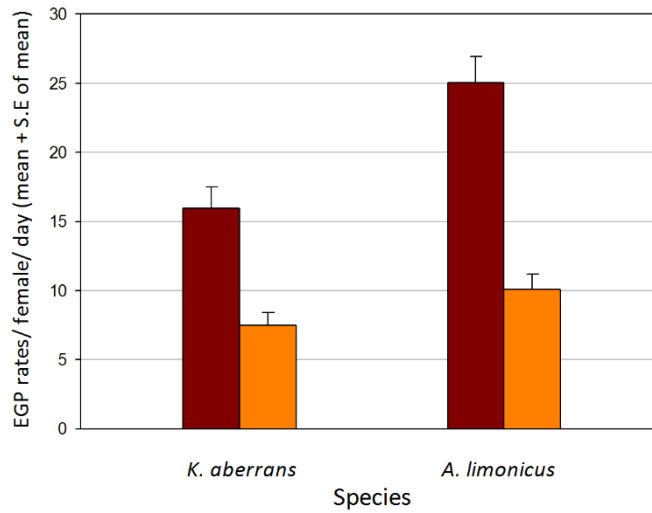


Fig. 6: Extraguild predation rates (EGP) in total (spider mite eggs) by *K. aberrans* and *A. limonicus* females after 24h (red bars) and 48h (orange bars).

The intraguild predation rates of the females significantly differed between the species ($X^2_1 = 580.857$, $P < 0.001$), but was also affected by the species*time interaction ($X^2_1 = 13.561$, $P < 0.001$). Pooled over time, *K. aberrans* females did not prey on *A. limonicus* eggs. Contrary, *A. limonicus* females preyed on *K. aberrans* eggs (1.41 eggs/female/day \pm 0.06 SE) and the IG predation rates of *A. limonicus* were higher on the first day ($P > 0.001$) (Fig. 7).

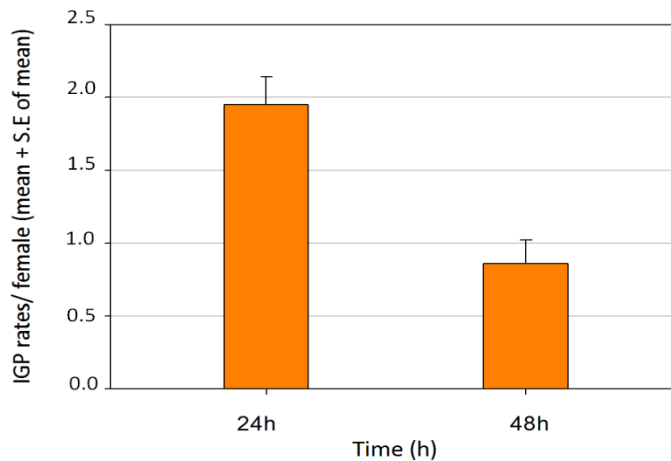


Fig. 7: Intraguild predation rates (IGP) of *A. limonicus* females on *K. aberrans* eggs after 24h and 48h.

3.2 Experiment 2: Oviposition site selection of naive and experienced *K. aberrans* & *A. limonicus* females in the presence of IG predator cues

3.2.1 Prey patch selection

Prey patch selection of *K. aberrans* and *A. limonicus* was not affected by experience (*K. aberrans*: $X^2_1 = 0.136$, $P = 0.713$; *A. limonicus*: $X^2_1 = 1.900$, $P = 0.168$) and the interaction between experience and time (*K. aberrans*: $X^2_1 = 12.205$, $P = 0.272$; *A. limonicus*: $X^2_1 = 16.150$, $P = 0.443$). In both species, the females were equally distributed between the prey patches with and without IG predator cues (Fig. 8, Fig. 9).

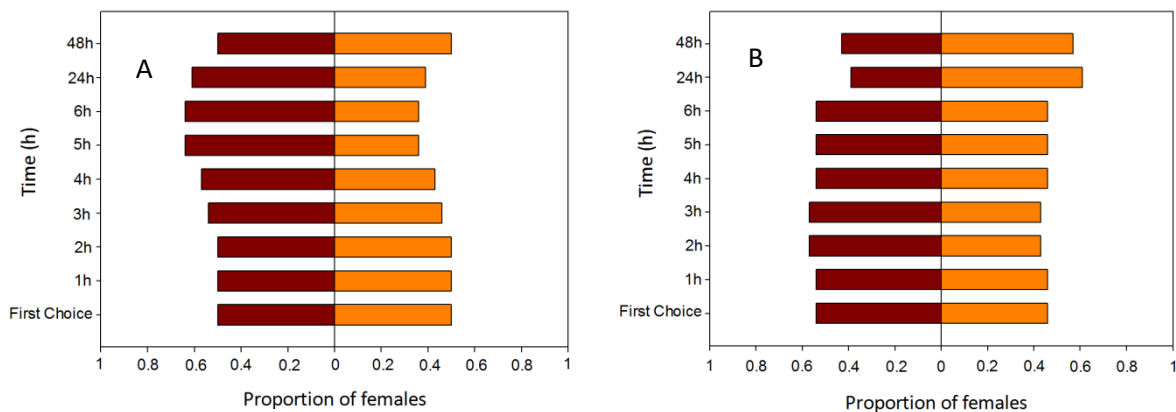


Fig. 8: Prey patch selection of experienced (A) and naïve (B) *K. aberrans* females given a choice between a prey patch with IG predator cues (red bars) or without IG predator cues (orange bars) after first choice, 1, 2, 3, 4, 5, 6, 24 and 48h.

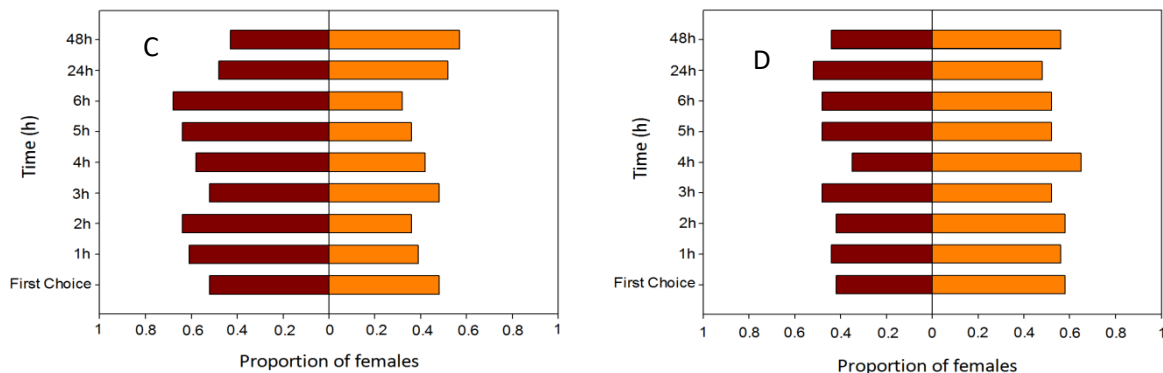


Fig. 9: Prey patch selection of experienced (C) and naïve (D) *A. limonicus* females given a choice between a prey patch with IG predator cues (red bars) or without IG predator cues (orange bars) after first choice, 1, 2, 3, 4, 5, 6, 24 and 48h.

3.2.2 Total egg production and oviposition site selection

The total egg production of *K. aberrans* and *A. limonicus* females was not influenced by experience (naïve versus experienced *K. aberrans*: $0.71 \text{ eggs}/24\text{h}/\text{female} \pm 0.06\text{SE}$ versus 0.75 ± 0.06 , $X^2_1 = 0.168$, $P = 0.682$; naïve versus experienced *A. limonicus*: 1.05 ± 0.09 versus 0.98 ± 0.09 , $X^2_1 = 0.329$, $P = 0.566$) and experience interacting with time (*K. aberrans*: $X^2_2 = 3.285$, $P = 0.193$; *A. limonicus*: $X^2_2 = 2.179$, $P = 0.336$).

The main factor experience (GEE: $X^2_1 = 0.423$, $P = 0.515$) and the interaction term experience * time (GEE: $X^2_2 = 2.309$, $P = 0.315$) had no significant influence on oviposition site selection of the *K. aberrans* females (Fig. 10).

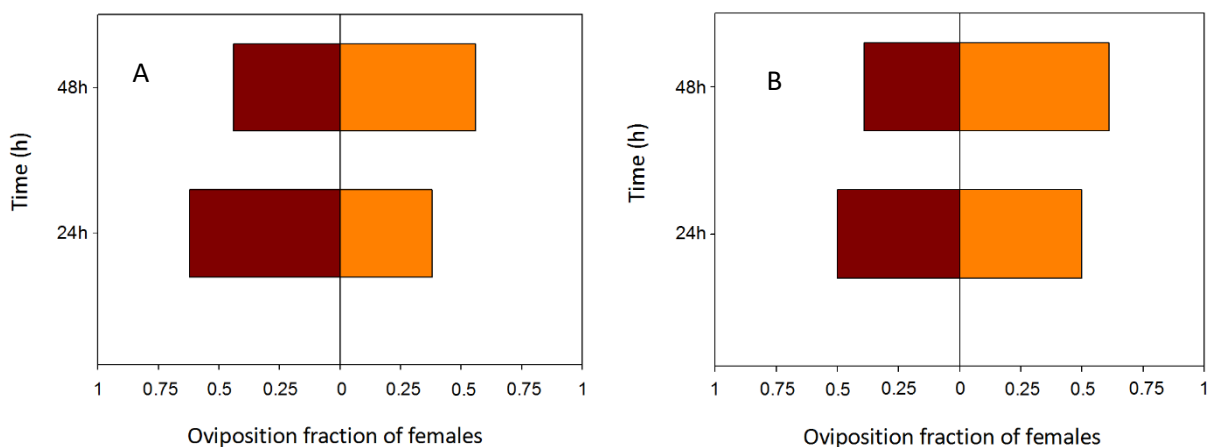


Fig. 10: Oviposition site selection of experienced (A) and naïve (B) *K. aberrans* females given a choice between a prey patch with heterospecific predator cues (red bars) or a prey patch without heterospecific predator cues (orange bars) after 24 and 48h.

Experience did not influence the oviposition site selection of *A. limonicus* females (GEE: Wald $X^2_1 = 0.003$; $P = 0.958$) but was marginally affected by experience * time (Wald $X^2_2 = 4.775$; $P = 0.092$). Both naïve and experienced females tended to deposit their eggs in prey patches with IG predator cues after 48h (pairwise LSD-tests, naïve females: $P = 0.092$; experienced females: $P = 0.127$) (Fig. 11).

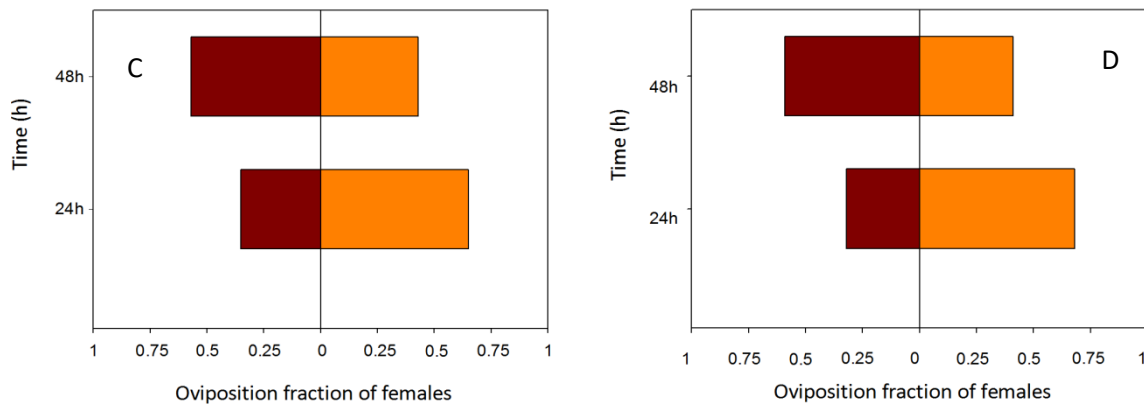


Fig. 11: Oviposition site selection of experienced (C) and control (D) *A. limonicus* females given a choice between a prey patch with heterospecific predator cues (red bars) or a prey patch without heterospecific predator cues (orange bars) after 24 and 48h.

3.2.3 Extra- and intraguild predation

The extra-guild predation rates (spider mite eggs) of *K. aberrans* and *A. limonicus* were not influenced by experience (*K. aberrans*: $X^2_1 = 0.816$, $P = 0.366$; *A. limonicus*: $X^2_1 = 0.084$, $P = 0.772$), but by experience interacting with time (*K. aberrans*: $X^2_2 = 17.772$, $P < 0.001$; *A. limonicus*: $X^2_2 = 21.110$, $P < 0.001$). Naive and experienced females of both species consumed more spider mite eggs on the first day compared to the second day (all pairwise LSD-tests: $P < 0.001$) (Fig. 12).

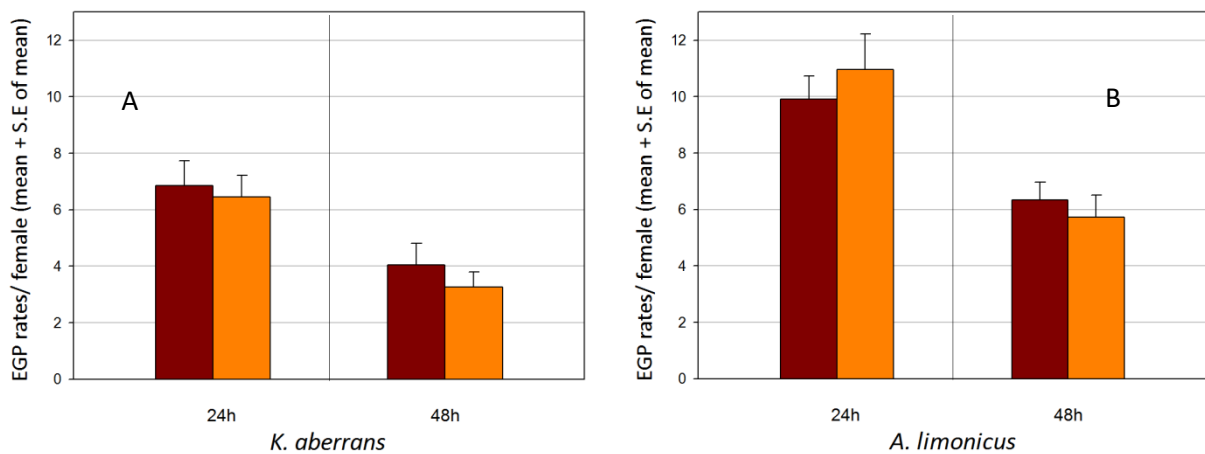


Fig. 12: Extraguild predation rates (EGP) in total (spider mite eggs) by *K. aberrans* (A) and *A. limonicus* (B) females (red bars= experienced; orange bars= naive) after 24 and 48h.

The intraguild predation (IGP) rates (eggs of *A. limonicus*) of *K. aberrans* were influenced by experience (GEE: Wald $X^2_1 = 31.664$; $P < 0.001$), but not by experience interacting with time (Wald $X^2_2 = 2.154$; $P = 0.341$). Irrespective of time, IGP rates of naïve females were negligible ($0.04 \text{ eggs/female/day} \pm 0.03$

SE), whereas experienced *K. aberrans* females frequently preyed on heterospecific eggs (0.43 eggs/female \pm 0.09 SE versus) (Fig. 13).

Conversely, experience did not significantly influence the IGP rates of *A. limonicus* on heterospecific eggs (GEE: Time: Wald $X^2_1= 0.979$; $P = 0.323$), but were marginally affected by experience interacting with time (GEE: Time: Wald $X^2_2= 5.418$; $P = 0.067$). The IGP rates of naïve and experienced *A. limonicus* females were similar at the first day ($P=0.293$). Then the IGP rates of the naïve females decreased on the second day ($P = 0.021$), whereas the corresponding data of the experienced females kept constant ($P = 0.750$). Consequently, experienced females consumed more eggs of *K. aberrans* than naïve females on the second day ($P = 0.029$) (Fig. 13).

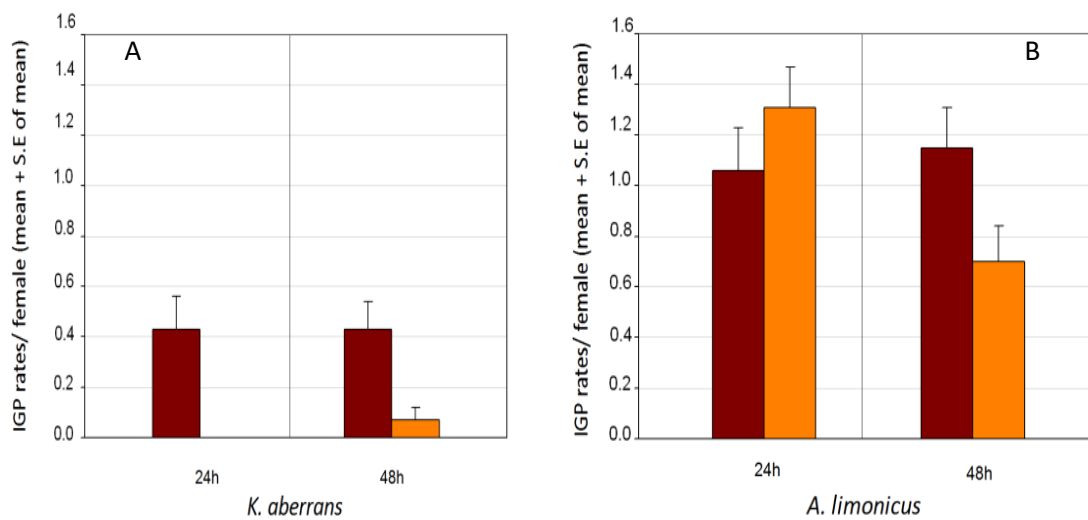


Fig. 13: Intraguild predation rates (IGP) in total (*A. limonicus*/*K. aberrans* eggs) of experienced (red bars) and naïve (orange bars) *K. aberrans* (A) and *A. limonicus* (B) females after 24h and 48h.

4 Discussion

To sum up all the results, native *K. aberrans* females did not prey on *A. limonicus* eggs, but preferred to stay and oviposit in prey patches free from IG predator cues. When having experience with IG predator cues, *K. aberrans* displayed strong IG predation on *A. limonicus* eggs, but no oviposition site selection. Contrary, independent of experience, *A. limonicus* females preferred the oviposition sites with IG predator cues of *K. aberrans* for oviposition and preyed on *K. aberrans* eggs.

4.1 *Kampimodromus aberrans* & *A. limonicus* females in the presence of IG predator cues

Kampimodromus aberrans females have proved to be careful mothers by reducing the risk for their offspring to be eaten by the aggressive IG predator *A. limonicus*. It is quite common that IG prey to oviposit in places away from IG predators when having the choice, as has also been shown in another study with *K. aberrans* females (Saitoh and Choh, 2018). Along the same line, females of *Phytoseiulus persimilis* and *Iphiseius degenerans* preferred to deposit their eggs in IG-predator free prey patches (Walzer et al. 2006; Choh et al., 2015). Interestingly, the native predator females did not kill any IG prey egg, which would have been a suitable way to minimize both a potential threat in form of a prospective IG predator and food competitor (Polis et al., 1989). Contrary to the introduced results, Schausberger (1997) showed in choice experiments that *K. aberrans* females prefer to feed on IG eggs than conspecific eggs. (Schausberger 1997). An explanation for these different findings might be that the offered IG eggs in this choice experiment were from native species (e.g. *Euseius finlandicus* and *Typhlodromus pyri*, Acari: Phytoseiidae), which co-occur with *K. aberrans*. Thus, the cues of *E. finlandicus* and *T. pyri* are known for *K. aberrans* indicating a potential threat for their offspring or/and an alternative prey for the females. In this case, the decision to prey on the IG eggs should be adaptive, because it reduces the offspring predation risk and offers the predator females a high-quality food (Walzer and Schausberger, 1999). However, the cues from the alien predator *A. limonicus* are unspecific for *K. aberrans* in the introduced choice experiments and cannot be allocated to a specific predator stage or food resource. In this case, it makes sense to avoid the alien IG eggs, which may represent an unknown danger for *K. aberrans* (Choh et al., 2015; Walzer et al., 2006).

In contrast to the native predator, the alien predator female deposited more eggs in the prey patch with the eggs of the native predator, which seems puzzling at a first glance. Usually, predatory mite females should choose oviposition sites that provide enough food for their juvenile offspring with additionally low risk to be eaten by potential IG predators (Choh et al., 2015; Walzer and Schausberger, 2012). However, the alien predator had chosen the prey patch with IG predator eggs, spider mite eggs and pollen, which potentially offers more food for both the predator and its offspring than the

alternative prey patch without IG predator eggs. IG prey eggs per se do not present an immediate predation risk for the offspring of the alien predator and they are a high quality food resource allowing juvenile development and reproduction in several predatory mite species (Walzer and Schausberger, 1999). Additionally, the alien predator females reduced the IGP risk of its offspring by consuming a large amount of the native IG eggs. Furthermore, in the case that alien juveniles are confronted with mobile stages of the native species *K. aberrans*, the risk of being killed by the native IG predator should be also small. The alien mobile stages are larger, moving faster and more aggressive than the corresponding mobile stages of the native predator (Walzer, personal observation). Moreover, the development time of *A. limonicus* from larva to adult is faster than as from *K. aberrans*, which is an advantage for *A. limonicus*, as larger immature stages of IG predators can more easily consume the smaller immature IG prey stages (Kasap, 2005; Polis et al., 1989; Samaras et al., 2015). As a consequence, the faster growing alien species is more likely to become a stronger food competitor and IG predator on the slowly development individuals of the native species than vice versa (Walzer and Schausberger, 2012). These findings indicate that the more aggressive *A. limonicus* females have also chosen an adequate place for their offspring, whereas the immature stages of *A. limonicus* were additionally offered nutritious food in form of IG prey (Choh et al., 2015).

The fact that *A. limonicus* females consumed more spider mite eggs (extraguild prey) than *K. aberrans*, may depend on the higher reproductive rate of *A. limonicus*, which demands a higher food supply for egg production (Helle, 1985; Schuster and Earl Pritchard, 1963). *Amblydromalus limonicus* produced more eggs than *K. aberrans* females throughout the time, which is typical for the alien biocontrol agent because of its larger body size and a higher reproductive potential compared to the native species *K. aberrans* (Helle, 1985; Schuster and Earl Pritchard, 1963). Furthermore, it is known that *A. limonicus* develops faster when feeding on animal prey than on pollen (Vangansbeke et al., 2014). The daily and total egg production rates of *K. aberrans* are the highest when fed with pollen or *T. urticae* + pollen compared than fed with IG prey like larva of *P. finitimus*, *A. andersoni* or *T. pyri* (Ahmad et al., 2015; Kasap, 2005). Therefore, the food supply was optimally for the needs of *K. aberrans* by adding pollen and spider mite eggs on both prey patches offered to the test females to guarantee high egg production rates during the experiment. Nonetheless, the reproductive output of the alien predator is significantly higher than for the native species.

4.2 Learning effects on the behavior of *K. aberrans* & *A. limonicus*

Learning is a behavioral change induced by experience to adapt to environmental changes and processes, which can also influence anti-predator behavior (Rahmani et al., 2009; Schausberger, 2018b; Seiter and Schausberger, 2015). In general, predatory mites can change their behavior long- or short-term depending on the development stage and age induced by environmental stimuli (experience). Such behavioral plasticity can occur within a generation, termed within-generational plasticity. However, parents can also modify the behavior of their offspring without changing the DNA-sequences, which is classified in transgenerational behavioral plasticity (Schausberger, 2018a). For example, it is well known that predatory mite females being confronted to predator risk during egg formation modified the defense behavior of their offspring against potential IG predators (Seiter and Schausberger, 2015). Here, only the within-generational plasticity effects on female behavior induced by learning were examined in the present study.

There are already some studies dealing with other predatory mites (*P. persimilis*, *N. californicus* and *A. andersoni*), which were in direct contact with different low- and high-risk IG predator females during juvenile development initiating a learning process. The experienced predatory mites of *P. persimilis* and *N. californicus*, but not *A. andersoni*, showed a higher tendency for the IG predator-free prey patches for preying and oviposition, but the females were also able to discriminate between high risk and low risk IG predators (Walzer and Schausberger, 2011; 2012). Here, independent on experience, the native predatory females of *K. aberrans* deposited an equal number of eggs in prey patches with and without IG predator eggs. These results for the naïve predator females are surprising, because the *K. aberrans* females selecting the predator-free prey patches for oviposition in the former choice experiment had also no experience with the alien predator. The most parsimonious explanation is that the learning procedure (exposed to an air flow containing IG predator cues) had stressed the naïve females lowering the sensitivity to alien IG predator cues. However, experienced *K. aberrans* showed a strong behavioral change by increased predation on the eggs of the alien predator. Such a behavior of the *K. aberrans* females is considered to be adaptive, because it reduces IGP-risk for their offspring and minimize potential food competitors while increasing their own fitness by food intake (Polis et al., 1989). Another benefit by the consumption of the eggs of the alien predatory mite *A. limonicus* is that several heterospecific predatory mites such as *Amblyseius andersoni*, *Typhlodromus pyri* and *Phytoseius finitimus* constitute a high quality food resource for *K. aberrans* allowing egg production (Ahmad et al., 2015). It is likely that the eggs of *A. limonicus* have a similar nutritional value for *K. aberrans*. Furthermore, the killing of IG predator eggs should be a strategy with lower costs compared to oviposition site selection, because the searching for IG predator-free prey patches provokes a high risk and is time-consuming under natural conditions. Thus, to kill eggs and probably also small mobile

developmental stages (larvae) of a dangerous IG predator is also an appropriate survival strategy to coexist with other IG predators (Choh et al., 2015).

Similar to the first choice experiment, the alien *A. limonicus* females tended to place more eggs in prey patches with IG predator cues, but this behavior was not affected by experience. However, the predation rates on IG predator eggs was higher in the experienced females. Obviously, the alien females perceive the cues of the IG predator *K. aberrans* as potential food for themselves and their offspring by depositing its eggs more often in prey patches containing eggs of *K. aberrans*. Furthermore, it is quite common for aggressive IG predators to prefer patches with cues of a low-risk IG predator to offer their offspring nutritious IG prey in addition to extraguild prey (Walzer and Schausberger, 2012).

Which cues might be responsible for the shifts in IGP behavior of the alien and native predatory females? During the learning phase the experienced females were exposed to volatile cues of the IG predator and IG prey. These cues were produced by IG predators that were in prey patches together with eggs and nymphs of the IG prey as exclusive food resource. In the case of an IG predator attack, prey often produce alarm pheromones to warn their conspecifics against a dangerous predator. Such responses are well documented in several arthropod taxa including mites (Chivers and Smith, 1998; Raspotnig, 2006). It is likely that the IG predator species, used for producing predator cues in the learning phase, behaved differently in relation to the prey, as it was demonstrated in the first choice experiment with naïve predator females. The alien predatory female probably attacked and killed IG prey frequently, whereas IG predation by native *K. aberrans* females was probably negligible. Thus, *K. aberrans* females also received alarm pheromones during the learning phase, but *A. limonicus* females not or in very low concentrations. In the choice experiments, the experienced females only perceived direct IG predator cues: eggs and predator traces. Obviously, experienced *K. aberrans* females associated the direct IG predator cues with prospective predation risk and did not leave the prey patch, but reduced the number of IG predator eggs by killing them. Contrary, both experienced and naïve alien predator females might associate direct cues from the native species with additional food.

Summing up, the behavioral responses of the native species *K. aberrans* to the alien predator *A. limonicus* may allow the co-existence of the two species in the case of the establishment of *A. limonicus* in the field. However, this assumption has to be verified with population experiments both under controlled and natural conditions.

5 Conclusions and Outlook

Independent of experience, *Amblydromalus limonicus* is the more aggressive predatory mite, when this alien predatory mite and the native predatory mite *K. aberrans* interact with each other. Nonetheless, the presented lab results indicate that *K. aberrans* might be able to co-exist on plants with the alien predator because of avoiding prey patches occupied by *A. limonicus* and by strong IGP. Under natural conditions, other aspects can increase the likelihood of co-existence between the alien and native predatory mite species. For example, the native predatory mite can establish itself spatially on a plant in a completely different way or colonize habitats that the alien predatory mite might not even accept. It is well known that the small predatory mite species *K. aberrans* prefers to establish itself on pubescent leaves, which are rather avoided by the other larger predatory mite species, including the alien predator *A. limonicus* (Escudero-Colomar and Chorazy, 2012; Kreiter et al., 2002; Seelmann, 2005). Additionally, the small body size allows *K. aberrans* to use leaves structures such as domatia as shelter against larger predators. Furthermore, in contrast to alien species, native species may have the great advantage that they are better able to cope with the extreme weather conditions, such as heat waves and cold spells, because they have been able to adapt over the decades (Blackburn et al., 2011). It is well known that the alien species *A. limonicus* is more heat-sensitive than the native species *K. aberrans* (Walzer et al., 2017a).

However, population experiments on different plant species under controlled and natural conditions should be conducted to evaluate the consequences of oviposition site selection, intraguild predation and learning on the population dynamics of the alien and native predatory mite species. Based on the climatic conditions in Austria, an escape of the alien predatory mite *A. limonicus* from the greenhouse in the field may result (1) only in the seasonal establishment of *A. limonicus* at severe winter conditions or (2) in the seasonal establishment and overwintering at mild winter conditions. In the former scenario, native species should have the advantage to occupy the plants earlier than the alien predator, which may arrive in early summer in the field. However, the alien predator can reach adulthood and reproduce eggs at temperatures of +10°C (Dittmann et al., 2016), whereas native species start with reproduction and development at temperatures above +14°C. Consequently, in the case of casual annual establishment of *A. limonicus* the alien predator arrives earlier on plants than native species, which may result in an advantage of the alien predator by dominating the resources early (inhibitory priority effect). Testing out these two scenarios at population level may provide a deeper insight, if the alien predator is able to outcompete locally native species in Austria or not.

6 References

- Ahmad S., Pozzebon A., Duso C. (2015) Predation on heterospecific larvae by adult females of *Kampimodromus aberrans*, *Amblyseius andersoni*, *Typhlodromus pyri* and *Phytoseius finitimus* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 67 (1):1-20.
- Blackburn T. M., Pyšek P., Bacher S., Carlton J. T., Duncan R. P., Jarošík V., Wilson J. R., Richardson D. M. (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* 26 (7):333-339.
- Broufas G. D., Pappas M. L., Koveos D. S. (2007) Development, survival, and reproduction of the predatory mite *Kampimodromus aberrans* (Acari : Phytoseiidae) at different constant temperatures. *Environmental Entomology* 36 (4):657-665.
- Chant D. A., McMurtry J. A. (2005) A review of the subfamily Amblyseiinae Muma (Acari : Phytoseiidae). *International Journal of Acarology* 31 (3):187-224.
- Chivers D. P., Smith R. J. F. (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. *Écoscience* 5 (3):338-352.
- Choh Y., Sabelis M. W., Janssen A. (2015) Distribution and oviposition site selection by predatory mites in the presence of intraguild predators. *Experimental and Applied Acarology* 67 (4):477-491.
- Chorąży A., Kropczyńska-Linkiewicz D., Sas D., Escudero-Colomar L. A. (2016) Distribution of *Amblydromalus limonicus* in northeastern Spain and diversity of phytoseiid mites (Acari: Phytoseiidae) in tomato and other vegetable crops after its introduction. *Experimental and Applied Acarology* 69 (4):465-478.
- Dittmann L., Walzer A., Schausberger P. (2016) Population-specific cold tolerance of the predatory mite *Amblydromalus limonicus*. In: 5th Meeting of the IOBC-WPRS Bulletin, Castello de la Plana, Spain, September 7-10, 2015. Working Group "Integrated Control of Pest Mites" (120), pp 10-12.
- Duso C., Fanti M., Pozzebon A., Angeli G. (2009) Is the predatory mite *Kampimodromus aberrans* a candidate for the control of phytophagous mites in European apple orchards? *BioControl* 54 (3):369-382.
- Escudero-Colomar L., Chorazy A. (2012) First record of *Amblydromalus limonicus* (Acari: Phytoseiidae) from Spain. *International Journal of Acarology* 38 (6):545-546.
- Helle W. (1985) Spider mites: B [Natural enemies of the Tetranychidae; damage and control] their biology, natural enemies and control. Amsterdam [u.a.], Elsevier.
- Hoogerbrugge H., van Houten Y., Knapp M., Bolckmans K. (2011) Biological control of thrips and whitefly on strawberries with *Amblydromalus limonicus* and *Amblyseius swirskii*:65-69.

Kasap I., Atlihan R. (2011) Consumption rate and functional response of the predaceous mite *Kampimodromus aberrans* to two-spotted spider mite *Tetranychus urticae* in the laboratory. *Experimental and Applied Acarology* 53 (3):253-261.

Kasap I. (2005) Life-history traits of the predaceous mite *Kampimodromus aberrans* (Oudemans) (Acarina: Phytoseiidae) on four different types of food. *Biological Control* 35 (1):40-45.

Knapp M., van Houten Y., Hoogerbrugge H., Bolckmans K. (2013) *Amblydromalus limonicus* (Acari: Phytoseiidae) as a biocontrol agent: Literature review and new Findings. *Acarologia* 53:191-202.

Kreiter S., Tixier M. S., Croft B. A., Auger P., Barret D. (2002) Plants and leaf characteristics influencing the predaceous mite *Kampimodromus aberrans* (Acari: Phytoseiidae) in habitats surrounding vineyards. *Environmental Entomology* 31 (4):648-660.

Lorenzon M., Pozzebon A., Duso C. (2012) Effects of potential food sources on biological and demographic parameters of the predatory mites *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni*. *Experimental and Applied Acarology* 58 (3):259-278.

McMurtry J. A., Johnson H. G., Badii M. H. (1984) Experiments to determine effects of predator releases on populations of *Oligonychus punicae* [Acarina: Tetranychidae] on avocado in California. *Entomophaga* 29 (1):11-19.

McMurtry J. A., Moraes G. J. D., Sourassou N. F. (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic and Applied Acarology* 18 (4):297.

McMurtry J. A., Scriven G. T. (1965) Life-History Studies of *Amblyseius limonicus*, with Comparative Observations on *Amblyseius hibisci* (Acarina: Phytoseiidae). *Annals of the Entomological Society of America* 58 (1):106-111.

McMurtry J. A., Scriven G. T. (1971) Predation by *Amblyseius limonicus* on *Oligonychus punicae* (Acarina): Effects of initial predator-prey ratios and prey distribution. *Annals of the Entomological Society of America* 64 (1):219-224.

Morosinotto C. (2012) Antipredator behaviours in prey and predators during breeding: from habitat selection to parental care. Department of Biology, University of Turku, Finland.

Negloh K., Hanna R., Schausberger P. (2012) Intraguild predation and cannibalism between the predatory mites *Neoseiulus neobaraki* and *N. paspalivorus*, natural enemies of the coconut mite *Aceria guerreronis*. *Experimental and Applied Acarology* 58 (3):235-246.

Polis G. A., Myers C. A., Holt R. D. (1989) The ecology and evolution of intraguild predation-Potential competitors that eat each other. *Annual Review of Ecology and Systematics* 20:297-330.

- Rahmani H., Hoffmann D., Schausberger P., Walzer A. (2009) Adaptive learning in the foraging behavior of the predatory mite *Phytoseiulus persimilis*. *Behavioral Ecology* 20 (5):946-950.
- Raspotnig G. (2006) Chemical alarm and defence in the oribatid mite *Collohmanna gigantea* (Acari: Oribatida). *Experimental & Applied Acarology* 39 (3):177-194.
- Root R. B. (1967) The niche exploitation pattern of the Blue-Gray Gnatcatcher. *Ecological Monographs* 37 (4):317-350.
- Saitoh F., Choh Y. (2018) Do intraguild prey protect their eggs from intraguild predators that share their oviposition site? *Animal Behaviour* 140:49-55.
- Samaras K., Pappas M. L., Fytas E., Broufas G. D. (2015) Pollen suitability for the development and reproduction of *Amblydromalus limonicus* (Acari: Phytoseiidae). *Biocontrol* 60 (6):773-782.
- Schausberger P. (1997) Inter- and intraspecific predation on immatures by adult females in *Euseius finlandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 21 (3):131-150.
- Schausberger P. (2018a) Behavioral plasticity of plant-inhabiting predatory mites shaped by early life experiences. In: 15th International Congress of Acarology, 2-8 September 2018, Antalya, Turkey.
- Schausberger P. (2018b) Benefit-cost trade-offs of early learning by predatory mites. *Journal of the Acarological Society of Japan* 28:45-46.
- Schausberger P., Croft B. A. (2000) Cannibalism and intraguild predation among phytoseiid mites: Are aggressiveness and prey preference related to diet specialization? *Experimental and Applied Acarology* 24 (9):709-725.
- Scheuerlein A., Van't Hof T., Gwinner E. (2001) Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268:1575-1582.
- Schuster R., Earl Pritchard A. (1963) Phytoseiid mites of California. *Hilgardia*:191-285.
- Seelmann L. (2005) Die Blattbehaarung reguliert Intraguild Predation zwischen den Raubmilben *Kampimodromus aberrans* und *Euseius finlandicus* auf Apfel. Masterthesis, University of Natural Resources and Life Sciences, Vienna.
- Seiter M., Schausberger P. (2015) Maternal intraguild predation risk affects offspring anti-predator behavior and learning in mites. *Scientific Reports* 5:15046.

Sengonca C., Drescher K. (2001) Laboratory studies on the suitability of *Thrips tabaci* Lindeman (Thysanoptera, Thripidae) as prey for the development, longevity, reproduction and predation of four predatory mite species of the genus *Amblyseius* (Acari, Phytoseiidae). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 108 (1):66-76.

van Houten Y. M., van Rijn P. C. J., Tanigoshi L. K., van Stratum P., Bruin J. (1995) Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomologia Experimentalis et Applicata* 74 (3):225-234.

Vangansbeke D., Nguyen D. T., Audenaert J., Verhoeven R., Gobin B., Tirry L., De Clercq P. (2014) Performance of the predatory mite *Amblydromalus limonicus* on factitious foods. *BioControl* 59 (1):67-77.

Venzon M., Janssen A., Pallini A., Sabelis M. W. (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. *Animal Behaviour* 60 (3):369-375.

Walzer A., Dittmann L., Schausberger P. (2016) Comparison of three *Amblydromalus limonicus* populations regarding their potential to overcome abiotic resistance of Austrian ecosystems under climate warming scenarios. In: 5th Meeting of the IOBC-WPRS Bulletin, Castello de la Plana, Spain, September 7-10, 2015. Working Group "Integrated Control of Pest Mites" (120), pp 85-86.

Walzer A., Dittmann L., Schausberger P. (2017a) Die thermale Sensitivität des exotischen Nützlings *Amblydromalus limonicus*. In: 72. ALVA Tagung, Waldkirchen am Wesen, May 22-23, 2017.

Walzer A., Lepp N., Dittmann L., Schausberger P. (2017b) Temperature-dependent intraguild predation between the exotic *Amblydromalus limonicus* and Austrian native predatory mites. In: IOBC Canada 2017 "Integrated control in protected crops, temperate climate", Niagara Falls, June 4-8, 2017.

Walzer A., Paulus H. F., Schausberger P. (2006) Oviposition behavior of interacting predatory mites: response to the presence of con- and heterospecific eggs. *Journal of Insect Behavior* 19 (3):305-320.

Walzer A., Schausberger P. (1999) Cannibalism and interspecific predation in the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*: predation rates and effects on reproduction and juvenile development. *BioControl* 43 (4):457-468.

Walzer A., Schausberger P. (2011) Threat-sensitive anti-intraguild predation behaviour: Maternal strategies to reduce offspring predation risk in mites. *Animal Behaviour* 81 (1):177-184.

Walzer A., Schausberger P. (2012) Integration of multiple intraguild predator cues for oviposition decisions by a predatory mite. *Animal Behaviour* 84 (6):1411-1417.

Ward N. L., Masters G. J. (2007) Linking climate change and species invasion: an illustration using insect herbivores. *Global Change Biology* 13 (8):1605-1615.

Zenni R., Nuñez M. (2013) The elephant in the room: The role of failed invasions in understanding invasion biology. *Oikos* 122(6): 801.815

7 List of figures

Fig. 1: Experimental unit for a single gravid female having the choice between a prey patch without carrying heterospecific predator cues (NO CUE) and a prey patch carrying heterospecific predator cues (CUE) for oviposition and prey patch selection.	15
Fig. 2: Each gravid test-female was stored in one of these acrylic for 3-4h before the start of the experiment.	16
Fig. 3: Experimental construction for confronting females of <i>K. aberrans</i> / <i>A. limonicus</i> with cues (1) from intraguild IG prey and IG predators and (2) from clean air. Females exposed to clean air were used as naïve females in the choice experiments, females exposed to IG prey and IG predator cues were used as experienced females.	18
Fig. 4: Prey patch selection after first choice, and 1, 2, 3, 4, 5, 6, 24 and 48h by <i>K. aberrans</i> (A) and <i>A. limonicus</i> (B) females when having the choice between prey patches with (red bars) or without IG predator cues (orange bars)	19
Fig. 5: Oviposition site selection of <i>K. aberrans</i> (A) and <i>A. limonicus</i> (B) females after 24h and 48h when having the choice between prey patches with (red bars) or without IG predator cues (orange bars).	20
Fig. 6: Extraguild predation rates (EGP) in total (spider mite eggs) by <i>K. aberrans</i> and <i>A. limonicus</i> females after 24h (red bars) and 48h (orange bars).	21
Fig. 7: Intraguild predation rates (IGP) of <i>A. limonicus</i> females on <i>K. aberrans</i> eggs after 24h and 48h.	21
Fig. 8: Prey patch selection of experienced (A) and naïve (B) <i>K. aberrans</i> females given a choice between a prey patch with IG predator cues (red bars) or without IG predator cues (orange bars) after first choice, 1, 2, 3, 4, 5, 6, 24 and 48h.	22
Fig. 9: Prey patch selection of experienced (C) and naïve (D) <i>A. limonicus</i> females given a choice between a prey patch with IG predator cues (red bars) or without IG predator cues (orange bars) after first choice, 1, 2, 3, 4, 5, 6, 24 and 48h.	22
Fig. 10: Oviposition site selection of experienced (A) and naïve (B) <i>K. aberrans</i> females given a choice between a prey patch with heterospecific predator cues (red bars) or a prey patch without heterospecific predator cues (orange bars) after 24 and 48h.	23

Fig. 11: Oviposition site selection of experienced (C) and control (D) *A. limonicus* females given a choice between a prey patch with heterospecific predator cues (red bars) or a prey patch without heterospecific predator cues (orange bars) after 24 and 48h. 24

Fig. 12: Extraguild predation rates (EGP) in total (spider mite eggs) by *K. aberrans* (A) and *A. limonicus* (B) females (red bars= experienced; orange bars= naive) after 24 and 48h. 24

Fig. 13: Intraguild predation rates (IGP) in total (*A. limonicus*/ *K. aberrans* eggs) of experienced (red bars) and naive (orange bars) *K. aberrans* (A) and *A. limonicus* (B) females after 24h and 48h. 25