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Master Thesis

Predation risk-mediated maternal effects in the spider mite

Tetranychus urticae

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Ich widme diese Masterarbeit allen Menschen die Spaß an Wissenschaft haben.

Dedicated to all people who have fun with science.

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1 Introduction

The two-spotted spider mite *Tetranychus urticae* Koch is worldwide one of the most important pests in cropping systems (Van Leeuwen et al., 2010). Spider mites degrade the plant's tissue, irritate its normal physiological processes, disturb its growth as well as its flowering and, consequently, have strong influence on yield amount and quality. *T. urticae* is a highly polyphagous species and able to quickly develop resistances to various insecticides and acaricides. *T. urticae* is the number one in the list of the top ten most resistant arthropod species worldwide (Van Leeuwen et al., 2010). The spider mites can quickly develop resistance against chemicals because of their short life cycle, numerous progeny and arrhenotokous reproduction (Van Leeuwen et al., 2010). In some cases resistance development is so severe that the spider mites cannot even be killed by application of several pesticides, making their control exceedingly challenging (Van Leeuwen et al., 2010). Spider mites actually became such serious pests worldwide because of the intensive use of chemical control. Lots of pesticides do not only adversely affect the pest animals but they also reduce the populations of their natural enemies, which are often more sensitive to the pesticide than the herbivores themselves. Furthermore, using chemical pest control may lead to other serious problems like contamination of the environment and negative impacts on human health, causing further challenges. Knowledge about those risks alerted farmers and customers to foster alternative forms of pest control and, as a consequence, biological control is receiving more and more attention (Van Driesche and Bellows, 1996). Biological control is a pest management technique that supports sustainable agriculture and commonly sustains or even enhances biodiversity (Bale et al., 2008).

Biological control is, by definition, the use of a species to lower another species' population number (Bale et al., 2008). Men try to control animals, weeds and diseases by mechanisms like predation, parasitism, pathogenicity or competition (Van Driesche and Bellows, 1996). Control without human intervention, just by naturally occurring enemies, is called natural control. Modern societies more and more use and establish biological control to reduce pests. Predatory mites, for example, are commonly used as biocontrol agents of mite and

insect pests. In specific terminology, larger natural enemies such as insects, spiders and mites are called macrobial agents, while smaller ones such as bacteria, fungi, nematodes or viruses are called microbials (Bale et al., 2008). Biological control can be categorized in three different major methods (Van Driesche and Bellows, 1996). Classical biological control is employed, when the goal is reducing exotic invasive plants and/or pests established in new countries. A usually small number of the natural enemies of the exotic pests in their native home countries are gathered and then imported and released in the new country. Under favourable abiotic conditions, long term environments like orchards or forests allow those enemies to establish and build up competitive populations. Augmentative biological control comes in use when natural enemies are missing or only present in low quantity. First, multiplication, often through commercial production, of biocontrol agents takes place, which is then followed by single or repeated releases of large numbers of enemies in the cultivation systems for pest control (Bale et al., 2008). Augmentation may be implemented irrespective of whether the target pests are indigenous or adventive species (Van Driesche and Bellows, 1996). Conservation biological control has the goal to enhance the activity of locally present natural enemies, by improving the environmental factors. It strengthens the action of naturally occurring beneficial organisms and by that way lessens or eradicates the pest populations of a certain area (Bale et al., 2008).

Depending on the crop, *T. urticae* may be controlled by either augmentative or conservation biological control. Regarding augmentative control, predatory mites of the family Phytoseiidae are the most important and most successfully used natural enemies of spider mites worldwide. There exists lots of evidence of the successful use of phytoseiid mites as biological control agents in pest management (McMurtry and Croft, 1997). Within the family Phytoseiidae, some species, predominantly of the genus *Phytoseiulus*, evolved a prey preference for spider mites. Diverse characteristics reflect the specialisation of *Phytoseiulus* sp. such as *P. persimilis* Athias-Henriot for tetranychid mites. For example, the predatory mites' long dorsal setae help them moving through the dense webbing of the spider mites. They also have a shorter developmental time than *T. urticae* and a rapid numerical response to spider mite populations. Worldwide programs to control *T. urticae* with *P. persimilis* exist since the 1960s. Commercial rearing and sale of *P. persimilis* as a regular product started in

1968 (Van Lenteren and Woets, 1988). Today there are about 20 different predatory mite species of the family Phytoseiidae for sale. In 2007 there were about 50 mass providers worldwide (Gerson and Weintraub, 2007) but the number of biocontrol manufacturers is still rising. According to Van Lenteren (2012), in 2012 there were about 85 producers worldwide and hundreds of state- or farmer-funded production units.

Individual predator-prey interactions have huge impacts on populations and communities. Predation is, by definition, the killing and consumption of another individual, resulting in the death of prey and so in its removal from the system. However, also non-lethal predator effects on prey (Lima, 1998) are an important interaction with respect to natural and biological pest control. Non-lethal predator effects may influence the physiology, morphology, and/or behaviour of prey individuals. Many prey show flexible habits when getting in contact with predators or when perceiving their cues and commonly perform specific behaviours allowing them to be not detected or to escape from and avoid being killed by their predators. These so called anti-predator behaviours are strategies that make it harder for the predator to detect, encounter, catch and/or kill the prey and therefore avoid successful attacks by the predator. Clearly, non-lethal predator effects also influence other prey life activities such as social living, foraging or mating (Lima and Dill, 1990). Assuming adaptive decision-making, animals should have evolved to arrive at an appropriate trade-off between the costs of losing time and energy for other fitness enhancing activities and the benefits of the avoidance of predators (Lima and Dill, 1990). More and more studies also highlight the importance of non-lethal predator effects for population and community dynamics (e.g. Nelson et al., 2004). In general, for a better understanding of predator-prey interactions both lethal and non-lethal effects require close scrutiny (Lima, 1998). Therefore, regarding pest management, the persistence of a pest population may be contingent not only on killing and consumption by predators but also on their non-lethal effects on prey.

My thesis is about anti-predator behaviour and changes in life history of the two-spotted spider mite *T. urticae* in response to its prime natural enemy, the predatory mite *P. persimilis*. Specifically, I asked whether maternal predation risk can induce maternal effects on offspring, such as how they develop, reproduce and behave under predation risk. Bernardo (1996), Mousseau and Fox (1998) or Wolf and Wade (2009) defined maternal

effects as phenotypic effects of the mother on the offspring phenotype that are unrelated to the offspring's genotype. For example, maternal effects can happen, when a mother experiences food shortage, predation risk or other diverse environmental factors and consequently influences the phenotype of her offspring (Mommer and Bell, 2013).

1.1 Anti-predator behaviour

Predation is a strong selective force shaping prey morphology, life history and behaviour (e.g. Lima and Dill, 1990; Fernández Ferrari and Schausberger, 2013). Anti-predator behaviour is every behavioural change an animal performs to avoid getting killed by a predator. Apart from behavioural changes, prey may also evolve morphological (cryptic and aposematic colouration, protective armour) or physiological adaptations (chemical defence) as long-lasting defence mechanisms. However, on a short time basis, anti-predator protection works by decision-making (Lima and Dill, 1990). Prey animals often have to differentiate between various hazard levels. Anti-predator behaviours like fleeing, avoiding, activity reduction or hiding, lower the risk of predation but also entail energy costs that could be used for other life activities such as feeding and/or reproduction. To find the optimal balance between energy uptake and energy investment for the appropriate behavioural response under predation risk, animals should be sensitive to the level of predation risk (Sih, 1982; Helfman, 1989). To correctly recognise predators and interpret the associated level of threat, many prey of diverse taxa evolved sophisticated recognition abilities, using various sensory modalities such as vision, touch, audition and/or chemical (olfaction, taste) senses.

In general, information cues indicating predation risk, including chemical cues, can be direct or indirect (Grostal und Dicke, 1999). Direct chemical cues are emitted by the predators themselves and are functionally working as kairomones (negative for sender, positive for receiver). Indirect chemical cues indicating predation risk emanate from the environment of the predator and may come from disturbed, injured or dead conspecifics and include, for example, alarm pheromones. Many animals, especially arthropods, may perform threat-sensitive anti-predator behaviours upon perception of direct and/or indirect chemical cues (Grostal und Dicke, 1999).

1.2 Chemical information in predator-prey interactions

Commonly, animals may perceive extrinsic information via three major sensory pathways, visual, mechanical and/or chemical. Chemical cues play an important role in enemy recognition in a wide diversity of animals. Studying the ways how arthropods, like mites, detect natural enemies and translation of this knowledge into practice is important for biological control (Grostal and Dicke, 1999). Regarding chemosensory cues, arthropod prey may perceive direct and/or indirect predator cues by olfaction and/or taste via volatile and non-volatile molecules (Grostal and Dicke, 1999; Dicke and Grostal, 2001). Olfaction means perception of molecules present in air or water. Taste is perception of molecules by direct contact.

Chemicals transferring information from one species to another are called allelochemicals, which can be further subcategorized regarding their effects on sender and receiver. Direct cues are produced by the predator, which thereby conveys information. The receiver, in this case the prey species, perceives the information and responds in a certain way. If the prey response is to the prey's disadvantage but to the benefit of the predator, the allelochemical is called allomone. If it benefits the prey but is disadvantageous for the predator it is called kairomone and if both organisms profit from the information it is called a synomone (Dicke and Grostal, 2001). Direct cues conveying information from predators to prey can be present on the body or in the exuviae, faeces, eggs, marking pheromones or any other product of the predator. Predator kairomones often alarm prey and induce anti-predator behaviours. In addition, direct predator cues can influence the life history and morphology of potential prey (Grostal and Dicke, 1999).

Lots of pertinent studies deal with the defense behaviours of aquatic prey and their reactions to predator kairomones and point out that activity and distribution of prey may be strongly modified under kairomone influence (Grostal and Dicke, 1999). For example, mayfly larvae *Baetis bicaudatus* decrease drift and hide more when perceiving the kairomones of their predator, the brook trout *Salvelinus fontinalis* (McIntosh and Peckarsky, 2001). Examples for terrestrial prey species come from the spider mite *T. urticae*, which, within plants, avoids leaves with predator kairomones of *P. persimilis* (Grostal und Dicke, 1999; Kriesch und Dicke,

1997) and even avoids the colonization of plants with cues of *P. persimilis* (Pallini et al., 1999).

Indirect predator cues are not products of the predator but may, for example, be pheromones emanating from injured or dead conspecifics or alarm pheromones sent out by conspecific individuals perceiving risk (Grostal and Dicke, 1999). Such indirect cues may affect the prey behavioural response similar to direct cues. However, due to the generality of indirect cues the behavioural changes brought about by their perception are largely unspecific. For example, when honey bees *Apis mellifera scutellata* perceive an alarm pheromone they prolong their stinging response (Dicke and Grostal, 2001). My study animal, *T. urticae*, does not only respond to direct predator cues but also indirect cues emanating from dead and injured conspecifics, causing reduced oviposition and foraging activities (e.g., Grostal and Dicke, 1999).

1.3 Maternal effects

Maternal effects are non-genetic effects that mothers exert on the phenotypes of their progeny without any relation to the progeny's own genotype (Bernardo, 1996; Mousseau and Fox, 1998). Maternal effects contribute to shaping the offspring phenotype, represent the influence of the mother's environmental experience but have nothing to do with the offspring's own genes or its own environmental experiences. Maternal effects are trans-generational and highly relevant for ecological and evolutionary processes like population dynamics, phenotypic plasticity, niche construction, life-history evolution and the evolutionary responses to selection (Wolf and Wade, 2009). Maternal effects can be mechanisms increasing maternal and offspring fitness if they render the offspring's phenotype better suited to its future environment (Bernardo, 1996). There exist numerous studies demonstrating maternal effects in a wide range of taxa. Plants, insects, amphibians or mammals can have adaptive as well as non- or mal-adaptive impacts on their young (Mommer and Bell, 2013). For instance of maternal effects under predation risk, the female lizard *Pseudomoia pagenstecheri* adaptively produces highly predator-sensitive offspring after living under strong predation pressure by *Drysdalia coronoides* (Shine and Downes,

1999). In contrast, barn swallows *Hirundo rustica* experiencing stress by cats negatively influence their offspring by a higher corticosterol concentration in the eggs, slowing down the development of the young (Saino et al., 2005).

Mothers of many animals have the ability to respond during offspring production or care to environmental factors like temperature, moisture, level of competition and predation risk and so on. All these factors may inform mothers about the status of the future habitat, such as food availability, for their offspring (Bernardo, 1996). When, where and how females produce and place their progeny may be determined by this information and these decisions may have great impact on offspring survival. For example, a risky environment can influence mothers to produce offspring with a changed phenotype, such as smaller or larger body size (Bernardo, 1996). In order to keep the balance between avoidance of predator attacks and foraging, animals often have to reduce food intake, which in turn has an impact on the propagules (Lima and Dill, 1990). Maternal effects are often nutritionally mediated: in general and for most animals, the supply of food resources during propagule production has impacts on propagule growth, development and survival (Mousseau and Fox, 1998). Mothers producing larger offspring often do so because of the availability of abundant highly nutritious food (Bernardo, 1996). The quality and quantity of a plant, a phytophagous spider mite mother develops on, has influence on the physical and chemical egg composition and therefore on the young emerging from the eggs (Bernardo, 1996). However, maternal effects can also be non-nutritionally mediated by pre- and post-natal influences. Mechanistically, maternal effects are initiated through environmental conditions, which influence the mothers' condition, behaviour, or physiological state and hence may affect the offspring via the yolk amount and composition, mRNAs, gene methylation, chemical cues, hormones etc..

1.4 General information about the spider mite *Tetranychus urticae*

The highly polyphagous two-spotted spider mite *Tetranychus urticae* (Figure 1) is one of about 1200 plant-feeding species of the cosmopolitan mite family of the true spider mites, the Tetranychidae (Table 1) (Fry, 1989; Gerson and Weintraub, 2007). *T. urticae* can attack more than 1000 host plant species belonging to different botanical families (Navajas, 1998)

and is able to quickly develop resistance against insecticides and acaricides (Van Leeuwen et al., 2010). *T. urticae* prefers to sit and feed on the lower side of the leaves (Gutierrez and Helle, 1985a) and injures its host plants by piercing the mesophyll cells and extracting the cell contents (Fry, 1989). Typical consequences of spider mite feeding are leaf wilting, deficits in yield quantity as well as quality and even total desiccation and death of the attacked plants (Gerson und Weintraub, 2007). Compared to non-webbing phytophagous mites, *T. urticae* is in a better position when killing its host plant because of its well-developed ability to move from one plant to another using the webs. The level of plant damage depends, among other factors, on the pest population size as well as the time of infestation, climatic factors and the host plant species (Helle and Sabelis, 1985a).

The production of threads and webs is significant for dispersal, protection from predators and adverse climatic factors, interspecific relationships and mate finding (Gerson, 1985a). Diverse sensory organs like setae, pedipalps, the first pair of legs, slit sense organs, eyes and one apodemal organ exist to experience their environment, obtain information and consequently affect their behaviour. Female spider mites are highly fertile and can produce about 10 eggs per day at 21 °C. The globular egg gives rise to the larva, which then molts to protonymph, to deutonymph and to adult. After each of the three mobile juvenile stages intervening periods of inactive quiescence, called chrysalis, happen to form a new cuticle (Feiertag-Koppen and Pijnacker, 1985a). *T. urticae* is arrhenotokous and thus able to produce male offspring without fertilization. Unfertilized haploid eggs produce males and fertilized diploid eggs produce females. Due to the short generation time (12 days at 21 °C), high fecundity (10 eggs per female per day at 21 °C) and arrhenotokous reproduction *T. urticae* is able to increase in population size in relatively short time. These characteristics make this highly adaptable species a severe economic threat and one of the most significant agricultural pests on earth (Helle and Sabelis, 1985a).

Table 1: Taxonomic classification of the spider mite *Tetranychus urticae* Koch 1836 (after Hoy, 2011)

Taxon	Name
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Acari
Order	Trombidiformes
Family	Tetranychidae
Genus	<i>Tetranychus</i>
Species	<i>Tetranychus urticae</i> Koch



Figure 1: Two adult *Tetranychus urticae* females, a six-legged larva and eggs (©www.photo.net).

1.5 General information about the predatory mite *Phytoseiulus persimilis*

Phytoseiulus persimilis Athias-Henriot (Figure 2) belongs to the family of the Phytoseiidae (Table 2); it is a type 1 specialised spider mite predator, which almost exclusively feeds on phytophagous spider mites such as *T. urticae* (McMurtry and Croft, 1997). This efficient and famous biocontrol agent is one of about 20 commercially mass-reared and sold phytoseiid species worldwide (Gerson et al., 2003). *P. persimilis* is highly specialised on tetranychid mites and lives on the preys' host plant in the midst of their colonies (McMurtry and Croft, 1997). Their predation and oviposition rates, aggregation, time spent in prey patches, a.s.o. all depend on the population size of their tetranychid prey colony. They sense, grasp and pierce the spider mites with their first pair of legs, chelicerae and pedipalps and extract the body fluids using their stylets (Chant, 1985b). *P. persimilis* can be used for biological control of spider mites on many crops and in many agro-ecosystems in temperate and subtropical regions (Gerson et al., 2003).

The ability to move quickly through the spider mites' dense web is an important feature of *P. persimilis*. Because of a reduced body surface and keeping the silk away by long, thin, flexible and backwards pointing setae moving through the webs is no problem (Sabelis and Bakker, 1992). Also the high consumption rate with spider mite prey is remarkable. Various studies have shown that *P. persimilis* is able to distinguish different tetranychid species by olfaction, i.e. perception of volatile chemical cues (McMurtry and Croft, 1997), and they can also discriminate between spider mite-colonized and non-colonized parts of a leaf (Sabelis, 1985b). Female predatory mites have a lower reproduction rate than their spider mite prey (~4 eggs per day at 25 °C) (McMurtry and Croft, 1997). Development proceeds from the oval-shaped egg to larva, protonymph, deutonymph and adult. *P. persimilis* is pseudo-arrhenotokous; the male is haploid in the end but develops initially also from a fertilized egg; without mating egg production is impossible. Developmental time depends on prey availability, humidity and temperature. At ample food, 21 °C and a relative humidity of 60 to 90 % it takes ~7 to 9 days (Overmeer, 1985b).

Table 2: Taxonomic classification of the predatory mite *Phytoseiulus persimilis* Athias-Henriot 1957
(after McMurtry and Croft, 1997).

Taxon	Name
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Acari
Superorder	Parasitiformes
Order	Mesostigmata
Family	Phytoseiidae
Genus	<i>Phytoseiulus</i>
Species	<i>Phytoseiulus persimilis</i> Athias-Henriot



Figure 2: Adult female *Phytoseiulus persimilis* (©www.naturalinsectcontrol.com).

1.6 Study aims and objectives

Quite a few studies have found that maternal predation risk can influence the mothers' physiology and behaviour (Lima 1990) and can consequently also, via maternal effects, influence the offspring produced during predation risk (Roche et al., 2012; Mommer and Bell, 2013). The primary aim of my study was to find out if different levels of predation risk (no predator cues, direct predator cues, direct predator cues plus dead conspecifics and direct predator cues plus dead conspecifics plus live predators) exerted by *P. persimilis* may induce maternal effects in *T. urticae* females and consequently change the life history and/or the anti-predator behaviour of their offspring. Based on the previously gained knowledge that spider mites are threat-sensitive, respond the strongest to the predator *P. persimilis* and the information that predator recognition may be either innate, learned or both (Fernández Ferrari and Schausberger, 2013; Hackl and Schausberger, 2014), I used direct and indirect cues of *P. persimilis* to create environments differing in predation risk for the spider mite mothers. In the first experiment, I investigated survival, development and anti-predator behaviour of spider mite offspring in their juvenile phase, while in the second experiment, I investigated activity, oviposition and anti-predator behaviour of spider mite offspring in the adult female stage.

2 Materials and methods

Organisms necessary for both experiments were the common bean plant *Phaseolus vulgaris*, the two-spotted spider mite *Tetranychus urticae* and the predatory mite *Phytoseiulus persimilis*.

2.1 Growing of *Phaseolus vulgaris*

The common bean plant *Phaseolus vulgaris* was used in two different forms. First, it served as a rearing plant for *T. urticae* and second, leaves of clean uninfested plants were used for the experiments. Whole plants were used to rear the stock population of *T. urticae*. Single leaflets of trifoliolate leaves of the clean plants were used for preparing all the leaf arenas in both experiments. Primary leaves were used for rearing the predatory mites. The potting substrate for all plants was a mixture consisting of 75 % commercial soil and 25 % expanded clay. Cultivation of plants used to rear *T. urticae* happened under room conditions 23 ± 2 °C, 50 ± 10 % relative humidity and a light/dark ratio of 16:8 h. Clean plants used for experiments were grown in a walk-in environmental chamber at 25 ± 1 °C, 60 ± 5 % relative humidity and light/dark ratio of 16:8 h.

2.2 Rearing of *Tetranychus urticae*

Adult females of the two-spotted spider mite *T. urticae* were used to produce experimental animals and mixed life stages were used as prey for rearing the predatory mite *P. persimilis*. The stock population of *T. urticae* was reared on whole bean plants at 23 ± 2 °C, 50 ± 10 % relative humidity and a light/dark ratio of 16:8 h. To generate experimental animals, adult *T. urticae* females were randomly picked from the plants and transferred to detached leaf arenas varying in the level of risk (stress) posed by the predatory mite *P. persimilis*. Each leaf arena consisted of an uninfested trifoliolate leaflet of *P. vulgaris* placed upside down on filter paper covering a moist foam cube (8 x 8 x 6 cm). The foam cube was kept in a plastic box (10

x 10 x 6 cm) half-filled with tap water. The leaf arenas were stored in an environmental chamber at 25 ± 1 °C , 60 ± 5 % relative humidity and 16 : 8 h light/dark.

2.3 Rearing of *Phytoseiulus persimilis*

Adult females of the predatory mite *P. persimilis* were needed to create graded stress levels for the adult spider mite females during egg production (giving rise to the experimental animals) and for the experimental animals during testing. To this end, a *P. persimilis* colony was established on a detached leaf arena consisting of a primary leaf of common bean resting upside down on a filter paper covering a moist foam cube kept in a plastic box (14 x 14 x 5 cm). The plastic box was half-filled with tap water and was placed itself into a larger plastic box half-filled with water and some dish-washing liquid to reduce the surface tension (Figure 3). The water served as a barrier preventing the mites from escaping. About 15 gravid female predatory mites were transferred from the base population of *P. persimilis* to the arena and fed every second day with mixed spider mite stages brushed onto the arena. This predatory mite arena was kept in an environmental chamber at 25 ± 1 °C , 60 ± 5 % relative humidity, and 16 : 8 h light/dark (Figure 3).



Figure 3: Leaf arena used to rear adult females of *Phytoseiulus persimilis* needed to create graded risk (stress) levels for spider mites.

2.4 Experiments

To assess how maternal stress during egg production affects the behaviour and life history of spider mite offspring during their juvenile and adult phase, two experiments were performed. In both experiments, experimental animals derived from four types of mothers exposed to graded stress levels. To this end, mother spider mites were held on one of four types of arenas representing differently stressful environments. Stress was defined by the risk associated with cues of the predatory mite *P. persimilis* on the arenas.

2.4.1 Production of differently stressed spider mites

Juvenile and adult spider mites, to be used in experiments, had to be produced by mothers held on four types of arenas (ca. 6 x 6 cm) with graded stress levels: (1) high stress – *P. persimilis* traces, killed spider mites and live *P. persimilis*, (2) medium stress - *P. persimilis* traces and killed spider mites, (3) low stress – only *P. persimilis* traces, and (4) no stress (control group) – no cues of *P. persimilis* and no killed spider mites. Leaf arenas type (1) and (2) first received mixed life stages of spider mites. Second, three gravid *P. persimilis* females were randomly picked from the stock colony and transferred to each leaf arena of type (1), (2) and (3). Leaf arena type (4) was left without any spider mites and predators (control group). Third, after storing the leaf arenas for 20 h in an environmental chamber (25 ± 1 °C , 60 ± 5 % relative humidity, 16 : 8 h L:D), the predatory mites were removed from arena types (2) and (3) and all arenas were ready to receive adult spider mite females. Six adult female spider mites, randomly chosen from the stock population, were transferred to each arena. Before preparing arena type (1) it was important to determine an adequate ratio between predatory mite females and spider mites. On one hand it was necessary, that the adult female spider mites experienced stress caused by physical presence of *P. persimilis*, on the other hand it was necessary to offer enough prey to the predators, so that the adult spider mites themselves were not killed (Figures 4 and 5).

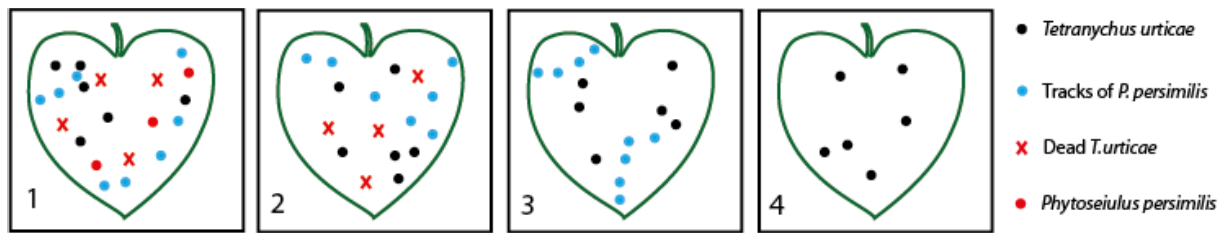


Figure 4: Characteristics of leaf arenas used to establish graded stress levels for *T. urticae* mothers

After being placed on the arenas, the spider mites had to experience their graded stress environments for 40 to 48 h. Arenas were stored in environmental chambers at $25 \pm 1^\circ\text{C}$, $60 \pm 5\%$ relative humidity, and 16: 8 h light/dark. Thereafter, the spider mite females were transferred to new clean leaf arenas for 3 to 4 h for oviposition of eggs giving rise to the experimental animals. Transfer to new clean arenas was needed to avoid that the eggs used for experiments were externally contaminated by cues of the predators or cues of killed conspecifics. After about 4 d, before eclosion, the eggs were transferred from the oviposition arenas to the experimental arenas. Each experimental arena ($\sim 2 \times 6$ cm) consisted of a rectangular leaf arena bearing traces of the predator *P. persimilis* or not (Figure 6). To create leaf arenas, trifoliolate bean leaves were placed upside down on filter paper on moist foam cubes kept in plastic boxes (10 x 10 x 6 cm) half-filled with water. Arenas were delimited by strips of moist tissue paper. To create arenas with *P. persimilis* traces, single adult *P. persimilis* females were placed on the arena for 16 h before the experiment and then removed again. In total, 48 arenas each were arranged with and without *P. persimilis* traces. In each series, twelve eggs from each of the four treatments 1, 2, 3 and 4 were used, of which 6 were singly placed on arenas with predator traces and the other 6 on arenas without any predator cues (Figure 6).



Figure 5: Leaf arenas used to expose the adult spider mite females to graded stress levels.



Figure 6: Experimental leaf arenas with or without *P. persimilis* traces.

2.4.2 Experiment 1

The first experiment examined the developmental time (from egg to larva, protonymph, deutonymph, and adult), position on the leaf (close to veins or on the leaf blade), movement (yes/no) and sex of *T. urticae*, emerging from the eggs of females exposed to graded stress levels. Juvenile spider mites from all four maternal environments were held on arenas with predator cues and on clean arenas without predator cues. Each spider mite egg was singly

placed on a small arena (2 x 6 cm) representing one replicate. Eight different treatments (defined by the combination of maternal stress and individual experience on arena with/without predator traces) were distinguished: (1) *T. urticae* egg from heavily stressed mother (type 1) on arena without predator cues, (2) *T. urticae* egg from heavily stressed mother (type 1) on arena with predator cues, (3) *T. urticae* egg from moderately stressed mother (type 2) on arena without predator cues, (4) *T. urticae* egg from moderately stressed mother (type 2) on arena with predator cues, (5) *T. urticae* egg from little stressed mother (type 3) on arena without predator cues, (6) *T. urticae* egg from little stressed mother (type 3) on arena with predator cues, (7) *T. urticae* egg from unstressed mother (type 4) on arena without predator cues, (8) *T. urticae* egg from unstressed mother (type 4) on arena with predator cues (Figure 7).

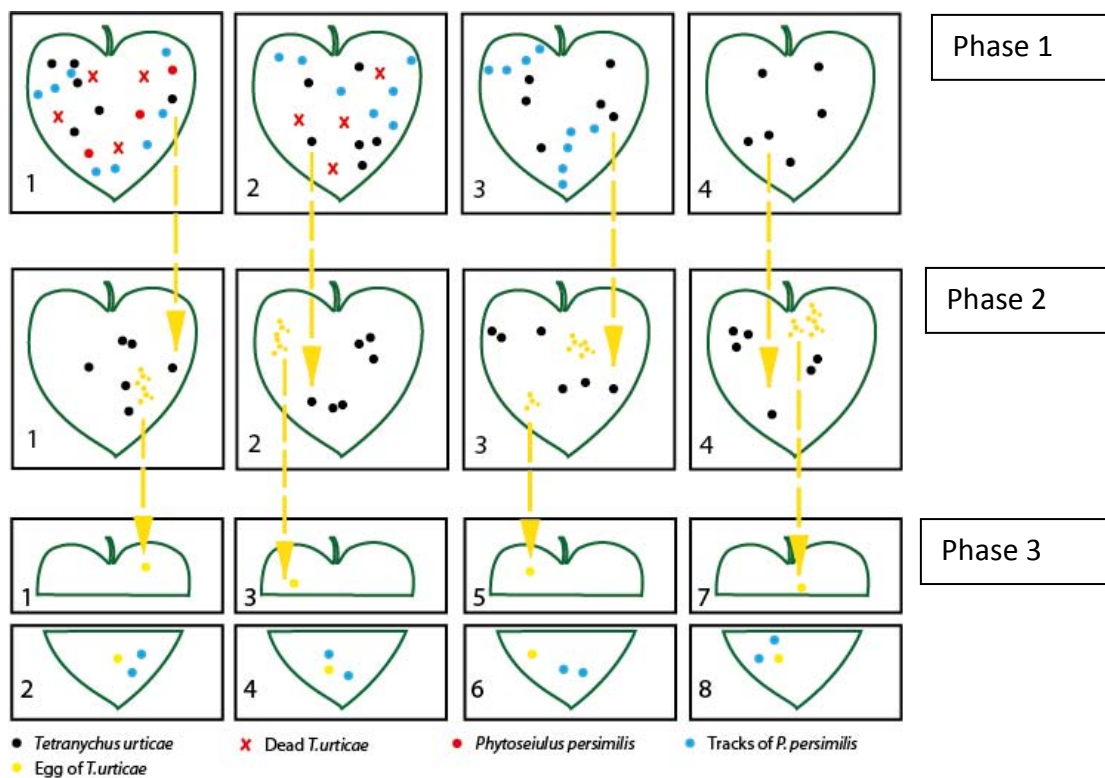


Figure 7: Characteristics of the creation of the eight different experimental treatments. In phase 1 the mothers were exposed to graded stress levels; in phase 2 they were transferred to clean leaves to lay eggs giving rise to experimental animals; in phase 3, representing the experimental phase, the experimental animals were singly held on leaf arenas with or without predator traces.

Each treatment was replicated 18 times. Starting with the day of eclosion the experimental animals were observed twice a day with 8 and 16 h intervals. After reaching the protonymphal stage all animals were transferred to fresh arenas with or without predator cues, because the predator cues are partially volatile and may thus decrease over time. Monitoring stopped when the spider mites had reached adulthood and their sex was determined. During the whole experiment the experimental arenas were stored in an environmental chamber at 25 ± 1 °C, 60 ± 5 % relative humidity, and 16 : 8 h light/dark.

2.4.3 Experiment 2

The second experiment assessed the behaviour and life history of adult female spider mites, which emerged from the eggs of females exposed to graded stress. At each observation the position of the adult female (close to veins or on leaf blade), mobility (stationary/moving), the place of egg deposition, and the quantity of eggs were recorded. Egg number and site were drawn on sketches on paper, which were then used to determine an aggregation index per laid egg. In contrast to the first experiment, all experimental females were raised together with males till the adult phase on clean leaf arenas without any stress, i.e. in absence of any cues associated with predation risk, and then singly transferred to experimental arenas. The eight treatments were the same as those described for experiment 1. Each adult female spider mite held on a small arena (2 x 6 cm) represented one replicate. Each treatment was replicated at least 7 times. In the first hour after placing the spider mite females on the arenas, they were monitored every 10 min. During the ensuing 2 h the observations were carried out every 20 min. After 24 h the arenas were observed again and the full amount of eggs was determined. The proportional area of each leaf arena used for oviposition divided by the number of eggs present on the arena, represented the aggregation index per laid egg.

2.4.4 Statistical analyses

SPSS Version 20 was used for all statistical analyses.

In the first experiment, the influence of maternal stress types (four types) and individual experience (on arena with/without predator cues) on total developmental time of spider mites was analyzed by a generalized linear model (GLM; normal distribution, identity link function). For subsequent pairwise comparisons between maternal stress levels least significant difference (LSD) tests were used. Similarly, the position of the mites on the leaf (close to veins or on the leaf blade) and the sex ratio as affected by maternal stress and individual experience (on arena with/without predator cues) were investigated by generalized linear models (GLM; binomial distribution, logistic-link function). Activity of the mites over time (used as inner subject variable) as influenced by maternal stress level and individual experience was analyzed by means of generalized estimating equations (GEE; binomial distribution, logistic link function).

In the second experiment, the influence of maternal stress type (four types) and individual experience (on arena with/without predator cues) on the position (on leaf blade or close to the leaf veins) of the *T. urticae* females over time (used as inner subject variable) was assessed by generalized estimating equations (GEE; binomial distribution, logistic link), and their activity (moving/stationary; data aggregated before analysis) by generalized linear models (GLM; binomial distribution, logit link). To compare the timing of the first egg laid (normal distribution, identity link) and the total number of eggs laid (Poisson distribution, log-link function), as influenced by maternal stress level and individual experience (on arena with/without predator cues), generalized linear models (GLMs) were used. Similarly, the effect of maternal stress type (four types) and individual experience (on arena with/without predator cues) on the aggregation index per laid egg was evaluated by a generalized linear model (GLM; normal distribution, identity link function).

3 Results

3.1 Experiment 1

3.1.1 Time of development

The total time of development was significantly affected by the maternal stress level (severe, medium, low or no stress) (Wald $\chi^2 = 13.286$, $df = 3$, $P = 0.004$). *T. urticae* from mothers held without stress had a significantly shorter developmental time on both leaf treatments (no cues or with cues) than individuals coming from the other three maternal groups (low, medium, severe stress). There was no evidence that the developmental times of the spider mites differed between leaves with and without predator cues (Wald $\chi^2 = 1.740$, $df = 1$, $P = 0.187$). However, GLM indicated that also the interaction between the maternal stress levels and individual stress was significant (Wald $\chi^2 = 10.127$, $df = 3$, $P = 0.018$). The pairwise comparisons (LSD) revealed that groups 1 (severe stress), 2 (medium stress) and 3 (low stress) did not differ among each other but all differed from group 4 (no stress) (Figure 8).

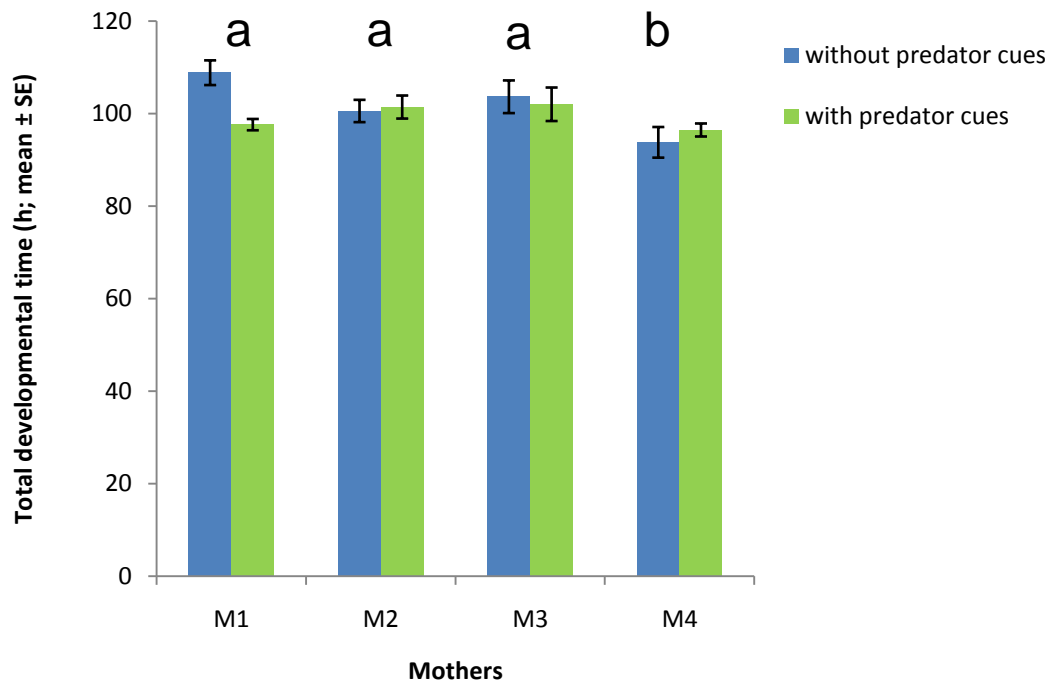


Figure 8: Time of development (egg to adult) of *T. urticae*, as influenced by maternal stress levels (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress) held on leaves with or without cues of *P. persimilis*. Different letters on top of pairs of bars indicate significant differences among the four maternal stress levels ($P < 0.05$; LSD tests following GLM).

3.1.2 Activity across time

There was a significant difference between the spider mites held on leaves with or without predator cues (Wald $\chi^2 = 5.058$, $df = 1$, $P = 0.025$). All in all, the mites were more active on leaves with than without cues. In contrast, maternal stress levels (Wald $\chi^2 = 1.313$, $df = 3$, $P = 0.726$) and the interaction of the two factors (Wald $\chi^2 = 0.081$, $df = 3$, $P = 0.994$) did not influence offspring activity (Figure 9).

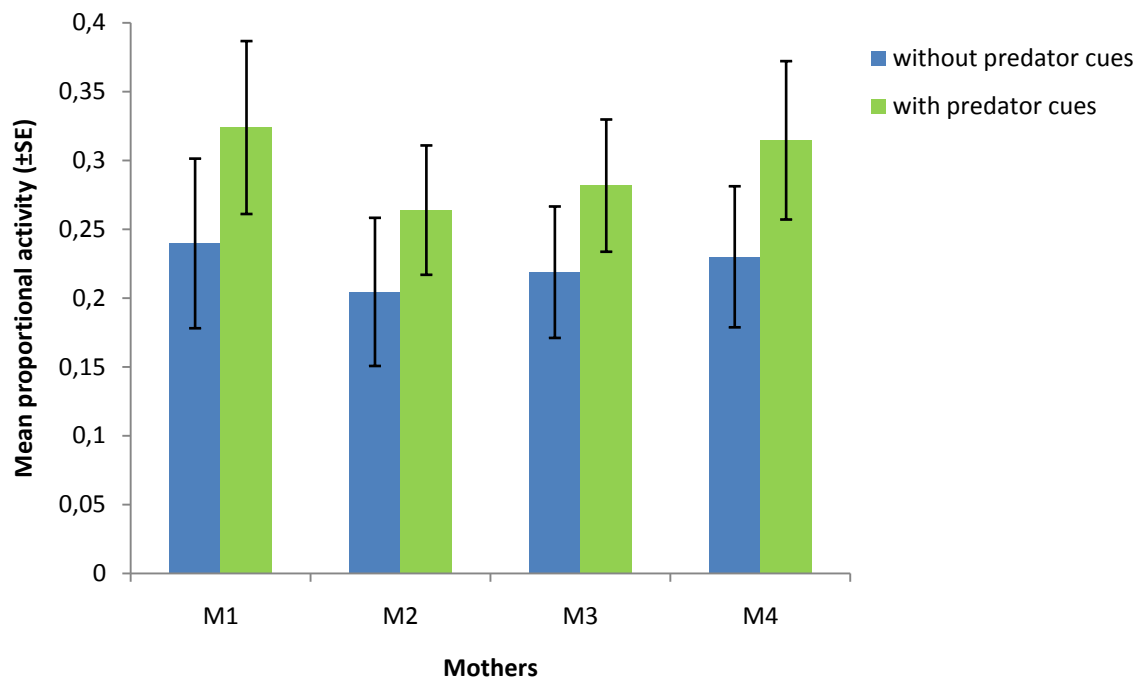


Figure 9: Mean proportional activity of *T. urticae* juveniles, as influenced by four types of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress), on leaves with or without *P. persimilis* cues during development.

3.1.3 Position on leaf over time

There was no statistical evidence that the position preferences of *T. urticae* juveniles - to sit on the vein or on the leaf blade – were influenced by maternal stress levels (Wald $\chi^2 = 3.212$, $df = 3$, $P = 0.360$) or individual experience – on leaf with or without predator cues (Wald $\chi^2 = 0.494$, $df = 1$, $P = 0.482$) or their interaction (Wald $\chi^2 = 3.533$, $df = 3$, $P = 0.316$) (Figure 10). The position preferences developed similarly over time, both among maternal stress levels (Wald $\chi^2 = 2.695$, $df = 3$, $P = 0.441$) and between individual experiences (Wald $\chi^2 = 1.723$, $df = 1$, $P = 0.189$).

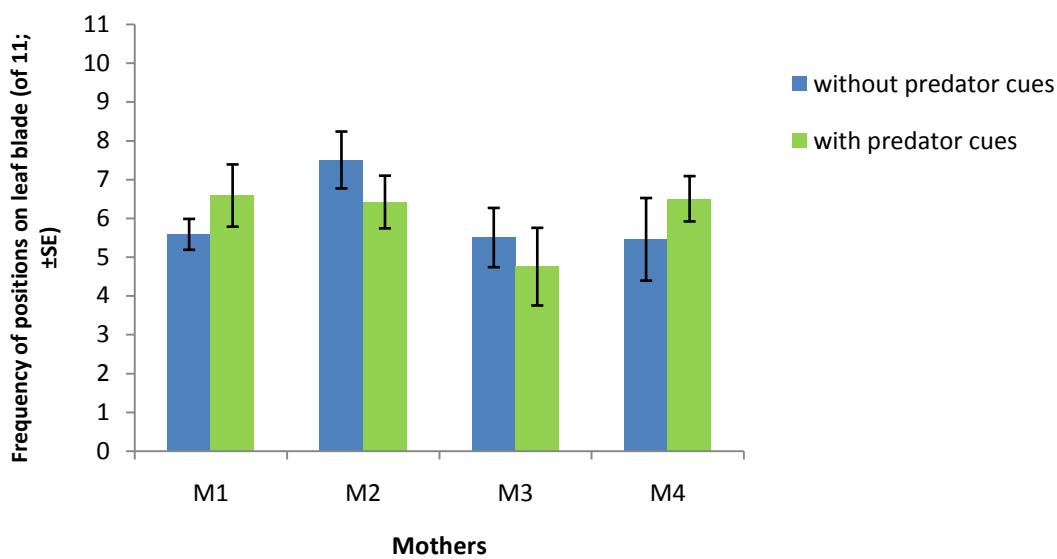


Figure 10: The number of positions on the leaf blade (out of total 11 observations per individual) of *T. urticae* juveniles, as influenced by four types of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress) on leaves with or without *P. persimilis* cues during development.

3.1.4 Sex ratio

GLM revealed no effect of maternal stress levels (Wald $\chi^2 = 2.180$, $df = 3$, $P = 0.536$) and individual experience (on leaf with or without predator cues) (Wald $\chi^2 = 1.322$, $df = 1$, $P = 0.250$) and their interaction (Wald $\chi^2 = 4.326$, $df = 3$, $P = 0.228$) on the offspring sex ratio (Figure 11).

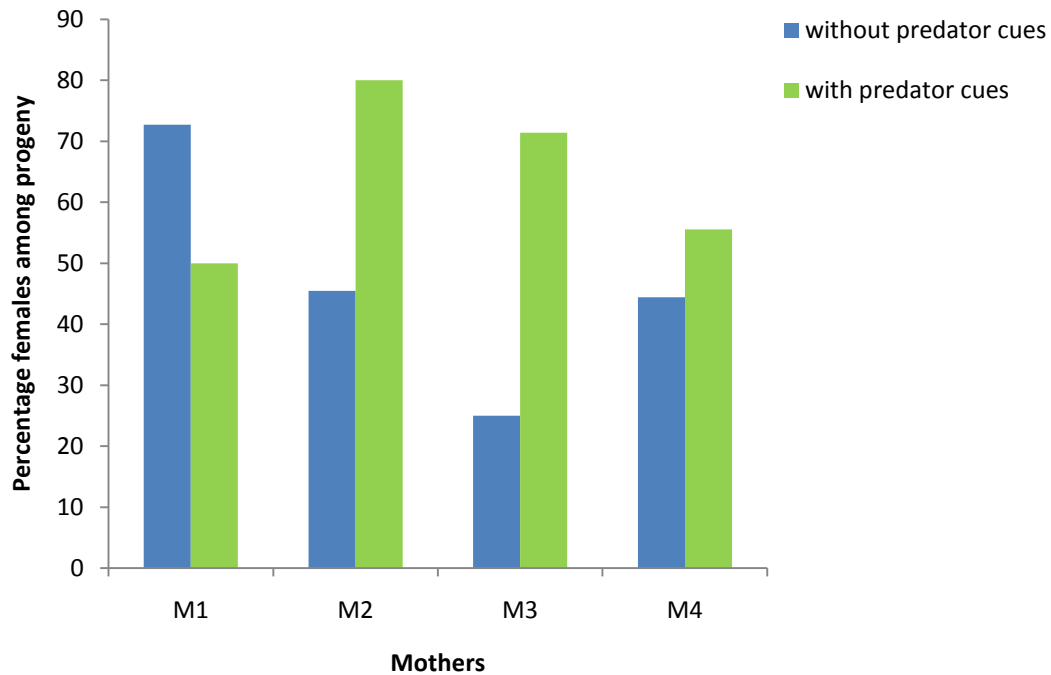


Figure 11: Percentage of females among progeny of *T. urticae* as influenced by four types of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress) and held on leaves with and without predator cues.

3.2 Experiment 2

3.2.1 Oviposition within 24 hours

Oviposition by *T. urticae* within 24 h was not affected by maternal stress levels (severe, medium, low or no stress) (Wald $\chi^2 = 3.307$, $df = 3$, $P = 0.347$) but by individual experience (on leaf with or without *P. persimilis* cues) (Wald $\chi^2 = 5.013$, $df = 1$, $P = 0.025$). The spider mite females produced more eggs on arenas without than with predator cues. There was no significant interaction between the maternal stress levels and individual experience (Wald $\chi^2 = 6.338$, $df = 3$; $P = 0.096$) (Figure 12).

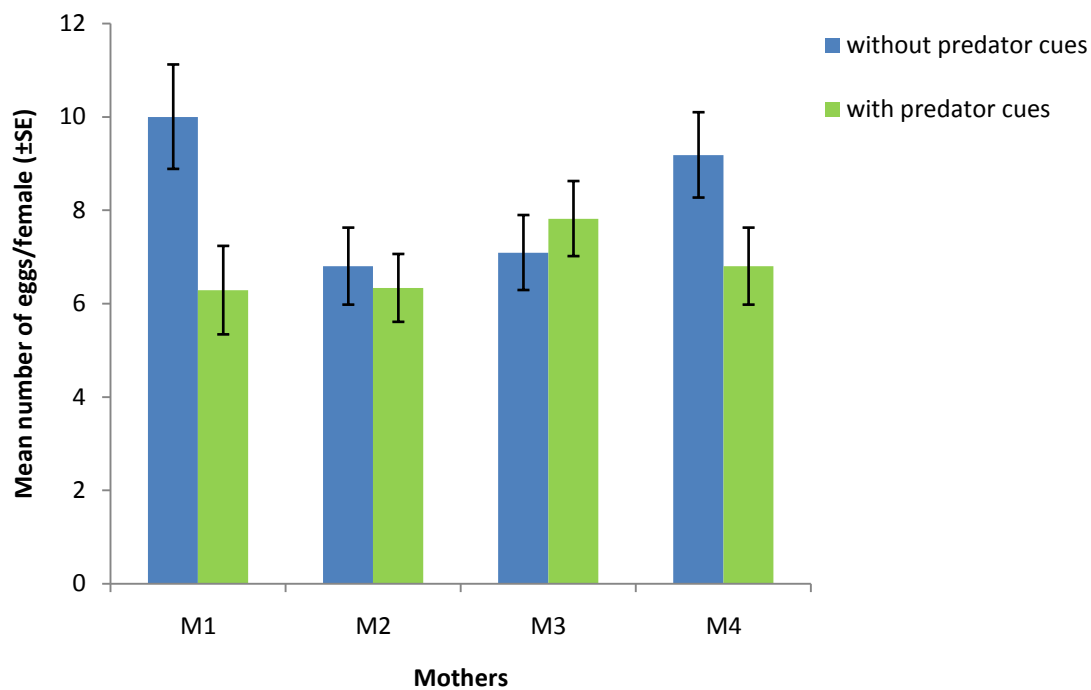


Figure 12: The total number of eggs laid by single adult *T. urticae* females, as influenced by maternal stress levels (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress), on leaf arenas with or without *P. persimilis* cues within 24h.

3.2.2 Time of first egg

Maternal stress levels (severe, medium, low or no stress) did not affect the timing of the first laid egg (Wald $\chi^2 = 0.978$, $df = 3$, $P = 0.807$). In contrast, there was a marginally significant effect of individual experience (on leaf with or without *P. persimilis* cues) on the time of the first egg laid (Wald $\chi^2 = 3.057$, $df = 1$, $P = 0.080$). Spider mites on leaves without predator cues laid their first egg earlier than those on leaves with cues. There was no significant interaction between the maternal stress levels and individual experience (Wald $\chi^2 = 2.555$, $df = 3$, $P = 0.465$) (Figure 13).

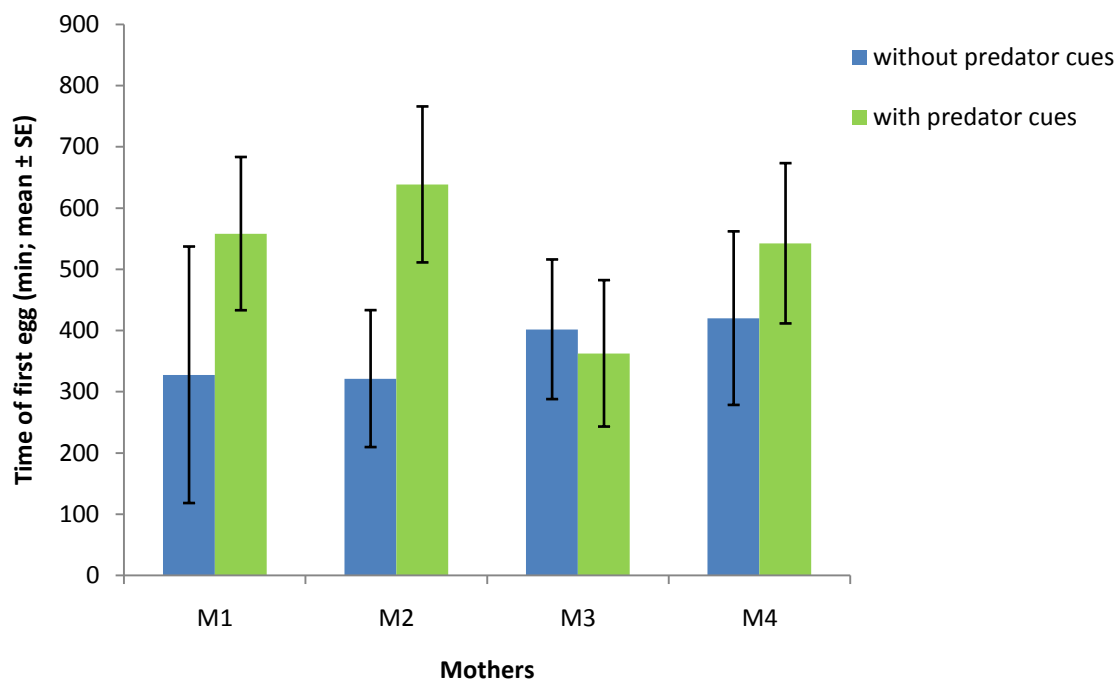


Figure 13: Timing of the first egg laid by *T. urticae* females, as influenced by four maternal stress levels (M1= high stress, M2 = medium stress, M3 = low stress, M4 = no stress), held on arenas with or without predator cues.

3.2.3 Activity

There was no significant influence of maternal stress level (severe, medium, low or no stress) (Wald $\chi^2 = 2.632$, $df = 3$, $P = 0.452$) and individual experience (on leaves with or without predator cues) (Wald $\chi^2 = 1.131$, $df = 1$, $P = 0.288$) on *T. urticae* activity (aggregated). There was also no significant interaction between maternal stress levels and individual experience (Wald $\chi^2 = 4.253$, $df = 3$, $P = 0.235$) (Figure 14).

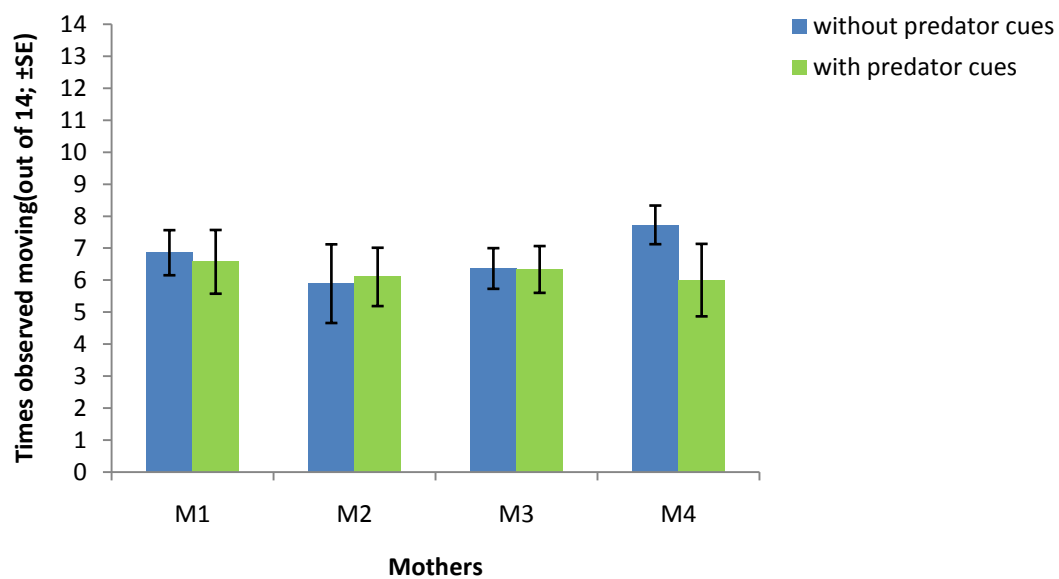


Figure 14: Cumulative activity (times observed moving) of *T. urticae* females, as influenced by four types of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress), held on leaves with or without predator cues.

3.2.4 Position over time

GLM did not indicate any influence of maternal stress levels (Wald $\chi^2 = 1.935$, $df = 3$, $P = 0.586$) and individual experience (on leaf with or without predator cues) (Wald $\chi^2 = 0.717$, $df = 1$, $P = 0.397$) on position (close to veins or on leaf blade) of *T. urticae* females. Also none of the interactions, between maternal stress levels and individual experience (Wald $\chi^2 = 2.173$, $df = 3$, $P = 0.537$), between time and maternal stress levels (Wald $\chi^2 = 0.288$, $df = 3$, $P = 0.962$) or between time and individual experience (Wald $\chi^2 = 0.176$, $df = 1$, $P = 0.675$), was significant (Figure 15).

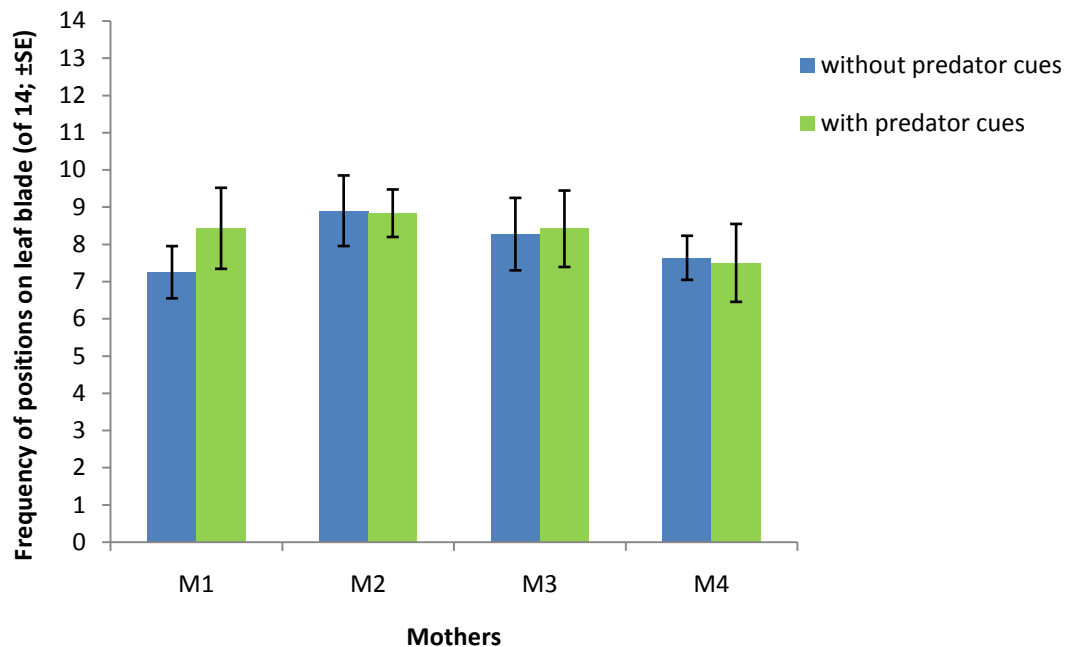


Figure 15: Frequency of positions on the leaf blade (out of 14) of *T. urticae* females, as influenced by four types of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress) on leaves with or without *P. persimilis* cues.

3.2.5 Aggregation index per laid egg

The maternal stress levels marginally influenced the egg aggregation index of *T. urticae* (Wald $\chi^2 = 7.368$, $df = 3$, $P = 0.061$), whereas individual experience (on leaves with or without predator cues) did not have an influence (Wald $\chi^2 = 1.453$, $df = 1$, $P = 0.228$). There was also no significant interaction between maternal stress levels and individual experience (Wald $\chi^2 = 2.197$, $df = 3$, $P = 0.533$) (Figure 16).

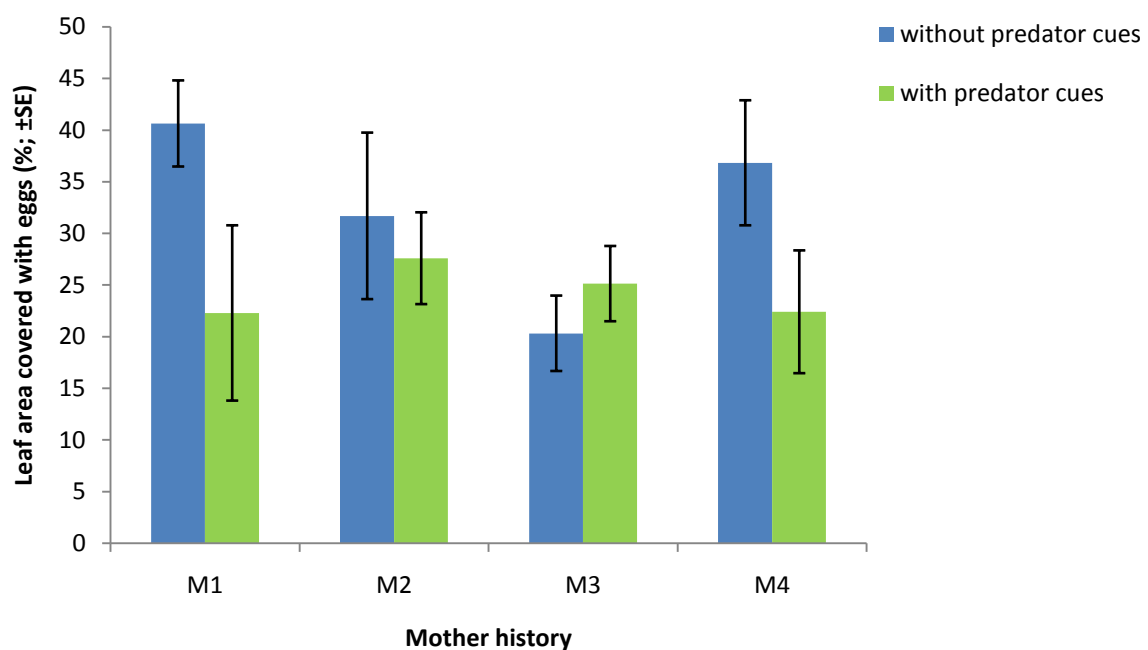


Figure 16: The percentage of leaf area used for oviposition by *T. urticae*, as influenced by four levels of maternal stress (M1 = high stress, M2 = medium stress, M3 = low stress, M4 = no stress) on leaves with or without *P. persimilis* cues over 24 h.

4 Discussion

Perception of environmental cues and signals, correct recognition of natural enemies and the appropriate behavioural response is essential for successful anti-predator behaviour and survival of prey (e.g., Grostal and Dicke, 1999). Different animals evolved different strategies to avoid being detected by or defend themselves against predators. Morphological, physiological, life historical and also behavioural anti-predator strategies have been studied (Kats and Dill, 1998). In general, anti-predator responses can be innate but can also be learned and sometimes both factors can work together (e.g., Walzer and Schausberger, 2011). Since the study by Grostal and Dicke (1999) we know that the study animals of my thesis, the spider mites *Tetranychus urticae*, innately recognise their enemies, predatory mites, and their cues, respectively, and respond accordingly. Hackl and Schausberger (2014) recently showed that the anti-predator behaviours of spider mites can be modulated by learning. In general, both innate and learned responses to predation risk can also be influenced by maternal effects (Bernardo, 1996; Stratman and Taborsky, 2014). When predation risk triggers maternal effects it is possible that the allocation of resources within the offspring changes, which in turn can influence their development. Maternal effects are often adaptive but can also result in a mismatch regarding the adjustment of offspring, when the environment of the mother differs from the environment of the offspring (Coslovsky and Richner, 2012).

Adaptive as well as maladaptive and non-adaptive maternal effects induced by predation risk have been observed in insects (Mousseau and Fox, 1998), fish (Roche et al., 2012) and reptiles (Robert et al., 2009) but there are no such studies for spider mites. For predatory mites it is known that the maternal diet during egg production may lead to maternal effects affecting the foraging behaviour of offspring (Peralta-Quesada and Schausberger, 2012; Ambichl, 2013). Anti-predator behaviours commonly influence food acquisition and utilization (Werner and Peacor, 2003).

To the best of my knowledge, my study is the first focusing on maternal effects induced by predation risk in mites. In detail, I asked if a risky, stressful environment, due to the presence

of predators, induces maternal effects in the herbivorous spider mite *Tetranychus urticae*. The main aim of my study was to find out if maternal experience with the predator *Phytoseiulus persimilis* influences the anti-predator behaviour and life history of the spider mites' offspring in the juvenile and adult phase.

Experiment 1

In the first experiment, I compared four groups of juvenile *T. urticae* derived from three groups of mothers exposed to graded stress levels, and from one control group of unstressed mothers, for their behavioural and life historical changes, on leaf arenas with and without predator cues. I investigated the time of development, the preferred positions on the leaf over time, and the activity levels over time. Most strikingly, I found a significant difference in developmental time between offspring from the three stressed groups and offspring from the unstressed group of spider mites. Time of development was quicker for offspring of unstressed *T. urticae* mothers, independently if the offspring developed on leaves with or without any predator cues. This is similar to the findings by Saino et al. (2005) on barn swallows, which showed that mothers exposed to threatening conditions lay eggs with an elevated concentration of a stress hormone retarding growth of offspring.

Activity over time only differed between the habitats with and without predator cues - mites were more active on leaves harbouring predator cues - but was not influenced by maternal predation risk. This result suggests that elevated activity levels represent an innate anti-predator response of the spider mites to chemical cues from predators. This is in accordance with the findings by Škaloudová et al. (2007), Fernández-Ferrari and Schausberger (2013) and Hackl and Schausberger (2014) that increased walking activity is a characteristic anti-predator response of the spider mites to chemical cues of their predator *P. persimilis*, indicating attempts to escape and leave the risky sites.

I did not observe any effect of maternal stress on position preference over time. The offspring did not change their preferences concerning the leaf blade or the vein, independent of the leaf treatment, with or without predator cues. In contrast, Oku and Yano (2008) found that quiescent females of the related spider mite *T. kanzawai* preferred staying

near veins without predators but tended to reside more likely on the leaf blade if there were predators present.

Maternal stress did also not have an effect on the offspring sex ratio of *T. urticae*. Spider mite females are generally able to adjust the offspring sex-ratio and shift it towards more males in unfavourable environments (for example, Jackson and Martin, 2010). Offspring sex can be influenced by biotic factors (food quality, density of conspecifics), abiotic factors (temperature, photoperiod, pH) and time and place of oviposition. Some species like *T. urticae* are able to manipulate the sex of their offspring due to arrhenotokous parthenogenesis (Helle and Pijnacker, 1985a). Fertilized eggs become diploid females and unfertilized eggs become haploid males. Because of mating delays and locale mate competition *T. urticae* has a female-biased sex ratio (male:female, 1:2 to 1:9) (Macke et al., 2010; Jackson and Martin, 2010). The Trivers-Willard hypothesis says, whenever there is a better environmental situation for one sex, this sex should be better provisioned with resources than the other sex (Jackson and Martin, 2010). Reasons for a shift towards males under unfavourable conditions could be that male *T. urticae* are smaller, are more dispersive, move more quickly, can flee faster and thus reach new safe habitats more easily than females. It may be that in my experiments the length of exposure of the mother mites to stressful conditions was too short to induce offspring sex-ratio adjustments.

Previous studies on spider mites researched similar anti predator-strategies as examined in my study but there is no previous study on spider mites that looked for a maternal influence on offspring anti-predator strategies. Regarding the detailed maternal effects, I will thus discuss my findings in relation to other topically relevant studies in this field with other animals. As mentioned above, my first experiment shows that maternal predation risk had an effect on development of spider mite offspring. Most of the known researches about maternal impacts on offspring morphology/life history measured offspring weight and/or body size. For example, Saino et al. (2005) examined the influence of maternal predation risk on egg quality of barn swallows *Hirundo rustica*. Due to stress exposure the level of the hormone glucocorticosteroid increased in egg-producing females. This hormone can be passed from the mother to her offspring and can have adverse effects on egg hatchability, juvenile growth and development of plumage. Saino et al. (2005) exposed one nest with a

female barn swallow to a predator (stuffed cat) and the other one to a non-predacious control animal (rabbit) and repeated the exposures whenever a new egg was laid. When the fourth egg appeared they were collected for analysis of corticosterone concentration. Saino et al. (2005) found out that exposure to cats raised the corticosterone concentration in the eggs compared to the rabbit exposure and high corticosterone levels are known to negatively influence bird development. The studies by McCormick (2009) showed a similar phenomenon in damselfish. McCormick (2009) revealed that female *Pomacentrus amboinensis* produce higher levels of the stress hormone cortisol when they experience predation pressure by con- or hetero-specific individuals. Also in this case cortisol was an important mediator of life history variability. This hormone influenced the developmental rhythm of the fish embryos, allowed to allocate less endogenous energy to growth and therefore resulted in larvae experiencing higher mortality and being smaller at time of hatching. Similar to these findings on birds and fish, in my experiments, maternal predation risk may have influenced the hormonal and nutritional composition of *T. urticae* eggs, which led to slower development and thereby extended the length of time being in the vulnerable juvenile stages. Detailed separate examination of the different juvenile developmental stages of the spider mites (larvae, protonymphs, deutonymphs) showed that a difference in offspring developmental time was only visible until the protonymphal stage but not in deutonymphs. In the later phases of development the offspring might thus have compensated their initial disadvantages by increased food intake, suggesting that the maternally influenced differences in the early developmental stages may weaken or disappear with increasing age.

The behavioural anti-predator strategies monitored in my work were activity and choice of position on the leaf, neither of which was influenced by maternal effects. However, presence of predator cues changed offspring activity. The rate of activity was higher for offspring on leaf arenas with than without direct predator cues. Škaloudová et al. (2007) examined if predation risk changes the activity of adult spider mite females. During their experiment, female spider mites were placed on two discs cut from a single bean leaf. One was a clean leaf disc used as a control and the other disc harboured predator cues, because it had been previously exposed to a *P. persimilis* female for 24 h. The method was pretty similar to the

one used in my experiment with the exception that I used larger leaf arenas. The results of Škaloudová et al. (2007), albeit with a different life stage, are similar to my findings that *T. urticae* increases its activity on leaves with predator cues. More recently, Fernández-Ferrari and Schausberger (2013) and Hackl and Schausberger (2014), using more or less the same experimental setup and same population of spider mites as in my experiments, reported similar observations. Kats and Dill (1998) explained in their review that many anti-predator behaviours such as fleeing may be induced by chemical predator cues, which often leads to increased running speed or higher overall activity levels. The spider mites apparently linked the perceived chemical cues with the presence of predators and hence a dangerous environment. Therefore, they wanted to escape from the risky environment and move away from the predator cues. In my experiment, the mites did not have the chance to escape because of the surrounding water barrier. Hence, they scanned the habitat as much and as quickly as possible to find out if there is an exit or a safe place or not. In any case, predation pressure must have been interpreted as high by the spider mites because I repeatedly observed drowned mites in the treatment with predator cues.

Experiment 2

In the second experiment, I assessed if female offspring from the four differently stressed mothers are still influenced by maternal effects in their adult stage when exposed to cues of predators or not. I examined the following characteristics: oviposition within 24 h, time of first egg laid, activity over time, position over time and an egg aggregation index. I found no impact of maternal effects on the time of first egg and the amount of eggs within 24 h. However, leaf treatment (with or without predator cues) resulted in a significant difference in oviposition behaviour, with more eggs laid earlier on leaves without any predator cues. This is similar to the recent study by Fernández-Ferrari and Schausberger (2013), which also showed that *T. urticae* deposit their first egg later and produce fewer eggs on leaves with predator cues of *P. persimilis*. Along the same line, Škaloudová et al. (2007) and Grostal and Dicke (1999) placed spider mite females on leaf discs with graded predation risk levels and watched their oviposition behaviour for 96 h. They found out that the higher the risk level the lower the oviposition rate of the spider mites. In contrast to my work, these two studies did not include and examine maternal effects. Proximately, constantly searching for safer

sites, increased fleeing tendencies and therefore reduced food intake are energetic costs that could possibly lower the amount of produced eggs. Ultimately, mothers preferentially deposit their eggs in safe environments to enhance their survival chances and thus increase their fitness. The lacking maternal effects in oviposition behaviour could be due to weakening of maternal effects with increasing age. Maternal effects were most pronounced in the early stages of development, when the spider mites are most vulnerable to predation, but later on weakened due to individual effects during ontogeny. During ontogeny, maternal effects can generally vary in magnitude and duration (Bernardo, 1996). For example, in the salamander *Ambystoma taploideum* offspring of stress-experienced mothers grew smaller than the control group till day 129 but this initial difference in size disappeared during later development, probably due to compensatory growth (Bernardo, 1996; Semlitsch and Gibbons, 1990).

The activity of the adult females was neither influenced by maternal effects nor by presence of predator cues. This is interesting because the first experiment revealed that juvenile offspring do react to predator cues on leaves with increased activity. Like in the first experiment position over time was neither influenced by maternal effects nor by presence of predator cues. It could be that the perceived risk of predation to cues alone, without physical predator presence, was too weak to influence the spider mites' choice of position. Possibly, living predators, like in the experiment of Oku and Yano (2008) would have been needed to trigger a change in the position preference of *T. urticae*. Alternatively, it could be that the whole experimental leaf disc was homogeneously covered by the cues left by the predators, rendering each microsite equally risky. Since also in the first experiment no maternal effects on activity and residence preference were detectable, those two aspects of the spider mites' anti-predator strategies might just not be influenced by maternal effects.

The egg aggregation index was marginally influenced by maternal stress levels. However, this result is difficult to interpret because no clear consistent trend was detectable regarding the intensity of maternal stress and egg aggregation. In general, in many group-living animals including spider mites (Yano, 2012; Clotuche et al., 2014), aggregation is a common strategy to reduce the individual risk of predation. Using a smaller area to deposit eggs could

therefore be a maternal anti-predator strategy in *T. urticae* but this requires further comprehensive investigations.

5 Conclusion

Altogether, I observed a maternal influence on offspring development but not on anti-predator behaviour of both juvenile and adult offspring of *T. urticae*. My experiments revealed that maternal predator experience influences the offspring in their juvenile stages whereas there is little to no influence of maternal predation experience on offspring in their adult stage. A possible explanation is that in the course of development, the offspring's own experiences and compensatory strategies during ontogeny counter-balance the initially acting maternal effects. The extended developmental times of offspring produced by stressed mothers, as compared to unstressed mothers, are unfavourable for the offspring themselves because leaving them longer in the vulnerable juvenile phase and granting delayed availability to potential mates. The long-term benefits/costs trade-off of these effects for the mothers themselves needs further investigations.

6 References

- Ambichl, A. (2013). Maternale Effekte bei der Raubmilbe *Neoseiulus californicus*: Einfluss der Mutternahrung auf die Nahrungspräferenz der Nachkommen. Diplomarbeit Universität für Bodenkultur Wien, 56 p.
- Bale, J. S., van Lenteren, J. C., Bigler, F. (2008). Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B* 363:761-776.
- Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist* 36:83-105.
- Chant, D. A. (1985b). External Anatomy. In: Helle, W., Sabelis, M. W. (1985b). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 5-10 pp.
- Clotuche, G., Yano, S., Akino, T., Amano, H. (2014). Chemical investigation of aggregation behaviour in two-spotted spider mite *Tetranychus urticae*. *Experimental and Applied Acarology* 63:377-387.
- Coslovsky, M., Richner, H. (2012). Preparing offspring for a dangerous world: potential costs of being wrong. *Plos One* 7:e48840.
- Dicke, M. Grostal, P. (2001). Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology* 32:1-23.
- Feiertag-Koppen, C. C. M., Pijnacker, L. P. (1985a). Oogenesis. In: Helle, W., Sabelis, M. W. (1985a). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 117-127 pp.
- Fry, J. D. (1989). Evolutionary adaption to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia* 81:559-565.
- Gerson, U., Smiley, R. L., Ochoa, R. (2003). Mites (Acari) for pest control. *Wiley Blackwell*.

Gerson, U., Weintraub, P. G. (2007). Mites for the control of pests in protected cultivation. *Pest Management Science* 63:658-676.

Grostal, P., Dicke, M. (1999). Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology* 10:422-427.

Gutierrez, J., Helle, W. (1985a). Evolutionary Changes in the Tetranychidae. In: Helle, W., Sabelis, M. W. (1985a). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 91-107 pp.

Hackl, T., Schausberger, P. (2014). Learned predation risk management by spider mites. *Frontiers in Ecology and Evolution* 2:58.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47-58.

Helle, W., Pijnacker, L.P. (1985a). Parthogenesis, Chromosomes and Sex. In: Helle, W., Sabelis, M. W. (1985a). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 129-139 pp.

Helle, W., Sabelis, M. W. (1985a). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*.

Helle, W., Sabelis, M.W. (1985b). Spider mites – their biology, natural enemies and control. World Crop Pests. Vol 1B, *Elsevier Science Publishers, Amsterdam*.

Jackson, D. E., Martin, S. (2010). Sex allocation: size matters for red spider mites. *Current Biology* 20:R 1080.

Kats, L., Dill, L. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361-394.

Kriesch, S., Dicke, M., (1997). Avoidance of predatory mites by the two-spotted spider mite *Tetranychus urticae* the role of infochemicals. *Proc. Exp. App. Entomol* 8:121-126.

Lima, S.L. (1998). Non-lethal effects in the ecology of predator-prey interactions – what are the ecological effects of anti-predator decision-making? *Bioscience* 48:25-34.

Lima, S. L., Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 98:619-659.

Macke, E., Magalhães, S., Khan, H., Luciano, A., Frantz, A., Facon, B., Olivieri, I. (2011). Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society B, Biological Sciences* 278:1054-1063.

McCormick, M. I. (2009). Indirect effects of heterospecific interactions on progeny size through maternal stress. *Oikos* 118:744-752.

McIntosh, A. R., Peckarsky, B. L. (1996). Differential behavioural responses of mayflies from streams with and without fish to trout odour. *Freshwater Biology* 35:141-48.

McMurtry, J.A., Croft, B. A. (1997). Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* 42:291-321.

Mommer, B. C., Bell, A. M. (2013). A test of maternal programming of offspring stress response to predation risk in threespine sticklebacks. *Physiology and Behavior* 122:222-227.

Mousseau, T. A., Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13:403-407.

Navajas, M. (1998). Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae). Insights from molecular phylogeography. *Experimental and Applied Acarology* 22:201-214.

Nelson, E., Matthews, C., Rosenheim, J. (2004). Predators reduce prey population growth by inducing changes in prey behavior. *Ecology* 85:1853-1858.

Oku, K., Yano, S. (2008). Effects on predation risk on mating behavior of the Kanzawa spider mite. *Journal of Ethology* 26:251-266.

- Overmeer, W. P. J. (1985b). Rearing and Handling. In: Helle, W., Sabelis, M. W. (1985b). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 163-170 pp.
- Pallini, A., Janssen, A., Sabelis, M. W. (1999). Spider mites avoid plants with predators. *Experimental and Applied Acarology* 23:803-815.
- Peralta-Quesada, P. C., Schausberger, P. (2012). Prenatal chemosensory learning by the predatory mite *Neoseiulus californicus*. *Plos One* 7:e53229.
- Roche, D. P., McGhee, K. E., Bell, A. M. (2012). Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. *Proceedings of the Royal Society B, Biological Sciences* 8:932-935.
- Robert, K. A., Vleck, C., Bronikowski, A. M. (2009). The effects of maternal corticosterone levels on offspring behavior in fast- and slow-growth garter snakes (*Thamnophis elegans*). *Hormones and Behavior* 55:24-32.
- Sabelis, M. W., (1985b). Predation on spider mites. In: Helle, W., Sabelis, M. W. (1985b). Spider mites – their biology, natural enemies and control. World Crop Pests, Vol 1A, *Elsevier Science Publishers, Amsterdam*, 103-127 pp.
- Sabelis, M. W., Bakker, F.M. (1992). How predatory mites cope with the web of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. *Experimental and Applied Acarology* 16:203-225.
- Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., Møller, A. P. (2005). Stressed mothers lay eggs with high corticosterone levels which produce low quality offspring. *Journal of Experimental Zoology* 303:998-1006.
- Semlitsch, R. D., Gibbons, J. W. (1990). Effects on egg size on success of larval salamanders in complex aquatic environments. *Ecology* 71:1789-1795.
- Shine, R., Downes, S. J. (1999). Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* 119:1-8.

Sih, A. (1982). Foraging strategies and the avoidance of predation by an aquatic insect, *Notonectahoffmanni*. *Ecology* 63:786-796.

Škaloudová, B., Zemek, R., Krivan, V. (2007). The effect of predation risk on an acarine system. *Animal Behaviour* 74: 813-821.

Stratman, A., Taborsky, A. (2014). Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids. *Functional Ecology* 28:944-953.

Van Driesche, R.G., Bellows, T. (1996). Biological Control. *Kluwer Academic Publishers Group*, Dordrecht, Netherlands.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology* 40:563-572.

Van Lenteren, J. C. (2012). IOBC Internet Book of Biological Control, Version 6. <http://www.iobc-global.org/> (accessed 19.11.2014).

Van Lenteren, J. C., Woets, J. (1988). Biological and integrated pest control in greenhouses. *Annual Review of Entomology* 33:239-269.

Walzer, A., Schausberger, P. (2011). Threat-sensitive anti-intraguild predation behaviour: maternal strategies to reduce offspring predation risk in mites. *Animal Behaviour* 88:177-184.

Werner, E., Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083-1100.

Wolf, J. B., Wade, M. J. (2009). What are maternal effects (and what are they not)? *Proceedings of the Royal Society B, Biological Sciences* 364:1107-1115.

Yano, S. (2012). Cooperative web sharing against predators promotes group living in spider mites. *Behavioral Ecology and Sociobiology* 66:845-853.

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9 Summary

Anti-predator behaviour is every behavioural change an animal performs to avoid getting killed by a predator. Recognising the enemy and the appropriate behavioural response is essential for survival. Predator recognition may be either innate, learned or both. Mothers of many animals have the ability to influence offspring predation risk via maternal effects. Maternal effects contribute to shaping the offspring phenotype, represent the influence of the mother's environmental factors but have nothing to do with the offspring's own genotype or its own environmental experiences. Maternal effects can be mechanisms increasing maternal and offspring fitness if they render the offspring's phenotype better suited to its environment. I tested if different levels of predation risk exerted by the predatory mite *Phytoseiulus persimilis* may induce maternal effects in females of the two-spotted spider mite *Tetranychus urticae* and change the life history and/or anti-predator behaviour of the spider mites' offspring in their juvenile and/or adult phase. First, I generated four types of differently stressed mothers by exposing them to various combinations of predator cues and no predator cues, respectively. Direct and indirect cues as well as living predators were used to create environments with differing risks. Then, I conducted two experiments to investigate the life history and anti-predator response of juvenile and adult spider mite offspring on leaf arenas with (dangerous) and without (safe) predator cues. In the first experiment, where I was watching the juvenile offspring, I measured the time of development, activity, position on the leaf and sex. Maternal effects had only an influence on the developmental time, which was shorter in offspring from unstressed mothers than in those from stressed mothers, independently if they developed on leaves with or without predator cues. In the second experiment, I observed behavioural changes of adult female offspring when exposed to predator cues or not but did not detect any significant maternal effects. Oviposition within 24 h, time of first egg laid, activity, position and an egg aggregation index was observed. Altogether, my experiments show that juvenile spider mites are more strongly affected by maternal effects than are adult offspring. My study represents the first documentation of maternal effects induced by predation risk in mites.

10 Zusammenfassung

Anti-Prädations Verhalten ist jede Verhaltensänderung von Beutetieren die darauf abzielt nicht getötet zu werden. Korrektes Erkennen der Räuber und dementsprechende Reaktion bei Prädationsrisiko sind ausschlaggebend für das Überleben der Beute. Erkennen der Räuber ist entweder angeboren, gelernt oder eine Kombination aus beidem. Von vielen Tierarten ist bekannt, dass Mütter das Prädationsrisiko ihrer Nachkommen durch maternale Effekte beeinflussen können. Maternale Effekte können den Phänotyp der Nachkommen verändern. Sie werden durch Umweltfaktoren der Mutter ausgelöst und sind unabhängig vom Genotyp und den eigenen Erfahrungen der Nachkommen. Maternale Effekte können adaptiv sein, wenn sie den Phänotyp der Nachkommen besser an die zukünftige Umwelt anpassen. Ich habe bei der Gemeinen Spinnmilbe *Tetranychus urticae* untersucht, ob Prädationsrisiko, ausgeübt durch die Raubmilbe *Phytoseiulus persimilis*, während der Eiproduktion maternale Effekte auf die Entwicklung und/oder das Anti-Prädations Verhalten der Nachkommen in der Juvenil- und Adultphase auslösen kann. Zuerst wurden vier Gruppen von unterschiedlich gestressten Müttern generiert. Dies erfolgte durch Konfrontation der Mütter mit unterschiedlichen Kombinationen von lebenden Räubern und deren direkten und indirekten Spuren. Daraufhin wurden zwei Experimente durchgeführt, um den Einfluss mütterlicher Effekte auf die Lebenszyklusparameter und das Anti-Prädations Verhalten von juvenilen und adulten Nachkommen auf Blattarenen, mit (gefährlich) und ohne (ungefährlich) Räuberspuren, zu testen. Im ersten Experiment wurden die juvenilen Nachkommen beobachtet. Es wurde die Entwicklungszeit, die Aktivität, die Position am Blatt sowie das Geschlecht untersucht. Maternale Effekte wirkten sich nur auf die Entwicklungszeit aus: Nachkommen von nicht-gestressten Müttern entwickelten sich rascher als jene von gestressten Müttern, unabhängig davon ob sie auf Blättern mit oder ohne Räuberspuren aufwuchsen. Im zweiten Experiment wurde die Veränderung des Anti-Prädations Verhalten von adulten Spinnmilbenweibchen auf Blattarenen, mit und ohne Räuberspuren, erforscht. Hier konnte ich keine signifikanten maternalen Effekte bezüglich der Gesamteiablage, Zeitpunkt der ersten Eiablage, Aktivität, Position und der Eiaggregation beobachten. Zusammengefasst zeigen meine Experimente, dass sich die maternalen Effekte

bei der Spinnmilbe *T. urticae* stärker auf die juvenilen Stadien als auf die adulte Nachkommen auswirken. Meine Studie ist die erste Dokumentation von, durch Prädationsrisiko induzierten, maternalen Effekten bei Milben.

11 Curriculum vitae

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