Diving deep into a tiny world

Effects of drought on the predatory mite *Phytoseiulus persimilis*



Sophie Le Hesran

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This research was conducted under the auspices of the Graduate School for Production Ecology and Resource Conservation.

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Effects of drought on the predatory mite
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Sophie LE HESRAN

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 20 March 2020
at 4 p.m. in the Aula.

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Diving deep into a tiny world – Effects of drought on the predatory mite *Phytoseiulus persimilis*, 146 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2020)
With references, with summary in English

ISBN 978-94-6395-300-9
DOI https://doi.org/10.18174/513144

"PS: please if you get a chanse put some flowers on Algernons grave in the bak yard."

Daniel Keyes, Flowers for Algernon

Dedicated to all my *Phytoseiulus persimilis*

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1

INTRODUCTION

1.1. THE UNIVERSAL SOLVENT

Drinking a glass of water when we are thirsty gives us invaluable comfort and relief: the relief of satisfying a vital need of our body. Water, also called the "universal solvent", is fundamental for the maintenance of life on our planet (Hodkinson et al., 1999; Everatt et al., 2015). Even beyond our planet, in space exploration, water has become a key element in the search for extra-terrestrial life (Franks, 2007). Many biochemical and physiological processes involved in the maintenance and reproduction of living organisms depend on water. As a consequence, drought – lack of water in the environment – is one of the greatest obstacles to the survival of land-living organisms (Alpert, 2006). For an organism, the ability to tolerate dehydration requires the continuous maintenance of adequate levels of body water by carefully balancing water gain and water loss, a relationship called water balance (Yoder, 1998).

1.2. DROUGHT STRESS IN ARTHROPODS

1.2.1. ARTHROPODS AND THEIR FUNCTIONS IN TERRESTRIAL ECOSYSTEMS

With more than a million described species, arthropods represent around 80% of all known animal species. They are invertebrate animals, such as insects, arachnids, crustaceans, centipedes, or millipedes, with an external skeleton (a cuticle made of chitin), a segmented body, and paired jointed appendages. Arthropods are present in every habitat on Earth, be it the equator, the poles, high mountains or deep ocean trenches, and are part of terrestrial and aquatic ecosystems (Chakravarthy and Sridhara, 2016). Terrestrial ecosystems include populations of living organisms (plants, animals, microorganisms) functioning together with their non-living environment on land (soil, water, temperature), through nutrient cycles and energy flows (Odum and Barrett, 1971). Arthropods are essential components of terrestrial ecosystems, in which they have important ecological functions. Herbivores, predators, decomposers, parasites, pollinators, or seed dispersers participate in diverse ecological processes such as nutrient cycling, seed dispersal, bioturbation (burrowing, ingestion and defecation of sediments) and pollination (Maleque et al., 2006; Nichols et al., 2008; Chakravarthy and Sridhara, 2016). The ecological functions of terrestrial arthropods are also significant in agricultural ecosystems, whether through herbivores eating the crops or through beneficial arthropods providing ecological services, such as crop pollination and pest control (Altieri, 1999; Isaacs et al., 2009). For example, several insect species like bees, wasps, butterflies and ants feed on nectar or pollen found in flowers. While insects feed in a flower, pollen grains will get attached to their body, and be 'accidentally' transferred to another flower when they visit the next plant, allowing it to reproduce (Chakravarthy and Sridhara, 2016). Additionally, many arthropod species, including predators and parasitoids, help controlling agricultural pest populations and reducing the need for synthetic pesticides.

1.2.2. WHY ARE TERRESTRIAL ARTHROPODS VULNERABLE TO DROUGHT?

Terrestrial arthropods face a serious problem with respect to drought and water balance, because of their small size (most of them are between 0.1 and 25 mm long), and because

their body has a high surface-area-to-volume ratio. In other words, they have a relatively large surface area through which they lose water that is available in a relatively small volume (Gibbs, 2002; Gefen et al., 2006). They can lose the little water they contain in many ways: cuticular transpiration, respiration, secretion from the mouth and anus, and excretion (Hadley, 1994; Berridge, 2012; Ghazy and Suzuki, 2014; Ghazy et al., 2016).

1.2.3. HOW DO TERRESTRIAL ARTHROPODS DEAL WITH DROUGHT STRESS?

In order to survive drought stress, terrestrial arthropods can increase their drought resistance through desiccation avoidance behaviours or physiological adaptations (Gibbs and Matzkin, 2001; Gefen et al., 2006; Bazinet et al., 2010). Behavioural strategies can be adopted to avoid desiccation, such as drinking from water sources, increasing feeding rate, absorbing water from the atmosphere, burrowing into the ground, or exhibiting nocturnal activity (Cloudsley-Thompson, 1975; Block et al., 1996; Gefen et al., 2006; Berridge, 2012; Ghazy and Suzuki, 2014). Alternatively, physiological adaptations at the organismal level can help resist drought stress via three main mechanisms, not mutually exclusive: (1) an increase in initial body water content, (2) a decrease in water loss rate, for example through changes in cuticular permeability, or (3) an increase in drought tolerance (Gibbs et al., 1997; Gefen et al., 2006; Bazinet et al., 2010). Drought-tolerant organisms have the capacity to tolerate the loss of a high percentage of body water before this loss becomes fatal. Depending on their environment and physiological condition, arthropods can withstand the loss of 17 to 89% of their body water content (Hadley, 1994). In extreme cases, arthropods can survive the loss of almost all body water by putting their metabolism on “standby” (a metabolic state called anhydrobiosis) until rehydration (Block et al., 1996; Gusev et al., 2014). For example, larvae of the midge *Polypedilum vanderplanki* Hinton can survive several years after losing 97% of their body water. Molecules such as Late Embryogenesis Abundant proteins (which protect other proteins from aggregation due to desiccation), trehalose, antioxidants and heat-shock proteins contribute to this extreme drought tolerance (Gusev et al., 2014). In some cases, physiological, behavioural, and morphological adjustments are associated with the onset of diapause: a state of developmental and metabolic interruption allowing some arthropod species to avoid desiccation (Ghazy and Suzuki, 2014). Diapausing arthropods search for protected sites with moderate temperatures and high relative humidity (RH). They also have reduced body water requirements because of a decrease in metabolic rate, and reduced water loss through the accumulation of osmolytes and cuticular hydrocarbons (Benoit, 2010). To conclude, terrestrial arthropods deal with drought stress at the individual level through many behavioural strategies and physiological adaptations. At a higher level, arthropod response to drought may also have an impact on their ecosystem. In agricultural ecosystems, where the ecological functions of arthropods are particularly significant, dry conditions can have serious consequences.

1.3. BIOLOGICAL PEST CONTROL IN DRY CONDITIONS

1.3.1. WHAT IS BIOLOGICAL PEST CONTROL?

Fruit, vegetable, cereal and flower fields can be attacked by many species of herbivorous arthropods, including insects and mites. To maintain their production, farmers need to protect their crops against herbivores. Over the last 75 years, they have relied mainly on chemical pest control (Brodeur et al., 2018). However, the need for alternative methods has become more and more urgent, because of the negative effects of synthetic pesticides on human health and our environment, and because certain pests have become resistant to synthetic pesticides. Biological pest control is a healthier, environmentally and economically sound alternative to chemical pest control (van Lenteren, 2012; Tracy, 2015; Brodeur et al., 2018). Biological control is present and active in all ecosystems, with or without (natural biological control) human intervention. Successfully practiced by humans for centuries, biological control is defined as the use of living organisms (called natural enemies or biocontrol agents) to suppress the population density or impact of a specific pest organism (Eilenberg et al., 2001). Over 1700 years ago, in the year 304, the weaver ant *Oecophylla smaragdina* (Fabricius) was used to protect citrus trees against insect damage in China (Peng, 1983). In biological control, different pests require different natural enemies: invertebrate pest populations can be controlled using predators, parasitoids and pathogens; while weeds can be managed using herbivores and pathogens, and plant pathogens can be suppressed using antagonistic microorganisms (Eilenberg et al., 2001). Natural enemies can be used in three ways: classical biological control (import of exotic species and their establishment in a new habitat), augmentative biological control (mass production of established species and periodic colonization), and conservation biological control (conservation of established species through manipulation of the environment) (DeBach and Rosen, 1991). Although classical biological control has been successful in many cases, the introduction of exotic species in a new habitat may present risks: they may disrupt established local populations (Howarth, 1991). Therefore, stricter regulations have been implemented recently regarding the export, shipment, import and release of exotic biocontrol agents (IPPC, 2005), hindering the use of classical biological control. Conservation biological control is often effective in supporting natural enemy populations, but it remains a challenge to direct biological control services of these populations to achieve suppression of the target pests (Begg et al., 2017). Alternatively, augmentative biological control has evolved rapidly with the development of biocontrol companies, and sales of mass-reared natural enemies (arthropods, nematodes and pathogens) have grown considerably (Collier and Van Steenwyk, 2004). Natural enemy species used in augmentative biological control may be indigenous or exotic. For the first time since 1960, more indigenous than exotic invertebrate natural enemy species were introduced to the European market in the last decade (van Lenteren, 2012).

1.3.2. EFFECTS OF DRY CONDITIONS ON AUGMENTATIVE BIOLOGICAL CONTROL

Despite these recent advances, biological control only represents 1-3% of the worldwide annual turnover of plant protection products nowadays, and augmentative biological control is only applied on about 0.4% of land under cultivation (Cock et al., 2010; Tracy, 2015; Heim-

pel and Mills, 2017; Brodeur et al., 2018). This situation is the result of many factors, such as entrenched commitment to synthetic pesticides, short-term economic interests, concerns about non-target environmental effects (natural enemies attacking non-target species), legislative barriers, relative complexity of use, and the low level of investment in research and development for improving biocontrol agents (Tracy, 2015; Brodeur et al., 2018). Ensuring the efficacy of biocontrol agents in augmentative biological control is not always easy. Their performance can be affected by many abiotic and biotic factors, like unfavourable climatic conditions (heat and drought especially), dispersal of biocontrol agents, predation on biocontrol agents by other predators, presence of synthetic pesticides, plant defense mechanisms, and potential deleterious effects of unwanted breeding selection and inbreeding in mass rearing programs (Collier and Van Steenwyk, 2004; Le Hesran et al., 2019b). Dry conditions, especially, can seriously hamper augmentative biological control, because drought stress is one of the most important factors limiting the efficacy of natural enemies (Van Dinh et al., 1988; English-Loeb, 1990; Bakker et al., 1993; Croft et al., 1993; Stiling, 1993; Collier and Van Steenwyk, 2004). Many aspects of arthropod life can be affected by drought stress: fecundity, developmental time, lifespan, juvenile survival, feeding rate etc. Moreover, depending on the species and the intensity of the stress, natural enemy and pest populations may be affected differently by dry conditions. Consequently, the effects of dry conditions on the success of augmentative biocontrol may be direct, such as actual death of the natural enemy from inability to cope with drought stress, or indirect, like the disruption of the synchrony between pest and predator populations (Stiling, 1993). For example, drought stress may promote herbivore outbreaks and plant damage for several reasons: (1) because biocontrol agents suffer a higher mortality in dry conditions, and therefore struggle to control herbivore populations, (2) because drought-stressed plants are more nutritious for herbivores, having higher nutritional quality and lower defenses, and/or (3) because drought stress reduces plant size, and smaller plants are damaged faster by herbivores (English-Loeb, 1990; Dale and Frank, 2017; Ximénez-Embún et al., 2017). So far, the effects of drought on natural enemy and pest species are not fully understood. Scientists have a major role to play in understanding the weaknesses of biocontrol agents in dry conditions and developing suitable solutions to improve augmentative biological control. A potential solution, which has received more attention recently, is the possibility to improve the performance of biocontrol agents in dry conditions through selective breeding.

1.4. IMPROVEMENT OF BIOCONTROL AGENTS

1.4.1. ARTIFICIAL SELECTION AND EXPERIMENTAL EVOLUTION

Artificial selection, also called selective breeding, has been used for centuries by farmers and breeders, by selecting only the plants and animals with desirable characteristics for reproduction. For example, farmers have artificially selected for specific traits in the wild mustard *Sinapis arvensis* Linnaeus to obtain cauliflowers and broccoli, and dairy cows have been 'improved' genetically to produce more milk. Although they have received less attention to date, biocontrol agents can also be selectively bred. Specific traits such as insecticide resistance, adaptation to heat, foraging behaviour, plant adaptation, desiccation resistance, diapause, or dispersal have been selected for in different insect and mite species for biologi-

cal control purposes (Lirakis and Magalhães, 2019). For example, artificial selection on wing truncation in a biocontrol agent of aphids (the ladybird beetle *Adalia bipunctata* Linnaeus) was conducted to ensure that it remains close to its place of release (Lommen et al., 2019). Another selection method, called experimental evolution, refers to a methodology in which populations are exposed to particular environments during several generations, and adaptation is tested by comparing trait values in those populations and in populations maintained in control environments (Lirakis and Magalhães, 2019). For instance, heat tolerance of the predatory mite *Neoseiulus barkeri* Hughes was significantly increased by combining experimental evolution (long-term heat acclimation) and artificial selection (frequent heat increases) (Zhang et al., 2018). Artificial selection and experimental evolution have been described as powerful tools which should be used more often to 'improve' biocontrol agents for augmentative biological control (Lommen et al., 2019; Lirakis and Magalhães, 2019).

1.4.2. SELECTION FOR INCREASED DROUGHT RESISTANCE IN BIOCONTROL AGENTS

Selection for increased drought resistance in biocontrol agents can be done at different levels. To select for the most drought-resistant biocontrol agent species, drought resistance should be compared between different species. Alternatively, to increase drought resistance of a specific biocontrol agent species, genetic variation for drought resistance within this species should be assessed (Wajnberg, 2004; Lommen et al., 2017). The presence of genetic variation for a trait within a species will provide the potential to select for this trait. Genetic variation for drought resistance within a species can be assessed by comparing drought resistance between different populations of this species, or different individuals within a population of this species. Subsequently, classical breeding methods, based on artificial selection of the most drought-resistant individuals, or experimental evolution, based on the 'semi-natural' evolution of a population under drought stress, can be used to improve the drought resistance of individuals in a population. The selection process could be facilitated by recent advances in the fields of genetics and genomics, for example by determining the genetic architecture of the drought-resistance trait and using genetic markers linked to it. However, this approach requires different skills and resources, because it relies on the availability of genetic markers, such as microsatellites, and the use of several molecular and statistical tools. Few selection experiments have been conducted to increase drought resistance in biocontrol agents, compared to selection for insecticide resistance or heat tolerance (Lirakis and Magalhães, 2019). So far, drought resistance has been successfully selected for in two species of entomopathogenic nematodes: *Heterorhabditis bacteriophora* Poinar and *Steinernema feltiae* Filipjev (Mukuka et al., 2010; Salame et al., 2010; Anbesse et al., 2013). Other important natural enemies, such as predatory mites, are highly sensitive to drought, and no reliable solution has yet been found to improve their performance as biocontrol agents in dry conditions.

1.5. THE PREDATORY MITE *Phytoseiulus persimilis* AND ITS PREY *Tetranychus urticae*

1.5.1. MITES IN AGRICULTURE

Mites are small arthropods (most of them are less than 1 mm in length) belonging to the class Arachnida. With their eight legs, they are related to spiders and scorpions. Many mite species live in the soil, on plants or in water, where they act as decomposers, herbivores, predators or parasites. Some 48,200 species of mites have been described, and there may be a million or more species still to be identified (Halliday et al., 2000). Although mites are best known for being parasites (e.g. scabies) or provoking allergies (e.g. dust mites), they play a fundamental role in agricultural ecosystems, as herbivores and predators.

1.5.2. A HERBIVOROUS MITE: *Tetranychus urticae*

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a major herbivorous pest that is present all around the world. Two-spotted spider mites feed on more than 1100 plant species, of which more than 100 are important agricultural crops (Amano and Chant, 1977; Mori, 1977; Sabelis, 1981; Marinosci et al., 2015; Amoah et al., 2016). The five life stages of *T. urticae* (egg, larva, protonymph, deutonymph, adult; with three quiescent molting stages at the end of the larval and both nymphal stages) live in self-produced protective silk webs, on the underside of the leaves. Two-spotted spider mites penetrate plant cells with their mouthparts to suck their contents, causing the appearance of chlorotic spots and falling leaves (Fig.1.1 and Fig.1.2). They are a difficult pest to control, because they have developed resistance to many synthetic pesticides, and because of their high reproductive potential and rapid development: at 25 °C, a young *T. urticae* female can lay up to 12 eggs per day, and up to 300 eggs in her lifetime; and a *T. urticae* egg will develop into an adult in approximately 9.4 days (males) to 10.8 days (females) (Sabelis, 1981; Helle and Sabelis, 1985). With an intrinsic rate of increase of around 0.28 day^{-1} at 25 °C, two-spotted spider mites double their population size every 2 to 4 days (population doubling time) (Helle and Sabelis, 1985; Sabelis et al., 2012; Flores et al., 2013).

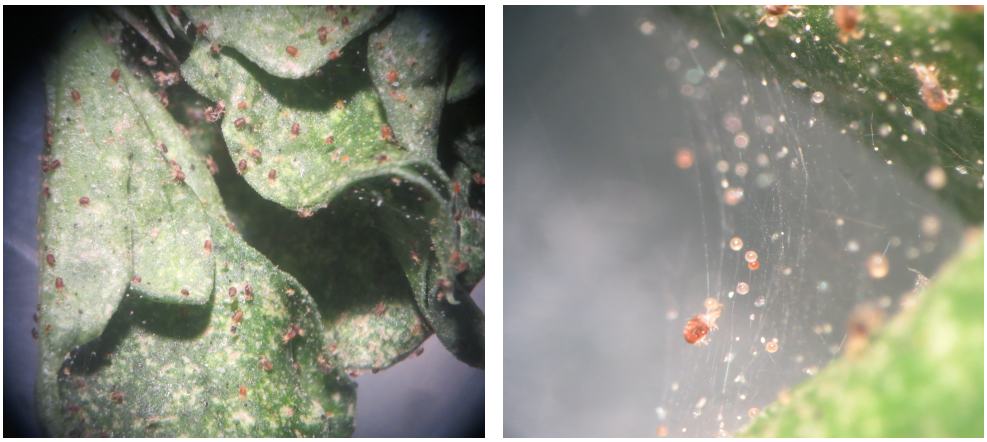


Figure 1.1: Bean leaves infested by *T. urticae* (left). *Tetranychus urticae* webs with *T. urticae* eggs (right). *Tetranychus urticae* eggs are spherical and their diameter varies from 0.11 to 0.15 mm. Photos: Sophie Le Hesran

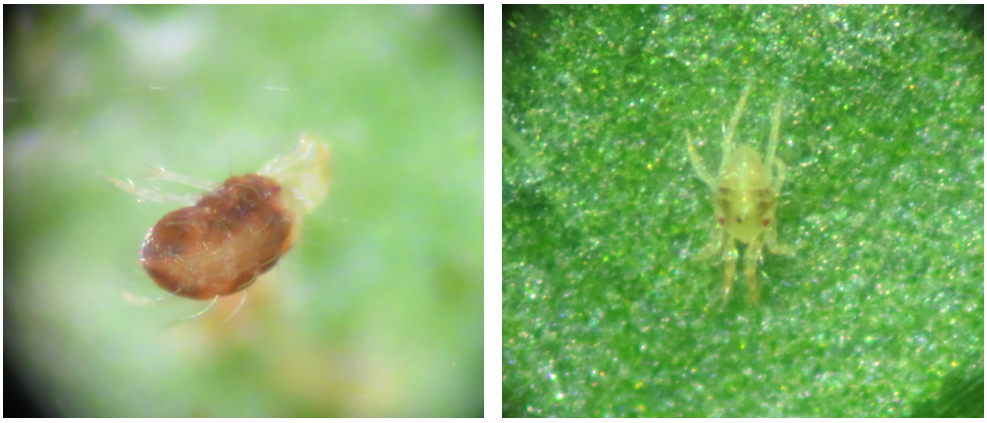


Figure 1.2: Adult female *T. urticae* in a web (left). Deutonymph *T. urticae* feeding on a leaf (right). An adult female *T. urticae* is on average 0.4 mm long. Photos: Sophie Le Hesran

1.5.3. A PREDATORY MITE: *Phytoseiulus persimilis*

Efficient biological control of two-spotted spider mites can be achieved with predatory mites from the family Phytoseiidae. The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), in particular, is a specialist predator of *Tetranychus* species (McMurtry et al., 2013). *Phytoseiulus persimilis* is native to Chile and North Africa (Mori and Chant, 1966), and has been used worldwide for decades as the most efficient natural enemy for aug-

mentative biological control of two-spotted spider mites (Weintraub and Palevsky, 2008). *Phytoseiulus persimilis* individuals go through five life stages: egg, larva, protonymph, deutonymph, and adult (with three short molting stages at the end of the larval and both nymphal stages). Females usually lay their eggs on top of leaf hairs, on leaf surfaces, or in the web strands produced by *T. urticae*. Larvae are non-feeding stages, protonymphs and deutonymphs feed on *T. urticae* eggs, larvae and protonymphs; and adults feed on all *T. urticae* life stages, with a preference for the egg stage. The high efficacy of *P. persimilis* as a biocontrol agent can be explained by its short developmental time and its high food-to-egg conversion rate. Developmental time of *P. persimilis* eggs is only half as long as that of *T. urticae* eggs: at 25 °C, a *P. persimilis* egg will develop into an adult in around 5.37 days (Takafuji and Chant, 1976). This short developmental time makes the population doubling time of *P. persimilis* similar to that of *T. urticae*, even though the fecundity of *P. persimilis* females (4 to 5 eggs per day) is around two times lower than that of *T. urticae* (Sabelis et al., 2012). Moreover, the mass of a *P. persimilis* egg is around two times larger than that of a *T. urticae* egg (Sabelis, 1981). To achieve their reproductive potential, *P. persimilis* females therefore have to eat large quantities of prey (Fig.1.3 and Fig.1.4).

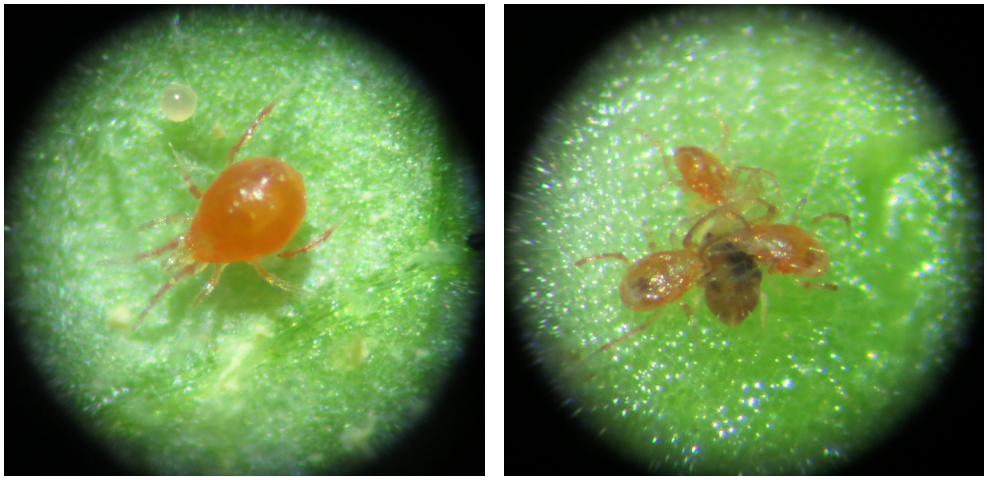


Figure 1.3: A *P. persimilis* adult female next to a *T. urticae* egg (left). Three *P. persimilis* individuals attacking a *T. urticae* female (right). A *P. persimilis* adult female is around 0.5 to 0.6 mm long. Photos: Sophie Le Hesran

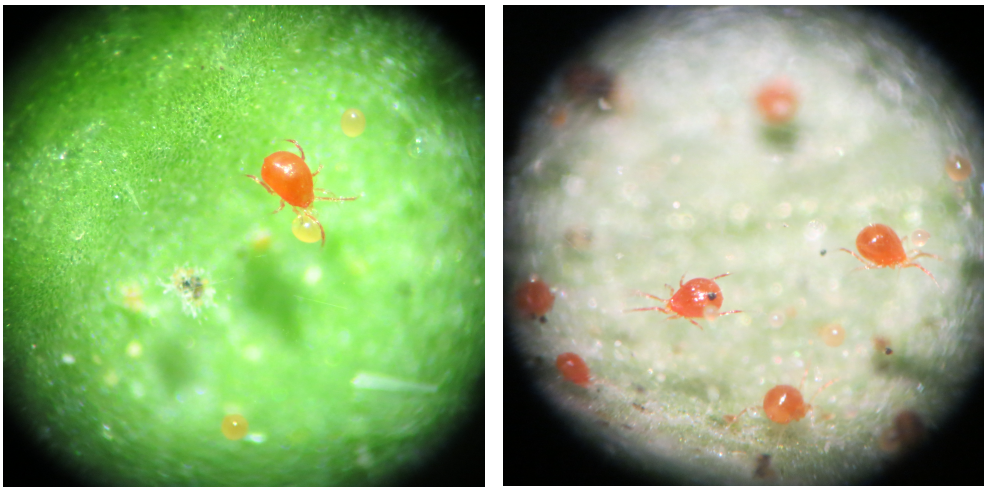


Figure 1.4: A *P. persimilis* adult female next to three *P. persimilis* eggs (left). *Phytoseiulus persimilis* adult females in a *T. urticae* web (right). *Phytoseiulus persimilis* eggs have an ovoid shape and an average maximum diameter of 0.24 mm. Photos: Sophie Le Hesran

1.5.4. BIOLOGICAL CONTROL OF TWO-SPOTTED SPIDER MITES IN DRY CONDITIONS

Atmospheric humidity and temperature are important factors affecting the water balance and performance of *P. persimilis*: to establish and thrive, *P. persimilis* requires a high relative humidity (above 70% RH) and a moderate temperature (lower than 30 °C) (Palevsky et al., 2008). In contrast, two-spotted spider mites develop faster and have a higher oviposition rate at low humidity (25-30% RH) than at high humidity (85-90% RH) (Helle and Sabelis, 1985). As a consequence, while outbreaks of *T. urticae* are favoured by hot and dry conditions, *P. persimilis* populations struggle to control *T. urticae* populations in these conditions (Sabelis, 1981; Bakker et al., 1993; Croft et al., 1993; Schausberger, 1998; Walzer et al., 2007; Weintraub and Palevsky, 2008). Although few studies have investigated the effects of dry conditions on *P. persimilis*, failures in biological control of *T. urticae* in dry conditions are often explained by the fact that *P. persimilis* eggs are highly sensitive to drought and do not survive in these conditions (Schausberger, 1998; Ferrero et al., 2010; Döker et al., 2016). Two studies have compared egg survival in constant dry conditions among different phytoseiid mite species, and concluded that *P. persimilis* eggs are among the most drought-sensitive (Williams et al., 2004; Ferrero et al., 2010). However, in the field, several factors may promote egg survival in plant systems even under dry ambient conditions. First, humidity and temperature regimes usually follow a diurnal cycle resulting in higher humidity conditions at night, which may increase egg survival (Audenaert et al., 2014). Second, *P. persimilis* eggs are often laid on the leaf surface or in *T. urticae* webs, where they may benefit from a microclimate with higher humidity than the ambient air. Third, phytoseiid females laying eggs may exhibit behavioural responses to changing humidity regimes, assuming that they are

able to perceive differences in humidity levels (Gaede, 1992). Consequently, two main questions remain to be answered: How is *P. persimilis* affected by drought? Why is biological control of *T. urticae* with *P. persimilis* particularly challenging in dry conditions?

1.6. OBJECTIVES AND OUTLINE OF THIS THESIS

Augmentative biological pest control is a promising alternative to chemical pest control. To encourage the development of an agriculture more respectful of our environment and our health, it is fundamental to conduct scientific research on the interactions between pests and their natural enemies. In that spirit, the European Union financed a project called BINGO (Breeding Invertebrates for Next Generation BioControl, Horizon 2020), involving senior researchers and PhD candidates from different countries and institutions, from 2015 to 2018. The aim of BINGO was to quantify, describe, and exploit natural genetic variation in different arthropod species to improve their performance as biocontrol agents. This thesis, part of the BINGO project, is focused on the predatory mite *P. persimilis*, biocontrol agent of the pest *T. urticae*. The objective of this thesis is to provide answers to the following questions:

1. How are *P. persimilis* eggs affected by relative humidity in their environment?

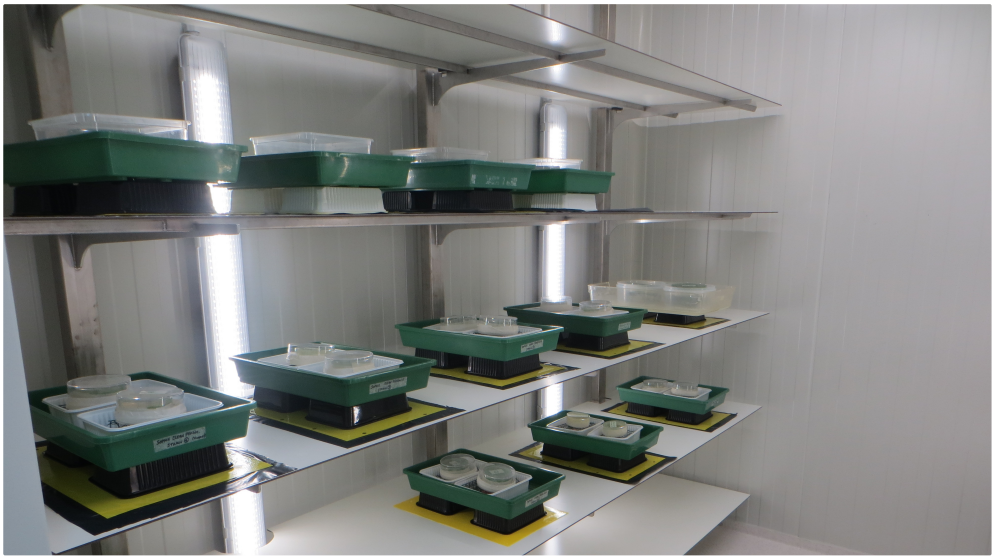
2. Can we explain failures in biological control of *T. urticae* in dry conditions by the drought-sensitivity of *P. persimilis* eggs?

3. How can biological control of *T. urticae* with *P. persimilis* be improved under dry conditions?

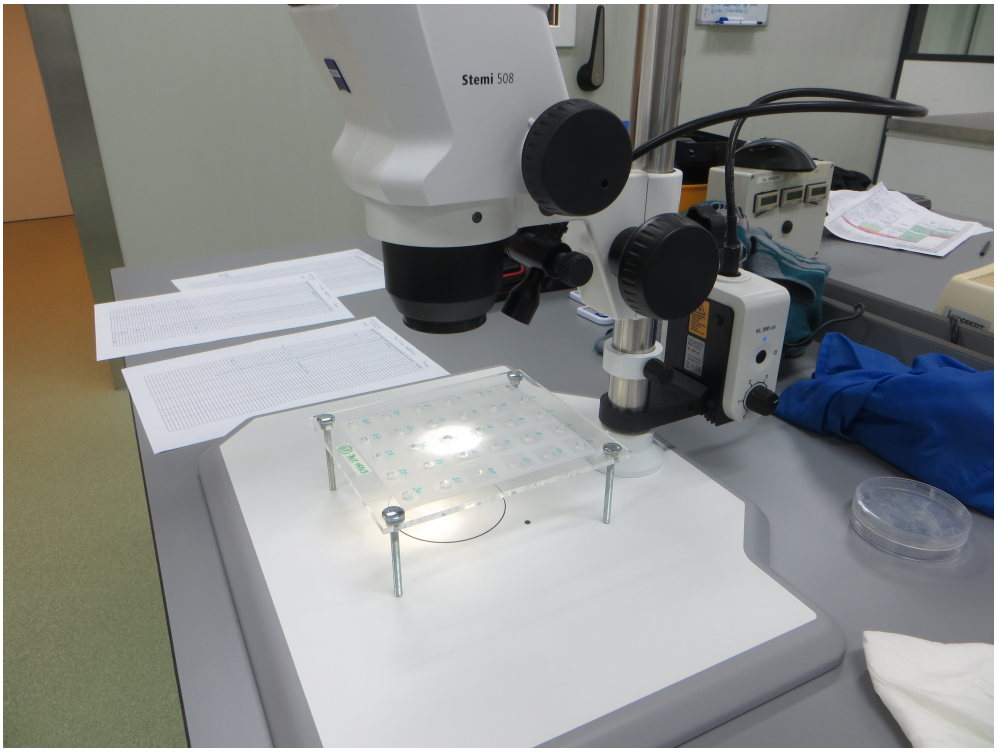
4. Is it possible to increase the drought resistance of *P. persimilis* through artificial selection or experimental evolution?

CHAPTER 2 focusses on the effects of different relative humidity conditions on *P. persimilis* eggs, because they are considered the most drought-sensitive life stage in this species. I started from the assumption that natural selection has resulted in genetic differentiation among *P. persimilis* populations from different geographical origins, with some populations producing drought-resistant eggs and others not. By comparing egg survival among five *P. persimilis* populations in different humidity conditions (constant low, constant high and variable), I investigated the presence of genetic variation for egg drought resistance in *P. persimilis*, and the potential sources (genetic or environmental) of phenotypic variation in this trait. In CHAPTER 3, I report on the possibilities to improve drought resistance of *P. persimilis* eggs. I conducted two selection experiments, based on artificial selection and experimental evolution procedures. I started both experiments with a base population made by mixing individuals from the five previously mentioned populations, to maximize the genetic variation and increase the chances of obtaining a response to selection. CHAPTER 4 presents a study on the effects of constant and variable humidity on the oviposition behaviour and the survival of *P. persimilis* adult females. More particularly, I was interested in assessing the drought resistance of *P. persimilis* eggs laid by females exposed to drought. I

tested the hypothesis that drought resistance of *P. persimilis* eggs is influenced by the humidity conditions encountered by their mother. In CHAPTER 5, I study the characteristics of drought-resistant *P. persimilis* eggs. I compared the volume, sex ratio, chemical composition, internal and external structure, and developmental time of drought-resistant and drought-sensitive *P. persimilis* eggs. My objective was to define the mechanisms of drought resistance in *P. persimilis* eggs, and to evaluate the energetic costs involved in the production of drought-resistant eggs. CHAPTER 6 presents a critical discussion of the results presented in this thesis. I also take some distance to place my results in a wider context, and make conclusions and recommendations regarding the improvement of *P. persimilis* as a biocontrol agent under dry conditions.



Phytoseiulus persimilis rearing described in this thesis



Plexiglass platform used to evaluate the hatching rate of *Phytoseiulus persimilis* eggs

2

PHENOTYPIC VARIATION IN EGG SURVIVAL IN THE PREDATORY MITE *Phytoseiulus persimilis* UNDER DRY CONDITIONS

The predatory mite Phytoseiulus persimilis *is widely used for augmentative biological control, as an effective predator of the spider mite* Tetranychus urticae. *However, the biocontrol efficacy of* P. persimilis *decreases under dry conditions. One of the reasons for this decline concerns* P. persimilis*' eggs, which are sensitive to low humidity. In this chapter, we investigated the possibility to select for a strain of* P. persimilis *adapted to dry conditions. To understand the potential sources of phenotypic variation in egg survival under dry conditions, we tested the effects of genetic and environmental factors on variation in this trait. We compared egg hatching of five* P. persimilis *populations, under constant as well as variable humidity conditions, at 25 °C. The results show no intraspecific genetic variation among the five tested populations in egg hatching under constant and variable humidity conditions. In all five populations, less than 20% of the eggs hatched when they were exposed to constant low (60% RH) humidity conditions. However, when eggs were exposed to successive cycles of low and high humidity, a common pattern observed in the field, significantly higher hatching rates were observed. Under variable humidity conditions, more than 73% of the eggs hatched successfully, even when exposure to high humidity was limited to only 13% of the egg developmental time. Although* P. persimilis *eggs suffered from a high rate of water loss under constant dry conditions, they were able to compensate for this water loss when exposed to high humidity conditions for a few hours during their development. A decreased biocontrol efficacy of* P. persimilis *under dry conditions may be explained by a higher egg mortality when relative humidity is constantly low. Yet, when relative humidity exhibits diurnal variation, periods of high humidity may mitigate the effects of periods of low humidity during development of* P. persimilis *eggs.*

Chapter 2 is based on the article: Le Hesran, S., Groot, T., Knapp, M., Bukovinszky, T., Forestier, T., Dicke, M., 2019. Phenotypic variation in egg survival in the predatory mite *Phytoseiulus persimilis* under dry conditions. *Biological Control*, **130**:88-94, doi:10.1016/j.biocontrol.2018.10.007.

2.1. INTRODUCTION

Terrestrial arthropods are particularly susceptible to desiccation, because they have a relatively large surface-area-to-volume ratio (Gibbs, 2002; Gefen et al., 2006). The sensitivity of insects and mites to desiccation partly explains why humidity and temperature are the most important factors limiting the effectiveness of augmentative biological control (Collier and Van Steenwyk, 2004). As relative humidity decreases and temperature increases at the leaf surface of drought-stressed plants, micro-environmental conditions impede survival and reproduction of natural enemies (Holtzer et al., 1988). Understanding how natural enemies and their prey are affected by arid conditions is, therefore, a necessary step to improve augmentative biocontrol strategies. The predatory mite *Phytoseiulus persimilis* and its prey *Tetranychus urticae* are affected differently by arid conditions, therefore constituting an interesting system to investigate the interactions between arid conditions and augmentative biocontrol. The two-spotted spider mite *T. urticae* is an important agricultural pest worldwide, in greenhouses and open fields (Helle and Sabelis, 1985; Grbić et al., 2011). In the Phytoseiidae family, *P. persimilis* is a specialized predator of *Tetranychus* species (McMurtry et al., 2013), and is used worldwide as a biological control agent of *T. urticae* (Zhang, 2003). The abiotic conditions for optimal performance of *P. persimilis* are temperatures between 15 °C and 27 °C, and a relative humidity of 60 to 90% (Stenseth, 1979). While it is an excellent predator under these climatic regimes, its efficacy under hot and dry conditions is often insufficient (Force, 1967; Nihoul, 1992; Skirvin and Fenlon, 2003; Escudero and Ferragut, 2005; Weintraub and Palevsky, 2008). Previous studies explained the poor performance of *P. persimilis* in arid conditions by the fact that many phytoseiid species have a lower egg viability under dry conditions (Schausberger, 1998; Walzer et al., 2007; Ferrero et al., 2010; Döker et al., 2016). Mite eggs have a large surface-area-to-volume ratio, which makes them vulnerable to water loss (Hinton, 1981), and, unlike the mobile life stages, they cannot move or feed to compensate for the water deficit (Ferrero et al., 2010). *Tetranychus urticae* populations, however, have a higher intrinsic rate of increase at high temperatures (30 °C) and low humidity (40% RH) (Boudreaux, 1958; Stenseth, 1979; Nihoul, 1992). This may be explained by the fact that plants become more nutritious for herbivores and have lower levels of defensive compounds when exposed to drought stress (English-Loeb, 1990; Ximénez-Embún et al., 2017). Consequently, because of the effects of high temperature and low humidity, biological control of two-spotted spider mites on crops grown in arid environments remains a serious problem, which is difficult to solve with the predatory mites that are currently commercially available (Walzer et al., 2007). There is a need to find or select a *P. persimilis* strain that is better adapted to dry conditions, using egg survival as an indicator of adaptation to drought. Intraspecific variation in egg survival at low humidity has been observed in *P. persimilis* and in *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) (Perring and Lackey, 1989; Walzer et al., 2007). In a study on *P. persimilis*, egg hatching rates of two populations, from Israel and California, were compared under different humidity conditions (Perring and Lackey, 1989). At 26.7 °C and 73% RH, 74% of the eggs from California and 26% of the eggs from Israel desiccated and died. These results suggest a genetic differentiation among *P. persimilis* populations in the field. Some populations may have adapted to dry conditions, producing eggs that can survive at low humidity. Adaptation to different abiotic conditions can be achieved by genetic differentiation or phenotypic plasticity (Mousseau and Roff, 1989; Koveos et al., 1993; Blanckenhorn, 1997). Separating the effects

of genetic and environmental factors on phenotypic variation in egg survival is necessary, if we want to understand how certain *P. persimilis* populations can cope with dry conditions. Moreover, it will allow us to determine whether this trait has the ability to respond to natural or artificial selection. To investigate the effects of genetic factors on intraspecific variation in egg survival, we compared the egg hatching rate of five populations of *P. persimilis* under different constant humidity conditions. We tested whether genotypic variation in drought tolerance was present by comparing populations that originated from different environments. Finding variation among populations in egg hatching rate under similar humidity conditions would support the hypothesis that genetic differentiation is involved. To complement this approach, we compared the nucleotidic sequences of two loci among the five populations. Intraspecific genetic distances have already been observed within three Phytoseiidae species (*Typhlodromus pyri* Scheuten, *Neoseiulella aceri* (Collyer) and *Phytoseiulus longipes* Evans) with two mitochondrial molecular markers, i.e. Cytb mtDNA and 12S rRNA, justifying their use to investigate intraspecific genotypic variation in *P. persimilis* (Kanouh et al., 2010; Tixier et al., 2010, 2012). Although intraspecific variation in egg survival has been observed under constant humidity conditions (Perring and Lackey, 1989; Walzer et al., 2007), we considered that this approach was not the most realistic one. In the field, relative humidity fluctuates diurnally, and a variable humidity may allow the eggs to rehydrate and better tolerate subsequent periods of drought. For example, under dry conditions, *Dermatophagoides pteronyssinus* Trouessart (Acari: Pyroglyphidae) eggs can lose over 80% of their mass in a period of 48 hours and still survive to develop and hatch, after being transferred to humid conditions (Colloff, 1987). Additionally, the sensitivity of eggs to low humidity may change during their development, leading to better chances of survival if they are exposed to drought during the 'less sensitive' period. It has been shown, for example, that the eggs of *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) were more sensitive to low humidity in the last stage of their development (Zhang and Kong, 1985). Therefore, exposing mite eggs to a constant low humidity may not reflect the real conditions in which they develop, and ignore the fact that they may have a plastic response to variations in humidity. To investigate the effects of environmental factors on intraspecific variation, we exposed the eggs of three *P. persimilis* populations to two different types of environment: constant humidity and variable humidity. Finding different egg survival rates between variable humidity and the corresponding average constant humidity within a population would indicate the presence of an environmental effect in phenotypic variation. We compared the three *P. persimilis* populations under variable humidity conditions to investigate whether populations differ in phenotypic variation. Finding variation among populations in egg hatching rate under similar variable humidity conditions would support the hypothesis that genetic differentiation is involved. In this study, we aimed at evaluating the phenotypic variation in egg survival under dry conditions among five populations of *P. persimilis*, and at understanding the potential sources of phenotypic variation in this trait. We tested the hypothesis that natural selection has resulted in genetic differentiation among *P. persimilis* populations.

2.2. MATERIALS AND METHODS

2.2.1. ORIGIN OF THE POPULATIONS AND REARING METHOD

Five populations of *P. persimilis* were tested for egg survival, four of which were field-collected populations from four different locations (France, Israel, Sicily and Turkey). These four field-collected populations were generously provided to us by Arnon Tabic (population from Israel, started with ca 30 individuals), Marie-Stéphane Tixier (population from France, started with ca 30 individuals), and Alexandra Revynthi (populations from Turkey and Sicily; for details see (Revynthi, 2017)). Although these four populations all come from the Mediterranean region, we expected differences in drought tolerance between them. The annual precipitation levels are higher in the French and the Turkish locations, with 585-1159 mm per year, against 10-585 mm per year for the Sicilian and the Israeli locations (`http://www.worldclim.org`, 2016). The mean temperature of the three driest months of the year is higher in the Israeli and the Turkish locations (25.0-34.2 °C) than in the French and Sicilian locations (15.8-25.0 °C). Therefore, we expected different responses to drought among the four populations. The fifth population was obtained from a commercial mass rearing (Koppert Biological Systems) (Table 2.1). The populations were reared separately in Petri dishes (ø 7.5 cm, 3 cm high) containing an agar layer (agar powder, VWR Chemicals, 1/100 diluted) on which a disk (ø 7 cm) of a cucumber leaf (*Cucumis sativus* 'Pyralis') infested with a mixture of all stages of *T. urticae* was placed, with the adaxial side facing the agar plate. Each Petri dish was closed with a lid, containing a 5 cm diameter hole covered by a 90-µm-mesh insect screen to allow air exchange while preventing the mites from escaping. Predatory mites were transferred to new dishes once a week and maintained in a climate chamber at 18 ± 1 °C, 65 ± 2% RH, and L16:D8 photoperiod. We assumed that the mites were exposed to a relative humidity higher than 65% because of the microclimate within the laminar layer at the leaf surface (Gaede, 1992), and the agar layer in the Petri dishes. Contamination between the populations was prevented by several barriers: each Petri dish was placed in a closed transparent circular plastic box (ø 10 cm, 4 cm high) with a 90-µm-mesh insect screen in the lid. Each plastic box was placed on a wire platform inside a bigger open box, in a tray filled with water and soap. A sticky trap (HORIVER, Koppert Biological Systems) was placed around each tray (Fig.2.1). Both experiments were carried out between April 2016 and January 2018.

Table 2.1: Origin of the *Phytoseiulus persimilis* populations used in the study.

Origin	Sampling Date	Host Plant
Mass rearing Koppert	August 2015	
Carnon, France (43°33'14"N, 4°00'26"E)	September 2014	*Datura spp.*
Karaçay, Turkey (36°0'8.683"N, 36°0'4.355"E)	July 2013	*Solanum melongena*
Alcamo, Sicily, Italy (37°58'40"N, 12°57'50"E)	June 2014	*Ricinus communis*
Ofer, Israel (32°37'17.1"N, 34°58'56.9"E)	February 2016	*Solanum lycopersicum*

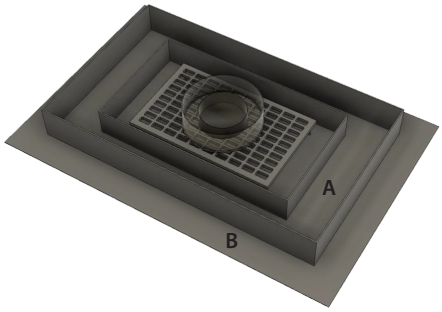


Figure 2.1: Rearing system used to avoid contamination between populations. A: tray filled with water and soap. B: sticky trap.

2.2.2. EGG HATCHING TESTS

2.2.2.1. VARIATION IN EGG HATCHING RATE AMONG POPULATIONS UNDER CONSTANT HUMIDITY CONDITIONS

We carried out a pilot test on the egg hatching rate of the Koppert population at 25 °C and different relative humidity levels: we found an egg hatching rate close to 0 at 60% RH, and close to 1 at 85% RH. Therefore, we decided to perform the experiment at the following four relative humidity levels: 60%, 65%, 70% and 85% RH; at 25 °C. We used the egg hatching rate at 85% RH as a positive control in all replicates.

For each population, around 40 young females (6 to 10 days old) were isolated to lay eggs during 6 h in a Petri dish with a *T. urticae* infested cucumber leaf disk on agar, at 25 °C and 65 ± 2% RH. After 6 h, 15-30 eggs were collected from each population. The eggs were placed with a brush in a hole (ø 0.7 cm, 0.4 cm deep) in a plexiglass platform (17.5 L x 15 W x 4.5 H cm) containing 30 holes (one egg per hole). Each hole had a 90-µm-mesh insect screen at the bottom, to ensure contact with ambient relative humidity. The hole was then covered with a coverslip to prevent the larvae from escaping after hatching. Five platforms (one platform per population) were placed in the same climate chamber (Panasonic, MLR-352H) at a constant relative humidity and 25 ± 1.7 °C for 72 h (L16:D8 photoperiod). In parallel, five platforms (one platform per population) containing 15 eggs each were placed in a second climate chamber at 85 ± 3.5% RH and 25 ± 0.4 °C for 72 h, as a positive control. The average developmental time of a *P. persimilis* egg, at 25 °C and a relative humidity between 75% and 90%, is 54.2 ± 0.48 h (Takafuji and Chant, 1976). We chose to incubate the eggs in the climate chambers during 72 h to be sure that they all had sufficient time to hatch. After 72 h, we counted the number of hatched eggs. For each relative humidity and each population, we performed between 4 and 8 replicates, each in parallel with a positive control at 85% RH. For each replicate, we switched the two climate chambers. In total, 3219 eggs were tested in this experiment. The temperature and relative humidity were continually monitored using calibrated data loggers (LogTag, HAXO-8).

2.2.2.2. VARIATION IN EGG HATCHING RATE AMONG POPULATIONS UNDER VARIABLE HUMIDITY CONDITIONS

We tested the egg hatching rate of three populations (Koppert, France, Israel) under variable humidity conditions at a constant temperature (25 °C). Throughout their development (54 h on average), the eggs were exposed to one of 7 different treatments (Fig.2.2). For each population, 15 to 30 eggs, from 0 to 6 h old, were collected in the same way as for the previous experiment. For the low humidity conditions, one climate chamber was set at 60 ± 1.6% RH and 25 ± 0.5 °C. For the high humidity conditions, another climate chamber was set at 75 ± 1.5% RH and 25 ± 0.6 °C. The platforms containing the eggs were moved from one climate chamber to the other when needed during the experiment (see Fig.2.2). Even if most of the eggs had hatched after 54 h, we decided to count the number of hatched eggs after 72 h, as in the previous experiment. Between 54 h and 72 h, the platforms stayed at the same humidity as they were at 54 h. Between 4 and 7 replicates per treatment and per population were carried out. In total, 2474 eggs were tested in this experiment.

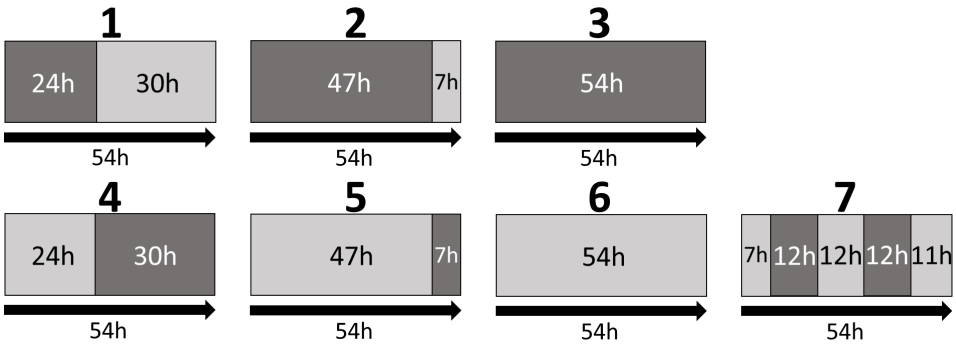


Figure 2.2: Seven humidity treatments to which the *Phytoseiulus persimilis* eggs were exposed during their developmental time (at 25 °C). Dark grey: 75% RH. Light grey: 60% RH. The numbers on top of each treatment represent treatment ID.

2.2.3. RELATIVE HUMIDITY AND VAPOUR PRESSURE DEFICIT

Stenseth (1979) reported that, as temperature increased, higher RH was needed to maintain *P. persimilis* egg vitality. This suggests that the eggs respond more to the absolute rather than the relative humidity (Perring and Lackey, 1989). Unlike relative humidity, vapour pressure deficit (VPD) gives a direct indication of the atmospheric moisture conditions, independent of the temperature (Anderson, 1936). To be more precise in terms of humidity experienced by the eggs, and to be able to compare our results with other studies, both relative humidity (%) and VPD (kPa) will be mentioned in the results of this chapter. VPD was calculated with the following equation:

$$VPD = SVP \cdot (1 - \frac{RH}{100}) \tag{2.1}$$

where the saturation vapour pressure (SVP) is a constant related to temperature and atmospheric pressure. Under our experimental conditions (25 °C, sea level), SVP equals 3.17 kPa.

2.2.4. SEQUENCING OF CYTB AND 12S GENES

To investigate intraspecific genotypic variation among our populations, we sequenced the mitochondrial *Cytochrome b* (Cytb) gene and the ribosomal RNA *12S* gene. DNA was extracted from single *P. persimilis* adult females with the DNeasy Blood and Tissue Kits method (Qiagen). Two to 6 mites per population were used for the DNA extraction. The mitochondrial Cytb region was amplified using the 5'TAWRAARTATCAYTCDGGTTKRATATG3' (forward) and 3'CCWTGAGGACAAATAWSWTTYTGAGG5' (reverse) primers (Vicente dos Santos and Tixier, 2017). The ribosomal RNA 12S region was amplified using the 5'TACTATGTTACGACTTAT3' (forward) and 3'AAACTAGGATTAGATACCC5' (reverse) primers (Vicente dos Santos and Tixier, 2017). For the PCR, we used 40 µL reaction volumes containing 16.4 µL of nuclease free water, 20 µL of MasterMix (OneTaq Quick-Load 2xMM with standard Buffer, New England BioLabs), 0.8 µL of each primer, and 2 µL of DNA sample. For Cytb, samples were denaturated at 94 °C for 3 min, and then PCR was carried out for 35 cycles of 20 s denaturation at 92 °C, 1 min annealing at 48 °C, 1 min extension at 72 °C, and a final extension step at 72 °C for 5 min (Vicente dos Santos and Tixier, 2017). For 12S, samples were denaturated at 95 °C for 1 min, and then PCR was carried out for 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 44 °C, 1 min extension at 72 °C, and a final extension step at 72 °C for 5 min (Vicente dos Santos and Tixier, 2017). The PCR products were visualized with UV light using a 1% agarose gel stained with GelRed (Biotium). Direct sequencing of PCR amplifications was done by BaseClear BV, using the same primers as for the PCR. The sequences were compared using the MEGA7 software.

2.2.5. STATISTICAL ANALYSIS

For both experiments, the response variable was expressed as a proportion (number of eggs hatched/number of eggs tested), for each replicate. For the experiment with constant relative humidity, we studied the influence of the factors population (categorical explanatory variable) and relative humidity (continuous explanatory variable) on the egg hatching rate. We used a generalized linear mixed effect model with a binomial error distribution. The variables population and relative humidity were expressed as fixed effects in the model. For the experiment with variable relative humidity, we studied the influence of the factors population (categorical explanatory variable) and treatment (categorical explanatory variable) on the egg hatching rate. We used a generalized linear mixed effect model with a binomial error distribution. The variables population and treatment were expressed as fixed effects in the model. To compare the different treatments, we used multiple pairwise comparisons. Within each humidity and treatment tested, two replicates which had been performed in an interval of no more than 7 days were assigned the same time of replicate value. For both experiments, the variable time of replicate was expressed as a random effect in the model. In order to correct for overdispersion in our data from both experiments, we introduced a "per-observation" random effect in the model. We used the model-fitting method of the

maximum likelihood, and all models were ranked based on their second-order Akaike information criterion (AIC). The best-fitting model was defined as the one minimizing the AIC. We used RStudio (version 3.2.3) for all the analyses.

2.3. RESULTS

2.3.1. VARIATION IN EGG HATCHING RATE AMONG POPULATIONS UNDER CONSTANT HUMIDITY CONDITIONS

Relative humidity had a statistically significant effect on egg hatching rate ($\chi^2 = 29.5; DF = 1; P = 6 \cdot 10^{-8}$, Fig.2.3). For all populations, the average egg hatching rate was lower than 0.2 at 60% RH (1.26 kPa), and was close to 1 at 85% RH (0.47 kPa). We did not find a statistically significant effect of the factor population on the egg hatching rate ($\chi^2 = 0.98; DF = 4; P = 0.91$) nor a significant interaction between population and relative humidity ($\chi^2 = 2.44; DF = 4; P = 0.65$). The most parsimonious fitted model was described by the following equation (p is egg hatching rate, rh is relative humidity, and ϵ is binomial error):

$$ln(\frac{p}{1-p}) = -17.51 + 0.26 \cdot rh + \epsilon \tag{2.2}$$

The RH_{50} of the fitted model (relative humidity at which 50% of the eggs died) was 66.8%, which corresponds to a VPD_{50} of 1.05 kPa. Between 65% (1.1 kPa) and 70% RH (0.95 kPa), the fitted egg hatching rate increased by a factor 1.8, from 0.39 to 0.71 respectively. Since the hatched eggs were counted 72 h after transferring them to the experimental conditions, we were able to observe that most of the larvae survived the larval stage and developed into nymphs.

2.3.2. VARIATION IN EGG HATCHING RATE AMONG POPULATIONS UNDER VARIABLE HUMIDITY CONDITIONS

Humidity treatment significantly affected egg hatching rate ($\chi^2 = 48.9; DF = 6; P = 8 \cdot 10^{-9}$, Fig.2.4). Population did not affect egg hatching rate ($\chi^2 = 0.23; DF = 2; P = 0.89$), and there was no significant interaction between population and treatment ($\chi^2 = 16.68; DF = 12; P = 0.16$). Treatment 6 (complete development at 60% RH - 1.26 kPa) had the strongest impact on the average hatching rate: the most parsimonious fitted model predicted an egg hatching rate of 0.08. These results correspond to what we observed in the first experiment, at 60% RH. The highest egg hatching rates were observed after treatments 2 and 3 (first 47 h of the development at 75% RH – 0.85 kPa; and full development at 75% RH – 0.79 kPa respectively), with egg hatching rates higher than 0.95. The fitted egg hatching rates in treatments 1, 4, 5 and 7 (first 24 h of the development at 75% RH – 1.05 kPa; first 24 h of the development at 60% RH – 1 kPa; first 47 h of the development at 60% RH – 1.2 kPa; and successive cycles at 60% and 75% RH – 1.05 kPa) were not significantly different.

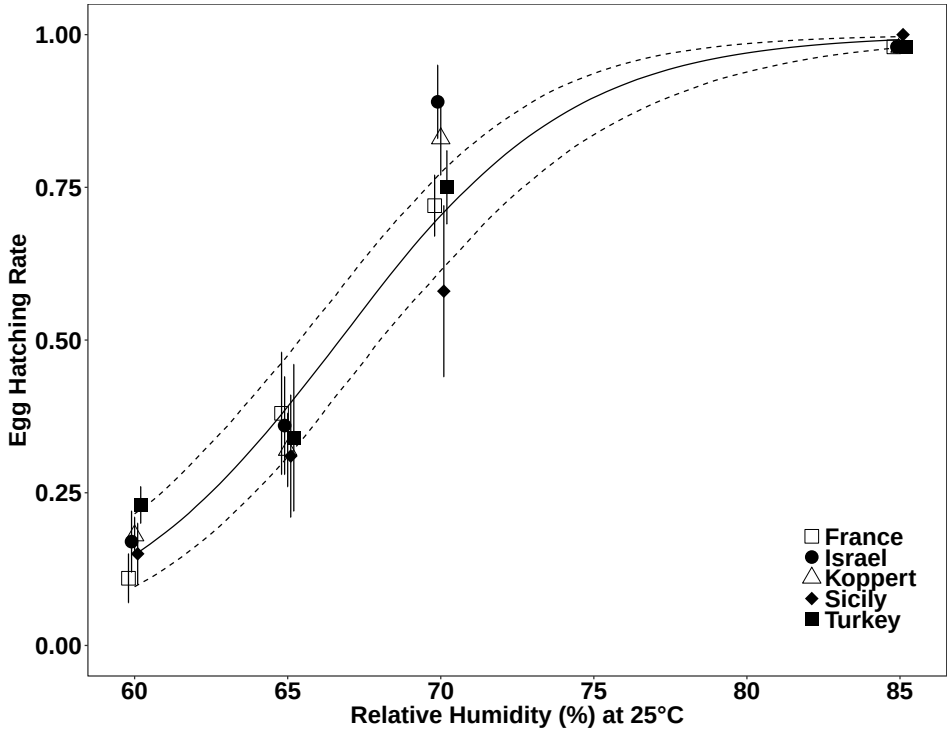


Figure 2.3: Observed (symbols) and fitted (solid line) egg hatch probabilities of five *Phytoseiulus persimilis* populations at different humidity levels at 25 °C. The dashed lines represent the 95% confidence intervals, and the error bars represent ± 1SD.

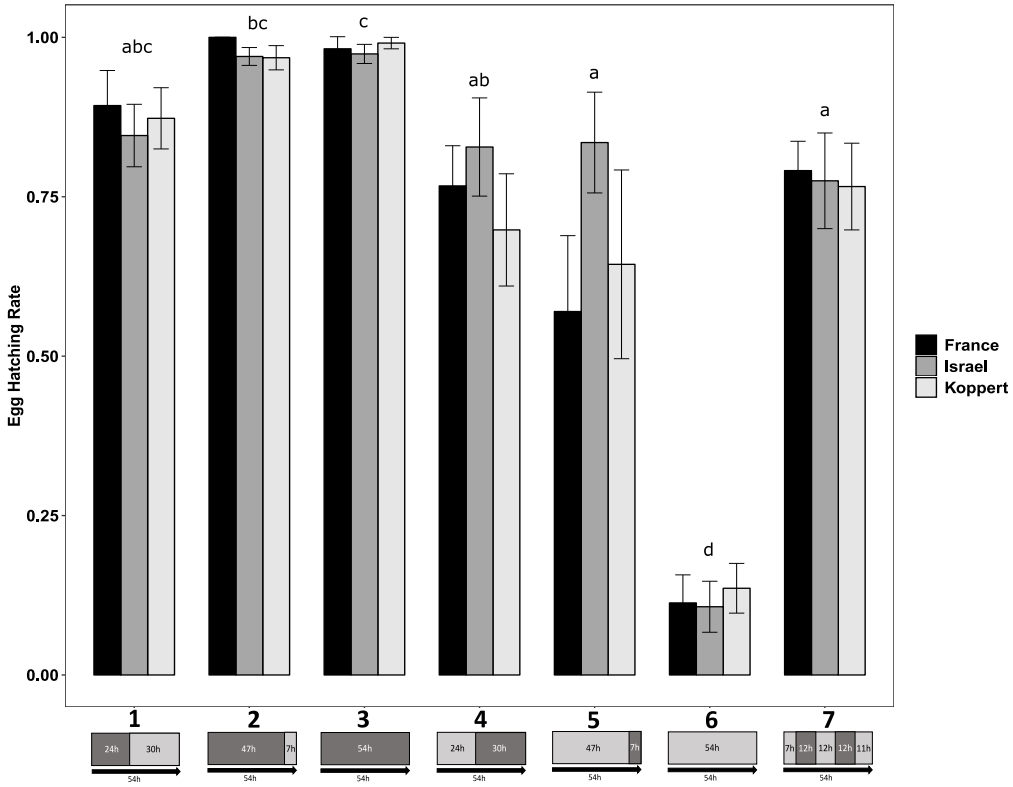


Figure 2.4: Observed egg hatching probabilities for three *Phytoseiulus persimilis* populations under seven RH treatments (dark grey: 75% RH, light grey: 60% RH), at 25 °C. The error bars represent ± 1SD. Different letters above triplets of bars indicate significant differences between treatments (multiple pairwise comparisons, $P < 0.05$).

2.3.3. COMPARISON OF EGG HATCHING RATES OBSERVED UNDER CONSTANT AND VARIABLE HUMIDITY CONDITIONS

We calculated the average relative humidity corresponding to each of the 7 treatments tested in the experiment under variable humidity conditions. We compared the egg hatching rates observed for each variable humidity treatment with the egg hatching rates fitted for each corresponding constant average relative humidity (Fig.2.5). At all humidity levels tested, except for treatment 6, the observed egg hatching rates under variable humidity conditions were higher than the ones expected under the corresponding constant humidity conditions. For treatments 1, 2, 4, 5 and 7, the observed egg hatching rates did not overlap with the 95% confidence bands of the fitted values under constant humidity. The greatest difference was observed in treatment 5, where the average observed egg hatching rate was 0.67, whereas the predicted egg hatching rate under the corresponding constant humidity was 0.22.

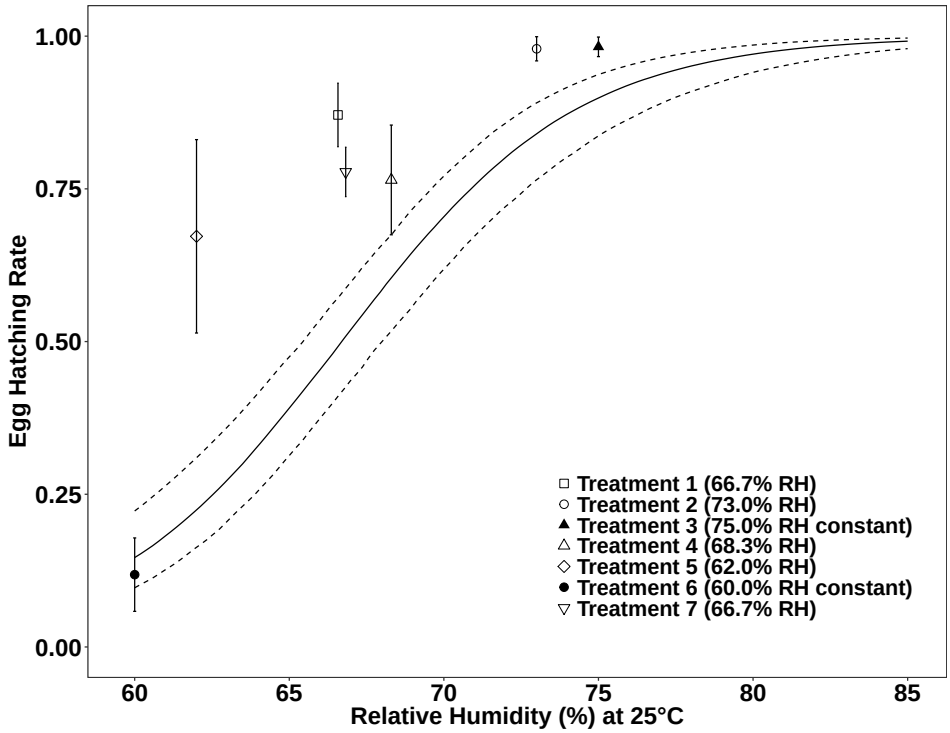


Figure 2.5: Fitted (solid line) and observed (symbols) egg hatching probabilities for *Phytoseiulus persimilis* under constant (solid line and full symbols) and variable (empty symbols) relative humidity conditions, at 25 °C. For explanation of treatments 1-7, see Fig.2.2. The error bars represent ± 1SD, and the dashed lines represent the 95% confidence intervals.

2.3.4. SEQUENCING OF CYTB AND 12S GENES

No differences among populations were found for the Cytb and 12S genes. For each of the two markers, all sequences were identical, corresponding to the GQ222414.1 entry in GenBank.

2.4. DISCUSSION

The objective of this study was to evaluate the phenotypic variation in egg survival under low humidity conditions among five populations of *P. persimilis*, and to understand the potential sources of variation in this trait. We did not find variation among populations for egg survival under constant and variable humidity conditions. The eggs of all tested populations were highly sensitive to constant dry conditions, especially below 60% RH, when the egg hatching rate was lower than 0.2. Survival rate of the eggs increased significantly when they were exposed to variable humidity conditions. Our results differ from the results of Perring and Lackey (1989). At 1 kPa (73% RH at 26.7 °C), they observed egg hatching rates of 0.74

and 0.26 for an Israeli and a Californian population respectively. At 1 kPa (68.4% at 25 °C), we observed an egg hatching rate of 0.60 ± 0.08, somewhat lower than for their Israeli population, and much higher than for their Californian population. In view of our results, there is no indication of differentiation between the five *P. persimilis* populations studied here. This observation is further supported by the fact that we did not find inter-population genotypic variation for the two sequenced loci. However, the use of more variable genetic markers, such as microsatellite DNA markers, is necessary to confirm this result. Although a genetic effect does not seem to be responsible for variation in egg survival under low humidity conditions in *P. persimilis*, this statement must be placed in its context. The four field-collected populations we studied each originated from a low number of founding mites, leading to a limited sampling of genetic diversity within populations. Furthermore, the populations had been reared in the laboratory at high humidity for many generations. It remains to be investigated whether this has resulted in a common adaptation to high humidity conditions. Yet, we did not observe any effect of the time spent by the populations in laboratory conditions on our results over two years of experimentation, and this supports data by Walzer et al. (2007). In their study on *N. californicus*, intraspecific variation in egg hatching rate was observed between several field-collected populations which had spent several years in laboratory conditions. In our study, in all treatments with variable humidity throughout egg development, the most parsimonious fitted model estimated an egg hatching rate higher than 0.73, suggesting an important plasticity in the response of *P. persimilis* eggs to humidity variations. Within each population, the eggs survived significantly better when exposed to variable humidity than to the corresponding average constant humidity, indicating that the observed variation in egg survival is caused by environmental effects. Similar results were found for the predatory mite *N. fallacis*. The immature stages of this species survived significantly better under variable than under constant humidity regimes (Kramer and Hain, 1989). One explanation for this higher survival under variable humidity could be that, after being brought back to high humidity conditions, water condenses on the surface of the egg chorion, and enters into the egg as a liquid, resulting in a water gain and greater hatching rate, as observed in the lone star tick *Amblyomma americanum* Linnaeus (Yoder et al., 2004). It seems that the most important factor for egg survival is not the average relative humidity during their development, but the occurrence and duration of high humidity exposure. We observed the same results for all *P. persimilis* populations studied, suggesting that there is no differentiation involved for egg survival in constant dry conditions in this species, but rather a common adaptive plasticity to variable humidity conditions. Several potential explanations may be considered. Firstly, under field conditions, many factors may promote egg survival in plant systems even under dry ambient conditions. Being able to cope with low humidity for a few hours during their development might already allow most *P. persimilis* eggs to survive. The humidity conditions in the leaf boundary layer, where the eggs are laid, may provide lower saturation deficits than the ambient air (Ferro and Southwick, 1984). For example, in the boundary layer of tomato leaves in a greenhouse, a considerable increase in relative humidity 5 mm from the underside of the leaves was observed, compared to the ambient relative humidity, particularly during day-time when crop transpiration was high (Boulard et al., 2002). This phenomenon is illustrated by our results: the most realistic conditions we tested simulated the diurnal variation in humidity (treatment 7). Under these conditions, the fitted model estimated an egg hatching rate higher than 0.8. These re-

sults indicate that the fluctuating humidity conditions in the field could affect egg hatching rate of *P. persimilis*, but not as much as under the corresponding average constant humidity (66.7% - 1.05 kPa), where egg hatching rate is expected to be only 0.48. Additionally, *P. persimilis* females may be able to detect low humidity levels, and change their oviposition behaviour accordingly, by selecting high humidity locations, like leaf domatia or two-spotted spider mite webbing, to lay their eggs (Gaede, 1992; Palevsky et al., 2008). If so, natural selection will favour females choosing oviposition sites with optimal humidity conditions, which might compensate for the morphological and physiological constraints of eggs that cause mortality under dry conditions (Walzer et al., 2007). Another potential explanation of why we did not find differences in egg survival between populations may be that the production of drought-resistant eggs is dependent on the humidity conditions experienced by the mother, rather than by the eggs. In oviparous mites, many different modes of behaviour can be observed during oviposition, with the most important involving egg protection. In some species, the female simply deposits the egg on the substrate surface, sometimes pushing it into depressions or cracks (Marquardt et al., 2016). In other species, like *Phytoseius hawaiiensis* Prasad (Acari: Phytoseiidae) females are able to retain their eggs until just before larvae hatch if the oviposition substrate is unfavourable for the eggs (Sanderson and McMurtry, 1984). In the American dog tick, *Dermacentor variabilis* Say (Acari: Ixodidae), desiccation resistance of larvae is determined by the relative humidity experienced by the mother rather than the moisture conditions encountered by eggs after they are laid (Yoder et al., 2006). The diapausing eggs of *Petrobia latens* Muller (Acari: Tetranychidae) are covered by an outer impermeable waxy envelope limiting the evaporation rate (Lees, 1961). Finally, it has also been suggested, for astigmatid mites, that the chorion (external layer of the egg) may be covered with additional material, called exochorion, to increase egg desiccation resistance. The exochorion would be deposited by the mother onto the egg surface during its passage through the oviduct (Witaliński, 1993). All eggs used in our study were produced by females exposed to high humidity conditions before and during oviposition. We hypothesize that *P. persimilis* females adopt a different oviposition behaviour if they are exposed to low humidity conditions, leading to different characteristics of the eggs they lay.

In conclusion, *P. persimilis* eggs are differentially affected by constant and variable humidity conditions. While *P. persimilis* eggs are highly sensitive to constant low humidity, they are able to hatch, if exposed to high humidity for a few hours, even after spending more than 80% of their development at low humidity. This plasticity, enhancing their chances of survival under variable humidity conditions in the field, is of adaptive significance and present in all five populations studied here. High phenotypic variation in egg survival within populations is likely of crucial importance for the survival of *P. persimilis* under dry conditions. It appears that this predatory mite is capable of dealing with low humidity conditions more effectively than previously thought. To better understand the effects of low humidity on *P. persimilis* and further improve its efficacy as a biocontrol agent in dry conditions, future studies should focus on oviposition behaviour under low and variable humidity conditions.

2.5. ACKNOWLEDGMENTS

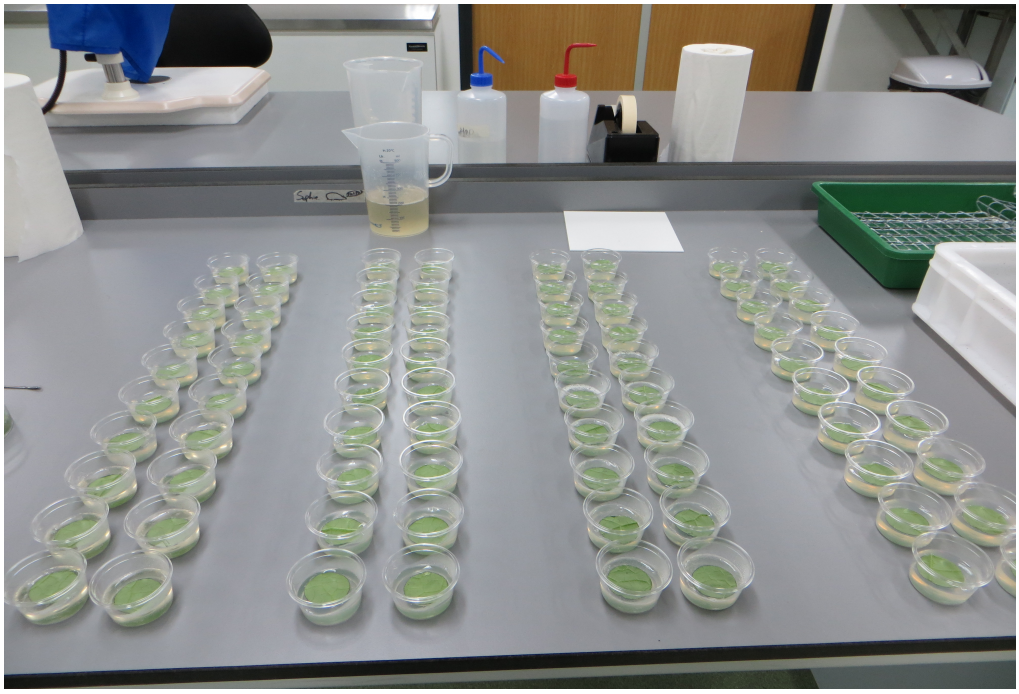
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 641456. The authors would like to thank Arnon Tabic, Marie-Stéphane Tixier and Alexandra Revynthi for collecting and sharing the *P. persimilis* populations from Israel, France, Turkey and Sicily. We finally thank two anonymous reviewers for insightful comments that helped to improve the manuscript.

2.6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.



Selection line on bean plants (experimental evolution trial Chapter 3)


Individual cups used for the oviposition tests (Chapter 3)

3

SELECTION FOR INCREASED DROUGHT RESISTANCE IN *Phytoseiulus persimilis* EGGS

Artificial selection and experimental evolution are promising tools to improve the efficacy of biocontrol agents in augmentative biological control. In this chapter, we tested these two methods to increase drought resistance in eggs of the predatory mite Phytoseiulus persimilis. *In an artificial selection trial, we exposed* P. persimilis *eggs from two selection lines to three selection rounds. In an experimental evolution trial, we exposed all life stages from two selection lines to a constant low humidity selection pressure. To evaluate the response to selection, we assessed egg survival at low humidity in both trials. At regular intervals during the trials, we also looked at oviposition rate of the females from each line, to check for potentially correlated responses to selection. We observed a significant increase in drought resistance of eggs in the experimental evolution trial. Already one month after the start of the trial, egg survival at low humidity had more than doubled. However, this increase in egg drought resistance disappeared within 15 days after we had removed the selection pressure from a group of adult females. In the artificial selection trial, no response to selection was observed after three selection rounds, and the selection process had no impact on oviposition rate. The results of this study indicate that drought resistance in* P. persimilis *eggs is a phenotypically plastic trait, regulated by their mother. This discovery raises new questions in the study of the effects of low humidity on* P. persimilis.

3.1. INTRODUCTION

Through the domestication of plants and animals, humans have carried out evolutionary experiments for millennia. Farmers and breeders, especially, have steered the evolution of many plant and animal species, by allowing only the individuals with desirable characteristics to reproduce. This process is called artificial selection, or selective breeding. Populations can also evolve more "naturally", when they are exposed by humans to new environments during several generations, and adapt to these environments. This process is called experimental evolution. Artificial selection and experimental evolution have both been used with insects and mites, the most famous examples being the domestication of silkworms and honey bees (Hoy, 1986). In addition to their use for the production of silk threads and honey, these two methodologies have the potential to improve biological control of agricultural pests (Lommen et al., 2017). Biological pest control relies on the use of living organisms (natural enemies), such as insects, mites and microbial pathogens, to suppress the population density or impact of a specific pest organism (Eilenberg et al., 2001). With the development of biocontrol companies over the last decades, the use of augmentative biocontrol, i.e. the augmentation of natural enemy species by mass production and repeated releases, has increased (van Lenteren, 2012). Despite the efficacy and the development of augmentative biocontrol worldwide, many challenges remain to be addressed. One of them is the difficulty to ensure the efficacy of natural enemies in varying environmental conditions. Their performance can be affected by many abiotic and biotic factors, including unfavourable climatic conditions, the presence of synthetic pesticides, potential attack by predators, or plant traits such as the presence of glandular trichomes (Le Hesran et al., 2019b). A solution to adverse conditions hampering the performance of biocontrol agents is to optimize some of their characteristics through selective breeding or experimental evolution. Successful genetic improvements have already been carried out for some natural enemy species (Lommen et al., 2017). For example, a strain of the predatory mite *Metaseiulus occidentalis* Nesbitt has been artificially selected for resistance to certain types of synthetic pesticides (Hoy, 1986). Moreover, tolerance to heat or desiccation has been genetically improved in several nematode, mite, and parasitic wasp species (White et al., 1970; Shapiro et al., 1997; Salame et al., 2010; Anbesse et al., 2012; Zhang et al., 2018). Selective breeding and experimental evolution are therefore promising methods to improve biocontrol agents. *Phytoseiulus persimilis* was one of the first natural enemies sold in Europe for augmentative biocontrol, as a specialized predator of *Tetranychus* mite species. The performance of *P. persimilis* as a biocontrol agent is negatively affected by low humidity, particularly because *P. persimilis* eggs are sensitive to drought (Zhang et al., 2018; Le Hesran et al., 2019a). Several studies have reported successful results from artificial selection or experimental evolution on *P. persimilis:* foraging behaviour, response to herbivore-induced plant volatiles, heat tolerance, adaptation to tomato plants, resistance to insecticides and dispersal rate were selected for in this species (Schulten et al., 1976; Voroshilov, 1979; Drukker et al., 1997; Margolies et al., 1997; Nachappa et al., 2010; Revynthi, 2017). However, to our knowledge, no selection experiments to improve drought resistance in *P. persimilis* have been reported. In this study, we investigated the possibility to select for enhanced drought resistance of *P. persimilis* eggs, since they are considered the most drought-sensitive life stage. We applied both artificial selection and experimental evolution methods, and measured the response to selection by assessing egg survival at low humidity, i.e. drought resistance of eggs. When

selecting for a specific trait, there are four important aspects of a relevant experimental design: (1) having extensive genetic variation in the base population (to maximize the chances of obtaining a response to selection), (2) creating replicated selection and control lines (to rule out effects of drift or a biased set-up), (3) controlling for environmental effects (to make sure a response to selection is due to a genetic change), and (4) check for the occurrence of correlated responses to selection, usually in the form of trade-offs (Lirakis and Magalhães, 2019). First, to increase genetic variation, we used a base population made of a mixture of five *P. persimilis* populations coming from different geographic locations. Although we did not find intraspecific genotypic variation between these populations for two tested markers (Cytb and 12S), this does not warrant the conclusion that there was no genetic variation between them (Le Hesran et al., 2019a). Second, we made sure to have replicated lines by creating two selection lines and two control lines for both selection trials. Third, we controlled for environmental effects at the end of the experimental evolution trial, by removing the selection pressure from individuals of the selection lines for 15 days, and evaluating the survival of their eggs at low humidity. And fourth, to determine whether trade-offs were present between egg drought resistance and other traits associated with fitness, we evaluated the oviposition rate of the females used in our trials, at regular time intervals during the selection process. This study is a first attempt to increase drought resistance of *P. persimilis* eggs, comparing two selection methods: artificial selection and experimental evolution.

3.2. MATERIALS AND METHODS

3.2.1. BASE POPULATION

A *P. persimilis* base population was created to start the selection trials. This base population was made by mixing five *P. persimilis* populations, originating from five different locations: France, Sicily, Turkey, Israel, and a commercial mass rearing (Koppert Biological Systems) (see CHAPTER 2 for more details on the geographical origins of the strains). To create the base population, 50 adult individuals from each population were placed together in a Petri dish (ø 12 cm, 3 cm high). The Petri dish contained an agar layer (1% agar, VWR Chemicals) on which a disk (ø 12 cm) of a cucumber leaf (*Cucumis sativus* 'Pyralis') infested with a mixture of all developmental stages of *T. urticae* was placed, with the adaxial side facing the agar plate. The Petri dish was closed with a lid, containing a 5 cm diameter hole covered by a 90-μm-mesh insect screen to allow air exchange while preventing the mites from escaping. The base population was maintained in a climate chamber at 18 ± 1 °C, 65 ± 2% RH and L16:D8 photoperiod, and mites were transferred to a new dish once a week. Individuals from all populations were able to interbreed in the base population for one month, before starting the selection trials. To ensure successful genetic crosses between the five populations, we had carried out separate crossings beforehand between individuals of all populations. These crosses confirmed that they could produce offspring.

3.2.2. ARTIFICIAL SELECTION

One month after creating the base population, two selection lines ($S1_{as}$, $S2_{as}$) and two control lines ($C1_{as}$, $C2_{as}$) were created for the artificial selection trial by collecting for each line

around 150 individuals from the base population. The selection lines and control lines were reared separately in Petri dishes (ø 12 cm, 3 cm high) in the same way as the base population, and kept in a climate chamber at 25 ± 1 °C, 75 ± 2% RH and L16:D8 photoperiod. Due to the higher temperature, the Petri dishes were replaced twice a week, because of deterioration of the leaf disks. Three selection rounds were conducted on the selection lines over a period of 145 days (selection round 1: 14 to 29 days after starting the trial; selection round 2: 66-82 days; and selection round 3: 129-145 days). For the first selection round, between 150 and 400 freshly laid eggs (0 to 6 hours old) were collected from the two selection lines. Because we could not collect so many eggs in one day, successive groups of eggs, or replicates, were collected for each selection line on different days (with 6 to 116 eggs per replicate). Eggs from the same line collected on the same day were part of the same replicate. For each replicate, the eggs were placed with a brush in holes (ø 0.7 cm, 0.4 cm deep, one egg per hole) in a plexiglass platform (17.5 L x 15 W x 4.5 H cm), each platform containing 30 holes. Each hole had a 90-μm-mesh insect screen at the bottom, to ensure contact with ambient relative humidity. The hole was then covered with a coverslip to prevent the larvae from escaping after hatching. The plexiglass platforms were placed in a climate cell at 65 ± 2% RH and 25 ± 1 °C for three days. After three days, the surviving individuals (larvae and nymphs) from each selection line were collected from the platforms, transferred to a new Petri dish with agar and leaf disk, and reared at 25 °C and 75% RH with *T. urticae* provided *ad libitum* (L16:D8 photoperiod). The first selection round was completed when 100 survivors per selection line were collected. These survivors were reared at 25 °C and 75% RH during approximately one month. The same process was repeated 66 days after starting the trial, using the offspring of the individuals selected in the first selection round: 100 survivors per line were collected again, to complete the second selection round (Fig.3.1). Finally, 129 days after starting the trial, the same process was repeated for the third selection round. For each replicate of each selection round, the egg hatching rate was assessed by counting the number of dead eggs (non-hatched eggs after three days) in the platforms. To compare the selection lines with the control lines, egg hatching rates of the control lines were assessed at 25 °C and 65% RH, in parallel with the selection lines. In the case of the control lines, no survivors were collected to complete a selection round (Fig.3.1).

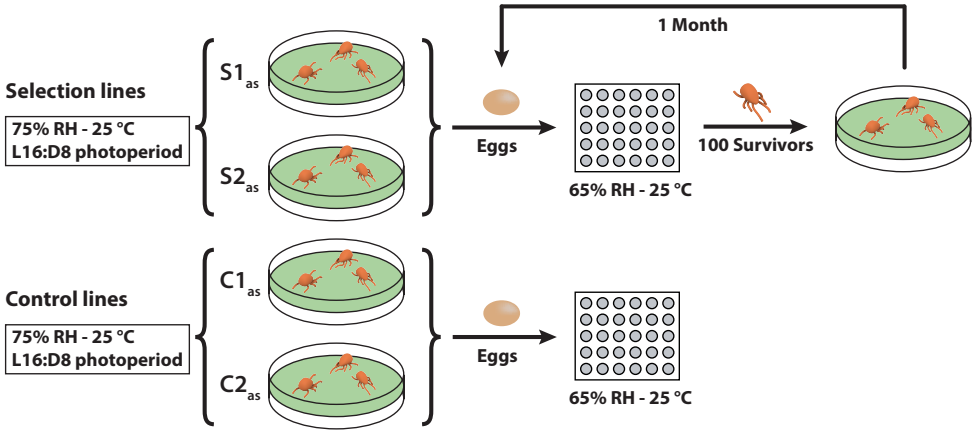


Figure 3.1: Set-up of the artificial selection trial for drought resistance of *Phytoseiulus persimilis* eggs. For the control lines, exposing the eggs to 65% RH was done exclusively to assess their drought resistance. No survivors were collected to create the next generations.

3.2.3. EXPERIMENTAL EVOLUTION

In parallel with the artificial selection trial, we carried out an experimental evolution trial. Two selection lines ($S1_{ee}$, $S2_{ee}$) and two control lines ($C1_{ee}$, $C2_{ee}$) were created by collecting around 150 individuals for each line from the base population. Each line was transferred to a separate container (14 L x 10 W x 13 H cm) with four living faba bean plants (*Vicia faba*) infested with *T. urticae*. The two selection lines were kept in a climate cell (Panasonic Versatile Environmental Test Chamber MLR-352) at 25 ± 1 °C and 55 ± 2% RH during the day (16 hours light), and 25 ± 1 °C and 65 ± 2% RH during the night (8 hours dark). The two control lines were kept in a climate chamber at 25 ± 1 °C and 75 ± 2% RH (L16:D8 photoperiod), inside an insect-proof cage (one line per cage). To prevent the mites from escaping and contamination between the lines, each container with a control line was placed inside a tray filled with water and soap, and each container with a selection line was placed inside a tray filled with sunflower oil (instead of water, to avoid increasing the relative humidity in the climate cell). The selection and control lines were transferred to new bean plants once a week. Hatching rates of the eggs laid by the females from each line were assessed at six time periods over 343 days: time period 1 (29-57 days after starting the trial), time period 2 (74-85 days), time period 3 (125-136 days), time period 4 (178-189 days), time period 5 (308-315 days), and time period 6 (after 343 days). To assess egg hatching rate at each time period, 30 adult females were collected from each line and placed in a Petri dish (one Petri dish per line) with agar and a cucumber leaf disk infested with *T. urticae*, at 25 ± 1 °C and 75 ± 2% RH. After six hours, the eggs laid by these females were collected and transferred to a plexiglass platform (one platform per line), in the same way as described in the artificial selection trial. The platforms with eggs were placed in a climate cell at 25 ± 1 °C and 65 ± 2% RH (time period 1); 62 ± 2% RH (time periods 2, 3) or 60 ± 2% RH (time periods 4, 5, 6) for three days (Fig.3.2). The number of hatched eggs was then recorded for each line. At time

periods 2, 3, 4 and 5, several replicates per line were conducted over several days: a group of eggs collected from one line on the same day formed one replicate.

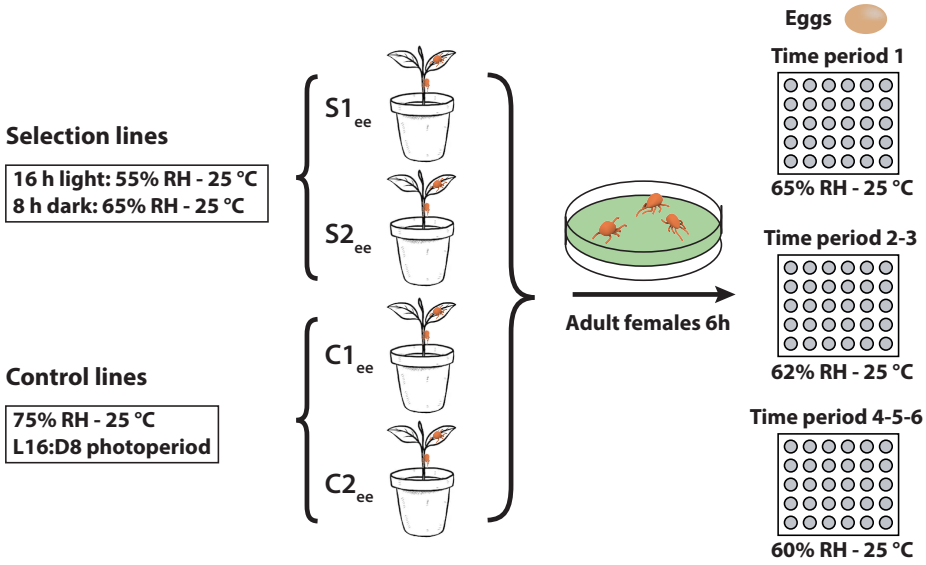


Figure 3.2: Set-up of the experimental evolution trial for drought resistance of *Phytoseiulus persimilis* eggs.

To control for environmental effects, 10 months after the start of the trial, 30 adult females from each selection line were collected and placed in a Petri dish (one Petri dish per line) with agar and a cucumber leaf disk infested with *T. urticae*, at 25 ± 1 °C and 75 ± 2% RH for 15 days. After 15 days of released selection pressure, young eggs (0 to 6 hours old) laid by these females were collected and placed in holes of a plexiglass platform, in a climate cell at 25 ± 1 °C and 60 ± 2% RH. After three days, the egg hatching rate was recorded.

3.2.4. OVIPOSITION TESTS

For the control and selection lines of the artificial selection trial, an oviposition test was carried out right after the completion of each selection round. For the lines of the experimental evolution trial, an oviposition test was carried out 32, 145, and 194 days after starting the trial. For each oviposition test, 20 adult females from each line were randomly collected and placed in individual plastic cups (ø 3.5 cm, 2.8 cm deep), covered by a lid with a 90-μm-mesh insect screen. At the bottom of each cup, an agar layer covered by a cucumber leaf disk (adaxial side facing the agar) infested with *T. urticae* provided a favorable substrate for the females to lay their eggs. During the oviposition tests, the cups with females were kept in a climate chamber at 25 ± 1 °C and 75 ± 2% RH (L16:D8 photoperiod) for three days. During these three days, the number of eggs laid by each female was recorded every 24 hours.

3.2.5. STATISTICAL ANALYSIS

For the artificial selection trial, we studied the effect of the factors treatment (control, selection) and selection round (1, 2, 3) on egg hatching rate at 65% RH, as well as the interaction between these two factors. For the experimental evolution trial, we studied the effect of the factors treatment (control, selection) and time period (1, 2, 3, 4, 5, 6) on egg hatching rate, as well as the interaction between these two factors. In both analyses, the response variable was expressed as a proportion (number of eggs hatched/number of eggs tested) for each replicate. To account for overdispersion in the data of both trials, we used a generalized linear mixed model with Template Model Builder (glmmTMB) with a betabinomial error distribution and a logit link function. The variables treatment and selection round (artificial selection trial) and treatment and time period (experimental evolution trial) were expressed as fixed effects in both models. The variable line was expressed as a random effect in both models, because of its collinearity with the variable treatment (line was nested into treatment). We carried out pairwise comparisons of means (estimated marginal means) to assess differences within selection round and time period levels. In the experimental evolution trial, we compared egg hatching rates at 60% RH of both selection lines at time period 5 (after 10 months of selection pressure), with egg hatching rates at 60% RH after 10 months of selection pressure followed by 15 days without selection pressure, and with an expected egg hatching rate at 60% RH for a *P. persimilis* line which has not gone through any selection pressure (see Le Hesran et al. 2019a). We investigated the effect of the factor treatment ("after 10 months of selection pressure", "after 10 months of selection pressure followed by 15 days without selection pressure", "no selection pressure") on egg hatching rate. We used a glmmTMB with a betabinomial error distribution and a logit link function. The factor treatment was expressed as a fixed effect in the model, and the factor line was expressed as a random effect. In the same way, for the artificial selection trial, we compared egg hatching rates at 65% RH of both selection lines after three selection rounds with an expected egg hatching rate at 65% RH for a *P. persimilis* line which has not gone through any selection pressure (see Le Hesran et al. 2019a). For the oviposition tests, we analysed the effects of the factors treatment (selection, control), line (C1, C2, S1, S2), and selection round (for artificial selection trial) or time (for experimental evolution trial) on the oviposition rate per female over three days. For each trial, we used a generalized linear model with a Poisson error distribution and a log link function. We considered a multiplicative dispersion parameter in the variance (quasi-Poisson error distribution), and we found an estimated parameter smaller than 1, therefore we decided upon two Poisson models with dispersion parameter equal to 1, leading to more conservative conclusions. The variables treatment, line and selection round/time were included as fixed effects in the two models. Although there was a collinearity between the variables treatment and line, we could not express the variable line as a random effect in the models, because we had only one replicate per line for each selection round/time level. We carried out pairwise comparisons of means (Tukey test) to assess differences within selection round and time levels. For all analyses, we used likelihood-ratio tests to select the most parsimonious models. The statistical analyses were performed in R (R version 3.5.1).

3.3. RESULTS

3.3.1. ARTIFICIAL SELECTION

In this trial, we investigated the possibility to increase the drought resistance of *P. persimilis* eggs after three rounds of selective breeding. Selection round had a significant effect on egg hatching rate ($\chi^2 = 13.44; DF = 2; P = 0.001$; Fig.3.3). For both treatments (selection and control), egg hatching rate at selection round 2 was significantly lower than egg hatching rate at selection round 1 (P = 0.001). Egg hatching rate at selection round 3 was not significantly different from egg hatching rates at round 1 (P = 0.07) and round 2 (P = 0.2). Treatment had no significant effect on egg hatching rate ($\chi^2 = 1.15; DF = 1; P = 0.28$): egg hatching rates of the selection lines were not significantly different from those of the control lines. Moreover, after three selection rounds, the estimated egg hatching rate at 65% RH for the two selection lines (0.38 ± 0.09) was not significantly different from the expected egg hatching rate for a *P. persimilis* line that has not experienced any selection pressure (0.39 ± 0.08) ($\chi^2 = 0.49; DF = 1; P = 0.48$). Finally, there was no significant interaction between the effects of selection round and treatment ($\chi^2 = 0.57; DF = 2; P = 0.75$). We mentioned earlier the importance of creating replicated selection and control lines to rule out effects of drift or a biased set-up. In our model, the variable line was expressed as a random effect. The estimated among-line standard deviation ($3 \cdot 10^{-5}$) was negligible compared to the magnitude of the largest selection round effect (-1.17), meaning that we can reasonably discard the hypothesis of a drift or a biased set-up effect. To conclude, three rounds of selective breeding at low humidity did not result in an increase in drought resistance of *P. persimilis* eggs.

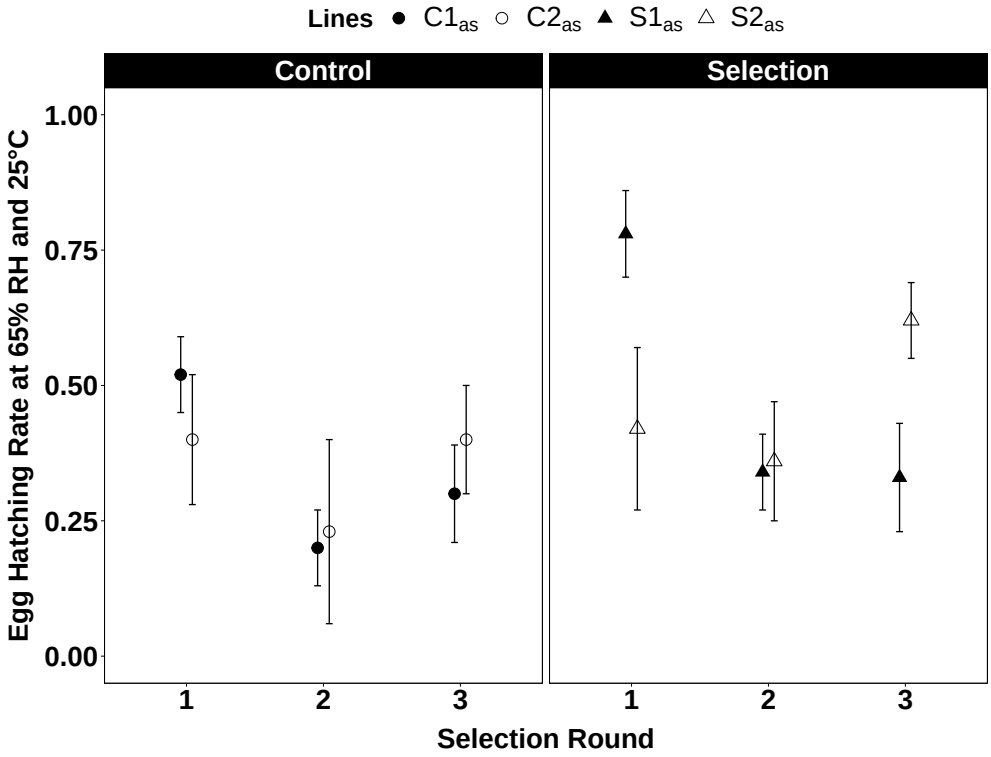


Figure 3.3: Egg hatching rate at 65% RH and 25 °C for eggs laid by *Phytoseiulus persimilis* females from four different lines in an artificial selection trial. Two lines went through three selection rounds (selection lines $S1_{as}$ and $S2_{as}$), and two lines were not exposed to any selection pressure during these three rounds (control lines $C1_{as}$ and $C2_{as}$). Symbols represent weighted average egg hatching rates for each selection round (3 to 8 replicates per selection round, n = 6 to 116 eggs per replicate). Error bars represent ± 1 weighted SD.

3.3.2. EXPERIMENTAL EVOLUTION

In this trial, we investigated the possibility to increase the drought resistance of *P. persimilis* eggs through experimental evolution over a period of 343 days. Treatment had a significant effect on egg hatching rate ($\chi^2 = 5.06; DF = 1; P = 0.02$; Fig.3.4): egg hatching rate at low RH was significantly higher for eggs from the selection treatment than for eggs from the control treatment, especially at the end of the trial, at time periods 5 and 6. Egg hatching rate of the selection lines was on average 0.52 ± 0.12 (time period 5) and 0.57 ± 0.19 (time period 6); while egg hatching rate of the control lines was on average 0.39 ± 0.12 (time period 5) and 0.44 ± 0.19 (time period 6) (estimated values ± 95% CI). Time period also had a significant effect on egg hatching rate ($\chi^2 = 250.5; DF = 6; P = 2 \cdot 10^{-16}$). Significantly higher egg hatching rates were observed at time periods 1 (P = 0.005), 3 (P < 0.0001) and 4 (P = 0.01) than at time period 5. Additionally, egg hatching rate was significantly higher at time period 3 than at time period 6 (P = 0.006). These differences are probably due to the fact that egg hatching

rate was evaluated at lower humidity levels at time periods 5 and 6 than at time periods 1 to 4. Finally, there was no significant interaction between the effects of treatment and time period ($\chi^2 = 6.9; DF = 5; P = 0.22$). In our model, the factor line was expressed as a random effect. The estimated among-line standard deviation ($1.9 \cdot 10^{-5}$) was negligible compared to the magnitude of the largest time period effect (-1.9), meaning that we can reasonably discard the hypothesis of a drift or a biased set-up effect.

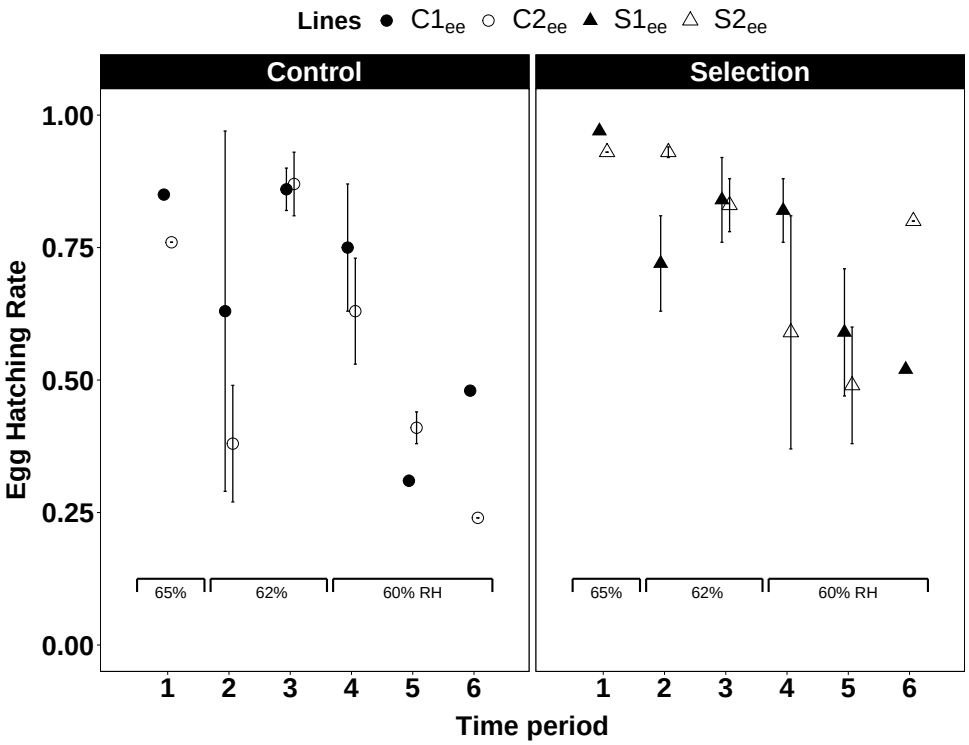


Figure 3.4: Egg hatching rate for eggs laid by *P. persimilis* females from four different lines, at different time periods, in an experimental evolution trial. Two lines were exposed to a constant selection pressure (55% RH during the day and 65% RH during the night) during 343 days (selection lines $S1_{ee}$ and $S2_{ee}$), and two lines were not exposed to low humidity selection pressure (75% RH) during 343 days (control lines $C1_{ee}$ and $C2_{ee}$). The three brackets represent the three humidity conditions under which egg hatching rate was measured. Symbols represent the weighted average egg hatching rate for each time period (1 to 5 replicates per time period, n = 9 to 48 eggs per replicate). Error bars represent ± 1 weighted SD.

Ten months after the start of the experimental evolution trial, we removed the selection pressure from a group of adult females from each selection line for 15 days, to control for environmental effects on egg survival at low humidity. Removing the selection pressure from the females had a significant effect on the hatching rate of their eggs at 60% RH ($\chi^2 = 12.4; DF = 1; P = 4 \cdot 10^{-4}$; Fig.3.5). Ten months after the start of the trial, egg hatch-

ing rate at 60% RH of the two selection lines was 0.59 ± 0.12 (line $S1_{ee}$) and 0.49 ± 0.11 (line $S2_{ee}$). After removing the selection pressure for 15 days, egg hatching rate at 60% RH was significantly lower: 0.18 ± 0.05 (line $S1_{ee}$) and 0.16 ± 0.07 (line $S2_{ee}$). These two lower egg hatching rates correspond to the expected values for a *P. persimilis* line that has not experienced any selection pressure (Fig.3.5). To conclude, we observed a response to the selection pressure in the experimental evolution trial: survival of *P. persimilis* eggs at low humidity was significantly increased. However, this response to selection disappeared shortly after we placed *P. persimilis* adult females back to high humidity: their eggs were not drought resistant anymore.

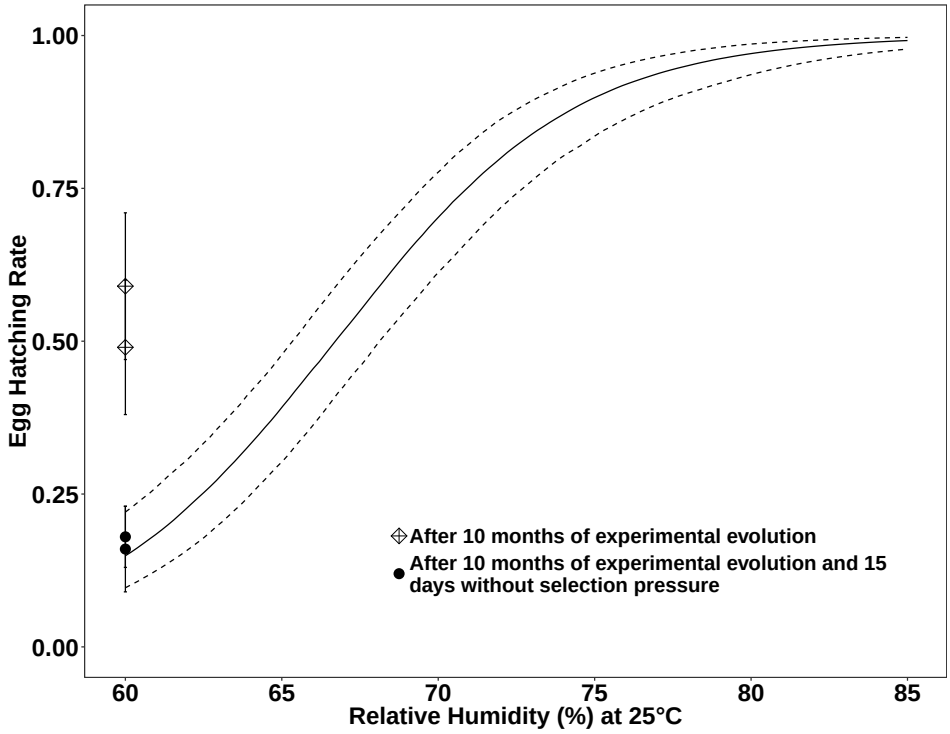


Figure 3.5: Comparison between the estimated (solid line, see CHAPTER 2) and the observed (symbols) hatching rates of *Phytoseiulus persimilis* eggs under different humidity conditions. Symbols represent the weighted average egg hatching rates after two different humidity treatments, error bars represent ± 1 weighted SD. Dashed lines represent the 95% confidence intervals around the estimated values.

3.3.3. OVIPOSITION TESTS

3.3.3.1. ARTIFICIAL SELECTION

Selection round had a significant effect on oviposition rate over three days ($\chi^2 = 7.01; DF = 2; P = 0.03$; Fig.3.6). Oviposition rate after selection round 3 was significantly higher than

after selection round 1 (P = 0.049): females laid on average 16.3 ± 1.0 eggs over three days after selection round 3, while they laid on average 14.6 ± 1.0 eggs after selection round 1 (estimated means ± 95% CI). Treatment (selection vs control) had no effect on oviposition rate ($\chi^2 = 0.0005; DF = 1; P = 0.98$), line had no effect on oviposition rate ($\chi^2 = 0.77; DF = 2; P = 0.68$), and there was no significant interaction between the effects of treatment and selection round ($\chi^2 = 0.74; DF = 2; P = 0.68$).

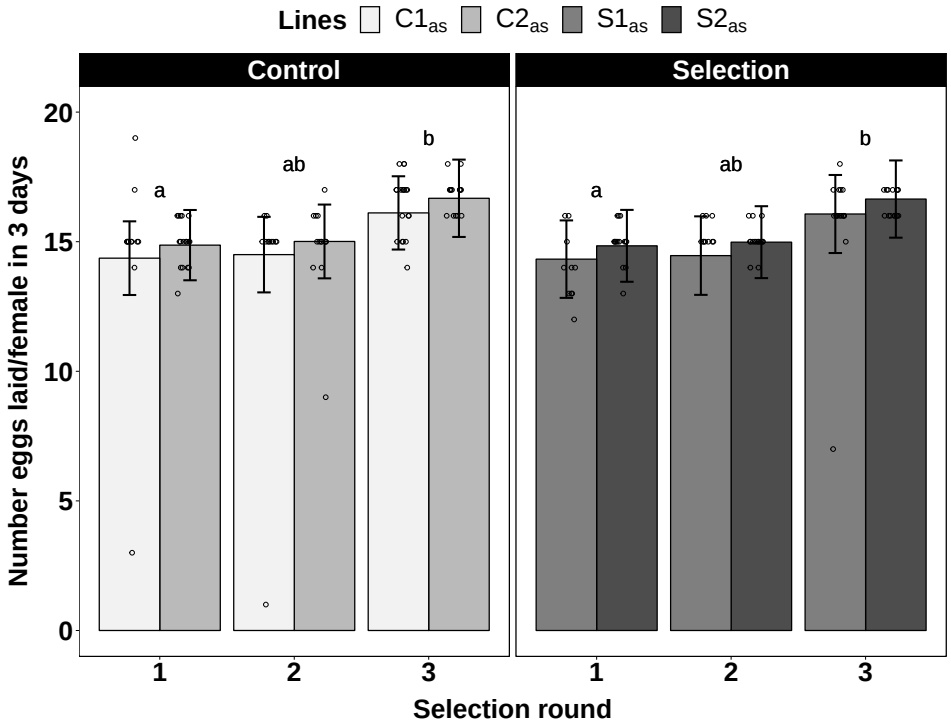


Figure 3.6: Estimated means (GLM) of the total number of eggs laid by *Phytoseiulus persimilis* females over three days, when exposed to two treatments in an artificial selection trial (control, selection), for four lines (light grey: control lines $C1_{as}$ and $C2_{as}$, dark grey: selection lines $S1_{as}$ and $S2_{as}$). Each dot represents the observed mean for one female. Error bars represent the 95% confidence intervals of the estimated means. Different letters above bars indicate significant differences between selection round levels (P < 0.05).

3.3.3.2. EXPERIMENTAL EVOLUTION

Time had a significant effect on oviposition rate over three days ($\chi^2 = 11.34; DF = 2; P = 0.003$; Fig.3.7). Females laid significantly more eggs 145 days after starting the trial (on average 14.6 ± 0.9 eggs) than 194 days after starting the trial (12.7 ± 0.8 eggs) (P = 0.002; estimated means ± 95% CI). Treatment had no effect on oviposition rate: ($\chi^2 = 0.56; DF = 1; P = 0.45$), line had no effect on oviposition rate ($\chi^2 = 0.17; DF = 2; P = 0.9$), and there was no significant interaction between effects of treatment and time ($\chi^2 = 0.96; DF = 2; P = 0.6$).

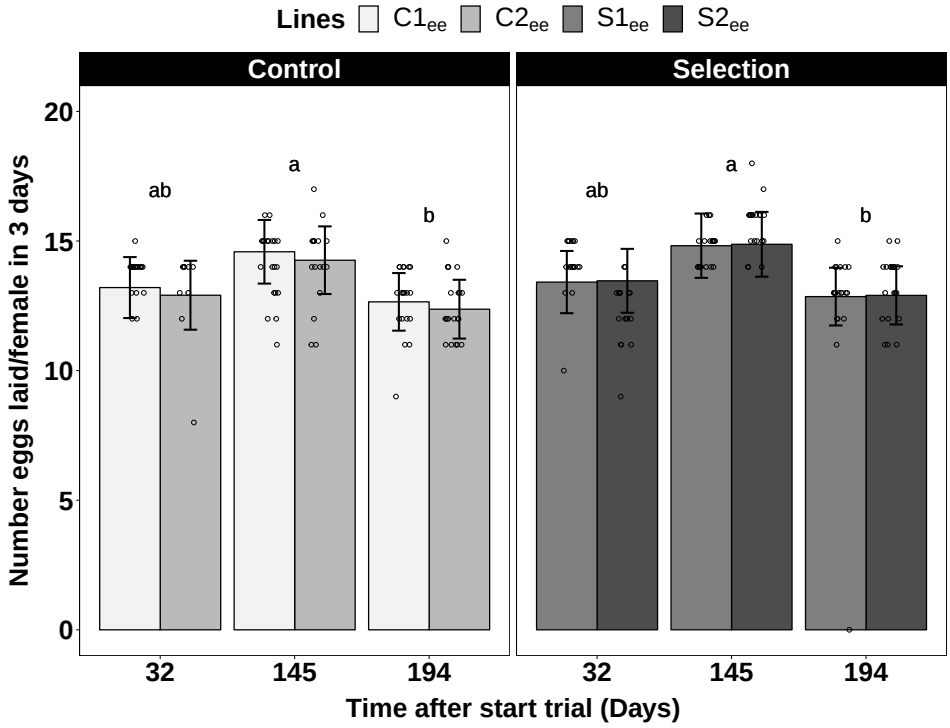


Figure 3.7: Estimated means (GLM) of the total number of eggs laid by *Phytoseiulus persimilis* females over three days, when exposed to two treatments in an experimental evolution trial (control, selection), for four lines (light grey: control lines $C1_{ee}$ and $C2_{ee}$, dark grey: selection lines $S1_{ee}$ and $S2_{ee}$). Each dot represents the observed mean for one female. Error bars represent the 95% confidence intervals of the estimated means. Different letters above bars indicate significant differences between time levels ($P < 0.05$).

3.4. DISCUSSION

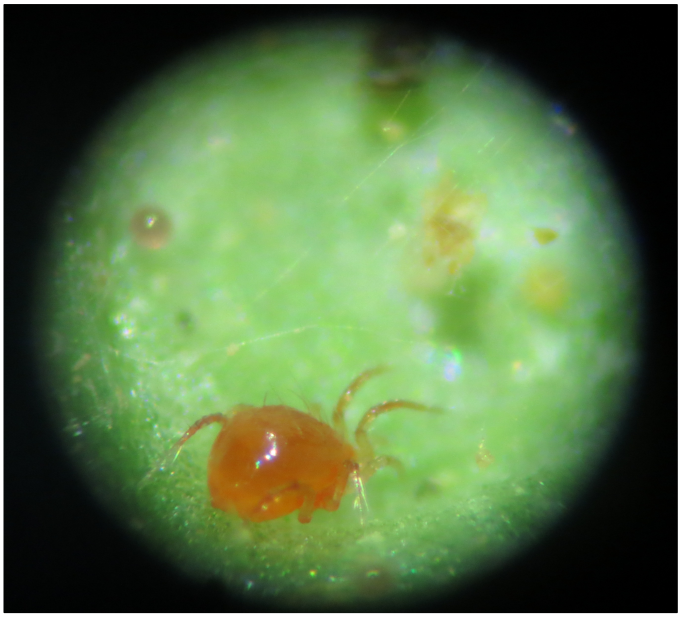
Our data show that drought resistance of *P. persimilis* eggs was differentially affected by the two types of selection trials conducted in this study: artificial selection and experimental evolution. Already one month after starting the experimental evolution trial, the estimated egg hatching rate at 65% RH was 0.88 ± 0.1 for the selection lines. This value is more than two times higher than the expected hatching rate of 0.39 ± 0.08 for *P. persimilis* eggs from a non-selected line exposed to 65% RH at 25 °C (Fig.3.5; see CHAPTER 2). On the other hand, in the artificial selection trial, the estimated egg hatching rate at 65% RH was below 0.55 during the three selection rounds, and there was no significant difference between the control and the selection lines. In the experimental evolution trial, surprisingly, not only the selection lines had a higher egg hatching rate at low RH, but also the control lines. It is possible that the humidity conditions experienced by the control lines were also stressful for the mites. In fact, before being transferred to the bean plants, individuals from the control lines came

from the base population and were reared in closed Petri dishes, with a leaf disk fixed to an agar layer. Relative humidity in the Petri dishes was probably very high (around 90% RH), compared to the actual RH on a bean plant at 75% RH conditions. Even though there might be a microclimate with higher RH on the surface of bean leaves (Pincebourde and Woods, 2012), this transition from a closed Petri dish to an "open air" environment might have been perceived as stressful for individuals of the control lines. Nevertheless, we can still assume that the selection pressure was higher on the selection lines, because their egg hatching rate at 60% RH was significantly higher than that of the control lines after ten months of selection. The question now is why drought resistance of eggs significantly increased in the experimental evolution trial, but not in the artificial selection trial? Various reasons could explain why a trait does not respond to selection. It may be that the evolution of this trait is constrained by the pleiotropic effects of genetic correlations with other traits, or that there has been insufficient genetic variation or selection pressure to affect the trait (Gibbs et al., 1997). The lack of genetic variation or the presence of pleiotropic effects do not seem to be good explanations for the absence of response in our artificial selection trial, since the base population and the trait of interest were the same for both trials. Instead, there were two main differences between the two trials conducted in this study. First, in the artificial selection trial, only the egg stage was exposed to a selection pressure, whereas in the experimental evolution trial, all life stages were constantly exposed to a selection pressure. When only the egg stage was exposed to a selection pressure, no significant increase in egg drought resistance occurred. When all life stages were exposed to the low humidity selection pressure, drought resistance of eggs significantly increased within a short time. Moreover, in the experimental evolution trial, when the selection pressure was removed from a group of adult females from both selection lines for only 15 days, the hatching rate of their eggs at 60% RH significantly decreased. It became equivalent to the expected egg hatching rate for a *P. persimilis* line that has not experienced any low humidity selection pressure. Ten months of selection were undone in 15 days. These observations lead to two conclusions. First, another life stage than the eggs was responding to the selection pressure in the experimental evolution trial: the adult females. Second, since drought resistance of eggs significantly decreased when the females returned to higher humidity conditions, the response of adult females was probably due to environmental effects rather than a genetic change in the selection lines. Drought resistance of *P. persimilis* eggs seems to be a phenotypically plastic trait, regulated by their mothers, depending on the relative humidity in their environment. This transgenerational phenotypic plasticity, called environmental maternal effect, has also been observed in a selection experiment on dispersal distance in *T. urticae*. No response to selection was observed after 10 generations of artificial selection, because maternal effects induced phenotypic plasticity for dispersal distance: the population density experienced by the mother influenced the dispersal distance of her offspring (Bitume et al., 2011). In our study, applying the selection pressure to eggs only, like in the artificial selection trial, was therefore not an adequate selection method to increase drought resistance of eggs. The second important difference between the two selection trials relates to the rearing conditions: in the artificial selection trial, the mites were reared in Petri dishes on cucumber leaves, while in the experimental selection trial they were reared on live bean plants. We can wonder if the use of two different plant species had an effect on egg drought resistance, since the morphology and the biochemistry of a plant species can affect the response of a preda-

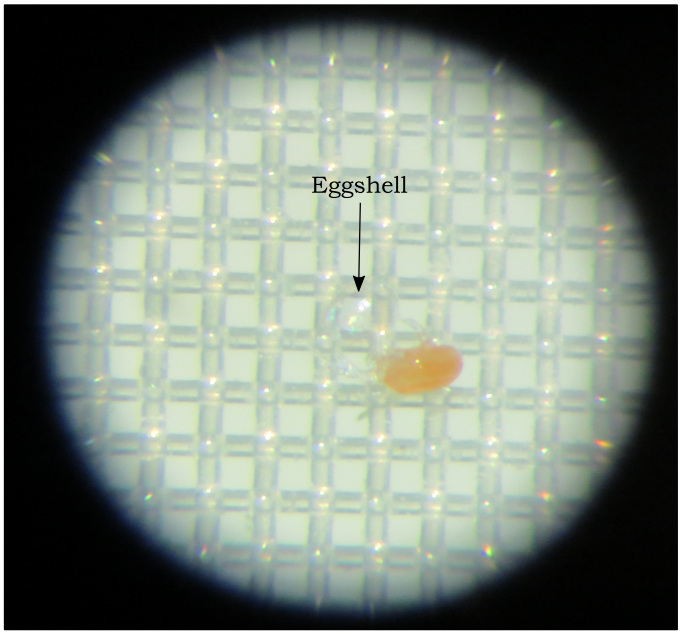
tor and its prey, through direct and indirect effects (Takabayashi and Dicke, 1992; Skirvin and Fenlon, 2001; Schmidt, 2014). For example, leaf trichomes of certain plant species can have an impact on biocontrol agents: *Phytoseiulus persimilis* females lay fewer eggs and have a shorter lifespan on tomato leaves than on bean leaves, because tomato leaves are full of toxic and sticky trichomes (Gillespie and Quiring, 1994; Drukker et al., 1997). In our trials, the morphologies of the two plant species were similar, and probably had a negligible impact on *P. persimilis*: the bean plants had glabrous leaves and stems, and the cucumber leaves had very few trichomes on their surface. Regarding biochemistry, infested plants can produce herbivore-induced volatiles that attract predators, or secrete toxins as a defense against herbivores. The composition of the blend of these volatiles or toxins depends on the plant species and the herbivore attacking the plant (Takabayashi et al., 1991). For example, bean plants infested with herbivorous spider mites emit volatile chemicals that attract *P. persimilis* (Dicke et al., 1990). In our study, we cannot say with certainty whether the volatiles or toxins produced by cucumber leaves and bean plants had an effect on drought resistance of eggs. However, in both trials, egg drought resistance was evaluated in the same way: females were first transferred to Petri dishes with cucumber leaves infested with *T. urticae*, where they did not have to rely on volatile chemicals to find their prey (due to the small size of the Petri dish and the big quantity of prey), and their eggs were collected for the egg hatching test six hours later. Therefore, the effect of volatile chemicals or toxins on our results, if it exists, was probably negligible. In parallel with the two selection trials, we tested for the occurrence of correlated responses to selection, more specifically in terms of oviposition rate. Under optimal conditions, between 25 and 30 °C, a *P. persimilis* female can lay around 4 to 5 eggs per day (Schulten et al., 1978; Helle and Sabelis, 1985). For the artificial selection trial, there was no negative effect of the selection process on oviposition rate. Oviposition rate after selection round 3 (16.3 eggs in three days, equivalent to 5.4 eggs per day) was even significantly higher than that after selection round 1 (14.6 eggs in three days, equivalent to 4.8 eggs per day). However, this change happened for both the selection lines and the control lines. It was therefore probably due to an environmental effect during the trial, rather than to the selection process. For the experimental evolution trial, oviposition rate was similar for both the selection and the control lines. Oviposition rate was the same at the beginning of the trial (after 32 days) and after 194 days: 12.7 eggs in three days, equivalent to 4.2 eggs per day. Interestingly, females from the experimental evolution trial laid less eggs than females from the artificial selection trial: 12.7 eggs in three days after 194 days of experimental evolution, compared to 16.3 eggs in three days after three rounds of artificial selection. This difference could be due to the fact that adult females from the experimental evolution trial were exposed to a low humidity selection pressure, contrary to females from the artificial selection trial. This drought stress just before the oviposition tests could have weakened the females from the experimental evolution trial, and affected their oviposition rate, at least during the three days of the oviposition tests. As a consequence, the lower oviposition rate in the experimental evolution trial was probably more the result of a reversible effect of drought stress on the females rather than a correlated response to selection.

To conclude, these two trials allowed a deeper understanding of the effects of low humidity on *P. persimilis*. Although the egg stage is considered the most drought-sensitive life stage in this species, it appears that *P. persimilis* eggs have two major resources to deal

with low humidity conditions. First, we showed in CHAPTER 2 that *P. persimilis* eggs have a plastic response to humidity variations: they are able to survive long periods of drought if they are exposed to high humidity for a few hours of their development. This 'flexibility' enhances their chances of survival in dry conditions. Second, in the present study, we showed that drought resistance of *P. persimilis* eggs can be significantly increased by their mothers, depending on the humidity in their environment. Drought resistance in *P. persimilis* eggs seems to be a phenotypically plastic trait triggered by low humidity conditions in their mothers' environment, rather than a trait genetically determined in eggs. It is now a necessary step to study further the effects of low humidity on *P. persimilis* adult females and their oviposition behaviour.



A *Phytoseiulus persimilis* adult female (back view)



A *Phytoseiulus persimilis* larva freshly hatched from its eggshell

4

MATERNAL EFFECT DETERMINES DROUGHT RESISTANCE OF EGGS IN THE PREDATORY MITE *Phytoseiulus persimilis*

The ability of an organism to adapt to short-term environmental changes within its lifetime is of fundamental importance. This adaptation may occur through phenotypic plasticity. Insects and mites, in particular, are sensitive to changes in temperature and humidity, especially during the juvenile stages. In this chapter, we studied the role of phenotypic plasticity in the adaptation of eggs to different relative humidity conditions, in the predatory mite Phytoseiulus persimilis, *used worldwide as a biological control agent of the spider mite* Tetranychus urticae. *The biocontrol efficacy of* P. persimilis *decreases under dry conditions, partly because* P. persimilis *eggs are sensitive to drought. We exposed* P. persimilis *adult females from two different strains to constant and variable humidity regimes, and evaluated the hatching rate of their eggs in dry conditions, as well as the survival and oviposition rates of these females. Whereas the eggs laid by* P. persimilis *females exposed to constant high humidity did not survive in dry conditions, females exposed to constant low humidity started laying drought-resistant eggs after 24 hours of exposure. Survival and oviposition rates of the females were affected by humidity: females laid fewer eggs under constant low humidity, and had a shorter lifespan under constant high and constant low humidity. The humidity regimes tested had similar effects across the two* P. persimilis *strains. Our results demonstrate that transgenerational phenotypic plasticity, called maternal effect, allows* P. persimilis *females to prepare their offspring for dry conditions.*

Chapter 4 is based on the article: Le Hesran, S., Groot, T., Knapp, M., Bukovinszky, T., Nugroho, J.E., Beretta, G., Dicke, M. (2020). Maternal effect determines drought resistance of eggs in the predatory mite *Phytoseiulus persimilis*. *Oecologia*, **192**:29-41, doi:10.1007/s00442-019-04556-0.

4.1. INTRODUCTION

Long-term adaptations, through natural selection, may lead to a match between organisms and their environment (Darwin, 1859). Short-term variation in environmental factors, however, can disrupt this match and negatively affect the survival and fitness of an organism (Nussey et al., 2007; Whitman and Agrawal, 2009). Understanding how organisms adapt to short-term environmental changes, and the consequences of this adaptation at the population level is necessary to predict population dynamics in stressful environments. Terrestrial insects and mites, in particular, are highly sensitive to changes in temperature and humidity, because they are poikilothermic organisms (Gotoh et al., 2014) with small body size and a large surface-area-to-volume ratio (Gibbs, 2002; Gefen et al., 2006). Under variable abiotic conditions, they face two main physiological challenges: avoiding harmful body temperatures, and retaining sufficient water while maintaining gas exchange (Potter and Woods, 2012). One solution to these challenges is phenotypic plasticity, defined as the ability of an individual to display a range of different phenotypes in multiple environments (DeWitt et al., 1998). Phenotypic plasticity allows individuals to adjust to environmental changes in real time (Whitman and Agrawal, 2009), and includes morphological, behavioural, physiological, and molecular adaptations (Price et al., 2003). These plastic adaptations can vary significantly depending on many factors, like the developmental stages of insects and mites (Whitman and Agrawal, 2009; Potter and Woods, 2012; Ghazy et al., 2016; Fischer and Kirste, 2018; Mutamiswa et al., 2019). Early developmental stages such as eggs and larvae are often considered more vulnerable to environmental stresses, because of their limited dispersal ability and their small size (Schausberger, 1998; Montserrat et al., 2007; Walzer et al., 2007; Ferrero et al., 2010; Potter and Woods, 2012; Döker et al., 2016; Torres-Campos et al., 2016). Although many studies on insects and mites have focused on the sensitivity of the egg stage to extreme temperature and humidity conditions (Colloff, 1987; Sota and Mogi, 1992; Schausberger, 1998; Williams et al., 2004; Yoder et al., 2004; Walzer et al., 2007; Ferrero et al., 2010; Potter and Woods, 2012; Le Hesran et al., 2019a), most of these studies have only exposed the eggs to stressful abiotic conditions, while the females that produced them were kept under favourable conditions. In various species, it has been shown that a mother can change the type of eggs that she lays or can program a developmental switch in her offspring, so that it may better endure adverse environmental conditions (Saunders, 1966; Margolies and Wrensch, 1996; Mousseau and Dingle, 1991; Fox et al., 1999; Fischer et al., 2003; Rahman et al., 2004; Yoder et al., 2006; Montserrat et al., 2007; Ross et al., 2011). For example, when females of the parasitic wasp *Nasonia vitripennis* (Walker) are exposed to short day length and low temperature, the majority of their larval offspring will enter diapause (Saunders, 1966). This special case of transgenerational phenotypic plasticity is called maternal effect. It is defined as the causal influence of the maternal genotype or phenotype on the offspring's phenotype (Wolf and Wade, 2009). A maternal effect can also be considered as a 'shared phenotype' that affects both maternal and offspring fitness simultaneously (Rossiter, 1991; Marshall and Uller, 2007; Walzer and Schausberger, 2015). How females find the most 'adaptive' strategy to ensure the survival of their offspring as well as their own survival in stressful conditions is therefore an essential question when studying maternal effects. Moreover, these maternal adjustments of offspring phenotype may vary within and between populations, due to genetic variation for plasticity (Pigliucci, 2005). For example, populations that experience the greatest extent of variability in humidity condi-

tions are expected to be more plastic in traits that mitigate humidity stress (Valladares et al., 2014). Finally, although maternal effects are increasingly recognized for their role in adaptation to variable environments (Lorenzon et al., 2001; Marshall and Uller, 2007; Van Asch et al., 2010), little is known about their impact on insects and mites under extreme temperature and humidity conditions. In the present study, we focus on the effects of low relative humidity on the phytoseiid predatory mite *Phytoseiulus persimilis*. *Phytoseiulus persimilis* is the most frequently applied predator for biological control of two-spotted spider mites (*Tetranychus urticae*), and its efficacy is dependent on temperature and humidity conditions (Weintraub and Palevsky, 2008). This predatory mite goes through five developmental stages: egg, larva, protonymph, deutonymph, and adult (Sabelis, 1981). The egg stage is expected to be the most drought-sensitive life stage, because eggs can neither move, nor feed, nor drink to compensate for water deficit. Eggs of *P. persimilis* do not survive at constant low humidity. This sensitivity of the egg stage is considered to be partly responsible for the low efficacy of *P. persimilis* as a biocontrol agent in dry conditions (Helle and Sabelis, 1985; Croft et al., 1993; Schausberger, 1998; Walzer et al., 2007; Ferrero et al., 2010; Döker et al., 2016). However, the effects of drought on other life stages of this predator are still unclear. To our knowledge, no study has focused on the impact of drought stress on adult females in *P. persimilis* or other phytoseiid mite species, and more specifically on the effects of this drought stress on the drought sensitivity of their eggs. The three main objectives of this study were to: (i) investigate whether maternal strategies enhance the survival of *P. persimilis* eggs under stressful humidity conditions, and estimate the plasticity of these strategies (ii) evaluate the effects of different humidity levels on *P. persimilis* adult females and (iii) evaluate the degree of genetic variation for potential maternal strategies in *P. persimilis*. We showed in CHAPTER 2 that *P. persimilis* eggs have a different sensitivity to constant and variable humidity conditions (Le Hesran et al., 2019a). We therefore exposed *P. persimilis* females to constant low, constant high, and variable humidity conditions. To investigate the potential maternal strategies promoting egg survival under dry conditions, we assessed, under low humidity, the hatching rate of the eggs laid by these females. To estimate the plasticity of these maternal strategies, we exposed the females to a sudden change in humidity conditions (see humidity treatment "variable 2"). To evaluate the effects of different humidity levels on *P. persimilis* females, we focused on their oviposition and survival rates, two traits determining population growth and biocontrol efficacy. As there may be trade-offs between fecundity and survivorship (Biro and Stamps, 2008), it is important to study them simultaneously. Although *P. persimilis* females can live for more than 60 days (Amano and Chant, 1977), we studied their survival rate over a period of 20 days (from 7 days old to 27 days old). Egg production of *P. persimilis* females at 25 °C starts within 24 hours after mating, and will continue for a period of 15-20 days (Schulten et al., 1978). Therefore, we considered these first 20 days as the most important part of a *P. persimilis* female's life. We studied their oviposition rate over periods of 4 and 10 days. Finally, to evaluate the degree of genetic variation for potential maternal strategies, we compared the egg hatching, oviposition and survival rates of two different strains of *P. persimilis*, with different geographical origins and reared under different humidity conditions.

4.2. MATERIALS AND METHODS

4.2.1. PREDATORY MITES

Two *P. persimilis* strains were used: a "commercial strain", and a "mixed strain" (line $S1_{ee}$ from CHAPTER 3). The commercial strain was obtained from a commercial mass rearing (Koppert Biological Systems), and reared in two Petri dishes, containing around 100 individuals each (see Le Hesran et al. 2019a). The mixed strain was created by mixing individuals from five different populations (50 individuals per population). Four of these populations were field-collected in France, Sicily, Israel, and Turkey (see Le Hesran et al. 2019a). The fifth one was obtained from the same commercial mass rearing as the commercial strain (Koppert Biological Systems). In November 2016, one month after mixing these five populations, 150 individuals were randomly collected from this mix and transferred to a container with four faba bean plants (*Vicia faba* L.) infested with *T. urticae*. The container was kept in a climate cell (Panasonic Versatile Environmental Test Chamber MLR-352) at 55 ± 2% RH during the day (16 hours) and 65 ± 2% RH during the night (8 hours), at 25 °C (photoperiod L16:D8). The bean plants were replaced once a week, and two-spotted spider mites were provided as food twice a week. The container with bean plants was placed in a tray filled with sunflower oil, to prevent mite dispersal. The mixed strain was reared in these conditions during 17 months before the experiments started. To control for the influence of *P. persimilis* female age, we used even-aged cohorts of young adult females (seven days since the egg stage) for all experiments. These females were collected as eggs from the two strains, and kept for seven days in two separate Petri dishes, in a climate cell at 70 ± 2% RH and 25 ± 1 °C. Inside the Petri dishes, an agar layer (agar powder, VWR Chemicals, 1/100 diluted) and a cucumber leaf disk infested with two-spotted spider mites provided optimal conditions for the development of these eggs to adults.

4.2.2. HUMIDITY TREATMENTS

We tested 4 humidity treatments: "constant low" (65% RH), "constant high" (95% RH), "variable 1" (successive cycles of 8 hours at 65% RH and 16 hours at 95% RH), and "variable 2" (78 hours at 65% RH followed by 24 hours at 95% RH) (Fig.4.1). We showed in CHAPTER 2 that only 39% of *P. persimilis* eggs survive at 65% RH and 25 °C (Le Hesran et al., 2019a). We therefore considered that 65% RH was stressful enough for *P. persimilis* eggs, and that these humidity conditions were likely to trigger a maternal effect in *P. persimilis* females. One replicate consisted of 15 to 20 adult females per treatment and per strain. The females were placed in individual plastic cups (ø 3.5 cm, 2.8 cm deep), covered by a lid with a fine gauze (gauze-width 90 µm). To provide females with an oviposition substrate, a thin layer of cardboard with hairy surface facing up was fixed with a double-sided tape (Tesa®) at the bottom of each cup. For humidity treatment "constant low", the cups were placed upside down on top of a wire platform (2.5 x 2.5 cm spacing) inside a closed plastic box (40 L x 25.5 W x 16.5 H cm). The relative humidity inside the box was regulated with a humidifier (Cigar Oasis Excel), to achieve constant 65% RH (average 64.7 ± 1.6% RH). For humidity treatment "constant high", the cups were placed upside down on an agar layer at the bottom of a closed plastic box, in which the relative humidity was maintained at 95% RH (average 96.2 ± 1.7% RH). For humidity treatment "variable 1", two additional plastic boxes were set up using the

same methods: one box at 65% RH and one box at 95% RH. The cups were kept 8 hours per day in the box at 65% RH (photoperiod L8:D0), and 16 hours per day in the box at 95% RH (photoperiod L8:D8). For humidity treatment "variable 2", the cups were kept during 78 hours in the same box as humidity treatment "constant low", followed by 24 hours in the same box as humidity treatment "constant high". All boxes containing the cups were placed in a climate cell at 70 ± 2% RH and 25 ± 1 °C (L16:D8 photoperiod). The females were provided with fresh *T. urticae* (larvae, nymphs and adults) *ad libitum* every day.

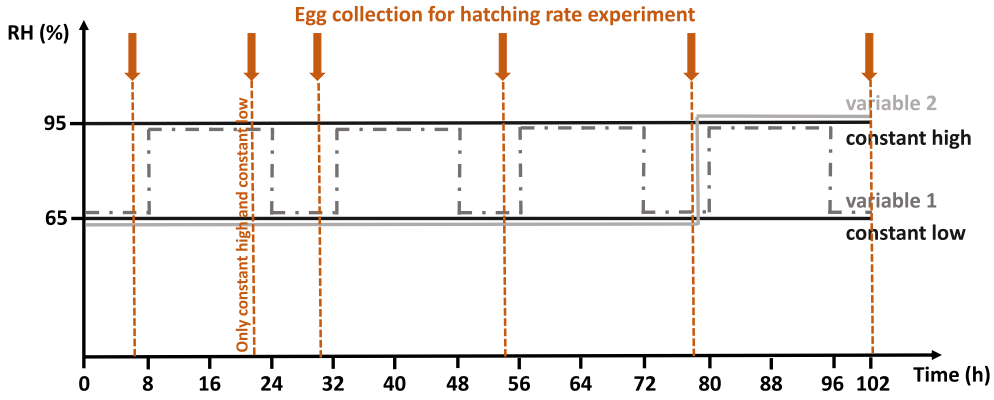


Figure 4.1: Four humidity treatments tested in this study. The arrows represent the time points when eggs were collected for the hatching rate experiment.

4.2.3. EGG HATCHING

We collected freshly laid *P. persimilis* eggs (between 0 and 6 hours old) after 6, 21, 30, 54, 78, and 102 hours of female exposure to each humidity treatment (for humidity treatments "variable 1" and "variable 2", we did not collect eggs after 21 hours of female exposure). To achieve this, all eggs present in each cup were removed 6 hours before collecting the eggs. Six hours later, the freshly laid eggs were collected from each cup and placed with a brush in a hole (ø 0.7 cm, 0.4 cm deep) in a platform made of polymethyl methacrylate (PMMA) (17.5 L x 15 W x 4.5 H cm) containing 30 holes (one egg per hole). Each hole had a fine gauze at the bottom (gauze-width 90 µm), to ensure contact with ambient air, and was then covered with a coverslip to prevent the larvae from escaping after hatching. The PMMA platforms (one platform per collection time, per strain, per humidity treatment) were placed in a climate chamber at 60 ± 1.6% RH, 25 ± 1.7 °C and L16:D8 photoperiod. Egg hatching rate (number of hatched eggs divided by total number of eggs in the platform) was recorded 72 hours after placing the platforms in the climate chamber. A data logger (LogTag TRIX 8) was placed in the climate chamber and in each box containing the cups, to measure relative humidity and temperature. For each humidity treatment and each strain, this protocol was repeated 5 to 7 times.

4.2.4. OVIPOSITION

We assessed the oviposition rate of the females exposed to the 4 humidity treatments over 4 days. We counted the number of eggs laid by each female after 24, 48, 72, and 96 hours of exposure. For each humidity treatment and each strain, the oviposition experiment over 4 days was repeated 5 to 10 times, with 15 to 20 females per replicate. We also assessed the oviposition rate of females exposed to humidity treatments "constant low", "constant high", and "variable 1" during 10 days. Females from the commercial strain were exposed to the 3 humidity treatments, while females from the mixed strain were exposed to treatment "variable 1" only. One replicate consisted of 20 females, and we carried out 3 replicates per treatment and per strain. On days 6 and 7, females exposed to humidity treatment "variable 1" spent 5 hours at low humidity (photoperiod L5:D0) and 19 hours at high humidity (photoperiod L11:D8), instead of 8 hours at low humidity and 16 hours at high humidity, for logistic reasons. The cups from humidity treatment "constant high" were changed after five days, to avoid development of fungi inside the cups.

4.2.5. SURVIVAL

The same females used in the oviposition experiment over 10 days were used for the survival experiment: after an exposure of 10 days to humidity treatments "constant low", "constant high", and "variable 1", females were kept under the same conditions during 10 additional days. Their survival rate was assessed over these 20 days. Females were supplied every day with ample fresh *T. urticae* (larvae, nymphs and adults) as food. On days 6, 7, 13, 14, and 20, females exposed to humidity treatment "variable 1" spent 5 hours at low humidity (photoperiod L5:D0) and 19 hours at high humidity (photoperiod L11:D8), instead of 8 hours at low humidity and 16 hours at high humidity, for logistic reasons. The cups from humidity treatment "constant high" were changed every 5 days, and the cups from humidity treatment "variable 1" were changed after 10 days, to avoid development of fungi inside the cups. To make sure that the potential stress caused by the transfer of the females to new cups did not affect their survival or oviposition rate, we also changed the cups of humidity treatments "constant low" and "variable 1" every 5 days during 20 days, for one replicate. We did not observe an influence of changing cups on the survival or oviposition of the females.

4.2.6. STATISTICAL ANALYSIS

For the egg hatching experiment, we carried out three analyses. In a first model, we studied the effect of the factors humidity treatment ("constant low", "constant high", and "variable 1"), strain, and exposure time of females on the hatching rate of *P. persimilis* eggs during 102 hours. We also looked at the following interactions, "humidity treatment x exposure time of females" and "humidity treatment x strain". In a second model, we compared the effects of the humidity treatments "constant low" and "variable 2", as well as the factors strain and exposure time of females, on the egg hatching rate during the first 78 hours (in both treatments, females were exposed to constant low humidity during the first 78 hours). We also looked at the interaction "humidity treatment x strain". We then compared, in a third model, the effects of the two humidity treatments "constant low" and "variable 2", as well as the factor strain, on the egg hatching rate after 102 hours of female exposure (between 78 and

102 hours of exposure, humidity increased in treatment "variable 2"). We also looked at the interaction "humidity treatment x strain". In all three analyses, the response variable was expressed as a proportion (number of eggs hatched/number of eggs tested) for each replicate. For all analyses, we used a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function. The variables humidity treatment, exposure time of females, and strain were expressed as fixed effects in the models. The replicates which had been performed at the same date were assigned to the same replicate number. The variable replicate was expressed as a random effect in the models (by-replicate random intercept), to take into account the fact that individuals within the same replicate were potentially correlated. Since there was overdispersion in the data for the first two models, we introduced a "per-observation" random effect. For all analyses, we used the model-fitting method of the maximum likelihood (Laplace approximation), and used likelihood-ratio tests to select the most parsimonious models. For the oviposition experiment, we first analysed the total number of eggs laid per female over four days, comparing humidity treatments "constant low", "constant high", and "variable 1" for both strains. We also looked at the interaction "humidity treatment x strain". Data from females which died before the fourth day of the experiment were not used. In total, data from 586 females were analysed. Thereafter, we analysed the total number of eggs laid per female over 10 days, comparing the 3 humidity treatments ("constant low", "constant high", "variable 1") for the commercial strain, and comparing both strains for humidity treatment "variable 1". Data from females which died before the tenth day of the experiment were not used. In total, data from 135 females were analysed for the commercial strain, and from 53 females for the mixed strain. For both analyses, we used a generalized linear model (GLM) with a Poisson error distribution and a log link function. We considered a multiplicative dispersion parameter in the variance (quasi-Poisson error distribution), and we found an estimated parameter smaller than 1, therefore we decided upon a Poisson model with dispersion parameter equal to 1, leading to more conservative conclusions. The variables humidity treatment, strain and replicate were included as fixed effects in the two models. In the analysis of oviposition rate over 10 days, we had only 3 replicates for each combination of humidity treatment and strain, making a reliable estimate of variance between replicates tenuous (Crawley, 2002). Therefore, we specified the variable replicate as a fixed rather than a random effect in the model. Similarly, in the analysis of oviposition rate over 4 days, we included the variable replicate as a fixed effect. We carried out pairwise comparisons of means (Tukey test) to assess differences within strain and humidity treatment levels. For both models, the estimated values for each humidity treatment and strain were obtained by calculating the weighted average of the estimated values of all replicates. The third part of the analysis was about the oviposition experiment over 4 days. For both strains, we calculated for each female the ratio of eggs laid on day 4 divided by the total number of eggs laid over 4 days. We then studied the effects of humidity treatment ("constant low", "constant high", "variable 1", and "variable 2") and strain on this ratio. We also looked at the interaction "humidity treatment x strain". Data from females which died before the fourth day of the experiment were not used. In total, data from 728 females were analysed. We used a GLM with a binomial error distribution and a logit link function. The variables humidity treatment, strain and replicate were expressed as fixed effects in the model. The estimated values for each humidity treatment were obtained by calculating the weighted average of the estimated values of all the replicates. We carried

out pairwise comparisons of means (Tukey test) to assess differences within humidity treatment levels. For the survival experiment, we studied the influence of the factors humidity treatment and strain on the survival probability ("time to death", observed right-censored data) of *P. persimilis* females, during 20 days. The data was right-censored because the females that were still alive at day 20 or died from "handling accidents" during the experiment were censored. For each humidity treatment and each strain, all females that were observed during the same 20 days were grouped under the same replicate number. To account for a possible correlation between observations grouped in the same replicate, we used a shared gamma frailty model, with gamma-distributed shared frailties at replicate level (Rondeau et al., 2012). We plotted the estimated survival curves for each humidity treatment using the Kaplan-Meier method (packages survival and survminer in R). The statistical analysis was performed in R (version 3.5.1).

4.3. RESULTS

4.3.1. EGG HATCHING

Humidity treatment to which *P. persimilis* females were exposed significantly affected the hatching rate of their eggs at 60% RH ($\chi^2 = 7.38; DF = 2; P = 0.02$, Fig.4.2). This effect became stronger with duration of female exposure, and the interaction between humidity treatment and duration of exposure to the treatment was statistically significant ($\chi^2 = 75.62; DF = 2; P < 2 \cdot 10^{-16}$). While the hatching rate of eggs laid by females exposed to constant high humidity remained between 0.03 (-0.02 to +0.04) and 0.06 (-0.03 to +0.08) (estimated values ± asymmetrical 95% CI) during the whole experiment, hatching rate of eggs laid by females exposed to constant low humidity increased from 0.11 (-0.06 to +0.12) after 1 hour of exposure, to 0.98 (-0.04 to +0.01) after 102 hours of exposure. The hatching rate of eggs laid by females exposed to treatment "variable 1" increased to a lesser extent: from 0.04 (-0.03 to +0.05) after 1 hour of exposure to 0.43 (-0.18 to +0.20) after 102 hours of exposure. Strain did not significantly affect egg hatching rate ($\chi^2 = 0.33; DF = 1; P = 0.57$), and there was no significant interaction between effects of humidity treatment and strain ($\chi^2 = 4.89; DF = 2; P = 0.09$).

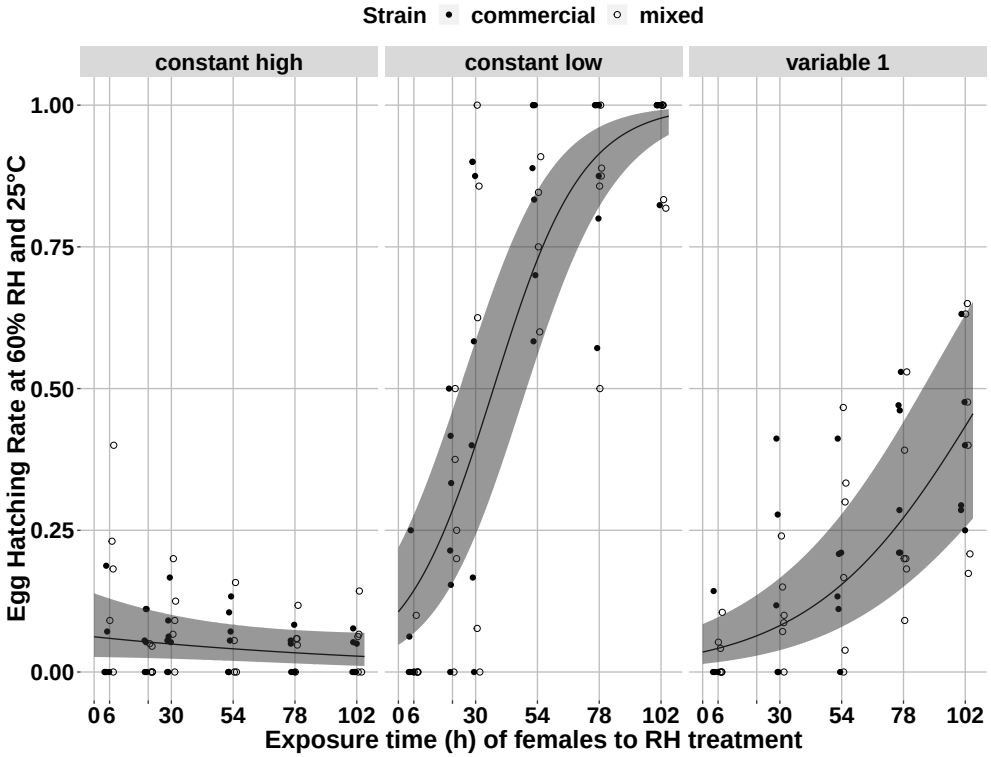


Figure 4.2: Observed (symbols) and estimated (curves, GLMM) egg hatching rates at 60% RH in relation to the exposure time of *Phytoseiulus persimilis* females to three humidity treatments. One symbol represents one replicate. Full symbols: commercial strain, empty symbols: mixed strain. Symbols are jittered around each exposure time point for more clarity. The shaded areas represent the 95% confidence intervals.

For humidity treatment "variable 2", during the first 78 hours of exposure, egg hatching rate was the same as for humidity treatment "constant low" ($\chi^2 = 0.23; DF = 1; P = 0.63$, Fig.4.3), and strain had no effect on egg hatching rate ($\chi^2 = 0.16; DF = 1; P = 0.68$). However, after 102 hours of female exposure to treatment "variable 2" (24 hours after the females had been transferred from low to high humidity), egg hatching rate suddenly decreased. After 102 hours of female exposure, hatching rate of eggs laid in treatment "variable 2" (0.48; -0.29 to +0.3) was significantly lower ($\chi^2 = 72.62; DF = 1; P < 2 \cdot 10^{-16}$) than hatching rate of eggs laid in treatment "constant low" (0.94; -0.15 to +0.05) (estimated values ± asymmetrical 95% CI, Fig.4.3). Strain did not significantly affect egg hatching rate after 102 hours of exposure ($\chi^2 = 2.45; DF = 1; P = 0.12$), and there was no significant interaction between effects of humidity treatment and strain ($\chi^2 = 0.24; DF = 1; P = 0.62$). It is important to notice that, in the egg hatching experiment, we collected around one egg per female after 6, 21, 30, 30, 54, 78, and 102 hours of exposure to each humidity treatment. Thus, the proportion of drought-resistant eggs reported here (egg hatching rate at 60% RH) strongly correlates with

the percentage of females that laid drought-resistant eggs.

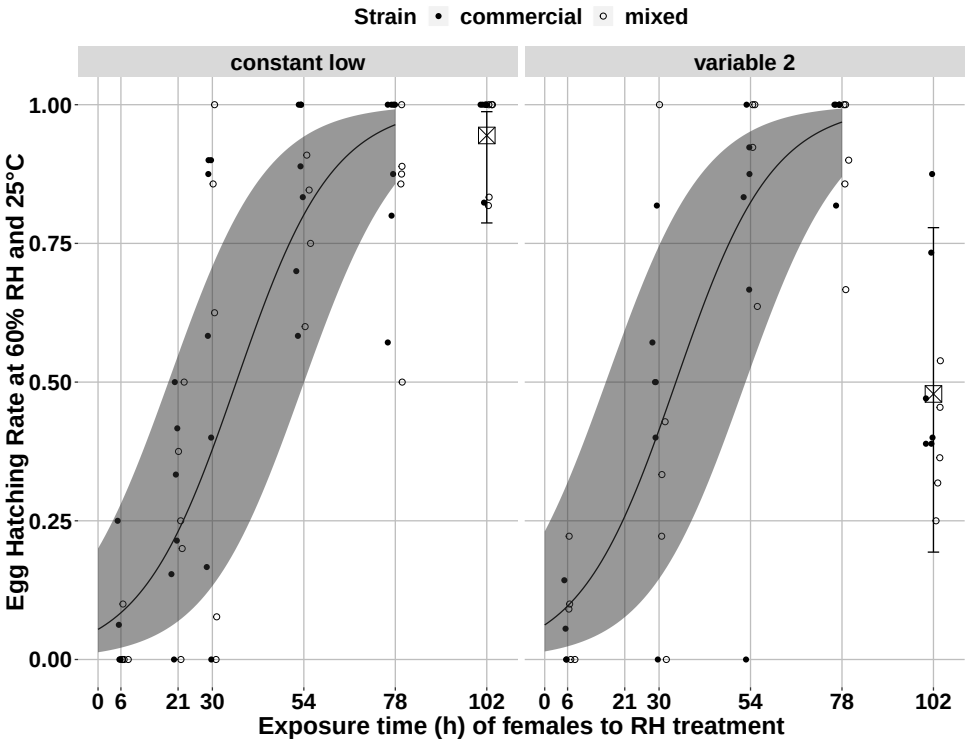


Figure 4.3: Observed (circular symbols) and estimated (2 curves and 2 square symbols, GLMM) egg hatching rates at 60% RH in relation to the exposure time of *Phytoseiulus persimilis* females to two humidity treatments. One symbol represents one replicate. Full circular symbols: commercial strain, empty circular symbols: mixed strain. Symbols are jittered around each exposure time point for more clarity. The shaded areas represent the 95% confidence intervals.

4.3.2. OVIPOSITION RATE

Humidity treatment significantly affected the oviposition rate of *P. persimilis* females over 4 days ($\chi^2 = 430.28; DF = 2; P < 2 \cdot 10^{-16}$, Fig.4.4 and Fig.4.5) and the oviposition rate over 10 days ($\chi^2 = 136.02; DF = 2; P < 2 \cdot 10^{-16}$, Fig.4.4 and Appendix Fig.4.7). On average, over 10 days, a female from the commercial strain laid 30.9 ± 2.5 eggs under constant low humidity, 46.2 ± 3.2 eggs under constant high humidity, and 39.5 ± 2.4 eggs under humidity treatment "variable 1" (estimated values ± 95% CI). Strain had a statistically significant effect on the oviposition rate over 4 days ($\chi^2 = 8.27; DF = 1; P = 0.004$), and there was no significant interaction between effects of humidity treatment and strain ($\chi^2 = 0.07; DF = 2; P = 0.97$). On average, over 4 days of exposure to humidity treatment "variable 1", females from the mixed strain laid significantly more eggs than females from the commercial strain (P = 0.046). To investigate further this strain effect on oviposition rate, we compared the oviposition rate of

the two strains over 10 days for humidity treatment "variable 1". The factor strain also had a significant effect on the oviposition rate over 10 days ($\chi^2 = 7.33; DF = 1; P = 0.007$). On average, over 10 days under humidity treatment "variable 1", a female from the mixed strain laid 3.4 eggs more than a female from the commercial strain.

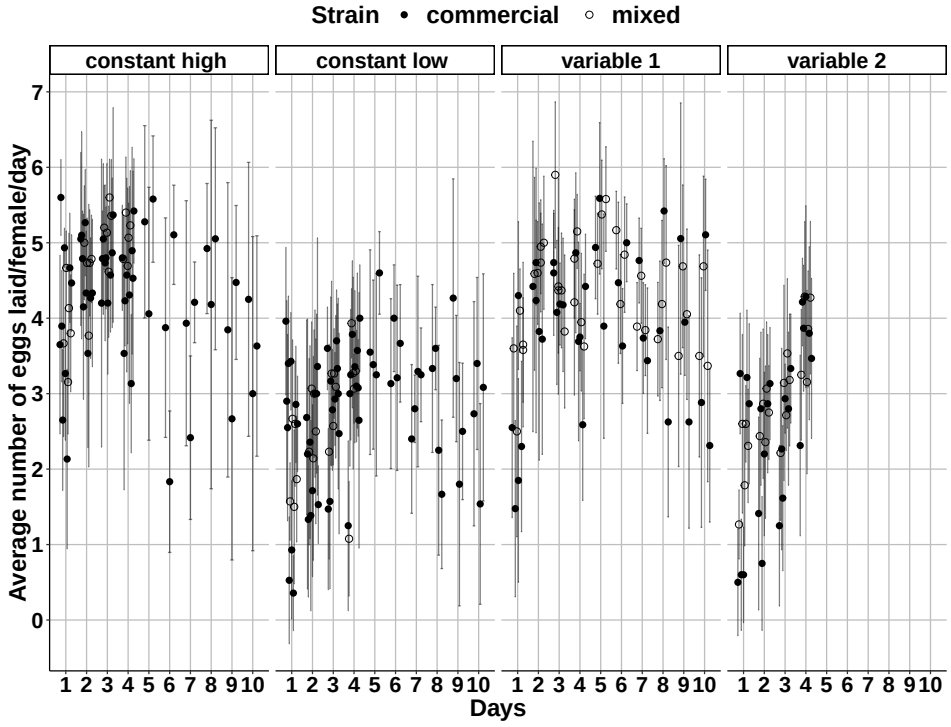


Figure 4.4: Observed values of the average number of eggs laid by *Phytoseiulus persimilis* females over 10 days and 4 days, when exposed to four humidity treatments at 25 °C, for two strains (full symbols: commercial strain, empty symbols: mixed strain). Each symbol represents one replicate of 15 to 20 females. The error bars represent ± 1 SD.

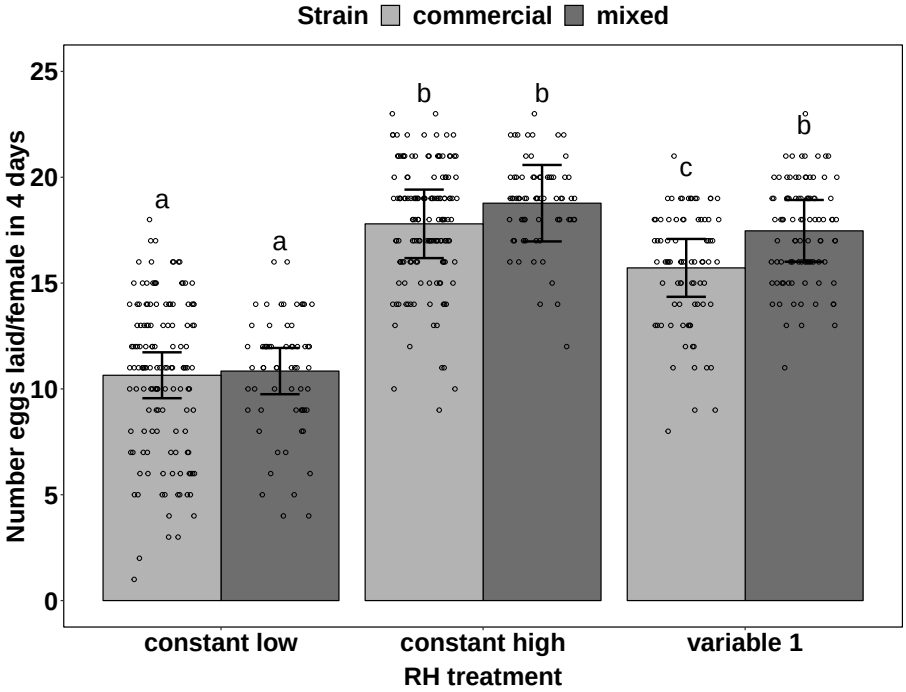


Figure 4.5: Estimated means (GLM) of the total number of eggs laid by *Phytoseiulus persimilis* females over 4 days, when exposed to three humidity treatments at 25 °C, for two strains (light grey: commercial strain, dark grey: mixed strain). Each dot represents the observed mean for one female. The error bars represent the 95% confidence intervals of the estimated means. Different letters above bars indicate significant differences between treatments and strains ($P < 0.05$).

Finally, humidity treatment had a statistically significant effect on the ratio "number of eggs laid on Day 4 / number of eggs laid over 4 days" ($\chi^2 = 47.39; DF = 3; P = 2 \cdot 10^{-10}$). More specifically, this ratio was significantly different between humidity treatment "variable 2" and the three other treatments ("constant high", $P < 0.001$; "constant low", $P = 0.007$; "variable 1", $P < 0.001$). Females exposed to humidity treatment "variable 2" laid a significantly higher number of eggs during the last 24 hours compared to the first 78 hours, whereas for the three other treatments the number of eggs produced stayed relatively constant over 4 days. On average, 34% (-4% to +5%) of the eggs laid by a female exposed to humidity treatment "variable 2" were laid during the last 24 hours, while between 25% and 29% of the eggs laid by a female exposed to humidity treatments "constant low" (29%; -5% to +4%), "constant high" (26%; -4% to +3%), or "variable 1" (25%; -4% to +3%) were laid during the last 24 hours (estimated values ± asymmetrical 95% CI). Strain had no effect on this ratio ($\chi^2 = 3.05; DF = 1; P = 0.08$), and there was no significant interaction between effects of humidity treatment and strain ($\chi^2 = 7,8; DF = 3; P = 0.05$). Overall, the oviposition rate experiment showed that *P. persimilis* females lay fewer eggs when exposed to constant low humidity than when exposed to constant high or variable humidity.

4.3.3. ADULT SURVIVAL

Humidity treatment had a statistically significant effect on the survival of *P. persimilis* females over 20 days ($\chi^2 = 31.22; DF = 2; P = 2 \cdot 10^{-7}$, Fig.4.6). Females exposed to humidity treatment "variable 1" survived significantly longer than females exposed to constant low and constant high humidity (P = $1 \cdot 10^{-4}$ for "constant low"; P = $2 \cdot 10^{-8}$ for "constant high"). Around 70% of *P. persimilis* females were still alive after 20 days under humidity treatment "variable 1", whereas only less than 35% and 25% of the females survived after 20 days under constant low and constant high humidity respectively. Females exposed to constant low humidity survived a bit longer than the ones exposed to constant high humidity, however this difference was not statistically significant (P = 0.07). Strain did not affect survival of the females under humidity treatment "variable 1" (P = 0.32). Overall, the survival experiment showed that the lifespan of *P. persimilis* females is shorter under constant low and constant high humidity, compared to variable humidity conditions.

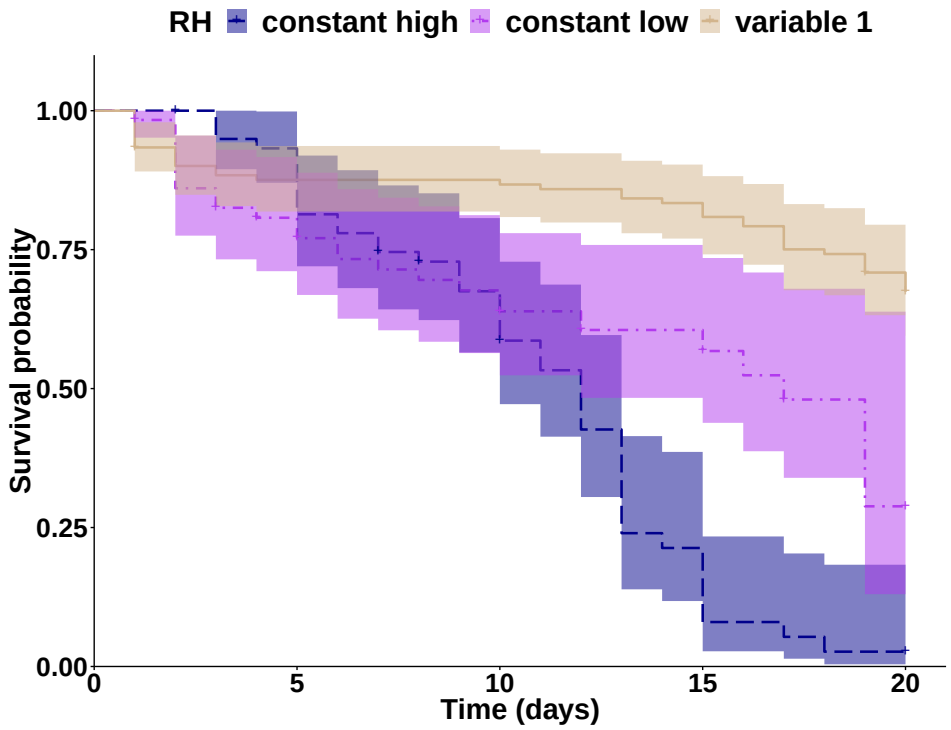


Figure 4.6: Survival probabilities for *Phytoseiulus persimilis* females exposed to three humidity treatments at 25 °C during 20 days. Three replicates per treatment, 20 females per replicate (Kaplan-Meier method, with 95% CI).

4.4. DISCUSSION

Our data show that the relative humidity experienced by *P. persimilis* females has a strong effect on drought resistance of their eggs. The size of this effect depends on the duration of female exposure. When females are exposed to constant low relative humidity (65% RH at 25 °C) during 102 hours, almost all females lay drought-resistant eggs. However, when females are exposed to constant high relative humidity (95% RH at 25 °C) during 102 hours, only 3 to 6% lay drought-resistant eggs. These results demonstrate a strong phenotypic plasticity in *P. persimilis* females with regard to relative humidity: they are capable of sensing unfavourable humidity conditions, and prenatally prepare their offspring to cope with drought. This mechanism for transgenerational phenotypic plasticity, or maternal effect, is defined as the ability of a female to alter its offspring's phenotype, allowing it to survive in a specific environment (Bernardo, 1996; Mousseau and Fox, 1998; Fox et al., 1999; Freinschlag and Schausberger, 2016). In phytoseiid mites, maternal strategies promoting offspring survival have been associated with food deprivation, risk of intraguild predation, and risk of sibling cannibalism (Toyoshima and Amano, 1998; Schausberger and Hoffmann, 2008; Walzer and Schausberger, 2011, 2015; Seiter and Schausberger, 2015). To our knowledge, only one published study has reported a maternal effect associated with relative humidity in mites: in larvae of the American dog tick *Dermacentor variabilis* (Say) (Acari: Ixodidae), the ability to absorb water vapor from the air is under maternal control (Yoder et al., 2006). This maternal effect has the adaptive significance of enabling larvae to maintain adequate levels of body water, by preventing dehydration and over-hydration. In our study, the maternal effect observed in *P. persimilis* females takes the form of a discrete, or switched response. When *P. persimilis* females exposed to low humidity for 78 hours were transferred back to high humidity for 24 hours, the percentage of females laying drought-resistant eggs significantly decreased in the last 24 hours, from 90% to 48%. This sudden drop suggests that the production of drought-resistant eggs in *P. persimilis* females is the result of a maternal investment that can quickly be 'switched' on and off. From there, two questions arise: how does the production of drought-resistant eggs affect *P. persimilis* females, more particularly their oviposition and survival rates? And how do *P. persimilis* females find the most 'adaptive' strategy to ensure the survival of their eggs as well as their own survival in dry conditions? Our data show that the oviposition rate of *P. persimilis* females depends on relative humidity conditions in their environment. Most females exposed to low humidity for 78 hours significantly increased their oviposition rate after being transferred to high humidity for 24 hours. Moreover, females exposed to constant low humidity had the lowest oviposition rate. We propose two non-exclusive explanations for this. First, this may relate to the costs of laying drought-resistant eggs. Females exposed to constant low humidity laid the highest proportion of drought-resistant eggs. Drought resistance in arthropods can be achieved through three main mechanisms: an increase in initial body water content, a decreased water loss rate, or a higher drought tolerance, i.e. the capacity to tolerate the loss of a higher percentage of water prior to death (Gefen et al., 2006; Bazinet et al., 2010). Although the mechanisms making *P. persimilis* eggs drought resistant remain to be elucidated, the production of such eggs may represent a higher energetic cost for the females, resulting in a trade-off between quality and quantity of offspring (Fox et al., 1999; Moczek, 2010). Second, the low oviposition rate under constant low humidity may relate to investment of the females in their own survival. *Phytoseiulus persimilis* females can carry only one mature egg

at a time, since it requires a considerable amount of energy and resources: a *P. persimilis* egg weighs more than 20% of the female body weight (Sabelis, 1981). Therefore, the decrease we observed in oviposition rate under low humidity may also be the result of a reallocation of the resources to somatic maintenance, instead of reproduction (Montserrat et al., 2007). For example, when prey density varies, *P. persimilis* females adjust the number of eggs deposited in a prey patch to their own nutritional needs, enhancing adult survival and reproduction, and to the needs of their progeny, enhancing immature survival and development (Vanas et al., 2006). Under humidity treatment "variable 1" (exposure to low humidity for 1/3 of the time), around 43% of the females laid drought-resistant eggs after 102 hours of exposure. Interestingly, oviposition rate under treatment "variable 1" was not significantly different (mixed strain) or slightly significantly lower (commercial strain) compared to oviposition rate of females exposed to constant high humidity, which did not lay drought-resistant eggs. These results suggest that fecundity is not proportionally affected by the exposure time to low humidity. Moreover, females exposed to humidity treatment "variable 1" probably did not suffer from dehydration and did not need to reallocate resources to their own survival. Their oviposition rate was, therefore, only affected by the production of a limited proportion of drought-resistant eggs, and modified to a lesser extent. On the contrary, females exposed to humidity treatment "constant low" had to deal with the combination of a severe drought stress and the production of a high proportion of drought-resistant eggs, resulting in a significant decrease in oviposition rate. The only difference we observed between the two strains tested was in the oviposition rate over 10 days under humidity treatment "variable 1": the mixed strain laid on average 3.4 eggs more than the commercial strain. However, the biological significance of these 3.4 eggs over 10 days in terms of impact on population dynamics is likely limited. Despite various differences between these two strains (rearing conditions, origin), they had a similar response to the relative humidity treatments tested in this study. These results indicate that there was probably little genetic variation for the production of drought-resistant eggs between these strains, and that there might be little room for improvement of the adaptive maternal strategies described previously. We also showed that *P. persimilis* females' lifespan depends on relative humidity conditions in their environment. We recorded a shorter lifespan for *P. persimilis* females exposed to constant low humidity. More surprisingly, females exposed to constant high humidity had the shortest lifespan. A previous study showed that when both *T. urticae* and *P. persimilis* were exposed to 100% RH, their activity diminished gradually with time, and more than 90% of the mites ceased activity after 4 hours (Mori and Chant, 1966). For *T. urticae*, water loss by evaporation through the cuticle is necessary because it allows the ingestion of large quantities of plant liquids and the concentration of the nutrients in the mite's body (Boudreaux, 1958). Extremely high relative humidity (95-100%), therefore, has a negative effect on feeding of *T. urticae*, by preventing loss of moisture from the body by evaporation. In our experiment, *P. persimilis* females exposed to 95% RH did not cease activity, since they had the highest oviposition rate of all three treatments. However, they might have decreased their predation rate, due to a water retention problem, similar to that of *T. urticae*. This water retention problem, combined with other unknown physiological effects, probably contributed to shorten the lifespan of *P. persimilis* females under extreme high humidity. After 102 hours of exposure to humidity treatment "variable 1", around 43% of the females laid drought-resistant eggs. This observation is somewhat surprising, considering the humidity conditions needed for a drought-sensitive

P. persimilis egg to successfully hatch. More than 50% of *P. persimilis* eggs laid under high humidity conditions successfully hatch under low humidity (60% RH at 25 °C) if these eggs are exposed to high humidity (85% RH at 25 °C) for at least 7 hours during their development (Le Hesran et al., 2019a). Moreover, under successive cycles of 12 hours at low and 12 hours at high humidity, more than 75% of *P. persimilis* eggs hatch (Le Hesran et al., 2019a). Therefore, in our experiment, *P. persimilis* females exposed to humidity treatment "variable 1" should not have perceived these conditions as particularly unfavourable for their eggs. Still, 43% of them switched and started laying drought-resistant eggs after 102 hours of exposure. Possibly, a small risk of mortality in *P. persimilis* females' offspring is already enough to change the type of eggs they lay. Moreover, environmental variations are highly stochastic. For a female experiencing some unpredictability in the environment, it may be more adaptive to prepare her offspring for the worst conditions, therefore laying drought-resistant eggs even when relative humidity conditions are not so unfavourable. Theory shows that a 'bet-hedging' strategy is successful when facing unpredictability. This strategy uses the idea of "not putting all your eggs in the same basket", and results in limited variation in long-term offspring success (Rossiter et al., 1993). Under variable humidity conditions, *P. persimilis* females may use this strategy and produce drought-resistant and drought-sensitive eggs alternately, to maximize their contribution to the next generation. We can therefore hypothesize that the 43% females who laid drought-resistant eggs after 102 hours under treatment "variable 1" may have laid a drought-sensitive egg subsequently. This strategy of alternating between drought-resistant and drought-sensitive eggs under variable humidity may explain why around half of the eggs hatched under these conditions. Under constant low humidity conditions, where *P. persimilis* eggs are expected to die, a different strategy is adopted by *P. persimilis* females, which seems to be the most adaptive for both offspring and maternal fitness: laying drought-resistant eggs only, while maintaining the health of the mother through a decrease in oviposition rate. When relative humidity rises again, a high level of plasticity in the production of drought-resistant eggs is adaptive for *P. persimilis* females. Under constant high humidity, most *P. persimilis* eggs are expected to hatch successfully, and *P. persimilis* females have no need to lay drought-resistant eggs. Although these maternal strategies seem to be beneficial for *P. persimilis* females and their offspring, we observed an interesting phenomenon: not all females started laying drought-resistant eggs, or switched, at the same time. The observation that females from the same strain, same age, and exposed to the same conditions responded differentially is intriguing, and raises two questions: first, what is the humidity threshold separating the production of drought-sensitive eggs from the production of drought-resistant eggs in *P. persimilis* females? Second, is this threshold the same for all individuals? Between 6 and 21 hours of exposure to constant low humidity, the percentage of females laying drought-resistant eggs increased from 14.4% to 28.5%. Under humidity treatment "variable 1", however, where females spent 8 hours per day at low humidity, it took 80 hours of exposure to reach the same proportion of females laying drought-resistant eggs. We therefore hypothesize that, in terms of threshold, *P. persimilis* females need a minimum of 6 to 21 hours of constant exposure to low humidity (65% RH at 25 °C) to start laying drought-resistant eggs, and that an interruption of this exposure will delay the response of the females. The second question was on the variation of the humidity threshold separating the production of drought-sensitive eggs from the production of drought-resistant eggs between females. Why did some females start laying

drought-resistant eggs after 24 hours of exposure to low humidity, whereas others only did so after 78 hours of exposure? There could be two reasons for this. First, each female may respond to variation in humidity according to her own physiological limits, and her own degree of plasticity. However, much of the behavioural variation within populations cannot be attributed to within-individual plasticity and physiology alone (Dingemanse et al., 2010). A second reason for this behavioural variation could be that each *P. persimilis* female has her own life-experience and personality, influencing her behaviour (Gosling, 2001; Dall et al., 2004; Biro and Stamps, 2008; McNamara et al., 2008; Wolf, 2009). The concept of personality has been used for *P. persimilis*. Early social isolation, for example, has proven to impair development, mate choice and grouping behaviour of *P. persimilis*, and therefore to be an important determinant in shaping *P. persimilis* individual personality (Schausberger et al., 2017). In conclusion, our study provides new insight into the effects of relative humidity on the predatory mite *P. persimilis*. We show that a maternal effect of *P. persimilis* females determines egg survival when females are exposed to constant low and variable humidity conditions: they produce drought-resistant eggs. The production of drought-resistant *P. persimilis* eggs is a phenotypically highly plastic trait, and the mechanisms making them drought resistant still remain to be elucidated.

4.5. ACKNOWLEDGMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 641456. We thank two anonymous reviewers for their comments that helped improve this manuscript. The experiments of this chapter have been carried out with the help of a Master student (Nugroho, J.E.) who was on a mobility stay funded by the Erasmus+ - KA1 Erasmus Mundus Joint Master Degrees Programme of the European Commission under the PLANT HEALTH Project.

4.6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

4.7. APPENDIX

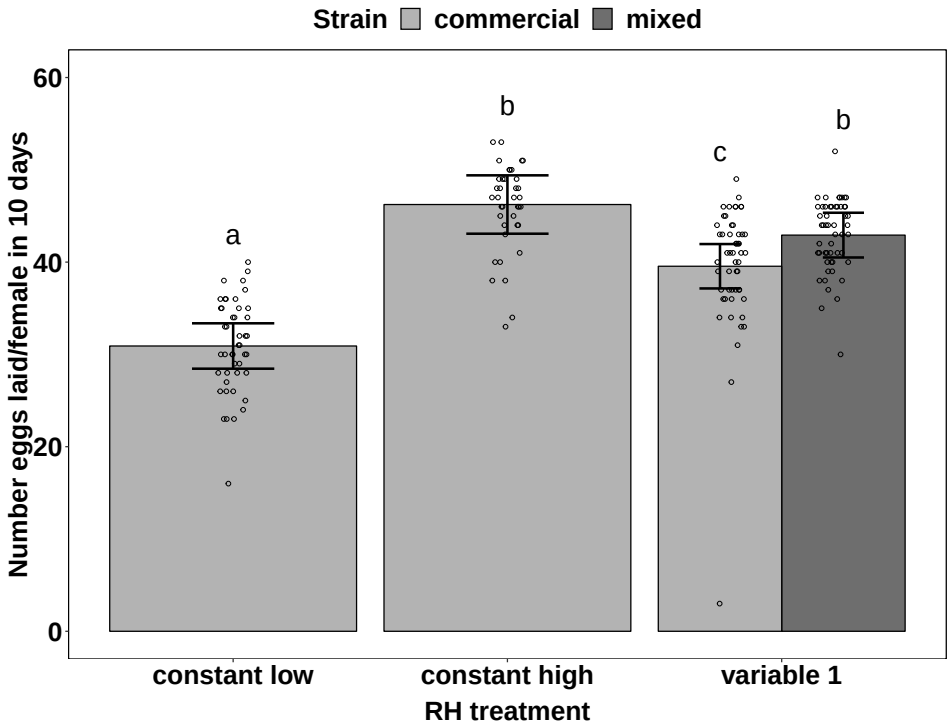
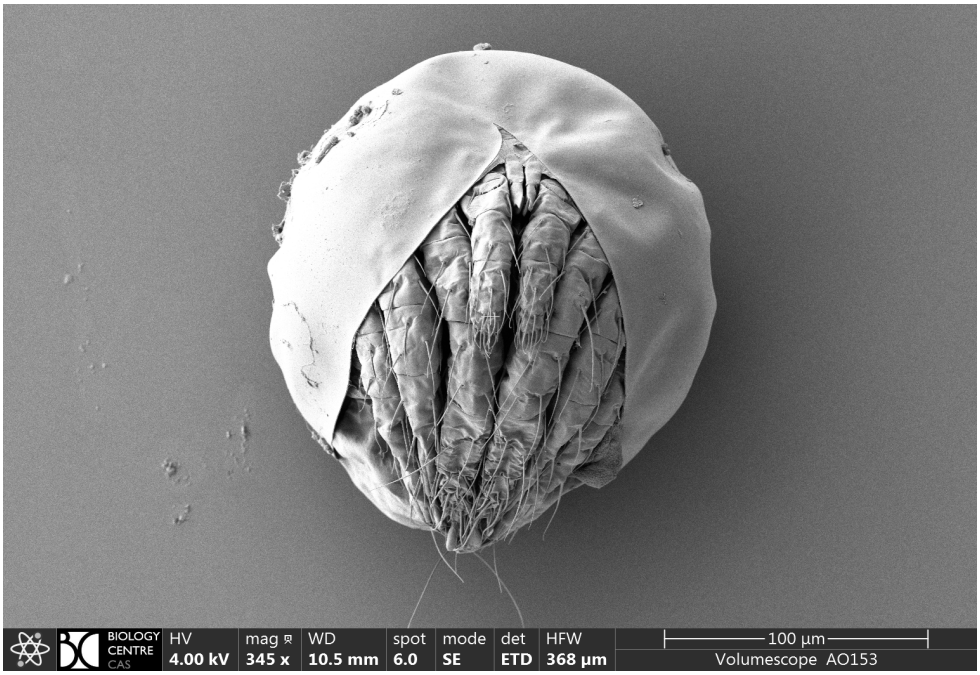


Figure 4.7: Estimated means (GLM) of the total number of eggs laid by *Phytoseiulus persimilis* females over 10 days, when exposed to three humidity treatments, for two strains (light grey: commercial strain, dark grey: mixed strain). Each dot represents the observed mean for one female. The error bars represent the 95% confidence intervals of the estimated means. Different letters above bars indicate significant differences between treatments and strains ($P < 0.05$).



Phytoseiulus persimilis larva hatching from an egg (SEM image)



Scanning electron microscope used in Chapter 5

5

PROXIMATE MECHANISMS OF DROUGHT RESISTANCE IN *Phytoseiulus persimilis* EGGS

Under drought stress, Phytoseiulus persimilis *females are able to lay drought-resistant eggs through an adaptive maternal effect. The mechanisms making these eggs drought resistant still remain to be investigated. For this purpose, we studied in this chapter the physiological differences between drought-resistant and drought-sensitive eggs. We compared the volume and the surface-area-to-volume ratio (SA:V) of the eggs, their sex ratio, their chemical composition (by gas chromatography-mass spectrometry), their internal and external structure (by scanning electron microscope and transmission electron microscope images), and their developmental time. Our results show that drought-resistant and drought-sensitive eggs have a different chemical composition: drought-resistant eggs contain more compatible solutes (free amino acids and sugar alcohols) and saturated hydrocarbons than drought-sensitive eggs. This difference may contribute to reducing water loss in drought-resistant eggs. Moreover, drought-resistant eggs are on average 8.4% larger in volume, and have a 2.4% smaller SA:V than drought-sensitive eggs. This larger volume and smaller SA:V, probably the result of a higher water content, may make drought-resistant eggs less vulnerable to water loss. We did not find any difference in sex ratio, internal or external structure nor developmental time between drought-resistant and drought-sensitive eggs. These results mark the first step in the understanding of the strategies and the energetic costs involved in the production of drought-resistant eggs in* P. persimilis *females.*

Chapter 5 is based on the article: Le Hesran, S., Groot, T., Knapp, M., Nugroho, J.E., Beretta, G., Salomé-Abarca, L.F., Choi, Y.H., Vancová, M., Moreno-Rodenas, A.M., Dicke, M. (2019). Proximate mechanisms of drought resistance in *Phytoseiulus persimilis* eggs. *Experimental and Applied Acarology*, **79**:279-298, doi:10.1007/s10493-019-00442-9.

5.1. INTRODUCTION

Parental care is a behavioural strategy through which parents increase the survival and growth of their offspring, often at a cost to their own survival and reproduction (Royle et al., 2012). Some insect and arachnid species demonstrate elaborate forms of parental care, and a broad diversity in extent and forms of care (Costa, 2006; Wong et al., 2013; Gilbert and Manica, 2015). For example, the burying beetle *Nicrophorus vespilloides* Herbst provides food to its larvae via regurgitation, and acacia thrips in Australia (*Dunatothrips aneurae* Mound) build small niches, called "domiciles", to protect their larvae from desiccation (Wong et al., 2013; Gilbert, 2014). In some spider and mite species, eggs are covered with silk layers or debris, to protect them against predators or stressful abiotic conditions (Marquardt et al., 2016; Vicente dos Santos and Tixier, 2017). Besides these parental traits enhancing offspring fitness, parental care can also benefit offspring through adaptive maternal effects (Benowitz et al., 2015). Adaptive maternal effects are defined as mechanisms of transgenerational phenotypic plasticity whereby, in response to a predictive environmental cue, a mother can change the phenotype of her offspring, and prepare it for the environmental conditions predicted by the cue (Mousseau and Fox, 1998; Fox et al., 1999). The key characteristic of adaptive maternal effect phenotypes is their plasticity, providing organisms with the ability to respond to environmental variations. Whereas the consequences of adaptive maternal effects are often clear, i.e. an increase in offspring fitness in a specific environment, the physiological adaptations responsible for this higher fitness are more complicated to discern. Understanding the physiological adaptations involved in adaptive maternal effects is key to better estimate their consequences for offspring performance, as well as the costs of such maternal effects for the mothers, and finally the constraints to their evolution. Here, we focus on an adaptive maternal effect triggered by low relative humidity conditions in the predatory mite *Phytoseiulus persimilis*. In CHAPTER 4, we showed that *P. persimilis* females start laying drought-resistant eggs when they are exposed to constant low RH (65% RH) (Le Hesran et al., 2020). The physiological adaptations making these eggs drought-resistant remain to be investigated. Protection against desiccation in insects and mites includes different non-mutually exclusive strategies. One strategy consists in increasing the initial body water content, mainly stored in the haemolymph and the tissues (Gibbs et al., 1997; Gray and Bradley, 2005; Gefen et al., 2006). For example, body water content increased by 30% after selection for drought resistance in populations of *Drosophila melanogaster* Meigen (Gibbs et al., 1997). Insect and mite eggs have a large surface-area-to-volume ratio (SA:V): they contain a small volume of water, that can be lost through a relatively large surface. Although the relationship between egg composition and egg volume is not clear, we can expect that an increase in initial water content would result in an increase in egg volume and a decrease in SA:V. In the butterfly *Bicyclus anynana* (Butler), for example, two lines of females were artificially selected: a line producing large eggs (L) and a line producing smaller eggs (S). Eggs from the L line contained more water and lipids, and had a higher hatching success when reared at low RH (Karl et al., 2007). Another strategy for drought resistance consists in reducing water loss rate (Gibbs et al., 1997; Gray and Bradley, 2005; Gefen et al., 2006). Water loss occurs mainly via evaporation through the cuticle, and to a lesser extent via respiration and excretion (Dittrich and Streibert, 1969; Kühsel et al., 2017). A reduction in water loss rate can be achieved via changes in the chemical composition of the cuticle. For example, lipid analysis after drought acclimation in the springtail *Folsomia candida* Willem revealed

changes in membrane fatty acids (Bayley et al., 2001). Furthermore, microbial symbionts confer desiccation resistance to the saw-toothed grain beetle *Oryzaephilus surinamensis* (Linnaeus) by changing the cuticle thickness, melanisation and hydrocarbon profile (Engl et al., 2018). Instead of a cuticle, insect and mite eggs are surrounded by a chorion (or eggshell), which should reconcile, even under drought stress, the opposing demands of water retention and respiration (Lees, 1961). To make their eggs more waterproof and reduce water loss rate, some terrestrial arthropods change the physical structure of the chorion. In the mite *Petrobia latens* (Müller), females add an external coating of hard wax to their eggs to protect them from desiccation during diapause (Beament, 1959; Lees, 1961). Alternatively, in other mite species, the egg is covered with a protective exochorion layer, formed before or during oviposition (Marquardt et al., 2016). In *Tyrophagus perniciosus* Zakhvatkin, for example, the exochorion takes the form of locular chambers on the surface of the egg, and may contribute to reduction of water loss (Witaliński, 1993). A third strategy to protect eggs against desiccation might be egg retention, leading to a shorter egg developmental time. In the phytoseiid mite *Phytoseius hawaiiensis* Prasad, females retain their eggs until just before the larvae hatch, when the substrate is unsuitable for oviposition. Extreme variations in egg hatching time were observed in this species, from ten minutes to over four days (Sanderson and McMurtry, 1984). Similarly, females of *Neoseiulus cucumeris* (Oudemans) reduce oviposition when exposed to a predator by retaining the egg inside their body, gaining some time to search for less risky oviposition sites, and leading to a shorter egg developmental time (Montserrat et al., 2007). With the aim to understand the physiological adaptations providing drought resistance to *P. persimilis* eggs, we investigated *P. persimilis* eggs laid by females exposed to constant low (drought-resistant eggs) and to constant high humidity (drought-sensitive eggs). We focused on the three strategies described previously: increase in initial egg water content, reduction of water loss rate through the chorion, and egg retention. We first measured the volume and the SA:V of the two groups of eggs. In *P. persimilis*, a change in egg volume could reflect a change in water content, a thicker chorion, but also a change in offspring sex ratio, since female eggs are larger than male eggs (Toyoshima and Amano, 1998; Walzer and Schausberger, 2015). In phytoseiid mites, short-term variations in sex ratio are related to temperature, humidity, wind speed, mating duration, and prey density (Amano and Chant, 1977; Dyer and Swift, 1979; Toyoshima and Amano, 1998; Lü et al., 2019). Therefore, in parallel with egg volume, we assessed the sex ratio of the offspring coming from drought-resistant and drought-sensitive eggs. Secondly, we analysed the chemical composition of the two groups of eggs, by Gas Chromatography-Mass Spectrometry (GC-MS), and we studied the internal and external structure of the eggs, by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Finally, we compared the developmental time of drought-resistant and drought-sensitive eggs.

5.2. MATERIALS AND METHODS

5.2.1. PREDATORY MITES

The *P. persimilis* strain used in this study was obtained from a mass rearing (Koppert Biological Systems), and reared in closed Petri dishes containing around 100 individuals each. Each Petri dish (ø 7.5 cm, 3 cm high) contained an agar layer (agar powder, VWR Chemicals,

Amsterdam, the Netherlands, 1/100 diluted) on which a disk (ø 7 cm) of a cucumber leaf (*Cucumis sativus* 'Pyralis') infested with a mixture of all stages of spider mites (*Tetranychus urticae*) was placed, with the adaxial side facing the agar plate. Predatory mites were transferred to new Petri dishes once a week, and maintained in a climate chamber at 18 ± 1 °C and 65 ± 2% RH, with L16:D8 photoperiod (see Le Hesran et al. 2019a for details). To control for the influence of *P. persimilis* female age, we used even-aged cohorts of young adult females (7 days since the egg stage). These females were collected as eggs and kept for 7 days in a closed Petri dish in a climate chamber at 70 ± 2% RH and 25 ± 1 °C. Inside the Petri dish, a cucumber leaf disk infested with spider mites was placed upside down on an agar layer, to provide optimal conditions for the development of these eggs to adults.

5.2.2. HUMIDITY TREATMENTS

For each replicate, 15 to 20 *P. persimilis* females were exposed to either of two humidity treatments: "constant low" (65% RH) or "constant high" (95% RH). For this, females were placed in individual plastic cups (ø 3.5 cm, 2.8 cm deep), covered by a lid with a fine gauze (gauze width 90 µm). To provide them with an oviposition substrate, a thin layer of cardboard with hairy surface facing up was fixed to the bottom of the cup with double-sided tape (Tesa®). For humidity treatment "constant low", 15 to 20 cups were placed upside down on top of a wire platform (2.5 x 2.5 cm spacing) inside a closed plastic box (40 L x 25.5 W x 16.5 H cm). The relative humidity inside the box was regulated with a humidifier (Cigar Oasis Excel, New York, USA), to achieve constant humidity of 65% RH (average 64.7 ± 1.6% RH). For humidity treatment "constant high", 15 to 20 cups were placed upside down on an agar layer at the bottom of a closed plastic box. The relative humidity inside the box was maintained at 95% RH (average 96.2 ± 1.7% RH). The two boxes containing the cups were placed in a climate chamber at 70 ± 2% RH and 25 ± 1 °C (L16:D8 photoperiod). The females were provided with fresh *T. urticae* (larvae, nymphs and adults) *ad libitum* every day. A data logger (LogTag TRIX 8) was placed in each box containing the cups, to measure relative humidity and temperature. For logistic reasons, in the five experiments described hereafter, *P. persimilis* females were exposed to the two humidity treatments for different time periods (from 48 h to 120 h). After 48 h exposure to constant low RH, around 60% of *P. persimilis* females lay drought-resistant eggs, which is high enough to detect a difference between eggs from both treatments (Le Hesran et al., 2020). Moreover, the percentage of females laying drought-resistant eggs increases with time exposure to low RH. It is, therefore, unlikely that this difference in exposure time prevented us from detecting differences between drought-resistant and drought-sensitive eggs.

5.2.3. EGG VOLUME AND SURFACE-AREA-TO-VOLUME RATIO

Phytoseiulus persimilis females were exposed to either of both humidity treatments for 72 to 102 h, and 0-to-6-h-old eggs laid by these females were collected. For this, we removed all eggs from each cup after 72 h of exposure, and collected the freshly laid eggs 6 h later, i.e. after 78 h of exposure. We repeated the same procedure between 96 and 102 h of exposure. The collected eggs were placed on a smooth black PVC plate (8 L x 4 W x 0.5 H cm) in such a way that the longest semi-major axis was facing up (Fig.5.1a). A picture of each egg was taken,

using a Dino-Lite Digital Microscope installed on a stereo microscope (Stemi 508) with KL 300 LED light source at level 3 (ZEISS Nederland, Breda, the Netherlands). The camera was re-calibrated for each block. A computer vision algorithm (OpenCV, Python) was developed to automate the estimation of geometrical characteristics from egg images. This consisted of a colour-based segmentation algorithm to derive the egg contour. Then, the maximum egg diameter was estimated (D_{max}). The egg top-view projection was divided into two symmetrical lobes (Fig.5.1b), for which the cross-section area ($A^i_{l/2}$) and outer perimeter ($P^i_{l/2}$) were computed. The three-dimensional geometry of the egg was derived from a single image under the assumption that the egg is approximated by a solid of revolution and that the computed D_{max} represents the symmetry axis. The first and second Pappus's centroid theorems were used to estimate the volume t(V^i_{egg}) and surface area (S^i_{egg}) of each egg as:

$$V^i_{egg} = 2\pi \cdot \delta^i_{GA} \cdot A^i_{l/2} \tag{5.1}$$

$$S^i_{egg} = 2\pi \cdot \delta^i_{GP} \cdot P^i_{l/2} \tag{5.2}$$

where δ^i_{GA} represents the distance between the axis of symmetry and the centroid of $A^i_{l/2}$, and δ^i_{GP} is the distance between the axis of symmetry and the centroid of the egg semi-perimeter (Fig.5.1b). Egg volume and surface area were derived as the mean from those computed from both symmetrical lobes (Fig.5.1c).

For each egg, the maximum diameter, volume, surface area and SA:V were estimated. We carried out 7 replicates for humidity treatment "constant low" and 7 replicates for humidity treatment "constant high", with 6 to 46 eggs per replicate. In total, 238 eggs were examined (127 eggs for treatment "constant high", 111 eggs for treatment "constant low"). An error analysis was conducted to assess the measurement repeatability and the influence of relative positioning misalignments (see Appendix Table 5.1 and Fig.5.10). Successive observations of samples produced standard deviations of σ_{Dmax} = 0.0044 mm; σ_V = 0.00024 mm^3 and $\sigma_{SA:V}$ = 0.55 mm^{-1}, and coefficients of variation of 0.92%; 2.62% and 0.95% respectively.

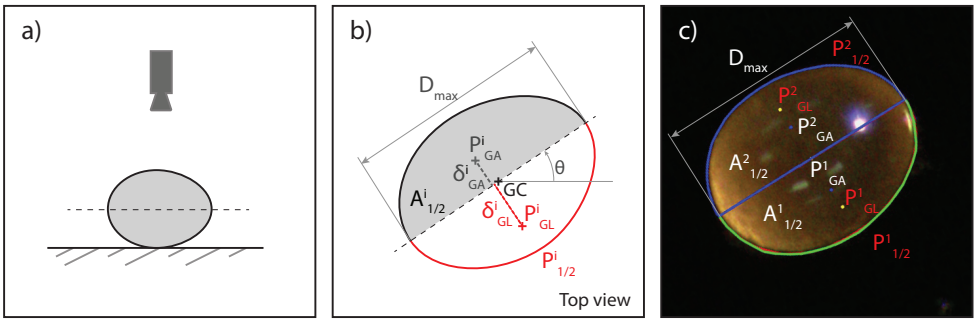


Figure 5.1: Scheme of the automated *Phytoseiulus persimilis* egg geometry estimation. a) Egg placed on the observation surface. b) Derived characteristics of the top-view egg section; maximum diameter (D_{max}), geometrical center of the area (P^i_{GA}) and of the outer perimeter (P^i_{GL}) at each symmetrical lobe, and distance from the geometrical centers and the main symmetry axis (δ^i_{GA} and δ^i_{GL}). c) Example of a post-processed egg image.

5

5.2.4. OFFSPRING SEX RATIO

Phytoseiulus persimilis females were exposed to either of both humidity treatments for 78 to 102 h. During this time interval, we collected 0-to-18-h-old eggs from each treatment, and placed them in two Petri dishes containing an agar layer, a cucumber leaf disk and enough spider mites to guarantee their development to adults. One week after egg collection, we counted the number of males and females present in each Petri dish. In case of any doubt about an individual, we made a glass slide preparation and placed the mite in a drop of lactic acid. The glass slide was then covered with a cover slip and placed on a heated plate (33 °C) for 72 h. After 72 h, males and females could be differentiated by the external morphology of the anal region: *P. persimilis* females have the anus inside a circular shield; while males do not have such a shield structure (see Appendix Fig.5.13). We carried out 7 replicates for treatment "constant high", and 9 replicates for treatment "constant low", with 15 to 20 females and 15 to 56 offspring individuals per replicate.

5.2.5. CHEMICAL COMPOSITION OF EGGS

Adult females were exposed to either of both humidity treatments for 72 to 102 h. During this time interval, we collected 0-to-18-h-old eggs. For each replicate, 100 eggs from each humidity treatment were placed in two Eppendorf tubes (one tube per treatment) and stored at -18 °C. We carried out 10 replicates per treatment.

5.2.5.1. SAMPLE PREPARATION

Each sample (Eppendorf tube) was extracted in 1 mL methanol under ultra-sonication for 20 min. Subsequently, the samples were centrifuged at 13,000 rpm for 10 min. The supernatant (500 µL) was collected for each sample, transferred to a new micro-tube and dried completely in a centrivap concentrator (LABCONCO). The dried extracts were then re-dissolved in 100 µL pyridine under ultra-sonication for 5 min. Then, 100 µL of *N,O-*

Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS (99:1), Sigma-Aldrich Chemie N.V., Zwijndrecht, the Netherlands) were added to the samples and they were incubated at 80 °C for 50 min. The samples were cooled down to room temperature and methyl palmitate was added as internal standard with a final concentration of 20 ng/µL. Finally, the samples were transferred to micro-inserts for GC-MS analysis.

5.2.5.2. GC-MS ANALYSIS AND DATA EXTRACTION

The samples were analysed with an Agilent 7890A gas chromatograph equipped with a 7693 automatic sampler and coupled to a 5975C mass single-quadrupole detector (Agilent, Folsom, CA, USA). The samples were separated using a DB-5 GC column (30 m x 0.25 mm, 0.25 µm film, J&W Science, Folsom, CA, USA) and He (99.9% purity) as a carrier gas at a flow rate of 1.2 mL/min. The injection port in splitless mode was set at 270 °C and the sample injection volume was 1 µL. The oven temperature was programmed to start at 60 °C, kept for 1 min, then increased to 202 °C at 7 °C/min and kept again for 1 min. The temperature then increased to 212 °C at 4 °C/min, was held for 1 min, and then finally increased to 290 °C at 7 °C/min and was held for 3 min. The interface temperature was 280 °C, and the ion source and quadrupole temperature of the mass detector were 230 °C and 150 °C, respectively. The ionization energy in EI mode was 70 eV and peaks were identified by comparison of their ion spectra with the NIST library (version 2008), or by comparison of their retention time with standard compounds. For GC-MS data acquisition, all of the total ion chromatograms were automatically integrated and peaks were identified using Mass Hunter Qualitative Analysis software version B.07.00 (Agilent). The area of the identified compounds was normalized to the area of the internal standard (20 ng/µL of methyl palmitate).

5.2.6. INTERNAL AND EXTERNAL STRUCTURE OF EGGS

Phytoseiulus persimilis females were exposed to either of both humidity treatments for 120 h. After 120 h, 0-to-24-h-old eggs were collected from each treatment. To analyse the internal and external structure of the eggs, we used SEM and TEM.

5.2.6.1. SEM

Non-dehydrated samples and dehydrated samples were used for the SEM analysis. For the non-dehydrated samples, we used two specimen preparation protocols. First, eggs were either high pressure frozen (HPF) using Leica EM PACT or frozen by plunging in liquid nitrogen slush, using Cryo ALTO 2500 (Gatan). Just before the HPF, we placed 3 *P. persimilis* eggs from each humidity treatment on 2 copper specimen carriers (one carrier per humidity treatment, ø 1.5 mm, Leica), that were then filled in with 20% Bovine Serum Albumin (BSA). For the plunging method, we placed the eggs onto a SEM specimen holder (aluminium) covered with Tissue-Tek® (EMS). After freezing, samples were transferred into a liquid nitrogen bath where they were longitudinally fractured with a scalpel under optical microscope control. Some eggs from the plunging method were kept intact, to analyse their external structure. The eggs were then transferred under vacuum to a Cryo ALTO 2500 chamber of a JEOL 7401F SEM, cooled at -140 °C. Inside the chamber, sublimation was conducted at -95 °C for 5 min. Temperature was then decreased to -140 °C and the samples were sputter coated with

platinum-palladium for 100 seconds. Finally, samples were examined by JEOL 7401F SEM at 1-2 kV using the Everhart-Thornley detector of secondary electrons. For the dehydrated samples, eggs were fixed in 2.5% glutaraldehyde in 0.1M Phosphate Buffer (PB) overnight, at 4 °C, then washed in 0.1M PB with 4% glucose three times for 15 min and post-fixed in 2% OsO4 for 2 h. The samples were washed and dehydrated in a graded acetone series (30%, 50%, 70%, 80%, 90%, 95% and 100% acetone in water), for 15 min in each solution. The samples were dried using Critical Point Dryer (CPD2, Pelco), mounted using a carbon tape on aluminium stub and sputter coated with gold (Bal-Tec). Specimens were observed using a JEOL 7401F and SEM Apreo (Thermo Fisher Scientific) at 4 kV.

5.2.6.2. TEM

For TEM, the samples were high pressure frozen (see details above) and transferred to the freeze substitution media containing 2% OsO4 in 100% acetone cooled at -90 °C (Leica AFS). After 96 h, the temperature was increased (5 °C/h) to -20 °C and, after the next 24 h, to 4 °C. At room temperature, the samples were rinsed three times in 100% acetone (15 min each) and infiltrated in 25%, 50% and 75% Embed 812 resin/acetone solutions, for 1 h in each solution. Finally, samples were incubated in 100% resin overnight in a vacuum desiccator. Samples were polymerized at 60 °C. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined using a JEOL 1010 TEM.

5.2.6.3. TEM IMAGES ANALYSIS

The ImageJ program (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018) was used to measure thickness of the exochorion on two TEM images. On each image, the exochorion thickness was measured 10 times, at 10 random locations, and the average and standard deviation of these 10 measurements were calculated.

5.2.7. EGG DEVELOPMENTAL TIME

Phytoseiulus persimilis females were exposed to either of both humidity treatments for 48 to 54 h. After 48 h of exposure, all eggs were removed from each cup, and the freshly laid eggs (0 to 6 h old) were collected 6 h later. Each egg was placed with a brush in a hole (ø 0.7 cm, 0.4 cm deep) in a platform made of polymethyl methacrylate (PMMA) (17.5 L x 15 W x 4.5 H cm) containing 30 holes (one egg per hole). Each hole had a fine gauze at the bottom (gauze-width 90 µm), to ensure contact with ambient air. The hole was then covered with a coverslip to prevent the larvae from escaping after hatching. The PMMA platforms (one platform per humidity treatment, per replicate) were placed in a climate chamber at 85 ± 2% RH, 25 ± 1 °C and L16:D8 photoperiod. The number of hatched larvae was recorded after 24, 41, 43, 45, 47, and 49 h. For each humidity treatment, we carried out 8 replicates, with 9 to 22 eggs per replicate. In total, we assessed the developmental time of 257 eggs.

5.2.8. STATISTICAL ANALYSIS

The statistical analysis was performed in R (v.3.5.1) and SIMCA P (v.14.1, Umeå, Sweden).

5.2.8.1. EGG VOLUME AND SURFACE-AREA-TO-VOLUME RATIO

To compare egg volume and SA:V between humidity treatments "constant high" and "constant low", we used a linear mixed effect model with Gaussian error distribution for both analyses. The variable humidity treatment was expressed as a fixed effect in the model. The replicates which had been performed at the same date were assigned to the same replicate number, and the replicate factor was expressed as a random effect in both models (by-replicate random intercept). We used the model-fitting method of the maximum likelihood, and used likelihood-ratio tests to select the most parsimonious models.

5.2.8.2. OFFSPRING SEX RATIO

To compare the proportion of males in the offspring of females exposed to the humidity treatments "constant high" and "constant low", we used a generalized linear mixed model with a binomial error distribution and logit link function. Humidity treatment was expressed as a fixed effect in the model, and replicate was expressed as a random effect (by- replicate random intercept). We used the model-fitting method of the maximum likelihood, and used likelihood-ratio tests to select the most parsimonious model.

5.2.8.3. EGG CHEMICAL COMPOSITION

Multivariate data analysis (MVDA) was performed using SIMCA P (v.14.1, Umeå, Sweden). Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were performed for GC-MS data with two classes (constant low and constant high humidity). For this model, a unit-variance (UV) scaling method was used to suppress the effects of other random factors and identify differences caused by humidity. All normalized data was scaled by UV method for PCA and PLS-DA models. An area value corresponding to half of the lowest area detected in the whole analysis was assigned to the compounds that were not detected in some samples. The correlated compounds to each treatment were selected by interpretation of the PLS-DA loading plot and variable importance for the projection (VIP) plot.

5.2.8.4. EGG DEVELOPMENTAL TIME

For the analysis of egg developmental time, we studied the influence of the factor humidity treatment on hatching probability ("time to hatching", observed right-censored data) of *P. persimilis* eggs, during 49 h. For each humidity treatment, eggs that were observed during the same 49 h were grouped under the same replicate number. We used a mixed-effects Cox model, with humidity treatment as a fixed effect, and replicate as a random effect. We used likelihood-ratio tests to select the most parsimonious model, and plotted the estimated survival curves for each RH treatment using the Kaplan-Meier method (packages survival and survminer in R).

5.3. RESULTS

5.3.1. EGG VOLUME AND SURFACE-AREA-TO-VOLUME RATIO

Humidity treatment had a significant effect on egg volume ($\chi^2 = 31.6; DF = 1; P = 2 \cdot 10^{-8}$; Fig.5.2). On average, eggs laid by females exposed to constant low humidity had a bigger volume ($4.8 \cdot 10^{-3} \pm 3 \cdot 10^{-4}$ mm^3) than eggs laid by females exposed to constant high humidity ($4.4 \cdot 10^{-3} \pm 3 \cdot 10^{-4}$ mm^3) (estimated values ± 95% CI). Humidity treatment also had a significant effect on egg SA:V ($\chi^2 = 24.9; DF = 1; P = 6 \cdot 10^{-7}$; see Appendix Fig.5.11). On average, the eggs laid by females exposed to constant low humidity had a smaller SA:V (29.1 ± 0.51 mm^{-1}) than those laid by females exposed to constant high humidity (29.8 ± 0.51 mm^{-1}) (estimated values ± 95% CI). The measured values for maximum diameter and surface area of the eggs are presented in Appendix Fig.5.12

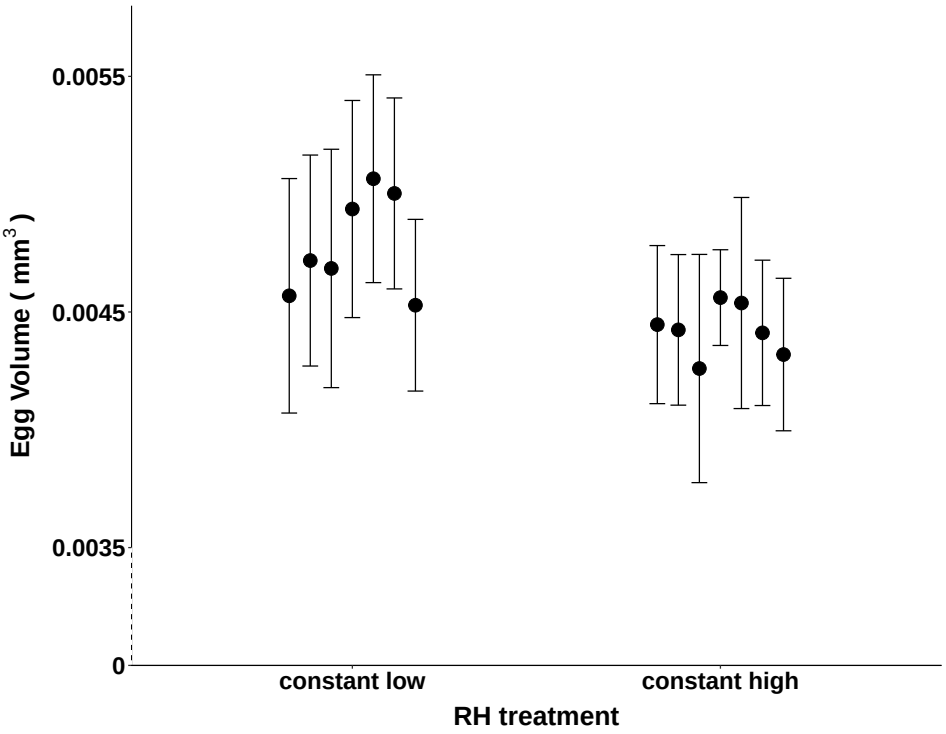


Figure 5.2: Observed egg volume (mm^3), for eggs laid by *Phytoseiulus persimilis* females exposed to two humidity treatments (constant high (95%) RH: 127 eggs, and constant low (65%) RH: 111 eggs). Each symbol represents the average egg volume for one replicate (n=37;15;19;9;14;11;6 eggs for constant low RH and n=46;15;18;14;9;13;12 eggs for constant high RH). Error bars represent ± 1 SD.

5.3.2. OFFSPRING SEX RATIO

Relative humidity significantly affected the sex ratio of offspring ($\chi^2 = 3.87; DF = 1; P = 0.049$; Fig.5.3). When females were exposed to constant low humidity, the proportion of males in their offspring was on average 0.19 (-0.08 to +0.13). For females exposed to constant high humidity, the proportion of males in their offspring was on average 0.12 (-0.06 to +0.11) (estimated values ± asymmetric 95% CI).

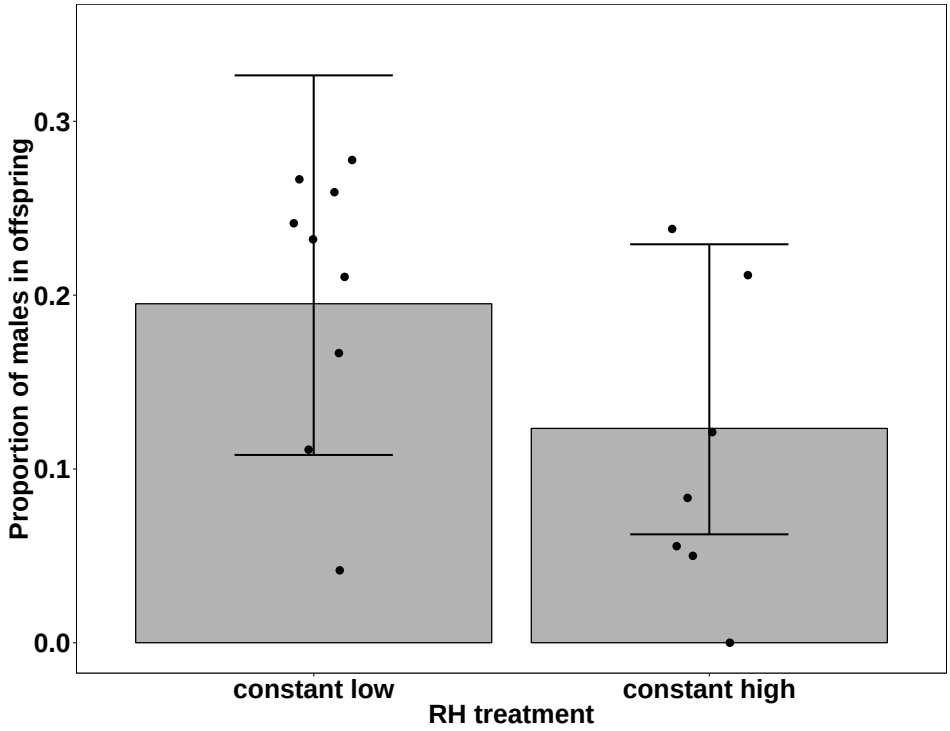


Figure 5.3: Observed (symbols) and estimated (bars) sex ratio (proportion of males) in offspring from *Phytoseiulus persimilis* females exposed to two humidity treatments (constant high (95%) and constant low (65%) RH). Each symbol represents one replicate, with offspring from 15 to 20 females (n=15;24;29;18;18;19;18;27;56 for constant low RH and n=18;24;33;20;18;21;52 for constant high RH). Error bars represent the 95% CI of the estimated values.

5.3.3. CHEMICAL COMPOSITION OF EGGS

Through GC-MS analysis we detected primary metabolites such as amino acids, fatty acids, monoglycerides, sugars, alkanes, and phosphoric acid. There were qualitative and quantitative differences in chemical composition between eggs from constant low and constant high humidity. In the PCA model (see Appendix Fig.5.14), the samples were principally separated by humidity treatment ($R^2 = 0.47$). However, there was a certain degree of overlap between the two groups, which was eliminated when a third principal component was added to the

model. Therefore, even if humidity treatment has an effect on the chemical composition of the egg extracts, there are other unknown factors influencing the plot separation. To obtain a clearer separation by a supervised method, a PLS-DA model was applied to the same dataset and it showed a clear effect of humidity on the chemical composition of the samples (Q^2= 0.46, P = 0.03 by CV-ANOVA) (Fig.5.4).

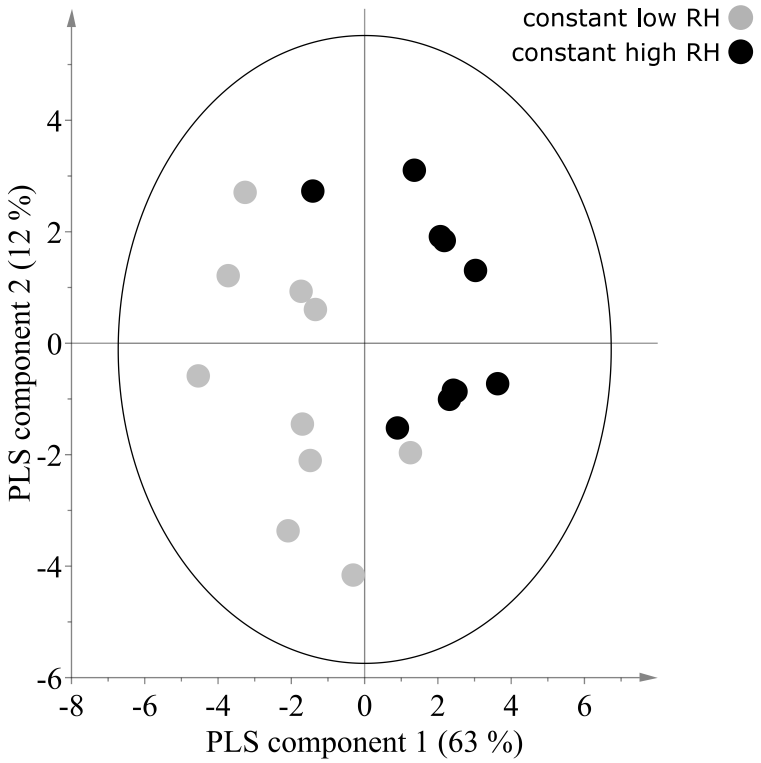


Figure 5.4: PLS-DA plot for chemical composition of eggs laid by *Phytoseiulus persimilis* females exposed to each of two humidity treatments (constant low (65%) and constant high (95%) RH) (Q^2 = 0.46, P = 0.03). The ellipse represents the Hotelling's T2 (95%) border. Grey symbols represent 10 replicates for constant low humidity, black symbols represent 10 replicates for constant high humidity (n=100 eggs per replicate).

Metabolites more associated to extracts of eggs laid under constant low humidity were amino acids, sugars, one alkane, one fatty acid, and phosphoric acid. Metabolites more associated to extracts of eggs from constant high humidity were two fatty acids, one sugar and one monoglyceride (Fig.5.5 and Appendix Table **??**). Serine is the metabolite that accounts most for eggs from constant low humidity, and *trans*-β-octadecenoic acid is the metabolite that accounts most for eggs from constant high humidity. Both groups of eggs contained serine, which could indicate quantitative differences in this metabolite. The *trans*-β-octadecenoic acid, however, was detected only in eggs from constant high humidity.

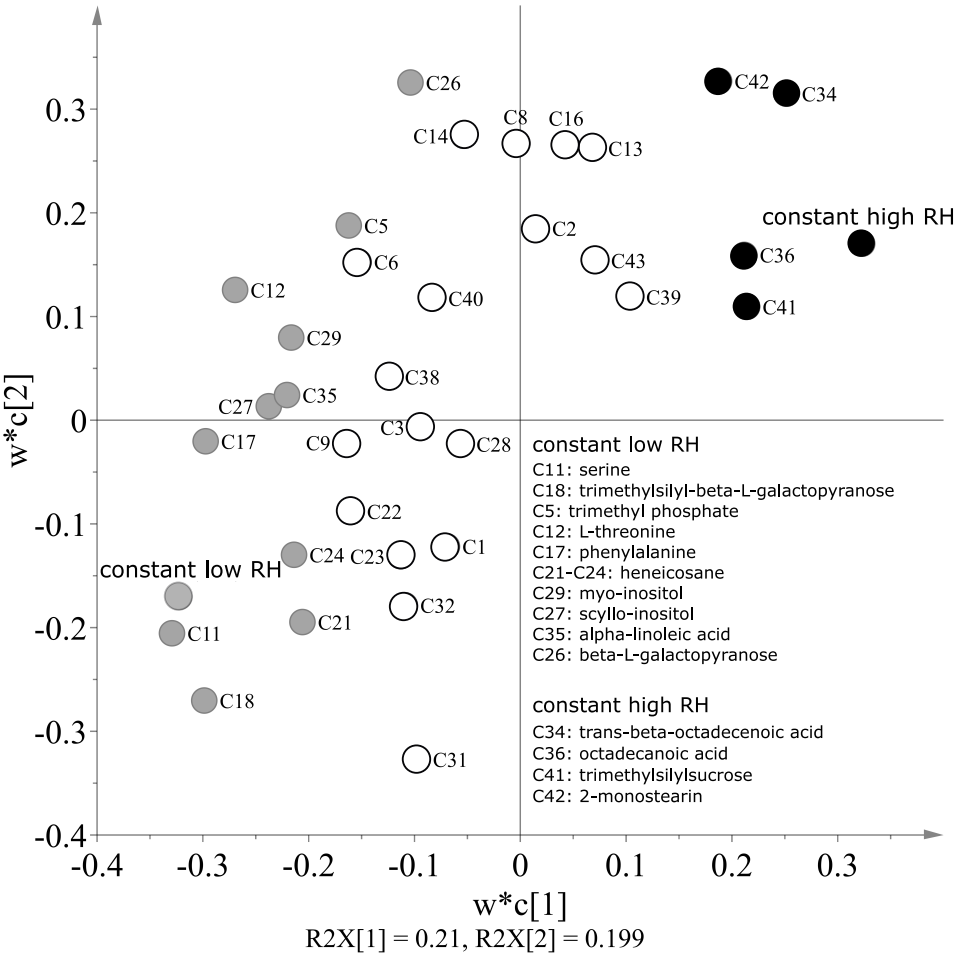


Figure 5.5: Loading plot from PLS-DA analysis for *Phytoseiulus persimilis* eggs laid by females exposed to two humidity treatments (constant low (65%) and constant high (95%) RH). Eleven metabolites more correlated (loading value higher than 1 on the VIP plot) to eggs laid under constant low humidity are represented by grey symbols. Four metabolites more correlated (loading value higher than 1 on the VIP plot) to eggs laid under constant high humidity are represented by black symbols. White symbols represent metabolites that are not correlated to any humidity treatment. The full names of the chemical compounds corresponding to each labelled dot are listed in Table **??** in Appendix.

5.3.4. INTERNAL AND EXTERNAL STRUCTURE OF EGGS

We did not observe any clear structural difference between eggs laid under constant low and constant high humidity conditions. The external surface of all eggs was similar: smooth and without any external structure or pattern (Fig.5.6a and 5.7a). Zooming in (x 10,000) on the external surface of the eggs did not reveal any visible respiratory pores. Regarding the

internal structure, all eggs were covered with three layers: an exochorion, an endochorion, and a third fibrous layer, probably made of proteins (Fig.5.6, 5.7, and 5.8). The most external layer, the exochorion, was thick and compact. Closely attached to the exochorion, was the endochorion, around 10 times thinner than the exochorion. Between the endochorion and the embryo tissue was a fibrous layer, thicker than the endochorion, but thinner and less compact than the exochorion. This fibrous layer is the last layer torn by the hatching larva (Fig.5.7b and 5.7c). On average (for 10 measurements on one egg), the exochorion was 0.33 ± 0.007 µm thick for an egg laid under constant low humidity, and 0.43 ± 0.009 µm thick for an egg laid under constant high humidity (Fig.5.8b and 5.8d). Due to the limited number of TEM images, we were not able to measure more than one egg for each humidity treatment.

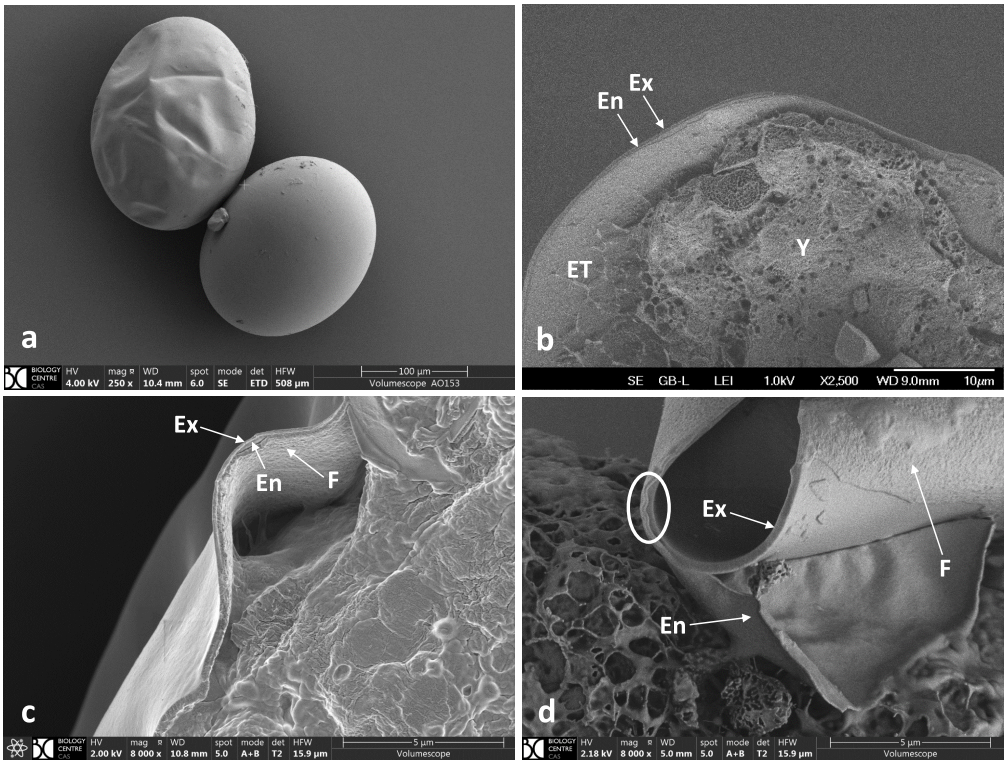


Figure 5.6: SEM images of *Phytoseiulus persimilis* eggs laid by females exposed to constant low (65%) RH. (a) External view of a dehydrated egg (left), and a full egg (right). (b) Egg section, with exochorion, endochorion, embryo tissue and yolk. (c, d) Egg sections with external layers (exochorion, endochorion, fibrous layer) peeled off, due to dehydration. The circle in (d) highlights the three external layers. Ex: exochorion; En: endochorion; ET: embryo tissue; Y: yolk; F: fibrous layer.

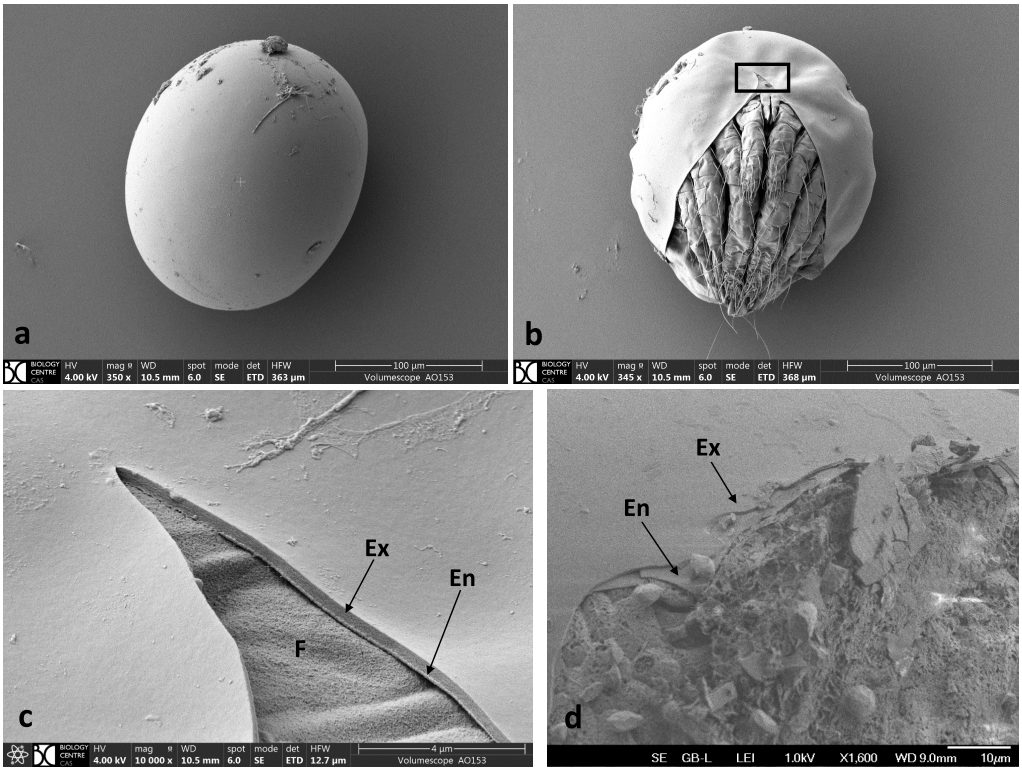


Figure 5.7: SEM images of *Phytoseiulus persimilis* eggs laid by females exposed to constant high (95%) RH. (a) External view of a full egg. (b, c) External view of a larva hatching from an egg, (c) is an enlargement of a section (black rectangle) from image (b), where the eggshell splits, at the top of the egg. (d) Egg section, with exochorion and endochorion. Ex: exochorion; En: endochorion; F: fibrous layer.

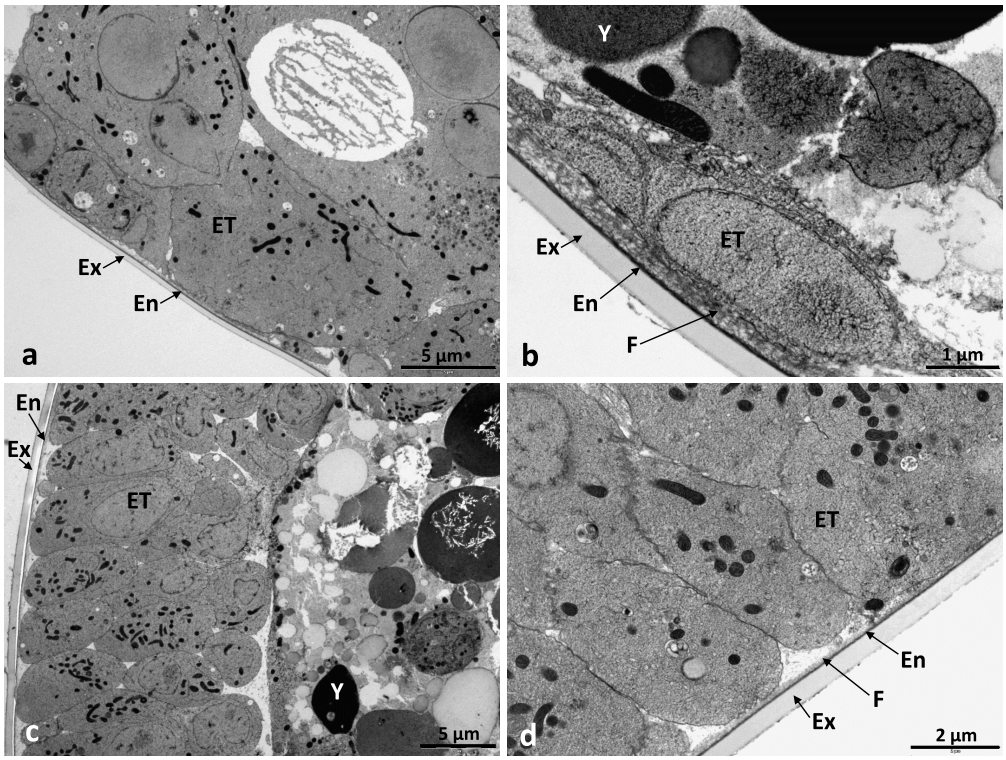


Figure 5.8: TEM images (cross sections) of *Phytoseiulus persimilis* eggs laid by females exposed to constant low RH (a,b; 65% RH) and constant high RH (c,d; 95% RH). The embryo tissue and yolk are covered with three layers: exochorion, endochorion, and fibrous layer. In image (b), the exochorion is 0.33 ± 0.007 µm thick (10 measurements). In image (d), the exochorion is 0.43 ± 0.009 µm thick (10 measurements). Ex: exochorion; En: endochorion; ET: embryo tissue; Y: yolk; F: fibrous layer.

5.3.5. DEVELOPMENTAL TIME

Relative humidity did not influence egg developmental time ($\chi^2 = 0.01; DF = 1; P = 0.9$; Fig.5.9). For both humidity treatments, more than 50% of the eggs hatched between 24 and 41 h after egg collection, and at least 98% of the eggs had hatched 49 h after egg collection. Since the eggs were between 0 and 6 h old when they were collected, the real developmental time of the eggs could be up to 3 h longer than the developmental time presented in Fig.5.9 (the 0-to-6-h difference was averaged to 3 h since multiple eggs were collected randomly between 0 and 6 h after laying).

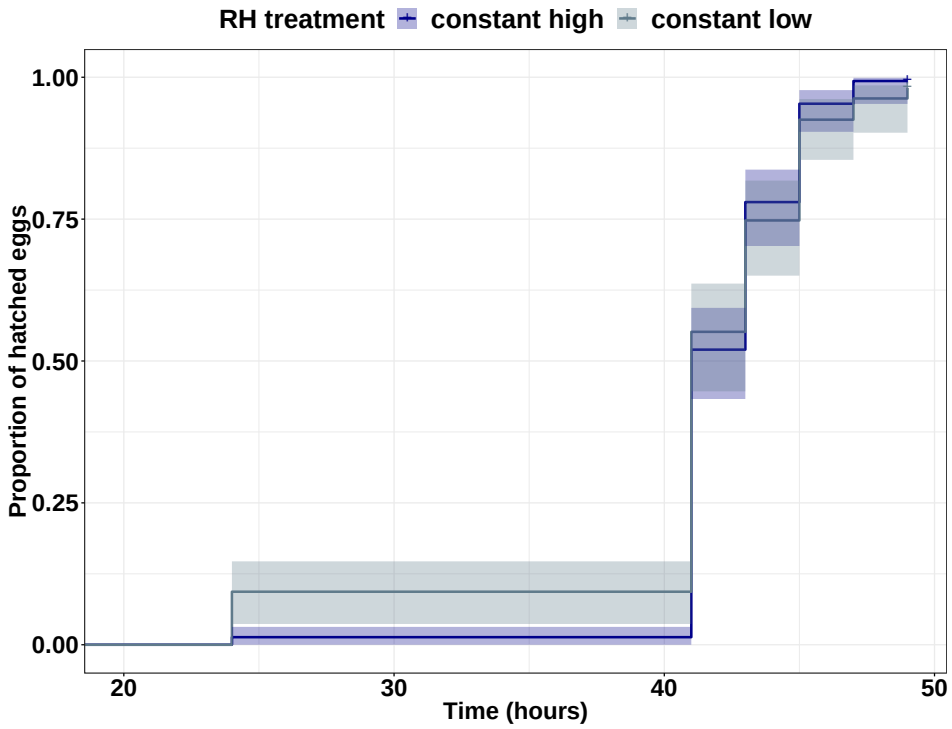


Figure 5.9: Developmental time of eggs laid by *Phytoseiulus persimilis* females exposed to two different humidity treatments at 25 °C for 48 to 54 hours (dark blue: constant high (95%) RH, light blue: constant low (65%) RH). Eight replicates per treatment, 9 to 22 eggs per replicate (Kaplan-Meier method, with 95% CI).

5.4. DISCUSSION

The objective of our study was to better understand the physiological adaptations making *P. persimilis* eggs drought resistant. We show that humidity conditions experienced by adult *P. persimilis* females influence the chemical composition of their eggs. Drought-resistant eggs, laid under constant low humidity, contain higher levels of free amino acids, such as serine, phenylalanine, and threonine, higher levels of sugar alcohols, such as *myo*-inositol and *scyllo*-inositol, and higher levels of heneicosane, a saturated hydrocarbon. In water-stressed arthropods, the ability to regulate osmotic pressure of body fluids and to maintain cell volume is based on the accumulation of organic osmolytes, also called compatible solutes, such as sugar alcohols, free amino acids, and polyols (Miles, 1966; Yancey et al., 1982; Holmstrup and Bayley, 2013; Holmstrup et al., 2015; Holmstrup and Slotsbo, 2018). Compatible solutes are small organic molecules with neutral charge and low toxicity at high concentrations, that act as osmolytes and help organisms survive extreme osmotic stress (Lang, 2007). For example, when exposed to drought stress, the soil-dwelling springtail *F. candida* actively produces *myo*-inositol and glucose to increase its osmotic pressure and stop water efflux

(Bayley and Holmstrup, 1999). Furthermore, insects are able to adapt to climate variation by modifying the composition of their cuticle, which serves a variety of functions, including chemical communication and waterproofing (Nation et al., 1992; Woodrow et al., 2000). The cuticle of insects contains a wide range of organic compounds including fatty acids, alcohols, esters, ketones, glycerides, sterols, aldehydes and hydrocarbons. Hydrocarbons are the primary components of the lipid layer that are responsible for waterproofing the cuticle (Hadley, 1978). In the mosquito *Aedes albopictus* (Skuse), for example, diapause eggs contain one-third more surface hydrocarbons and have a 50% lower water loss rate compared to non-diapause eggs (Urbanski et al., 2010). Our results suggest that the same mechanisms are involved in drought resistance of *P. persimilis* eggs: they contain higher levels of compatible solutes and hydrocarbons, likely reducing their water loss rate under dry conditions. The compatible solutes may be located inside the egg, in the tissues and the haemolymph, and the hydrocarbons may be located in the chorion. These metabolites are probably produced and allocated to the egg by the mother before laying, through a maternal effect triggered by constant low humidity. We previously showed that *P. persimilis* females start laying drought-resistant eggs after 6 to 24 h of exposure to low humidity (65% RH), and that the production of drought-resistant eggs can stop within 24 h if the females are transferred back to high humidity (Le Hesran et al., 2020). These observations suggest that *P. persimilis* females start allocating more compatible solutes and hydrocarbons to their eggs shortly after the start of an exposure to constant low humidity. Second, our data show that *P. persimilis* females exposed to constant low humidity lay larger eggs (on average 8.4% larger volume), with a 2.4% smaller SA:V than eggs laid by females exposed to constant high humidity. For an egg, a smaller SA:V results in a relatively smaller area from which water is lost, allowing the egg to be less vulnerable to water loss and dehydration stress (Gibbs and Matzkin, 2001; Kühsel et al., 2017). Drought-resistant *P. persimilis* eggs could have a larger volume for three main reasons: a higher initial water content, a thicker chorion, or a shift in offspring sex ratio. The production of larger eggs could be due to the fact that *P. persimilis* females produce more female offspring under drought stress (Toyoshima and Amano, 1998; Walzer and Schausberger, 2015). However, we show that females exposed to constant low humidity produce more males (male proportion: 0.19) than females exposed to constant high humidity (male proportion: 0.12). Therefore, the larger volume of eggs laid under dry conditions cannot be explained by a change in offspring sex ratio. Under optimal conditions, the percentage of females in *P. persimilis* offspring is 83.7% (Toyoshima and Amano, 1998), and this percentage can decrease with maternal stress, such as food deprivation (Walzer and Schausberger, 2015). In our study, the percentage of female offspring under maternal drought stress was 81%, lower than the 88% female offspring produced under high humidity conditions. This reduction in the percentage of female offspring under dry conditions could be due to the higher production costs of large female eggs than smaller male eggs. Regarding the chorion, we would have expected to see a thicker exochorion or an extra wax layer covering drought-resistant eggs, because many terrestrial arthropods rely on hard waxes for waterproofing (Beament, 1959). However, we did not observe any structural difference between chorions of drought-resistant and drought-sensitive eggs. All eggs were covered with three layers: a thick exochorion, a thin endochorion, and a fibrous layer, probably attaching the embryo to the endochorion. We are not able to draw strong conclusions regarding exochorion thickness. However, on the basis of the two TEM pictures on which it was mea-

sured, the exochorion of the drought-resistant egg (0.33 ± 0.007 µm) was not thicker, but rather thinner than the exochorion of the drought-sensitive egg (0.43 ± 0.009 µm). More observations are needed to investigate potential differences in exochorion thickness between drought-resistant and drought-sensitive eggs. Ultimately, a higher initial water content is a plausible explanation for the larger volume of drought-resistant *P. persimilis* eggs. A higher initial water content, combined with a decreased water loss rate due to a different chemical composition and a lower SA:V ratio, may explain how drought-resistant *P. persimilis* eggs survive under constant dry conditions. Previous studies on insects suggest that a decreased water loss rate plays a more important role in desiccation resistance than a higher water content. For example, desert *Drosophila* species have adapted to arid conditions primarily by reducing rates of water loss rather than by increasing the amount of water in their body or by tolerating the loss of a greater percentage of body water (Gibbs and Matzkin, 2001). Similarly, in the moth *Manduca sexta* (Linnaeus), egg adaptation to drought stress was explained by the modification of eggshell conductance rather than by a greater amount of water in the eggs (Potter and Woods, 2012). Which mechanism contributes most to drought resistance in *P. persimilis* eggs remains to be investigated. Egg retention does not seem to be involved in the mechanisms allowing *P. persimilis* eggs to be drought-resistant. Drought-resistant and drought-sensitive eggs had the same developmental time under high humidity, confirming that *P. persimilis* females do not retain their eggs longer in their body under constant low humidity. Regarding the costs of laying drought-resistant eggs, we showed in CHAPTER 4 that *P. persimilis* females lay fewer eggs under constant low humidity (Le Hesran et al., 2020). This lower oviposition rate is probably due to high energy costs of producing drought-resistant eggs, combined with a reallocation of the female's resources to somatic maintenance instead of reproduction. For *P. persimilis* females, drought-resistant eggs could be costly to produce because of their larger volume, or because of their different chemical composition. Laying larger eggs likely costs more energy, and the selection for larger eggs in a population generally results in a lower fecundity (Roff, 1993; Fox, 2000). For example, in the butterfly *B. anynana*, oviposition temperature induces a plastic response in egg size: at 20 °C, females lay significantly larger but fewer eggs than their sisters at 27 °C (Fischer et al., 2003). However, the assumption of a trade-off between egg size and egg number is challenged by the results of another study on *B. anynana*: despite an increase in egg size through artificial selection, there was no evidence for any correlated change in fecundity of *B. anynana* females. Due to adjustments in egg composition, females producing larger eggs did not have to sacrifice fecundity (Karl et al., 2007). For water-stressed *P. persimilis* females, allocating more water to their eggs probably represents an important sacrifice, at the expense of water availability to themselves. The lower fecundity observed in *P. persimilis* females under low humidity is probably due to a lack of water to satisfy both the production of larger eggs and the physiological needs of the females. Finally, during our research on the mechanisms of drought resistance in *P. persimilis* eggs, an important question emerged: is dry air fatal for *P. persimilis* eggs because the embryo dehydrates until it dies, or because the chorion becomes hard and impenetrable? When eggs of *Haematopinus asini* (Linnaeus) are exposed to dry air, the embryo dies before development is finished (Bacot and Linzell, 1919). But in other cases, death is due to the inability of the larva to penetrate the chorion and hatch (Buxton, 1932). The mechanical properties of the sternal cuticle of the locust were investigated under dry and wet conditions. The results show that water has a major impact on the mechanical

properties of the cuticle (measured in GPa): after drying, the endocuticle became harder by a factor of up to 9 (Klocke and Schmitz, 2011). Similarly, a decrease in water content of only a few percent can cause a 10-fold increase in stiffness in the cuticle of a maggot in *Calliphora erythrocephala* Meigen (Vincent, 2004). During our experiments, several developed larvae were observed inside drought-sensitive eggs trying to hatch under dry conditions, without success. After approximately 15 min of struggling to break the chorion, the larvae would die inside the eggs (Le Hesran personal observation). There may be two explanations for this: first, under constant low humidity, the chorion of *P. persimilis* eggs becomes too hard to break for the fully developed larva inside. Second, under constant low humidity, the embryo suffers from dehydration and at the end of its development, the larva inside the egg is too weak to break the chorion. In both cases, the larvae do not fail to hatch because the embryo inside is dead from dehydration, but rather because they cannot emerge. If this is the case, the higher water content in drought-resistant *P. persimilis* eggs could prevent the hardening of the chorion or the dehydration of the larva, by compensating the decrease in water content due to dry conditions. Alternatively, the exochorion of drought-resistant eggs may be easier to break because it is thinner, even after hardening due to desiccation. In conclusion, two main mechanisms seem to be responsible for the drought resistance of *P. persimilis* eggs laid under low humidity: they are larger, probably because of a higher water content, and contain more compatible solutes (free amino acids and sugar alcohols) and hydrocarbons than drought-sensitive eggs. Why *P. persimilis* eggs do not hatch under dry conditions remains to be further investigated, but our observations suggest that the embryo fully develops in *P. persimilis* eggs under dry conditions, whereas the larva cannot break the eggshell, and dies inside the egg.

5.5. ACKNOWLEDGMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 641456. We thank the Laboratory of Electron Microscopy (Institute of Parasitology), in Biology Center CAS in České Budějovice, supported by the MEYS CR (LM2015062 Czech-BioImaging) and ERDF (No. CZ.02.1.01/0.0/0.0/16_013/0001775) for their support in obtaining the TEM and SEM images presented in this chapter. We also thank Maurijn van der Zee and Gerda Lamers (Institute of Biology, Leiden University, the Netherlands) and Wojciech Witalinski (Jagiellonian University, Krakow, Poland) for their help in interpreting the TEM and SEM images. The experiments of this chapter have been carried out with the help of a Master student (Nugroho, J.E.) who was on a mobility stay funded by the Erasmus+ - KA1 Erasmus Mundus Joint Master Degrees Programme of the European Commission under the PLANT HEALTH Project.

5.6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

5.7. APPENDIX

Table 5.1: Error analysis of the automated *Phytoseiulus persimilis* egg geometry extraction. Four replicates (R0, R1, R2, R3) were carried out in which the same egg was measured 10 times. After each measurement, the egg was removed and placed in a different position. σ is the standard deviation for the 10 measurements of each replicate, and CV is the coefficient of variation.

	Maximum Diameter			**Volume**			**SA:V**		
	Mean (mm)	σ (mm)	CV (%)	Mean (mm^3)	σ (mm^3)	CV (%)	Mean (mm^{-1})	σ (mm^{-1})	CV (%)
R0	0.230	0.0062	1.36	0.00425	0.00021	2.42	30.16	0.512	0.85
R1	0.243	0.0031	0.64	0.00510	0.00022	2.12	28.35	0.432	0.76
R2	0.228	0.0034	0.64	0.00421	0.00028	3.30	30.23	0.747	1.23
R3	0.247	0.0052	1.05	0.00502	0.00027	2.64	28.55	0.547	0.96

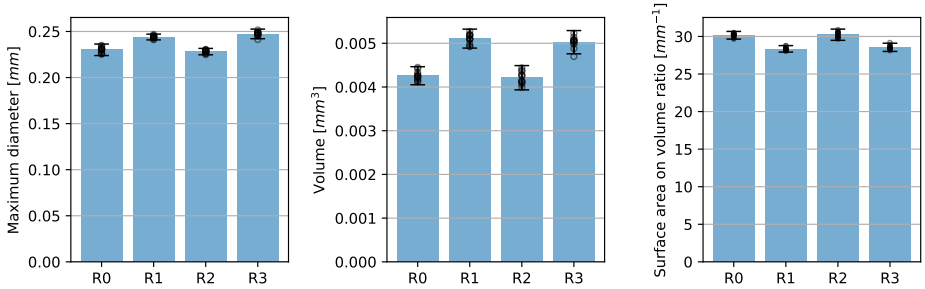


Figure 5.10: Error analysis of the automated *Phytoseiulus persimilis* egg geometry extraction. Plots corresponding to Table 5.1, representing the mean Maximum diameter, Volume and SA:V, for the four replicates. Error bars represent ± 2 σ.

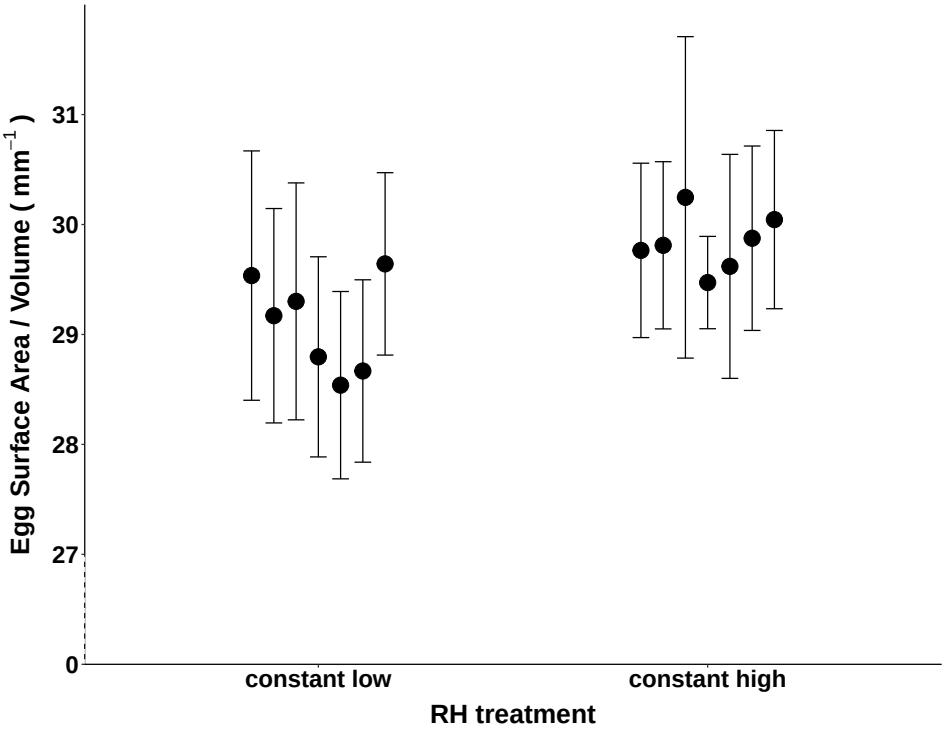
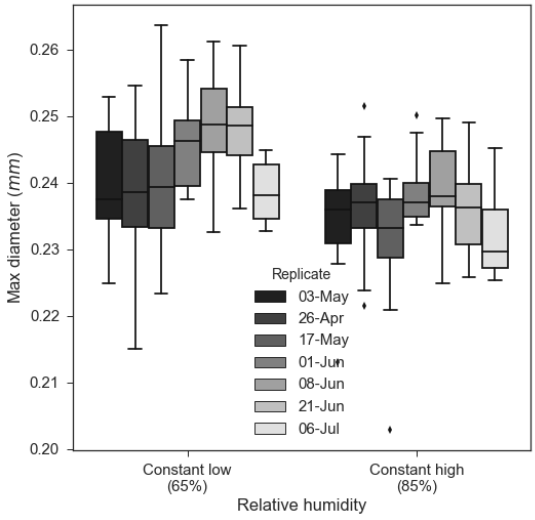
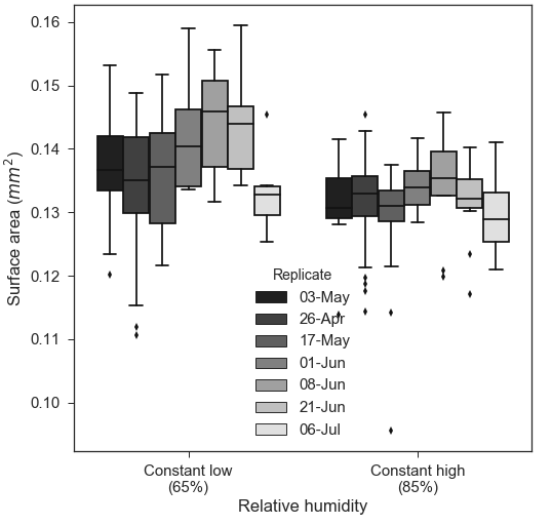


Figure 5.11: Observed SA:V (mm^{-1}), for eggs laid by *Phytoseiulus persimilis* females exposed to two humidity treatments (constant high (95%) RH: 127 eggs, and constant low (65%) RH: 111 eggs). Each symbol represents the average egg SA:V for one replicate (n=37;15;19;9;14;11;6 eggs for constant low RH and n=46;15;18;14;9;13;12 eggs for constant high RH). Error bars represent ± 1 SD.



Replicate	Max diameter (mm)
01-Jun	0.2412
03-May	0.2369
06-Jul	0.2342
08-Jun	0.2448
17-May	0.2354
21-Jun	0.2414
26-Apr	0.2370



Replicate	Surface area (mm^2)
01-Jun	0.1372
03-May	0.1344
06-Jul	0.1307
08-Jun	0.1402
17-May	0.1325
21-Jun	0.1368
26-Apr	0.1331

Figure 5.12: Observed maximum diameter (mm) and surface area (mm^2) for eggs laid by *Phytoseiulus persimilis* females exposed to two humidity treatments (constant high RH (95%): 127 eggs, and constant low RH (65%): 111 eggs). Each bar represents the average egg maximum diameter or surface area for one replicate (n=37;15;19;9;14;11;6 eggs for constant low RH and n=46;15;18;14;9;13;12 eggs for constant high RH). Error bars represent ± 1 SD.

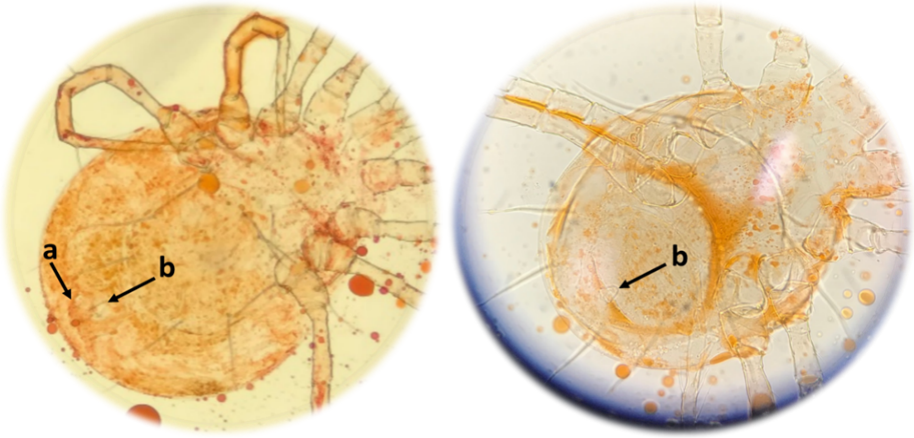


Figure 5.13: Visual cues used for the sex ratio experiment, to differentiate between male and female *Phytoseiulus persimilis*. Left picture: adult female *P. persimilis* anal structure (b) surrounded by a circular shield (a). Right picture: adult male *P. persimilis* anal structure (b), without the shield. Optical microscope (x400).

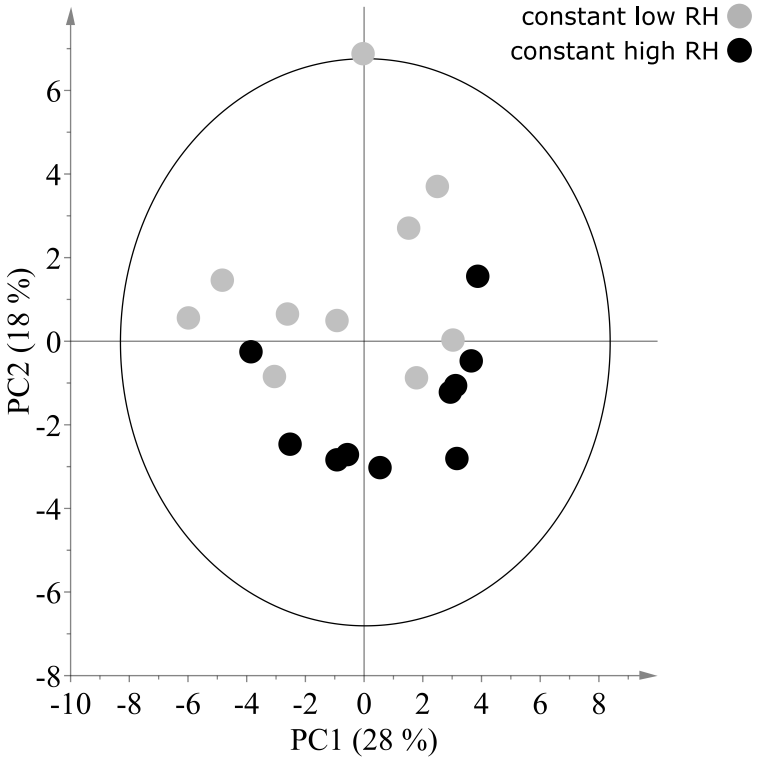


Figure 5.14: PCA plot for *Phytoseiulus persimilis* eggs laid under two humidity treatments (grey symbols: constant low (65%) RH, black symbols: constant high (95%) RH), using the first two principal components of the model. The ellipse represents the Hotelling's T2 (95%) border. Ten symbols per humidity treatment represent ten replicates, with 100 eggs per replicate.

Table 5.2: List of chemical compounds corresponding to the labelled dots in the loading plot from the PLS-DA analysis for Phytoseiulus persimilis eggs (Fig.5.5).

Chemical compound	Dot label (Fig.5.5)
2-sec-Butyl-3-methyl-1-pentene	C1
D-(-)-Lactic acid, trimethylsilyl ether, trimethylsilyl ester	C2
Nonane, 2,2,4,4,6,8,8-heptamethyl-	C3
Silanol, trimethyl-, phosphate (3:1)	C5
Trimethylsilyl ether of glycerol	C6
Glycine, N,N-bis(trimethylsilyl)-, trimethylsilyl ester	C8
Dodecane, 2,6,11-trimethyl-	C9
L-Serine, N,O-bis(trimethylsilyl)-, trimethylsilyl ester	C11
N,O,O-Tris(trimethylsilyl)-L-threonine	C12
Tetradecane	C13
L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester	C14
Hexadecane	C16
N,O-Bis-(trimethylsilyl)phenylalanine	C17
.beta.-L-Galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)-	C18
Heneicosane	C21-C23-C24-C31-C32-C39
Tetradecanoic acid, trimethylsilyl ester	C22
.beta.-L-Galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)-	C26
Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-	C27
Hexadecanoic acid, trimethylsilyl ester	C28
Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	C29
trans-13-Octadecenoic acid, trimethylsilyl ester	C34
.alpha.-Linolenic acid, trimethylsilyl ester	C35
Octadecanoic acid, trimethylsilyl ester	C36
2-Monopalmitin trimethylsilyl ether	C38
Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	C40
.alpha.-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-.beta.-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	C41
2-Monostearin trimethylsilyl ether	C42
Bis(trimethylsilyl)monostearin	C43

6

General Discussion

6.1. GENERAL CONTEXT

Terrestrial arthropods face a serious problem with respect to drought and water balance. In agricultural ecosystems, where arthropods play an important role, dry conditions can have differential effects on herbivorous pests and their natural enemies, making biological pest control more challenging. In this thesis, I focused on the particular case of the predatory mite *P. persimilis* and its prey *T. urticae*. While *T. urticae* populations thrive in dry conditions, *P. persimilis* populations suffer from drought stress. When I started this research project, most studies published on the effects of low humidity on *P. persimilis* were focused on the egg stage. The drought sensitivity of *P. persimilis* eggs was presented as the main reason for the low performance of this biocontrol agent in dry conditions (Schausberger, 1998; Ferrero et al., 2010; Döker et al., 2016). After four years of investigation, this thesis brings new perspectives on the effects of relative humidity on *P. persimilis* eggs as well as adult females, and provides answers to fundamental questions regarding the low efficacy of *P. persimilis* in dry conditions: How is *P. persimilis* affected by drought stress? How can biological control of spider mites with *P. persimilis* be improved under dry conditions?

6.2. EFFECTS OF DROUGHT STRESS ON *P. persimilis*

6.2.1. SOME CLARIFICATIONS ON THE DEFINITION OF DROUGHT STRESS

When studying the effects of drought stress on *P. persimilis*, one of the first questions I wish to answer is: which environmental conditions are perceived as dry and stressful by *P. persimilis*? Since humidity conditions experienced by a living organism depend on the temperature in its environment, setting a "drought-stress limit" for *P. persimilis* is not easy. For example, *P. persimilis* eggs are more sensitive to low relative humidity when temperature increases (Stenseth, 1979). To have a more direct indication of the atmospheric moisture conditions experienced by an organism, independently of the temperature, relative humidity can also be expressed as Vapour Pressure Deficit (VPD, in kPa; see CHAPTER 2). In CHAPTER 4, I used 65% RH and 25 °C as stressful conditions for *P. persimilis* adult females. The VPD corresponding to 65% RH and 25 °C is equal to 1.1 kPa. To have the same VPD at 20 °C or 30 °C, i.e. to experience the same moisture conditions at 20 °C or 30 °C, relative humidity should be equal to 53% or 74% respectively. This means that for *P. persimilis*, environmental conditions such as 65% RH at 25 °C are probably as stressful as 53% RH at 20 °C, or as 74% RH at 30 °C. When considering drought, it is therefore important to remember that the definition of drought stress is dependent on both relative humidity and temperature. In this thesis, humidity conditions were expressed in terms of relative humidity, in % (except in CHAPTER 2 where I introduced the concept of VPD), because it is easier for most people to 'visualize' what represents a relative humidity expressed in % than a VPD expressed in kPa. All experiments presented in this thesis were carried out at a constant temperature of 25 °C, conditions which are not stressful for *P. persimilis*. I therefore conclude that the results presented in this thesis were primarily influenced by relative humidity.

6.2.2. HOW ARE *P. persimilis* EGGS AFFECTED BY RELATIVE HUMIDITY IN THEIR ENVIRONMENT?

CHAPTER 2 confirmed that *P. persimilis* eggs are highly sensitive to constant low relative humidity: less than 20% of them survive at 60% RH and 25 °C. Even eggs from *P. persimilis* populations originating from dry locations in the Mediterranean region were sensitive to constant low humidity. However, survival of *P. persimilis* eggs increases significantly under variable humidity conditions: a short period at high humidity allows the eggs to recover from a long period of drought and to survive (CHAPTER 2). This capacity to recover enhances their chances of survival under variable humidity conditions in fields and greenhouses. *Phytoseiulus persimilis* eggs are, therefore, probably not as vulnerable as was previously thought regarding relative humidity in their environment. Moreover, if *P. persimilis* eggs are only vulnerable to constant low humidity, one might even wonder if they are ever exposed to constant low humidity during the approximately 54 hours of their development. First, because humidity and temperature (and therefore VPD) fluctuations are common in fields and greenhouses, for example between day and night. Second, because *P. persimilis* eggs are mostly laid on leaf surfaces or in *T. urticae* webs, where they probably benefit from a microclimate with higher humidity than in the atmosphere. On tomato plants, for example, the relative humidity at a 5 mm distance from the underside leaf surface can be 30 to 40% greater than in the ambient air (Boulard et al., 2002). CHAPTER 3 provides information indicating that *P. persimilis* eggs are indeed likely to be exposed to humidity conditions that are unfavourable for their survival, even on live plants infested with *T. urticae*. In the experimental evolution trial, *P. persimilis* females apparently perceived the humidity conditions as unfavourable for the survival of their eggs and laid drought-resistant eggs. *Phytoseiulus persimilis* eggs are therefore likely to suffer from low humidity conditions in their environment, at least based on the decisions made by their mother. With these observations, CHAPTER 3 marked a turning point in my thesis: even though the selection experiments did not lead to a permanent increase in drought resistance of *P. persimilis* eggs, they allowed me to change my focus from *P. persimilis* eggs to *P. persimilis* adult females.

6.2.3. THE FORESIGHT OF A MOTHER: HOW *P. persimilis* FEMALES PROTECT THEIR EGGS FROM DESICCATION

In CHAPTER 4, I investigated how *P. persimilis* adult females respond to humidity variations in their environment. *Phytoseiulus persimilis* females have a major influence on the survival of their eggs: if humidity conditions threaten the survival of their eggs, *P. persimilis* females can produce drought-resistant eggs through a maternal effect. The production of drought-resistant eggs is a highly plastic trait, which females can "switch" on and off in less than 24 hours. Drought-resistant eggs can survive at constant 60% RH and 25 °C, are 8.4% bigger in volume and have a different chemical composition compared to drought-sensitive eggs (CHAPTER 5). This bigger volume is probably the result of a higher water content, making drought-resistant eggs less vulnerable to water loss. Moreover, with higher concentrations of compatible solutes (free amino acids and sugar alcohols) and saturated hydrocarbons, drought-resistant eggs may benefit from a reduced water loss rate in dry conditions. Chorion thickness could also have an influence on water loss rate in drought-resistant eggs. However, the results presented in CHAPTER 5 on chorion thickness are preliminary, and

this aspect deserves to be studied more intensively. Even though the exact causes explaining why *P. persimilis* eggs do not survive in dry conditions have not yet been elucidated, I observed an interesting phenomenon. My hypothesis is that the embryo inside a *P. persimilis* egg is able to complete its development even when exposed to constant drought stress. However, at the end of the embryo development, the larva inside the egg appears to be unable to break through the eggshell and will finally die inside the egg. The drought sensitivity of *P. persimilis* eggs could therefore be due to mechanical reasons: the eggshell becomes so hard after desiccation that the larva inside does not have the strength to break it and hatch. Therefore, the greater water content and reduced water loss rate in drought-resistant eggs might help avoiding extreme desiccation and hardening of the eggshell, allowing the larva inside to break through the eggshell and hatch. Measuring hardness of the chorion of *P. persimilis* eggs after exposure to high and low humidity would be a way to test this hypothesis. My initial perspective on drought sensitivity of *P. persimilis* eggs has changed significantly as the investigations progressed. First, because *P. persimilis* eggs can recover from a long exposure to low humidity (60% RH at 25 °C) after a few hours exposure to high humidity (75% RH at 25 °C) (CHAPTER 2). Second, because even in the case of constant low humidity, *P. persimilis* females are able to lay drought-resistant eggs that will survive in these conditions (CHAPTER 4). Therefore, it seems that drought sensitivity of *P. persimilis* eggs is not the main reason why *P. persimilis* populations struggle to control *T. urticae* populations in dry conditions.

6.3. BIOLOGICAL CONTROL OF TWO-SPOTTED SPIDER MITES IN DRY CONDITIONS

6.3.1. WHY IS *P. persimilis* LESS EFFICIENT AS A BIOCONTROL AGENT IN DRY CONDITIONS?

Phytoseiulus persimilis females lay significantly fewer eggs in constant dry conditions than in constant high or variable humidity conditions (CHAPTER 4). Drought stress, combined with the energetically costly production of drought-resistant eggs, causes a significant decrease in oviposition rate of *P. persimilis* females. This may explain why, in CHAPTER 3, females from the experimental evolution trial had a lower oviposition rate compared to females from the artificial selection trial. Females from the experimental evolution trial were exposed to low humidity before the oviposition test, and probably continued laying drought-resistant eggs during the first day of the oviposition test. As a consequence, they laid fewer eggs over three days than females from the artificial selection trial. Moreover, *P. persimilis* females have a significantly shorter lifespan in constant dry conditions than in variable humidity conditions (CHAPTER 4). The lower oviposition rate combined with a shorter lifespan of *P. persimilis* females could, therefore, be the main problem regarding biological control of spider mites in constant dry conditions. Other *P. persimilis* life stages than eggs and adult females may also be affected by low humidity, however probably to a lesser extent. Survival of *P. persimilis* larvae does not seem to be affected by drought, since their developmental time is very short: around 15-20 hours at 25 °C (Sabelis, 1981). In the egg hatching tests in CHAPTER 2, I observed that all hatched larvae developed into nymphs even when exposed to constant 60% RH. I do not expect *P. persimilis* nymphs either

to be particularly drought-sensitive, because they can eat, move or drink to avoid desiccation stress. Finally, the impact of drought stress on *P. persimilis* adult males is not known. *Phytoseiulus persimilis* males probably suffer from drought stress to a lesser extent than *P. persimilis* females, because they do not have to spend energy on laying drought-resistant eggs in these conditions. However, it is not known whether drought stress could have a negative impact on their reproductive capacity, for example, through a slower activity. I observed in CHAPTER 5 that *P. persimilis* females exposed to constant low RH produce more males (male proportion: 0.19) than females exposed to constant high RH (male proportion: 0.12). This increase in the percentage of male offspring under dry conditions could be due to the higher production costs of large female eggs than smaller male eggs. Alternatively, *P. persimilis* females might produce more males under dry conditions to compensate for lower reproductive capacities of *P. persimilis* males in these conditions. This aspect still remains to be investigated. In conclusion, the relationship between *P. persimilis* females and their eggs seems to be a key element in the lower efficacy of *P. persimilis* as a biocontrol agent of *T. urticae* in dry conditions. However, another essential factor should be taken into account: the positive impact of low relative humidity on *T. urticae* populations.

6.3.2. HOW ARE *T. urticae* POPULATIONS AFFECTED BY DRY CONDITIONS?

Unlike *P. persimilis* populations, *T. urticae* populations thrive in hot and dry conditions (Palevsky et al., 2008). At low relative humidity (35% RH at 25-35 °C), two-spotted spider mites eat more, lay more eggs, live longer, and have a shorter developmental time than at high relative humidity (95% RH at 25-35 °C) (Sabelis, 1981). Moreover, hatching of *T. urticae* eggs is not significantly affected by extremely low relative humidity (Boudreaux, 1958). *Tetranychus urticae* eggs are protected against water loss in two ways. First, they have a very thin wax layer around the inner surface of their shell (Sabelis, 1981). Second, *T. urticae* eggs have developed a unique respiratory system allowing them to breathe and minimize water loss at the same time: two embryonic stigmata, connected to the intermediate membrane covering the embryo, pierce the eggshell. Through perforations in the intermediate membrane, an air plastron of 0.2-0.3 µm thick is present between the shell and the embryo, allowing air exchange with the outside while minimizing water loss (Dittrich and Streibert, 1969). Consequently, failures in augmentative biological control of two-spotted spider mites in dry conditions are probably due to the combination of two phenomena: *T. urticae* populations perform significantly better at low relative humidity, and *P. persimilis* populations have a lower intrinsic rate of increase in these conditions. If I would have had more time to conduct an additional experiment, I would have studied the intrinsic rates of increase of *P. persimilis* and *T. urticae* populations on live plants, in low and high humidity conditions, and compared them. This experiment would hopefully bring responses to a remaining question: how big is the gap between *P. persimilis* and *T. urticae* population growth rates in dry conditions?

6.3.3. RECOMMENDATIONS TO IMPROVE BIOLOGICAL CONTROL OF TWO-SPOTTED SPIDER MITES IN DRY CONDITIONS

I propose here four potential solutions to improve augmentative biological control of two-spotted spider mites by *P. persimilis* in dry conditions.

1. Releasing more *P. persimilis* predators.

   In the case of a *T. urticae* infestation in dry conditions, releasing more *P. persimilis* individuals could compensate for the lower oviposition rate and shorter lifespan of *P. persimilis* females. The gap between *P. persimilis* and *T. urticae* population growth rates would then be smaller and the latter would be under control. Modelling population growth rates of both species in different humidity conditions would help estimating how many additional *P. persimilis* are needed to control a two-spotted spider mite population under specific humidity conditions. However, this solution implies that growers would have to pay a higher price to buy more natural enemies.

2. Optimizing the humidity conditions.

   This solution is applicable to greenhouses only. In case of a drought period, increasing the relative humidity inside the greenhouse could help reducing the impact of two-spotted spider mites on the crop, and promoting *P. persimilis* population growth. As *P. persimilis* populations perform quite well in variable humidity conditions, a substantial increase of relative humidity during at least a few hours every day might help reducing the stress on *P. persimilis* populations. Increasing the relative humidity in a greenhouse a few hours per day should be relatively easy to implement. Again, modelling population growth rates of *P. persimilis* and *T. urticae* in variable humidity conditions could help estimate how humidity conditions should be optimized in the case of drought, to reduce the gap between population growth rates of these two species.

3. Combining the use of *P. persimilis* with a more drought-resistant biocontrol agent.

   When *P. persimilis* struggles too much in controlling *T. urticae* populations in dry conditions, other native biocontrol agent species could be used in combination. For example, the predatory mite *Neoseiulus californicus* McGregor is known to be more drought resistant than *P. persimilis*. Depending on the host plant, *N. californicus* can sometimes provide better control of *T. urticae* populations in dry conditions than *P. persimilis* (Palevsky et al., 2008; Weintraub and Palevsky, 2008). These two natural enemies could be released in combination: *P. persimilis* would provide an immediate and short-term suppression of *T. urticae* populations, while *N. californicus* would be used for both prophylactic and long-term control, as it can survive by feeding on alternate food sources such as pollen. However, combining two phytoseiid predators is not always a good solution, as there can be a risk of intraguild predation (Schausberger and Croft, 2000). Therefore, preliminary field and greenhouse trials would be necessary to assess the efficacy of this solution before offering it to growers, because it requires to buy more biocontrol agents and is therefore more costly.

4. Artificial selection.

   In CHAPTER 3, I tried to artificially select for more drought-resistant *P. persimilis* eggs. I did not succeed, probably because the production of drought-resistant eggs is a highly phenotypically plastic trait, regulated by *P. persimilis* females. Another potential explanation is that there was not enough genetic variation for egg drought resistance in the base population used for the selection trials. Since *P. persimilis* females lay fewer eggs in constant dry conditions, selecting for *P. persimilis* females with a higher oviposition rate at low relative humidity could be a better approach to improve the performance of this biocontrol agent in dry conditions. For this, new populations of *P. persimilis* should be collected from different locations with dry climates. Genetic variation for drought resistance in females, i.e. a high oviposition rate in dry conditions, should be assessed among these populations, to select for the best performing females. Nevertheless, I have some reservations about this solution. In the experimental evolution trial in CHAPTER 3, even though *P. persimilis* females were exposed to variable low humidity conditions for 17 months, their oviposition rate in dry conditions was not significantly higher than that of *P. persimilis* females which had not gone through any selection pressure. It may be that there was not enough genetic variation for drought resistance in the base population we used in CHAPTER 3, or that the selection pressure on the females was not high enough. Alternatively, it is possible that *P. persimilis* females are already 'optimized' to lay a maximum amount of drought-resistant eggs under dry conditions, leaving little room for an increase in oviposition rate. In any case, this solution is worth exploring, because genetic variation for drought resistance in *P. persimilis* females has not been investigated yet.

Among these four potential solutions, the first two about releasing more *P. persimilis* and optimizing the humidity conditions in greenhouses seem to be the easiest to apply in the short term. However, in the long term, more research on artificial selection or the combination of *P. persimilis* with a drought-resistant biocontrol agent could lead to improvement of augmentative biological control of *T. urticae* in dry conditions.

6.4. CONCLUSION

This thesis provides essential information on the effects of dry conditions on *P. persimilis*. I started from the assumption that the drought sensitivity of *P. persimilis* eggs was the main reason for the low efficacy of this biocontrol agent in dry conditions. However, during this research project, I discovered that two main factors significantly enhance the survival of *P. persimilis* eggs in dry conditions. First, they are sensitive to drought only if they are exposed to it during their whole development, and they are able to recover from long periods of drought after a short exposure to high humidity. Second, *P. persimilis* females can protect their eggs from desiccation. Through a maternal effect, adult females can lay drought-resistant eggs if the humidity conditions are unfavourable for their survival. Therefore, it seems that drought sensitivity of *P. persimilis* eggs is not the main cause of failure of biological control of two-spotted spider mites in dry conditions. Instead, I discovered that *P. persimilis* females have a lower oviposition rate and a shorter lifespan in constant dry conditions. Because of the physiological stress generated by drought, and because of the en-

ergetic costs of producing drought-resistant eggs, *P. persimilis* females lay fewer eggs in dry conditions. Consequently, the lower growth rate of *P. persimilis* populations, combined with a higher growth rate of *T. urticae* populations in dry conditions, are probably key elements affecting the efficacy of augmentative biological control of two-spotted spider mites in dry conditions. The research presented in this thesis provides a big step in the comprehension of the effects of drought on *P. persimilis*, and the mechanisms affecting the efficacy of this biocontrol agent in dry conditions. This thesis gives new perspectives to future research on the performance of *P. persimilis* in harsh environmental conditions.

6.5. CONTRIBUTIONS OF THIS THESIS TO THE SCIENTIFIC LITERATURE

In the context of global climate change and increasing variation in rainfall, the effects of drought on terrestrial arthropods is a particularly relevant topic. Drought stress has been the subject of many studies on insect species, which have demonstrated that cuticular water loss rate is the main factor accounting for variation in insect desiccation resistance (Chown et al., 2011). In comparison, far fewer studies have been published on this topic about mites. The effects of drought stress have been especially studied in spider mites, because they are able to lay diapausing eggs which are resistant to desiccation, and because diapausing spider mites have a high tolerance to desiccation stress (Lees, 1961; Dittrich and Streibert, 1969; Ghazy and Suzuki, 2014). I found only one published study reporting a maternal effect associated with relative humidity in mites; in larvae of the American dog tick *Dermacentor variabilis*, the ability to absorb water vapour from the air is under maternal control (Yoder et al., 2006). To my knowledge, my thesis provides the first proof that a maternal effect associated with relative humidity exists in a phytoseiid mite species. This discovery is of particular importance for biological pest control, since phytoseiid mites include highly efficient predators used worldwide, such as *Amblyseius swirskii* Athias-Henriot and *N. californicus*. Previous studies had also taken up the challenge of studying mite eggs with scanning and transmission electron microscope images (Marchiondo, 1981; Mazzini and Baiocchi, 1983; Witalinski, 1987; Witaliñski, 1993). However, these studies were conducted more than 25 years ago, and none of them was on phytoseiid mites. This thesis presents the first study on internal and external structure, as well as chemical composition of eggs, in a phytoseiid mite. The detailed study of *P. persimilis* eggs allowed me to begin to unravel the mystery of drought-resistance mechanisms in this species. The results reported in this thesis should inspire and guide further studies on phytoseiid mites and their responses to environmental conditions.

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SUMMARY

Terrestrial arthropods are particularly vulnerable to drought stress, because of their small size, and because their body has a high surface-area-to-volume ratio (SA:V). In agricultural ecosystems, where the ecological functions of terrestrial arthropods are significant (herbivores, pollinators, predators), this sensitivity to drought can have serious consequences. Biological pest control with arthropod predators, in particular, is strongly affected by relative humidity conditions. Understanding how terrestrial arthropods respond to changes in humidity in their environment is, therefore, essential to ensure the success of biological control programs. In this thesis, I investigated the effects of drought stress on the predatory mite *Phytoseiulus persimilis*. This mite is widely used in augmentative biological control as an effective predator of the two-spotted spider mite *Tetranychus urticae*. The biocontrol efficacy of *P. persimilis* decreases under dry conditions, and this lower efficacy has often been explained as the consequence of the drought sensitivity of *P. persimilis* eggs. In CHAPTER 2, I studied the effects of relative humidity on *P. persimilis* eggs. I investigated the presence of genetic variation for egg drought resistance in *P. persimilis*, and the potential sources (genetic or environmental) of phenotypic variation in this trait, by comparing egg-hatching rates among five *P. persimilis* populations in different humidity conditions (constant low, constant high, and variable humidity). I found no intraspecific genetic variation among the five tested populations in egg hatching under constant and variable humidity conditions. In all five populations, less than 20% of the eggs hatched when they were exposed to constant low (60% RH) humidity conditions at 25 °C. However, when eggs were exposed to successive cycles of low and high humidity, significantly higher hatching rates were observed. Under variable humidity conditions, more than 73% of the eggs hatched successfully, even when exposure to high humidity was limited to only 13% of the egg developmental time. Although *P. persimilis* eggs suffered from a high rate of water loss under constant dry conditions, they were able to compensate for this water loss when exposed to high humidity conditions for a few hours during their development. These results changed my initial perspective on the drought sensitivity of *P. persimilis* eggs: it appears that they are capable of dealing with harsh humidity conditions more effectively than previously thought. In CHAPTER 3, I investigated the possibilities to select for increased drought resistance in *P. persimilis* eggs, through artificial selection and experimental evolution. In an artificial selection trial, *P. persimilis* eggs from two selection lines were exposed to three selection rounds. In an experimental evolution trial, all *P. persimilis* life stages from two selection lines were exposed to a constant low humidity selection pressure. To evaluate the response to selection, egg hatching rate at low humidity was assessed in both trials. At regular intervals during the trials, I also looked at oviposition rate of the females from each line, to check for potentially correlated responses to selection. A significant increase in drought resistance of eggs occurred in the experimental evolution trial. Already one month after the start of the trial, egg survival at low humidity had more than doubled. However, this increase in egg drought resistance disappeared

within 15 days after I had removed the selection pressure from a group of adult females. In the artificial selection trial, no response to selection was observed after three selection rounds, and the selection process had no impact on oviposition rate. The results of this study indicate that drought resistance in *P. persimilis* eggs is a phenotypically plastic trait, regulated by their mother. This discovery raised new questions and made me change my focus from *P. persimilis* eggs to *P. persimilis* females. In CHAPTER 4, I studied the role of phenotypic plasticity in the adaptation of *P. persimilis* eggs to different relative humidity conditions. For this, I exposed *P. persimilis* adult females from two different strains to constant and variable humidity regimes, and evaluated the hatching rate of their eggs in dry conditions, as well as the survival and oviposition rates of these females. Whereas the eggs laid by *P. persimilis* females exposed to constant high humidity did not survive in dry conditions, females exposed to constant low humidity started laying drought-resistant eggs after 24 hours of exposure. Around 43% of the females exposed to variable humidity conditions laid drought-resistant eggs after 102 hours of exposure. Survival and oviposition rates of the females were affected by humidity: females laid fewer eggs under constant low humidity, and had a shorter lifespan under constant high and constant low humidity. The humidity regimes tested had similar effects across the two *P. persimilis* strains. These results demonstrate that transgenerational phenotypic plasticity, called maternal effect, allows *P. persimilis* females to prepare their offspring for dry conditions, by laying drought-resistant eggs. In constant dry conditions, *P. persimilis* females lay fewer eggs, due to desiccation stress and the production of drought-resistant eggs. In CHAPTER 5, I investigated the mechanisms underlying drought resistance of *P. persimilis* eggs, by studying the physiological differences between drought-resistant and drought-sensitive eggs. I compared the volume and the SA:V of the eggs, their sex ratio, their chemical composition (by gas chromatography-mass spectrometry), their internal and external structure (by scanning electron microscope and transmission electron microscope images), and their developmental time. The results showed that drought-resistant and drought-sensitive eggs have a different chemical composition: drought-resistant eggs contain more free amino acids, sugar alcohols, and saturated hydrocarbons than drought-sensitive eggs. This difference may contribute to reducing water loss in drought-resistant eggs. Moreover, drought-resistant eggs are on average 8.4% larger in volume, and have a 2.4% smaller SA:V than drought-sensitive eggs. This larger volume and smaller SA:V, probably the result of a higher water content, may make drought-resistant eggs less vulnerable to water loss. I did not find difference in sex ratio, internal or external structure nor developmental time between drought-resistant and drought-sensitive eggs. I also made observations that led me to the following hypothesis: drought-sensitive eggs do not survive at low humidity as a result of mechanical aspects. It seems that the embryo inside a drought-sensitive egg completes its development even under constant low humidity conditions, but the larva cannot break the chorion and dies inside the egg. These results mark the first step in the understanding of the strategies and the energetic costs involved in the production of drought-resistant eggs in *P. persimilis* females.

In conclusion, this thesis provides new perspectives to the understanding of the effects of drought on *P. persimilis*. I demonstrated that *P. persimilis* eggs can deal with harsh humidity conditions better than we previously thought. First, they are able to recover from a long exposure to drought if they are exposed to high humidity for only a few hours. Second, through a maternal effect, *P. persimilis* females are able to protect their eggs against

desiccation stress and lay drought-resistant eggs. As a consequence, the lower efficacy of *P. persimilis* in dry conditions may rather be due to a lower oviposition rate and a shorter lifespan of *P. persimilis* females than to the drought sensitivity of their eggs. Finally, this thesis is also a first step in understanding the internal egg structure in a phytoseiid mite species.

ACKNOWLEDGMENTS

Today, 20 March 2020, I crossed the finish line of a marathon, and gave birth to a baby I have dreamed of since July 15, 2015: my PhD thesis.

From my first to my last stride, I have been guided by three brilliant supervisors: Marcel Dicke, Tom Groot, and Markus Knapp. Three different personalities, who formed a remarkable team devoted to help me cross the finish line. I would like to thank all three of you for your kindness, and for finding the perfect balance between supporting me and giving me enough freedom to carry out this research project.

Marcel, you guided me gently but surely towards the completion of this thesis. Although I was not physically located in Wageningen, I always felt closely supported by your encouragements, your calm and your positivity. Tom, you gave me continuous attention, and always took care to make me feel good at Koppert. Thank you for your enthusiasm, your flexibility, and for trusting me. Markus, thank you for being a caring, comprehensive, and cheerful supervisor during these four years. I really appreciated your presence in the stressful moments: you helped me to calm down (even though you probably did not notice).

Hartelijk dank aan jullie alle drie!

In addition to my three supervisors, I would like to thank Tibor Bukovinszky for his essential contribution to this thesis. Passionate, curious, full of ideas, and always enjoying his crazy experiments! Tibor, you were a great colleague, and a precious reviewer for my manuscripts.

I started this PhD thesis in July 2015, with a group of 14 fellow PhDs: the BINGO team. Angeliki, Milena, Wouter, Kim, Shuwen, Manolis, Erica, Sophie, Sander, Kelley, Judith, Simone, Alejandra, Panagiota: I enjoyed every moment in your company, and will always remember our trips to Germany, Spain, Switzerland, the Netherlands, Austria, Japan, Italy, and Turkey (Angeliki!). I take this opportunity to greet the rest of the BINGO team, especially Bart and Margreet.

Along these four years, I had the pleasure to supervise four Master students: Francesco, Thomas, Jovano and Giuditta. The good work provided by each of them contributed to the completion of this thesis. Francesco, thank you for your efficient work and your funny stories! Thomas, it was a pleasure to collaborate with a kind, open-minded and serene person like you. Jovano, you were a great and reliable team partner. I was impressed by your flawless organization. Giuditta, apart from the fact that I loved all your earrings, you were the perfect combination between working seriously and having fun.

During my thesis, I learned how to do statistics with R. Three good statisticians have helped me, directly or indirectly, to carry out my statistical analyses: Eric Wajnberg, Bas Engel, and Ben Bolker. Eric and Bas, thank you for taking the time to reply to my emails full of questions. Ben, we never met and you do not know me, but you helped me through your

excellent explanations on the statistics forums I consulted.

It took a lot of time and effort to obtain the electron microscope images and the chemical composition of *Phytoseiulus persimilis* eggs (Chapter 5). Fortunately, for these experiments, I was guided by many excellent scientists. From Leiden University: Maurijn van der Zee, Gerda Lamers, Luis Francisco Salomé-Abarca and Young Choi. From the Biology Center in Ceske Budejovice (Czech Republic): Marie Vancová, Jirka, Martina, and Frantisek Marec. Thank you all for your patience and for offering your expertise to this research project.

Although I was a PhD student from Wageningen University, I carried out this thesis at Koppert Biological Systems, in Berkel en Rodenrijs. I would like to thank here all my Koppert friends and colleagues, who made me feel at home in the Netherlands.

Marloes, you are the one who taught me everything about the rearing methods of *P. persimilis* and the PCR when I arrived at Koppert. Throughout my thesis, you have always been a person of good advice, ready to help with a smile. I chose you as a paranymph today, as my friend, but also because I consider that you largely contributed to the success of this thesis. Thank you for helping me during my experiments, for taking care of my rearing when I was on holidays, for being funny, for your little gifts, cards, and attentions (especially the tipi for my guinea pigs and the guinea pigs cups!), and for coming to Delft with Otis to eat an éclair au chocolat!

Kirsten, I wish you were here today. I know you would have brought a huge bouquet of flowers to congratulate me. I had the privilege to meet you on my first day at Koppert, on 15 July 2015. I remember your smile full of kindness. With you I walked from Holwerd to Ameland, I cycled in the dunes of Meijendel, I surfed in Scheveningen, I visited your home in Diepenheim, I cooked and made cocktails in your kitchen while drinking a cup of Christmas tea, I tasted new beers in nice breweries… Thank you for being so caring and making my life in the Netherlands so special. Since 21 June 2018, I keep precious memories of you, beautiful Kirsten…

Sem, I am glad I met someone as special and sensitive as you. You are a discrete person, but one cannot miss your presence. I really like talking with you, about deep things, but also about cats and guinea pigs (by the way, I love how you draw guinea pigs!). Musician, philosopher, and above all an attentive observer, thank you for being a dear friend!

Robbie, you bring kindness and happiness every day to the lab, and this is so important! Always in for a chat, a coffee break, watching cat videos or playing Mario Kart. Thank you for being a caring friend, but also our fitness coach during lunch breaks, a wizard (beginner) sometimes, a literature consultant, and most importantly a cookie supplier (good shit Robbie!).

Anna-Petra, Jolein, José, Anton, Marika, Nakita, Ruben, Caroline, Léonie, Elmer, Firouz, Radbout, Nils, Jan, Suzanne, Yvonne, Martine, Hans, Mikkel, Tom, Joël, Katy, André, and Martijn: thank you all for making me feel comfortable and welcome at Koppert.

I also made exotic friends in the R&D Microbiology, the Business Intelligence and the Marketing teams at Koppert: Pablo (and Garry 23!), Iñaki, Marcela, Felipe, Jeroen, Ritesh, Zhivko, Clara, Antonin, David, Nacho, Ruth (you are almost from Koppert!), Andrea. Thank you all for the joyful moments at Koppert and the crazy parties in Rotterdam, Delft, or

Utrecht. You really spiced up my life here!

I also owe a big thank you to all those who did not directly contribute to this thesis, but rather to my happiness in my life outside the PhD.

To my friends in Delft:

Adithya and Lucia, a couple of adventurous guinea pigs. A mixture of India and Italy, what could be more delicious? I learned to enjoy spicy food with Adi, and I experienced heaven with Lucia's tiramisu and the canestrelli. With them, I went to India, dressed in sari and walked barefoot in Hindu temples, ate on banana leaves, drank infinite amounts of chai latte and lassi, visited Chennai and Pondicherry, and freaked out when our chauffeur reversed in the middle of a highway. Thank you for all these magic moments and for your precious friendship.

Mathilde and Antonio, thank you for being faithful friends, always ready for new adventures. I am looking forward to our next salsa parties, hiking trips, and surprise birthday parties in Bruxelles!

Sotiria and Jesus, thank you for the dinners at the real greek (souvlaki kotopoulo!), the movies at the cinema, the beers at Doerak, the music recording session, the Easter ceremony in a Greek church in Rotterdam, the pulpo a la gallega, the barbecues... You made Delft a cozy place!

Monica, thank you for pushing us to do so many crazy things! With a special dedication to "money penny" and "Monica, vete a la mierda!".

Magela, you made me taste the best lasagnas in the world! Thank you for sharing Boterbrug with us and for being such a nice flatmate.

Dani, thank you for the philosophical and scientific discussions accompanied by your famous salmonete and chinese noodles (and the white rabbit candies!). And also for introducing us to the magic African and Asian nights of IHE!

Jessica and Angelos, I am happy I met you in Delft 6 months ago, and hope to share more good times with you!

Thank you to Michel, Peter, Afshin and Maedeh, with whom I learned to speak Dutch in a cozy atmosphere every Monday.

Finally, I want to thank my four guinea pigs (the real ones): Alan Papaya, Steeve Gomez, Rodney Doolittle and Monico Garcia. These little fluffy balls give (and gave) me affection every day.

To my friends in France:

Anne-So, mon choux, merci d'être une amie si fidèle! Bientôt 16 ans que l'on se connait, et je me rappelle encore ce premier jour de seconde oú tu étais assise devant moi en cours. Quelle chance d'avoir croisé ton chemin! J'ai beaucoup de souvenirs mémorables en tête, parmi eux cette soirée surréaliste à Toulouse, oú nous avons tenté d'entrer dans une cumette... Merci pour toutes ces années d'amitié mon Anne-Sofiotte!

Maud, tout a commencé pendant notre Erasmus à Valencia en 2012, oú l'on s'est vues tous les jours sans exception pendant 6 mois! Comme tu l'as si bien dit: quand nos chemins

se sont séparés à notre retour en France, j'ai eu l'impression que l'on m'arrachait une partie de moi. Tu as définitivement laissé une marque dans ma vie! Merci pour tous les souvenirs heureux que j'ai avec toi, et pour tous ceux qui viendront!

Eléonore, Sibylle, Lucie, mes fantastiques colocs angevines! Que d'aventures ensemble! Je suis fière de voir comment nous avons chacune suivi notre petit bout de chemin et évolué, tout en restant toujours aussi proches au fil des années et malgré la distance. Merci pour ces belles années d'amitié et pour toutes celles à venir!

Noémie, Caroline, Michel, mes petits! J'adore nos week-ends à Paris, aux Pays-Bas ou en Savoie! Continuons à organiser toutes ces retrouvailles précieuses, à chaque fois c'est trop bien!!

Laure, merci d'être venue depuis la Suisse pour assister à ma soutenance! J'ai regardé nos vieilles photos de prépa d'il y a 10 ans en écrivant ces lignes, et ça m'a fait plaisir de voir quel bout de chemin on a parcouru!

To my family:

J'ai la chance de faire partie d'une famille géniale. Maman, Papa, merci d'avoir fondé une si jolie famille et de m'avoir toujours guidée avec beaucoup de sagesse. Vous m'avez appris l'empathie, l'humilité, la curiosité, et l'esprit d'aventure... C'est grâce à vous si je me sens à ma place aujourd'hui!

Erwann, Anne-Lise, Delphine, Corentin: quelle chance d'être entourée de 4 frères et sœurs aussi géniaux! Winib, merci d'avoir toujours été un grand frère attentionné (sauf quand tu me volais mes cacahuètes dans ma chambre!). Anne-Loutre, la force tranquille. Merci pour ta sérénité inspiratrice. Je repense à ton premier téléphone portable et les petits mots que j'écrivais à papa et maman pour me plaindre... Delcroute, merci d'être devenue mon amie malgré nos innombrables conflits sororaux passés (spéciale dédicace au combat pour l'aspirateur dans les escaliers à Hillion). Coquito, merci d'être un modèle de gentillesse et d'être sur la même longueur d'onde que moi lorsqu'il s'agit de donner un nom à tous les conducteurs que l'on croise ou de faire des battle de hip-hop. Eliott, merci d'être si choux! Et pour finir, une pensée pour mon chien Snoopy, avec qui j'ai grandi jusqu'à mes 14 ans.

Je remercie aussi Mamie, Papi, Minette et Gros Loup, qui sont (ou seraient), je l'espère, fiers de moi aujourd'hui.

To my family-in-law:

Llanos, Manuel y Pablo: Gracias por haberme acogido en vuestra familia, por siempre tratarme tan bien, y por todos los buenos momentos que he pasado con vosotros en España y en Países Bajos!

To my other half:

Antonio, mi minion! Tantas cosas que decir sobre ti! Como ya lo sabes, sin ti sería como una naranja sin su piel, como un M&M's sin su cacahuete. No sé cómo hacemos para llevarnos tan bien, seguramente estamos hechos para estar juntos. Gracias por ser tan buena persona, tan emocionalmente inteligente. Siempre me empujas a ir más lejos (no solo con LaTeX!), y gracias a ti hoy he cruzado la línea final. Mi fiel compañero, todavía tenemos un largo y feliz camino por recorrer. Ven conmigo para el próximo paso minion!



PUBLICATIONS

Peer-reviewed journal publications

Le Hesran, S., Groot, T., Knapp, M., Bukovinszky, T., Nugroho, J.E., Beretta, G., Dicke, M. (2020). Maternal effect determines drought resistance of eggs in the predatory mite *Phytoseiulus persimilis*. *Oecologia*, **192**:29-41, doi:10.1007/s00442-019-04556-0.

Le Hesran, S., Groot, T., Knapp, M., Nugroho, J.E., Beretta, G., Salomé-Abarca, L.F., Choi, Y.H., Vancová, M., Moreno-Rodenas, A.M., Dicke, M. (2019). Proximate mechanisms of drought resistance in *Phytoseiulus persimilis* eggs. *Experimental and Applied Acarology*, **79**:279-298, doi:10.1007/s10493-019-00442-9.

Le Hesran, S., Groot, T., Knapp, M., Bukovinszky, T., Forestier, T., Dicke, M. (2019). Phenotypic variation in egg survival in the predatory mite *Phytoseiulus persimilis* under dry conditions. *Biological Control*, **130**:88-94, doi:10.1016/j.biocontrol.2018.10.007.

Le Hesran, S., Ras, E., Wajnberg, E., Beukeboom, L.W. (2019). Next generation biological control – an introduction. *Entomologia Experimentalis et Applicata*, **167**:579-583, doi:10.1111/eea.12805.

Arganda, S., Bouchebti, S., Bazazi, S., **Le Hesran, S.**, Puga, C., Latil, G., Simpson, S.J., Dussutour, A. (2017). Parsing the life-shortening effects of dietary protein: effects of individual amino acids. *Proceedings of the Royal Society B: Biological Sciences*, **284**, 20162052. doi: 10.1098/rspb.2016.2052.

Submitted manuscripts

Leung, K., Ras, E., Ferguson, K., Ariens, S., Babendreier, D. B., Bijma, P., Bourtzis, K., Brodeur, J., Bruins, M., Centurión, A., Chattington, S., Chinchilla-Ramírez, M., Dicke, M., Fatouros, N., González Cabrera, J., Groot, T., Haye, T., Knapp, M., Koskinioti, P., **Le Hesran, S.**, Lirakis, M., Paspati, A., Pérez-Hedo, M., Plouvier, W., Schlötterer, C., Stahl, J., Thiel, A., Urbaneja, A., van de Zande, L., Verhulst, E., Vet, L., Visser, S., Werren, J., Xia, S., Zwaan, B., Magalhães, S., Beukeboom, L., Pannebakker, B. (2019). Next Generation Biological Control: The Need for Integrating Genetics and Evolution. Preprints 2019, 2019110300. doi: 10.20944/preprints201911.0300.v1.

About the Author



Sophie Le Hesran was born on 22 June 1990, in Saint-Brieuc (France). At one month old, she flew to South America, and spent one year in French Guyana. She then grew up in Africa, in Cameroon during 4 years and in Senegal during 6 years. At the age of 13 years, she came back to France with her family, to live on the north coast of Brittany, facing the sea.

After finishing high school, she started her studies in Rennes (France). There, she spent three years in a preparatory course (classe préparatoire BCPST), to join an engineering school in agronomy. In 2010, she was admitted to Agrocampus Ouest (Angers), an engineering school specialized in horticulture and landscape. During her first year of the Master programme, in 2012, she went to Valencia (Spain) for a six-months Erasmus stay at the Universidad Politécnica de Valencia. In 2013, she chose a specialization in crop protection for her second year of the Master programme, and followed courses in Montpellier (Montpellier SupAgro), Rennes (Agrocampus Ouest) and Paris (AgroParisTech). In 2014, for her Master thesis, she spent six months in Sevilla (Spain) with the CSIC (Higher Council for Scientific Research). There, she studied the impact of an invasive ant species (*Linepithema humile*) on cork oak trees in the Doñana National Park. After graduating with a Master's degree in crop protection, she started her PhD thesis on the predatory mite *Phytoseiulus persimilis* in July 2015, at Wageningen University and Koppert Biological Systems (the Netherlands). Her PhD project was part of the International Training Network called BINGO (Breeding Invertebrates for Next Generation BioControl), financed by the European Union (Marie Skłodowska-Curie grant). On the 20th of March 2020, Sophie defended her PhD thesis at Wageningen University.

This book contains the fruit of four years and five months of passion, perseverance and patience.

PE&RC Training and Education Statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Next generation biological control – an introduction

Writing of project proposal (4.5 ECTS)

- Expanding the range of uses of a predatory mite in biological pest control

Post-graduate courses (11.4 ECTS)

- Summer school; BINGO, Germany (2015)
- Summer school; BINGO, the Netherlands (2016)
- Survival analysis; PE&RC / SENSE, the Netherlands (2016)
- Basic statistics; PE&RC, the Netherlands (2016)
- Summer school; BINGO, Austria (2017)
- Mixed linear models; PE&RC, the Netherlands (2018)

Laboratory training and working visits (25.7 ECTS)

- Effects of dry conditions on *P. persimilis* populations in a greenhouse; IVIA, Valencia, Spain (2017)
- SEM and TEM images of *P. persimilis* eggs; Biology Center, České Budějovice, Czech Republic (2019)

Competence strengthening / skills courses (3.1 ECTS)

- Scientific publishing; WGS (2015)
- Communication with the media and the general public; WGS (2016)
- Scientific writing; Wageningen in'to Languages (2017)

PE&RC Annual meetings, seminars and the PE&RC weekend (3.3 ECTS)

- PE&RC First years weekend (2015)
- PE&RC Day (2015, 2016, 2017)
- 3rd Wageningen PhD symposium (2016)
- PhD Workshop carousel (2016)
- PE&RC Last years weekend (2018)

Discussion groups / local seminars / other scientific meetings (7.2 ECTS)

- Entomologendag; the Netherlands (2015, 2016, 2017, 2018)
- BINGO Workshop; Spain (2016)
- BINGO Workshop; Switzerland (2017)
- BINGO Workshop; the Netherlands (2018)

International symposia, workshops and conferences (8.8 ECTS)

- 5th International Entomophagous Insects Conference; oral presentation; Kyoto, Japan (2017)
- XI European Congress of Entomology; oral presentation; Napoli, Italy (2018)

- XV International Congress of Acarology; oral presentation; Antalya, Turkey (2018)
- IOBC Working group "Integrated control of plant-feeding mites"; oral presentation; Vienna, Austria (2019)

Societally relevant exposure (2.5 ECTS)

- FameLab science communication competition (Dutch final) (2017)
- Production of a popular science video published on YouTube: Biological control in agriculture – the invisible world of mites (2019)

Lecturing / supervision of practicals / tutorials (0.6 ECTS)

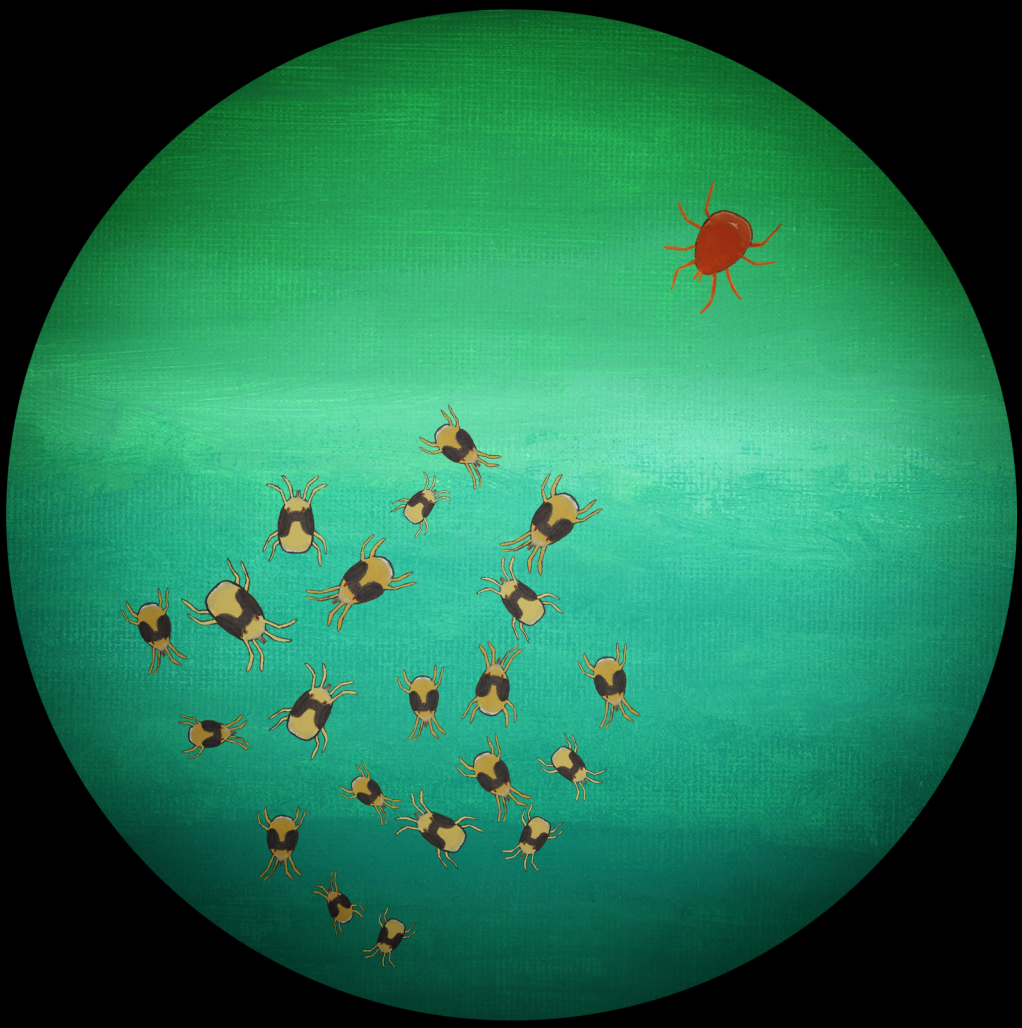
- Insects and mites in biological control

Supervision of MSc students (9 ECTS)

- Selective breeding for drought tolerance in *P. persimilis*
- Effects of variable relative humidity regimes on egg survival in *P. persimilis*
- Effects of low relative humidity on *P. persimilis* gravid females and their eggs
- Effects of low relative humidity on the predatory mite *P. persimilis*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 641456.

Colophon
Cover design and chapter figures: Sophie Le Hesran
Printed by: GVO drukkers & vormgevers B.V, Ede, the Netherlands



Propositions

1. Biological control of *Tetranychus urticae* in dry conditions is a challenge, less due to *Phytoseiulus persimilis* struggling but rather due to *T. urticae* thriving.
(this thesis)

2. The egg is the developmental stage best protected against dry conditions in *Phytoseiulus persimilis*.
(this thesis)

3. Rather than 'flying rats', pigeons in cities are precious to researchers as indicators of urban pollution.

4. Although many scientists prefer to have few co-authors on their publications, having many co-authors indicates that several fields of expertise have joined forces to produce a higher quality work.

5. Mites for augmentative biological pest control are like cleaning employees for hotels: essential but nearly invisible.

6. In scientific conferences, the quality of research tends to be assessed by the proficiency of English, putting non-native English speakers at a disadvantage.

7. The two most valuable assets of scientists working for private companies are their patience and their ability to consider the long term.

8. The lack of resources allocated to public services for child protection is a major cause of violence and unhappiness in society.

Propositions belonging to the thesis entitled:

Diving deep into a tiny world - Effects of drought on the predatory mite *Phytoseiulus persimilis*.

Sophie Le Hesran
Wageningen, 20 March 2020