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Phenotypic variation in egg survival in the predatory mite *Phytoseiulus persimilis* under dry conditions

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ABSTRACT

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 study, 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* strains, under constant as well as variable humidity conditions, at 25 °C. The results show no intraspecific genetic variation among the five tested strains in egg hatching under constant and variable humidity conditions. In all five strains, 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.

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* Athias-Henriot (Acari: Phytoseiidae) and its prey *Tetranychus urticae* Koch (Acari: Tetranychidae) 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 60–90% (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

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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; Doker 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 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 strains, 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 the Californian strain and 26% of the eggs from the Israeli strain 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 strains of *P. persimilis* under different constant humidity conditions. We tested whether genotypic variation in drought tolerance was present by comparing strains established from populations that originated from different environments. Finding variation among strains 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 strains. 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, Tixier et al., 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 h 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* strains 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 strain would indicate the presence of an environmental effect in phenotypic variation. We compared the three *P. persimilis* strains under variable humidity conditions to investigate whether populations differ in phenotypic variation. Finding variation among strains 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 strains 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. Materials and methods

2.1. Origin of the strains and rearing method

Five strains of *P. persimilis* were tested for egg survival, four of which were field-collected strains from four different locations (France, Israel, Sicily and Turkey). These four field-collected strains were generously provided to us by Arnon Tabic (strain from Israel, started with ca 30 individuals), Marie-Stéphane Tixier (strain from France, started with ca 30 individuals), and Alexandra Revynthi (strains from Turkey and Sicily; for details see Revynthi, 2017). Although these four strains 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 strains. The fifth strain was obtained from a commercial mass-rearing (Koppert Biological Systems) (Table 1). The strains were reared separately in Petri dishes (7.5 cm diameter, 3 cm high) containing an agar layer (agar powder, VWR Chemicals, 1/100 diluted) on which a disk (7 cm diameter) 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

Table 1
Origin of the *P. persimilis* strains 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*

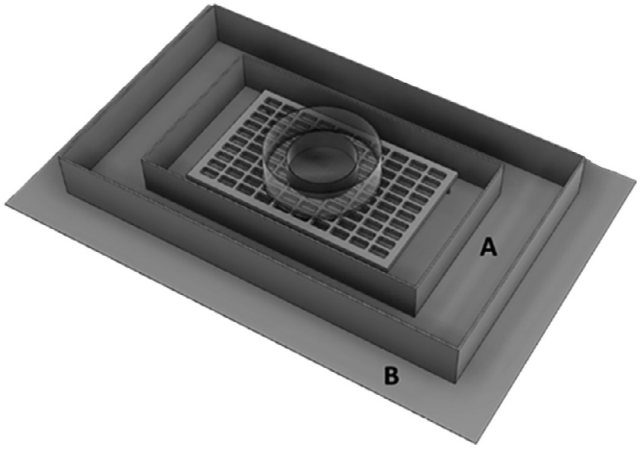

Fig. 1. Rearing system used to avoid contamination between strains. A: tray filled with water and soap. B: sticky trap.

closed with a lid, containing a 5 cm diameter hole covered by a 90 µm meshed 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 strains was prevented by several barriers: each Petri dish was placed in a closed transparent circular plastic box (10 cm diameter, 4 cm high) with a 90 µm meshed 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. 1). Both experiments were carried out between April 2016 and January 2018.

2.2. Egg hatching tests

2.2.1. Variation in egg hatching among strains under constant humidity conditions

We carried out a pilot test on the egg hatching of the Koppert strain 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 strain, around 40 young females (6–10 days old) were isolated to lay eggs during 6 h in a Petri dish with a spider mite infested cucumber leaf disk on agar, at 25 °C and 65 ± 2% RH. After 6 h, 15–30 eggs were collected from each strain. The eggs were placed with a brush in a hole (0.7 cm in diameter, 0.4 cm deep) in a Plexiglas platform (15 cm wide and 17.5 cm long) containing 30 holes (one egg per hole). Each hole had a 90 µm meshed 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 egg hatching. Five platforms (one platform per strain) 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 strain) 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 strain, we performed between four and eight 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. Variation in egg hatching among strains under variable humidity conditions

We tested the egg hatching rate of three strains (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 seven different treatments (Fig. 2). For each strain, 15–30 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). 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 four and seven replicates per treatment and per strain were carried out. In total, 2474 eggs were tested in this experiment.

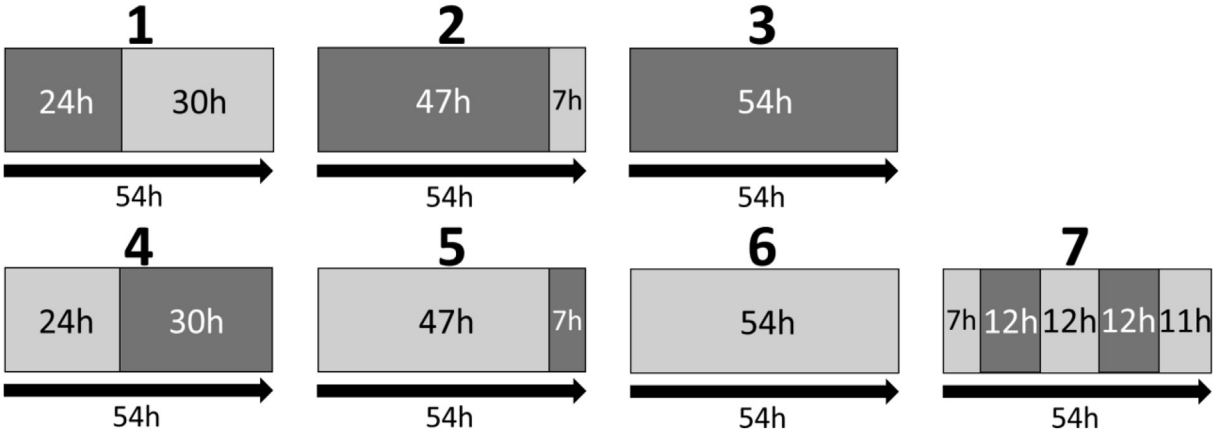

Fig. 2. Seven treatments to which the eggs were exposed during their developmental time. Dark grey: 75% RH. Light grey: 60% RH. The numbers on top of each treatment represent treatment ID.

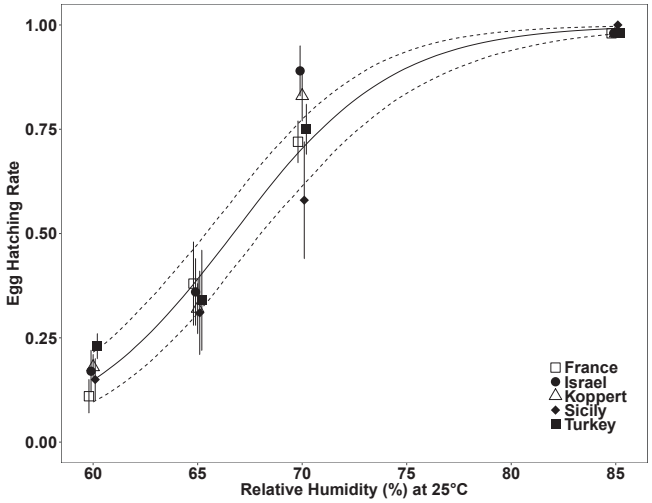

Fig. 3. Observed (symbols) and fitted (solid line) egg hatch probabilities of five *P. persimilis* strains at different humidity levels at 25 °C. The dashed lines represent the 95% confidence intervals, and the error bars represent ± 1SD.

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 study. VPD was calculated with the following equation:

$$VPD = SVP \cdot \left(1 - \frac{RH}{100}\right)$$

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.

2.4. Sequencing of Cytb and 12S genes

To investigate intraspecific genotypic variation among our strains, we sequenced the mitochondrial *Cytochrome b* (Cytb) gene and the ribosomal RNA *12S* gene. DNA was extracted from single adult females of *P. persimilis* with the DNeasy Blood and Tissue Kits method (Qiagen). Two to six mites per strain 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′TACTATGT TACGACTTAT3′ (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.

2.5. 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 strain (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 strain 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 strain (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 strain 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 seven 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.

3. Results

3.1. Variation in egg hatching among strains under constant humidity conditions

Relative humidity had a statistically significant effect on egg hatching rate ($\chi^2 = 29.5$; DF = 1; P = $6*10^{-8}$, Fig. 3). For all strains, 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 strain on the egg hatching rate ($\chi^2 = 0.98$; DF = 4; P = 0.91) nor a significant interaction between strain 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):

$$\ln\left(\frac{p}{1-p}\right) = -17.51 + 0.26 \times rh + \varepsilon$$

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.

3.2. Variation in egg hatching among strains under variable humidity conditions

Treatment significantly affected egg hatching rate ($\chi^2 = 48.9$; DF = 6; P = $8*10^{-9}$, Fig. 4). Strain did not affect egg hatching rate ($\chi^2 = 0.23$; DF = 2; P = 0.89), and there was no significant interaction

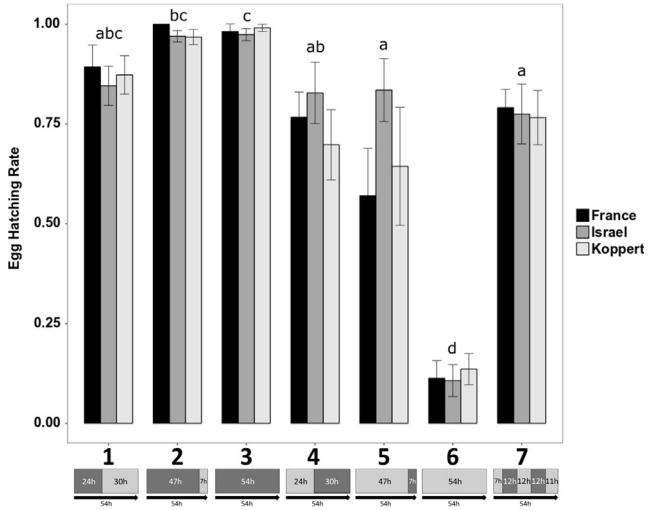

Fig. 4. Observed egg hatching probabilities for three P. persimilis strains under seven variable relative humidity treatments, at 25 °C. The error bars represent ± 1SD. Different letters above triplets of bars indicate significant differences between treatments (multiple pairwise comparisons, $P < 0.05$).

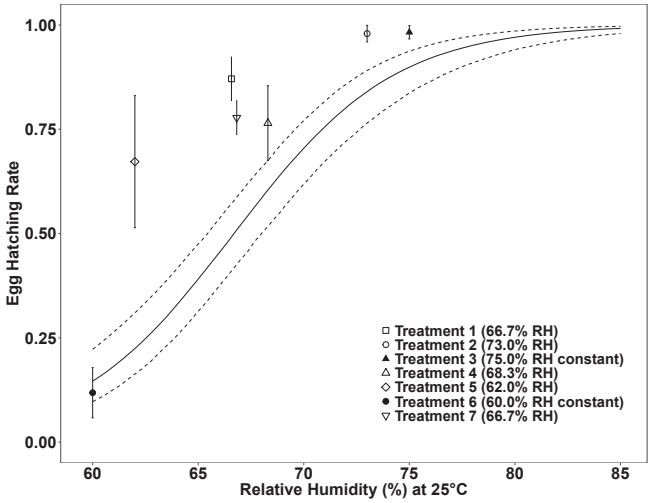

Fig. 5. Fitted (solid line) and observed (symbols) egg hatching probabilities under constant (solid line and full symbols) and variable (empty symbols) relative humidity conditions, at 25 °C. For explanation of treatments 1–7 see Fig. 2. The error bars represent ± 1SD, and the dashed lines represent the 95% confidence intervals.

between strain 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.

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 seven 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. 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.

3.4. Sequencing of Cytb and 12S genes

No differences among strains 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.

4. Discussion

The objective of this study was to evaluate the phenotypic variation in egg survival under low humidity conditions among five strains of *P. persimilis*, and to understand the potential sources of variation in this trait.

We did not find variation among strains for egg survival under constant and variable humidity conditions. The eggs of all tested strains 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 strain 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 strain, and much higher than for their Californian strain. In view of our results, there is no indication of differentiation between the five *P. persimilis* strains studied here. This observation is further supported by the fact that we did not find inter-strain 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 strains we studied each originated from a low number of founding mites, leading to a limited sampling of genetic diversity within strains. Furthermore, the strains 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 strains 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 strains which had spent several years in laboratory conditions.

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 strain, 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* strains 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 plants 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 results 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 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 strains 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 unfavorable for the eggs (Sanderson and McMurtry, 1984). In the American dog tick, *Dermacentor variabilis* Say (Acari: Ixodidae), the 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.

Insects and mites have two main strategies to deal with desiccation: tolerance and resistance. Desiccation tolerance is the ability to tolerate the loss of a significant proportion of body water, without negative consequences for survival or reproduction. Desiccation resistance is a mechanism to avoid dehydration, and may involve behavioural, physiological, biochemical, or morphological adjustments such as a change in respiration or evaporation through the integument, to reduce water loss (Edney, 1977; Hadley, 1994; Gibbs et al., 1997, Gibbs et al., 2003). Investigating which mechanism is used in the case of *P. persimilis* eggs to survive drought periods will be an interesting topic for further study.

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 strains 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.

Author contribution

SLH, TG, MK and MD conceived the experiments, SLH and TF collected the data, SLH, TB, TG, MK and MD analyzed the data, SLH prepared the manuscript with input from TB, TG, MK and MD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2018.10.007.

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