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Short-time effects of water sprinkling on the two-spotted spider mite predator *Phytoseiulus persimilis* in strawberry

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Preface

This master thesis is written as an enclosure of my Master`s degree in Biology at the Norwegian University of Life Science together with the Norwegian Institute of Bioeconomy Research. The last year has been challenging but most of all educating and a valuable experience. The time on NMBU is unforgettable, filled with good memories.

Primary, I would like to thank supervisors Dr. Nina Trandem of the Faculty of Environmental Sciences and Natural Recourse Management and Dr. Belachew Asalf Tadesse of Norwegian Institute of Bioeconomy Research for giving good guidance and always being available for discussions and regular meetings. I am grateful for the opportunity to be a part of the project SMARTCROP with project manager Ingeborg Klingen.

I am truly grateful for all the people helping with knowledge, equipment and mite supplies. Thank you Venche Talgø for lending me the plastic tunnel, Andrew Dobson for installing the water sprinklers, Chloé Grieu and Rodrigo Onofre helping with the strawberry plants, Karin Westrum for mite supplies, Torfinn Torp for statistical help, Anne Sverdrup-Thygeson and Tone Birkemoe for master meetings and fellow student Camilla G. Auberg for good cooperation in the same field. I am also grateful for support from my parents, brothers, cohabitant and friends. This would not have been the same without you.

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Abstract

Combining two tools for an integrated pest management (IPM) was the reason for investigating the potential use of predatory mites (*Phytoseiulus persimilis*) in addition to water sprinklers in order to reduce spider mite (*Tetranychus urticae*) populations in tunnel grown strawberries. To determine the short-time effects of irrigation on the predatory mite, 26 strawberry plants were infested with spider mites as a prey, and 2 weeks later predatory mites were introduced. Leaves were sampled before, immediately after, and twenty-four hours after, irrigation of one minute from overhead water sprinklers in a tunnel. The number of predatory mites was counted on the abaxial of the leaves sampled. On a control plant, not receiving irrigation, the number of mites was counted at the same times as in the experiment. This experiment was repeated four times. To compare the water tolerance of spider mites and predatory mites, leaves with both spider mites and predatory mites were hand sprayed in a laboratory experiment. Number of mites and eggs was counted before and after spraying. The water sprinkling in tunnel significantly increased predatory mite populations up to nearly 2-fold, compared to before irrigation. Predatory mite eggs increased statistically different from before irrigation to one minute after irrigation, while total mobile stages (larva, nymphs and adults) larvae and nymphs increased significantly after twenty-four hours. Control plants had no significant increase, except from larvae. In the laboratory experiment, predatory mites (average of 11 % reduction of mobile stages and eggs) tolerated water spraying to a greater extent than spider mites (average of 22 % reduction of mobile stages and eggs). Both mites had higher reductions when sprayed on the abaxial side, compared to sprayed on the adaxial side of leaves. The findings confirm that water sprinklers combined with biological control by predatory mites is a promising IPM strategy to suppress spider mites in tunnel grown strawberries.

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Introduction

Integrated pest management (IPM) is a pest control strategy making use of tools and techniques that minimize the risk of harm to the environment, ecosystem and humans, simultaneously maintaining the pest population below levels causing economic injury (Dent 1995). In IPM, several tools and methods are often combined to control pests, and the possibility of combining two IPM tools is the idea behind the current study. IPM is used on a global scale (Maredia et al. 2003), where several studies have shown successful use (Kenmore et al. 1995), especially when biological control is one of the tools (Waage 2012). Unfortunately, pesticides are still an essential part of pest control (Pimentel et al. 1992; Wilson & Tisdell 2001), therefore other methods are often required to be compatible with pesticides. Also, in some cases the pest has developed resistance to pesticides, so alternatives to pesticides are needed (Poe 1972; Wolfenbarger 1968). IPM has become a requirement in the EU-directive coming into force in 2014 in EU, and in June 2015 in Norway (Forskrift om plantevernmidler 2015). NIBIO, the Norwegian Institute of Bioeconomy, is working with IPM by exploring alternative methods and techniques (IPM tools) to minimize the use of chemical pesticides (Norsk institutt for bioøkonomi 2015). IPM tools need to be tested for a successful implementation of IPM, and the project SMARTCROP, run by NIBIO, addresses these challenges (Norsk institutt for bioøkonomi 2016b).

Strawberry (*Fragaria x ananassa*) is one of many cultivated plant species where IPM is appropriate to use, because of frequent damage by pests – resulting in yield losses (Sorensen 1997). Cultivation of strawberries has great economic value, and over 8.1 million tons of strawberries are produced each year worldwide (Statista 2017). The biggest producer is United States with a total farm gate value of 1.8 million dollar a year, measured in 2008 (Bolda et al. 2010). Even in northern temperate areas, like Norway, the income from strawberry production in 2017 was about 335 mill NOK (Budsjettnemnda for jordbruket 2017), and about 4.5K tons of strawberries are produced for commercial sale each year (Opplysningskontoret for frukt og grønt 2016). Commercial strawberries are grown in most parts of Norway, mostly in open fields or high plastic tunnels.

Cultivation of strawberries in plastic tunnel gives higher yield than cultivation in open fields(Kadir et al. 2006), therefore the trend in strawberry production is shifting from open field to tunnel production. Tunnels provide a number of advantages like protection from rain, warmer conditions, reducing fungal attacks and extending the growing season (Døving et al. 2011). Unfortunately, plastic tunnels create a dry and warm condition, which is favourable for pests like the two-spotted spider mite (*Tetranychus urticae*) (Figure 1) and dowdery mildew (*Podospharea aphains*). *T. urticae* can cause big production losses, by reducing photosynthetic rate in the leaves (Park & Lee 2002). To offer farmers a non-chemical alternative to manage two-spotted spider mites, the SMARTCROP project have been testing irrigation (water sprinklers) as an IPM tool to control the spider mites(Norsk institutt for bioøkonomi 2016c). Water sprinkling was initially deployed to control powdery mildew (*Sphaerotheca aphanis)* but the side-effects of water sprinkling on spider mites is reported a century ago (Trägårdh 1915). A recent study confirmed the positive effect of water on the management of spider mites in strawberry under tunnel condition (Asalf et al. 2016). “A minute of irrigation four times a day aimed at controlling powdery mildew, also reduced leaf damage of two-spotted spider mites with about 38 % in relation to the untreated control” (Norsk institutt for bioøkonomi 2016a). Hence, use of water sprinklers is a promising tool to control spider mites, but more data is needed and are being investigated right now in the same projects at NIBIO (Auberg 2018).

Figure 1: Two-spotted spider mites *T. urticae* on a strawberry leaf. Photo: Camilla G. Auberg.

The use of predatory mites (Phytoseiidae) is a widespread and effective method used to keep spider mites populations down (Oatman et al. 1977). In a Californian experiment, they tested nineteen predators, including predatory mites, to control population of spider mites. The results showed that predatory mites in high enough numbers could be a major factor to control the spider mites populations, and *Phytoseiulus persimilis* and *Galendromus occidentalis* were considered the most important species in suppression of spider mites (Oatman et al. 1985). Similar results are reported on the effect of predatory mites on spider mites in cucumbers (*Cucumis sativus*) (Stenseth 1979b) and strawberries in Norway (Stenseth 1979a), and strawberries in the UK (*P. persimilis)* (Easterbrook 1991). *P. persimilis* (Figure 2) is a specialist on predation on two-spotted spider mites. It can handle webbing from spider mites because of their long legs.

Figure 2: Predatory mite *P. persimilis (orange)* eating a two-spotted spider mite *T. urticae* (bright with black spots). Photo: Belachew Asalf Tadesse.

The success of the biological control is dependent on suitable conditions for the predatory mites. This includes access to prey (spider mites in this case) in addition to an environment offering suitable temperature and humidity and possibilities of dispersal. In a study from 1984, Stenseth concluded that a moderate temperature (20 °C) and high humidity (90 %) gives the best viability for the predatory mite *P. persimilis.* Earlier research has shown that relatively high humidity promotes egg hatching, and larval and nymphal survival in predatory mites (Ferrero et al. 2010). This gives reason to believe that irrigation may enhance biological control by *P. persimilis* in hot periods. The question is whether the advantages of a higher humidity is off-set by the potentially negative effects from exposure to free water. This potential negative effect can get the predatory mites washed away or actively avoid free water. If the mites get wash away, mechanical forces from the water droplets pushing mites of the plant or droplets killing the mite, can to be expected. Immersion in water could potentially be detrimental for predatory mites' survival after irrigation, because their legs could get stuck to their body by water droplets (Helle & Sabelis 1985), and when large quantities of webbing from spider mites, droplets more frequent remain on leaves. More droplets can cause bigger chances of immersion. A study on ornamental plants showed a significantly reduced population size of *P. persimilis*, with overhead irrigation (Opit et al. 2006).

To use a combination of predatory mites and water sprinklers to keep spider mite populations lower, we need to understand the effects of water sprinkling on predatory mites. In earlier studies, predatory mites have shown small or no avoidance of high humidity patches, unlike the spider mites that always avoid the very humid places (Mori & Chant 1966). If the same effect applies for direct water from the sprinklers, combining predatory mites and irrigation can be a good solution for the spider mite problem.

If the predatory mites get washed away, a reduction in their number immediately after sprinkling is to be expected. It seems no studies on short-term effect of water sprinklers has been done, so the effect is not known. The predatory mite development goes from egg to larva to nymph to adult mite (Figure 3) (Helle & Sabelis 1985). The smallest and the youngest life stages are often more vulnerable for external forces, because they are less mobile and have shorter and weaker legs to stick to the leaf. Eggs and larvae, unless disturbance of larva, are inactive stages (Helle & Sabelis 1985), in addition, larvae have only three pair of legs, and these two stages may therefore get more affected by the water sprinklers. It is of interest to look at a potential direct effect immediately after water sprinkling, but also a day after the sprinkling.

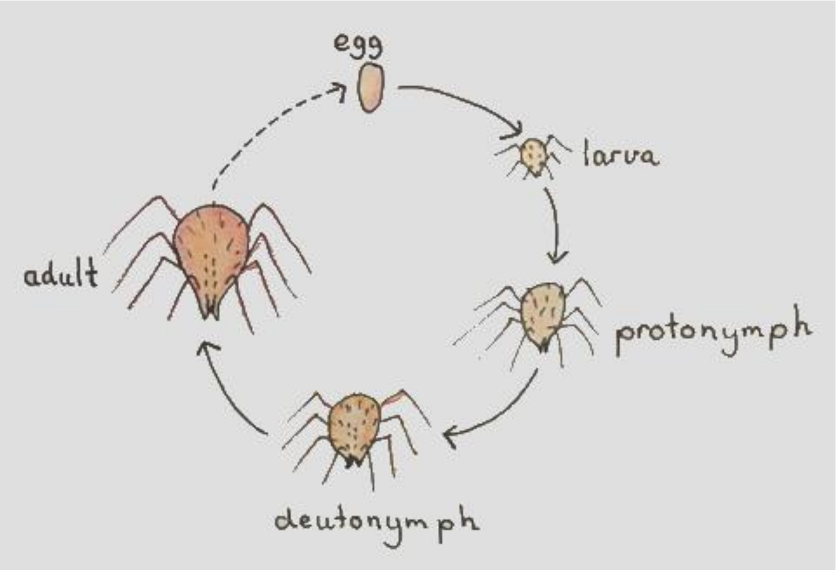


Figure 3: Life cycle of *P. persimilis*. Solid arrows show the molting process. Dashed arrow is when females oviposit. Drawing: Josefine Danielsen.

The overall aim of this thesis is to investigate the potential use of predatory mites in addition to water sprinklers in order to reduce spider mite populations in tunnel grown strawberries.

Research questions

I set out to examine the direct, short-term, effect of water sprinklers on populations of predatory mites (Phytoseiidae), more specifically *Phytoseiulus persimilis*. The effect was measured by observing the numbers of *P. persimilis* on strawberry plants before and after water sprinkling. All different life stages (egg, larva, nymph and adult) were examined. In addition, the tolerance to water spraying in *P. persimillis* and *T. urticae* was compared by counting the number of mites before and after handspraying water on strawberry leaflets in a controlled laboratory experiment. More specifically I tried to answer the following questions:

1.1) How does irrigation by water sprinkling for one minute affect the different stages of predatory mite on strawberry plants? Are some predatory mite stages less affected than others? Is there a difference in the effect of water sprinklers one minute after and twenty-four hours after irrigation?

1.2) Does the plant position relative to the water sprinklers affect the number of predatory mites?

2) Do predatory mites stay on the leaf to a larger degree than spider mites when exposed to direct spraying of water for one minute in the laboratory?

My overall prediction was that irrigation does not influence predatory mites negatively, measured as remaining population size (i. e., minimal reduction in population size per plant expected).

However, if there should be a negative effect I expected early life stages of predatory mites to be more affected than adults as they are more vulnerable to external forces. I also predicted that the plant position does not affect the number of predatory mites if all plants are equally sprinkled. Lastly, I predicted that predatory mites would be more tolerant to sprayed water than spider mites in the laboratory experiment.

Materials and methods

To investigate the effect of water sprinkling on predatory mites, I used two rows of potted strawberry plants (“environment-plants”) and water sprinklers installed in a plastic tunnel. Shortly before each sprinkling test, one random plant in both rows was replaced with a plant with spider mites and predatory mites (“experiment-plant”), and all plants sprinkled for one minute. To monitor the effect of water sprinklers on population size and different life stages of the predatory mite, one leaflet (Figure 7) was cut from each of two sides of the experiment-plants just before irrigation, right after irrigation and twenty-four hours after irrigation.

To compare the tolerance of water spraying in spider mites and predatory mites in more detail, leaflets were handsprayed in the laboratory, and mites was counted before and after spraying.

Study facilities

The plastic tunnel (Figure 4) was placed at Kirkejordet in Ås. It was 3 meter high, 4 meters wide and 15 meters long, and had new woven plastic. At each end of the tunnel a door was opened through the whole experiment. The side skirts were opened when the temperature was too high (>30 °C).

The water sprinklers (Figure 5 c)) were of the brand Bowsmith, model Fan-Jet overhead microsprinklers with nozzle size 50 (green) and spray pattern J2 (full circle). The water used was from Årungen, a lake in Ås.

Figure 4: **a)** The plastic tunnel with the door open from the west side **b)** and the plastic tunnel with the door closed from the east side. Photos: Josefine Danielsen.

Plant material

Strawberry plants of the Korona (*Fragaria x ananassa Tamella x Induka)* variety were used for the experiments. Plug plants were delivered by Sagaplant AS, Sauherad in Telemark, and the plants were free from pests ("elite plants" quality). The plants were first kept in a small quarantine room (K9 greenhouse at the Center for climate-regulated plant research on NMBU), to make sure that the plants were healthy and free from pests. Plants were watered every other day. Later the plants were repotted into 1.5 liter potts (13 cm tall), placed outside the plastic tunnel and watered once a day if needed, and fertilized twice a week. The plants were infested with spider mites and predatory mites when they were more than 15 cm tall. The strawberry plants were never watered on the leaves during the establishment of spider mites and predatory mites.

Mites

The two-spotted spider mite, *Tetranychus urticae*, was introduced manually by placing infested strawberry leaves on every experiment plants 16 days before first water sprinkler experiment. This was to ensure that the predatory mite studied would establish on the plants. Spider mites were taken from a culture that have been held in a culture room in NIBIO for three years (Auberg 2018). *T. urticae* suck cell contents out of the plant cells (Osborne, L. et al. 1985) and they can readily be separated visually from the predatory mites by looking for two black spots on their body (Figure 1).

The predatory mite, *Phytoseiulus persimilis*, was provided by LOG AS, a Norwegian gardener- and horticultural-industry company. *P. persimilis* was delivered in small tubes, from two different brands, EWH BioProduction ApS (Biofyto) and Koppert B.V. (Spidex). They were introduced manually by strewing an amount of wood chips estimated to contain ten predatory mites per experiment-plant infested with spider mites, per introduction (Figure 7). The predatory mites are orange of colour, and measure about 0.5 mm, with a globose body shape (Figure 2). Predatory mites develop through egg, larva, protonymph, and deutonymph to adult stage. They consume both spider mite adults, nymphs, larvae and eggs (Figure 3) (Oatman et al. 1985).

Experimental design – water sprinkler test in the plastic tunnel

The water sprinklers and environment-plants were placed in the middle of the plastic tunnel (Figure 5 a)). The environment-plants ensured a normal environment of a strawberry crop.

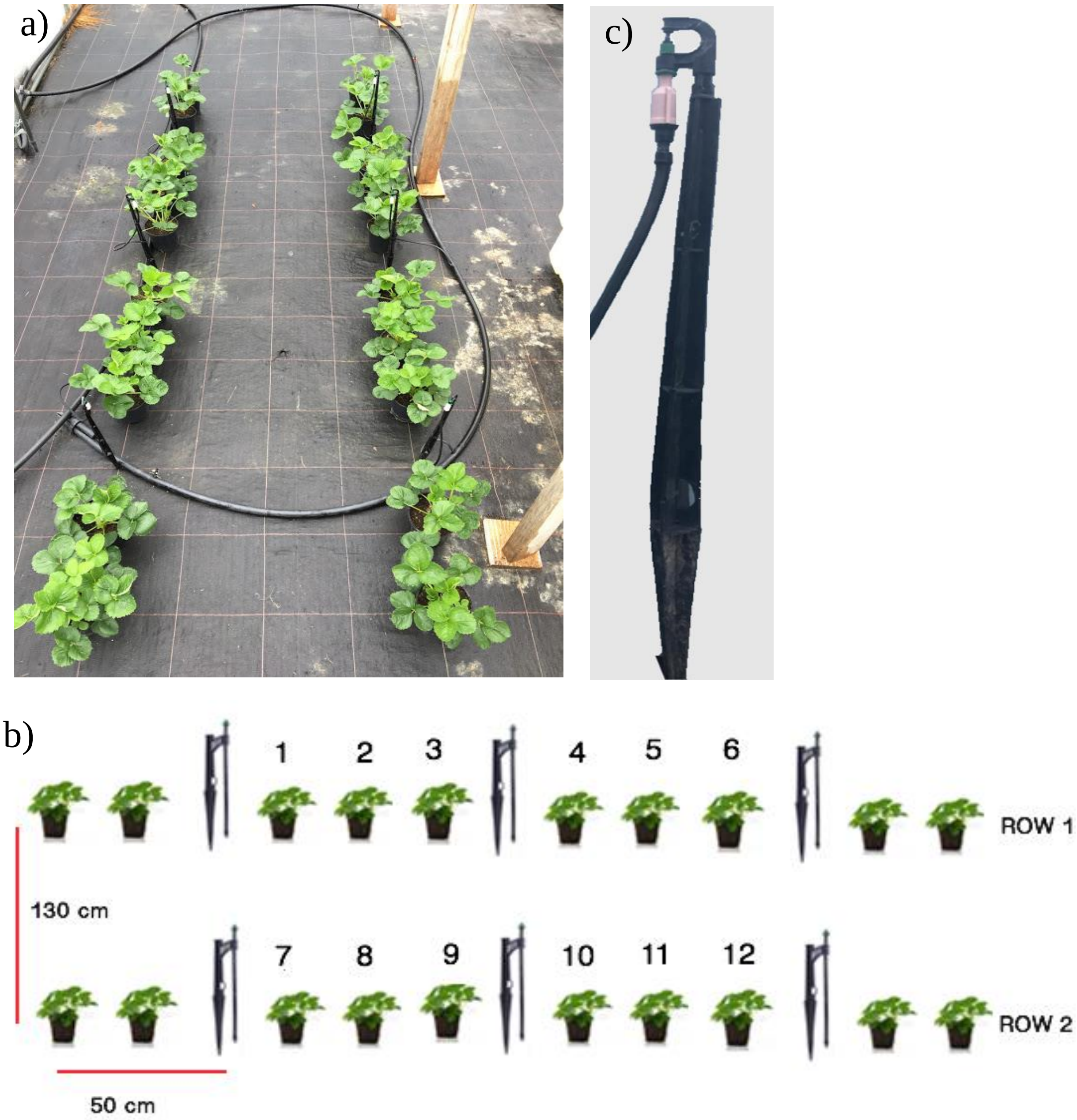


Figure 5: a) Setup with environment-plants and water sprinklers in the plastic tunnel. **b)** Illustration of setup for the water sprinkler test in the plastic tunnel. The environment-plants and water sprinklers were placed with 25 cm distance in the row, measured from the center of plants and water sprinklers. The total length of each row was 3 m. The distance between the rows was 130 cm. Numbers 1-12 denote plant positions where environment-plants could be replaced with experiment-plants in the sprinkler tests. Mean height of environment-plants was 32 cm. **c)** One of the water sprinklers used in the experiment. The length of water sprinklers was 54 cm. Sprinklers provided 4.15mm water per minute to plant positions (average of 16 measurements in in different positions, using a 40 mm garden rain gauge). Photos and illustration: Josefine Danielsen.

One environment-plant in each row was randomly replaced with an experiment-plant (plants with spider mites and predatory mites) shortly before each water sprinkler test. One water sprinkler test consisted of one minute of water sprinkling. One experiment consisted of 26 experiment-plants being tested (13 x 2 rows). The experiment was repeated four times, each repeat defined as one run (Table 1). Spider mites and predatory mites were put on the experiment-plants several times through the whole experiment (Figure 6) when kept outside the plastic tunnel, to ensure enough predatory mites present to assess the irrigation effect.

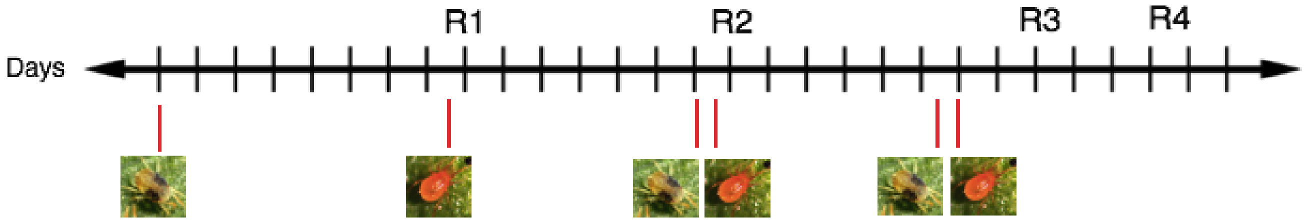


Figure 6: Timeline for all four runs (R) and introductions of spider mites (*T. urticae)* and predatory mites (*P. persimilis)* on plants, starting with the first introductions of spider mites *(*left) and ending with last run(right). Distance between each bar corresponds to two days. Yellow mite with black spots =spider mites. Orange mite=predatory mites. 1R= first run. 2R= second run. 3R= third run. 4R=fourth run. Illustration: Josefine Danielsen.

Table 1: The date, number of experiment-plants, population density of *P. persimilis* per leaf, number of introduction of both mites, temperature on sprinkler test day and relative humidity on sprinkler test day for each run.

Run	Date	Number of plants	Population density per leaf	Number of introductions of both *T. urticae* and *P. persimilis*	Temperature on sprinkler test day (°C)	Relative humidity on sprinkler test day (%)
R1	03.07.18	26	Low	1	27	45
R2	15.07.18	26	Low	2	24	60
R3	31.07.18	26	High	3	28	43
R4	07.08.18	26	High	3	27	55

When one environment-plant in each row was replaced with an experiment-plant, one of the experiment-plants was placed next to a water sprinkler (see figure 5 b): position 1, 3, 4, 6, 7, 9, 10 and 12) and the other midway between two sprinklers (see figure 5 b): position 2, 5, 8 and 11). The position of environment-plants at the end of the row was never used for experimental-plants because the effect from the water sprinklers should be from both side.

Data sampling - water sprinkler test in the plastic tunnel

After one of the environment-creating plants in each row was replaced with an experiment-plants the water sprinkler test was done. The following procedure was done each time testing:

I. Two leaflets (Figure 7), one on each side of the plant, were randomly selected for monitoring (to estimate population number of predator mites before irrigation) and cut off.
II. 1 minute of irrigation from the water sprinklers (all 6 sprinklers employed).
III. Plant left for 1 minute to allow most water to drain off the leaves.
IV. Another random leaflet from the same leaves was cut (to estimate change in population number one minute after irrigation).
V. Experiment plants placed separately in trays with water covering the bottom of the pot, for twenty-four hours.
VI. Last leaflet cut from the two leaves selected at the start of the test (population number twenty-four hours after irrigation).

Figure 7: A strawberry leaf consists of three leaflets. Photo: Josefine Danielsen.

Thus 6 leaflets from each experiment-plant was collected (2 before irrigation, 2 shortly after irrigation and 2 twenty-four hours after irrigation). Each leaflet was cut by a scissor and immediately put in a plastic zipbag. Maximum 30 minutes after harvest, the zipbags with leaflets were put in a freezer at temperature -20 degrees. The frozen leaflets were examined visually in the laboratory by a stereo microscope, to count the number of predatory mites on the lower side of the leaflet and register other factors (Table 2).

Table 2: Characteristics registered for each leaflet cut in tunnel sprinkler test. Altogether 624 leaflets were examined.

Number of *Phytoseiulus persimilis*	Eggs, larvae, nymphs and adults counted separately.
Time after irrigation	Leaflets cut before=0, leaflets cut one minute after=1, leaflets cut twenty-four hours after=24.
Number of *Tetranychus urticae* (including eggs)	Number of eggs and mobile stages in three categories: 0-20=0, 20-50=1, >50=2.
Plant position	Random placement for each plant in one of twelve positions: 1-12.
Run	Four categories: First run=one day after *P. persimilis* introduced, Second run=fifteen days after first introduced, Third run=thirty-one days after first introduced, Fourth run=thirty-eight days after first introduced.

One plant with mites that did not receive water from water sprinklers was used as control plant in each run. One leaflet from both sides of the control plants was cut at the same time as the first experiment-plant in each run; before irrigation, right after irrigation and twenty-four hours after irrigation.

Water tolerance – laboratory experiment

The objective of the lab experiment was to do a more detailed study on the ability to stick on leaves when spraying water for one minute, comprising both predatory mites and spider mites. Three laboratory-experiment 'Korona' plants were kept in cylindrical plastic-tubes with insect net on top, and infested with spider mites and predatory mites, one week apart. After two more days, 30 leaflets were cut off the plants. Numbers of living mites and eggs (both *T. urticae* and *P. persimilis*) on the abaxial of the leaflet were counted using a stereo microscope, just before the leaflet was handsprayed for one minute on the abaxial (lower side) with a spray bottle containing water (Figure 8), with 30 cm distance between nozzle and leaflet. Immediately after the water spraying, mites and eggs (*T. urticae* and *P. persimilis*) were re-counted on each leaflet immediately after the water spraying, using the stereo microscope. After two more days, the same procedure was repeated for 30 new leaflets, but this time the leaflets were sprayed on the on the adaxial (upper side) of the leaflet.

Figure 8: Spray bottle with water used in laboratory experiment. Photo: Josefine Danielsen.

Statistical analysis and data processing

All data from the tunnel experiments were analysed in GLM - ANOVA in MiniTab 16 (MiniTab, inc. 2016). Variables used to explain changing predatory mite population size were: time after irrigation (levels: 0, 1 and 24), number of spider mites (levels: 0, 1 and 2), run (levels: 1, 2, 3 and 4) and plant position (random variable levels: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) (Table 2). Data from the water sprinkler experiment in the plastic tunnel were logarithmically transformed and checked for normality before ANOVA, and all explanation variables were included in each model analyzed. Differences among the three times (before and after irrigation) for different predatory mite stages were analyzed within the model, with Tukey´s range tests. The control plant data are presented, but are too few to be included in the ANOVA models. Differences among the three times (before and after irrigation) for different predatory mite stages was analyzed with Kruskal-Wallis test. The result from models and tests was interpreted with a significance level of 0.05.

Data from the laboratory-experiment was analysed as percent reduction in mite population on leaves after spraying. Differences between mite groups were analysed using 2 sample t-test in MiniTab 16. The results from tests was interpreted with a significance level of 0.05.

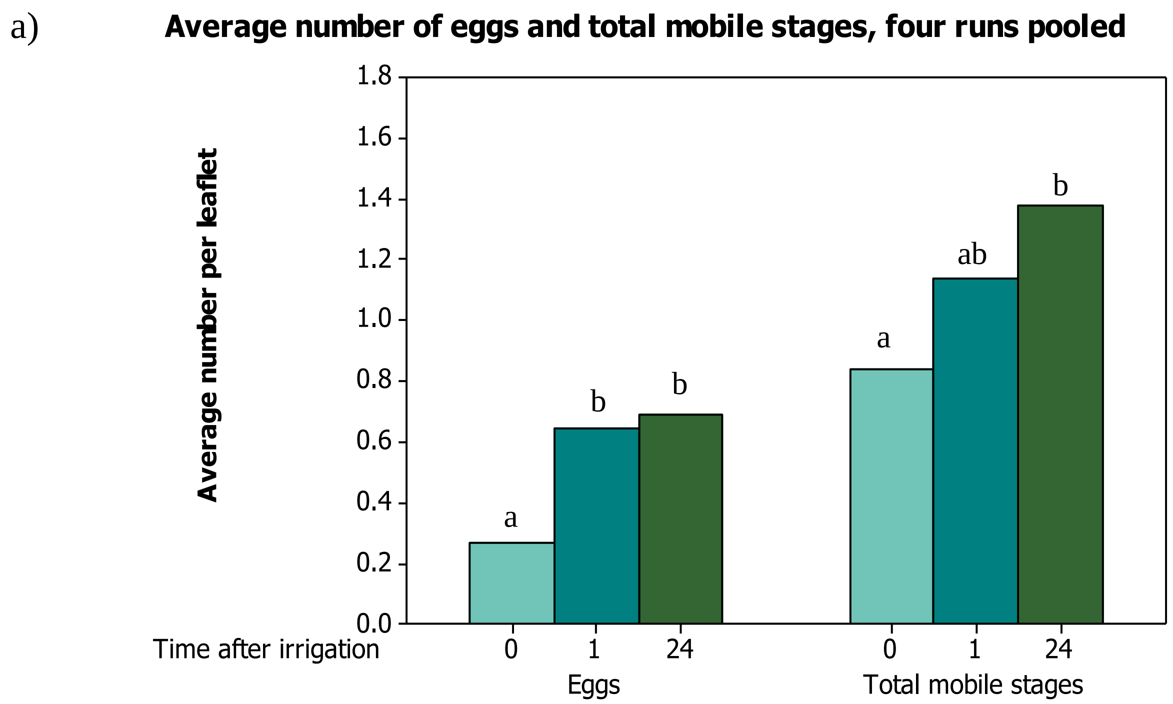
Results

1.1) How does irrigation by water sprinkling for one minute affect the different stages of predatory mite on strawberry plants? Are some predatory mite stages less affected than others? Is there a difference in the effect of water sprinklers one minute after and twenty-four hours after irrigation?

Time after irrigation significantly affected both the number of eggs ($F_{2,593}$ = 13.99, P<0.0005) and the total number of larvae, nymphs and adults, hereafter referred to as total mobile stages ($F_{2,593}$ = 6.38, P=0.002). The effect seems to be an increase in population size both one minute (total 67.9 % increase, all stages included) and twenty-four hours (total 96.2 % increase, all stages included) after irrigation (Figure 9 a)). For eggs, one minute after sprinkling and twenty-four hours were not statistically different, while for total mobiles the increase was not significant until twenty-four hours after irrigation (Figure 9 a)). Separating the different mobile stages, the effect is not statistically significant when analyzing number of adults only ($F_{2,593}$ =1.26, P=0.284) (Figure 10 a)), but it was statistically significant for both larvae ($F_{2,593}$ =7.49, P=0.001) and nymphs ($F_{2,593}$ =7.86, P<0.0005), and in both cases twenty-four hour after water sprinkling was significantly different from the two other sampling times (Figure 10 a)).

Run, the amount of spider mites present, and the interaction between the two effected the number of predatory mites for all stages significantly (Attachment 1). The interaction between run and time after irrigation effected number of eggs ($F_{6,593}$=2.94, P=0.008), larvae ($F_{6,593}$=4.64, P<0.0005) and nymphs ($F_{6,593}$=2.63, P=0.016) significantly.

For the control plants, the number of eggs and total mobile stages was stable through all three times sampled, with the exception of a large increase in mobile stages at twenty-four hours, concurrent with a slight fall inn egg number (Figure 9 b)). The juvenile stages (nymphs and larvae) constituted the majority of the increase in mobile stages after same timepoint as twenty-four hours after irrigation, were larvae is significantly increased after twenty-four hours (H_2 =6.54, p=0.038) (Figure 10 b)).



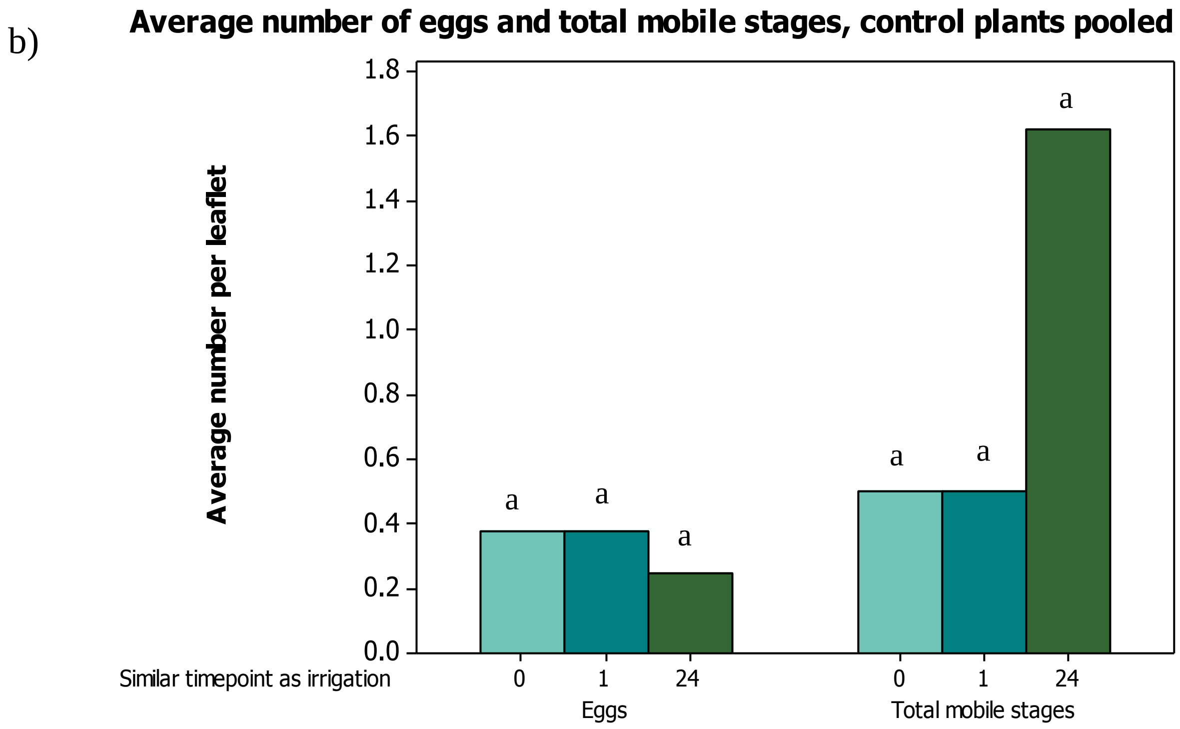


Figure 9: Average number of eggs and total mobile stages (larvae, nymphs and adults) of *Phytoseiulus persimilis* per strawberry leaflet in the tunnel experiment with plants water-sprinkled for one minute: **a)** for all four experimental runs pooled, and **b)** for pooled sample of control plants (no water sprinkling). Time after irrigation given as 0 (just before water sprinkling), 1 (one minute after) and 24 (twenty-four hours after irrigation). Different letters (a and b) above each bar in a) indicate significant differences ($p < 0.05$) between times after irrigation (Tukey´s range tests within a model including all significant factors), and letters in b) indicate significant differences ($p < 0.05$) between similar timepoint as irrigation (Kruskal-Wallis test).

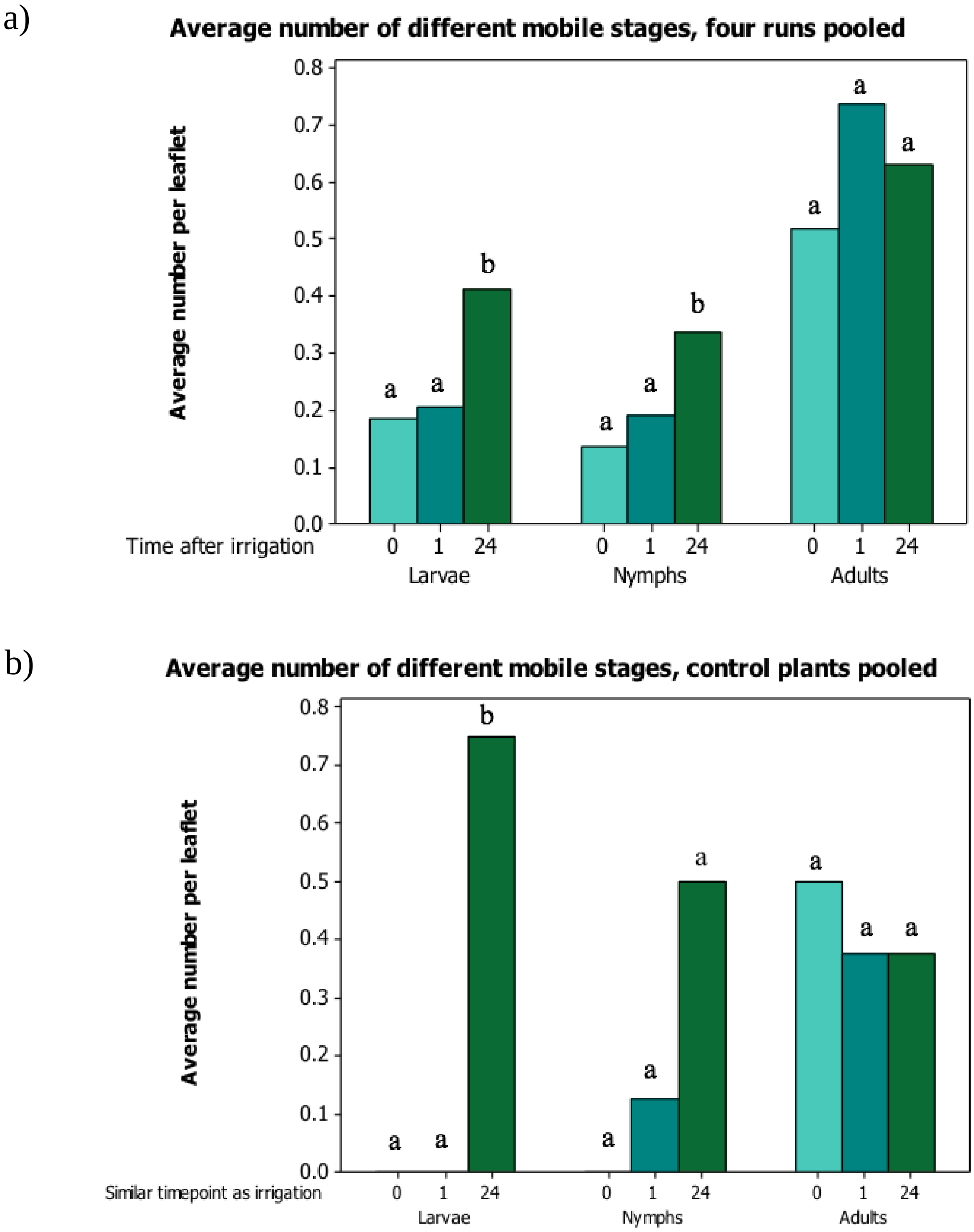


Figure 10: Average number of each mobile stage (larvae, nymphs and adults) of *Phytoseiulus persimilis* per strawberry leaflet in the tunnel experiment: **a)** for all four runs pooled with plants water-sprinkled for one minute, and **b)** for pooled sample of control plants (no water sprinkling). Time after irrigation given as 0 (just before water sprinkling), 1 (one minute after) and 24 (twenty-four hours after irrigation). Different letters (a and b) above each bar in a) indicate significant differences ($p < 0.05$) between times after irrigation (Tukey´s range tests within a model including all significant factors) and b) indicate significant differences ($p < 0.05$) between similar timepoint as irrigation (Kruskal-Wallis test).

1.2) Does the plant position relative to the water sprinklers affect the number of predatory mites?

The position of the plants in the water sprinkler test was not significant for the number of total mobile stages ($F_{11,\,593}$ =0.24, P=0.994), dissimilar to eggs, where plant position had an effect ($F_{11,\,593}$ = 2.03, P=0.028).

2) Do predatory mites stay on the leaf to a larger degree than spider mites when exposed to direct spraying of water for one minute in the laboratory?

Both mite species had a reduction in average number (Figure 11), predatory mites with an average of 11 % reduction of mobile stages and eggs and spider mites with an average of 22 % reduction of mobile stages and eggs. The average reduction was significantly lower for mobile stages of predatory mites (14 %) than for spider mites (27 %) when water was sprayed on the lower side of leaflets (t_{53}=4.27, P<0.0005). The decline in spider mite eggs (23 %) was comparable to that in spider mite mobile stages, but could not be tested against the effect on predatory mite eggs since no such eggs were present when this experiment was run.

Also when sprayed on the upper side, there was a significantly lower decline in mobile predatory mites (6 %) than in spider mites (18 %) (t_{40}=4.88, P<0.0005). In contrast, there were no significant differences between predatory mite eggs (13 %) and spider mite eggs (15 %) (t_{47}=0.30, P=0.763) (Figure 11).

When comparing the two spraying areas (upper side to lower side of leaflets) there was a higher average reduction in mobile stages when spraying on the lower side, both for predatory mites (t_{45}=3.80, P<0.0005), and spider mites (t_{58}=2.75, P=0.008). No such difference in spider mite eggs was erident (t_{34}=1.41, P=0.167) (Figure 11).

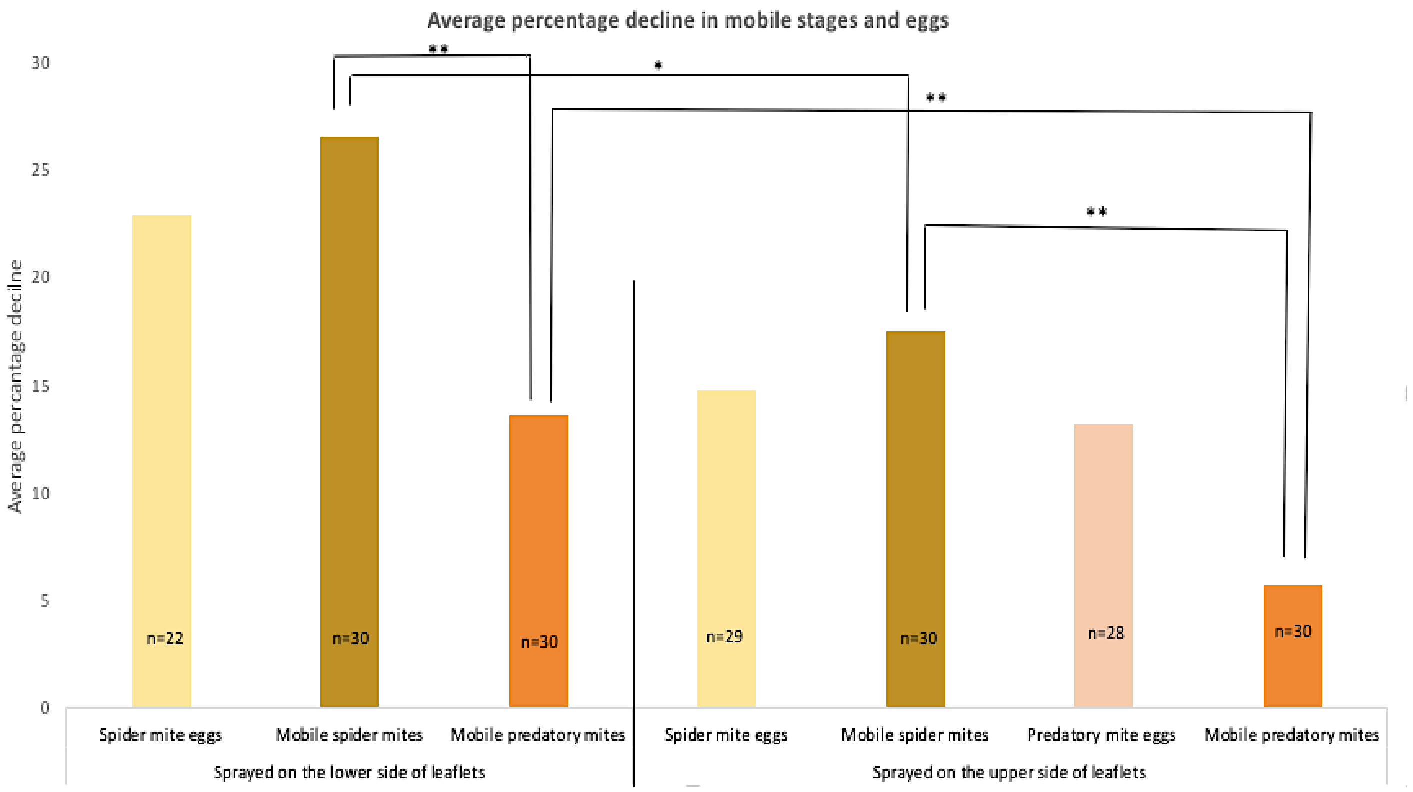


Figure 11: Average percentage decline in eggs and mobile stages of spider mites and predatory mites on the lower side of leaflets in the laboratory experiment, after hand spraying with water on the lower side and the upper side of the leaflets, respectively. *= p<0.05 and **= p<0.005 (two-sample t-tests). n=number of leaflets which had the organism present. There were no leaflets with predatory mite eggs in the lower side experiment.

Discussion

The present study was made to investigate the short-time effect of water sprinklers on the predatory mite *P. persimilis* and examine direct water tolerance for both predatory mites and spider mites. The results showed that all life stages of predatory mites were positively significantly affected by water sprinkling and had a clear increase in population number after water sprinkling. Number of predatory mites immediately after or a day after sprinkling was significantly different from before water sprinkling, except from predatory mite adults. Position relative to the water sprinklers affected number off eggs, but not number of mobile stages of predatory mites. Control plants had no significant increase, except from larvae. In the laboratory experiment, predatory mites had higher tolerance to water spraying compared to spider mites. Both mites spices were more affected when spraying on the lower side of leaflet.

An increase in population number for *P. persimilis* after irrigation in tunnel experiment is a new outcome in this field, and most certainly excludes a washing away effect, immersion resulting in death or behavior of actively avoidance of water moving from the leaves in this case. Rather the opposite is conceivable, where predatory mites gets attracted to the leaves after irrigation. Suggestion for the population dynamics with interest in number of predatory mites before and after water sprinkling is explained under.

Explaining the increase in predatory mite eggs

Increase in eggs (Figure 9 a)) is a result of either increased oviposition rate or more females present. An explanation can be a positive stress response to the sudden increase in moisture or water, resulting in more eggs laid on a short time period. *P. persimilis* lays approximately 2.6 times more eggs/female with relative humidity at 90 % compared to 50 % at the same temperature(20 °C) (Stenseth 1984). He found an average productivity of 1.78 eggs/day/female at 90 % humidity, and 1.58 eggs/day/female at 50 % humidity. With even higher humidity at 93 % and 20 °C, female can lay up to 2.67 eggs/day/female according to Pralavorio & Almaguel-Rojas (1980). For the female predatory mites to been able to double the oviposition rate after the irrigation (Figure 9 a)), the actual time from before irrigation to the one minute after irrigation leaflet was freezed, needs to be taken into account. A maximum of 35 minutes was the actual time from irrigation started to the leaflets were put into the freezer (Table 3), and therefore the actual time for predatory mites to oviposit from “before irrigation” to “one minute after irrigation”. Predatory females produce one egg at the time because of the size of the egg relative to the body of *P. persimilis* and can therefore lay only one egg at the time. If a positive stress response (in addition to more females present) is the explanation for the increased eggs, the predatory mite females have spent the 35 minutes laying the eggs they had ready.

Table 3: The actual time for females to lay eggs and other life stages to develop from the start of irrigation to the leaflets sampled one minute after irrigation being put into the freezer. Time given in minutes.

Actual time for leaflets sampled one minute after irrigation	Irrigation	Water draining off leaves	Sampling all 4 leaflets	Time in plastic zipbags before putting in the freezer	Total time from start of irrigation to freezing
	1 min	Approx. 1 min	Approx. 2 min	Maximum 30 min	**Maximum 35 min**

The dry conditions on the experiment days are not optimal for the predatory mite. A study was done on *P. persimilis* when in state of water deficit, and this demonstrated a positive hygrotactic behavior for the mobile stages (Gaede 1992), which possible can confirm the suddenly appearance of predatory mite eggs, were female moving from shaded places to the leaflets starting to lay eggs. The significant effect of position of the plant relative to water sprinklers on eggs, can explain the importance of water or humidity in eggs. Oviposition may be especially dependent of water, indicating increasing in eggs after irrigation as a result of females choosing places and timing in state of water surplus. Plant position number 1 stood out with the lowest population size one minute after irrigation. This observation was also done in a master theses on the same project at SMARTCROP, using the same method investigate sprinkling effect on spider mites (Auberg 2018).

Explaining the increase in predatory mite larvae and nymphs

Twenty-four hours after irrigation a significant increase happened for both larvae and nymphs (Figure 10 a)). The larva is almost inactive unless disturbed, so this means that eggs must have hatched on the leaflets for them to appear. Egg hatching is sensitive to low humidity (Ferrero et al. 2010; Williams et al. 2004), and when the water sprinklers increased the humidity, this could have caused egg to hatch, resulting in more larvae. Another explanation is that female predatory mites can hold back their eggs if the conditions are unfavorable, like in this case with low relative humidity before irrigation, and then lay eggs that immediately hatches to a larva if the conditions are getting more favorable, like when the irrigation creates higher humidity (Helle & Sabelis 1985). This means that they can “oviposit” fully developed larvae, explaining the increasing larvae population twenty-four hours after irrigation, however the phenomenon is rarely seen.

Predatory mite nymphs can move around on the strawberry plants, so this life stage could also have had a positive hygrotactic behavior, moving from near the soil or stalk to the leaves in the twenty-four hours after irrigation. If the nymphs did not come from other places to the leaves, larvae must have molted to nymphs. In a study from 1985 it was reported that relative humidity <70 % can reduce the ability of develop from a stage to another (Pralavorio & Almaguel-Rojas 1980). So, with the sudden higher humidity after irrigation in this study, larvae may have molted to nymphs within the twenty-four hours after irrigation.

The trend of increase in predatory mite adults

There is a trend towards an increasing predatory mite adult population size in all four runs, thus not significant, after irrigation (Figure 10 a)). This trend can be explained by behavior of movement towards water, caused by the hot and dry conditions on the sprinkler test days. *P. persimilis* is known to avoid dry places and rather reside in more shaded places near the soil or along the stalk when it is too dray (Bernstein 1983; Mori & Chant 1966). The temperature ranged from 24 °C to 28 °C and relative humidity from 43 % to 60 % on the sprinkler test days (Table 1), and this is dryer than *P. persimilis* prefer and probably affect the behavior of predatory mites (Williams et al. 2004). Predatory mites may therefore have stayed in the shaded places before irrigation and moved to the leaves when irrigation occurred. Adults of predatory mites are the most numerous (Osborne, L. S. et al. 1985) and active (Helle & Sabelis 1985) stage, where mites can move quickly from place to place. This means that most variation among stages in where they stay on the plant, is to be expected. On the other hand, a study showed less activity of *P. persimilis* in high humidity (Mori & Chant 1966) which exclude high movement to the leaves in adult populations, which again excluding high increases in adults population after irrigation, like in this experiment.

Comparing water sprinkling with the control plants

The control plants showed no difference in similar timepoint as irrigation, except from larvae after twenty-four hours (Figure 10 b)). This is contrasting to the result from the experiment-plants, with biggest interest of the immediately increase in population size one minute after irrigation in eggs. A growing predatory mite population is dependent on the birth rate where the population increase with time, to a certain point (Helle & Sabelis 1985). The time it takes to increase the population is dependent on the conditions, like humidity or water. For the control plants in this experiment, number of total mobile stages and eggs was stable from the same timepoint as before irrigation to same timepoint as one minute after irrigation (Figure 9 b)), as expected for this short period of time without changing the conditions. After a day (same timepoint as twenty-four hours in experiment-plants) an increase was detected in adults, naturally explained by time, and a reduction in number off eggs, possibly explained by the significantly increase in larvae. The fact that control plants population changes is most certainly explained by time, and experiment-plants population changes seems to be an effect from irrigation, indicate that the experiment confirms the overall prediction with no negatively effect of water sprinklers on predatory mites, and rather the opposite is proven were changes in the microclimate by water sprinklers is an advantage for predatory mites.

Irrigation and predatory mites number affected by population size?

Significant effect of run on number of predatory mites in all stages (Attachment 1) can easily be explained by a general increased population size from first run to fourth and last run. The same effect goes for the amount of spider mites present (Attachment 1). In cases with more than 50 spider mites present (level 2, highest) on a leaflet, the average of predatory mite mobile stages or eggs is higher compared to lower levels of spider mites. However, the interaction between run and spider mites explain that there is variation in the effect of amount of spider mites present on the number of predatory mites in different runs.

Number of the juvenile stages (eggs, larva and nymphs) were affected by the interaction between run and time after irrigation (Attachment 1). The interaction can be interpreted as different effect of water on the predatory mite number in the different runs, possibly explained by different population sizes of predatory mites in each run.

Even if the overall picture looks like an increased predatory mite population size after irrigation, the difference in eggs from one minute after irrigation to twenty-four hours after irrigation stands out with decrease in run number 1, 2 and 3, but high increase in run number 4 (Figure 12). This suggests that it was variations in the effect of sprinkling, although the main picture look like increase in number after water sprinkling. Number of larva and nymphs could also have been affected differently after irrigation in each run, but no such effect is clear from the figure 12. The interaction could also indicate other conditions which are different, and necessary not are measured in this experiment. The effect of water sprinkling on different population size of predatory mites needs to be further investigated.

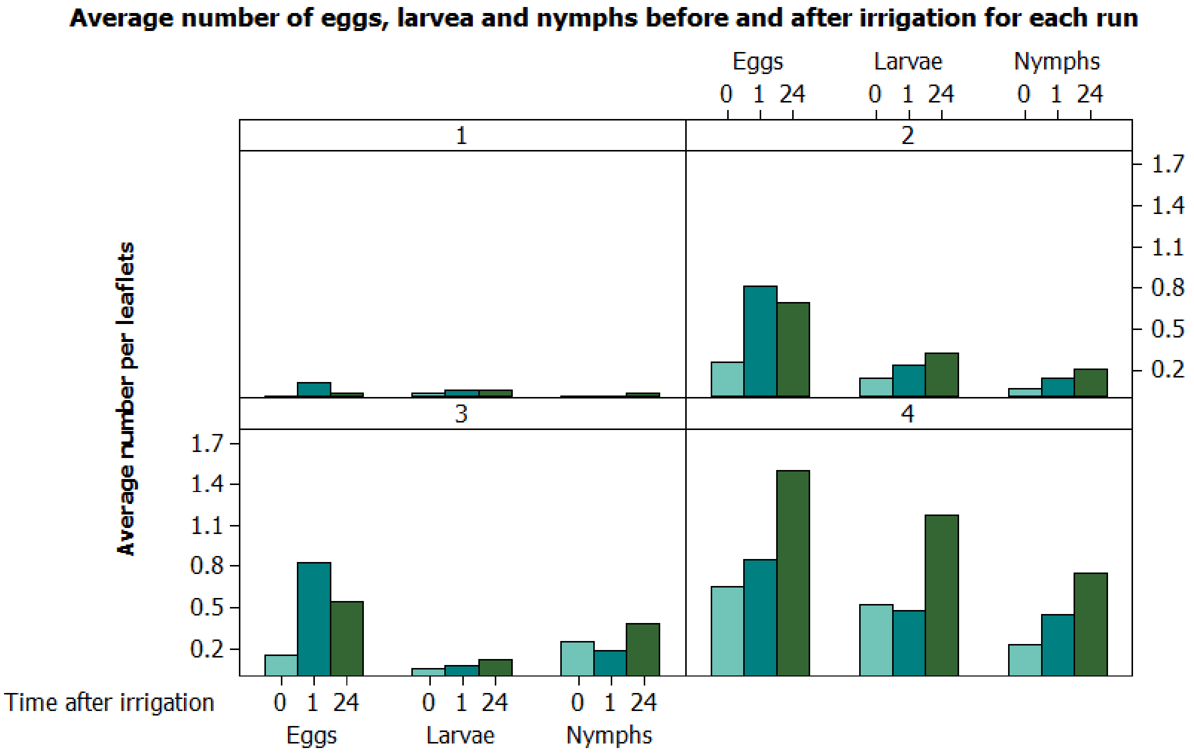


Figure 12: Average number of *Phytoseiulus persimilis* per strawberry leaflet in the tunnel experiment of stages that was affected by the interaction run*time after irrigation. Time after irrigation given as 0 (just before water sprinkling), 1 (one minute after) and 24 (twenty-four hours after irrigation). Different runs shown in panels, 1=first run, 2=second run, 3=third run, 4=fourth run.

Water tolerance in context of the possibilities of using water sprinklers

Results from the laboratory experiment showed that mites actually get washed away by mechanical forces from water droplets (Figure 11), pushing mites off the leaflets. This is a critical perspective to take into account if irrigation is going to be a part of IPM combined with predatory mites, supressing spider mites. There was observed legs of the predatory mite captured or glued in water droplets for a little while after the laboratory experiment was completed. This reduced mobility can prevent the predatory mites from controlling spider mites in the time period after irrigation, and until they become dry again. In the case of avoidance of free water, the predatory mite may get prevented or distracted from controlling spider mites. Additionally, the increased population size demonstrated in this tunnel experiment contrast the study that showed reduced predatory mites populations with overhead irrigation (Opit et al. 2006). The differences between this tunnel study and Opit et al. is that they used overhead watering by hand on the foliage canopy, compared to water sprinklers in this study. Opit et al. also did the irrigation once a day over three weeks, compared to one time in this study. Even though the report showed reduced population sizes with irrigation, they showed significantly fewer spider mites and spider mite damage when irrigation was used in addition to predatory mites, compared to irrigation without predatory mites. This means that even if some predatory mites get washed off or do not thrive, they still have big enough numbers to suppress spider mites together with overhead irrigation. As a matter of fact, since the predatory mites are washed off the leaves shown in the laboratory experiment, but increased in populations in the tunnel experiment, there is a possibility that they crawl back on the leaves or do not get completely washed off the leaflets. Besides, predatory mites were washed off the leaflets in a lower degree compared to spider mites, indicates that they still may be enough individuals controlling spider mites. The mechanisms on how predatory mites manage themselves after water sprinkling need to be investigated in the future. If the differences in water tolerance is not off-set by the negative washing off effect, combination of water sprinklers and biological control by predatory mites may be potential IPM tools together.

Conclusion

One minute of water sprinkling had a desired effect on the population size of predatory mite *P. persimilis* on strawberries in the tunnel experiment. The total population increased significantly after water sprinkling, contrasting the control plant not receiving irrigation with no significant increase, except from larvae. The laboratory experiment showed higher water spraying tolerance in predatory mites compared with spider mites (*T. urticae*). Both species had a higher reduction in mobile life stages when sprayed on the lower side. Further studies are needed to understand why *P. persimilis* increased in population size after water sprinkling, especially the sudden increase in eggs immediately after irrigation. Combining water sprinkling and predatory mite to control spider mites, looks promising for the strawberry farmers and is a natural and gently IPM option.

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Attachment 1

ANOVA results from the statistical analyses done for all life stages of predatory mite in the tunnel experiment. df= degrees of freedom. df2=593 for all explanatory variables tested for each response variables. Results with p<0.05 marked in bold.

Response variable:	**Total mobile stages**			**Eggs**			**Larva**			**Nymphs**			**Adults**		
Explanation variables	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
Time after irrigation	2	6.38	**0.002**	2	13.99	**0.000**	2	7.49	**0.001**	2	7.86	**0.000**	2	1.26	0.284
Run	3	71.07	**0.000**	3	34.09	**0.000**	3	32.10	**0.000**	3	22.99	**0.000**	3	41.75	**0.000**
Spider mites	2	45.64	**0.000**	2	22.44	**0.000**	2	11.57	**0.000**	2	7.62	**0.001**	2	30.81	**0.000**
Plant position (random)	11	0.24	0.994	11	2.03	**0.028**	11	0.60	0.831	11	1.06	0.396	11	0.69	0.745
Run * Spider mites	6	2.95	**0.008**	6	2.48	**0.023**	6	2.64	**0.015**	6	4.41	**0.000**	6	3.21	**0.004**
Run*Time after irrigation	6	1.68	0.124	6	2.94	**0.008**	6	4.64	**0.000**	6	2.63	**0.016**	6	0.39	0.886

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