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Short-term effect of water sprinkling on the two-spotted spider mite *(Tetranychus urticae)* in strawberry

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Preface

Through my five years at NMBU, I have had several courses in biology and ecology and I was introduced to courses in entomology which caught my interest. I was lucky enough to get in contact with Nina Trandem who presented several interesting research proposals, including the effect of water sprinkling on two-spotted spider mites on strawberry plants, which is the subject of this thesis.

Today I am very grateful for having had the pleasure to learn from and work with the experienced people at NIBIO and those who have cooperated through the SMARTCROP project, among others Ingeborg Klingen. They have provided me with everything I needed for my experiments and I have learned so much in the process. I would like to thank my amazing and hard-working supervisor Nina Trandem, who has been organizing regular meetings, answering mail, helping me get in touch with people who could aid me with my experiments and giving me indispensable feedback and guidance on my thesis. I am very grateful for everything she has done for me. I also want to thank my co-supervisor Belachew Asalf Tadesse for feedback, input and valuable help with Minitab16.

I would like to thank the people at Kirkejordet, SKP (Center for plant research in controlled climate): Andrew Dobson for helping me with assembling and testing the water sprinklers used in the experiments and Rodrigo Onofre and Chloé Grieu for aiding me with caring of the strawberry plants. I also want to thank Torfinn Torp for much needed statistical help and analysis of my data, Karin Westrum for providing the stock cultures of two-spotted spider mites and Tone Birkemoe and Anne Sverdrup-Thygeson for organizing group meetings for masterstudents in ecology where we evaluated each other’s papers and progress.

Finally, I would like to thank my family, friends and boyfriend for motivation and support and last, but not least my “masterbuddy” Josefine Danielsen. We had the same supervisors and similar experiments but with different focus and we have been cooperating on several stages from start to finish in this journey, which has been both helpful and clarifying.

Ås, May 7, 2018

Camilla G. Auberg

Abstract

The two-spotted spider mite, *Tetranychus urticae,* is a common pest on strawberry plants and especially when cultivating strawberry plants in tunnels due to the mites' preference for warm and dry conditions. A common control method is to apply pesticides, but it is advantageous to use measures that do not entail dangerous substances. This can be achieved by using IPM (integrated pest management) methods. This study addresses water sprinkling as an IPM method to reduce spider mite populations, focusing on the short-term effect of one minute of irrigation. The spider mites were studied through three experimental runs where leaflets from strawberry plants were sampled before irrigation, one minute after irrigation and 24 hours after irrigation of whole plants. The experimental runs were done on a growing spider mite population and revealed an interaction between the effect of the water sprinkling and run, probably due to the differences in the spider mite population size. The total number of all individuals in mobile life stages showed no effect of the water sprinkling, comparing the three times sampled (i.e. before, 1 minute after and 24 hours after irrigation). However, when analyzing each life stage (eggs, larvae, nymphs, adult females and adult males) separately, time after irrigation had an effect in all life stages. The results suggested that there was a short-term effect of the water sprinkling 1 minute after irrigation where the number of nymphs and adult males was reduced, but also a short-term effect 24 hours after irrigation resulting in a reduction of eggs, larvae, nymphs, adult females and adult males, all in which the numbers of spider mites decreased for one of the two experimental runs with a high number of mites. One of the 12 possible positions for the experimental plants where the leaflets were sampled differed from the other positions by having the fewest mean number of spider mites at all three sampling times.
In conclusion, the short-term effect of one minute of irrigation was not clear-cut and because one minute of irrigation per day was not enough, water sprinkling should be used with other IPM methods to increase the effect.

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Introduction

Pests feeding on crops are, and always have been, a key issue in agriculture. Pests reduce crop productivity, and farmers consequently try to keep their crops free from these organisms. Now, more than ever, it is important to find efficient and sustainable pest management methods because of the ever-increasing human population and the increased demand for food. Pest management includes physical, chemical and biological measures (Oerke 2006). The common chemical method is to apply pesticides, but these chemicals can be harmful for the crops, for instance when using herbicides, and can also have a negative effect on other organisms and surrounding areas in contact with these chemicals. Therefore, it is advantageous to use measures that do not entail dangerous substances but are still able to control the pests and keep the crops healthy and growing. A central concept in this is integrated pest management, also known as IPM (Mitrev et al. 2017). The main principle of IPM is to combine (integrate) several methods rather than just relying on one method alone. IPM has several benefits: it is more effective than using pesticides alone, it reduces the chance of pesticide resistance in organisms involved and reduces the risk of human and environmental damage.

As of today, IPM methods must be implemented by all professional users, according to EU Directive 2009/128/EC (Parliament 2009), which was implemented in the EU in 2014 and in Norway in 2015 (Lovdata 2015). This directive includes 8 IPM principles (Fig.1.1). One important aspect of IPM is the aim to keep the use of pesticides, and other forms of intervention, to levels that are economically and ecologically acceptable. This level is the economic injury level (EIL). By using all available information, tools and methods, organisms can be prevented and/or suppressed from harming plants with an integrated approach through these 8 principles. SMARTCROP (Norsk Institutt for Bioøkonomi (NIBIO) 2016a) is an IPM research project started in 2015 with a goal to find alternatives to the use of pesticides, including non-chemical methods such as the use of natural enemies or mechanical removal of pest organisms.

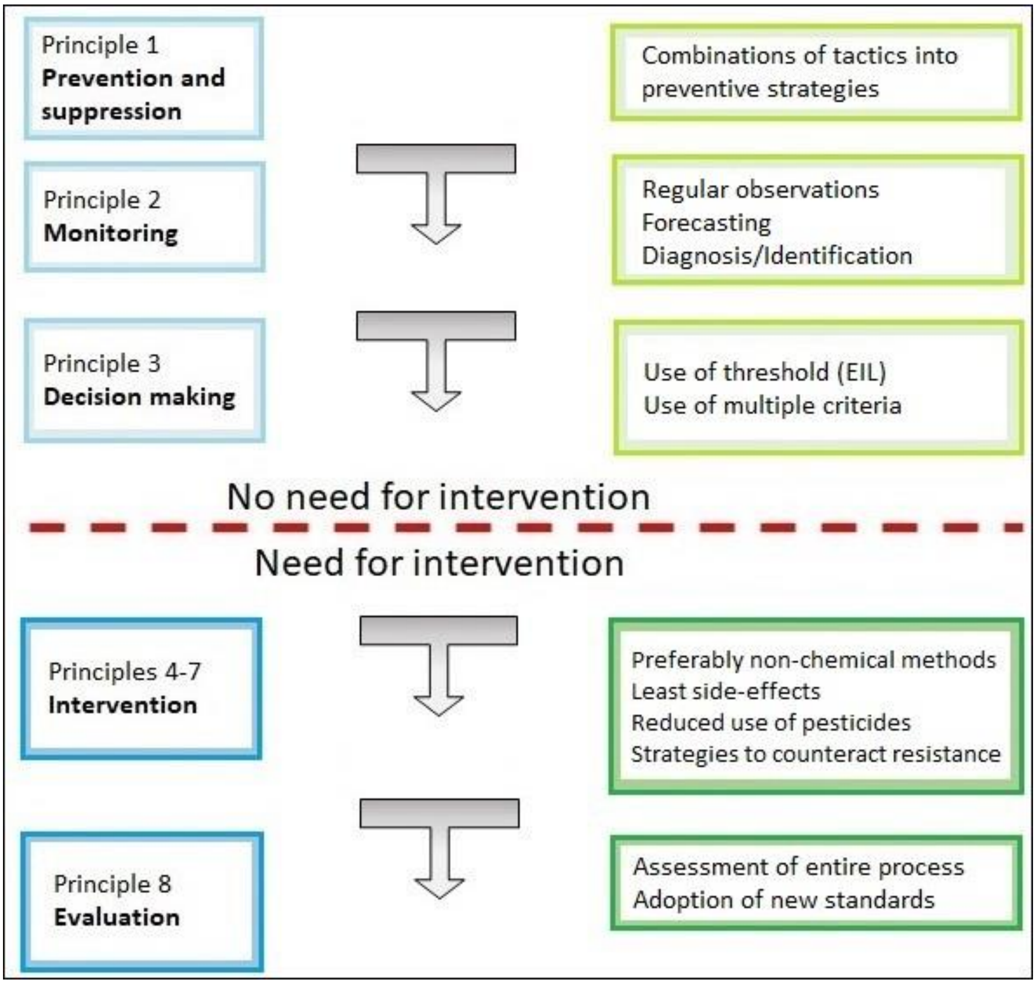


Fig.1.1 The eight principles of IPM as specified by EU Directive 2009/128/EC. It is mandatory for all professional users of pesticides to apply IPM through these principles. Adapted from Barzman et al. (2015)

SMARTCROP uses strawberry as a model for studying IPM in a perennial crop. Two-spotted spider mite, *Tetranychus urticae*, is a common pest on cultivated strawberry plants everywhere, living underneath the leaves and sucking the plant cells and filling them with air. This creates bright spots on the upper side of the leaves which later turn grayish (Stenseth 1990). It is also common to see white stippling on the upper side of the leaves, dark stippling or scarring on the undersides of the leaves and bronzing and eventually dead leaves (Shanks & Barriti 1975). Spider mites produce a web, found along the edges of the leaves and on the underside of the leaf (Fig.1.2a). This web protects both individual mites and colonies from being blown away by wind (Davis 1952) and from getting wet during rain because the web repels water (Linke 1953). The web also gives several other advantages for the mites. It protects eggs, secures that individuals are attached to the leaf when they are transitioning from one life stage to the next and works as a barrier against predators.

An increasing population of spider mites can be a large threat to the strawberry plants over time because more web in principle means more protection for the mites, and also, plants will be more stressed by the increased feeding activity (Sances, F. V. et al. 1982). Stress can decrease the growth and health of the strawberry plants, and high levels of spider mite populations reduces the photosynthesis and transpiration (Sances et al. 1981) and affect fruit production so that less fruit is produced. The extent of injury to the strawberry plants is largely determined by the amount of stress and the time of season when the stress occurs (Sances et al. 1979; Sances, F. et al. 1982; Sances, F. V. et al. 1982). The two-spotted spider mite develops from egg to adult in approximately seven days at 30°C, resulting in multiple overlapping generations throughout the summer (Fig.1.2b). The adult spider mite has a lifespan of about 21 days at 30°C (Herbert 1981) during which the female can lay 100-200 eggs (Stenseth 1990). The fast developmental rate and high egg production can lead to extremely rapid increases in spider mite populations.

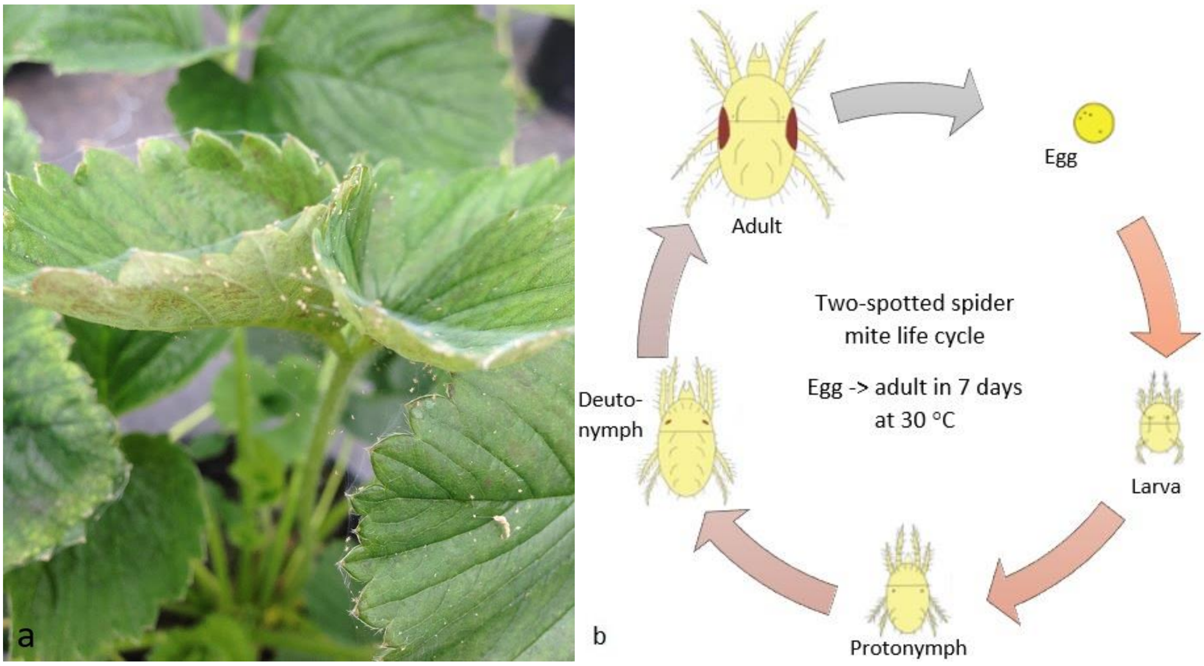


Fig 1.2 a) Spider mite (*Tetranychus urticae*) web on strawberry leaves. Mite damage can be seen on the underside of the leaves as darkened, brown areas. Photo by Camilla Auberg.
b) The spider mite life cycle from egg to adult. Developmental rate depends on temperature, relative humidity, age and quality of the host plant. The ratio between female and male is usually 3:1, due to large amounts of unfertilized eggs resulting in males (asexual reproduction). Adapted from Broughton (2013)

Cultivation of strawberries in plastic tunnels gives an advantage compared to cultivation in open fields because of higher yields in tunnels (Kadir et al. 2006). Tunnels provide protection from rain, warmer conditions, reduced fungal attacks and extending growing season (Døving et al. 2011), all of which are reasons why the strawberry production is shifting from open field to tunnel production. This development is good for strawberry production but increases the problems regarding spider mites, which thrive in the warm and dry environment provided by tunnels.

We know from previous studies in the SMARTCROP project that using water sprinkling as an IPM method in tunnel-grown strawberry plants significantly can reduce the spider mite population (Norsk Institutt for Bioøkonomi (NIBIO) 2016b). Plants were exposed to one minute of watering four times a day, and this reduced the damage done by the spider mites. The water sprinkling was initially used to control powdery mildew, and so it seems that water sprinkling can be a very helpful tool in strawberry plant protection (Asalf et al. 2016).

There have also been several earlier studies on how to control spider mites using methods involving water. The earliest study (Trägårdh 1915) reported that water sprinkling with a garden hose keeps mites in check. Other earlier studies also include: hot-water treatment (Gotoh et al. 2013), drip-tube irrigation and biological control (Opit et al. 2006), fogging (Duso et al. 2004) and biological control (Force 1967; Sabelis 1982; Xu et al. 2006). The latter often includes the predatory mite *Phytoseiulus persimilis* which is a specialist on spider mites and can deal with the spider mite web without getting entangled (Sabelis 1982). *P. persimilis* is therefore often used in biological control against two-spotted spider mites. An on-going study in SMARTCROP is investigating *P. persimilis* as a biological control on two-spotted spider mites in combination with water sprinkling (Danielsen 2018). All these studies show that water can help inhibit the development of large two-spotted spider mite populations, but none of them investigates if water affects each life stage differently or the short-term effect of water sprinkling. This would be interesting to examine. Hence, water sprinkling as an IPM method shows promise for spider mite control on strawberry plants in tunnels.

Objective of the study and research questions

There has been little research on the mechanisms behind the effects of water sprinkling on two-spotted spider mites. The objective of this study was therefore to study the short-term effect of one minute of water sprinkling on populations of two-spotted spider mites, where the ultimate goal was to see how this could be used to facilitate water sprinkling as a part of an IPM strategy.

Therefore, I formulated these research questions:

- i) Will the number of spider mites decrease after one minute of water sprinkling?
- ii) Has water sprinkling a different impact on different life stages (egg, larvae, nymphs and adult)?
- iii) What is the difference in the effect of water sprinkling right after, and after 24 hours?
- iv) Does the plant position affect number of spider mites present?

I predicted that i) the number of spider mites will decrease after one minute of water sprinkling, and that ii) the water sprinkling will have different effects on the different spider mite life stages. I would also expect that iii) there will be a difference in the effect of water sprinkling measured one minute and 24 hours after sprinkling. Finally, I predicted that iv) plant position will not affect the number of spider mites present.

Materials and Methods

Study facilities

The study was performed at Kirkejordet, Ås (59º42" N, 10º 44" E), a part of the Center for plant research in controlled climate (SKP). I used a greenhouse to grow the experimental plants and a high plastic tunnel to perform the sprinkler-tests. The plastic tunnel is shown in Fig.2.1.

Strawberry plants

The plants used were of cv. Korona. These plants of certified "elite plant" quality, free of pests and diseases, were bought as plug plants from Sagaplant AS (Sauherad in Telemark) ultimo April 2017. They were immediately placed in a small greenhouse "K9" at SKP in Ås, free of mites and insects, to allow the plants to grow isolated in a "clean" environment. The plug plants were eventually transferred into 1.5-liter pots with drainage holes and then placed in the plastic tunnel. A total of 98 strawberry plants were used in my experiments, 78 experimental plants (to be infested with spider mites) and 20 environmental plants (non-infested strawberry plants). The experiment was divided into three runs (repeats) where 26 experiment plants were used in each run (Table 1). The water used to water the plants was from Årungen, a lake near Kirkejordet. Plants were watered with fertilized water two times a week and clean water for the rest of the week. The plants were not treated with acaricides or any other pesticides before or during the experiment.

Source of two-spotted spider mites

A stock culture of *T. urticae* was maintained on strawberry plants in a climatic chamber at 20 ± 1ºC, 60 % Relative Humidity and photoperiod of 16 light: 8 darkness at NIBIO. To develop spider mite populations for my experiments, strawberry plants were placed for two weeks in one of the cultivation rooms at NIBIO at 20 °C, until May 29, when spider mites were introduced on the experimental plants. Leaves were inspected for spider mites, particularly females, in a stereo microscope for viable populations before they were transported to the experimental plants in the tunnel. Each leaflet with spider mites was cut in two and one half leaflet was placed in the crown of each of the 78 strawberry plants. The infested plants were kept in the southeast corner of the tunnel (Fig.2.1), away from the environmental plants, preventing unwanted infestation from the infested plants to the setup. The stock culture was originated from *T. urticae* collected from a strawberry field at Kirkejordet, Ås, in 2015, a few meters from where the experiments took place.

Fig.2.1 a) The plastic tunnel from the entrance on the west side. b) The tunnel had new woven plastic and measured 15 m long, 4 meters wide and 3 m high. c) The side skirts could be opened by rolling them up along the sides of the tunnel for necessary ventilation.
a and b photos by Camilla Auberg.

Experimental design

Two rows, each consisting of 10 non-infested strawberry plants, were placed in the experiment tunnel (Fig.2.2). Sprinklers were placed between every three plants. The sprinkler provided a full circle of overhead water sprinkling to all plants in the row (Fig.2.3).
The environmental plants were free of spider mites at the start of the experiment (i.e., were not infested on purpose as the experiment progressed), but were watered (also sprinkled), cared for and treated the same as the infested plants. The purpose of these "environmental plants" were to create two rows of plants, i.e. mimicking the plot used in former sprinkler experiments (Asalf et al. 2016) and to imitate the general environment of a strawberry crop since mites on solitary plants may be more exposed to the water than plants in a row. To test the effect of water sprinkling, one environmental plant in each row was randomly replaced with a spider-mite infested plant before sprinkling all 20 plants present in the plot. The exact position of each experimental plant was randomized by drawing pieces of paper with numbers from 1 to 12, indicating the position (Fig. 2.2).
Each sprinklertest consisted of one minute of irrigation. In each sprinklertest, one infested plant was placed next to a random water sprinkler in row 1, while the other infested plant was placed in the middle of two sprinklers in row 2. Because there were two rows, two infested plants could be tested at the same time, which made the experiments more time efficient. As

soon as an infested plant had been through the sprinklertest and all leaflets had been collected, it was removed from the tunnel.

Table 1: Events of experimental plants and associated spider mite population in the three experimental runs. All experimental plants were infested with spider mites May 29.

Run number	Date when run was conducted	Number of experiment plants in run	Density of the spider mite population	Amount of webbing
1	June 15	26	Low	Low
2	June 22	26	High	High
3	June 29	26	Very high	Very high

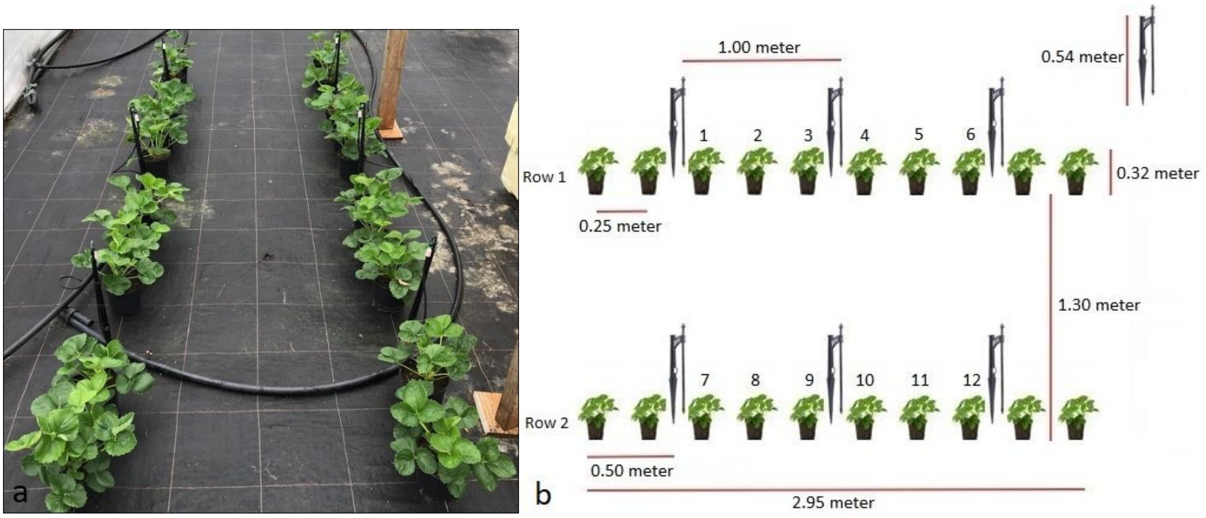
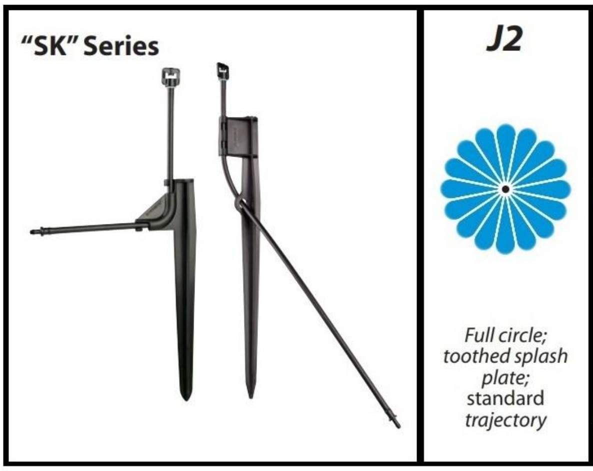


Fig.2.2 a) Experimental setup in the tunnel with the 20 “environmental plants” and 6 water sprinklers. b) Sketch indicating plant distance and possible plant positions from 1-12 for the experimental plants throughout the experiments.



Nozzle Size	Pressure (PSI)	Flow (GPH)	Approx. Diameter*
50 (GREEN)	10	11.8	14
	15	14.4	15
	20	16.7	16
	25	18.6	17
	30	20.4	18

* Pattern dimension data shown in feet, with 6" riser height.

Fig.2.3 The water sprinkler used in the experiments, from the brand Bowsmith. The model used was a Fan-Jet Microsprinkler with full circle overhead sprinkling with a green nozzle. Adapted from Bowsmith (2017). Irrigation time per sprinklertest was one minute and the amount of water was measured with two rain gauges in each row four times with a mean of 4.15 mm water per sprinkler per minute in the plot.

Deployment of water sprinkling and sampling of leaves

Leaflets were sampled (cut by a scissor) three times per run for each experimental plant: before irrigation (“0 minute”), one minute after irrigation and 24 hours after irrigation. The procedure for each experimental plant was:

1. Spider mite-infested plant was placed in the row (after the placement was decided by drawing the plant position).
2. Each strawberry leaf consists of three smaller leaves, called leaflets. One random leaflet was sampled from each side of the plant (to examine the start population before irrigation)
3. 1 minute of irrigation from the water sprinklers
4. The plant was left untouched for 1 minute to allow the water to drain off the leaves. A second leaflet was sampled from the same leaves as before irrigation (to examine effect after 1 minute after irrigation)
5. The plants were then moved to a corner in the tunnel, with good distance from each other (to minimize movement and transfer of spider mite from plant to plant) for 24 hours before the last leaflets were sampled from the two leaves per plant (to examine effect after 24 hours after irrigation)

The experimental plants used for each run were destroyed after leaflets had been sampled after 24 hours (as there would be new plants for the next upcoming runs). The environmental plants were kept for upcoming runs and was placed in a sheltered place in the tunnel when they were not in use as they were still a part of the experiments until all three runs were completed and all leaflets were sampled.

Handling of leaf samples

Altogether, 6 leaflets were sampled per plant in all three runs, giving a total of 468 individual leaflets. These were put in individual plastic zipbags and frozen at -20 °C after a maximum of 30 minutes since harvest. Later, leaflets were examined in a stereo microscope and all spider mites life stages (eggs, larvae, nymphs, adult males and adult females,) were counted on all leaflets sampled. Abiotic factors like temperature and humidity were not measured in this experiment because I was only interested in looking at the short-term effect of water itself and how the spider mites responded to this.

Statistical analysis and data processing

The statistical analysis was done using Minitab 16. Data was modeled with GLM (general linear model) with a significance level of 0.05. The model used in all analysis included the explanatory variables run, plant position and time after irrigation (Table 2), and the interaction between run and time after irrigation. Other interactions were also tested but not included in the model. Amount of webbing was originally a part of the model but was later excluded because amount of webbing and run were not independent factors.
The comparison-tests were done by using the Tukey test for each run separately, taken all significant factors into account, to compare the effect of time after irrigation. Data was not pooled because there was a significant effect of run.
The response variables used were number of spider mite eggs, larvae, nymphs, adult females, adult males and total mobile spider mites (all mites excluding eggs). Total number of all spider mites were also used. The models were made with transformed data by adding +1 to all mite counts and log-transforming them (using LOG10) in order to get a normal distribution of the data. The original (actual data, not log-transformed) numbers are presented in the figures.

Table 2: Explanatory factors used in the ANOVA regression (General linear model).

Factor	Type	Levels	Values	Description
Run	Fixed	3	1; 2; 3	The experiment was divided into three runs, spread over four weeks.
Plant position	Random	12	1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12	Indicates the position of the plant and how it is placed according to the water sprinklers (next to or between two)
Time after irrigation	Fixed	3	0; 1; 24	Samples of leaflets collected after 0 minutes (before irrigation), 1 minute after irrigation, and 24 hours after irrigation

Results

The results presented below are answers to the research questions, written in the order presented in the introduction.

i) Will the number of spider mites decrease after one minute of water sprinkling?

Due to the large number of eggs compared with mobile spider mites (twice as many eggs as mobile individuals), eggs were first separated from the other spider mite life stages when looking at the development of spider mite populations after irrigation. Time after irrigation was a significant factor for eggs ($F_{2,448}$=8.50, p<0.0005), but not for the total mobile individuals of spider mites ($F_{2,448}$=0.98, p=0.375) (Fig.3.1A & B). Run was significant for all response variables: eggs ($F_{2,448}$=114.71, p<0.0005), larvae ($F_{2,448}$=53.15, p<0.0005), nymphs ($F_{2,448}$=11.46, p<0.0005), adult females ($F_{2,448}$=208.27, p<0.0005), adult males ($F_{2,448}$=105.83, p<0.0005) and total mobile spider mites ($F_{2,448}$=175.34, p<0.0005). The interaction between run and time after irrigation was significant for eggs ($F_{4,448}$=7.25, p<0.0005), and this was a strong trend also for the mobile stages ($F_{4,448}$=2.38, p=0.051).
If the individuals of all spider mite stages were summed, time after irrigation was a significant factor ($F_{2,448}$=5.95, p=0.003), showing that the water sprinkling had an overall effect on the number of spider mites after irrigation, but as noted above the effects were different when separating the spider mites and looking at the life stages individually.
The answer to the question addressed is therefore that the number of spider mites did decrease after one minute of water sprinkling but not consistently in all runs. Hence, the overall effect varied for both eggs and the total mobile spider mite.

ii) Has water sprinkling a different impact on different life stages (eggs, larvae, nymphs, adult females and adult males)?

As already mentioned, time after irrigation was significant for eggs, and even though time after irrigation did not significantly explain the total number of mobile individuals, this factor was significant when each mobile stage was analyzed separately: larvae ($F_{2,448}$=9.87, p<0.0005), nymphs ($F_{2,448}$=4.00, p=0.019), adult females ($F_{2,448}$=5.70, p=0.004) and adult males ($F_{2,448}$=5.65, p=0.004).

The water sprinkling had different impact on the different life stages, meaning, the water sprinkling affected the number of spider mite eggs, nymphs, larvae, adult females and adult males differently (Fig.3.1). The interaction between run and time after irrigation was not only significant for eggs, as mentioned earlier, but also for the other juvenile life stages larvae ($F_{4,448}$=5.45, p<0.0005) and nymphs ($F_{4,448}$=8.02, p<0.0005). For the adult life stages, the interaction was significant for the males ($F_{4,448}$=9.62, p<0.0005) but not for the females ($F_{4,448}$=1.59, p=0.177). Nymphs and adult males decreased during run 3, as opposed to eggs and adult females which decreased during run 2. The number of larvae increased in run 2 after one minute of water sprinkling.

The water sprinkling had a different impact on each life stage where some numbers of mites increased, and others decreased. Each life stage had a significant effect for either 1 minute after irrigation or 24 hours after irrigation for one of the runs (Fig.3.1).

iii) What is the difference in the effect of water sprinkling right after, and after 24 hours?

The effect of water sprinkling was both positive (decrease of spider mites) and negative (increase of spider mites) for the different spider mite life stages after irrigation within the three experimental runs. The number of eggs, adult females and adult males remained relatively stable in run 1 (Fig.3.1). In the two other runs, all life stages significantly changed at least in one case. In total, the most consistent result (i.e. observed in all three runs) seems to be a longer-term effect (after 24 hours) for the mobile life stages that move the most: nymphs, adult females and adult males (Fig.3.1). They all had a significant decrease in numbers 24 hours after irrigation.

There was a greater decline for eggs and adult females 24 hours after irrigation in run 2, in opposition to adult males which had a greater decline 1 minute after irrigation in run 3 where the spider mite population size was bigger compared to run 2. The number of larvae had the greatest increase 1 minute after irrigation and was the only life stage that had a significant increase in numbers and showed no sign of decreasing.

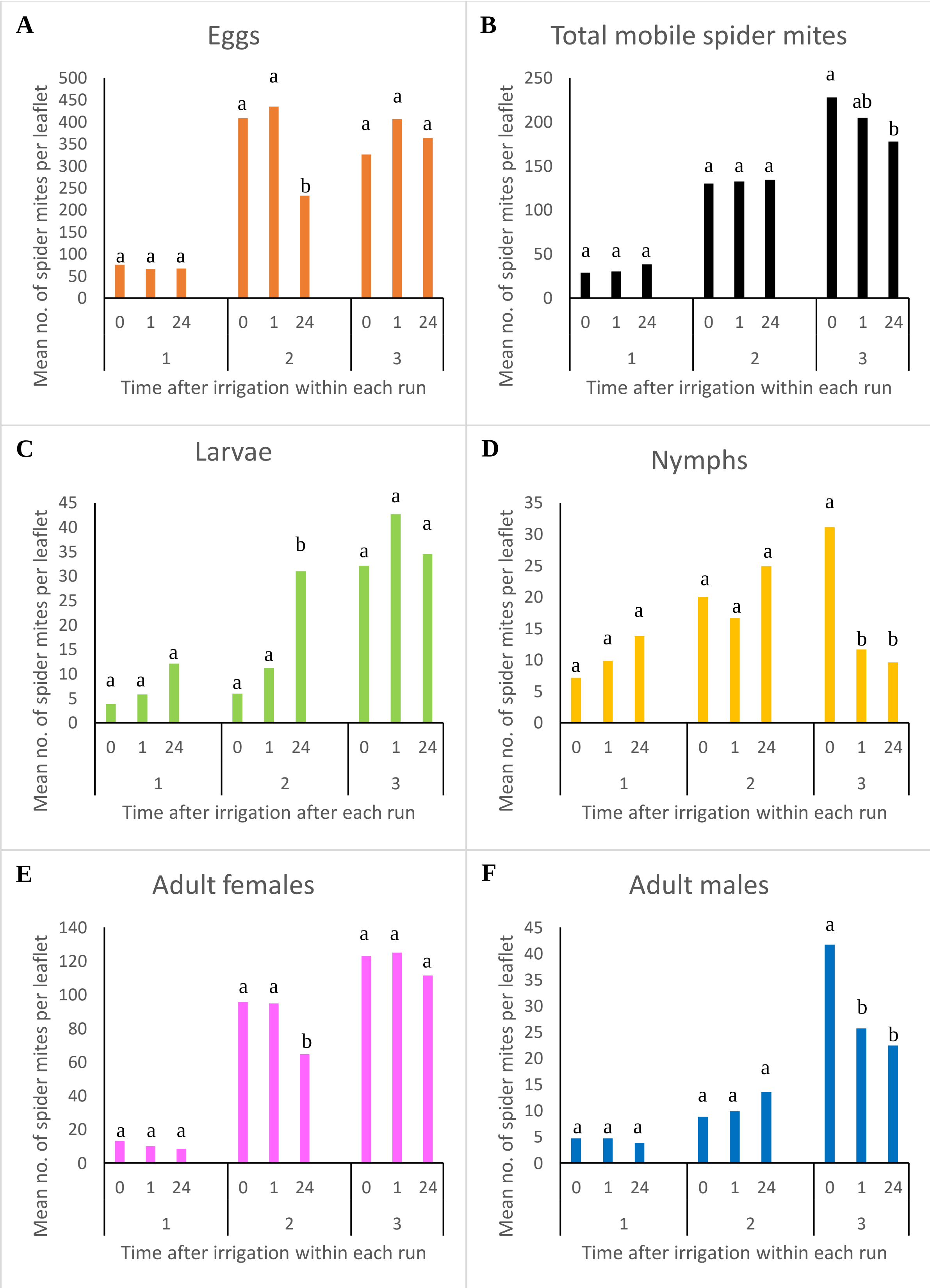


Fig.3.1 Mean number of spider mite eggs, larvae, nymphs, adult females, adult males and total mobile individuals per leaflet in the three experimental runs, before irrigation (0), one minute after irrigation (1) and 24 hours after irrigation (24). Letters were created by doing a Tukey comparison test for each run separately, taken all significant factors into account. Means that do not share a letter are significantly different.

iv) Does the plant position affect number of spider mites present?

Plant position was significant for eggs ($F_{11,448}$ = 2.39, p = 0.007), larvae ($F_{11,448}$ = 2.54, p = 0.004) and nymphs ($F_{11,448}$ = 2.28, p = 0.010). Plant position 1 differed from the other positions by having the fewest mean number of spider mites at all three sampling times (Fig.3.2). Plant position was not significant for adult females ($F_{11,448}$ = 1.64, p = 0.086) and adult males (F = $_{11,448}$ = 1.48, p = 0.136), but the trend was also here that plant position 1 had lower numbers of spider mites compared to the other positions.

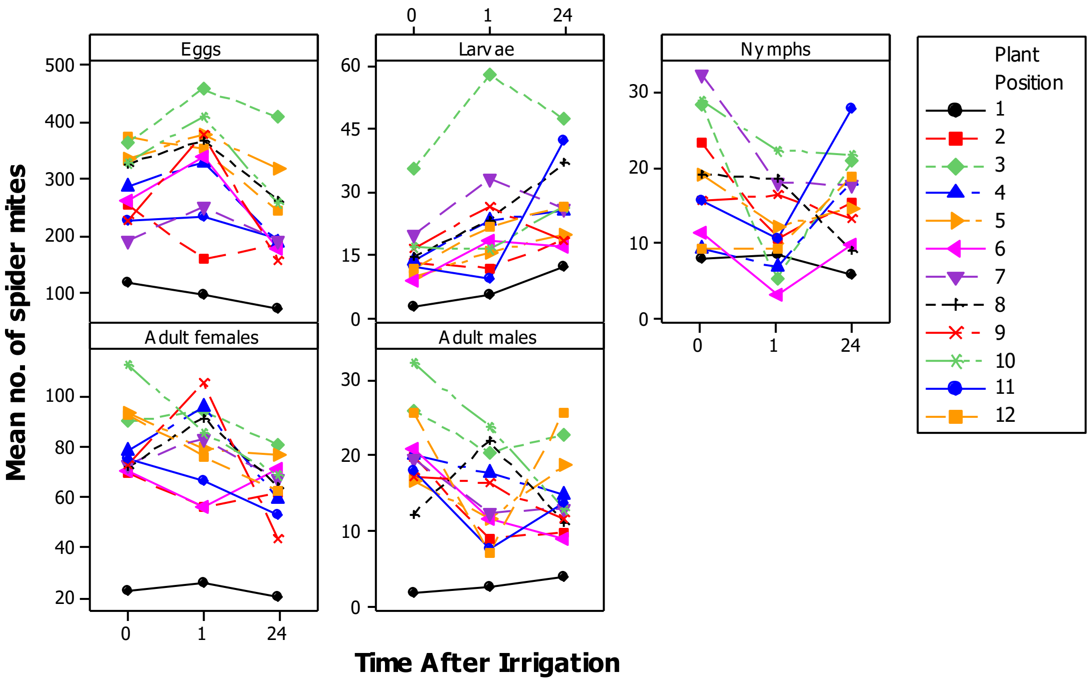


Fig.3.2 The mean number of spider mites present on each plant position before, after one minute and after 24 hours after irrigation.

Discussion

Although the short-term effect was a subtle effect, my results indicate that using water sprinklers on two-spotted spider mites to decrease their numbers have a positive effect. Combining all mobile life stages of spider mites showed no effect for the water sprinkling before, 1 minute and 24 hours after irrigation. However, analyzing eggs, larvae, nymphs, adult females and adult males separately had effect for time after irrigation for all life stages. Spider mite life stages were affected differently where both increases and decreases of spider mites were present. Nymphs and adult males decreased during run 3, while larvae increased in run 2, as opposed to eggs and adult females which decreased during run 2. While the number of eggs, adult females and adult males remained relatively stable in run 1, all life stages significantly changed at least in one case in the two other runs. There were not only differences of effects in water sprinkling between each run, but also within each run for the three times sampled (i.e. before, 1 minute after and 24 hours after irrigation). All life stages had a significant effect in either 1 minute or 24 hours after irrigation in one of the two runs with a high spider mite population where the number of spider mites decrease expect for larvae which increased. Among the 12 different plant positions, position 1 had the fewest number of spider mites compared with the other plant positions.

Adding all the mobile stages of spider mite into total mobile individuals had no effect on time after irrigation. By adding them together, both positive and negative effects within each mite stage were lost due to positive and negative effects equalizing each other. It is therefore favorable to divide the spider mites into life stages for future research because desirable effects may not present themselves if all life stages are summed. The effects might be small but still be significant for the water sprinkling. Nevertheless, one minute with water sprinkling once a day is not enough to keep the population of spider mites under desirable conditions. In the previous study done with powdery mildew, spider mites and water sprinkling, they used one minute of irrigation four times a day (Asalf et al. 2016). This showed a larger positive effect on the spider mites, compared to the effects presented in this paper which gives a good indication to increase the irrigation period or number of irrigations.

The immediate short-term effect, which was not clear-cut, could be explained by missing factors that was not measured in this study, such as temperature and humidity. The short-term effect in this context is related to the wash-off effect happening between 0 and 1 minute after irrigation where the spider mites are simply washed away when encountering the water from the water sprinklers. This effect may be effective in some cases, but not always as demonstrated here. In this study is was effective for adult males and nymphs where the number of mites decreased 1 minute after irrigation. The short-term effect after 24 hours is related to the behavior of the spider mites, happening between 1 minute and 24 hours after irrigation, and their reaction to the water where the change in climate on the plant probably causes them to leave the leaflet due to discomfort (Helle & Sabelis 1985). This applies to the mobile life stages as the eggs are attached to the leaflet, surrounded by web made from the spider mites, and may therefore not be affected to the same extent as the mobile life stages. Moist conditions can also make the spider mites sluggish and almost immobile as a result of the water sprinkling (Mori & Chant 1966). Even though the immediate short-term effect was unclear, the delayed short-term effect is desirable for the strawberry farmer in the long run. Nymphs, adult females and adult males showed a significant decrease in number of spider mites 24 hours after irrigation, where the natural explanation for this is that they do not like water. Spider mites thrive in warm and dry conditions and adding water from water sprinklers can alter these conditions, resulting in a change in behavior of the spider mites. The total mobile spider mites also had a significant decrease in spider mites 24 hours after irrigation in run 3 which may be due to the large decline of males in run 3. Eggs and larvae showed no tendency to being washed off 1 minute after irrigation. As a contrast to the other mobile life stages that showed decrease in numbers, larvae were the only life stage that showed no tendency of decrease. 24 hours after irrigation, eggs decreased while larvae increased. This could have happened during the 24 hours, during the 30-minute period were the leaflets were in the zipbag after sampling. Eggs may have developed to larvae before they were frozen and counted which can explain why eggs decrease and larvae increase in run 2, 24 hours after irrigation.

The short-term effect for all irrigation samples also varied between each run. The mean number of spider mites showed a natural increase from run 1 to run 3 for all spider mite life stages and for total mobile spider mites. This can be explained by the fact that all the experimental plants participating in the three runs were infested at the same time but was tested at different times (one week apart), which means the population size increased as the runs continued. This natural increase is also to be expected in a biological experiment such as this one. In conclusion, this short-term effect is not a simple linear effect, which could be caused by the web produced because of the increasing spider mite population.

The main result from this from study is the fact that the interaction between run and time after irrigation was significant for all stages and that all life stages had a significant change either in run 2 or run 3 where the spider mite population was high. However, there was not an overall consistent short-term effect of the waters sprinkling. A way to increase the effect of water sprinkling is to combine the water sprinkling with other IPM methods. My study only uses water as an IPM method but according to studies done with predatory mites (Force 1967; Opit et al. 2006; Sabelis 1982), biological control would be favorable as a combination with water to increase the effect of the already positive water sprinkling effect where the number of spider mites decrease. Populations of spider mites was significantly reduced with both water sprinkling and the use of predatory mites present in a recent study done in the same field with the same conditions as presented in this experiment (Danielsen 2018), which shows promise of an increased effect by combining water sprinkling and biological control.

Out of all the plant positions, plant position 1 stood out as the one position with the fewest individuals of spider mites. This was also the case in the experiment with water sprinkling and use of *P. persimilis* as a biological control (Danielsen 2018). This experiment had the exact same setup. Plant position 1 stood out among the 12 different position by having fewer individuals before and one minute after irrigation and 24 hours after irrigation, except from nymphs which only stood out 24 hours after irrigation. There could be several explanations for this. Spider mites use wind as a way of dispersing by making threads of web used to drift along the air streams (Fleschner et al. 1956). The mites could have used these threads to disperse from plant position 1 to another position, which would explain the low density of spider mite in this plant position compared to the rest. The water could also cause stress or discomfort to the spider mites, causing them to leave one leaflet and move to another. If position 1 was more exposed to water, causing the mites to move, this would explain the increase in mite density for all plant positions except position 1.
As all plants received water from several sprinklers, one plant position could potentially have received more water from more than one sprinkler. Since there were 6 plants with 3 water sprinklers in each row, the effect from the one-minute irrigation may vary if one plant received water from more than one sprinkler. Plant position could also just show the variation one can expect from water sprinkling, especially considering that the use of one type of sprinkler (like the one used in this experiment) can vary from another type of sprinkler. The use of different types of sprinkler may therefore give different effects and therefore different results.

Temperature and humidity are two important factors, especially when looking at spider mites because these can have a huge impact on the growth of the spider mite population and its further development (Helle & Sabelis 1985). The spider mites' responses to their external factors were not included in this study but are important factors which should be included in future studies when using water as a controlling factor. The change in temperature affects the behavior and activity of the spider mites to a great extent (Mori 1961). Increasing temperatures from 15°C to 21°C increased the number of immatures reaching adulthood and it increased the longevity for both females and males (Herbert 1981). In addition, Herbert (1981) explains that immature spider mites may tolerate higher temperature better than adults do which could explain why the number of immature spider mites live longer and therefore increases in numbers over time, showing lack of decrease from water sprinkling, in this study. This especially applies to eggs and larvae.

Besides from temperature, humidity and air movement, the web made by the mites is also an important factor to consider in this study. There were significant differences between run 1 and run 3 related to the spider mite webbing and that this influenced the spider mite population. Run 1 with a smaller population had a greater positive effect of water sprinkling than run 3 with a larger population. The overall effect was dependent on the spider mite population because the experimental runs were done with a growing spider mite population, which resulted in an interaction effect where the water sprinkling probably were dependent on the spider mite population. The web could therefore be a reason why a larger population has more protection than a small population. As a population of spider mite develop, more web is produced and over time this will give the mites more protection from outside threats, including water sprinkling. The web works as a protectant and provide protection against both biotic and abiotic stress (Oku et al. 2009). The survival of webbed colonies on the strawberry plants under adverse climate conditions contributes to their subsequent rapid population increase (Davis 1952), which in this case is caused by the water sprinkling. If this is the case, a reduction in number of spider mites is to be expected and because of heavy web formation in large population, small populations could be more affected by changes to the environment than large populations (Silvy 2012). Web does not only work as protection for the eggs but could also increase the egg production (Oku et al. 2009). The presence of already existing web increased the oviposition rate of *T. urticae* females resulting in more eggs being produced which could explain the differences in number of spider mites between runs and within runs. Water sprinkling influenced the population size, but the mechanisms behind this has not been properly addressed but it could be interesting to investigate the effect of water sprinkling on population size in the future.

The effects related to the decrease or increase of the spider mite population mentioned so far has been direct effects of water sprinkling, but it is important to address the indirect effect as well. One very relevant indirect effect is of irrigation in microclimate in the canopy of the strawberry plant which may affect the spider mite reproduction. As mentioned before, spider mites thrive in a dry and warm environment which are normal conditions inside cultivation tunnels. By using water and water sprinkling, the microclimate in the strawberry plant changes. The water can decrease the temperature and increase the relative humidity, which could affect the movement and dispersal of spider mites by making the mites move because of discomfort or simple because they are washed off the leaves as a result of the sprinkling. If they were to fall of the leaf, it would take time for the mites to get back up and they would possibly move to another leaf than the original leaf. As mentioned earlier, temperature can affect the oviposition, and this is linked to new changes in microclimate that water sprinkling triggers. Therefore, changes in microclimate affect the temperature which again affects the oviposition of the spider mite females.
The effect of rainfall has also been observed on spider mite populations (AGProfessional 2011). The reproductive rate of mite population was slowed down, but the mites did not go away. They simply hid until the conditions improved. However, one rain event will not be enough to reduce high spider mite numbers below the desired threshold (Cowan 2012). This also requires an increase in humidity and colder weather, or else the spider mite populations are likely to continue to increase.

Conclusion

Water sprinkling had an overall effect on the total number of spider mites but separating the mites into life stages revealed different responses to the minute of irrigation. The experimental runs were done with a growing spider mite population, which resulted in an interaction effect where the water sprinkling probably were dependent on the spider mite population. The results suggested that there was a short-term effect 1 minute after irrigation where number of nymphs and adult males was reduced, but also a short-term effect 24 hours after irrigation resulting in a reduction of eggs, larvae, nymphs, adult females and adult males, all in which the numbers of spider mites decreased for one of the two experimental runs with a high number of mites. However, the short-term effect 1 minute and 24 hours after irrigation, showed trends but not a clear-cut effect and using only one minute of irrigation per day was not enough to control the spider mite population. Considering an earlier study using one minute of irrigation four times a day for several weeks showed an effect, the subtle effects I have demonstrated for one minute of irrigation probably add up, resulting in more consistent impact on the spider mite population, which is desirable for cultivation of strawberry plants in tunnels and for the farmers growing strawberry plants.

In conclusion the mechanisms underlying the water effect presently was unclear. The use of one minute of water sprinkling as an IPM method should therefore not be used alone, but be combined with other methods, such as biological control, to increase the effect.

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