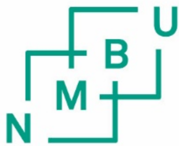


Norwegian University
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Faculty of Biosciences, Department of Plant Sciences (IPV)

# The Effect of UV Radiation on *Myzus persicae* and the Biological Control Agent *Aphidius colemani*

Runa Gidske
M.sc. Plant Sciences – Plant protection

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# Acknowledgement

It did not take long after I started at NMBU before I got bitten by the “entomology bug”, but it still took some time before I understood that crop protection was the field I wanted to pursue. I am grateful for the opportunity to spend over a year on this project, no matter how challenging it has been at certain points. To finish my formal education in plant sciences in the middle of the international year for plant health feels somewhat special, even though I know this is just the beginning my journey within the field of crop protection.

Most of all I would like to thank my supervisors Nina Svae Johansen, who for over a year has patiently and steadily guided me through this process, and Aruppillai Suthaparan, who spent hours with me mounting equipment and doing UV measurements. I am also ever so thankful for the always helpful technicians and engineers at NIBIO and SKP, NMBU and their knowledge, support and assistance. This project would not have been possible without you. This goes especially for Marta Bosque Fajardo who amongst other sat through hours of aphid counting, Elisa Gauslå for keeping an extra eye on my plants and Lars Opseth who always has been at service for any construction related obstacle at hand. I am also grateful for the support from Florian, for always being there for me and helping me through the roughest parts of the experiment workload. I am also lucky to get access to the time and great minds of Vera and Guro, who helped proofreading and gave valuable input in the finishing process.

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# Abstract

Greenhouse light technologies are advancing, giving new opportunities in pest management such as UV treatments against pathogenic fungi. Little is known about how these methods affect other relevant organisms in protected cropping systems, such as arthropod pests and biological control agents. This study investigated the effect of broad-spectrum UV ($\lambda_{peak}$=313 nm) and UV-C ($\lambda_{peak}$=254 nm) on *Myzus persicae,* and the aphid parasitoid, *Aphidius colemani.* Two independent experiments were conducted with *M. persicae* on leaf disks and with *M. persicae* with and without presence of *A. colemani* on whole plants of sweet pepper. In the leaf disk experiment broad-spectrum UV (0,314 $kJ/m^2$/day) and UV-C (0,169 and 0,371 $kJ/m^2$/day) treatments were given. UV-C (0,149 and 0,321 $kJ/m^2$/day) treatments were given in the experiment on whole plants. Significant effects of night-time UV treatments were seen on the lifespan (p=0,000) and development (p=0,000) of *M. persicae* on leaf disks. Average lifespan of *M. persicae* nymphs exposed to UV irradiation ranged from 4,5-7,2 days, compared to 36,7 days in the non-irradiated control. 88-100% of the UV irradiated aphid nymphs died before reaching the imaginal stage. On whole plants a significant effect of UV-C treatments was seen on *M. persicae* population size (p=0,006). After five weeks this study found a 91-90% reduction of aphids on leaves exposed to a higher dose of UV-C, compared to the non-irradiated control. This reduction was similar with biological control alone. UV-C treatments did also affect the spatial distribution of aphids on the plants (p=0,000). An average of 74-77% of aphids sat on the adaxial leaf side in the higher UV-C dose, but no difference was seen on spatial distribution in the non-irradiated controls. No effects of UV-treatments were seen on the parasitism rate by *A. colemani*. Due to high variation and lower averages than in the control, it is still reason to suspect that night-time UV treatments reduce the success of biological control. This study shows that UV treatments can reduce the pest pressure of *M. persicae*, but it is not believed to control established populations to under the economic threshold. Further investigation is needed of how biological control with *A. colemani* can be facilitated where UV treatments are in use.

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# Introduction

Aphids (Hemiptera: Aphididae) are  important insect pests with over 250 species feeding on agricultural or horticultural crops, of which about 100 species are of significant economic importance (Blackman & Eastop, 2007b). Whereas aphid feeding behaviour can damage crops directly, especially when densely populated, are the main crop damages are caused by transmission of viruse diseases. Aphids serve as the largest group of insect-vectors where 192 species have been registered of transferring 275 insect borne plant viruses (Nault, 1997). One of the most notorious vectors is the green peach aphid, *Myzus persicae* Sulzer. This is an extremely polyphagous species, with host plants in over 40 taxonomical families and acting as a vector for over 100 plant viruses (Blackman & Eastop, 2007a). *Myzus persicae* is found worldwide and the wide host range makes it an important pest in field and greenhouse grown crops.

As a greenhouse pest, *M. persicae* is found to be feeding on all species dominating the cut flower market and high value vegetable crops, such as tomatoes, peppers and cucumbers (Blackman & Eastop, 2000; CABI, 2020; van Liemt, 1999). The greenhouse climate is beneficial for the development of *M. persicae.* The aphid in its viviparous[1] stage can have a generation time between one to two weeks, leading to a rapid population growth. In favourable conditions female *M. persicae* can produce up to around 60 female offspring; As they reproduce parthenogenically[2], the infestation of one single individual may lead to a population of more than hundred individuals within a couple of weeks (Barbosa et al., 2011; Blackman, 1974; La Rossa et al., 2013; Ozgokce et al., 2018).

*M. persicae* has been reported to show resistance to most groups of insecticides, making it one of the most resistant insect pests in the world. This is due to many years of intensive insecticide use in combination of high reproductive rate and relatively high genetical diversity, even for individuals of asexual reproduction (Bass et al., 2014; Loxdale, 2009). Development of insecticide resistance, in combination with socioecological concerns demand alternatives for management of *M. persicae*.

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[1] **Vivipary:** Giving birth to living young
[2] **Parthenogenically reproduction:** Reproduction without fertilization of eggs

An important biocontrol agent used against *M. persicae* is the parasitoid[3] wasp *Aphidius colemani* Viereck (Hymenoptera: Braconidae). This parasitoid has a wide host range, consisting of about 40 aphid species. The female *A. colemani* oviposits a single egg in its host, in which the larva feed and pupate (*A. colemani* pupa is hereby referred to as *aphid mummies*), killing the aphid in the process before an adult *A. colemani* emerge from the mummy (Stary, 1975). *Aphidius colemani* is used worldwide for biological control of aphids in field and greenhouse crop production. It is one of our most economical important augmentative biocontrol agents, and has been mass reared for commercial sale since the early 1990's (Benelli et al., 2014; Prado et al., 2015; van Lenteren, 2012).

The use of light in protected crop production systems is being advanced parallel with advancement of artificial lightning. Crop growth and development can be regulated with management of the optical environment in the crop production system. In addition, the optical environment can also be used in management of pests and diseases. Automized UV-treatments at night is one of these novel pest management strategies and has been shown to be effective against many powdery mildew pathosystems. Brief night-time broad-spectrum UV ($\lambda_{peak}$=313 nm) treatments on a variety of greenhouse crops decreased the infestation significantly, as much as up to 99%, compared to non-treated plants (Suthaparan et al., 2012; Suthaparan et al., 2014; Suthaparan et al., 2016). The reason for night-time treatments is that UV exposure after dark prevents the DNA repairing effect of photoreactivation that has been observed in both fungi and spider mites (Galland, 1996; Murata & Osakabe, 2014). Commercial greenhouse trials in Norway have shown that similar treatments kept the powdery mildew infestation well under the economic threshold, even though it did not remove the powdery mildew entirely (Johansen et al., 2017). This kind of technology is on the verge of commercialization. Consequently, it is crucial to develop knowledge on how this technology may affect other organisms relevant for greenhouse production, such as arthropod pests and biological control agents.

UV radiation is known for its potentially harmful effect on living organisms. Depending on wavelength, irradiance level, duration of exposure, as well as the complexity of the organisms, UV can induce mutations and/or kill simple organisms if it is absorbed by DNA (Sutherland, 1995). The energy intensive, short wavelength in UV radiation promotes the formation of reactive oxygen species (ROS) in the cells by excitation of water. The highly

[3] **Parasitoid:** "An insect whose larvae live as parasites which eventually kill their hosts" – Oxford dictionary

reactive potential of ROS coupled with concentration may harm components in the cell, such as proteins, lipidic membranes and DNA (Shindo et al., 1994). It is suggested that small organisms are more fragile for UV mediated cell damage, due to their large surface to weight ratio (Suzuki et al., 2009). This makes it relevant to question if UV treatments can be suitable to control arthropod pests, and whether the optimized UV application affects the efficiency of beneficial species used against pests.

The earlier mentioned trials conducted in Norwegian commercial greenhouses showed no negative effect of UV treatments on population density of predatory mites *Neoseiulus cucumeri, Phytoseiulus persimilis* and the predatory bug *Macrolophus pygmaeus,* who all are biological control agents. However, a reduction of spider mites *(Tetranychidae ssp.)* compared to the untreated plants was observed (Johansen et al., 2019b). A similar trend was seen on two-spotted spider mites (*Tetranychus urticae*) in laboratory experiments done on cucumber; The mortality of spider-mites was significantly higher on plants treated with a combination of reflective ground cover, green light and broad-spectrum UV ($\lambda_{peak}$=313 nm 0,288 ± 36 kJ/m$^2$/day), compared to in the control without any light treatment (Øyri, 2017). One time broad-spectrum UV (280-315 nm) treatments in combination with other light has showed a mixed effect on English grain aphid (*Sitobion avenae)* on barley, dependent on the dose; Whereas aphids treated with lower dosages of broad-spectrum UV (216 kJ/m$^2$) had a shorter development time and higher fecundity than the untreated control, the aphids treated with higher doses (432 and 864 kJ/m$^2$) showed an extended development time of nymphs, as well as reduced the weight and fecundity of adults (Hu et al., 2013a). Contrasting effects have been reported in studies on solar UV-B and aphid fitness. While solar radiation with no UV -B filtration showed higher fecundity of *M. persicae* on lettuce, an opposite effect has been reported in cabbage aphids (*Brevicoryne brassicae*) on broccoli (Kuhlmann & Muller, 2010; Paul et al., 2012).

Limited studies have shown how some parasitoid wasps have higher reproductive success in UV-B treated hosts or prefer UV-B treated over un-treated hosts when given a choice (Edwin et al., 2016; Foggo et al., 2007; Van Atta et al., 2015). Van Atta et al. (2015) found that *Trichogramma ssp.* had higher parasitism activity in UV-B irradiated ($\lambda_{peak}$=302 nm, 1,5 W/m$^2$) environments, in spite of a reduction of wasps emerging from host eggs exposed to UV-B irradiation (21,6 kJ/m$^2$/day for four days) compared to the non-UV control. On the other hand, there are no published studies available of how additional UV-C treatments affects parasitoid wasps directly. A pilot study on *A. colemani* and *M. persicae* conducted by Johansen et al.

(2019a), showed a tendency of reduced mummy production and hatching in treatments with daily UV-C ($\lambda_{peak}$=254 nm, 0,270 kJ/m$^2$/day) exposure. In the same study broad-spectrum UV treatment ($\lambda_{peak}$=313 nm, 0,929 kJ/m$^2$/day) appeared not to differ from the non-irradiated control in regard of mummy production or hatching. However, there was a reduction in adult *A. colemani* longevity noticed in both UV treatments. Even though the experiment was conducted on leaf disks and is therefore not representable to greenhouse conditions, these results may still indicate that the effect of biological control by *A. colemani* can be compromised when exposed to UV treatments against fungal pathogens. To get a clearer picture of the effect of UV treatment on *M. persicae* and *A. colemani* and how this affects pest pressure and biological control, more knowledge is needed at a whole plant level. This will tell us more about the compatibility of UV treatments against pathogenic fungi and parasitic wasps as biocontrol agents in an integrated pest management (IPM) system.

### Research objectives and study questions

The main objective of this study is to investigate if UV treatment schemes used against powdery mildew can be used in control of *Myzus persicae,* with and without the biological control agent *Aphidius colemani*. Secondary objectives are (1) to study if UV exposure changes the spatial distribution of *M. persicae* and aphid mummies on the host plant and (2) investigate on an individual level in what degree UV treatments affect the survival, development time and fecundity of *M. persicae*.

## Materials and methods

Two independent experiments were done in this study: (1) direct exposure of UV on *M. persicae* on leaf disks in medicine cups (hereby referred to as *the leaf disk experiment*), and (2) UV exposure on whole plants infected with *M. persicae*, with or without presence of the biocontrol agent *A. colemani* (hereby referred to as *the cage experiment*). In the leaf disk experiment developmental time, fecundity and longevity under different UV-treatments were investigated. In the cage experiment *M. persicae* population size and – growth, parasitism rate by *A. colemani* and the hatching success of aphid mummies, as well as spatial distribution of *M. persicae* and aphid mummies on the plant were investigated.

## Organisms used in the experiments

### Plants

Sweet pepper plants (*Capsicum annuum 'Laerte'*) cv. Enza Zaden were used for the experiments, as well as for rearing *M. persicae*. Seeds were sown in petri dishes containing 90% peat and 10% fine sand (Go'jord, Degernes torvstrøfabrikk) and incubated in a controlled environment chamber with air temperature and relative humidity of 22°C and 70%, and with a daily light cycle of 16 h with an irradiance of 66 ± 5 µmol $m^{-2}$ $s^{-1}$. The germinated seedlings with 3-5 cm in height were transferred to 12 cm diameter plastic pots filled with similar substrate mixture (one plant per pot). The transplants were maintained in a controlled environment chamber with similar environmental conditions described above. Plants were irrigated three times per week with tap water. Plants grown in this condition were used for leaves for the leaf disk experiment and whole plants for the cage experiment.

### Aphids

A laboratory strain of *M. persicae* was used in both experiments. The strain was established by individuals collected from Napa cabbage (*Brassica rapa subsp. Pekinesis*) in year 2000 in Rogaland (Norway) and has since been reared on *C. annuum* (and *C. annuum 'Laerte'* for over a year before the beginning of the experiment) in a controlled environment chamber with air temperature and relative humidity of 22°C and 60%, and a daily light cycle of 16 h with an irradiance of 52 ± 9 µmol/$m^2$/s. The laboratory strain has not previously been exposed to UV since collection from the field.

Daily light cycle and irradiance was provided by fluorescent tubes (Philips, Master TL-D 90 Graphica 36W/965)[4] in both chambers rearing *C. annuum* and *M. persicae*. The irradiation was measured with LI-250 light meter with Li-190R quantum sensor (LI-COR).

### Parasitoid wasps

The parasitoid wasp, *A. colemani*, used in the cage experiment was the commercial product Aphiline (Bioline) delivered as pupae in aphid mummies (batch 4344 21027). The mummies were separated in vials to hatch separately and then sorted by sex. The vials were kept closed

[4] See **Feil! Fant ikke referansekilden.** in appendix for spectral emission of the growth lights used in this study.

and each wasp was offered honey (Flytende økologisk honning, Honningcentralen) diluted to 10% in water, by a moist piece of cotton in the vial opening.  All individuals of *A. colemani* used in the experiment was hatched within the same 24 hours.

## UV radiation sources, spectral distribution and level of irradiance

Both broad-spectrum UV ($\lambda_{peak}$=313 nm) (Philips Broadband TL 20W/12 RS Ultraviolet-B) and germicidal UV-C ($\lambda_{peak}$=254 nm) (LightTech GHO600T5L/MBP DE) fluorescent tubes were used in the leaf disk experiment. In the cage experiment, only UV-C ($\lambda_{peak}$=254 nm) (OSRAM Germicidal lamps, HNS 15 W G13) fluorescent tubes were used. Spectral emission of the UV radiation sources (Figure 1) was characterized with an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, FL). The level of UV irradiance was measured at every second, both directly under the lamp and between the lamps, at different heights throughout the experiment (Figure 2). Measurements were done using UV-C (PMA 1122, Solar Light Company Inc, PA) and UV-B (SKU 430, Skye Instruments Ltd, UK) sensors (calibrated against measurement with Optronic model 756 spectroradiometer) connected with a data logger (CR1000X, Campbell Scientific, UK). Daily doses of UV (Table 1, Figure 3) were calculated by integrating irradiance level measured every second within the exposed duration.[5]

All UV-treatments in the experiments started five minutes after onset of the dark period and were automized with digital electrical timer switches.

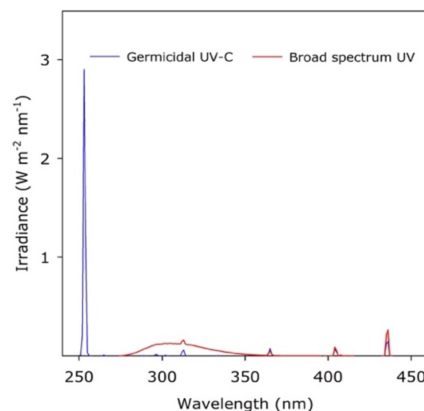



*Figure 1: Spectral distribution of the UV radiation sources (used in this study) measured at 1 nm wavelength intervals. Germicidal ultraviolet radiation sources with peak emission at 254 nm (Germicidal UV-C) and the broad-spectrum ultraviolet radiation source (broad spectrum UV) with spectral emission peat at 313 nm. Peaks within UVA and blue light overlap for both radiation sources.*

[5] See figure 2 in appendix for W/m$^2$ measurements at every second of the UV exposure in each treatment.

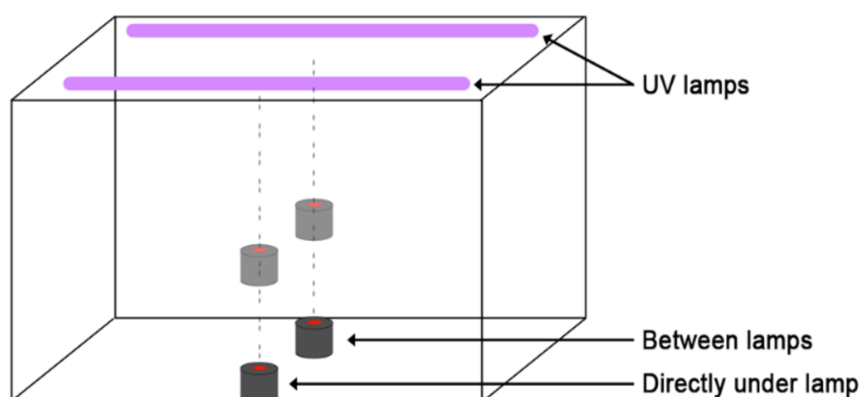
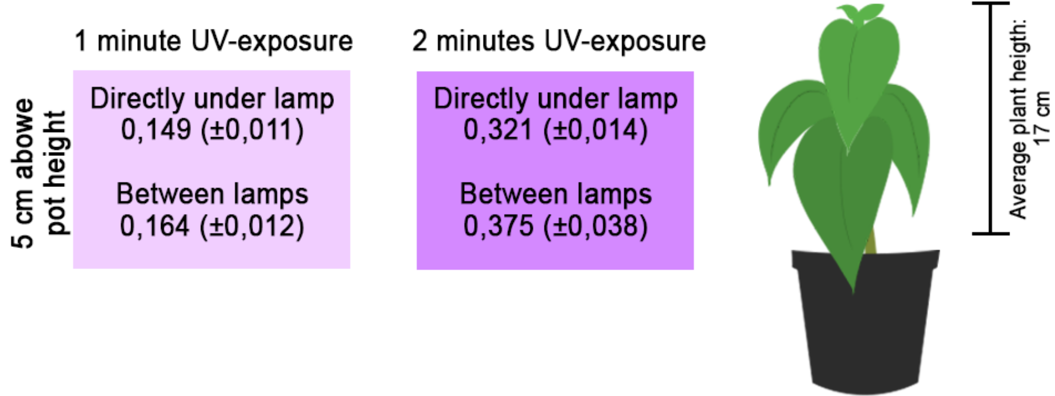



*Figure 2: The UV radiation dose measurements were done by placing the UV sensor at two different positions: directly under the lamp and between the lamps. At the beginning of the experiment the measurements were done at 5 cm above pot level. At the end of the experiment they were done at 5 cm and 22 cm above pot level (average plant height at the end of the experiment).*

*Table 1: Irradiation dose for each UV-treatment given in the leaf disk experiment. Irradiation was measured at the leaf disk height. Dosages were calculated by integrating irradiance level measured every second within the exposed duration, then adjusted to 85% filtration by medicine cup lid.*

| UV type | Wavelength (nm) | Duration of exposure (minutes) | Dosage per 24 hours ($kJ/m^2/day$) |
|---|---|---|---|
| Broad-spectrum UV | 250 nm – 315 nm | 2 | 0,314 |
| UV-C | 250 nm – 280 nm | 1 | 0,169 |
| UV-C | 250 nm – 280 nm | 2 | 0,371 |
| No UV | - | - | - |



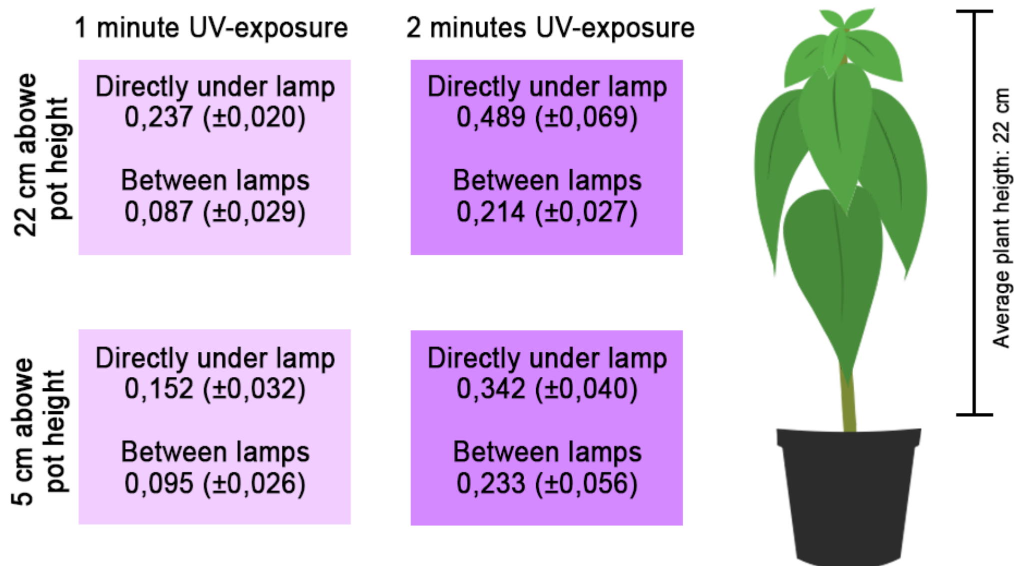




*Figure 3: Average UV-C irradiation dosages (kJ/m$^2$/day) at different heights at A) the beginning and B) the end of the cage experiment. All dosages were measured under and between the lamps.*

## Experimental design

### Leaf disk experiment

The leaf disk experiment was conducted at NIBIO, Ås (Norway). Leaf disks with a 24 mm diameter was cut from plants of *C. annum* and placed in medicine cups with agar prepared as described below.

30 ml medicine cups (Figure 4) were filled with 25 ml 1,5% water agar containing 3,76 µM kinetin to promote leaf freshness. The ~32 mm of the centre of the 40 mm medicine cup lid was removed, and the lid was covered with UV transparent (>85% UV transmission) plastic film (F-CLEAN; AGC Inc, Tokyo, Japan). The film was punctuated with small holes to allow airflow.

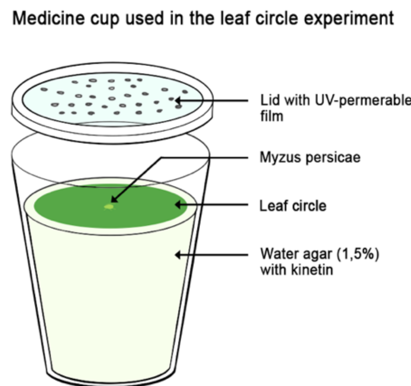



*Figure 4:Medicine cup containing 1,5% water agar with 3,76µM kinetin and leaf disk from Capsicum annuum (24 mm diameter) as used in the leaf disk experiment. One individual of M. persicae was kept on the leaf disk in each cup.*

A drop of distilled water was used to fix the leaf disk to the agar surface. One one-day old (< 24 hours) *M. persicae* nymphs were placed on the leaf disk with a brush. The samples were transferred to chambers with air temperature and relative humidity of 22 ± 5 °C and 60%, respectively. The daily light cycles of 16 h with an irradiance of 29 ± 8 µmol/m$^2$/s were supplied with fluorescent lamps (Philips, MASTER TL-D 90 Graphica 36W/965). UV-treatment was started the same day. Groups of 14-16 randomly chosen medicine cups containing one aphid each was treated with one of four treatments (1) Two minutes of daily exposure with broad-spectrum UV, (2) one minute of daily exposure with UV-C, (3) two minutes of daily exposure with UV-C, and (4) No UV (control) (Table 1).

It was not possible to completely randomize the treatments, due to the space between the shelves at the experimental site, and the different distance needed from the UV-lamps to the leaf disks to obtain correct UV dosage. As a result, both the UV-C treatments was given on the

upper shelf, whereas the broad-spectrum UV was given on the second shelf. To assess if placement had an effect on the results was one control placed on each shelf and treated as different treatments. The placement of the treatments within each shelf was randomized (Figure 5). The experiment was repeated twice.

Daily registrations of survival, developmental stage and number of offspring were done until the day of death for each individual of *M. persicae.* An individual was registered as dead if it had lost the grip of the leaf surface and showed no response to touch.

| | Entrance → | | |
|---|---|---|---|
| Upper shelf | UV-C 2 min | UV-C 1 min | Control |
| Lower shelf | | Control | UV-B 2 min |

*Figure 5: The experimental setup for the leaf disk experiment. N=14-16 medicine cups containing M. persicae nymphs were placed within each treatment. The experiment was repeated twice.*

**Cage experiment**

The cage experiment with randomized complete block design (RCBD) was conducted in three controlled environment growth chambers (as block) at Centre for controlled environment plant research (SKP), NMBU, Ås (Norway). Air temperature inside these chambers were 22 ± 5 °C with no control in relative humidity. Daily light cycle of 16 h with an irradiance level of 93 ± 5.5 $\mu mol/m^2/s$ (measured with LI-250 light meter with Li-190R quantum sensor, LI-COR) was supplied with warm white fluorescent lamps (OSRAM, L 58 W/840 LUMILUX).

Plants with an average height of 17 ± 2 cm and bearing 6-7 fully developed leaves were placed as five in cages (105 x 150 x 55 cm in size) constructed with fine viscose mesh stapled to frames of wood on metal shelves (Figure 7). Due to low percentage of sprouting in preparation of the cage experiment plants were sown in two rounds, two weeks apart, whereas the biggest plants were set in 17°C to slow down the growth the last two weeks before aphid infection. This was done to promote a homogenous height of all plants. A similar composition of the two plant groups were put in each cage. During the last three weeks of the experiment the plants in the cage experiment were irrigated with a fertilizer-water solution of 1,25 ml Superba (Nordic garden AS) per litre of water.

Plants were then infected with two one-week old individuals of *M. persicae* placed on the $6^{th}$ or $7^{th}$ true leaf. All aphids used for infection were in $4^{th}$ or $5^{th}$ instar, respectively last nymphal stage or imago. Both aphids were placed, within a clip cage, on the adaxial side of the leaf

(Figure 6) to make it possible to control establishment and reproduction. One aphid died after one day and was replaced by a same age individual. After three days, when all aphids were established, and reproducing, the clip cages were removed for the aphids to move freely on the plants. Seven days after infection, the following treatments were started. The aphid infected plants were given a total of six separate treatments, consisting of three UV-C treatments from irradiation sources fixed to the roof of the cage (1 minute, 2 minutes or no UV) in combination with presence or absence of the biocontrol agent *A. colemani*. (Figure 8). The treatments were randomized and replicated three times (Figure 9).

In the cages where presence of *A.* colemani was included in the treatment, three females and two males were released per cage one hour before starting the UV treatments. The wasps had hatched 7 ≤ 31 hours before release.

*Figure 6: Clip cages as shown in the picture were used to control establishment and reproduction of M. persicae on each plant in the in the cage experiment.*

*Figure 7: The cage experiment was conducted in three growth chambers, each holding one replication of the experiment. Each chamber contained six cages, three of which are seen in the above picture. Each cage holds five plants of Capsicum annum infected with M. persicae, and one of six different treatments are given in each cage.*

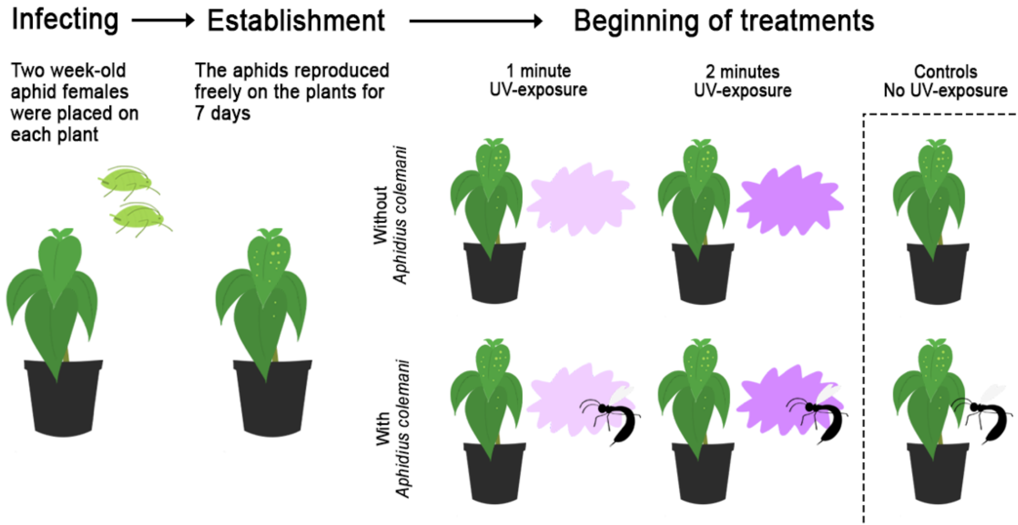



*Figure 8: Procedure for infection and establishment of M. persicae on C. annuum plants followed by the six different treatments, as given in the cage experiment.*

| Block 1 | | | | | Block 2 | | | | | Block 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | | | | | | | W | | | | | W | |
| W | | | | W | W | | | | W | W | | | W | |
| Upper shelf | Lower shelf | Door | Lower shelf | Upper shelf | Upper shelf | Lower shelf | Door | Lower shelf | Upper shelf | Upper shelf | Lower shelf | Door | Lower shelf | Upper shelf |

*Figure 9: Experimental setup for the cage experiment, where one cell represents one cage. Each cage contained groups of five plants of C. annuum infected with M. persicae. The treatments given to in the cage experiment were all possible combinations of three UV treatments (no UV, 1 minute UV-C and 2 minutes UV-C) and presence or absence of the biocontrol agent A. colemani, giving a total of six different treatments. Light purple represents 1 minute of UV exposure per 24 hours and dark purple is 2 minutes of UV exposure. White cells were not exposed to UV. Cells marked with W represent a treatment including the parasitic wasp A. colemani. Grey areas show empty shelves and floor space.*

Registrations of *M. persicae* population size, age distribution (nymphs vs. imagines) and distribution on the plant (abaxial leaf side vs. adaxial leaf side) were done after two and five weeks. Registrations were also done for the number of *A. colemani* mummies, if they were hatched or not and their distribution on the plant. Some plant characteristics were also registered, being plant height and number of leaves with marks of UV damage. The number of leaves on the plant was only registered at the five week registration.

## Statistics

Data was analysed in MiniTab (version 19.2). Mixed model ANOVA was used to analyse the effect of the different treatments to the response variables in both experiments. In the leaf disk experiment the response variables were 1) lifespan and 2) reached instar level. The response variables in the cage experiment were 1) population size, 2) population change over time, 3) parasitism rate, 4) percentage hatched aphid mummies, 5) *M. persicae* age distribution, 6) spatial distribution *of M. persicae* and *A. colemani* on the plant, and 7) leaf damage on the plants. Replication was modelled as a random factor in both experiments, whereas registration time was modelled as an additional fixed factor in the cage experiment.

When a significant effect of the treatments on the response variables was found, the treatments were grouped using Tukey's pairwise comparisons. Significance level of 95% was used for all statistical tests. The data was checked for normal distribution and homogeneous variance using the usual residual plots, and no critical deviations from these assumptions were found. All analyses were done with untransformed values of the response variables. Some simple modifications of the raw data were done before the statistical analyses. They are all described as follows.

**Definitions used in the statistical analyses**

Mortality at each day for each treatment in the leaf disk experiment was found using the following equation:

$$Mortality_{day(x)} = \frac{\sum_{i=1}^{x} dead\ aphids_{x}}{Initial\ number\ of\ aphids} \times 100$$

In the cage experiment *M. persicae* spatial distribution and age composition were found by dividing the investigated factor (adult aphids or aphids sitting on the adaxial leaf side) by the total number of aphids and multiplied with 100, as following:

$$\%\ Aphids\ on\ the\ adaxial\ leaf\ side = \frac{Total\ aphids\ on\ the\ adaxial\ leaf\ side}{Total\ live\ aphids} \times 100$$

For calculating the percentage of hatched aphid mummies was the number of hatched mummies divided by the total number of mummies. The parasitism rate was calculated by dividing the total number of mummies by the by the sum of the total number of mummies and live aphids. The last equation is illustrated below.

$$Parasitism\ rate\ (\%) = \frac{Total\ aphid\ mummies}{Total\ aphid\ mumimes + Total\ live\ aphids} \times 100$$

# Results

## Leaf disk experiment

Due to technical implications mentioned earlier there were two separate controls in the leaf disk experiment, one on each shelf. The results from both controls were tested statistically as two treatments to test if shelf placement had an effect on survival, development and fecundity of *M. persicae*. No significant effects of shelf were found on these response variables (all p-values ≥ 0,373) and the two controls were merged to one treatment for the further statistical analysis.

**Shorter lifespan in UV treatments**

UV exposure had a significant effect on the average lifespan (birth to death) of *M. persicae* (p=0,000). Longevity was significantly lower for all the UV treatments, compared to the control (Figure 10). Where the individuals in the control live for a bit more than a month in average, were the ones exposed to UV only living for a week or less, with a tendency of life length reduced in by shorter wavelengths treatments. It was also seen a large variation in the age of the longest living individual between the treatments (Table 2). Where all individuals were dead after seven and nine days of UV-C exposure in the 1-minute and 2-minute treatment, respectively, did the longest living individual in the control live for two months. The longest living individual in the 2-minute broad-spectrum UV treatment lived 25 days longer than the average in this treatment and twice as long as second oldest individual (Table 2, Figure 11).

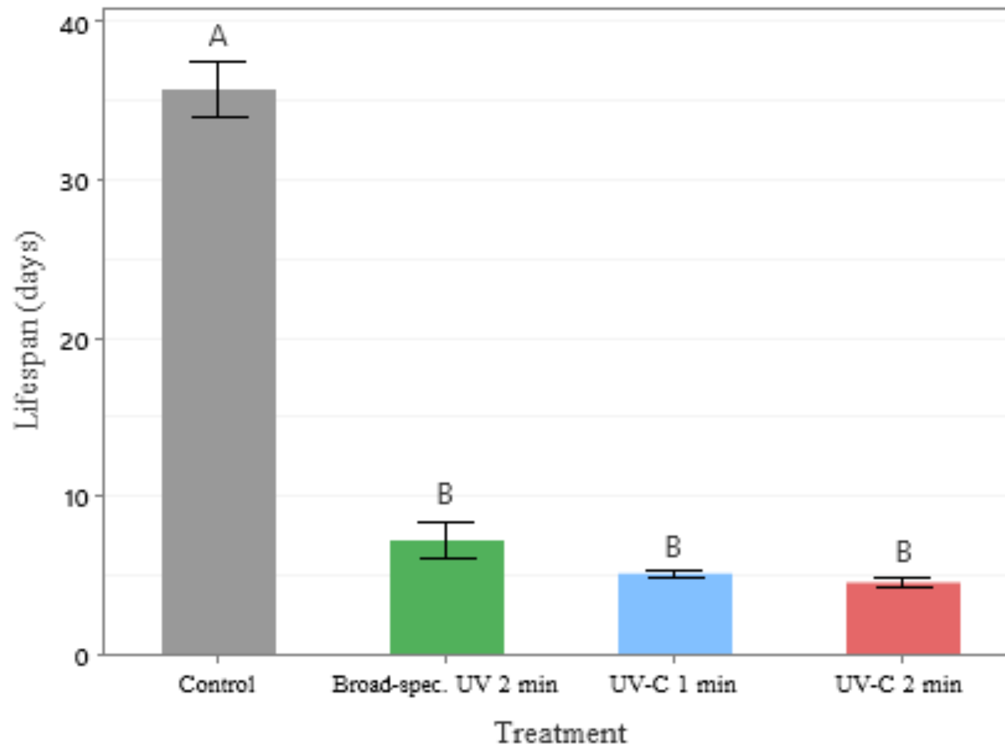



*Figure 10: Average lifespan in days for M. persicae kept on leaf disks in medicine cups under different UV treatments from ≤ 24 hours after birth and until they died. Effect of treatment on life expectancy was significant (p=0,000). The letters over the bars illustrate grouping done with Tukey's pairwise comparison. Average values that do not share a common letter are significantly different. Interval bar show 1 SD calculated for each average value. (n=28-59 per treatment)*

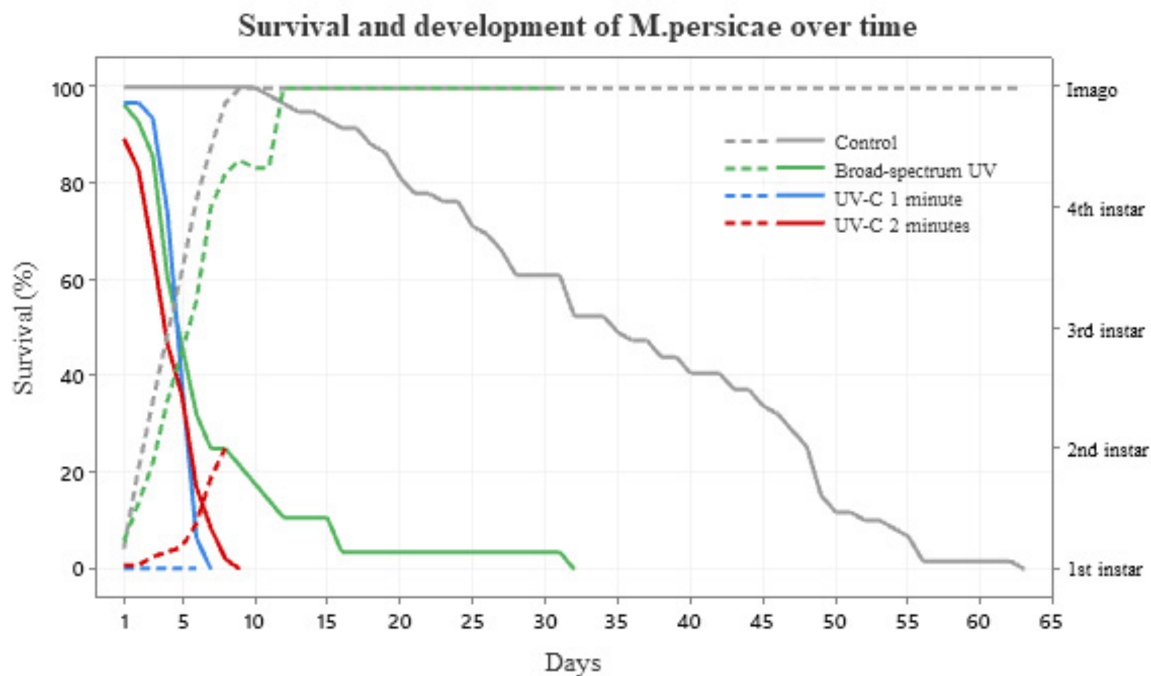



*Figure 11: Change over time (days) in percentage of survival (whole lines and primary y-axis) and average development stage (dotted lines and secondary y-axis) for M. persicae kept on leaf disks in medicine cups under different UV treatments from ≤ 24 hours after birth. Similar colours represent similar treatments. (n=28-59 per treatment)*

### Development time and reproduction

An effect on aphid development, with reduced development in the *M. persicae* exposed to UV, was also found (Table 2, Figure 12). Only 12% of the *M. persicae* in the broad-spectrum treatment developed to imagines. In the control group all did. None of the nymphs exposed to UV-C developed to the imaginal stage (Table 2). The average instar level reached in both the UV-C treatments was between the first and second instar, whereas the average for the broad-spectrum UV treatment was between the second and the third instar level (Table 3). The effect of treatment on the average instar level reached by *M. persicae* is significant (p=0,000), and the average instar levels reached are significantly different between the control and the UV treatments, as well as between the broad-spectrum and UV-C treatments.

Of the 12% individuals reaching the imaginal stage in the 2-minute broad-spectrum UV treatment the average number of offspring produced per imago was 19, much lower than for the control that produced 63 offspring (Table 2). However, a clear deviation was seen in the longest living individual in the 2-minute broad-spectrum UV treatment, which got 85 offspring.

*Table 2: The effect of direct exposure by broad-spectrum UV and UV-C on longevity, development time and fecundity in M. persicae on leaf disks Due to the low percentage of individuals reaching the imaginal stage in the broad-spectrum UV treatment mixed model ANOVA was not used to compare development time and average number of offspring per imago.*

| | **Control** | **Broad-spec. UV 2 minutes** | **UV-C 1 minute** | **UV-C 2 minutes** |
|---|---|---|---|---|
| **% aphid mortality by the point of average longevity** | 54% | 79% | 61% | 53% |
| **Longest living individual (days)** | 63 | 32 | 7 | 9 |
| **% aphids reaching the imaginal stage** | 100% | 12% | No nymphs developed to imaginal stage | |
| **Average development time to imaginal stage (days)** | 7 (± 0,8) | 9 (± 2) | | |
| **Average number of offspring per imago** | 63 (± 21) | 19 (± 37) | | |

*Table 3: Average instar level reached for M. persicae in the different treatments in the leaf disk experiment. The letters illustrate grouping done with Tukey's pairwise comparison, where average values that do not share a letter are significantly different. Values in bold are significantly different from the control.*

| | Control | Broad-spec. UV 2 minutes | UV-C 1 minute | UV-C 2 minutes | P-value |
|---|---|---|---|---|---|
| **Average instar level reached** | 5,00 A | **2,57 B** | **1,04 C** | **1,03 C** | p=0,000 |

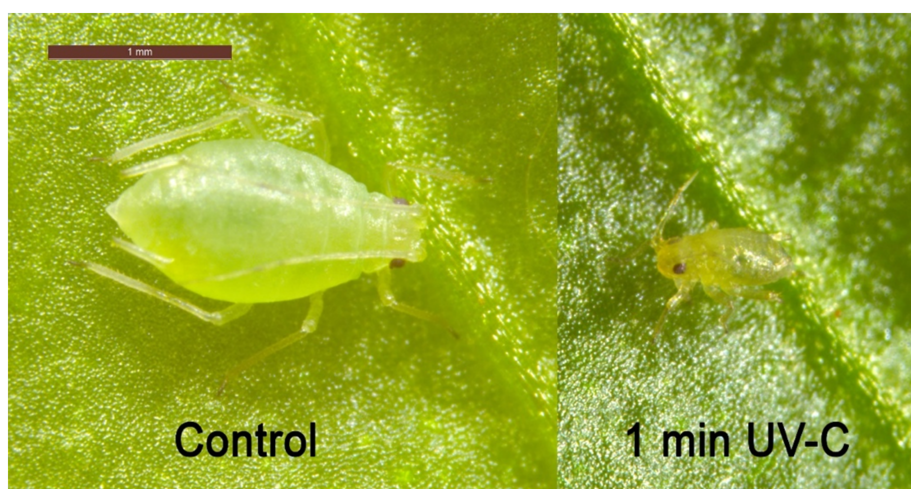



*Figure 12: Two individuals of M. persicae 7 days after birth. The one to the left (control) has not been exposed to any UV and the one to the right has been exposed to one minute of UV-C (0,169 kJ/m²/d) treatment every night, 5 minutes after onset of dark period. Both have been kept on leaf in the same environmental conditions. Look and development stage is representable for both individuals, but it is worth noting that the individual exposed to 1 minute of UV-C is the longest living individual in this treatment. (1 mm reference bar)*

### Other observations

It was observed that many of the *M. persicae* individuals treated with UV had an abnormal behaviour. Where the individuals in the control mostly would be found attached and feeding on the leaf disk, many of the UV treated aphids would be found lying on their back on the leaf or the water agar. This behaviour was not registered continuously throughout the experiment, but at day four of one replication 72% of the individuals in the 1-minute UV-C treatment were observed lying on their back.

*Figure 13: Example of misshapen antennaes in an individual in the 2-minute broad-spectrum UV treatment (right) compared to the non-irradiated control (left). Both individuals are 15 days old and in the imaginal stage. The individual in the control is representable for this group, whereas the individual exposed to broad-spectrum UV is only representable for the individuals reaching this age. (1 mm reference bar)*

Misshaped antennaes were also observed in the treatments with UV exposure (Figure 13). This phenomenon was not registered throughout the experiment, but it was noted that this occurred in later developmental stages. These misshapen antennaes were shorter than usual and often with darker colour and uneven structure.

## Cage experiment

### Aphid population size

It was seen a clear effect of treatment on *M. persicae* population size, both for the total aphid number in the cage (p=0,001) (Figure 14) and for the number of aphids per leaf (p=0,006) (Figure 15-Figure 16). After two weeks the number of aphids per cage was significantly lower in all the treatments including UV than the treatments without UV exposure (Figure 14). However, this was shifted after 5 weeks. The shift was due to a steep population growth in the treatment with 1 minute of UV and without *A. colemani,* and a reduction in the population size for the treatment without UV and presence of *A. colemani* A reduction in population size from the first to the last registration was only seen in the latter (Figure 17). Even though the highest aphid number was in the control treatment, the largest population growth from the first to the last registration was seen in both the treatments with 1-minute UV exposure (Figure 14, Figure 17). When only comparing the treatments with presence of *A. colemani* is the highest number of aphids in the 1-minute UV treatment; The population size in the treatment with no UV and 2 minutes UV are similar. For the treatments without *A. colemani* were the population size reduced with higher UV-C doses.

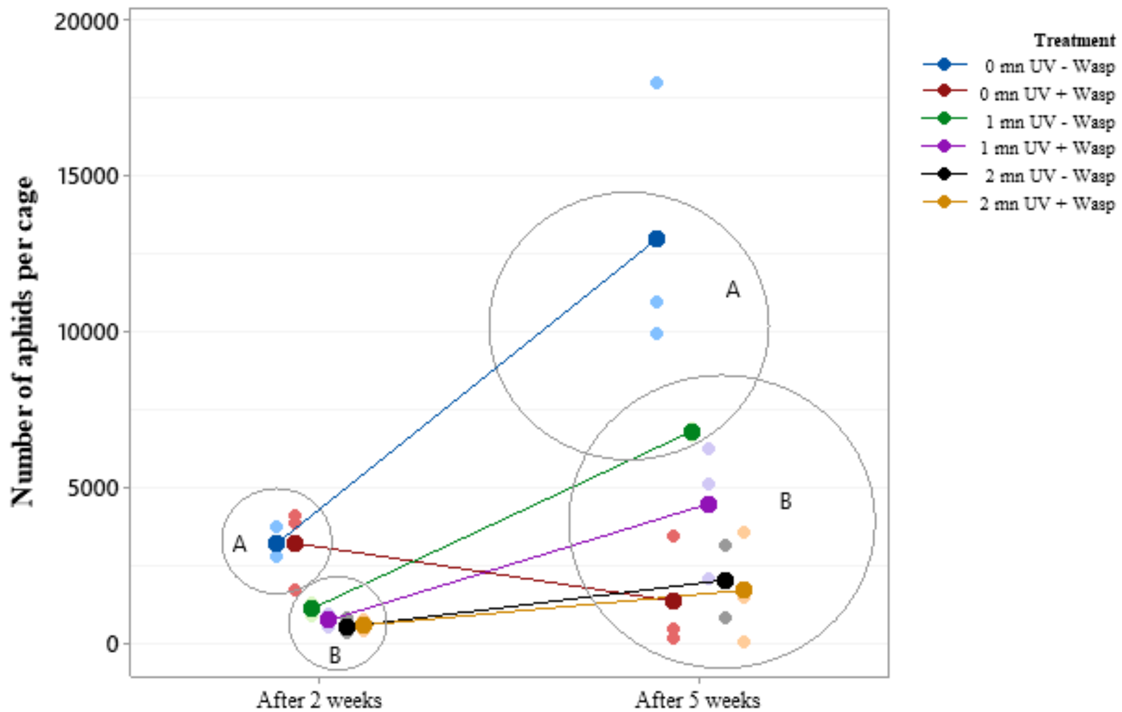



*Figure 14: Number of M. persicae per cage for each treatment in the cage experiment at 2 weeks and 5 weeks after the start of the experiment. The circles illustrate grouping of treatments by Tukey's pairwise comparison at each time point. Average values that do not share a circle are significantly different. The effect of treatment on population size is significant (p=0,001).*

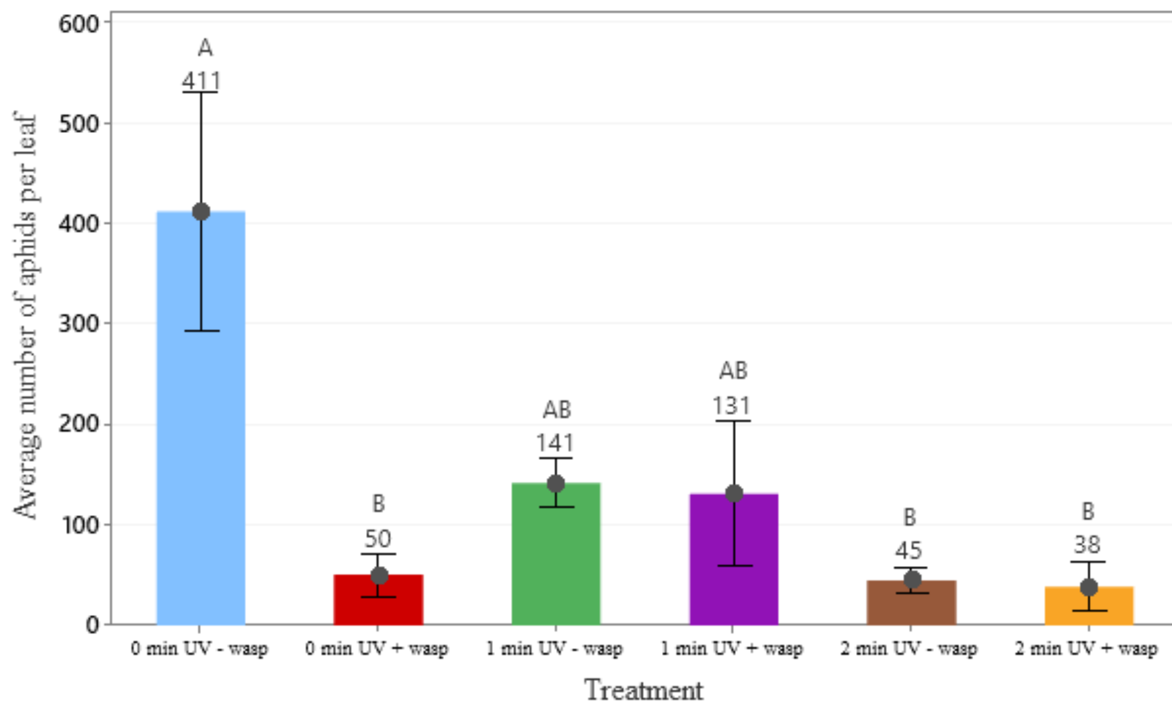



*Figure 15: The average number of M. persicae per leaf for each treatment in the cage experiment. The effect of treatment on aphid number per leaf is significant (p=0,006). The letters over the bars illustrate grouping done with Tukey's pairwise comparison. Average values that do not share a common letter are significantly different. Interval bar show 1 SD calculated for each average value.*

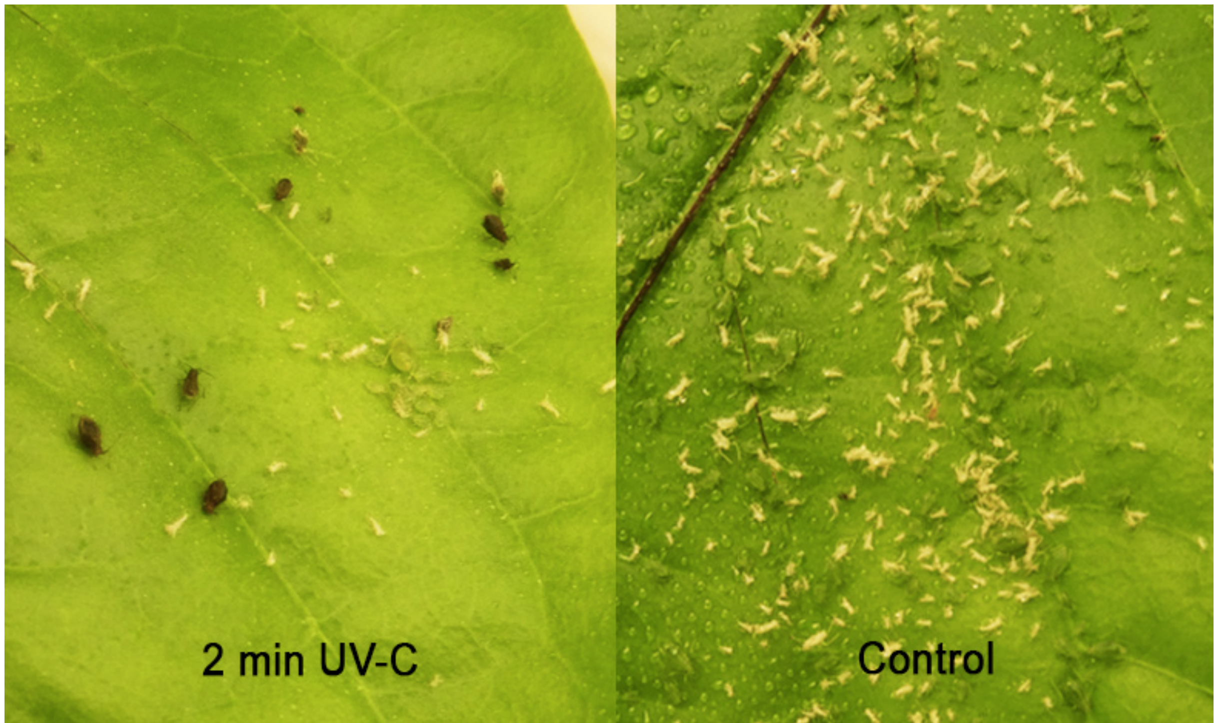



*Figure 16: Example of abaxial leaf side of leaves from C. annuum exposed to 2 minutes of UV-C treatment (left) and a control without UV irradiation (right). In the control is the M. persicae population density high, whereas fewer aphids are seen on the irradiated leaf. Dark aphids are dead.*

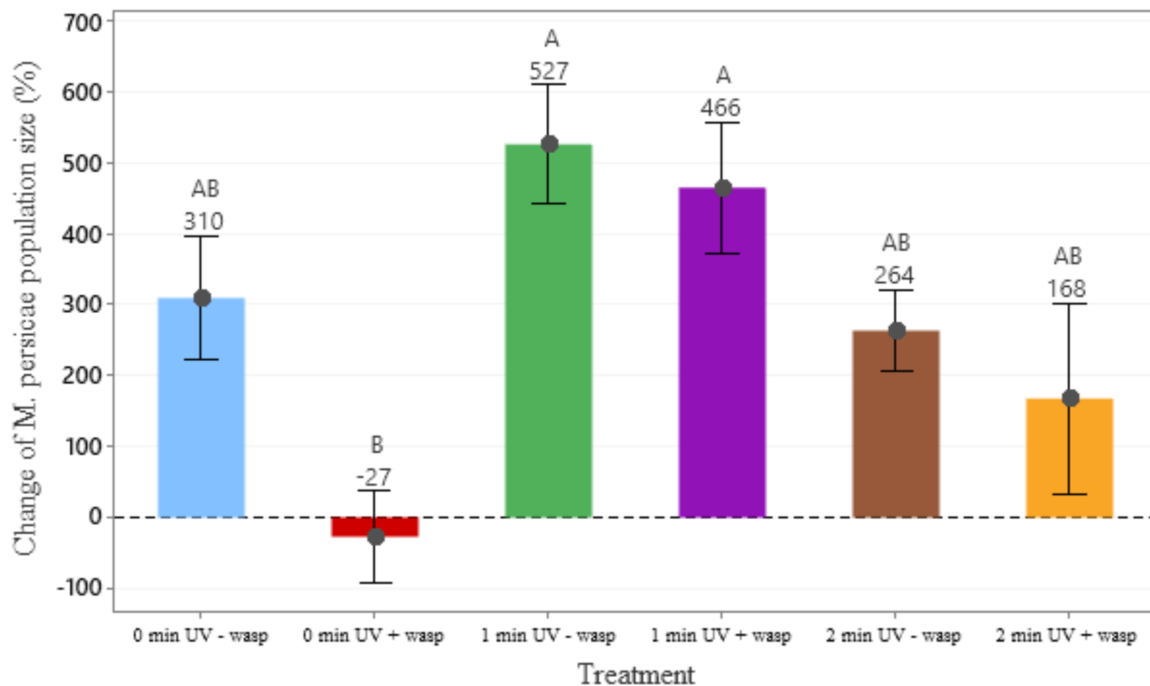



*Figure 17: Average change (in %) for M. persicae population size per cage from the first registration (after 2 weeks) to the last registration (after 5 weeks) for each treatment in the cage experiment. The effect of change in population size between the two timepoints is significant (p=0,007). The letters over the bars illustrate grouping done with Tukey's test. Averages that do not share a common letter are significantly different. Interval bar show 1 SD calculated for each average value.*

The effect of UV-treatment on the population structure, regarding percentage of imagines in the population, was significant (p=0,042). The highest average percentage of imagoes was found in the treatment with 2-minute UV exposure and *A. colemani* (22%), whereas the lowest was found in the treatment with 1-minute UV exposure and *A. colemani* (6,3%), followed by the control without *A. colemani* (6,7%). However, none of the groups were found to be significantly different when tested with Tukey's pairwise comparison.

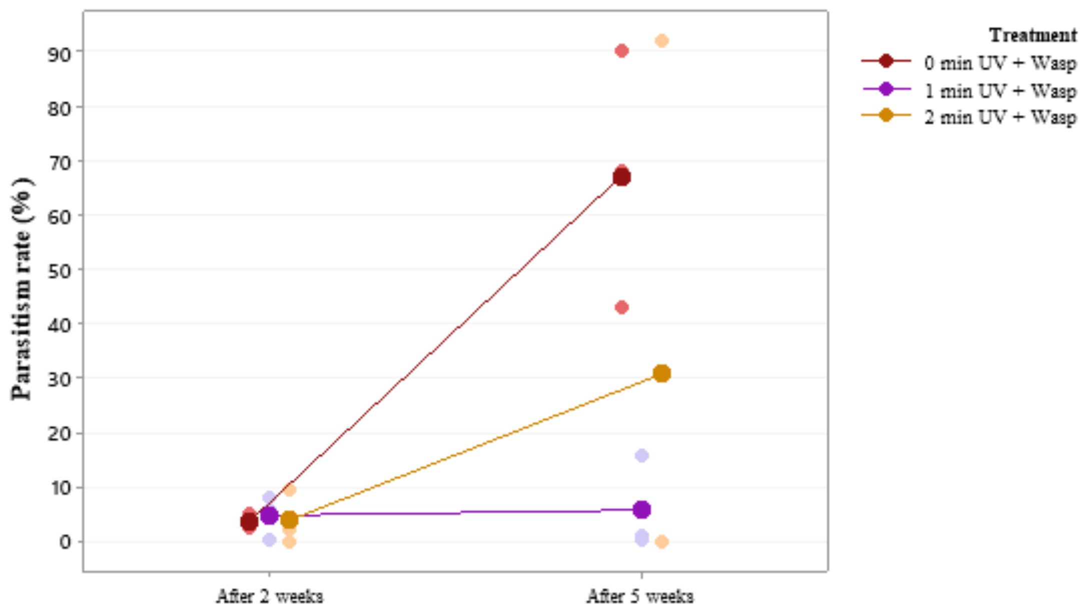



*Figure 18: Average parasitism rate by A. colemani on M. persicae when exposed to no, 1 and 2 minutes of UV radiation after 2 weeks and 5 weeks of the beginning of the experiment. The UV-exposure did not have a significant effect on parasitism rate (p=0,169).*

**Parasitism rate**

For the treatments including *A. colemani* the parasitism rate was very similar between all treatments at the first registration. This changed by the five week registration where it was a higher average parasitism rate in the control treatment (no UV) compared to the UV treatments (Figure 18). This is also the case for the percentage of unhatched mummies. The rate of unhatched mummies was around 50% for all treatments at the first registration, followed by an increase of 16% in the control and decreases between 37% and 41% in both the treatments with UV at the last registration. However, no significant effect of UV-treatment was seen on the parasitism rate (p=0,169), nor on the percentage of hatched aphid mummies (p=0,549). Large variation was seen within the 2 minute UV-C treatment, with SD=58 for parasitism rate (in %).

## Spatial distribution on the plants

A significant effect of treatment was also seen on the spatial distribution of *M. persicae* on the plant, measured by the percentage of aphids sitting on the adaxial leaf side (p=0,000).

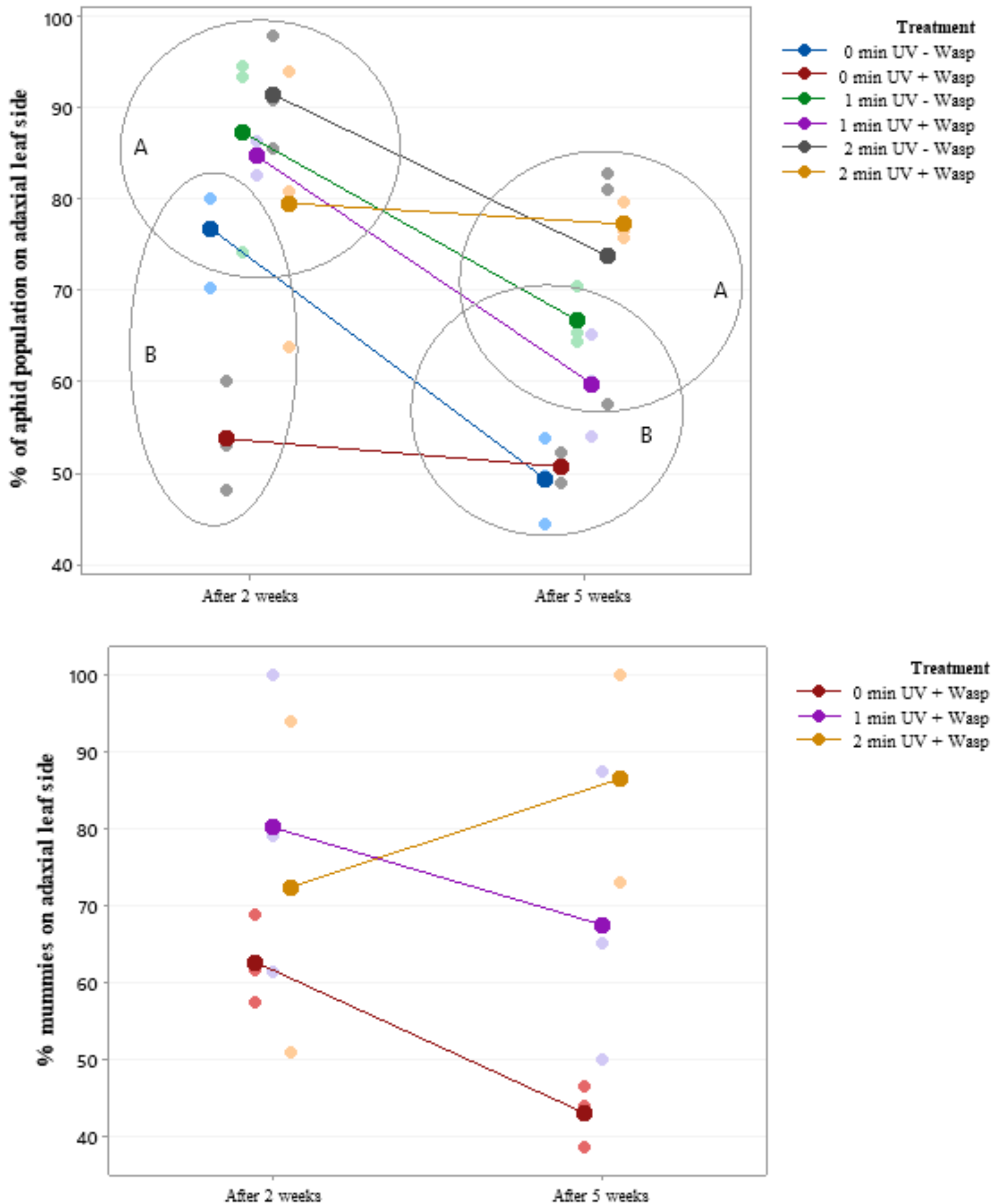



*Figure 19: Average spatial distribution of (A) M. persicae and (B) A. colemani on C. annuum plants (represented by % of total number placed on the adaxial leaf side) for each treatment in the cage experiment at registration times (2 weeks and 5 weeks after the start of the experiment). The effect of treatment on spatial distribution is significant (p=0,000) for M. persicae and not significant (p=0,069) for A. colemani. The circles illustrate grouping of treatments by Tukey's pairwise comparison at each time point. Average values that do not share a circle are significantly different.*

After 2 weeks the majority of the aphids were sitting on the adaxial leaf side in all treatments, but for the treatments with UV the percentage was higher than the treatments without UV (Figure 19). The differences got clearer with time and after 5 weeks the distribution was significantly different between the treatments without UV and the 2-minute UV treatments, with respectively around 50% and 75% of the aphids situated on the adaxial leaf side.

For the treatments with *A. colemani* higher percentages of the aphid mummies situated on the adaxial leaf side in the treatments with UV were also seen. Again, the differences got larger with time (Figure 19). However, the effect of UV on the distribution of aphid mummies on the plant was not significant[6].

**Plant damage**

UV damage in form of dark spots on the leaves (Figure 20) was registered in the plants that had been exposed to UV-C (Figure 21). The number of leaves with such damage was significantly higher in the plants that had been exposed to 2-minute UV-C treatments than the 1-minute and no UV-C treatments. No damage was observed in the 1-minute UV-C treatment before the second registration time.

Figure 20: Examples of UV damage seen on plants. This example is from plants treated with 2 minutes UV-C.

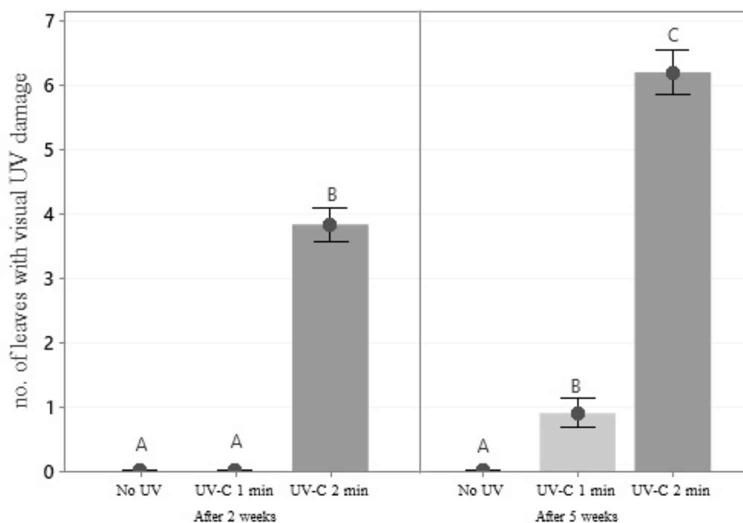



*Figure 21: Average number of leaves with visually observed UV damage per plant for each UV treatment at each registration time. The effect of UV treatment on leaf damage was significant (p=0,000) at each registration time. The letters over the bars illustrate grouping done with Tukey's pairwise comparison, Average values that do not share a letter are significantly different. Interval bar show 1 SD calculated for each average value*

[6] ANOVA tables for all tested effects of UV treatmnets on *A. colemani* are in table 2 in the appendix, as goes for most of the tests of treatment on *M. persicae* (table 1)

# Discussion

This aim of this study was to investigate if UV treatments schemes effective against powdery mildew can control *M. persicae,* and if it affects biological control of this insect pest by the parasitoid wasp *A. colemani*. This was done by studying the population size of *M. persicae* and parasitism rate by *A. colemani* on whole plants of *C. annuum*, exposed to one minute and two minutes of UV-C exposure. In addition, I wanted to investigate if the UV exposure had an effect on the spatial distribution of aphids and aphid mummies on the plants. This was done by surveying the percentage of the total number of aphids and mummies situated on the abaxial leaf side. To better understand how UV exposure affects the longevity, development and fecundity of *M. persicae* experiments with direct UV exposure on single individuals on *C. annuum* leaf disks were also conducted.

The experiments showed that UV treatments used against powdery mildew have a negative effect on the fitness and survival of *M. persicae*. Direct broad-spectrum UV exposure of 0,314 kJ/m$^2$/day and UV-C exposure with dosages of 0,169 and 0,371 kJ/m$^2$/day in the leaf disk experiment decreased the survival of *M. persicae*. This result is supported by Petillon et al. (2017). However, they observed a survival rate higher than in the current study. Petillon et al. found a survival rate of 85% after 10 days with a UV treatment of 0,288 kJ/m$^2$/day ($\lambda_{peak}$=313). In the current study the observed rate at the same time point was 18% and 0% for treatments with broad-spectrum UV and UV-C, respectively (Figure 11). Similar trends are seen in studies done spider mites. A study by Øyri (2017) showed a significantly higher mortality of eggs, larvae and nymphs of *T. urticae* in treatments with UV ($\lambda_{peak}$ =313 nm, 0,288 ± 36 kJ/m$^2$/day) compared to treatments without UV.

Direct UV exposure did also delay, and in many cases hinder, the development of *M. persicae* nymphs. This is in contrast to the results of Petillon et al. (2017), who saw no significant effect of UV exposure on aphid development. Hu et al. (2013a), on the other hand, did find a similar trend for *S. avenae* on barley. The one-time UV dose given in their study was much higher (432 – 864 kJ/m$^2$) than the dose used every night in the current study. They do not specify the wavelength peak of the UV irradiation and the treated aphids are placed in growth light after the treatment, giving an opportunity for photoreactivation. Therefore, it is difficult to compare the dosages of their and the current study. There are no available studies on photoreactivation in aphids, but due to findings in other arthropods is it reason to suspect that this may also occur in this insect group (Guo et al., 2019; Murata & Osakabe, 2017b).

A clear difference in the spatial distribution of *M. persicae* on the plants with respect to placement on leaf was seen in both the treatments with highest UV dose compared to the non-irradiated controls. A similar number of aphids were found on the adaxial and abdaxial leafsides the control, whereas around 80% of the aphids in the 2-minute UV treatments were situated on the adaxial leaf side. This could be caused by UV irradiation avoidance by the aphids. However, the significant overall lower number of *M. persicae* on these plants compared to the control gives reason to believe that lower fitness on the UV exposed abaxial side is the main cause of this observation. Øyri (2017) found that reflectors placed on the underside of the plant increased mortality of *T. urticae* compared to UV-irradiation from above alone. Reflectors can increase the area exposed to UV irradiation, thus minimizing microhabitats that can act as safe havens for the pests. As all walls in the cages used in the cage experiment were constructed by fabric, the overall UV reflection is minimal. However, it should be noted that there was no data collection to control any potential reflection in the current study.

A clear suppression of population size of *M. persicae* was seen on whole plants in both the 2-minute UV-C treatments (with and without presence of *A. colemani*) and the treatment without UV and with *A. colemani.* For all these three treatments the average number of aphids per leaf did not differ. However, the treatment without UV and with *A. colemani* was the only one showing a reduction in population size between the two registration times. This shows that control by *A. colemani* may use some time to show results but can be highly effective when established. The pattern of increased *M. persicae* population control by *A. colemani* from week 2 to 5 fits well with the life table parameters for these organisms found by Khatri et al. (2018). They observed a generation time of 17.05 ± 0.02 days for *A. colemani*, 33% longer than of *M. persicae*. Anyhow, this was of little hindrance of the overall control as the population growth of *A. colemani* was twice as big as for healthy aphids.

It was somewhat more surprising to find no significant effect of UV treatments on the parasitism rate. Especially as there were no difference of aphid number per leaf for neither of the two dosages of UV-C, regardless the presence of *A. colemani* (Figure 15). It was, on the other hand, observed lower average parasitism rates in the UV treatments compared to the non-UV control. The variation was very large, especially in the 2-minute UV-C treatment, that had a parasitism rate that ranged from 91% to no parasitism (0%) in the last registration. This variation may indicate little survival of *A. colemani* in some of the replications. With these results in mind can it be suggested that UV-C treatments used against powdery mildew somewhat reduces the effect of biocontrol by inhibiting the establishment of *A. colemani*. The reason for this tendency is not known, but potential reasons will be discussed as follows.

It has been shown that *A. colemani* performs as well in the presence of solar UV as in habitats covered by UV blocking filters, in contrast of the parasitoid wasp *Eretmocerus mundus* (Chiel et al., 2006). The latter was not able to localize and parasitize hosts in a UV deficient environment. However, *A. colemani* has shown to have a strong attraction to light containing solar UV. It is not known if *A. colemani* has a similar response to UV-C, but the lamps used in the cage experiment also emit some irradiation in both the UV-B and UV-A section of the light-spectra (Figure 1). Thus, it is reason to believe that the emission from these lamps may have some attractant effect on *A. colemani*, which again can increase the overall UV exposure.

Another explanation for the large variation of parasitism could be the small number of *A. colemani* released in each cage. It is a well-established ecological principle that small isolated populations are more fragile for eradication due to stochastic events. Such events could be disruption of mating or cause low survival of the released wasps. Due to separate vials used for both hatching and release none of the released *A. colemani* females were mated when released in the cages. This would not have been the case with normal use of commercial *A. colemani*, since some individuals of both sexes often hatch inside the container the organisms are delivered in before release. As s single *A. colemani* female can parasitize 220 individuals of *M. persicae* in one week, the number of wasps released in the current study would, in theory, be enough to control the aphid population on the plants (Khatri et al., 2017). This explanation supported by the small population of *M. persicae* registered in the treatment without UV exposure and with *A. colemani*, compared to treatment without UV exposure and biological control.

There are many possible reasons for the negative effects of the conducted UV treatments on *M. persicae* survival and population size. Misshapen antennas are a probable sign of cell damage and it is natural to believe that UV induced formation of ROS also has a negative effect on survival and development if cell structures or DNA is harmed. Similar damage was seen by Petillon et al. (2017), whereas abnormal shaped eggs and larvae have been observed in *T. urticae* on UV-B exposed plants (Murata & Osakabe, 2017a; Øyri, 2017). Harm to sensory organs may also affect the orientation and behaviour of *M. persicae*, which could explain the disoriented individuals observed in the UV-treatments of the leaf disk experiment. UV exposure may also alter the chemical composition of the host plant which again may affect herbivorous insects. However, studies in this field are conflicting and there are examples of how increased production of secondary metabolites can affect aphid fitness both positively and negatively, depending on host plant and aphid species (Kuhlmann & Muller, 2010; Paul et al., 2012; Rechner & Poehling, 2014). Another implication of UV-B treatment to aphid feeding was seen

by Hu et al. (2013b), who registered less probing behaviour on barley by *S. avenae* when exposed to enhanced UV-B radiation (120 $kJ/m^2/d$).

The results of this study suggest that UV treatments alone cannot control established *M. persicae* populations on *C. annuum* to under the economic threshold, without causing some kind of UV damage to the plant. The suppressive effect of the UV treatments, however, still has potential to be a part of *M. persicae* control in IPM systems. Even though no significant effect is seen on the parasitism rate by *A. colemani* it is reason to believe that growers using this biological control agent parallel with UV treatments against pathogenic fungi should monitor parasitoid establishment closely. Larger or frequent parasitoid releases could potentially be beneficial for the effect of biocontrol under such conditions to make up for respectively low parasitism rates or unsuccessful establishment.

It should be noted that in the current study UV treatments were given every night, which is more often than what is proven to be necessary to achieve sufficient effect against powdery mildew (Suthaparan et al., 2016). Fewer UV applications may give different outcomes for the organisms studied in these experiments. In addition, due to plant growth combined with fixed UV sources, the UV irradiation doses changed throughout the experiment. This caused undesirable high doses in the upper parts of the canopy by the end of the cage experiment. With automation of distance and passing speed of UV sources to the crop more precise dosages can be given compared to the system studied. Such robot technology is already taken in to use by some growers.

# Conclusion

This study shows that UV treatments used against powdery mildew can have a suppressing effect on *M. persicae* populations on *C. annuum*. Still these UV treatments are as, or less as, efficient in controlling *M. persicae* as biological control by *A. colemani* in absence of UV treatments. Due to large variation in parasitism rates in treatments that included UV, it is reason to believe that UV treatments used against pathogenic fungi may have implications on biological control with *A. colemani*. Anyhow, more research is needed to get a better understanding of how *A. colemani* is affected by these UV treatments, and if measures can be taken to facilitate biological control with *A. colemani* where UV treatments are in use. Further research should study aphid mummies of A. colemani under UV treatments to see if it affects emergence rates, as well as test for preferences between UV radiated and unirradiated hosts. It would also be valuable to investigate how parasitoid release frequency or use of unirradiated banker plants with non-pest hosts can support biological control of *M. persicae* by *A. colemani*. It is also relevant to study the colonisation success of aphids in crops where regular night-time UV treatments are given, as this could answer if UV treatments can be used as a preventive measure against aphid infestation.

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# Appendix

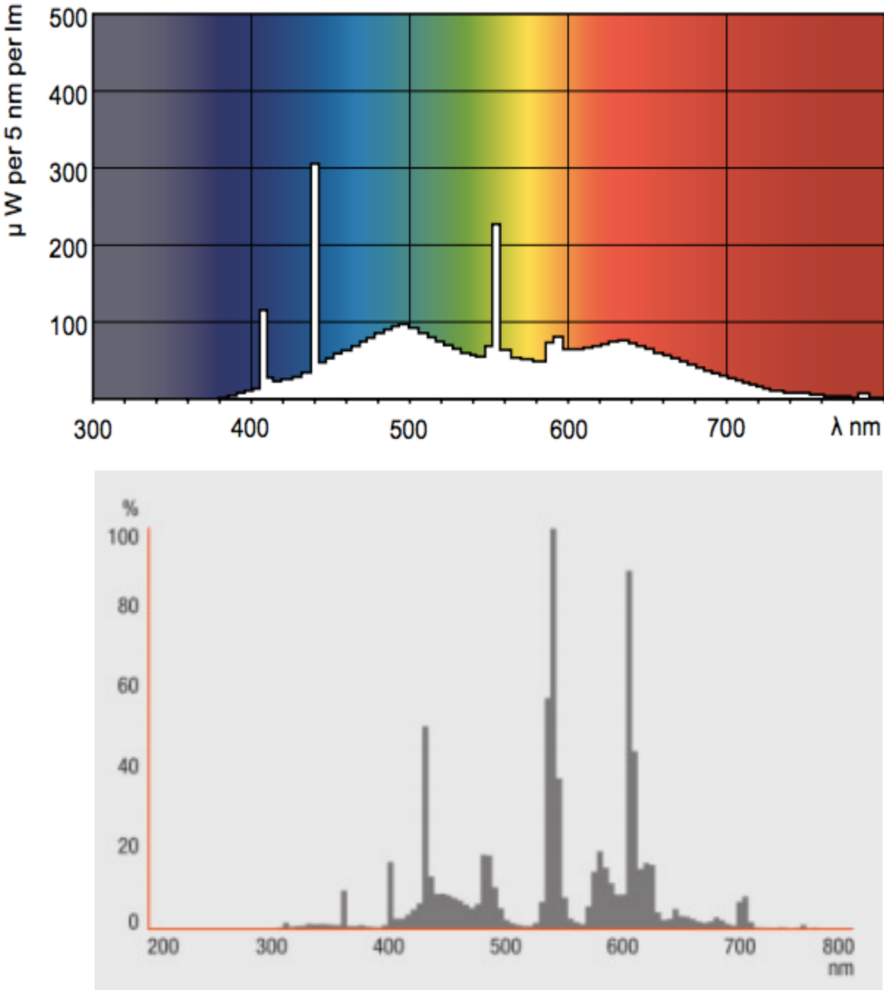



*Figure 1: Spectral emission from growth lights used in rearing of plants, aphids and in the leaf disk experiment (upper) and in the cage experiment (lower) (source: Philips and OSRAM)*

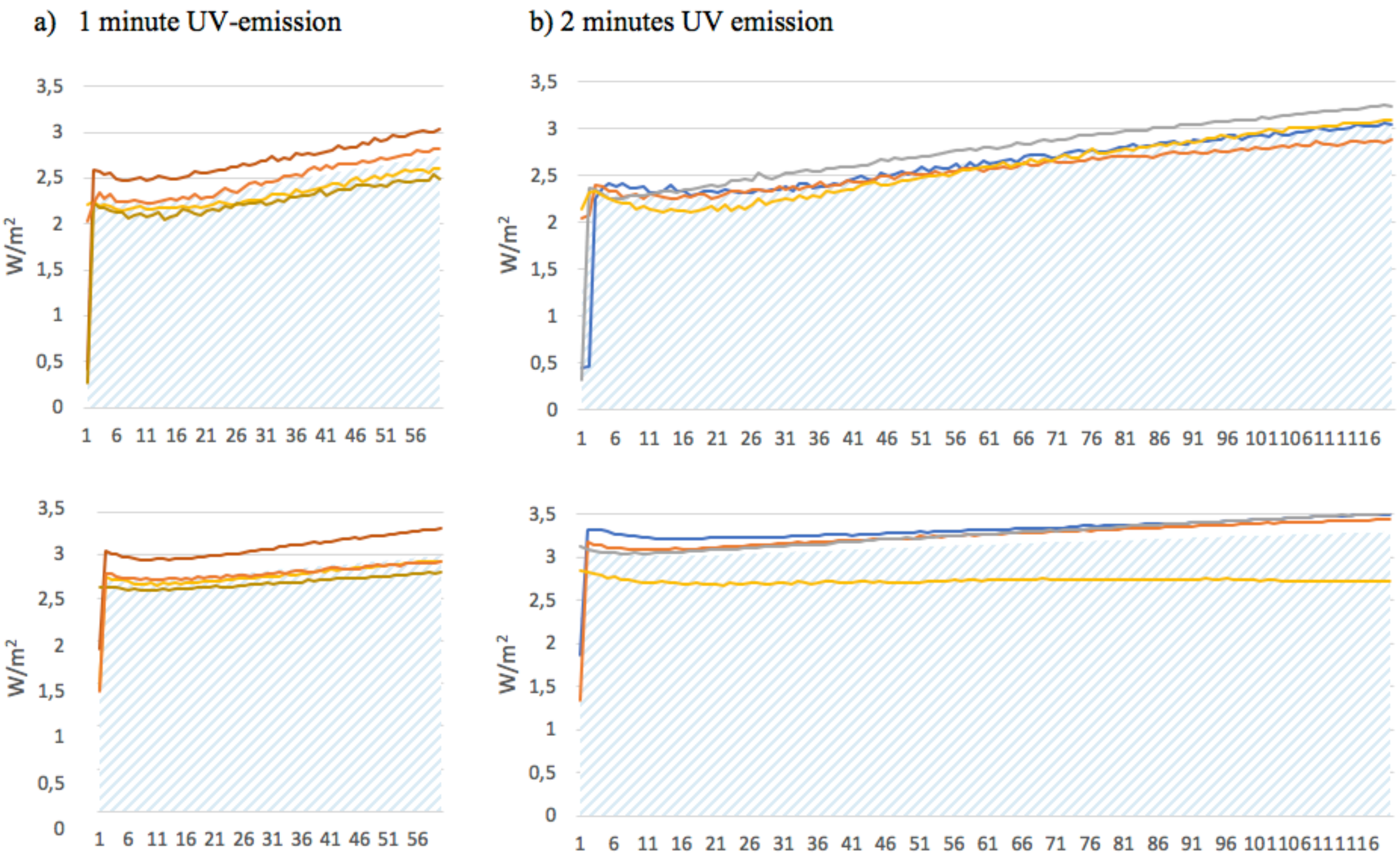



*Figure 2: Change of UV irradiation in W/m² (x-axis) measured every second (y-axis) over A) 1 minute and B) 2 minutes. Measurements were done 5 cm above pot height a) directly under the lamp and b between lamps at the beginning of the experiment. The lines represent single measurements and the shaded areas represent average accumulated UV-dose (A: (a) 148,5 J/m²/day and (b) 164,4 J/m²/day and B: (a) 321,4 J/m²/day and (b) 374,5 J/m²/day)*

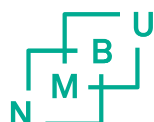
*Table 1: ANOVA mixed model with replication as random factors and treatment as fixed factors. In the categories marked * was registration time also added as a fixed factor. Tukey-tests were used for comparison, where values that do not share a letter are significantly different. P-values are marked as bold where treatment had a significant effect, whereas values in bold are significantly different from the control (No UV – wasp). P-values ≤ 0,005 show a significant effect of treatment at confidence level: 95%*

**The effect of treatment on the *Myzus persicae* populations in the cage experiment**

| | N | No UV<br>- wasp | No UV<br>+ wasp | 1 min UV<br>- wasp | 1 min UV<br>+ wasp | 2 min UV<br>- wasp | 2 min UV<br>+ wasp | P-value |
|---|---|---|---|---|---|---|---|---|
| **Population size** | | | | | | | | |
| After 2 weeks | 3 | 3213,0 A | 3232,3 A | **1119,7 B** | **759,0 B** | **595,3 B** | **541,0 B** | 0,000 |
| After 5 weeks | 3 | 12969,0 A | **1364,0 B** | 6804,7 AB | **4488,3 B** | **2044,0 B** | **1715,0 B** | 0,001 |
| Both registrations * | 6 | 8091,0 A | **2298,2 B** | 3962,2 AB | **2623,7 B** | **1292,5 B** | **1155,2 B** | 0,001 |
| % change from week 2 to 5 | 3 | 310,2 AB | -27,1B | 527,4 A | 466,2 A | 264,1 AB | 168,25 AB | 0,007 |
| **Aphids per leaf** | | | | | | | | |
| After 5 weeks | 3 | 411,4 A | **50,3 B** | 141,3 AB | 130,8 AB | **44,8 B** | **38,4 B** | 0,006 |
| **Distribution on the plant (% sitting on abaxial leaf side)** | | | | | | | | |
| After 2 weeks | 3 | 76,8 AB | 53,8 B | 87,3 A | 84,7 A | 91,5 A | 79,6 A | 0,003 |
| After 5 weeks | 3 | 49,4 B | 50,8 B | 66,8 AB | 59,8 AB | **73,8 A** | **77,3 A** | 0,001 |
| Both registrations * | 6 | 63,1 BC | 52,3 C | 77,1 AB | 72,2 AB | **82,6 A** | 78,4 AB | 0,000 |
| Change from week 2 to 5 | 3 | -27,4 A | -3,0 A | -20,5 A | -24,9 A | -17,6 A | -2,3 A | 0,062 |
| **Age distribution (% imagines)** | | | | | | | | |
| **On the whole plant** | | | | | | | | |
| After 2 weeks | 3 | 7,0 A | 6,5 A | 6,2 A | 10,4 A | 10,9 A | 15,5 A | 0,003 |
| After 5 weeks | 3 | 6,4 A | 31,9 A | 6,4 A | 12,3 A | 12,4 A | 28,5 A | 0,053 |
| Both registrations * | 6 | 6,7 A | 19,2 A | 6,3 A | 11,3 A | 11,6 A | 22,0 A | 0,042 |
| Change from week 2 to 5 | 3 | -0,6 A | 25,4 A | 0,2 A | 1,9 A | 1,5 A | 12,9 A | 0,218 |
| **Adaxial leaf side** | | | | | | | | |
| After 2 weeks | 3 | 9,6 A | 7,1 A | 10,4 A | 24,0 A | 18,8 A | 36,2 A | 0,483 |
| After 5 weeks | 3 | 5,5 B | 34,7 A | 10,7 AB | 19,8 AB | 19,1 AB | 23,2 AB | 0,055 |
| Both registrations * | 6 | 7,6 A | 20,9 A | 10,6 A | 21,9 A | 19,0 A | 29,7 A | 0,220 |
| Change from week 2 to 5 | 3 | -4,1 A | 27,7 A | 0,3 A | -4,2 A | 0,3 A | -13,0 A | 0,253 |
| **Abaxial leaf side** | | | | | | | | |
| After 2 weeks | 3 | 6,2 A | 6,2 A | 6,1 A | 8,2 A | 10,4 A | 11,0 A | 0,426 |
| After 5 weeks | 3 | 7,2 A | 29,2 A | 4,3 A | 7,2 A | 10,6 A | 30,2 A | 0,072 |
| Both registrations * | 6 | 6,7 A | 17,7 A | 5,2 A | 7,7 A | 10,5 A | 20,6 A | 0,051 |
| Change from week 2 to 5 | 3 | 1,1 A | 23,0 A | -1,8 A | -0,9 A | 0,2 A | 19,2 A | 0,168 |

*Table 2: ANOVA mixed model with replication as random factors and treatment as fixed factors. In the categories marked * was registration time also added as a fixed factor. Tukey-tests were used for comparison, where values that do not share a letter are significantly different.*

| **The effect of treatment on *A. colemani* mummies** | | | | | |
|---|---|---|---|---|---|
| | N | No UV | 1 min | 2 min | P-value |
| **Parasitism rate** | | | | | |
| After 2 weeks | 3 | 3,6 A | 4,7 A | 3,8 A | 0,936 |
| After 5 weeks | 3 | 67,2 A | 5,7 A | 30,7 A | 0,153 |
| Both registrations * | 6 | 35,4 A | 5,17 A | 17,2 A | 0,169 |
| % change from week 2 to 5 | 3 | 63,5 A | 1,0 A | 26,9 A | 0,137 |
| **% unhatched mummies** | | | | | |
| **On the whole plant** | | | | | |
| After 2 weeks | 3 | 47,4 A | 58,2 A | 55,8 A | 0,928 |
| After 5 weeks | 3 | 63,4 A | 21,4 A | 15,11 A | 0,081 |
| Both registrations * | 6 | 55,4 A | 39,8 A | 35,4 A | 0,549 |
| Change from week 2 to 5 | 3 | 16,1 A | -36,8 A | -40,7 A | 0,443 |
| **Adaxial leaf side** | | | | | |
| After 2 weeks | 3 | 38,1 A | 90,1 A | 62,5 A | 0,194 |
| After 5 weeks | 3 | 62,1 A | 29,6 A | 29,8 A | 0,264 |
| Both registrations * | 6 | 50,1 A | 50,6 A | 52,8 A | 0,992 |
| Change from week 2 to 5 | 3 | 24,0 A | -60,8 A | 1,2 A | 0,107 |
| **Abaxial leaf side** | | | | | |
| After 2 weeks | 3 | 58,8 A | 96,2 A | 67,0 A | 0,488 |
| After 5 weeks | 3 | 57,2 A | 41,8 A | 54,1 A | 0,800 |
| Both registrations * | 6 | 58,0 A | 61,8 A | 63,7 A | 0,950 |
| Change from week 2 to 5 | 3 | -1,6 A | -60,1 A | 16,6 A | 0,209 |
| **Mummy distribution on the plant (% on adaxial leaf side)** | | | | | |
| After 2 weeks | 3 | 62,7 A | 80,2 A | 70,0 A | 0,490 |
| After 5 weeks | 3 | 43,1 A | 67,6 A | 86,6 A | 0,057 |
| Both registrations * | 6 | 52,9 A | 73,9 A | 76,8 A | 0,069 |
| % change from week 2 to 5 | 3 | -19,6 A | -12,6 A | 14,2 A | 0,144 |



**Norges miljø- og biovitenskapelige universitet**
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway