WAGENINGEN UR
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# Validation of UV treatment with MIPS

Senternovem TeMa project 'UV-c gewasbescherming'

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# 1 Introduction

Crop treatment with UV-c light has the potential to reduce the development of plant pathogens. Recently Clean Light BV has introduced the technique in protected cultivation. In the SenterNovem TeMa project *UV-c Crop Protection* the development of UV-c crop protection applications in open cultivation is investigated. The sub-project *Validatie UV behandeling met MIPS* specifically deals with the demonstration of the effectiveness of UV-c light to prevent or stop the development of *Phytophthora infestans* inoculum on potato leaves.

## 1.1 Crop protection with UV-c

In a previous study (Kessel & Förch, 2006) the effect of UV-c light on germination of *P. infestans* sporangia on agar medium was studied. It was shown that the maximum dose rate applied (9.5mJ/cm$^2$) completely inhibited direct germination of the sporangia on water agar. The $ED_{95}$ was about 7mJ/cm$^2$.

## 1.2 MIPS - Multiple imaging of Plant Stress

MIPS is a vision technique to measure plant stress. Figure 1 shows an example of the development of a *P. infestans* infection on potato. The MIPS images visualise the response of plant photosynthesis on the infection. With image analysis tools the infection can be quantified.

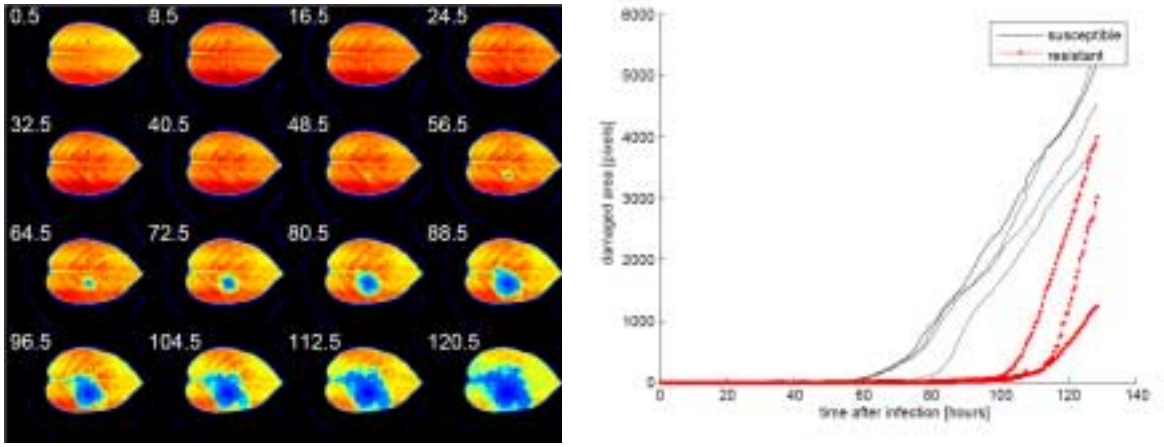


*Figure 1. Left: example of MIPS images of P. infestans infection development on a potato leaf on agar medium in a petridish. Chlorophyll fluorescence images were recorded with the MIPS at the indicated time (in h) after inoculation. Two droplets can be seen in the images. The upper droplet is the control without sporangia; the lower contains the sporangia. Right: Time course of development infected area of a series of infected leaves of susceptible and resistant potato cultivars. The infected area was estimated via automatic thresholding. Data from G. Polder (2006).*

The MIPS-Pro at WUR Greenhouse Horticulture consists of a MIPS camera mounted on an industrial robot. The MIPS-Pro can monitor the infection in up to 110 petridishes. With the MIPS-Pro the effects of UV-c can be quantified and validated.

## 1.3 Validation of UV-c on *Phytophthora infestans* development on potato leaves

In this project the role of the MIPS is to monitor and quantify the development of *P. infestans* infection on detached leaves of potato to i) demonstrate the effectiveness of UV-c treatment on inhibition of infection, and ii) quantify possible negative effects of UV-c on the leaf (phytotoxicity).

# 2 Experimental

## Plant material

Leaves of potato plants (*Solanum tuberosum* cv Frieslander and cv Agria) were collected on the morning of the first day of the experiment at the Organic Experimental and Training Farm Droevendaal in Wageningen. Fully developed leaves (4th leaf layer from the top) were picked and transported inside plastic boxes to the Phytotron at Born-zuid in Wageningen. Leaflets were selected and placed in 9cm agar-filled petridishes, lower side up.

## Culturing and application of sporangial suspensions

*P. infestans* isolate VK98014 was grown on detached potato leaves (cv Bintje) for one week. After this period, the sporulating leaflets were washed with tap water producing a sporangial suspension which was adjusted to $2x10^4$ sporangia/ml. Per leaf 10µl of this suspension was applied as a droplet on the lower side of the leaf near the central vein

## UV-c treatment

UV-c was measured with a calibrated RM-21 UV-c sensor (Dr. Gröbel UV-Elektronik GmbH, Ettlingen, Germany). The sensor was placed in the center of the petridishes to be treated. Before exposing the leaflets in the petridishes to UV, the lids were removed. The UV-lightsource (TUV PL-L 36W/4P 1CT) was switched on allowed to warm up at least 2 minutes before starting the exposure. The UV-lightsource was moved horizontally at a height of about 50cm above the petridishes until the UV-c sensor indicated that the target UV dose (5 mJ $cm^{-2}$ or 10 mJ $cm^{-2}$) was reached. It was ascertained that the lids of the petridishes did not transmit more than 1% UV-c. With this experimental protocol the error in the UV-c dose of a leaf in a petri is estimated to be maximally 20%.

## MIPS

The MIPS-Pro facility at Wageningen Greenhouse Horticulture captures time series of fluorescence images of plants. The MIPS system consists of a Kawasaki industrial robot with a sensor head containing imaging sensors for chlorophyll fluorescence and visual reflection. The images are recorded by a single camera with a rotating filter wheel. The main imaging sensor is a cooled CCD (Texas Instruments TC-237) at 5°C. The spatial resolution is 640×480 pixels with a dynamic range of 14 bits. A 4-position filter wheel contains red, green and blue filters for RGB reflection measurements and a 725 nm interference filter for detection of chlorophyll fluorescence. To record chlorophyll fluorescence, the plant is excited by a modulated, scanning red laser in the sensor head. The wavelength of the laser is 670 nm (Polder *et al.*, 2006). Photosystem II activity is derived from chlorophyll fluorescence measurements measured with a novel patented light saturating technique (Jilin *et al.*, 2004). Photosynthetic efficiency (PE) images can be calculated from the ambient light recording (f0) and the saturating light recording (Fm) as PE = ((Fm –F0)/Fm). Leaf reflection is measured in three bands with the RGB filters using either ambient room light or light from a LED ring attached to the sensor head.

*Figure 2.* *MIPS-Pro in action during the opening of the MIPS facility in 2002. The MIPS-Pro consists of a sensor head (black box) mounted on an industrial Kaawasaki robot. A plant table provides space for up to 85 petridishes.*

## Experimental setup

The MIPS-Pro and the table were placed in a growth chamber at 16°C in the dark. Two leaflets were placed in each petridish. The petridishes were closed and put on the table (see white circles in Fig. 3) and the leaves were allowed to adapt to the conditions in the petridish. To prevent reflection of the laserbeam, the petridishes were slightly tilted vertically by placing them with one side on a plastic lid. The experiments were carried out in the dark.
Inoculation with *P. infestans* was carried out at day 0. After inoculation table and petridishes were put for 24 hours under a black plastic cover and a water layer was put on the table to achieve 100% relative humidity to facilitate germination of the sporangia.

The MIPS measurements started after 24 hours after removal of the black plastic cover (day 1).

The following treatments were given.

*Table 1.* *Summary of treatments. The numbers in the table indicate the numbers of the petridishes in Figure 3.*

| | UV-c treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5 mJ cm$^{-2}$ | | | 10 mJ cm$^{-2}$ | | |
| Inoculation | no UV | day 0 | day 0+2 | day 0+2+4 | day 0 | day 0+2 | day 0+2+4 |
| no | 1, 2, 31, 32, 33, 34, 63, 64 | | | 3, 4, 29, 30, 35, 36, 61, 62 | | | 5, 6, 27, 28, 37, 38, 59, 60 |
| yes | 7, 14, 15, 16, 17, 18, 19, 26, 39, 46, 47, 48, 49, 50, 51, 58 | 8, 25, 40, 57 | 10, 23, 42, 55 | 12, 21, 44, 53 | 9, 24, 41, 56 | 11, 22, 43, 54 | 13, 20, 45, 52 |

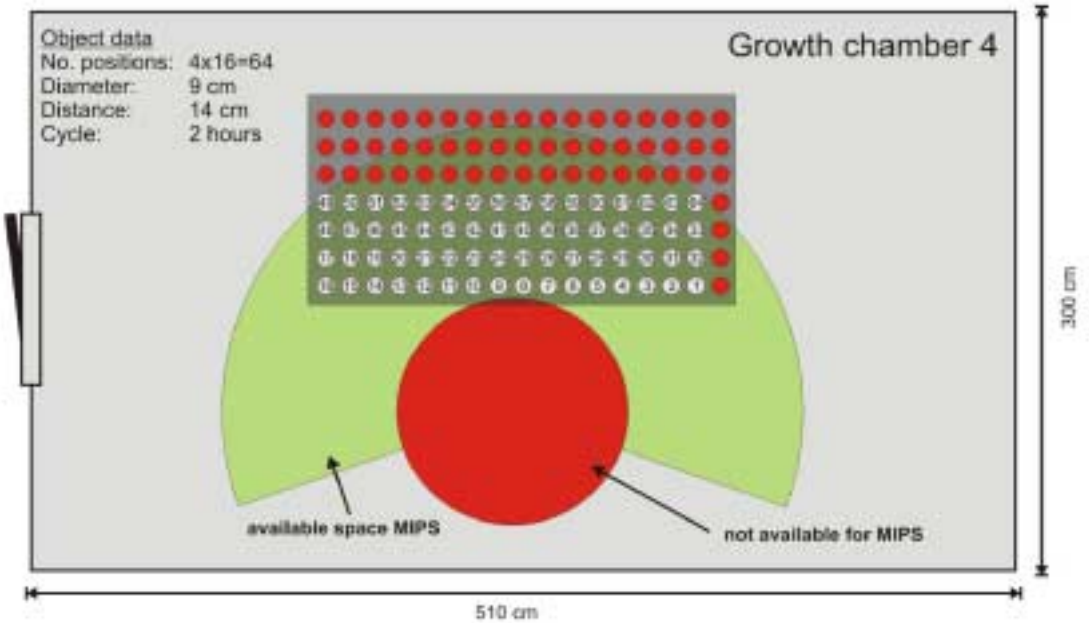


*Figure 3.* *Scheme of the MIPS experimental setup (Top View). The large green circle segment indicates the area the can be monitored with the MIPS camera on the robot arm. The large red circle indicates the area that is too close to the center and not available for monitoring. The white cirkels indicate the positions occupied by the 9 cm petridishes in the experiment. The numbers in the white cirkels indicate the number of the petridish.*

## Analysis

The fluorescence images were analysed by the home-made software MIPS-analyser. The infected area was calculated for each leaf. The relative area of the infected part of the plant was determined by counting the plant pixels with PE<40% and division by the total number of plant pixels. The result is expressed as a percentage.

Curve fitting of the area growth data to an exponential function was carried out in MathCad 14 using a least squares approach (Fig. 4).

# 3 Results

## 3.1 Effect of UV-c on symptom development

The quantification of infection symptoms of *P. infestans* on *S. tuberosum* cv Agria were not reliable, and therefore omitted from this report. All data shown were obtained l with cv Frieslander.

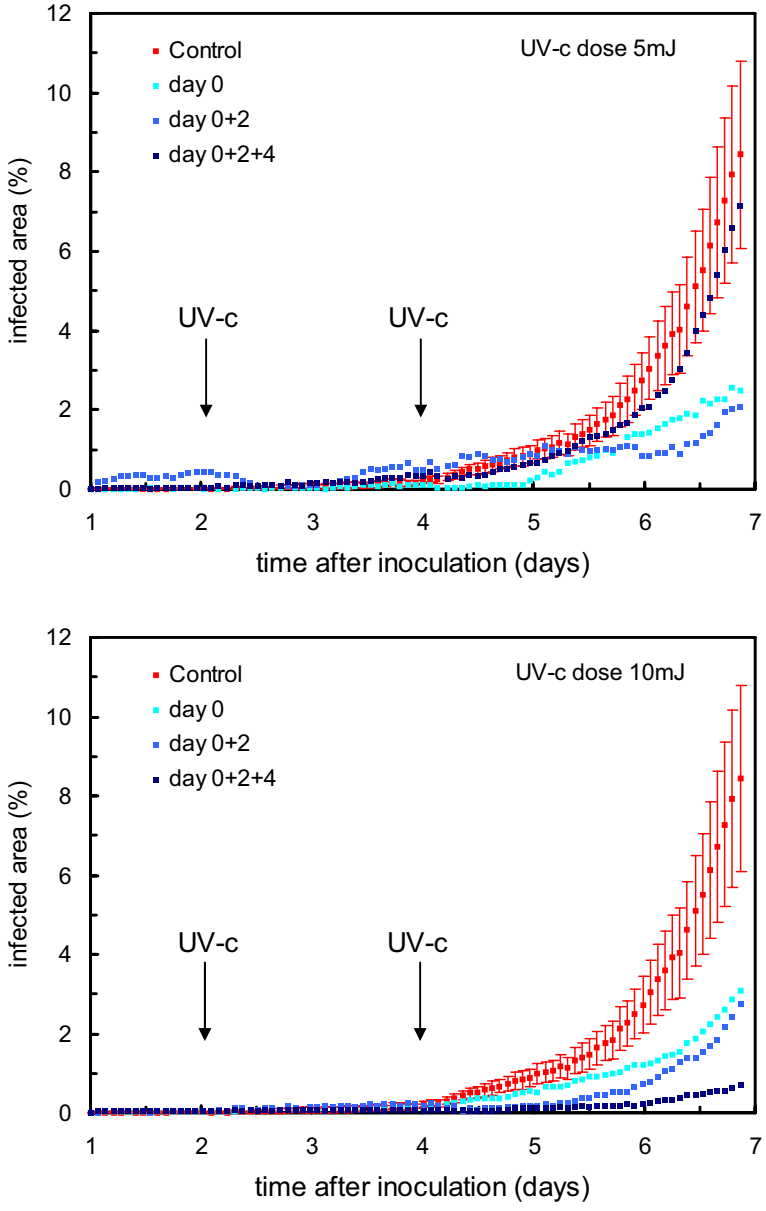


*Figure 4.* *Development of P. infestans infection on control and UV-c treated detached S. tuberosum cv Frieslander leaves at 5 mJ cm$^2$ (top) and 10 mJ cm$^2$ dose (bottom). The control is the average of 10 leaves. The UV-treatment datapoints are the average of 4 leaves. The error bars indicate the standard error. The area growth of the control is described by the function $Y=a*e^{b*x}$ with $a=1.538 \times 10^3$ and $b=1.256$ (Spearman correlation coefficient: 0.9995). For the treatment day 0+2+4 $a=4.368 \times 10^4$ and $b=1.069$ (Spearman correlation coefficient: 0.9963).*

Figure 4 shows that UV-c at both 5 and 10 mJ $cm^{-2}$ leads to a strong suppression of the infection development. At 7 days after inoculation the infected area is typically reduced by 60-70% and up to 92% by applying 10 mJ $cm^{-2}$ UV-c at days 0, 2 and 4. The only exception is the treatment with 5 mJ $cm^{-2}$ UV-c at days 0, 2 and 4. This relatively low suppression was caused by a single leaf with the largest infection observed in the experiment. Omitting this one leaf from the dataset leads to a result similar to the treatments with UV-c at day 0 and days 0 and 2.

The growth of the infected area under our conditions increased exponentially (Fig. 4). The slope of the lines shows that the rate of infection is only slightly (14%) lowered by UV-c treatment. The main effect of UV-c treatment is a decrease in the initial area of the infection (72%). This reduction in initial area could be caused by a reduction of the number of succesfull infections or established lesions.

## 3.2 Effect of UV-c on photosynthesis efficiency

The MIPS measures the photosynthesis efficiency (PE) of the leaf. To check whether UV-c may have a negative effect on the leaf, the PE of UV-c treated leaves was compared to the PE of control leaves.

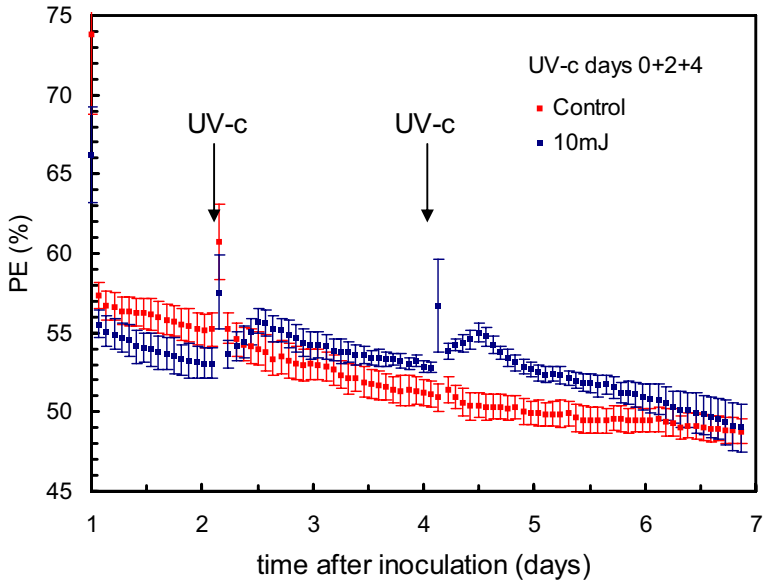


*Figure 5.* *Photosynthesis efficiency (PE) of control and UV-c treated detached S. tuberosum cv Frieslander leaves during the course of the experiment. The leaves were not inoculated. Datapoints are the average of 8 leaves. 4. Error bars indicate the standard error.*

Figure 5 shows that the PE of detached control leaves is slowly decreasing over a period of 7 days. The PE of UV-c treated detached leaves shows a similar trend, but UV-c effects can be seen as short stimulations of photosynthesis after the UV-c treatment. At the end of the experiment the PE of control and UV-c treated detached leaves are similar.

# 4 Discussion and Conclusions

The results of this study show that infection of *P. infestans* on *S. tuberosum* cv Frieslander can be suppressed by treatment with UV-C. Treatment with 10 mJ $cm^{-2}$ UV-c leads to a suppression of up to 93%. In *S. tuberosum* cv Agria, a more tolerant cultivar. the effects of the infection on symptoms as measured by chlorophyll fluorescence were less severe than in cv Frieslander. A similar difference in severity of symptoms between an sensitive and a resistant observation was made by Polder (2007). With more sophisticated image analysis these symptoms might be quantified. In the framework of this project development of such software was not possible.

The growth of the infected area appears to be exponential (Fig. 4) in our conditions. The rate constants ) is more or less similar in the control and the various UV-c treatments. This is most easily explained by the fact that UV-c kills most of the sporangia in the droplet with the inoculum. Subsequent infection by the surviving sporangia is delayed but once the infection is established, it colonizes the leaf at an identical rate as compared to the non treated objects.

The temporary increase in photosynthesis efficiency upon UV-c treatment was somewhat unexpected. The increase in photosynthesis efficiency is not an artefect caused by the opening of the lid during UV-c treatment or by the low amount of light required to carry out the UV-c treatment. This is shown by the fact that leaves in petridishes in control leaves that were opened for a brief period to simulate the UV-c treatment did not show a temporary increase in photosynthesis efficiency. It is not to be expected that UV-c will penetrate deep enough into the leaf to damage the chloroplasts in the mesophyl. The effect must, therefore, either involve some kind of signalling from the damaged cuticle to the mesophyl cells or be the result of small (<3%) amounts of other wavelengths (UV-B, UV-A, VIS) present in the UV lightsource.

We conclude that 10 mJ $cm^{-2}$ UV-c treatment suppresses *P. infestans* lesion growth without lasting effects on leaf photosynthesis efficiency.

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