PLANT RESEARCH INTERNATIONAL
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Plant Research International B.V., Wageningen
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Inhoudsopgave

1. Introduction and scope of the research

The oomycete *Phytophthora infestans*, the cause of late blight in potato and tomato, is considered one of the most important pathogens of potatoes worldwide (Hooker 1981). *P. infestans* affects foliage and stems, reducing the photosynthetic capacity of the crop and therefore leading to yield reduction. In addition, it affects tubers which reduces both, yield quantity and quality.

In the past, crop losses due to late blight have been estimated to account for 10 to 15 percent of the total global annual potato production (Anonymous, 1996). The economic value of the crop lost, plus the cost of crop protection amount to US $ three billion annually (Duncan, 1999). In the Netherlands, the cost of crop protection amounts to approximately 40 million euro annually on a total of 160 -180 thousand hectares with an average yield of 45 tonnes fresh weight per hectare.

The life cycle of *P. infestans* can be separated into an asexual cycle and a sexual cycle. The asexual cycle is the driving force behind rapid polycyclic epidemics that can be observed in potato crops during the growing season. Numerous sporangia are produced on infected leaflets and stems. Sporangia are released into the atmosphere under dry conditions or they can be washed into the ridge by rain. When released into the atmosphere sporangia may cause new foliar infections in the same crop or neighbouring crops. When washed into the soil, sporangia may cause tuber infections. In both cases, the ambient temperature determines whether the sporangium germinates directly (optimum at ±23°C) or indirectly (optimum at ±12°C). Direct germination results in formation of a germ tube. Indirect germination results in formation of motile zoospores. When zoospores loose their flagellae, they become cystospores which germinate and infect through a germ tube.

P. infestans is a heterothallic oomycete with two compatibility groups, referred to as mating types A1 and A2. The sexual cycle is completed only once per growing season. Oospores are produced in plant tissue when two compatible strains of opposite mating type interact. In Mexico, the presumed centre of origin of *P. infestans*, both mating types are present in approximately equal frequencies, and oospores are commonly found in infected potato crops. Prior to the 1980's, the *P. infestans* population in most other parts of the world consisted of a single clonal lineage (US-1) only containing the A1 mating type. During the 1980's and despite the frequent use of fungicides, late

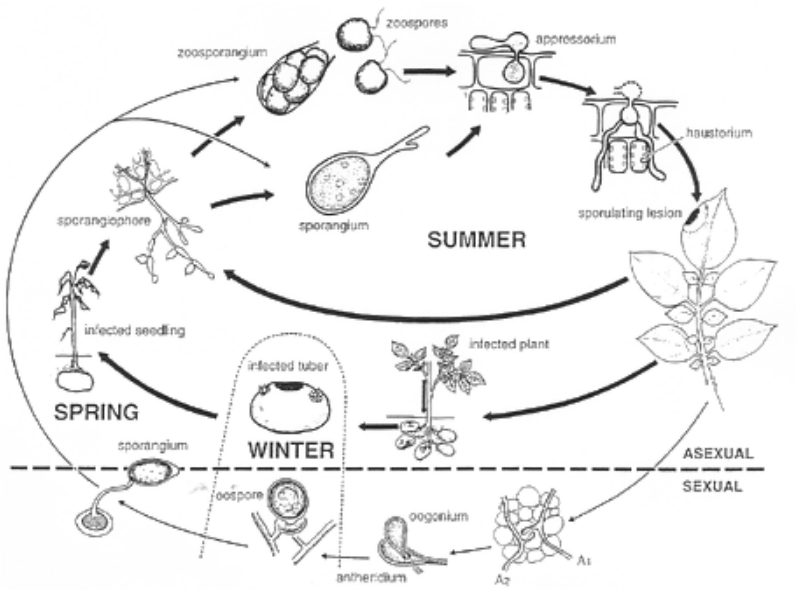

Figure 1. Life cycle of Phytophthora infestans, causal organism of late blight in potato and tomato.

blight epidemics have proven increasingly more difficult to control (e.g. Goodwin et al., 1995). This increased problem with controlling potato late blight coincides with the displacement of the old population (US-1 clonal lineage) by a new, genetically more variable population containing both mating types in many parts of the world. In regions where both mating types have been found, such as the Netherlands, evidence accumulates that sexual reproduction takes place and functional oospores are formed (e.g. Drenth et al., 1994).

In the Netherlands, conventional potato growers control potato late blight using a preventive control strategy. Typically, protectant fungicides are applied just before an infection period, a period of high infection risk due to suitable weather and availability of inoculum. When, for whatever reason, an infection occurs, curative or eradicant fungicides are applied to try and regain control. In organic crops only cultivation techniques are employed in an attempt to prevent or control late blight in potato. Here, an infection of the crop usually ends the growing season prematurely and thus significantly reduces yield and profitability of the crop.

UV illumination of a potato crop, the envisaged application, is aimed at killing *P. infestans* propagules (zoosporangia, zoospores and mycelium) present on the leaf surface.

Scope of the research

The research described in this report was carried out in March 2006 by Plant Research International by request of Clean Light as outlined in contract 06/PRI-0482. Clean Light (P.O.Box 271, 6700 AG Wageningen, the Netherlands, www.cleanlight.nl) is the developer/owner of the UV Crop Protection Technology described in this report. Modification of the experimental procedure with respect to the range of UV dose rates applied, was carried out based on the results of a pilot experiment and approved by Clean Light. The new procedure covered a range from 0 to 10mJ/cm^2.

The aim was to determine a dose response curve for the effect of UV-exposure of *P. infestans* sporangia on water agar on subsequent direct germination of the sporangia.
A 75 Watt UV source (Philips TUV 64T5 4P SE, Figure 2) was received from Clean Light and used for the experiments. VK98014, the *P. infestans* isolate used in the experiments was isolated in the Netherlands in 1998 and is representative for the current, new, population of *P. infestans* in the Netherlands.

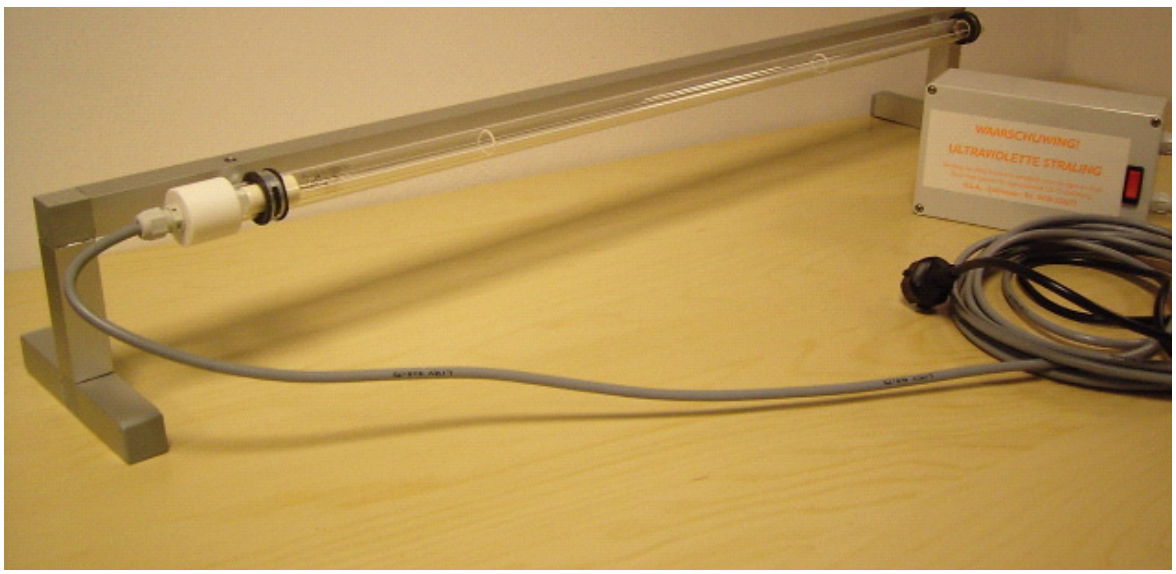

Figure 2. The 75 Watt UV source (Philips TUV 64T5 4P SE) as used in the experiment.

2. Materials and methods

The aim of the experiments was to determine a dose response curve for the effect of UV-exposure on direct germination of *P. infestans* sporangia on water agar. The UV source was mounted on a spray boom inside a spray cabinet at PRI. Spray boom speed and distance to the UV source are adjustable and thus the UV dose rate applied can be varied according to the desired intensity. Four experiments were carried out:

- Base line experiment to determine the dose rate UV received for the different speeds of the spray boom and for a range of distances from the UV source.
- Pilot experiment to determine the range of UV dose rates effective against germination of *P. infestans* sporangia. The range of dose rates applied in the "dose response curve experiments" was determined based on the results of this experiments.
- Two experiments to determine the dose response curve for UV exposure on direct germination of zoosporangia.

Culturing and preparation of sporangial suspensions

P. infestans isolate VK98014 was grown on pea agar for two weeks. After this period, the cultures were flooded with sterile water producing a sporangial suspension which was adjusted to $1x10^5$ sporangia/ml. 200 µl of this suspension was distributed over the entire surface area of Petri dishes containing 1,5% water agar. The petri dishes containing water agar and sporangia were exposed to UV illumination during the experiments.

Base line experiment

A base line experiment was carried out to fine tune the experimental set up to deliver the dose rates required. For this purpose, the dose rate received was measured using a Lutron UVC-254 UV light meter (Figure 3). UV intensity was measured for a range of spray boom velocities and distances from the UV source. The effect of "distances from the source" on the dose rate received was determined with a static (non moving) UV source. The experimental set up is schematically represented in Figure 4.
The effect of the speed of the spray boom on the dose rate received was determined with the sensor at 24.5 or 50.5 cm distance from the source with spray boom velocities of 0.36, 0.49, 0.61, 0.74, 0.86, 0.97, 1.08 and 1.19 m/s.
To determine the effect of distance from the source on the dose rate received, the sensor was placed at 76.5, 70, 61, 54.5, 48, 41.5, 35, 28.5, 22, and 15.5 cm from the source using a static source in position "B" (Figure 4).

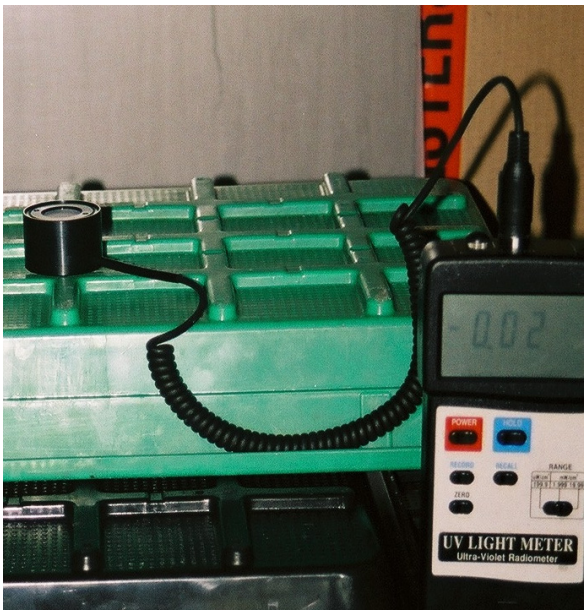

Figure 3. UV light meter as used in the base line experiment to determine the effect of spray boom velocity and distance from the source on the dose rate received.

Pilot experiment

The pilot experiment was carried out to determine the appropriate range of UV dose rates for the "dose response curve experiment". Petri dishes containing water agar and *P. infestans* sporangia were exposed to a range of UV

dose rates from 0 – 10 mJ/cm^2 . Two replicate petri dishes were included for each UV dose rate applied. After treatment, Petri dishes were incubated at 15°C for 24 hours. Germination was then determined by scoring germination for 100 sporangia per replicate petri dish using a microscope at 100x magnification. A sporangium was considered to be germinated when the germ tube was longer than half the diameter of the sporangium.

UV Dose response curve experiments

Petri dishes containing water agar and *P. infestans* zoosporangia were exposed to a range of UV dose rates. The UV source was switched on at least 2 minutes before starting the experiment. The lamp was used in a static position (B in Figure 4) to achieve the required dose rate range.

For each treatment, the UV source was placed in "position B" and kept there for 0, 1, 2, 3, 4, 5, 6, 8 or 10 s resulting in a UV dose rate range of 0, 0.95, 1.9, 2.85, 3.8, 4.75, 5.7, 7.6 and 9.5 mJ/cm^2.
The experiment was carried out on 20 March 2006 and replicated the same day using a different batch of *P. infestans* cultures and water agar petri dishes.

Statistical analysis

Logistic (symmetrical sigmoid) curves were fitted to the data using the Genstat FITCURVE directive. This directive produces estimates for the parameters describing the sigmoid curve including the corresponding standard error. The general mathematical form of a sigmoid curve is given in Equation 1. For the current dataset, "y" represents the percentage of germinated sporangia, "x" the UV dose rate received, "c" the upper asymptote i.e. the percentage germination for non-UV treated sporangia and "a" the lower asymptote i.e. the minimum percentage germination after UV treatment. "b" determines the steepness of the curve, "m" gives the UV dose rate reducing germination by 50% (the ED_{50}). For experiment two, the data obtained at 1.9 mJ/cm^2 were not included in fitting the sigmoid curve. Germination at 1.9 mJ/cm^2 was higher than at 0 mJ/cm^2 making it impossible to fit a suitable sigmoid curve.

$$y = a + \frac{c}{(1 + e^{-b(x-m)})}$$ Equation 1

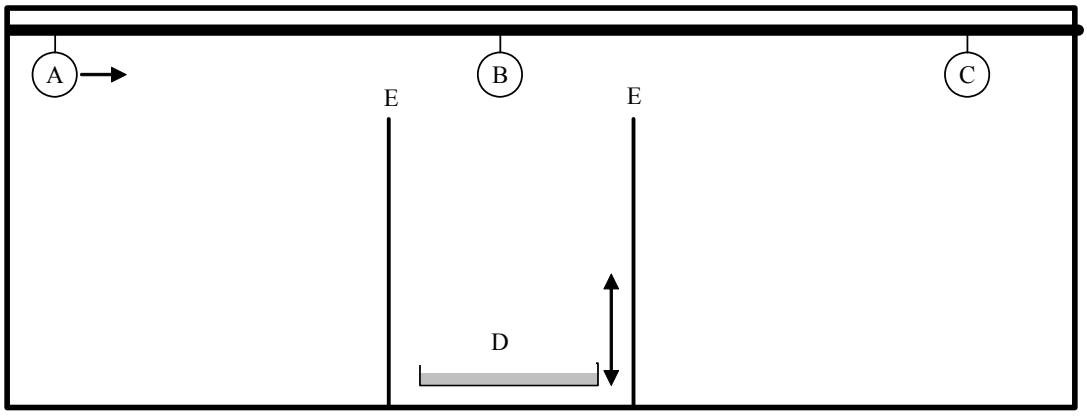

Figure 4. *Schematic of the experimental set up. Petri dish "D" containing water agar + P. infestans sporangia on top of the agar is exposed to UV from a source mounted on a rail and moving from its start position (A) via position "B" where the object receives the maximum UV dose rate to its end position (C). Two screens (E) are placed to protect the object from UV illumination when the source is in its start or end position. The UV source is not switched of during the experiment to ensure a stable UV output of the source. The spray cabinet measures 5x1x1m (LxHxW). Speed of the spray boom and distance of the Petri dish to the UV source are adjustable.*

3. Results

Base line experiment

Results of the measurements on the effect of distance from the UV source on UV intensity given in Figure 5. The relationship between UV intensity (mW/cm^2) and distance from the source can be mathematically described by the exponential decline function of Equation 2.
The relationship between the UV dose rate received and spray boom velocity could only be approximated since the UV sensor measured the instantaneous UV dose rate, not the cumulative UV dose rate. Since the experiments only used the UV source in a static position above the objects, these data are not included in this report.

$$y = 61.43x^{-1.33} \qquad \text{Equation 2}$$

Pilot experiment

The UV dose rate received for the different treatments included in the pilot experiment (2 distances to the source, 24.5 and 50.5 cm), 8 velocities of the spray boom + UV source (0.36, 0.49, 0.61, 0.74, 0.86, 0.97, 1.08 and 1.19 m/s) and two stationary settings (5 and 10 s) were translated to an estimated UV dose rate received. This resulted in a range from 0 – 9.5 mJ/cm^2 UV received. The effects on germination of *P. infestans* sporangia is summarized in Figure 6. Especially the higher UV dose rates applied resulted in a significant reduction of direct germination of the sporangia. Based on these results, the range of choice for the dose response experiments was set to 0 – 10 mJ/cm^2.

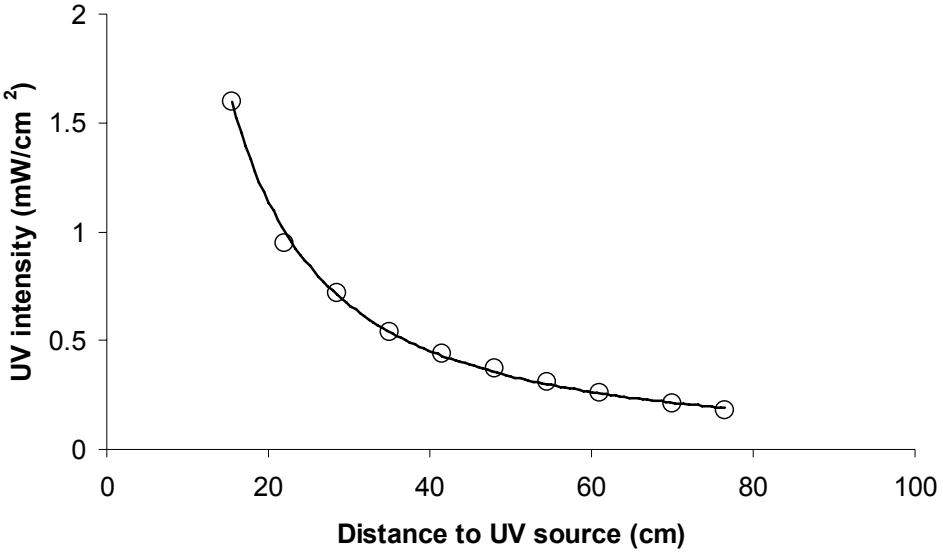

Figure 5. Effect of "distance from the source" on UV intensity as measured in the base line experiment using a Lutron UVC-254 UV light meter. The regression line is mathematically described by Equation 2.

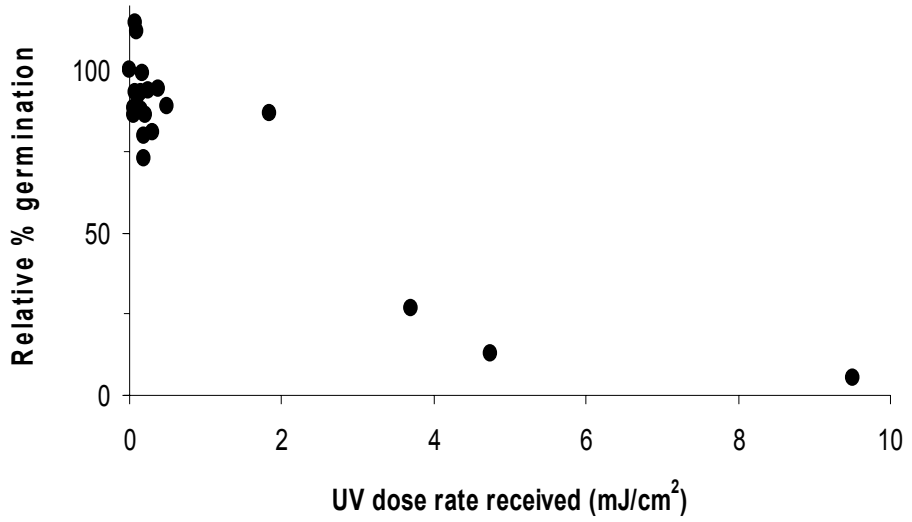

Figure 6. Results of the pilot experiment, effect of a range of UV dose rates on direct germination of P. infestans sporangia.

UV dose response curve

Results of both dose response curve experiments are summarized in Figure 7. Average germination in the non illuminated control treatment was 23 and 18% respectively for experiment 1 and 2. Regression analysis resulted in the similar Equation 3 and Equation 4 for the resulting dose response curves of experiment 1 and 2 respectively. The dose rate reducing germination by 50%, the ED_{50}, is calculated at 5.00 and 4.80 mJ/cm^2 for experiment 1 and 2 respectively.

Germination just before exposure to the UV source was checked and found to be 1.2% and 2.1% in experiment 1 and 2 respectively. Germination following the maximum dose rate of 9.5 mJ/cm^2 was 1.4% and 1.6% and not significantly different from germination found prior to UV exposure. It can be concluded that in both experiments the maximum dose rate applied completely inhibited direct germination of the sporangia on water agar.
The ED_{95} can be approximated as 8.3 mJ/cm^2 and 6.2 mJ/cm^2 for experiment 1 and 2 respectively.

Eight days after the experiment, the sporangia were checked for germination a second time for 0 (experiment 1), 8 and 9.5 mJ/cm^2 UV received. Germination after eight days incubation on water agar following UV treatment was found to be 49% for 0 mJ/cm^2, 50% and 32% for 8 mJ/cm^2 in experiment 1 and 2 respectively and 32% and 3.6% for 9.5 mJ/cm^2. It can be concluded that for most UV dose rates, the sporangia were not killed by the UV treatment. Up to 8 mJ/cm^2 the germination level after 8 days is approximately equal to that found for 0 mJ/cm^2 after eight days incubation. At 9.5 mJ/cm^2 the germination levels found after eight days incubation are significantly lower than those found at 0 mJ/cm^2 indicating a lasting affect of UV exposure on germination. Practical implications of the recovery effect observed are likely to be very limited since *P. infestans* sporangia are not likely to survive prolonged periods of several days on leaf surfaces in potato canopies.

$$y = 3.46 + \frac{23.85}{(1 + e^{0.95(x-5.00)})}$$ Equation 3

$$y = 1.82 \frac{20.56}{(1 + e^{2.18(x-4.80)})}$$ Equation 4

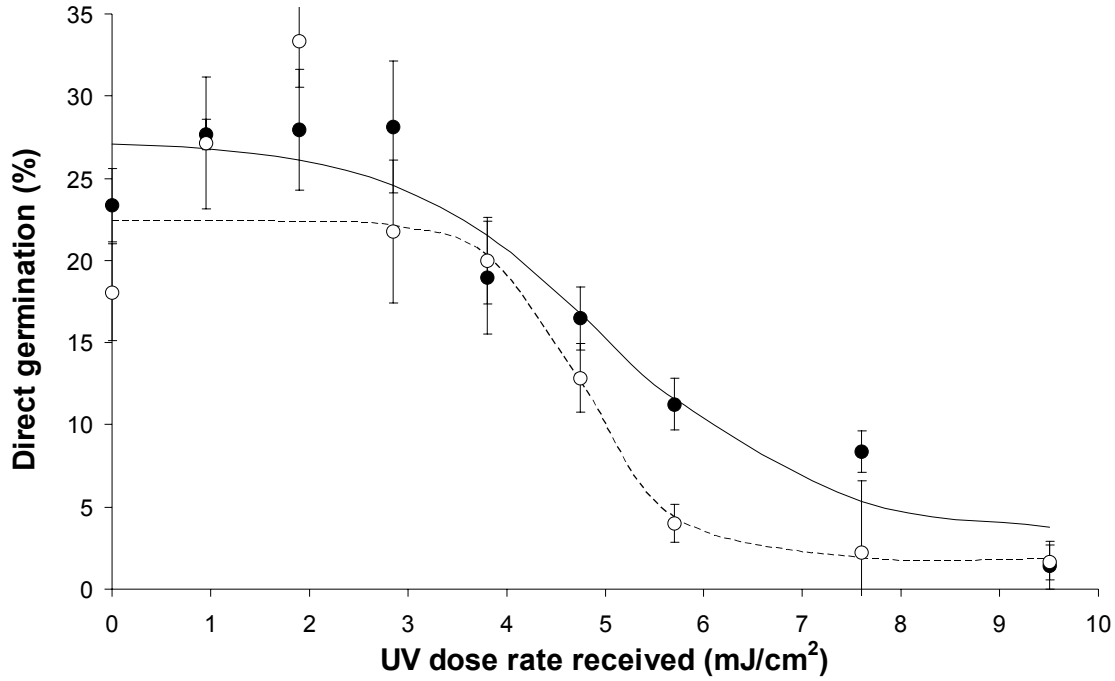

Figure 7. *Results of two experiments designed to determine the dose response curve for the effect of UV exposure on direct germination of Phytophthora infestans sporangia on water agar. Markers ● and ○ represent average germination levels for experiment 1 and 2 respectively. Error bars represent the standard deviation for the corresponding average. The solid regression line represents the calculated sigmoid dose response curve based on the results of experiment 1 whereas the dashed regression line represents the calculated sigmoid dose response curve based on results from experiment two. Equations 3 and 4 mathematically describe both regression lines.*

4. Discussion

The aim of the research described in this report was to determine dose response curves for UV exposure on direct germination of sporangia of *P. infestans*, causal agent of potato and tomato late blight. For this purpose, the spray boom of an experimental spray cabinet was modified to carry a Philips TUV 64T5 4P SE UV source. Using the results of two preliminary experiments, the experimental set up was fine tuned to deliver a range of UV dose rates resulting in "no effect on germination" for the lowest dose rates to "complete inhibition of germination" for the highest dose rates. A replicated experiment was the carried out, using a stationary UV source above the target, resulting in two dose response curves given in Figure 7, mathematically described by Equations 3 and 4. UV exposure strongly reduces sporangial germination for the higher UV dose rates used in the experiments (5 – 10 mJ/cm^2).

During the current experiments *P. infestans* sporangia were exposed to UV on a water agar surface. The effect of this matrix, if any, on the experimental results is unknown. It is however safe to assume that a potato leaf is a more harsh environment in which sporangia are less likely to survive and germinate than on water agar. On the other hand, *P. infestans* was grown on artificial medium (pea agar) under constant laboratory conditions which might result in sporangia that are slightly more susceptible to UV than sporangia formed under natural conditions on potato crops.
A slight increase of germination was observed after exposure to low dose rates of UV (1 – 3 mJ/cm^2). Although this increase in germination was not tested for statistical significance, it is known from experiments with fungicides that low dose rates of fungicides can stimulate germination instead of reducing it. Here we may be observing a similar phenomenon, as a last attempt of the organism to escape an unsuitable environment.

Germination was determined 24 hours after UV exposure resulting in the data as reported. When germination was determined a second time for a limited number of treatments and replicates, eight days after UV exposure, the level of germination was higher than during the first observation. Apparently, the sporangia were not killed by most of the dose rates applied although germination was significantly delayed. Germination of sporangia exposed to 8 mJ/cm^2 UV recovered after eight days to almost the level observed in the untreated petri dishes. This was not the case at 9.5 mJ/cm^2 UV received, after which recovery was incomplete (experiment 1) or almost absent (experiment2). For practical purposes, the recovery of germination observed under laboratory conditions is probably insignificant because it is unlikely that *P. infestans* sporangia (which have been exposed to UV treatment) survive for 24 hours or more under the harsh conditions of the potato phyllosphere.

Overall, exposure of *P. infestans* sporangia on water agar at 10mJ/cm^2 , while a low dose, appears to be effective at killing these propagules of Phytophthora infestans".

Appendix I.
References

Anonymous 1996.
Research needed to halt rapidly spreading late blight strains. In: CIP in 1995. The international potato center annual report. Lima, Peru 10-13.

Drenth, A. Turkensteen, L.J. and Govers, F. 1994.
The occurrence of the A2 mating type of Phytophthora infestans in the Netherlands: Significance and consequences. Netherlands Journal of Plant-Pathology 99: 57-67.

Duncan, J.M. 1999.
Phytophthora – an abiding threat to our crops. Microbiology today 26:114-116.

Goodwin, S.B., Sujkowski, L.S, Dyer, A.T., Fry, B.A. and Fry, W.E., 1995.
Direct detection of gene flow and probable sexual reproduction of Phytophthora infestans in Northern North America. Phytopathology 85: 473 – 479.

Hooker, W.J. 1981.
Compendium of potato diseases. American Phytopathological Society, St. Paul, MN, USA.