# UV-c radiation as an alternative tool to control powdery mildew on apple and strawberry

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**Abstract**

*The most used and effective strategy to control fungal diseases in fruit growing is the application of fungicides. The use of fungicides, however, can have a negative impact on the environment and its frequent use enhances the risk of fungicide resistance. Moreover, stronger limits concerning the maximum residue levels (MRLs) on fruits are set up by retailers and the export industry. In the future it will be a great challenge to produce fruits of high quality with a minimal input of pesticides. As such, a search for alternative methods, like biological control agents or physical treatments, which are able to efficiently reduce fruit diseases is required.*

*To this end a physical technique, ultraviolet light in particular, was developed by Clean Light Inc. of The Netherlands.*

*This technology was tested for its capacity to reduce powdery mildew infestation on apple and strawberry leaves. The applied exposure doses ranged from 5 to 60 mJ/cm$^2$. The effect of UV-c radiation was tested on the establishment of powdery mildew on leaves of apple seedlings and strawberry plants. For both apple and strawberry the powdery mildew infestation was significantly reduced by exposing the leaves of both plant species to UV-c with a dose of 30 mJ/cm$^2$, without negative effects on plant performance. The obtained efficacy levels were comparable with specific fungicide treatments. A regular application over time was better than using higher dose rates with longer intervals between applications.*

**Keywords:** Powdery mildew, UV-c light, physical control measurement

## Introduction

During the last decade fruit growers face a dilemma in the control of pathogens on their crops. Export countries, governments and consumers demand a product free of pathogens but on the other hand they want a minimal level of pesticide residues on the final product.

To this end, alternative techniques, like biological control, use of natural products and sanitary and physical measurements to help growers in controlling pathogen attack with a minimal input of chemical products are being developed (Tripathy and Dubey, 2004; Pal et al., 2006).

One specific technique is the use of ultraviolet light (UV-c) to control pathogen development on crops. This technique is owned by Cleanlight BV of the Netherlands (www.cleanlight.nl, PO Box 271, 6700 AG Wageningen). It is based on the fact that fungi are already killed by low doses of ultraviolet light, while plant cells can tolerate much higher doses of UV-c light. The uniqueness of this patented technology is based on the use of doses that are substantially lower than hitherto determined as being effective in the scientific world. The fact that these doses are so low, make it possible to kill fungi on a crop while damage to the crop is avoided.

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The effect of application of UV-light to reduce the impact of pathogens is already tested with succes on golf courses, most specifically golf greens (www.clean-greens.com) and for the control of postharvest decay (Marquenie, 2002; Pan *et al*., 2004; González-Aguilar *et al*., 2006; Stevens *et al*., 2009). Furthermore, the Clean Light Technology has also been tested on onions, potatoes, tomatoes, green peppers, cucumbers, lavender, etc.

In this article, the efficacy of UV-c light for the control of powdery mildew on apple seedlings and on strawberry plants is investigated.

## Material and Methods

Plant material

Seeds of *Malus sylvestris* were sown on soil and covered with a thin soil layer. A small amount of captan was applied to reduce fungal infestation of seeds during storage. The seeds were stratified at 4°C for 3 months. After the stratification the seeds were placed in the greenhouse for germination. Thereafter the plants were potted in small trays (9 x 9 cm) and put at 20°C and a period of 14 h daylight for growth.

Cold stored strawberry plants of the cultivar Elsanta were grown in the greenhouse for about 3 weeks prior to the analysis.

Inoculation of plants

Apple seedlings were inoculated with *Podosphaera leucotricha* by rubbing over the seedling leaves with powdery mildew infected apple leaves. The inoculated seedlings were placed at 20°C and 100% RH for 48 h in mini greenhouses. Thereafter the plants were placed in the greenhouse at 20°C and 60%RH for 2 weeks.

Strawberry plants were planted in trays (10 plants/tray) and were inoculated with *Sphaerotheca macularis* by rubbing over the strawberry leaves with powdery mildew infected strawberry leaves. The inoculated plants were placed at 20°C and 100% RH for 48 h in mini greenhouses. Thereafter the plants were placed in the greenhouse at 20°C and 60%RH for 2 weeks.

Application of UV-c

Three UV-c lights as produced by UV Source of Nijmegen, Netherlands (www.uvsource.eu) were used. These lights differ from commonly used water desinfection lights in that they radiate only the required wavelength, and no unnecessary others, which could cause the formation of dangerous ozon, or which could cause damage to the crop. The lights were installed above a mobile table, which was used for the application of the UV-c light. One light was installed above the plants, one at the left hand side and one on the right hand side (Figure 1). A correlation was made between the speed of the table and the UV-c dosis applied. As such the correct speed of the table to apply a certain dose of UV-c light could be determined. On the issue of dose we should provide additional explanation. The doses were measured using a UVc dosage meter, perpendicular to the source of the light. As such we measured fairly high doses. In fact, the surface of the plant is usually not perpendicular to the source of light, since a plant or flower is hardly a flat surface. This means that the dose recieved by the plant surface, or by the fungus in question is factually much lower than indicated in this trial.

As shown in table 1, different UV-c doses were applied to the plants in different time intervals. For the test on apple seedlings, each object consisted of 15 individual apple seedlings. For the test on strawberry plants, each object consisted of 10 strawberry plants (1 tray). Based on the results from the trial with the apple seedlings, some object were withdrawn from the test with strawberry plants. Chemical control in the strawberry trial consisted of an application with Fortress at 2 days post inoculation (dpi) and a treatment with Signum (boscalid & pyraclostrobine) at 9 dpi, both applied at the registered dose rate. The trials were performed within a time frame of 16 days and were evaluated the day after the final UV-c treatment.

Both tests were evaluated by scoring each individual leaf of the plants according to the “good experimental practice” (GEP) guidelines. From each plant, each leaf was evaluated by giving a score from 0 to 3. Score 0: no powdery mildew symptoms; 1: low powdery mildew infestation (<10% of the surface infested); 2: moderate powdery mildew infestation (10< x <50%); 3: severe powdery mildew infestation (> 50%).

Statistical analysis

Statistical analyses were performed on %A using the Unistat Statistical Package, version 5.5 (Unistat Ltd. 1998). Means were compared using Duncan’s multiple range test or Friedman multiple comparison test.

Table 1: Trial set-up

| object | apple seedlings | strawberry plants |
|---|---|---|
| 1 | untreated | untreated |
| 2 | 30mJ 2hpi | 30mJ 2 and 24hpi |
| | **starting 72h after inoculation** | **starting 48h after inoculation** |
| 3 | 30mJ 2hpi + 30mJ every 48h | 30mJ every 24h |
| 4 | 30mJ every 24h | 30mJ every 48h |
| 5 | 30mJ every 48h | 30mJ every 72h |
| 6 | 30mJ every 72h | 10mJ every 24h |
| 7 | 5mJ every 24h | 15mJ every 24h |
| 8 | 10mJ every 24h | 60mJ every 24h |
| 9 | 15mJ every day | 30mJ with air flow every 24h |
| 10 | 60mJ every day | chemical control (2 and 9 dpi) |
| 11 | 30mJ with air flow every 24h | |
| | **starting 7dpi** | |
| 12 | 30mJ every 24h | |
| 13 | 30mJ every 72h | |

hpi: hours post infection; dpi: days post infection.

## Results

In the summer of 2009 two lab trials to test the efficacy of UV-c light against powdery mildew on apple seedlings and on strawberry was set up. In Figure 1, the assembly of the lights on a motorised table is shown.

As can be seen from Table 2 and Figure 2(A), the efficacy of the different UV-c treatments against powdery mildew was high as compared to the untreated control plants. The effect of the UV-c radiation was dependent on the dosis of UV-c applied to the plants as the efficacy of 5mJ was much lower than when 30mJ was applied to the plants. Enhancing the UV-c dosis to 60mJ had no additional effect on the efficacy towards powdery mildew.

Furthermore, variation in the application interval (1, 2 or 3 days) only greatly reduced the efficacy when UV-c application took only place every 72h. Application of air flow in order to reach a higher percentage of the leaf area with the UV-c light did not enhance the effect of the UV-c light. The highest efficacy was obtained by application of 30 to 60mJ every day.

Figure 1: Representation of trial set-up with the motorised table. The black arrows indicate the position of the three different lights.

Table 3 and Figure 2B show the efficacy of UV-c light against powdery mildew on strawberry.

The same conclusions as for apple seedlings could be drawn from this test. Application of UV-c light significantly reduced powdery mildew infestation as compared to the untreated control plants. The efficacy of some applications was at least similar to that of the chemical control. In this trial, the highest efficacy of UV-c light is also obtained by application of 30 to 60mJ every day.

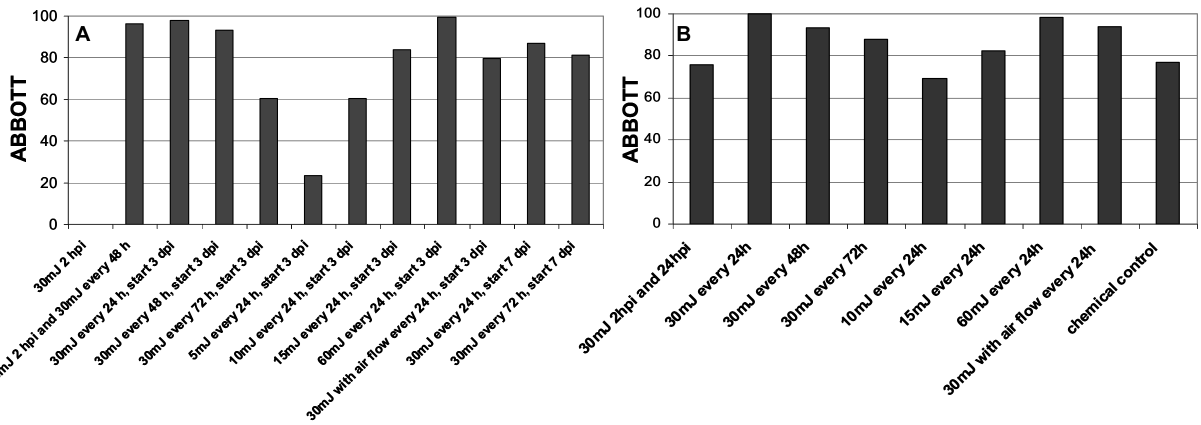


Figure 2: Efficacy of the UV-c treatments against powdery mildew. (A) Trial performed on apple seedlings; (B) trial performed on strawberry plants. dpi: days post infection.

Table 2: Powdery mildew infestation on the apple seedlings and the efficacy (ABBOTT) of the different treatments against powdery mildew

| TREATMENT | % infestation | STAT* | TH3 | EFFICACY (ABBOTT) |
|---|---|---|---|---|
| Untreated | 81,66 | g | 1,93 | |
| 30mJ 2 hpi | 92,17 | g | 2,01 | -4,00 |
| 30mJ 2 hpi and 30mJ every 48 h | 7,43 | ab | 0,07 | 96,15 |
| 30mJ every 24 h, start 3 dpi | 4,15 | a | 0,04 | 97,85 |
| 30mJ every 48 h, start 3 dpi | 13,56 | bc | 0,14 | 92,98 |
| 30mJ every 72 h, start 3 dpi | 57,45 | ef | 0,76 | 60,53 |
| 5mJ every 24 h, start 3 dpi | 84,78 | g | 1,48 | 23,65 |
| 10mJ every 24 h, start 3 dpi | 54,86 | f | 0,76 | 60,56 |
| 15mJ every 24 h, start 3 dpi | 28,35 | cd | 0,32 | 83,60 |
| 60mJ every 24 h, start 3 dpi | 0,83 | a | 0,01 | 99,57 |
| 30mJ with air flow every 24 h, start 3 dpi | 35,74 | de | 0,39 | 79,87 |
| 30mJ every 24 h, start 7 dpi | 21,27 | c | 0,25 | 87,02 |
| 30mJ every 72 h, start 7 dpi | 24,31 | c | 0,36 | 81,17 |

* different number indicated significantly different objects (p>0.05).

Table 3: Powdery mildew infestation on the strawberry plants and the efficacy (ABBOTT) of the different treatments against powdery mildew

| TREATMENT | % infestation | STAT* | TH3 | EFFICACY (ABBOTT) |
|---|---|---|---|---|
| Untreated | 93,57 | d | 2,24 | |
| 30mJ 2hpi and 24hpi | 30,47 | bc | 0,55 | 75,64 |
| 30mJ every 24h | 0 | a | 0 | 100,00 |
| 30mJ every 48h | 13,64 | a | 0,15 | 93,26 |
| 30mJ every 72h | 23,61 | bc | 0,27 | 87,79 |
| 10mJ every 24h | 45,40 | cd | 0,69 | 69,20 |
| 15mJ every 24h | 31,05 | bc | 0,40 | 82,23 |
| 60mJ every 24h | 4,17 | a | 0,04 | 98,14 |
| 30mJ with air flow every 24h | 13,67 | b | 0,14 | 93,91 |
| chemical control | 34,35 | bcd | 0,52 | 76,99 |

* different number indicated significantly different objects (p>0.05).

## Discussion

As stronger limits concerning MRLs on fruits are set-up by the government, retailers and the export industry, alternative techniques to control pathogen infestation are studied intensively over the past decade. One of the techniques investigated is the use of UV-c light. Up to now, most results are obtained in trials to test the postharvest lifetime of fruits. In these trials, application of UV-c seems to enhance the postharvest life time of strawberries and tomato's by reducing the Botrytis infestation (Pan *et al*., 2004; Charles *et al*., 2008). On the other hand, field application of the UV-c technique is also under investigation. The trials discussed here indicate that UV-c can be a promising technique to control powdery mildew in the field. However, field trials still need to be performed to confirm our observations. Furthermore, in greenhouse trials to control Botrytis stem rot, application of UV-c reduced the number of new Botrytis spots on the stem (Heuvelink, 2006).

Our results obtained here suggest also that for field trials, a regular application over time is better than using higher dose rates with longer intervals between the applications. The same observations were also done by Heuvelink (2006).

It is known that UV-c light has a direct effect on the pathogen by killing its mycelium. However, another not yet identified underlying effect can also play a role. In grapes, for example, UV-c application leads to the enhancement of defense related mechanisms (Bonomelli *et al.*, 2004). Futhermore, in tomato's application of UV-c results in fortification of the fruit surface. However, this effect alone could not explain the reduced colonization of the UV-treated fruit by the pathogen (Charles *et al*., 2008).

In this trial we simply killed the fungus, and it proved to be effective. The powdery mildew pathogen grows superficial and is as such easy to reach by UV-c light, which cannot penetrate the leaves. The additional benefits that are described in the literature refer almost exclusively to plants grown under glass i.e. plants grown in a UV starved environment. It is thus not expected that the additional benefits as described in the literature will be seen in outdoor crops such as apples. It is believed that the benefits to outdoor crops are simply limited to the fungus killing benefits as were observed in this trial.

In conclusion, up to now promising results are obtained with the use of UV-c light in controlling pathogen infestation. However, further investigation is still required.

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