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# Impact of UV-C radiation on the sensitivity of three strawberry plant cultivars (*Fragaria x ananassa*) against *Botrytis cinerea*

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ABSTRACT

Several studies suggest that UV-C radiation, known for its disinfecting effect, may also stimulate plant defenses. The objective of this study is to reduce the sensitivity of strawberry plants (*Fragaria x ananassa*) to *Botrytis cinerea* by application of non-deleterious doses of highly energetic UV-C light (254 nm) on leaves. Preliminary tests were carried out on strawberry plants: Cirafine, Charlotte and Candiss, to optimize the doses of UV-C to apply on plants and to test the sensitivity of these three cultivars to *B. cinerea*. The three cultivars showed different levels of susceptibility to *B. cinerea*: Cirafine was the most resistant followed by Charlotte and Candiss being the most sensitive. These observations were supported by histological examination and phenol levels in the leaves that indicated deeper penetration of *B. cinerea* into Candiss. Nine variations of treatments were applied to the plants, which were composed of varying UV-C doses and differing application frequencies. The treatment of UV-C applied at 0.85 and 1.70 kJ/m$^2$, four times every second day (p-value = 0.05), were shown to have a significant increase, around 25%, in the protection of Candiss against Bc1 strain of *B. cinerea*. Our observations show that exposing strawberry plants (Candiss) to low repeated doses of UV-C could improve their resistance against gray mold, while avoiding any apparent negative effects to the plants.

## 1. Introduction

The cultivated strawberry *Fragaria x ananassa* is one of the most important fruit crops worldwide. It is ranked first within berry crops with a worldwide fruit yield of 4.1 million tons per year (Flachowsky et al., 2011). Strawberries are produced in more than 70 countries and organic fruit production is becoming increasingly important (Wilbois et al., 2012).

Pathogen development on host plants, especially on plants producing edible fruits, has a major impact on agricultural production giving rise at once economical issue and phytosanitary issue. Several diseases can be particularly damaging for strawberry production, and among these, gray mold is a major concern for growers. Gray mold is an airborne disease, caused by the necrotrophic fungus *Botrytis cinerea*. The disease is difficult to control because the fungus can infect the plant as well as the fruits (Williamson et al., 2007). *B. cinerea* can also cause partial or total destruction of the host plant and its products like fruit, in pre- or post-harvest (Gullino, 1992). The most common and effective strategy to control gray mold is the application of fungicides. Unfortunately, the frequent use of fungicides can lead to the development of pathogen resistance (Fillinger and Walker, 2015). In addition, the excessive use of fungicides can have a negative impact on the environment as well as on human health, requiring the development of environmentally-friendly alternatives.

Therefore, there is a growing interest in alternative methods that could enabled the stimulation of plants defense mechanisms, based on the use of biotic or abiotic factors (Conrath, 2009). Unlike chemical products, abiotic mechanisms for pathogen control, such as light radiation, have been sparsely studied. Outside the visible light, infra-red light (IR) or ultraviolet light (UV) have specific properties which are exploited in agricultural sector and food industries. UV light corresponds to electromagnetic irradiations produced by the sun or by an

*Abbreviations:* APX, ascorbate peroxydase; AUDPC, area under the disease progress curve; AUFC, area under the curve of induction of the fluorescence of chlorophyll *a*; CAT, chloramphenicol acetyltransferase; CIREF, centre interrégional de recherche et d'expérimentation de la fraise; IR, infra-red light; PAL, phenylalanine ammonia lyase; PI, performance index; POD, peroxydase; PPO, polyphenol oxydase; PSII, photosystem II; ROS, reactive oxygen species; SOD, superoxyde dismutase; UV, ultraviolet light; PDA, potato dextrose agar

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artificial source. Three types of UV irradiations, corresponding to three ranges of wavelength, are distinguished according to their energy and biological activities. UV-A (315–400 nm) that are less energetic represent 95% of UV irradiations that arrive on earth surface, UV-B (280–315) that are moderately energetic are largely stopped by the ozone layer and UV-C (100–280 nm) that are the most energetic but they are integrally absorbed by the ozone layer.

Previous studies have shown that light is an important regulator of plant-pathogen interactions (Demkura and Ballare, 2012; Jenkins, 2009; Magerøy et al., 2010). In response to a sudden exposure to high light intensity or to UV light, plants respond by producing reactive oxygen species (ROS) that play a role in plant defenses against pests and diseases. Similarly the rapid accumulation of ROS at the pathogen attack site is toxic to pathogens directly (Lamb and Dixon, 1997) and can trigger signaling pathways that are responsible for the activation of other defense mechanisms (Dat et al., 2000; Grant and Loake, 2000). Part of these resistances can be attributed to changes in plant tissue metabolites induced by UV-B radiations, which include accumulation of protective phenolic compounds and enhancement of jasmonic acid dependent defense responses (Ballare et al., 1996; Demkura et al., 2010; Foggo et al., 2007; Izaguirre et al., 2003, 2007; Kuhlmann and Müller, 2009; Mazza et al., 1999; Rousseaux et al., 1998). Indeed, UV-B radiations stimulates transcription of defense genes, including those encoding for phenylalanine ammonia-lyase (PAL) and chalcone synthase, two key-enzymes controlling the synthesis of defence-related phenolic compounds, as well as pathogenesis-related proteins such as chitinase and ß-1,3-glucanase (El Ghaouth et al., 2003; Bonomelli et al., 2004; Borie et al., 2004).

There is however a major problem associated with UV-B light: it is generally effective only when delivered over rather extensive periods of time because of its lower photon energy, typically several hours or days. It is often difficult to consider exploiting UV-B light in practical terms. Hence the idea to using UV-C light which is capable to supply large amounts of energy in a very short period of time. The lethal effect of UV-C light has been exploited successfully to control postharvest diseases, thus extending shelf-life of fruits and vegetables (Liu et al., 2011; Maharaj et al., 1999; Mercier et al., 2001; Mercier et al., 1993a; Siddiqui et al., 2011). Previous studies have also defined hormetic doses that can stimulate plant defense without entailing negative side effects on the stored plant organs like fruits (Charles et al., 2008a,b,c,d; Charles et al., 2009; Ouhibi et al., 2015a,b; Pataro et al.,2015; Pinheiro et al., 2015; Sari et al., 2016; Mohamed et al., 2017). Numerous studies have tested different UV-C doses in order to find hormetic dose and doses were in the range of 0.125–9 kJ/m$^2$ (Pombo et al., 2011; Ouhibi et al., 2015a,b; Vasquez et al., 2017). Moreover, UV-C radiation stimulates production of pathogenesis-related proteins that play a significant role in plant defense such as chitinase and β-1,3-glucanase in strawberry leaves infected by *Colletotrichum acutatum* or in fruit infected by *B. cinerea* (Casado-Díaz et al., 2006; Jin et al., 2017). If a large number of studies have shown that UV-C radiation elicits defense responses in fruits, few studies have worked on treating plants during their growth. Treating *Arabidopsis*, *Pelargonium* or *Lettuce* plants during their growth with UV-C induces reduction of infection by *Hyaloperono sporaparasitica* and *B. cinerea* (Kunz et al., 2008; Darras et al., 2015; Vasquez et al., 2017). Xie et al. (2016) have shown that UV-C treatment applied in pre-harvest caused no leaf damage at the cumulative dose of 3.6 kJ/m$^2$, and fruits tend to be firmer and in some case redder. Unfortunately, there is still a lack of literature on crops destined for human consumption (Vasquez et al., 2017).

The objective of this study was to research the impact of non-damaging doses of UV-C radiation in strawberry plant on the sensitivity to *B. cinerea*. It was carried out on three strawberry cultivars (Cirafine, Charlotte and Candiss) and two strain of *B. cinerea* (Bc1 and Bc21). We proceeded in two steps. First, we tested the basal level of sensitivity of the three strawberry cultivars against *B. cinerea* by evaluating lesion development of the pathogen (histological experiment) and by measuring phenols content. Secondly, we tested the effect of single and repeated non-deleterious doses of UV-C radiation on lesion development of *B. cinerea* on leaves.

## 2. Material and methods

### 2.1. Plant material

Three cultivars of strawberry plants were used: Cirafine, Charlotte and Candiss. These cultivars have been developed and were provided by CIREF (Centre interrégional de recherche et d'expérimentation de la fraise, Dourville, France) as “frigo” plant and all have red fruits. Candiss corresponds to non-remontant plants, Charlotte and Cirafine are remontant plants.

Strawberry plants were transplanted in pots containing a horticulture compost mix (TS4 type, Klasmann and Deimann) in a glasshouse. The plants were fertilized with a standard commercial nutrient solution (Soluveg Parme, NPK 16-6-27 + 3 MgO + OE, Angibaud Derome) with a drip irrigation system (one dripper per pot) at a frequency adapted to the climatic demand. For each repetition, plants were randomly distributed and were grown for 2 months before treatment.

Three batches of plants were produced in 2016 to provide independent repetitions of the whole study. The first repetition (R1) was carried out in spring (from January to April 2016) with mean temperatures of 22.5 °C during the day and 13.5 °C during the night throughout the period of plant growth. The second repetition (R2) was carried out in summer (from April to June 2016) with mean temperatures of 27.0 °C during the day and 14.9 °C during the night. The third repetition (R3) was carried out in autumn (from August to October 2016) with mean temperatures of 27.2 °C during the day and 16.7 °C during the night.

### 2.2. UV-C treatment of plants

A closed box having a ceiling light with 9 UV-C lamps (DSP tube UV-C, OSRAM HNL, 24 W) of 254 nm (Fig. 1) was used to treat strawberry plants with UV-C radiation (Pascal et al., 2018). Four plants were processed at the same time in the box at a distance of 40 cm from the UV-C lamps (Pascal et al., 2018). Several doses and application frequencies were tested during a week (Ouhibi et al., 2015a, b; Vasquez et al., 2017):

- a single application of UV-C at 0.40, 0.85 or 1.70 kJ/m$^2$,
- a double application of UV-C at an interval of two days between each application at either 0.40 + 0.60 kJ/m$^2$ or 0.85 + 1.30 kJ/m$^2$,
- four successive applications of UV-C, every two days, at 0.40, 0.85 or 1.70 kJ/m$^2$.

The calculated UV-C dose applied on the plants depended on the fluence of the UV lamps and on the exposure time (Houghton et al., 2001). Light intensity measurements were performed with a radiometer positioned at 40 cm from the ceiling light and time of plant exposure to UV-C was calculated through the measurement of light intensity at a given time. The duration of UV-C radiation required was 49 s to obtain a final dose of 0.40 kJ/m$^2$, 1 min and 13 s to obtain 0.60 kJ/m$^2$, 1 min and 44 s to obtain 0.85 kJ/m$^2$, 2 min and 39 s to obtain 1.30 kJ/m$^2$ and 3 min and 28 s to obtain 1.70 kJ/m$^2$. Strawberry plants without any UV-C treatments were used as control. To avoid the photo-reactivation of white light (Mercier et al., 2001), plants were placed in the dark for 15 h after each UV-C treatment.

### 2.3. Chlorophyll a fluorescence

In order to characterize the impacts of UV-C treatment on the plant photosystem, the chlorophyll *a* fluorescence was measured (Stirbet and