Chapitre V

Impact des radiations UV-C combinées à des agents de lutte biologique sur la sensibilité du fraisier à *Botrytis cinerea*

I. Lutte biologique

1. Contexte

La protection biologique contre *B. cinerea* à l'aide de microorganismes antagonistes, tels que les champignons filamenteux, les levures et les bactéries, a été intensivement étudiée au cours des dernières décennies (Droby *et al.*, 2009 ; Elmer et Reglinski, 2006 ; Janisiewicz, 1998 ; Mari *et al.*, 2003; Paulitz et Belanger, 2001 ; Van Lenteren, 2000). Le contrôle biologique est également, en plus des rayonnements UV-C, une stratégie prometteuse pour lutter contre les agents pathogènes (Boff *et al.*, 2002 ; Card *et al.*, 2009 ; Cota *et al.*, 2009 ; Sutton *et al.*, 1997 ; Swadling et Jeffries, 1996) et contre *B. cinerea* en particulier (Nicot *et al.*, 2016). Des études ont démontré que le champignon *Microdochium dimerum*, souche L13, avait une bonne efficacité pour protéger les plaies d'effeuillage et les feuilles des plants de tomate contre les attaques de *B. cinerea* en culture sous abris (Bardin *et al.*, 2008 ; Nicot *et al.*, 2003). Un certain nombre de levures (dont *Pichia* et *Rhodotorula*) et de bactéries (dont *Bacillus* et *Pseudomonas*) ont également été signalées pour leur efficacité dans le contrôle de *B. cinerea* en post-récolte (Buck et Jeffers, 2004 ; De Meyer et Hofte, 1997 ; Elad *et al.*, 1994 ; Guetsky *et al.*, 2002).

Pour une efficacité maximale de protection des végétaux et dans le cas d'une gestion intégrée des maladies post-récolte, la combinaison de méthodes physiques (comme les traitements aux rayonnements UV-C) avec un agent de lutte biologique à l'aide de microorganismes antagonistes, a montré une meilleure efficacité de protection contre des maladies post-récolte des fruits, par rapport à un traitement seul (Janisiewicz et Conway 2010).

Nous émettons l'hypothèse que les traitements UV-C appliqués à la surface des feuilles de fraisier, en plus de leur effet inducteur des résistances de la fraise contre *B. cinerea*, pourraient favoriser l'accueil d'agents de lutte biologique

microbien en diminuant la quantité de microorganismes originellement présents sur les feuilles et en limitant ainsi la compétition avec ces microorganismes. Par leur action de désinfection de surface les rayonnements UV-C permettraient ainsi aux agents de lutte biologique de s'installer sur la feuille et donc de favoriser leur efficacité protectrice contre *B. cinerea*.

2. Principe de la lutte biologique

Nous parlons d'antibiose lorsque l'organisme antagoniste produit des métabolites secondaires toxiques pour l'agent pathogène cible. Des substances responsables de l'antibiose ont pu être caractérisées chez des souches appartenant à diverses espèces d'agents de lutte biologique (notamment *B. subtilis, Serratia plymuthica, P. fluorescens*), et les gènes impliqués dans la production de certaines de ces substances ont été identifiés (Duffy *et al.*, 2003 ; Raaijmakers *et al.*, 2002).

Nous parlons d'hyperparasitisme lorsque l'antagoniste est un parasite qui reconnaît spécifiquement sa cible. Il pénètre dans les cellules hôtes et entraîne sa destruction *via* la colonisation de ses organes. *Coniothyrium minitans* est par exemple un champignon hyperparasite de *Sclerotinia sclerotiorum*, capable de produire des enzymes dégradant les parois cellulaires de l'agent pathogène, telles que des chitinases ou des β-1,3-glucanases (Whipps et Gerlagh, 1992).

D'autres mécanismes d'action sont impliqués dans l'efficacité protectrice d'agents de lutte biologique, comme par exemple la compétition nutritive. Le champignon *B. cinerea* est très sensible à l'absence de nutriments : il a, par exemple, besoin de nutriments d'origine extérieur pour assurer la germination de ses spores (Elad, 1996). Certains microorganismes (bactéries, levures, champignons filamenteux) peuvent ainsi inhiber la germination des conidies de cet agent pathogène *via* la compétition pour des éléments nutritifs comme l'azote, le carbone, ou des macro-éléments ou micro-éléments présents dans le milieu (Blakeman et Fokkema, 1982 ; Elad et Stewart, 2004 ; Filonow, 1998). Le champignon antagoniste *Trichoderma harzianum*, souche T39, inhibe aussi la germination des

conidies de *B. cinerea* en rentrant en compétition pour les nutriments à des stades précoces de l'interaction (Zimand *et al.*, 1996).

3. Agents de lutte biologique utilisés dans cette étude

a. Sérénade® : Bacillus subtilis

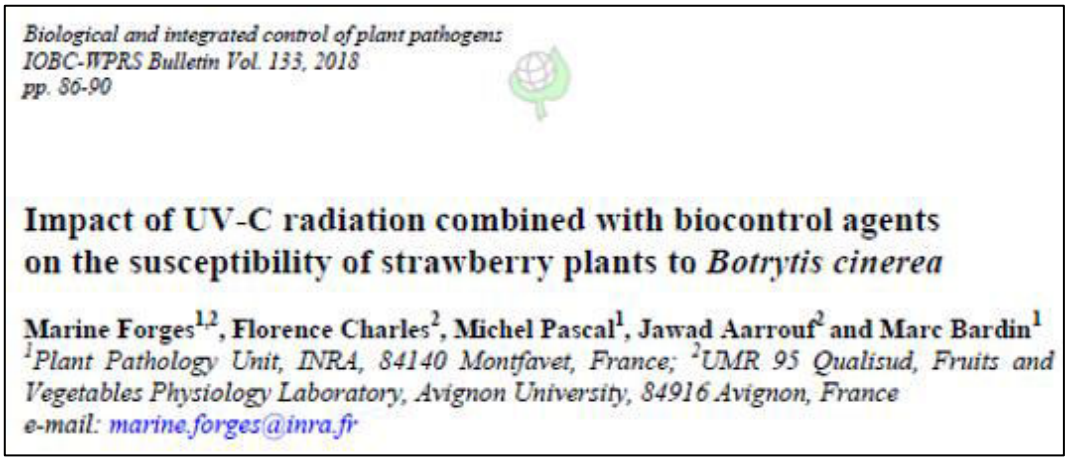
Bacillus subtilis est une bactérie GRAM-positif que nous retrouvons habituellement dans le sol et qui est ubiquitaire. Sérénade® (Bayer CropScience) est une préparation à base de la souche QST 713 de *B. subtilis*. Cette bactérie, en plus d'un effet direct antagoniste, confère une activité de stimulation des défenses des plantes à large spectre (données produit : www.bayer-agri.fr/produits/fiche/fongicides-serenade-max/). Sérénade n'est pas homologuée en France sur fraisier mais a montré une efficacité intéressante sur cette plante contre *B. cinerea* (Nicot *et al.*, 2013).

b. Prestop® : Gliocladium catenulatum

G. catenulatum est un champignon naturellement présent dans certains sols. Ce champignon a une action antagoniste directe vis-à-vis de *B. cinerea* (données produit : www.lallemandplantcare.com/products/prestop-hpm/). Le produit Prestop® (Lallemand) préparé à base de ce champignon est homologué en France pour contrôler la pourriture grise sur fraisier (https://ephy.anses.fr/).

II. Résultats et interprétation

Ce chapitre est rédigé sous la forme d'un article soumis lors du congrès IOBC-WPRS en 2018 (Figure 62).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Impact of UV-C radiation combined with biocontrol agents on the susceptibility of strawberry plants to *Botrytis cinerea*

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Figure 62 : **Article défendu au congrès IOBC-WPRS en 2018**.

1. Abstract

In order to enhance biocontrol efficacy against plant diseases, the combination of different control methods together with a given biocontrol agent can be achieved. In this study we have tested the effect of combining UV-C radiation with the application of biocontrol agents (*Bacillus subtilis* and *Gliocladium catenulatum* based products) against *Botrytis cinerea* on strawberry. We hypothesize that UV-C radiation applied on plants previously to biocontrol treatment, in addition to its induced resistance effect, will somehow disinfect leaves thus limiting the competition of inoculated biocontrol agents with the phyllosphere microorganisms and then promoting its development and its efficacy. We have shown that, despite the confirmed germicidal effect of UV-C radiation, this treatment applied on plants before treatment with biocontrol agent has no effect on

the level of efficacy of the biocontrol agents or even it tends to lower their efficacy. Work is in progress to understand mechanisms involved and to evaluate the effect of this combination of treatments on other plant species.

2. Introduction

Several microorganism-based products are now registered worldwide to control *Botrytis cinerea* on various crops, including strawberry (Nicot *et al*., 2016). However, the efficacy of biocontrol agents is generally considered as insufficient or inconsistent in field conditions thus promoting their use as components of an integrated disease management scheme. Increased biocontrol efficacy may then be achieved by combining various methods of protection.

Pre- or post-harvest treatment of plants or fruits with UV-C radiation has proven to be a promising tool for controlling plant pathogens. Results suggested for instance that UV-C treatment induced disease resistance against *B. cinerea* in lettuce (Vasquez *et al*., 2017), in pepper (Mercier *et al*., 2001) or in strawberry fruit (Jin *et al*., 2017). Combination of UV-C treatment with biocontrol agents has so far been successfully tested for the treatment of post-harvest diseases (Huang *et al*., 2015; Janisiewicz and Conway, 2010).

In this study, the objective was to evaluate the protective effect of the combination of UV-C radiation and biocontrol agents (*Bacillus subtilis* and *Gliocladium catenulatum* based products), both delivered on whole strawberry plants, against the development of *B. cinerea* on leaves. We hypothesize that UV-C radiation applied on the plants, in addition to its induced resistance effect, reduces the amount of microorganisms naturally existing on leaves, thus limiting the competition of the inoculated biocontrol agents with the phyllosphere microorganisms and then promoting its development and its efficacy.

3. Material and methods

a. UV-C treatments

The device used for the plant treatments with UV-C is a closed box having a ceiling light with 9 UV-C lamps (DSP tube UV-C, OSRAM HNL, 24 W) of 254 nm. Strawberry plants are placed in the box at 40 cm from UV-C lamps. UV-C dose calculation is done through measurements of light intensity at a given time, performed with a radiometer positioned at 40 cm from the ceiling light. The duration of UV-C radiation is 1 min and 44 sec to obtain 0.85 kJ/m² and 3 min and 28 sec to obtain 1.70 kJ/m². Plants were treated with UV-C radiation four times every other day. The last UV-C treatment was realized 2 days before inoculation of *B. cinerea*. Strawberry plants without any UV-C treatments were used as control. Four plants are processed at the same time in the box. To avoid the restorative effect of white light (Mercier *et al*., 2001), plants are placed in the dark for 15 hours after each UV-C treatment.

b. Estimation of phyllosphere microbial population

In order to estimate the total number of microorganisms (fungi and bacteria) present on leaves, upper-leaflet imprints (three leaflets per modality and medium) were realized on PDA medium (*Potato Dextrose Agar*, 39 g/L, Sigma-Aldrich) and on TSA medium (*Tryptic Soy Agar*, 40 g/L, Sigma-Aldrich). Colonies were numbered on both nutritive media after three days of incubation at 21 °C (14 hours of photoperiod at 114 µmol/s/m2). UV-C treated plants were compared to the non-treated control plants.

c. *Biocontrol agents*

The biocontrol agents tested were the fungus *Gliocladium catenulatum* (Prestop®, Lallemand) registered in France on strawberry against *B. cinerea* (https://ephy.anses.fr/), and prepared at a concentration of 1 % (m/V), and the bacterium *Bacillus subtilis* QST713 (Serenade®, Bayer CropScience) used at 8 g/L. This bacterium-based product has proved to be effective on strawberry leaves against *B. cinerea* (Nicot *et al*., 2013). Plants were sprayed once with a suspension of the commercialized product until run-off 2 days before inoculation. In the case of treatment combination, biocontrol agent was applied 4 hours after the last UV-C treatment.

d. *Assessing susceptibility of strawberry leaves to B. cinerea*

The strain Bc1 of *B. cinerea* was used throughout this study. It was grown 3 days on PDA medium in a growth chamber (21 °C, 14 hours of photoperiod at 114 µmol/s/m2) and mycelial plugs of 5 mm diameter taken from the growing margin of the culture were used as inoculum.

To evaluate the susceptibility of strawberry leaves, a test on detached-leaflets was realized. To this end, leaves were detached and leaflets were placed on moistened filter paper in transparent polystyrene boxes and inoculated with a mycelium plug in the center of the leaflet. Following inoculation with Bc1, leaflets were then placed in a growth chamber (21 °C, 14 hours of photoperiod at 114 µmol/s/m2). Leaflets were photographed every day between the third and the seventh days after inoculation and lesion areas were assessed with Image J software. The rate of lesion development (cm²/day) was calculated between the 3rd and the 6th day for each leaf. The area under the disease progress curve (*AUDPC*) was also calculated to determine the level of susceptibility of the strawberry plants. To compare the protection induced to the leaves by the different treatments realized, a protection index was computed as:

% Protection = 100 × (AUDPCuntreated – AUDPCtreated) / AUDPCuntreated

4. Results and discussion

a. Impact of UV-C radiation on phyllosphere microflora

After successive UV-C radiation of the plants, a decrease in the total number of microbial colonies (bacteria and fungi) was observed on both nutritive media PDA and TSA in a dose-dependent manner (ANOVA, p-value < 0.05; Figure 63). This suggests that UV-C radiation has a direct germicidal effect on phyllosphere microorganisms, thus partially degrading a part of the indigenous microbial community present on strawberry leaves.

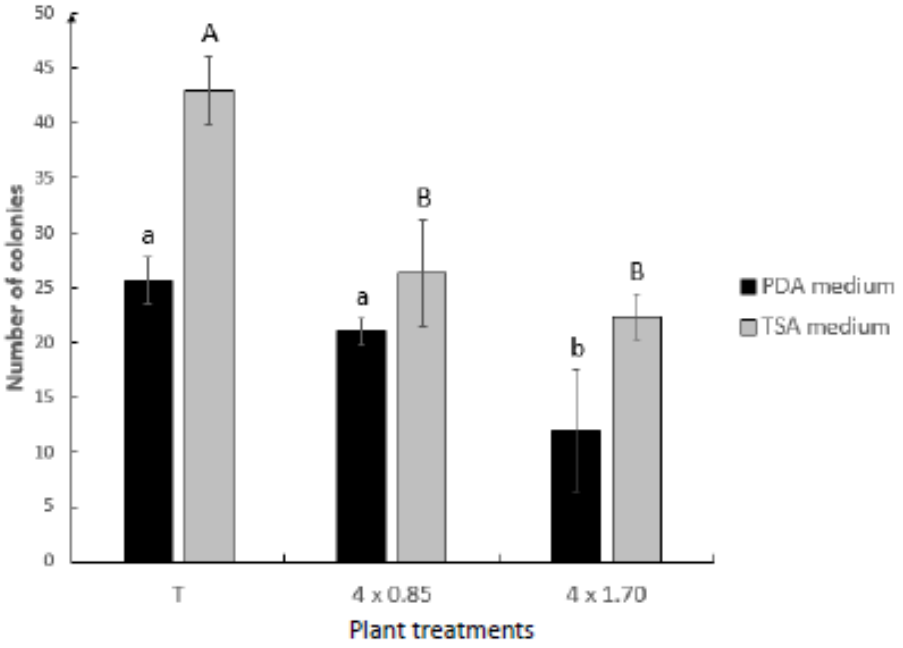

Figure 63: **Number of microbial colonies recovered from leaf imprints on two nutritive media (PDA and TSA) after successive UV-C treatments of the whole strawberry plants**. Plants were treated with UV-C radiation 4 times every two days at two different doses (0.85 and 1.70 kJ/m²) and untreated plants were used as control (T). Colonies were numbered 3 days after inoculation. The error bars show the standard error of the mean. Lower case letters indicate significant differences identified between different modalities tested on PDA medium and in upper case for modalities tested on TSA medium (standard error, NKT, p-value < 0.05).

b. *Impact of UV-C radiation combined with biocontrol agents on B. cinerea development*

A significant treatment effect was observed on the rate of lesion development (ANOVA, p-value < 0.0001) or on AUDPC values (Figure 64; ANOVA, p-value < 0.0001). UV-C radiation on the whole plant provides a slight but non-significant protection of the leaves towards *B. cinerea* (11 % and 16 %,

respectively for 1.70 and 0.85 kJ/m²). Protective efficacy against *B. cinerea* on strawberry leaves with Serenade and Prestop applied alone reaches 47 % and 72 %, respectively. The combination of both treatment (UV-C + biocontrol agent) provides a protective efficacy against *B. cinerea* of 19 % and 29 % with Serenade for 1.70 and 0.85 kJ/m² of UV-C radiation delivered on the plants, respectively. It provides a protective efficacy of 55 % and 20 % with Prestop, for 1.70 and 0.85 kJ/m² of UV-C radiation delivered, respectively. Therefore, UV-C treatments carried out before the biocontrol treatment did not increase the protection efficacy provided by the biocontrol agent. Rather, it systematically reduced the protective efficacy of the biocontrol agent used alone even if this effect was not always significant.

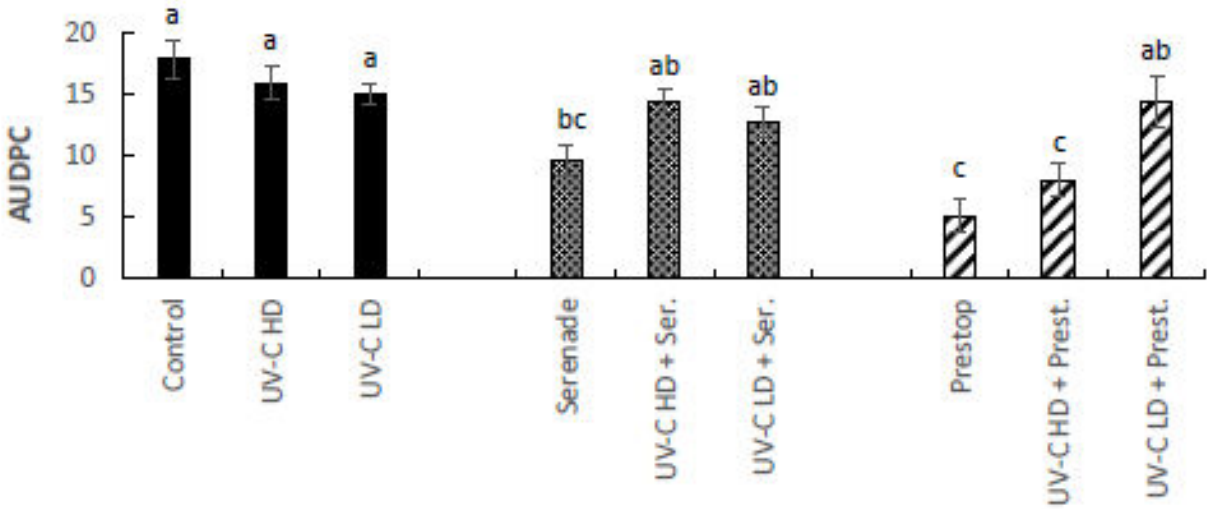

Figure 64: **Susceptibility of strawberry leaves to *B. cinerea* after UV-C radiation at high dose (HD, 4 × 1.70 kJ/m²) and at low dose (LD, 4 × 0.85 kJ/m²), after biocontrol treatment (Serenade, Prestop), and after the combination of UV-C radiation and biocontrol treatments**. The error bars show the standard error of the mean. Letters indicate significant differences identified between the different treatments (standard error, NKT, p-value < 0.05).

5. Conclusions and perspectives

In the present study, we evaluated the impact of UV-C radiation combined with biological control agents on the susceptibility of strawberry plants to *B. cinerea*.

In most cases, UV-C radiation applied on the plants before the treatment with a biocontrol agent has no significant effect on its efficacy. In one case it significantly lowers the level of efficacy of the biocontrol agent. It suggests that UV-C treatment, despite its germicidal effect on the phyllosphere microflora, does not favor the installation and the efficacy of the biocontrol agent. Different hypothesis may explain these results. Firstly, UV-C radiation applied on the plants induce the synthesis of antimicrobial defense metabolisms such as phytoalexins (Marti *et al*., 2014) that may have a direct effect on the installation of the biocontrol agents. Secondly the degradation of the superficial tissues of the plant due to UV-C treatment may prevent a proper installation of the biocontrol agents. Microscopic observations and metabolomics studies will be carried out to test these hypotheses.

To determine whether this phenomenon is universal, the combination of the two treatments will be tested on other plant species and against other plant pathogens.

6. Aknowledgments

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