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Biological and integrated control of plant pathogens
IOBC-WPRS Bulletin Vol. 133, 2018
pp. 86-90

Impact of UV-C radiation combined with biocontrol agents on the susceptibility of strawberry plants to *Botrytis cinerea*

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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.

Key words: Strawberry, *Botrytis cinerea*, UV-C, *Bacillus subtilis*, *Gliocladium catenulatum*

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 & 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.

Material and methods

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.

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/m^2). UV-C treated plants were compared to the non-treated control plants.

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.

***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/m^2) 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/m^2). 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 (cm^2/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:

$$\% \text{ Protection} = 100 \times (AUDPC_{untreated} - AUDPC_{treated}) / AUDPC_{untreated}$$

Results and discussion

Impact of UV-C radiation on phyllosphere microflora

After successive UV-C radiations 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, $p < 0.05$; Figure 1). 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.

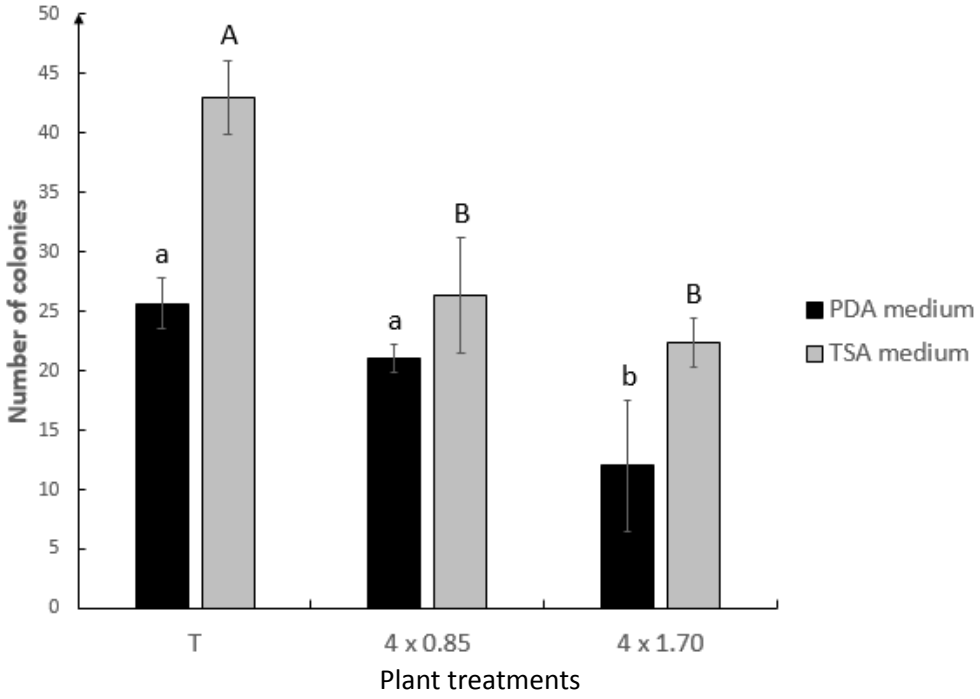

Figure 1. Number of microbial colonies recovered from leaf imprints on two nutritive media (PDA and TSA) after successive UV-C treatments of the whole strawberry plants. Plants were treated with UV-C radiation 4 times every two days at two different doses (0.85 and 1.70 kJ/m²) and untreated plants were 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 (Newman-Keuls, $p < 0.05$).

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, $p < 0.0001$) or on AUDPC values (Figure 2, ANOVA, $p < 0.0001$). 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.

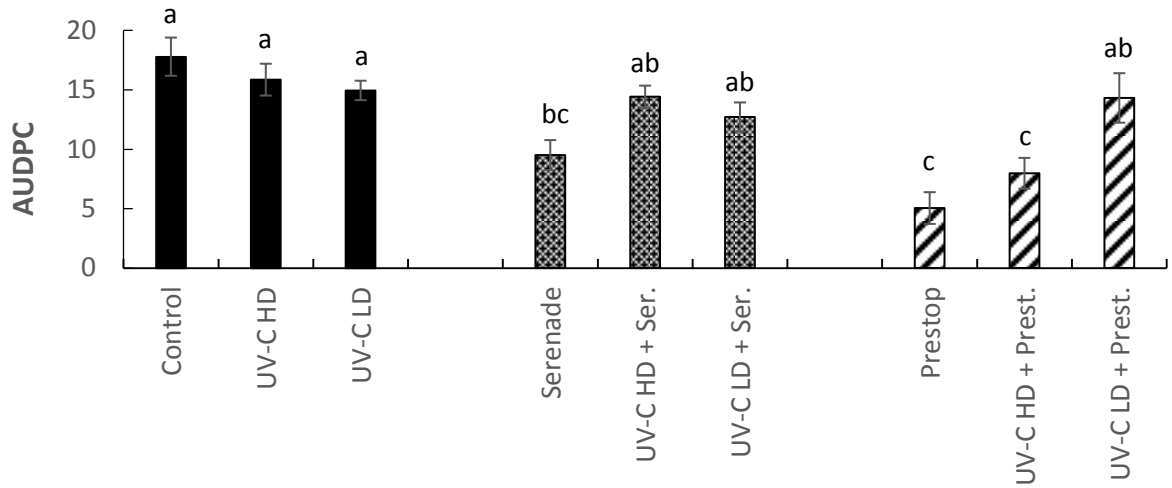

Figure 2. Susceptibility of strawberry leaves to *B. cinerea* after UV-C radiation at high dose (HD, 4 × 1.70 kJ/m²) and at low dose (LD, 4 × 0.85 kJ/m²), after biocontrol treatment (Serenade, Prestop), and after the combination of UV-C radiation and biocontrol treatments. The error bars show the standard error of the mean. Letters indicate significant differences identified between the different treatments (Newman-Keuls, $p < 0.05$).

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 radiations 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.

Acknowledgements

A PhD scholarship was provided by the TERSYS Research Federation (University of Avignon, France) for Marine Forges. Research on plant biology depends heavily on the cultivation of plants under experimental conditions that are controlled, monitored and repeatable. For ensuring a perfect plant production, the contribution of the Plant Production and Environmental Management Service of the Plant Pathology Research Unit is greatly appreciated.

References

Huang, K., Zou, Y., Luo, J. & Liu, Y.Q. 2015: Combining UV-C treatment with biocontrol yeast to control postharvest decay of melon. Environ. Science Poll. Res. 22: 14307-14313.

Janisiewicz, W. J. & Conway, W. S. 2010: Combining biological control with physical and chemical treatments to control fruit decay after harvest. Stewart Postharvest Rev. 1-3.

Jin, P., Wang, H. Y., Zhang, Y., Huang, Y. P., Wang, L. & Zheng, Y. H. 2017: UV-C enhances resistance against gray mold decay caused by *Botrytis cinerea* in strawberry fruit. Scientia Horticulturae 225: 106-111.

Marti, G., Schnee, S., Andrey, Y., Simoes-Pires, C., Carrupt, P. A., Wolfender, J. L. & Gindro, K. 2014: Study of Leaf Metabolome Modifications Induced by UV-C radiations in representative *Vitis*, *Cissus* and *Cannabis* species by LC-MS based metabolomics and antioxidant assays. Molecules 19: 14004-14021.

Mercier, J., Baka, M., Reddy, B., Corcuff, R. & Arul, J. 2001: Shortwave Ultraviolet irradiation for control of decay caused by *Botrytis cinerea* in bell pepper: induced resistance and germicidal effects. J. Am. Soc. Hortic. Sci. 126: 128-133.

Nicot, P., Bardin, M., Debruyne, F., Duffaud, M., Lecompte, F., Neu, L. & Pascal, M. 2013: Effect of nitrogen fertilisation of strawberry plants on the efficacy of defence-stimulating biocontrol products against *Botrytis cinerea*. IOBC-WPRS Bull. 88: 39-42.

Nicot, P., Stewart, A., Bardin, M., Elad, Y. 2016: Biological control and biopesticide suppression of *Botrytis*-incited diseases. In: *Botrytis* – the fungus, the pathogen and its management in agricultural systems (eds. Fillinger, S. & Elad, Y.): 165-187. Springer International Publishing, Switzerland.

Vasquez, H., Ouhibi, C., Lizzi, Y., Azzouz, N., Forges, M., Bardin, M., Nicot, P., Urban, L. & Aarrouf, J. 2017: Pre-harvest hormetic doses of UV-C radiation can decrease susceptibility of lettuce leaves (*Lactuca sativa* L.) to *Botrytis cinerea* L. Scientia Horticulturae 222: 32-39.