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Differential effect of resistance inducers on the susceptibility of lettuce cultivars to *Sclerotinia sclerotiorum* and *Botrytis cinerea*

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Abstract: The preventive use of resistance inducers has been shown to be an interesting method to reduce dependency on pesticides for plant protection. However, little is known on possible differences in the protective effects of such methods for different varieties of a given crop. In the present study, we assessed the effect of three compounds (acibenzolar-S-methyl, a calcium-based mineral compound and a yeast extract) for the protection of six lettuce cultivars against two major pathogens, *Sclerotinia sclerotiorum* and *Botrytis cinerea*. The compounds were sprayed on the plants three days before inoculation. A water spray was used as a control. The protective effect of the compounds was then assessed by comparing the size of lesions developing on inoculated leaves. For both pathogens, none of the compounds fully inhibited disease development. However, reduction in lesion size was observed on some of the leaves. The effect of the three compounds was different for the two pathogens. For tests with *B. cinerea*, effects of treatment were not statistically significant. In contrast, significant effects were found for cultivars inoculated with *S. sclerotiorum*. Overall, the yeast extract provided the highest level of protection against that pathogen. However, for all compounds, the extent of the protective effect depended on the cultivar. Furthermore, in some cases the effect the compound was opposite to that desired and disease was more severe on treated plants than on the water control. Possible consequences for field application of such methods will be discussed.

Key words: resistance stimulant, *Sclerotinia sclerotiorum*, *Botrytis cinerea, lettuce cultivars*

Introduction

Lettuce growers are often confronted to problems due to *Sclerotinia sclerotiorum* and *Botrytis cinerea*. Control of these two pathogens is economically important and relies mostly on the application of fungicides. The application of plant defense stimulators could provide an interesting alternative. Previous work has shown that several such preparations have a good potential to reduce the impact of downy mildew caused by *Bremia lactucae* (Maisonneuve *et al*., 2013). However, the protective effect of these preparations appeared to depend on the cultivar. The objectives of the present study were thus to evaluate the efficacy of three plant defense stimulators to protect six cultivars of lettuce against *S. sclerotiorum* and *B. cinerea*.

Material and methods

Plant production and treatments with resistance inducers

The tests were conducted between January and April 2014 with six cultivars of lettuce: 'Palomis' (Gautier Semences), 'Carlita' (Vilmorin), 'Blonde à bord rouge', abbreviated in the rest of this paper as "BBR" (old cv in public domain), 'Gloire du Dauphiné', abbreviated in the rest of this paper as "GDD" (old cv in public domain), 'Lasydo' (Syngenta), and 'Delsay' (Royal Sluis). Seedlings were produced in a heated nursery glasshouse and transferred to individual pots (9 x 9 x 8 cm) containing TS3 bedding plant substrate (Klasmann-Deilmann GmBH, Geeste, Germany). The plants were then grown in sanitary controlled conditions in the glasshouse (no pesticide treatments) and received a standard commercial nutrient solution once or twice a day, depending on needs.

Twenty-five days after seedling transplantation, batches of plants were treated with one of three preparations known for their defense-stimulating effect: acibenzolar-S-methyl supplied by Syngenta (Bion 50 WG®), a calcium-based preparation supplied by Ecoculture (CalFlux), and a yeast extract supplied by Jouffray-Drillaud (ABE IT56). All preparations were used as recommended by the manufacturers, at rates of 0.01%, 1% and 0.2%, respectively. A sterile water treatment was used as a control. Each plant was sprayed with approximately 25 ml of preparation, using a 2 l hand sprayer. For each lettuce cultivar, five plants were used per preparation.

Following treatment, the plants were kept in the greenhouse for three additional days until they were used for inoculations.

Inoculum production and inoculation

Strain SS2 of *S. sclerotiorum* and strain BC21 of *B. cinerea* were used in this study. Inoculum was produced in Petri plates containing Potato Dextrose Agar medium and incubated in a growth chamber (21 °C, 14 hours light). After three days of incubation, mycelial implants (5 mm in diameter) were excised from the growing margin of the colonies with a sterile cork-borer and used to inoculate lettuce leaves.

Three days after plant treatment, two leaves were excised from each plant and placed in clear polystyrene boxes over a sheet of wet paper. A mycelial implant was then placed in the center of each leaf and the boxes were closed and incubated in a growth chamber (21 °C, 14 h light).

Disease quantification and data analysis

Quantitative observations were carried out two days after inoculation (DAI) for leaves inoculated with *S. sclerotiorum* and 3 DAI for those inoculated with *B. cinerea*. Each leaf was photographed and the size of the lesion (in mm^2) was assessed by image analysis using ImageJ software.

The data were analyzed using Statistica software (Statsoft, Tulsa, OK, USA). The protective effect of a treatment was expressed as the percentage of reduction in lesion size on treated plants relative to the control plants sprayed with sterile water.

Results and discussion

Both pathogens incited lesions on all leaves of all lettuce cultivars, regardless of the treatments. However, the lesions caused by strain SS2 of *S. sclerotiorum* expanded much faster (to reach 400-2500 mm^2 in two days after inoculation; Figure 1) than those caused by strain BC21 of *B. cinerea* (which remained smaller than 700 mm^2 in three days after inoculation; Figure 2). These observations are compatible with earlier comparisons of the aggressiveness of those two pathogens (Amselem *et al.*, 2011; Lecompte *et al.*, 2013).

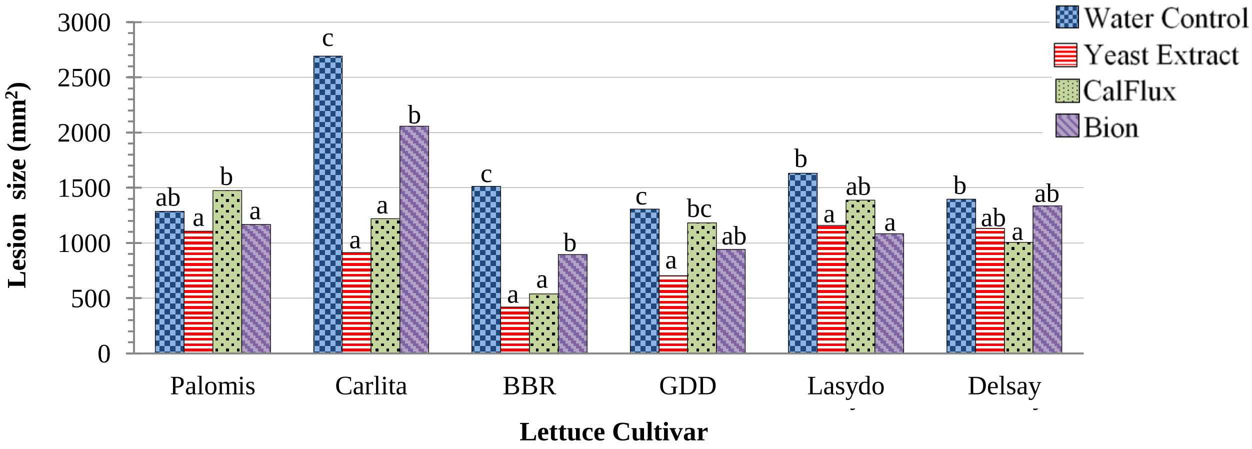

Figure 1. Effect of treatment with plant-defense stimulators on the development of *Sclerotinia sclerotiorum* on leaves of six lettuce cultivars (assessed 2 days after inoculation). For a given cultivar, letters indicate statistical differences ($P < 0.05$) among treatments according to Least Significant Difference tests.

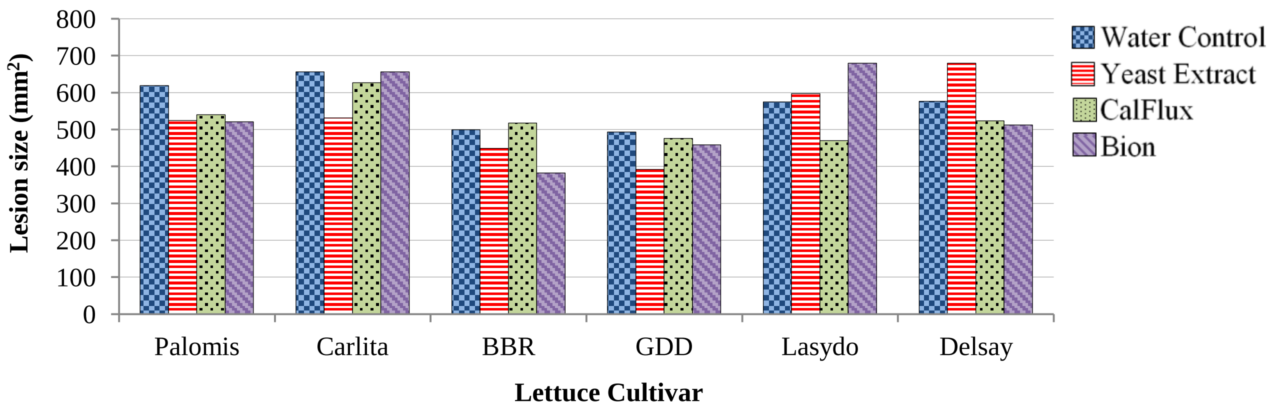

Figure 2. Effect of treatment with plant-defense stimulators on the development of *Botrytis cinerea* on leaves of six lettuce cultivars (assessed 3 days after inoculation). For a given cultivar, no significant differences were found ($P > 0.05$) among treatments.

Despite the lower aggressiveness of *B. cinerea*, none of the defense stimulators tested in this study significantly reduced lesion development by this pathogen (Figure 2). In tests with *S. sclerotiorum*, no complete inhibition of disease was achieved but the plant defense stimulators significantly reduced lesion development on at least one of the lettuce cultivars (Figure 1). The lack of complete disease inhibition on plants treated with defense stimulators appears to be a generalized phenomenon (Walters *et al.*, 2005). Although not much is known for lettuce, a difference in the protective efficacy of defenses stimulators against different types of pathogens have been reported before for other plants species. For example, Olivieri *et al.* (2009) have shown a differential effect of BABA treatment of potato plants on the accumulation of plant defense compounds in the tubers depending on whether they were infected with *Phytophthora infestans* or *Fusarium solani*. The protective efficacy of BABA against *P. infestans* also appeared to be slightly affected by the potato cultivar.

In our study, the protective efficacy of the defense stimulators against *S. sclerotiorum* was strongly dependent on the lettuce cultivar. For two of the six cultivars ('Palomis' and 'Delsay'), the reduction in lesion size provided by the defense stimulators was lower than 30% (and sometimes negative) and not statistically significant (Figure 3). The highest levels of protection were observed with the yeast extract and with Calflux on cultivars 'Carlita' and 'Blonde à Bord Rouge' (BBR). In earlier work, those two preparations were also found to provide high levels of protection on several lettuce cultivars against downy mildew caused by *Bremia lactucae* (Maisonneuve *et al*., 2013).

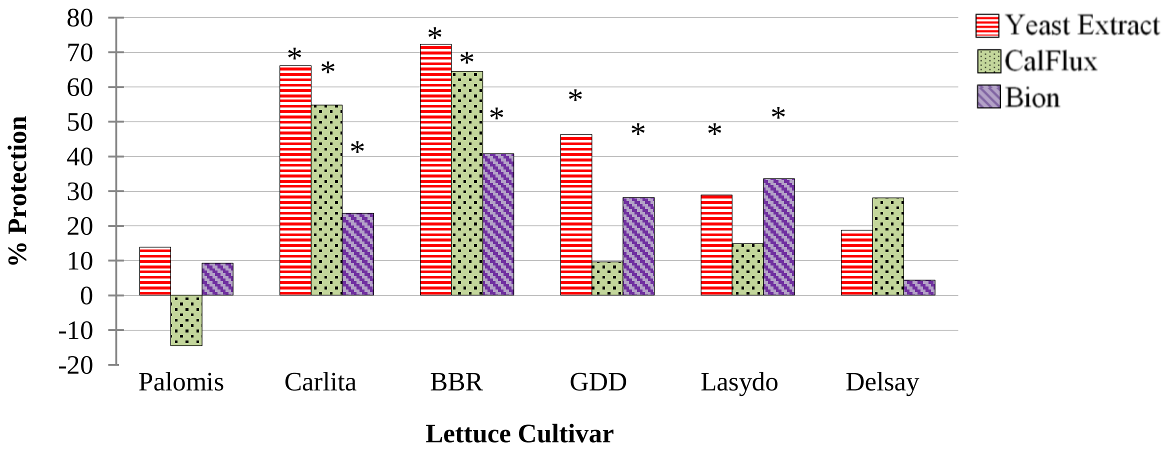

Figure 3. Protective efficacy of resistance inducers against *Sclerotinia sclerotiorum* on leaves of six lettuce cultivars. Stars (*) indicate significant reduction in lesion development between the water control and the treatment ($P < 0.05$).

We did not observe a clear relation between the protective efficacy of the plant defense stimulators and the original level of susceptibility of a cultivar to the pathogen. For example, 'Carlita' was the most susceptible to *S. sclerotiorum* among the six cultivars tested in our study, while 'BBR' had an average level of susceptibility. Other studies have reported similar results on other plant-pathogen systems. For example, following plant treatment with (defense-stimulating) benzothiadiazole, the highest level of resistance was observed for the susceptible cultivars of soybean and of winter wheat to *S. sclerotiorum* (Dann *et al*., 1998) and powdery mildew (Stadnik & Buchenauer, 1999), respectively. However, our results contrast sharply with previous reports of a higher efficacy of resistance induction on lettuce and on potato cultivars showing the lowest original levels of susceptibility to downy mildew (Maisonneuve *et al*., 2013) and to late blight and *Fusarium* rot (Olivieri *et al*., 2009), respectively.

In conclusion, the present study clearly showed that the efficacy of plant defense stimulators on lettuce against *Sclerotinia* was cultivar-dependent. Such situation, if occurring in other pathosystems, could partially explain the widely reported variability in field trials with resistance stimulators. It could make the field deployment of this type of control method complex. Further work is thus needed to gain a better understanding of the mechanisms underlying these contrasted results. Possible variability related to the behavior of different strains of the pathogen have been reported on other pathosystems (Bardin *et al*., 2013a, b; Maisonneuve *et al*., 2013) and should also be considered.

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References

Amselem, J., Cuomo, C. A., van Kan, J. A. L., Viaud, M., Benito, E. P., *et al*. 2011: Genomic Analysis of the Necrotrophic Fungal Pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet 7(8): e1002230. doi:10.1371/journal.pgen.1002230.

Bardin, M., Comby, M., Lenaerts, R. & Nicot, P. C. 2013: Diversity in susceptibility of *Botrytis cinerea* to biocontrol products inducing plant defence mechanisms. IOBC-WPRS Bull. 88: 45-49.

Dann, E., Diers, B., Byrum, J. & Hammerschmidt, R. 1998: Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. Eur. J. Plant Pathol. 104: 271-278.

Lecompte, F., Abro, M. A. & Nicot, P. C. 2013: Can plant sugars mediate the effect of nitrogen fertilization on lettuce susceptibility to two necrotrophic pathogens: *Botrytis cinerea* and *Sclerotinia sclerotiorum*? Plant Soil 369: 387-401.

Maisonneuve, B., Allen-Aubouard, C. & Pitrat, M. 2013: Effect of plant genotype on the efficacy of stimulators of plant defences in two horticultural pathosystems. IOBC-WPRS Bull. 89: 327-331.

Olivieri, E. P., Lobato, M. C., Gonzales Altamiranda, E., Daleo, G. R., Huarte, M., Guevara, M. G. & Andreu, A. B. 2009: BABA effects on the behaviour of potato cultivars infected by *Phytophthora infestans* and *Fusarium solani.* Eur. J. Plant Pathol. 123: 47-56.

Stadnik, M. J. & Buchenauer, H. 1999: Effects of benzothiadiazole, kinetin and urea on the severity of powdery mildew and yield of winter wheat. J. Plant Dis. Prot. 106: 476-489.

Walters, D. R., Walsh, D., Newton, A. & Lyon, G. 2005: Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology 95: 1368-1373.