Diversity in susceptibility of Botrytis cinerea to biocontrol products inducing plant defence mechanisms

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Induced resistance in plants against insects and diseases
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Diversity in susceptibility of *Botrytis cinerea* to biocontrol products inducing plant defence mechanisms

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Abstract: The development of plant defence stimulants to increase host resistance represents an attractive alternative to fungicides for the protection of crops against plant pathogens. In this study we evaluated the efficiency of 14 products presumed to induce plant defence mechanisms against *Botrytis cinerea* on tomato and lettuce. Two days after the application of the products, tomato and lettuce leaves were inoculated with *B. cinerea* and incubated in conditions conducive to disease development. Out of 14 products tested, Serenade Max® (*Bacillus subtilis* QST713) proved to have a significant protective efficacy against *B. cinerea* on both plants. To assess the presence of low susceptibility to Serenade Max in populations of *B. cinerea*, the protective efficacy of this product was evaluated against 20 strains differing in their geographic origin, host of isolation and level of aggressiveness. To this end, tomato and lettuce leaves were treated with Serenade Max two days before inoculation. The efficiency of the product was significantly influenced by the isolate of *B. cinerea* tested with protection levels ranging from 40% to 86% on tomato leaves and 0 to 80% on lettuce leaves. No correlation was observed between the level of aggressiveness of *B. cinerea* strains and the protection provided by the biocontrol agent.

Key words: biological control, induced resistance, durability, *Botrytis cinerea*

Introduction

The development of plant defence stimulants to increase host resistance represents an attractive alternative to fungicides for the protection of crops against plant pathogens. Various biotic and abiotic agents have been shown to induce defence mechanisms in different plant species against various plant pathogens and particularly against *Botrytis cinerea* (Elad and Stewart, 2004; Elmer and Reglinski, 2006). Although biocontrol preparations presumed to induce plant defence mechanisms against plant disease are now marketed, little is known on the durability of efficacy of these defence-stimulating biocontrol agents. To our knowledge, the diversity of the efficacy of resistance-inducing products against plant pathogen populations has not been studied. However, as for fungicides, knowledge on the baseline sensitivity of plant pathogens to resistance-inducing compounds appears to be necessary to determine the risk of possible adaptation of pathogen populations in response to selection pressure.

The purpose of the present study was (1) to evaluate the efficiency of various biocontrol preparations (microorganisms, plant extracts and organic products) presumed to induce plant defence mechanisms against *B. cinerea* on tomato and lettuce and (2) to estimate the diversity in susceptibility of *B. cinerea* to one of these biocontrol preparations (Serenade Max®) in order to detect any differences in sensitivity between isolates that might lead to development of resistance.

Material and methods

Collection of fungal isolates and inoculum production

To screen the effectiveness of biocontrol preparations, we used two strains of the pathogen (BC1 and BC21) known to differ for their aggressiveness on tomato (Ajouz *et al.*, 2010). To assess the presence of low susceptibility to Serenade Max® (*Bacillus subtilis* QST713) in populations of *B. cinerea*, the protective efficacy of this product was evaluated against 20 strains differing in their geographic origin, host of isolation and level of aggressiveness. All isolates were single-spored and conserved at -20°C before use. Inoculum was produced in Petri plates containing Potato Dextrose Agar medium and incubated in a growth chamber (21°C, 14 hours light) for 3 days.

Plant production and treatments

Seeds of tomato ('Monalbo') and lettuce ('Mantilia') were sown in compost and transplanted after 1 week in individual pots. Plants were grown in a glasshouse (for 7 weeks for tomato and 5 weeks for lettuce) where they received a standard commercial nutrient solution once or twice a day, depending on needs. For each plant species, a set of plants was selected at random and used as untreated control (no spray) or sprayed until run off with biocontrol preparations at the recommended dose. Fourteen preparations were tested on tomato and ten on lettuce including organic products, plant extracts and microorganisms.

Additional tests with Serenade Max were realised at two doses (2g/l and 8g/l).

Leaf inoculation

Two days after plant treatment, leaf disks (20mm diameter) of tomato and lettuce were placed on humid absorbent paper in clear polystyrene boxes. The centre of each leaf disk was inoculated with a mycelial plug (2mm diameter) taken from the growing margin of a 3-day old culture of *B. cinerea*. We used three replicate polystyrene boxes, each containing 4 leaf disks per treatment for each strain. Following inoculation, the leaf disks were incubated in a growth chamber in conditions conducive to disease development (21°C, 14h-photoperiod, 114µmol $s^{-1}m^{-2}$).

To evaluate the diversity of susceptibility of *B. cinerea* to Serenade® Max, we placed whole lettuce leaves in the polystyrene boxes instead of leaf disks. In this case, we used three replicate boxes, each containing 2 leaves per treatment for each strain.

Disease assessment and data analysis

The tomato leaf disks were photographed 24 and 48 hours after inoculation (HAI) and the lettuce leaves were photographed 24, 48, 72 and 96 HAI. The pictures were analysed, using the image analysis software Assess 2 (APS Press, St Paul Minnesota, USA), to quantify the surface of the necrotic lesions (in mm²). Analyses of variance were used (1) to test for an effect of treatment on disease development and (2) to test for an effect of strain on treatment efficacy. When appropriate, the means were compared with the test of Newman and Keuls.

To compare efficacy of the biocontrol agents, a protection index was computed as 100 * $(LS_{untreated} - LS_{biocontrol})/LS_{untreated}$, where LS was the average surface of the necrotic lesions for a given strain.

Results and discussion

Protective effect of the biocontrol preparations

Necrotic lesions became clearly visible mostly at 48 HAI on both tomato and lettuce leaves, regardless of plant treatment. Average lesion size was systematically larger for disks inoculated with strain BC1, which is highly aggressive on tomato, than for those inoculated with mildly aggressive strain BC21 (Figure 1). Among all products tested, Serenade Max proved to have a significant protective efficacy against both strains of **B. cinerea** on both host plants compared to the non-treated plants ($P < 0.01$).

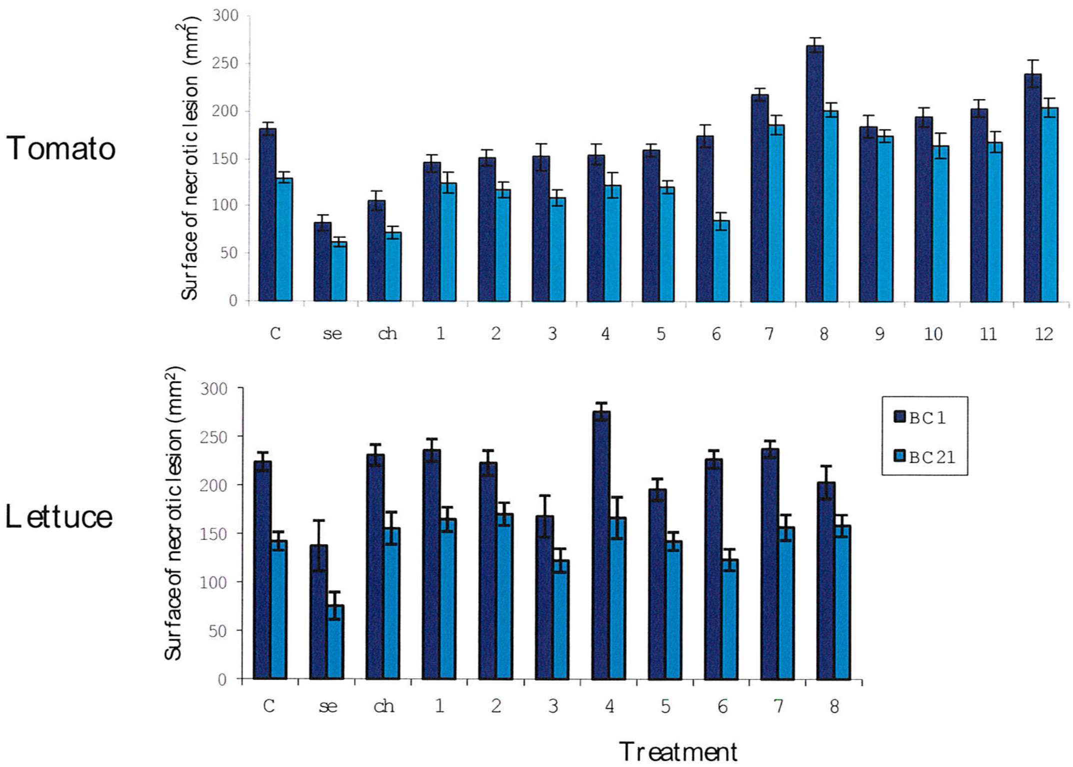

Figure 1. Effect of treatment of tomato and lettuce leaves with various biocontrol preparations on the development of two strains of **B. cinerea**.
se = Serenade Max®, ch = Chito Plant®, 1 to 12 = other products tested.

Protective effect of the Serenade Max preparation

Plant treatment with Serenade Max provided significant protection against **B. cinerea** on tomato ($P < 0.001$) and on lettuce ($P = 0.006$). Compared to the untreated control, Serenade Max provided a higher level of protection when applied at 0.8% than at 0.2% on both plant species (Figure 2). On tomato leaf disks, average protection was 37% for the low dose of product and 65% for the high dose ($P < 0.001$); on lettuce leaves, average protection was 23% and 45%, respectively for 0.2% and 0.8% of Serenade ($P = 0.004$). Protection was significantly higher on tomato than on lettuce at both application doses ($P = 0.001$ for 0.8% Serenade Max and $P = 0.043$ for 0.2% Serenade Max).

Effect of strain of *B. cinerea* on the efficacy of biocontrol

For both host species and both application doses, the protection provided by Serenade Max was significantly influenced by the strain of *B. cinerea* ($P < 0.001$). At the 0.8% application dose, the level of protection ranged from 40% to 86% on tomato and from 0% to 80% on lettuce, suggesting that some strains of *B. cinerea* are not efficiently controlled by Serenade Max, particularly on lettuce (Figure 2).

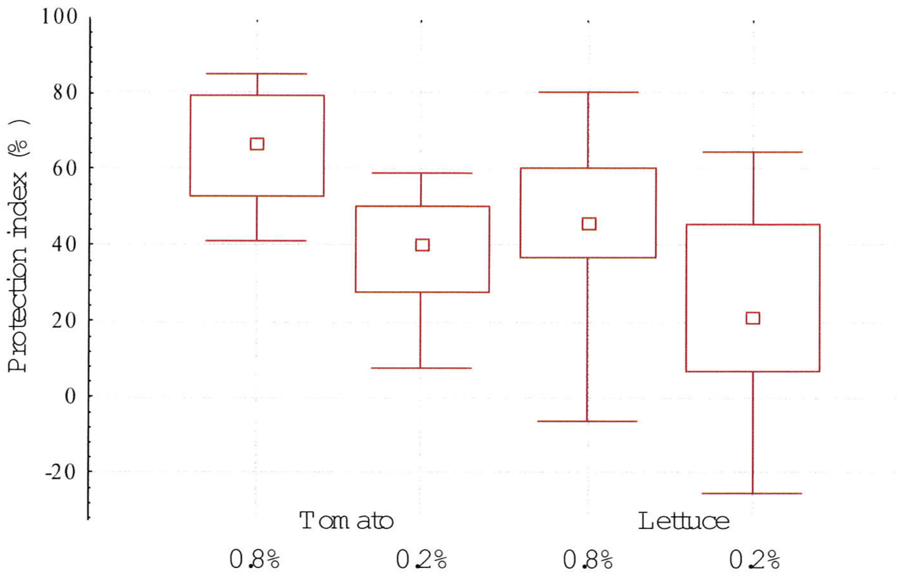

Figure 2. Distribution of the 20 strains of *B. cinerea* according to the level of protection of tomato and lettuce leaves with Serenade Max (0.8% and 0.2%). The boxplot represent the minimum, maximum, 25%-75% and median values of protection indices.

All *B. cinerea* strains grew on the control leaves of tomato and lettuce. The size of the lesions caused by the 20 strains ranged from 8 to 182mm^2 on tomato leaf disks at 48 HAI and from 20 to 762mm^2 on lettuce leaves at 72 HAI. Significant differences in aggressiveness were detected among strains on both plant species (ANOVA, $p < 0.001$). However, the level of protection conferred by Serenade Max was not correlated with the level of aggressiveness of *B. cinerea* strains.

The results of this study clearly show that the protective efficacy of resistance-inducing products can be influenced by the strain of *B. cinerea*. One may now wonder whether the use of this preparation on commercial fields or greenhouses could lead to the selection of more resistant strains which may eventually jeopardise the efficacy of this control method. These experiments should provide basic data for the risk assessment of resistance to such products.

Acknowledgements

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