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Abstract: Although Nitrogen (N) is a key component in many compounds implicated in host-pathogen interactions, little is known on the possible effect of N fertilisation of the plant on the efficacy of defence-stimulating biocontrol agents. In the present work we examined the effect of five levels of N nutrition on the susceptibility of strawberry leaves to *Botrytis cinerea* and on the protective efficacy of two biocontrol products presumed to induce plant defence mechanisms.

Two days after the application of the biocontrol products, batches of leaf discs were excised, inoculated with *B. cinerea* and incubated in conditions conducive to disease development. Plant fertilisation had a highly significant effect on disease development for both strains of *B. cinerea* tested and it significantly influenced the efficacy of the biocontrol products. Possible hypotheses and the relevance of these results for integrated protection are discussed.

Key words: biological control, *Bacillus subtilis*, chitosan, induced resistance

Introduction

Nitrogen (N) fertilisation is known to affect the susceptibility of host plants to certain pests and pathogens and the production of volatile secondary metabolites in response to attacks by herbivores (Hare, 2011; Huber & Thomson, 2007). Although N is a key component in many compounds implicated in host-pathogen interactions (Bolton, 2009), little is known on the possible effect of N fertilisation on the efficacy of defence-stimulating biocontrol agents.

The purpose of the present study was to evaluate the effect of a range of N fertilisation levels on the protection of strawberry leaves against *Botrytis cinerea* by two biocontrol preparations presumed to stimulate plant defence responses.

Material and methods

Plant production

Fully vernalized strawberry seedlings (cv Garriguette) were purchased from a commercial retailer and planted in a 1/1 mixture of pozzalana and vermiculite in a heated glasshouse. The plants were fertilised through a drip irrigation system. For an initial period of two months, they received a standard commercial nutrient solution. Then five regimes of N fertilisation were differentiated (0.5, 2, 5, 10 and 20mMol NO_3^- per litre of solution) in a randomized block design and the plants were grown for another six weeks before use.

Plant treatments
For each level of N fertilisation, three groups of four plants were selected at random and used as untreated control (no spray) or sprayed until run-off with preparations of either Serenade® Max (strain QST713 of *Bacillus subtilis*) or ChitoPlant® (soluble chitosan) at commercially recommended doses (8g/l and 10g/l, respectively).

Inoculum production and leaf inoculation
Two days after plant treatment, leaf discs (25mm diameter) were excised and placed on humid absorbent paper in transparent polystyrene boxes. The centre of each disc was inoculated with a mycelial plug (2mm diameter) taken from the growing margin of a 3-day old culture of *B. cinerea* on potato dextrose agar medium. We used two strains of the pathogen (BC1 and BC21) known to differ for their aggressiveness on tomato (Ajouz *et al.*, 2010; Lecompte *et al.*, 2010). For each combination of strain and plant fertilisation level, we used three replicate polystyrene boxes, each containing 4 leaf discs per plant treatment.

Following inoculation, the leaf discs were incubated in a growth chamber at 21°C under 14 hours of photoperiod (114µmol s^{-1} m^{-2}).

Disease assessment and data analysis
The leaf discs were photographed 48, 72 and 96 hours after inoculation (HAI) and the photos were analysed, using Assess 2 software (APS Press, St Paul Minnesota, USA), to quantify the surface of the necrotic lesions (in mm^2). Analysis of variance was used to test for an effect of fertilisation on disease development. 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 and N level combination.

Results and discussion

Disease development on the leaf discs
Although substantial superficial development of hyphae was observed on the leaf discs at 48 HAI, necrotic lesions became clearly visible mostly at 72 HAI, regardless of plant treatment and fertilisation regime. Average lesion size was systematically larger for discs inoculated with strain BC1, which is highly aggressive on tomato, than for those inoculated with mildly aggressive strain BC21 (Figure 1), suggesting a similar aggressiveness pattern on these very different host plants.

Effect of nitrogen fertilisation on disease
For both strains of *B. cinerea*, N fertilisation had a highly significant effect on disease regardless of the treatment applied to the plant (Figure 1). Lesion sizes were smallest and largest, respectively, on leaves from plants which had received the lowest and the highest N levels.

Effect of nitrogen fertilisation on the efficacy of biocontrol
Plant fertilisation significantly influenced the efficacy of the biocontrol products. Compared to the untreated control, Serenade® Max significantly reduced lesion development by either strain of *B. cinerea* on plants with low levels of N fertilisation (0.5 and 2mMol/l), but not on those that received higher doses (Figure 2). Some level of protection ($P = 0.10$) was also

observed against mildly aggressive strain BC21 on leaves from plants grown with intermediate N level 5mMol/l.

In contrast, no significant protection was provided by ChitoPlant® against aggressive strain BC1 regardless of the fertilisation regime (Figure 2). The compound provided a high level of protection (greater than 50%, $p < 0.01$) against mildly aggressive strain BC21, but only for plants with low N fertilisation levels (0.5 and 2mMol/l).

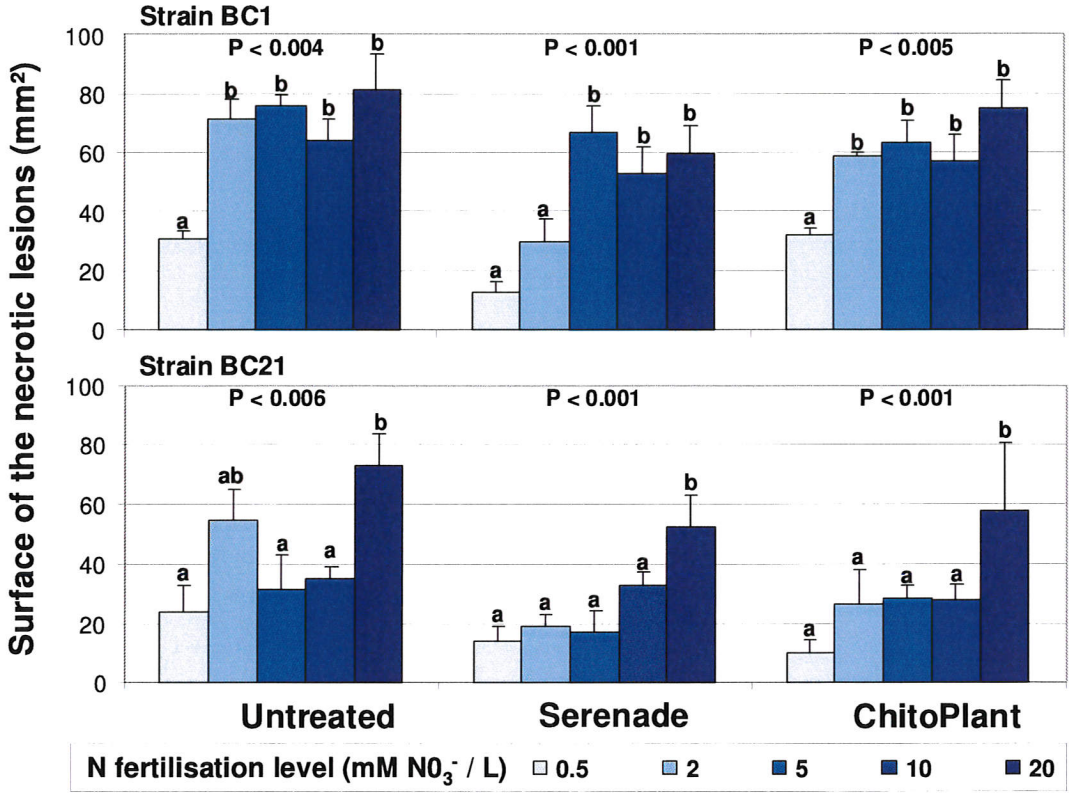

Figure 1. Effect of plant N fertilisation on the development of lesions caused by two strains of *Botrytis cinerea* (BC1 and BC21) 72 hours after the inoculation of strawberry leaf discs excised from plants treated with Serenade®, ChitoPlant®, or left untreated. The error bars show the standard error of the means. For a given strain and type of plant treatment, P values indicate a significant effect of N fertilisation on disease development; lesion size values associated with different letters are significantly different according to Newman & Keuls test for multiple comparisons of the means.

Conclusions and perspectives

The results of this study clearly show that both the susceptibility of strawberry leaves to *B. cinerea* and the protective efficacy of resistance-inducing products can be influenced by the level of N fertilisation provided to the plant. Contrarily to our observations on tomato with the same strains of the pathogen (Lecompte *et al.*, 2010), low N levels were associated with lower susceptibility of strawberry leaves. Further work is needed to elucidate the specific mechanisms/genes involved, possibly with well characterized resistance-inducing compounds. Particularly intriguing in our study is the difference in behaviour of the

biocontrol products against the two strains of *B. cinerea*, especially at low N levels. Among possible hypotheses, it could be speculated that other types of mechanisms may have been implicated in the protective effects, in addition to the stimulation of plant defence.

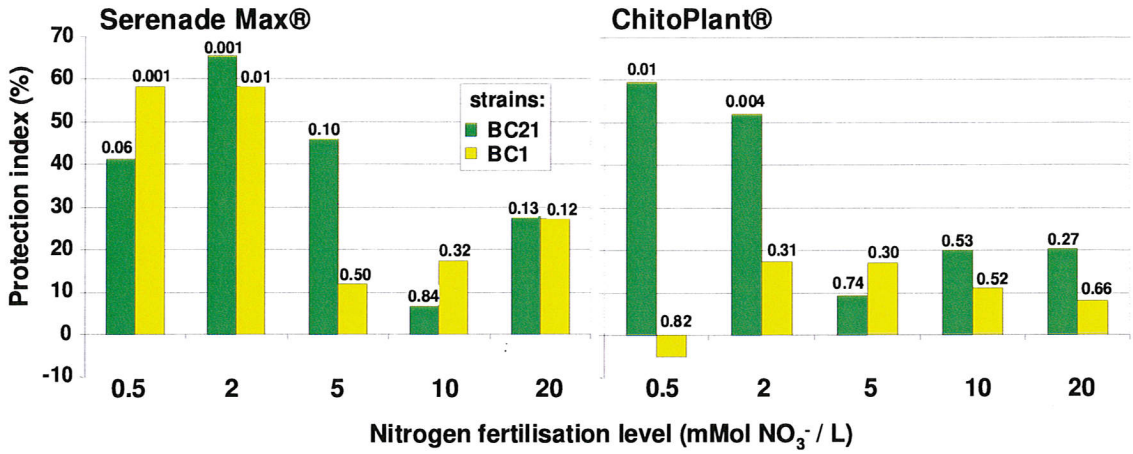

Figure 2. Effect of plant N fertilisation on the efficacy of leaf protection by two biocontrol agents against two strains of *Botrytis cinerea* (BC1 and BC21) 72 hours after inoculation. The values above each bar represent the P value for the comparison of lesion size on the leaves from treated and untreated plants.

As *Botrytis* mould is a key disease of strawberry, combining the application of resistance inducers with low nitrogen fertilisation may offer an interesting prospect for integrated protection. Field work is needed to validate the present results, but also to evaluate possible effects on other plant health aspects as well as on the yield and quality of the crop.

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