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Enhancement of biocontrol efficacy against *Botrytis cinerea* through the manipulation of nitrogen fertilization of tomato plants

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Abstract: Although nitrogen fertilization is known to affect plant susceptibility to certain pathogens, little is known on its possible effect on the efficacy of biological control. In the present study we examined the effect of five levels of NO_3^- nutrition on the efficacy of two biocontrol agents (*Trichoderma harzianum* and *Microdochium dimerum*) to protect pruning wounds of tomato against *Botrytis cinerea*. Plants were grown for two months in a greenhouse with a soil-less drip-irrigation system. Differential nitrogen nutrition was applied for the last four weeks prior to pruning, treatment of wounds with the biocontrol agents and inoculation with two strains of *B. cinerea*. They were then incubated in conditions conducive to disease development. Plant fertilization had a highly significant effect on disease development for both strains tested and it significantly influenced the efficacy of both biocontrol agents. High nitrogen fertilization generally decreased disease severity and also enhanced the efficacy biocontrol.

Key words: biological control, *Trichoderma harzianum*, *Microdochium dimerum*, nitrogen

Introduction

Grey mould, caused by *Botrytis cinerea*, is a major problem for may crops, including greenhouse tomato (*Solanum lycopersicum*) (Elad *et al*., 1995). Many biocontrol agents (BCAs) have been described against *B. cinerea* (Elad & Stewart, 2004; Nicot *et al*., 2011) including some with significant protective effects on pruning wounds on tomatoes (Bardin *et al.*, 2008). Nitrogen (N) fertilization is known to affect the susceptibility of host plants to pathogens (Huber & Haneklaus, 2007) including *B. cinerea* on tomato (Lecomte *et al*., 2010). In contrast, little is known on its possible effect on the efficacy of biocontrol agents against *B. cinerea*. The purpose of the present study was to evaluate the effect of a range of N fertilization levels on the efficacy of two biocontrol agents for the protection of pruning wounds of tomato against *B. cinerea.*

Material and methods

Plant production

Two batches of 175 tomato plants cv. Swanson were produced in a heated greenhouse. Seedlings were grown in rockwool cubes and watered daily with a standard nutrient solution. After one month, the seedlings were placed over pots filled with a 1/1 mixture of pozzalana and vermiculite and placed under conditions of differential N fertilization in a randomized block design. The plants were fertigated through a drip irrigation system with a solution containing 0.5, 2, 5, 10 or 20mMol NO_3^- per litre. Other major nutrients (P, K, Mg, Ca) and

oligo-elements (B, Cu, Mn, Mo, Zn) were kept constant in the solutions and the electric equilibrium was maintained by adjusting the concentration or $S0_4^{2-}$. The plants were grown for four weeks under these conditions before use.

Plant tissue analysis
For each level of N fertilization, five plants were selected randomly just before inoculation trials. Stems and leaves were weighed separately for each plant and dried for tissue analysis. Tissue content was analysed for total N, NO_3^- and carbon and amounts were expressed as mg/g of dry weight.

Plant treatments
Four leaves were removed from each plant, leaving 5-10mm petiole stubs on the stems, and the pruning wounds were treated with a spore suspension of *Trichoderma harzianum* or *Microdochium dimerum*, or left untreated to be used as controls. The suspensions of each BCA contained 10^7 conidia/ml and 10µl aliquots were applied on each wound. Immediately after treatment, the plants were inoculated with *B. cinerea* as described below.

Inoculum production and plant inoculation
We used two strains of *B. cinerea* with contrasted aggressiveness on tomato. Inoculum was produced on Potato Dextrose Agar medium in a growth chamber (21°C and 14h daylight). Conidia were collected in sterile distilled water from the surface of 14-day old cultures and suspensions adjusted to 10^6 and 10^7 conidia/ml were prepared for each strain. As the objective of the study was to investigate the possibility of manipulating N fertilization to improve the efficiency of biocontrol, high inoculum concentrations of *B. cinerea* were used purposefully to put the BCAs in a difficult situation. The wounds were inoculated with 10 µl aliquots and the plants were then incubated in a growth chamber at 21°C, 90% relative humidity under 14h of photoperiod.

Disease assessment and data analysis
The plants were examined daily from the 3rd to the 7th day after inoculation and the infection of petiole stubs, the initiation and length of resulting stem lesions were monitored. The area under the disease progress curve was computed as described by (Lecompte *et al*., 2010). The whole experiment was conducted twice independently. Analysis of variance was used to test for an effect of fertilization on disease development. When appropriate, the means were compared with the test of Newman and Keuls. To compare the efficacy of the BCAs, a protection index was computed as $100 \times (LL_{untreated} - LL_{biocontrol})/LL_{untreated}$, where LL was the average length of the stem lesions for a given strain and N level combination.

Results and discussion

Effect of nitrogen fertilization on plant tissue content
The N fertilization had a significant effect on the fresh and dry weight of both leaves and stems (Table 1). They increased significantly with increasing NO_3^- levels in the fertigation solution up to 10mMol/l. However, the N and NO_3^- tissue content (in mg/g of dry matter) continued to increase beyond 10mMol/l. In contrast, the carbon content in stems was not influenced by the fertilization level and it decreased in leaves beyond 10mMol/l.

Effect of nitrogen fertilization on disease severity

There was a significant effect ($P < 0.05$) of inoculum concentration and a highly significant effect ($P < 0.0001$) of N fertilization on the severity of disease (shown for strain BC1 in Fig. 1). It decreased with increasing N concentrations as observed in previous work (Lecompte et al., 2010). Wound treatment with either biocontrol agent generally reduced disease severity. The reduction in severity was significantly higher for *M. dimerum* than for *T. harzianum*. It was more pronounced for the plants subjected to the lower concentration of *B. cinerea* inoculum and for those grown under the higher levels of N fertilization.

Table 1: Effect of N fertilization on the tissue content of tomato leaves and stems.

Type of plant tissue	**N fertilization level (mMol/l)**	**Fresh weight* (g)**	**Dry weight (g)**	**N (mg/g dry weight)**	**NO_3^- (mg/g dry weight)**	**C (mg/g dry weight)**
Leaf	0.5	30.9 a	6.1 a	7.3 a	0.0 a	368.4 c
	2	48.8 b	8.4 b	11.2 b	0.3 a	371.0 c
	5	91.0 c	12.7 c	15.7 c	3.2 a	368.3 c
	10	136.3 d	14.7 d	25.3 d	25.1 b	351.3 b
	20	166.6 e	16.7 e	30.0 e	53.7 c	342.8 a
		P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Stem	0.5	22.1 a	3.3 a	11.9 a	0.1 a	392.1 a
	2	30.8 a	3.8 a	15.8 a	0.1 a	389.7 a
	5	60.9 b	6.4 b	22.8 a	0.8 a	385.2 a
	10	92.7 c	8.1 c	37.8 b	16.4 b	368.9 a
	20	99.1 c	8.0 c	48.8 c	33.9 c	392.5 a
		P < 0.001	P < 0.001	P < 0.001	P < 0.001	P = 0.275

* Data are means of five replicates per treatment. For a given column and type of plant tissue, numbers followed by different letters are significantly different (Newman-Keuls tests).

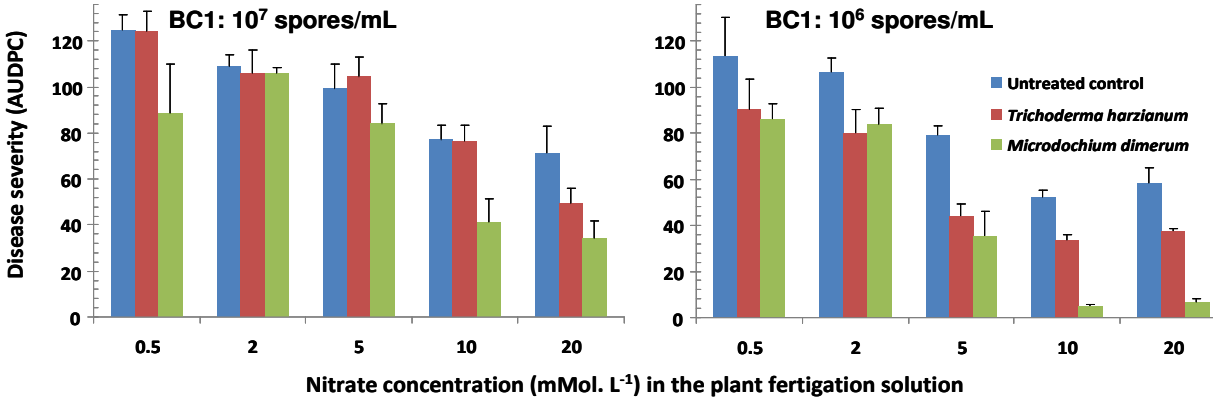

Figure 1. Effect of plant N fertilization and treatment with two BCAs on the disease severity (assessed as the Area Under the Disease Progress Curve) of *Botrytis cinerea*. The error bars show the standard error of the means.

Effect of nitrogen fertilization on the efficacy of biocontrol

Overall, high N fertilization enhanced the efficacy of the two BCAs, but efficacy was also affected by the strain of *B. cinerea* and by the concentration of the pathogen (Fig. 2). At low nitrogen doses, the efficacy of *M. dimerum* was more pronounced on less aggressive strain BC21 of *B. cinerea*, particularly at the lower inoculum dose of the pathogen. Regardless of the pathogen's strain, both BCAs were more effective against *B. cinerea* concentrations of 10^6 as compared to 10^7 conidia/ml. Regardless of the fertilization level, *M. dimerum* was generally more effective as compared to *T. harzianum* against *B. cinerea* for the protection of the pruning wounds on tomato stems.

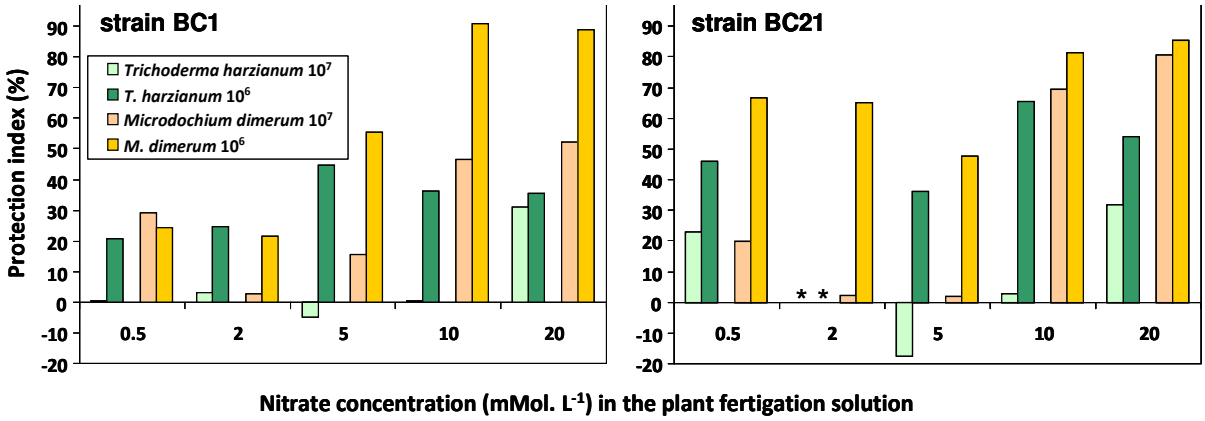

Figure 2. Effect of plant N fertilization on the efficacy of tomato wound protection by two biocontrol agents against two strains of *Botrytis cinerea*. The pathogen was applied at concentrations of 10^7 or 10^6 conidia/ml as indicated in the legend of the graph. *missing data.

Conclusions and perspectives

The results of this study indicated that both the susceptibility of tomato stems for the protection of pruning wounds to *B. cinerea* and the protective efficacy of two BCAs can be influenced by the level of N fertilization provided to the plant. High N fertilization enhanced the efficacy of both BCAs against *B. cinerea*. More research is needed in order to understand the mechanisms implicated in this effect at a cellular or molecular level. Further work also is also needed to validate the present results at field level.

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