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Nitrogen fertilization impacts biocontrol of tomato gray mold

Manzoor A. Abro · François Lecompte · Marc Bardin ·
Philippe C. Nicot

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Abstract Gray mold, caused by *Botrytis cinerea*, is a common threat for greenhouse production of tomatoes. Control of this disease can be difficult even with chemical treatments, and alternative methods are needed. Nitrogen (N) fertilization is known to modify the impact of pathogens on plants. However, there is scarce knowledge about the effect of fertilization on the efficacy of biocontrol. Here, we studied the effect of N fertilization on biocontrol agents *Trichoderma atroviride* and *Microdochium dimerum* that protect tomato against *B. cinerea*. Plants were grown for 2 months in a greenhouse with a soil-less drip irrigation system. Differential N fertilization (five concentrations of nitrate) was applied for the last 4 weeks prior to leaf pruning, biocontrol agent application, and *B. cinerea* inoculation. Results show that increasing N fertilization up to 10 mmol/L reduced disease by half for controls. High N fertilization also increased biocontrol, with a protection index rising from nearly 0 to up to 100 % depending on the biocontrol agent and the pressure of the pathogen. Indeed, high N fertilization delayed stem symptoms and slowed lesion expansion. To our knowledge, this is the first report of an effect of N fertilization on the efficacy of biocontrol against an airborne disease. Therefore, adapting N fertilization is a promising technique to protect greenhouse tomato.

Keywords Biological control · Tomato · *Trichoderma atroviride* · *Microdochium dimerum* · *Botrytis cinerea* · Nitrogen fertilization

M. A. Abro · M. Bardin · P. C. Nicot (✉)
INRA, UR407 Pathologie végétale, Domaine Saint Maurice, CS
60094, F-84143 Montfavet cedex, France
e-mail: philippe.nicot@avignon.inra.fr

F. Lecompte
INRA, UR 1115 Plantes et systèmes de cultures horticoles,
F-84914 Avignon cedex, France

1 Introduction

Reducing the reliance on pesticide use for crop protection is a generally sought after goal. Its achievement, however, requires the availability of efficacious alternative methods for growers to limit the risk of yield losses from pests and diseases. Much hope is placed on biological control, and commercial products are increasingly available against arthropod pests and more recently against diseases (van Lenteren 2012; Nicot 2011). For disease control, a lower and less predictable level of efficacy, compared to pesticides, is often cited as one of the obstacles to the commercial development of biological control agents and their wide adoption by growers (Nicot 2011). Much research has been dedicated to enhancing the efficacy and reliability of biocontrol agents. One approach consisted in improving the performance of the microbial biocontrol strains through mutagenesis or genetic manipulation (Clermont et al. 2011) or through optimizing nutrient substrate composition and conditions employed during biomass production (Jackson et al. 1991). Another approach may be to apply various compounds (including calcium salts, chitosan, amino acids, and carbohydrates) on the plants together with the biocontrol agent (El-Ghaouth et al. 2002; Guetsky et al. 2002; Janisiewicz et al. 1992). Soil amendments with various organic compounds such as chitin have improved biocontrol efficacy of some soilborne diseases (Barakat 2008; Lopez-Mondejar et al. 2012). Surprisingly, little information is available on the possible role of mineral plant fertilization on the efficacy of biocontrol agents, although its effect on susceptibility of the host plant to pathogens is well documented for a variety of crops and diseases (Datnoff et al. 2007).

In a recent study, we showed that the susceptibility of tomato to *Botrytis cinerea*, a fungus responsible for economically important diseases of a variety of crops, was significantly influenced by nitrogen (N) fertilization (Lecompte et al. 2010). N fertilization also had an impact on the abundance of spore production by *B. cinerea* on diseased plants

and on the pathogenicity of that secondary inoculum (Abro et al. 2013). For greenhouse tomato production, this fungus constitutes a recurrent menace (Fig. 1). Its management can be difficult despite chemical treatments and much effort is dedicated to developing alternative control methods and enhancing their efficacy.

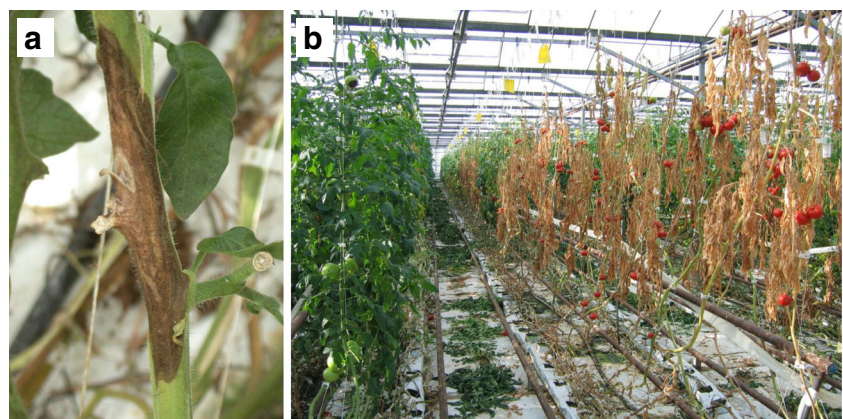
The objective of the present study was thus to investigate the potential of N fertilization to enhance the efficacy of two beneficial fungi shown in previous work to provide protection of tomato pruning wounds against *B. cinerea* (Nicot et al. 2003; Bardin et al. 2008). For both beneficial fungi, we observed enhanced biocontrol at high N fertilization levels, even in the presence of high inoculum levels of the pathogen.

2 Materials and methods

2.1 Plant production

Tomato plants were produced under five differential N nutrition regimes spanning a range from deficiency to excess (Lecompte et al. 2010). In short, seeds of cv Swanson were sown in 1 cm³ rockwool plugs in a greenhouse. Ten days after sowing, the plugs were transferred onto rockwool blocks and the plantlets (one per block) were fertigated twice a day with a standard commercial nutrient solution (Duclos International, Lunel, France). After 1 month, the plants (bearing three to four leaves) were placed on the top of 2 L pots filled with a mixture (1:1 v/v) of vermiculite and pozzolana (inert crushed volcanic rock) to start the nutrition treatments. Five levels of nitrate concentration were tested in the fertilization solution: 0.5, 2, 5, 10, and 20 mmol L⁻¹ (abbreviated as mM in this paper). The plants were fertigated with a drip irrigation system (one dripper per pot) at a frequency adapted to the climatic demand. Two batches of 200 plants (40 for each fertilization regime) were produced independently in 2009 and 2010 to provide independent repetitions of the whole study.

Fig. 1 Symptoms caused by *B. cinerea* on tomato plants in a heated greenhouse. Following the infection of pruning wounds by airborne spores of the fungus, stem cankers develop (a). The lesions can eventually girdle the stem and kill the plants (b), causing significant yield losses



2.2 Plant tissue analysis

From each batch of 200 plants, five were randomly selected for each N nutrition regime just before the inoculation assays. These samples were used to measure fresh and dry weight of aerial parts and for analyses of mineral content of stem tissues (Lecompte et al. 2010). Seven tissue components were measured: total nitrogen (N), total carbon (C), nitrate (NO₃⁻), phosphorous (P), potassium (K), magnesium (Mg), and calcium (Ca). In addition, glucose, fructose, and sucrose contents were determined with an enzymatic method (Gomez et al. 2007).

2.3 Biocontrol agents

We used two fungal species known from previous work in our laboratory for their protective effect against *B. cinerea* on tomato. Strain L13 of *Microdochium dimerum*, isolated from pruning wounds on tomato (Nicot et al. 2002), was grown on yeast peptone glucose agar containing per liter: 7 g Bacto™ yeast extract, 7 g BBL™ Gelysate™ peptone, 7 g glucose, and 15 g agar. Strain T1 of *Trichoderma atroviride*, kindly provided by Agrauxine, was produced on Difco™ potato dextrose agar. Both fungi were grown in a growth chamber at 21 °C with a 16-h photoperiod (162 μmole m⁻² s⁻¹). Spores were collected in sterile distilled water from the surface of 14-day-old cultures and suspensions (adjusted to 10⁷ spores mL⁻¹) were prepared for each strain.

2.4 Plant treatments with biocontrol agents

From each N fertilization regime, two groups of ten plants were treated with either biocontrol agent and one group was used as a control. On each plant, four leaves were removed, leaving 5–10 mm petiole stubs on the stems. The pruning wounds were either left untreated (control plants) or treated with 10 μL aliquots of a spore suspension of *T. atroviride* or *M. dimerum*. Immediately after treatment, the plants were inoculated with *B. cinerea*.

2.5 Production of *B. cinerea* inoculum

We used two strains of *B. cinerea* differing in aggressiveness on tomato. From previous work of the laboratory, strain BC1 was known to have a high level of aggressiveness on tomato, while strain BC21 had a medium to low level of aggressiveness (Lecompte et al. 2010). Inoculum was produced on potato dextrose agar in a growth chamber as described above. Spores were collected in sterile distilled water from the surface of 14-day-old cultures and suspensions were prepared and adjusted to 10^6 and 10^7 spores mL^{-1} 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 challenge the beneficial fungi.

2.6 Inoculations

For each treatment and N fertilization level, five plants were inoculated with strain BC1 and five with strain BC21. On a given plant, the pruning wounds of the third and fifth leaves (numbered from the bottom up) were inoculated with 10 μL of a suspension containing 10^7 spores mL^{-1} and the fourth and sixth leaves with a suspension containing 10^6 spores mL^{-1} . Following inoculation, the plants were incubated for 7 days in controlled conditions at 21 °C, 90 % relative humidity, and with 14 h of photoperiod (Lecompte et al. 2010). During this period, they were irrigated manually with the same fertilizer solutions as those used before inoculation.

2.7 Disease assessment and data analysis

The plants were examined daily from the third to the seventh day after inoculation for infection of the petiole stubs, and the initiation and length of resulting stem lesions were recorded. The latency period was calculated as the number of days between inoculation and lesion appearance on stems. If a lesion was visible on the third day after inoculation, the latency period was given a value of 3 days; if no lesion was visible on the last day of disease assessment, it was given a value of 8 days. On each inoculation site, the rate of lesion expansion (in millimeter per day) was calculated as the average daily increase in the length of the lesions between their appearance on the stem and the last day of measurement. It was thus estimated based on one to five measurements of lesion size depending on the day of lesion appearance. The area under the disease progress curve (AUDPC) was computed over the 5 days of disease rating as described by Lecompte et al. (2010). The whole experiment was conducted twice on independently produced batches of plants. Analysis of variance was used to compare the two trials and to test for an effect of fertilization on disease development. When appropriate, the means were compared

with the Newman and Keuls test. To compare the efficacy of the biocontrol agents, a protection index was computed as $100 \times (\text{AUDPC}_{\text{untreated}} - \text{AUDPC}_{\text{biocontrol}}) / \text{AUDPC}_{\text{untreated}}$, where $\text{AUDPC}_{\text{biocontrol}}$ and $\text{AUDPC}_{\text{untreated}}$ were the average AUDPC (for plants treated with a biocontrol agent or for control plants left untreated, respectively) computed for a given biocontrol agent and N fertilization level combination. The correlation between disease severity (AUDPC) and tissue content in the tomato stems for different minerals and sugars was investigated using Pearson's correlation coefficients. The Statistica software package (StatSoft Inc., Tulsa, OK, USA) was used for all data analyses.

3 Results and discussion

3.1 Effect of N fertilization on disease severity

The pathogen colonized petiole stubs and eventually developed stem cankers on all inoculation sites of the untreated control plants in both replicate experiments. In contrast, some of the wounds treated with either biocontrol agent were protected (despite the purposefully high level of *B. cinerea* inoculum used in the study), which resulted in no or reduced disease development. As expected from previous work (Lecompte et al. 2010), disease severity was significantly higher on plants inoculated with strain BC1 than BC21 of *B. cinerea*, regardless of inoculum concentration (Fig. 2). A global analysis of variance on the AUDPC (r^2 equal to 0.78, F equal to 108, P value lower than 0.0001, 1,199 degrees of freedom) for the five classification variables (*B. cinerea* strain, inoculum concentration, biocontrol, N fertilization level, and experiment) and their interactions yielded significant effects for all variables and interactions except the “concentration \times experiment” interaction. Although significant differences between experiments were observed, the contribution of this factor to the global model variance was low compared to the other ones. Therefore, for the sake of conciseness and clarity, the major effects described below will be presented with mixed data from the two experiments; however, differences between experiments will be pointed out when necessary.

Disease severity was significantly affected by the level of N fertilization provided to the plants for both strains of *B. cinerea* and both inoculum concentrations, in the two experiments. Disease severity on control plants with no biocontrol treatment decreased significantly (P values lower than 0.0001 in all situations) with increasing N fertilization level up to 5 mM, and in some cases at higher N rates (Fig. 2). These results are in agreement with those of earlier work showing that high N fertilization reduced the severity of *B. cinerea* on tomato (Hoffland et al. 1999; Lecompte et al. 2010). In the presence of either biocontrol agent (*M. dimerum* and *T. atroviride*), the AUDPC also decreased significantly with

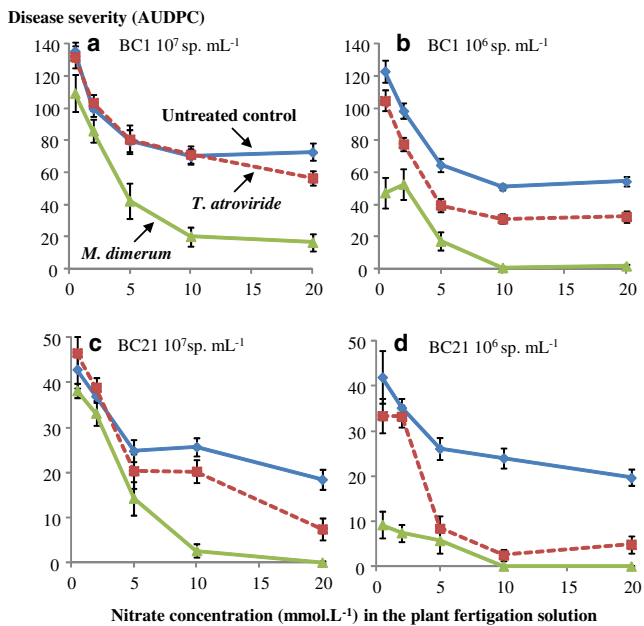


Fig. 2 Effect of nitrogen fertilization on the severity of disease caused by two strains of *B. cinerea* (a highly aggressive strain, BC1, and a mildly aggressive strain, BC21) on stems of tomato plants. The plants were inoculated with the pathogen alone (blue diamonds) at two different spore concentrations or together with biocontrol agent *T. atroviride* (red squares) or *M. dimerum* (green triangles). Disease severity (represented by the area under the disease progress curve) was differentially reduced in the presence of the biocontrol agents. In all cases, severity of disease decreased with increasing N fertilization. The bars represent the standard error of the means

increasing N fertilization (up to 5–20 mM, Fig. 2). Differences between experiments were observed for plants treated with *M. dimerum*. Decreased disease severity with increasing N levels was more pronounced in 2009 than in 2010 where a low severity was encountered.

3.2 Effect of N fertilization on the efficacy of biocontrol

The efficacy of biocontrol generally increased with increasing N fertilization. However, the degree of control depended on the strain and concentration of *B. cinerea* inoculum and on the species of biocontrol agent (Fig. 3). *M. dimerum* provided greater protection than *T. atroviride* and both biocontrol agents were more effective against the mildly aggressive than the highly aggressive strain of *B. cinerea*. In the presence of high *B. cinerea* inoculum, the efficacy of both biocontrol agents was low for plants grown at low N (Fig. 3a). The protection index of *M. dimerum* increased sharply with increasing N fertilization, to reach nearly 80 and 100 %, respectively, against the highly and the mildly aggressive strains of *B. cinerea*, while that of *T. atroviride* increased only moderately. At the lower inoculum concentration (Fig. 3b), *M. dimerum* provided good disease control at low N levels, and almost full protection against both strains of *B. cinerea* at high

N. Increasing N also improved the protective effect of *T. atroviride* against the mildly aggressive strain (BC21), but only limited protection was achieved against strain BC1, even at the high N rate.

Few studies have examined the effect of soil fertilization on biocontrol. Khattabi et al. (2004) reported that soil amendment with four types of N fertilizers decreased survival of sclerotia of soilborne pathogen *Sclerotium rolfsii* and that the antagonistic activity of biocontrol fungus *Trichoderma harzianum* against those sclerotia increased with increasing N doses. Nitrogen amendments in nutrient agar media have also been shown to increase in vitro activity of certain biocontrol microorganisms.

To gain a better understanding on the possible phenomena implicated in the similar effect observed in our study, two key steps of pathogenesis were examined more closely. The time needed for symptom development evaluated the first interactions between the plant, the pathogen, and the biocontrol agent, while the rate of stem lesion expansion evaluated interactions over several days between the three organisms.

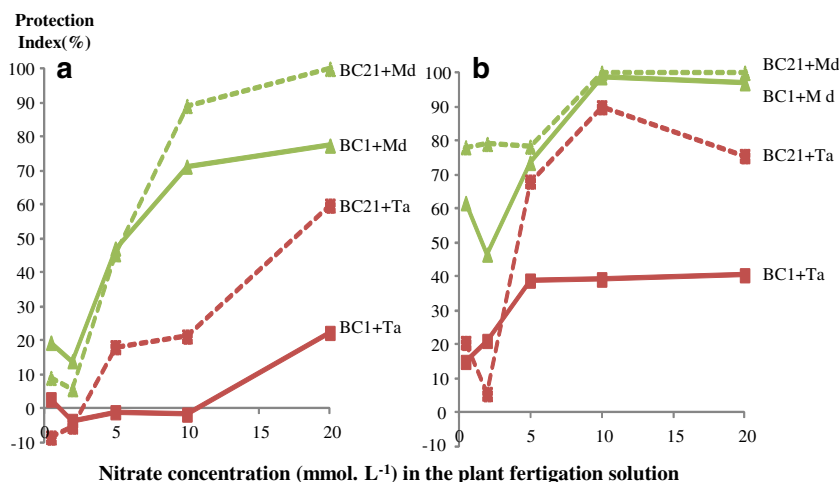
3.3 Effect of N fertilization on the latency period

The latency period on control plants increased significantly with increasing N fertilization, especially for the less aggressive strain BC21 (Fig. 4, *P* values lower than 0.0001 in all situations). This confirmed observations from our earlier work (Lecompte et al. 2010).

Both biocontrol agents increased the latency period as affected by N fertilization (Fig. 4, *P* values lower than 0.002 in all situations). In the presence of *M. dimerum*, the latency period for both strains of *B. cinerea* was significantly longer than on control plants at N levels of 5 mM and above when the inoculum concentration was high and at all N levels when the inoculum concentration was low. The latency period was similar on control plants and on those treated with *T. atroviride* at 0.5 and 2 mM NO_3^- . But it was significantly higher than the control on plants treated with *T. atroviride* at N levels of 5 mM and above, except for the highly aggressive strain (BC1) at high inoculum concentration.

This differential effect of N fertilization on the latent period, dependant both on the strain of *B. cinerea* and on the strain of biocontrol agent, reflects the outcome of early interactions between the three organisms that lead to the emergence of stem symptoms. The increase in the latency period with increasing N level generally paralleled the overall efficacy of both biocontrol agents. This suggests that the early interactions between the biocontrol agent, the plant, and the pathogen were important for the outcome of wound protection. These results are compatible with the observation of O'Neil et al. (1997) with tomato plants in the absence of any protective treatment, wherein the susceptibility of stems to infection by *B. cinerea* declined sharply with wound age within the first day after leaf removal.

Fig. 3 Effect of nitrogen fertilization of tomato plants on the efficacy of protection provided by two biocontrol agents against two strains of *B. cinerea* (BC1 and BC21). The plants were inoculated with the pathogen alone at two different spore concentrations (a, 10^7 spores mL^{-1} or b 10^6 spores mL^{-1}) or together with biocontrol agent *T. atroviride* (Ta, red squares) or *M. dimerum* (Md, green triangles). The efficacy of biocontrol was enhanced by high N fertilization



3.4 Effect of N fertilization on lesion expansion

The daily rate of lesion expansion decreased significantly on untreated control plants inoculated with the highly aggressive strain (BC1), with increasing N fertilization regardless of inoculum concentration (*P* values lower than 0.001 in both cases; no effect of inoculum concentration, *P* value greater than 0.58; averaged data shown in Table 1). Conversely, lesion expansion increased slightly with increasing N levels above 2 mM for strain BC21 (Table 1), as observed in earlier work contrasting highly and mildly aggressive strains of *B. cinerea* (Lecompte et al. 2010).

Lesion expansion was also significantly affected by N fertilization and the strain of *B. cinerea*, but not by the concentration of inoculum on plants treated with either biocontrol agent (*P* value greater than 0.80). Significant differences between the control and the treatments with biocontrol agents were mostly observed at high N levels for plants inoculated with the highly aggressive strain BC1, with reductions in lesion expansion amounting to 65–73 % for *M. dimerum* and nearly 50 % for *T. atroviride* (Table 1). Analyzing the influence of N fertilization on the reduction in lesion expansion by strain BC21 was complicated by the fact that no stem lesions developed at high N levels in the presence of *M. dimerum* and only in 2009 for *T. atroviride*. *T.*

Fig. 4 Effect of nitrogen fertilization on the latency period before symptom appearance on stems of tomato plants, following inoculation by two strains of *B. cinerea* (a highly aggressive strain, BC1, and a mildly aggressive strain, BC21). The plants were inoculated with the pathogen alone (blue bars) at two different spore concentrations or together with biocontrol agent *T. atroviride* (red bars) or *M. dimerum* (green bars). The latency period was differentially affected depending on the strain of the pathogen and the biocontrol agent. In all cases, the latency period was higher at high N fertilization levels. The bars represent the standard error of the means

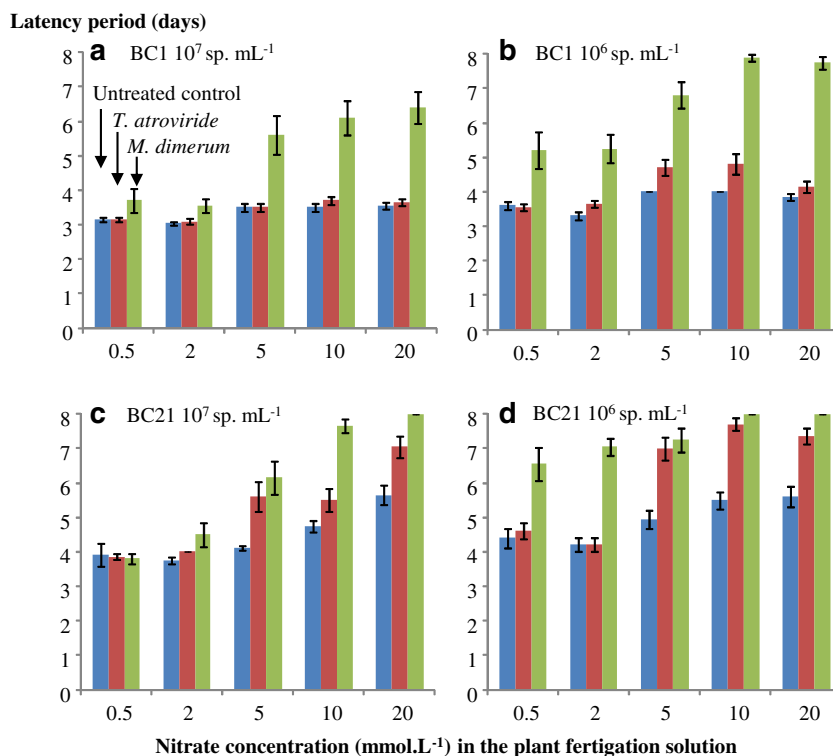


Table 1 Effect of nitrogen fertilization of tomato plants on expansion of stem lesions caused by *Botrytis cinerea*. The plants were inoculated with the pathogen alone (highly aggressive strain BC1 or mildly aggressive strain BC21) or together with either the biocontrol agent *T.*

atroviride or *M. dimerum*. Lesion expansion (in millimeter per day) was differentially affected depending on the strain of *B. cinerea* and the biocontrol agent. High N levels reduced lesion expansion and thus enhanced the protective effect of the biocontrol agents

Strain of <i>B. cinerea</i> and plant treatment	Nitrate concentration (mmol L ⁻¹) in the plant fertigation solution							
	0.5		2	5	10	20		
Strain BC1								
Untreated control	12.14	a ^a	9.33	5.89	3.44	a	3.35	a
<i>Trichoderma atroviride</i>	10.33	b	8.40	4.19	3.48	a	1.77	b
(% reduction) ^b	(15)		(10)	(29)	(-1)		(47)	
<i>Microdochium dimerum</i>	10.44	b	7.61	6.06	1.21 ^c	b	0.91 ^c	b
(% reduction)	(14)		(18)	(-3)	(65)		(73)	
<i>P</i> value ^d	0.015		0.095	0.085	0.035		0.000	
Strain BC21								
Untreated control	2.48		1.35	1.54	2.00		3.24	
<i>Trichoderma atroviride</i>	2.36		1.54	1.54	1.90		0.88 ^c	
(% reduction)	(5)		(-14)	(0)	(5)		(73)	
<i>Microdochium dimerum</i>	1.62		1.70	0.99	0 ^e		0 ^e	
(% reduction)	(35)		(-26)	(35)				
<i>P</i> value	0.068		0.165	0.227	0.728		0.000	

^a For a given strain of *B. cinerea* and nitrate fertilization level, numbers followed by different letters were significantly different (Newman and Keuls test; *P* value lower than 0.05)

^b Percent reduction in lesion expansion relative to the untreated control

^c Data for 2009 only (no stem lesion development in 2010)

^d Test of biocontrol treatment effect

^e No stem lesion development, due to full protection of the pruning wound

atroviride slowed lesion expansion by strain BC21 only at the highest N level (Table 1).

Although the major effect of N nutrition on biocontrol efficacy mostly occurred at the early infection phase, marked by a delay in symptom development, a reduction in lesion expansion contributed to increased biocontrol at high N when stems became infected.

3.5 Relationship between stem tissue content and the severity of disease

Nitrogen fertilization resulted in significant differences in fresh and dry weight (in gram) as well as tissue content of eight of the ten components quantified in the tomato stems (Table 2). Dry and fresh weight, total N, and NO₃⁻ increased with increasing nitrate up to 20 mM in the irrigation solution, while fructose increased significantly up to 5–10 mM, and C was not significantly influenced. These results were consistent with those of earlier work (Lecompte et al. 2010).

Highly significant correlations (*P* values lower than 0.001) were observed between disease severity (expressed as AUDPC) and the concentration in the stems (mg per gram dry matter) of some of the tissue components. Negative

correlations were observed with total N, NO₃⁻, and fructose, and positive correlations with sucrose (Table 3). However, these four tissue components were significantly autocorrelated, with highly positive correlations between N, NO₃⁻, and fructose content, and highly negative correlations between sucrose and the other three components, making it difficult to point to putative causal effects. Correlations between susceptibility or resistance to diseases and various indicators of nitrogen status of the host plant (amino acids, nucleic acids, phenols, total N, protein content, C/N, etc.) have been reported before in other plant–pathogen systems (Huber and Thompson 2007). Also, a relationship between plant sugar content and susceptibility has been suggested by Bolton (2009). Interestingly, no correlation was observed between disease severity and total soluble sugars, contrary to the results of Hoffland et al. (1999) with total soluble carbohydrates. This suggests that other compounds than those measured in our study (glucose, fructose, sucrose) may be implicated, or that only some of the soluble sugars, such as fructose and sucrose, are implicated in the response to infection. The recent description of a mutant of *B. cinerea* incapable of using fructose as a sole carbon source (Aguayo et al. 2011) should provide a useful tool to investigate this subject further.

Table 2 Effect of nitrogen fertilization on the fresh and dry weight and on tissue content of tomato stems. Increasing N fertilization resulted in a significant increase in fresh and dry weight, tissue content of total N, NO₃⁻, and fructose, and a significant decrease in sucrose

NO ₃ ⁻ (mM L ⁻¹)	Fresh weight (g)		Dry weight (g)		Tissue content (in mg per gram of dry matter)														
	N	NO ₃ ⁻	C	P	K	Mg	Ca	Sucrose	Glucose	Fructose									
0.5	21.5	a	4.1	a	7.5	a	0.1	a	369.7	7.2	54.6	3.2	6.1	32.8	c	83.6	a	7.9	a
2	33.6	a	5.6	a	11.3	a	0.2	a	374.5	6.8	53.3	3.5	5.9	29.6	c	110.8	b	9.7	a
5	66.1	b	8.1	ab	25.0	b	7.7	a	369.7	8.6	62.4	4.4	11.4	18.5	b	107.2	b	26.8	b
10	111.2	c	10.7	b	32.2	c	27.0	b	365.2	6.5	57.7	3.6	10.6	18.7	ab	104.2	b	33.9	c
20	126.6	c	11.9	b	34.7	c	41.9	c	350.9	6.2	57.5	3.4	14.8	13.7	a	73.1	a	26.4	b

Data are means of five replicates per treatment. For a given column the numbers followed by different letters are significantly different (Newman-Keuls tests; *P* value lower than 0.05)

Competition for nutrients is a common mode of action of biocontrol agents on plant surfaces. Although no information is available for the two beneficial fungi included in our study, competition for fructose and nitrate has been identified as a factor in the mode of action of certain biocontrol agents (Jacobsen 2006). However, in our study, repeated analyses of covariance on disease severity showed that there were no interactions between plant treatments (with either biocontrol agent or untreated controls) and the various plant components, since the slopes relating AUDPC to each of the individual plant components were similar for plants inoculated with *B. cinerea* alone or with *B. cinerea* and a biocontrol agent (graphs not shown). This indicated that the effects of these components were not different for plants inoculated with *B. cinerea* alone or with *B. cinerea* plus a biocontrol

agent and conflicted with the hypothesis of a direct effect of the plant components on biocontrol. Rather, the results suggest that the tissue content affected the capacity of the pathogen to infect the plant, and that the outcome of biocontrol was in turn affected by this modified interaction between the host and the pathogen. This hypothesis is in accordance with the observation that at each N regime, the efficacy of biocontrol was directly related to the pressure exerted by the pathogen, in terms of inoculum concentration and strain aggressiveness. Increasing plant N content either enhanced plant defenses or reduced the ability of the fungus to colonize the tissue, and by this direct reduction of fungal growth, indirectly facilitated the action of the biocontrol agents and hence their efficacy. Thus, the novel results presented here on the effect of N fertilization on the efficacy of two

Table 3 Correlation between the tissue content of tomato stems and the severity of disease on plants produced with various concentrations of nitrate in the irrigation solution. The tomato plants were inoculated with two strains of *B. cinerea* alone or co-inoculated with biocontrol agents

Tissue component	BC1 (control)		BC1 + <i>T. atroviride</i>		BC1 + <i>M. dimerum</i>		BC21 (control)		BC21 + <i>T. atroviride</i>		BC21 + <i>M. dimerum</i>	
	R ^a	<i>P</i> value	<i>R</i>	<i>P</i> value	<i>R</i>	<i>P</i> value	<i>R</i>	<i>P</i> value	<i>R</i>	<i>P</i> value	<i>R</i>	<i>P</i> value
N total	-0.81	0.000	-0.74	0.000	-0.76	0.000	-0.86	0.000	-0.81	0.000	-0.65	0.002
NO ₃ ⁻	-0.69	0.001	-0.66	0.001	-0.71	0.000	-0.80	0.000	-0.74	0.000	-0.63	0.002
C	0.39	0.088	0.40	0.074	0.45	0.046	0.53	0.014	0.49	0.026	0.41	0.071
P	0.09	0.677	0.10	0.659	-0.13	0.583	0.11	0.618	0.08	0.724	0.16	0.476
K	-0.30	0.185	-0.27	0.234	-0.28	0.229	-0.33	0.154	-0.33	0.150	-0.20	0.381
Mg	-0.30	0.192	-0.24	0.299	-0.17	0.451	-0.25	0.278	-0.23	0.316	-0.09	0.682
Ca	-0.39	0.084	-0.37	0.099	-0.38	0.092	-0.46	0.038	-0.43	0.055	-0.32	0.157
Sucrose	0.82	0.000	0.76	0.000	0.76	0.000	0.90	0.000	0.83	0.000	0.64	0.002
Glucose	-0.06	0.790	0.00	0.979	0.07	0.744	0.09	0.693	0.66	0.669	0.09	0.697
Fructose	-0.84	0.000	-0.74	0.000	-0.77	0.000	-0.82	0.000	-0.81	0.000	-0.64	0.002
Total sugars	0.03	0.875	0.05	0.817	0.04	0.861	0.09	0.703	0.05	0.806	0.04	0.844

^a Correlation analyses were performed on pooled data from two independent tests (2 × 5 replicates per modality). Tissue content was expressed in milligram per gram of dry matter and disease severity was represented by the area under the disease progress curve

biocontrol agents against *B. cinerea* should stimulate further work to better understand which mechanisms are involved at the cellular and molecular level.

4 Conclusions

The results of this study clearly indicated that the level of N fertilization provided to the plant can influence not only the susceptibility of tomato stems to *B. cinerea* but also the efficacy of biocontrol. High N fertilization enhanced the efficacy of both biocontrol agents tested against *B. cinerea*. To our knowledge, this is the first report of an effect of N fertilization on the efficacy of biocontrol against an airborne disease. It suggests that the manipulation of N fertilization could provide a new useful tool for integrated protection of greenhouse tomato. To devise specific recommendations for growers, further work is needed to validate the present results in conditions of commercial production and to evaluate possible side effects on other diseases or pests in the tomato greenhouse.

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