



HAL
open science

Contrasted responses of *Botrytis cinerea* isolates developing on tomato plants grown under different nitrogen nutrition regimes

François Lecompte, Manzoor Ali Abro, Philippe C. Nicot

► **To cite this version:**

François Lecompte, Manzoor Ali Abro, Philippe C. Nicot. Contrasted responses of *Botrytis cinerea* isolates developing on tomato plants grown under different nitrogen nutrition regimes. *Plant Pathology*, 2010, 59 (5), pp.891-899. 10.1111/j.1365-3059.2010.02320.x . hal-02663420

HAL Id: hal-02663420

<https://hal.inrae.fr/hal-02663420>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Contrasted responses of *Botrytis cinerea* isolates developing on tomato plants grown under different nitrogen nutrition regimes

F. Lecompte^{a*}, M. A. Abro^b and P.C. Nicot^b

^a INRA, UR 1115 Plantes et Systèmes de Cultures Horticoles, Domaine Saint Paul, Site Agroparc, F-84914, Avignon cedex, France

^b INRA, UR407 Pathologie végétale, Domaine Saint Maurice, BP94, F-84143 Montfavet cedex, France

* Corresponding author: francois.lecompte@avignon.inra.fr

Abstract

The nutritional status of a plant is known to influence its susceptibility to pathogens. In the case of *Botrytis cinerea* the role of nitrogen fertilization of various host plants on disease development appears to be variable. This study was carried out to characterize possible variability associated with strains and inoculum density of *B. cinerea* in its ability to infect leaf-pruning wounds and to develop stem lesions on tomato plants as affected by the nitrogen input. Six strains differing in their aggressiveness to tomato were compared. They all had similar reaction patterns *in vitro* in response to differential nitrogen levels. In tests on plants grown with contrasted regimes of nitrate fertilization, overall disease severity was lower for all strains on plants with higher nitrogen inputs, regardless of inoculum concentration. However, differences among strains were observed in the effect of plant nitrogen nutrition on infection and on lesion expansion. Disease onset was delayed on all plants with higher nitrogen inputs, but the response was greater for strains with lower aggressiveness on tomato. The highest contrast among strains was observed with the colonization of stems. The daily rate of stem lesion expansion decreased with increasing nitrogen fertilization levels for the more aggressive strains, while it increased for the less aggressive strain. Hypotheses to explain these results are discussed in light of the possible physiological effects of nitrogen fertilization on nutrient availability for the pathogen in the host tissue and of possible production of defence metabolites by the plant.

Additional key words

Solanum lycopersicum, *Lycopersicon esculentum*, gray mould, host resistance

Introduction

It has long been recognized that the nutritional status of a plant can play a role in its susceptibility to pathogenic fungi. Nitrogen, in particular, is deemed to strongly influence the host-pathogen interactions (Huber & Watson, 1974, Huber & Thompson, 2007). However, the nitrogen (referred to as "N" in the rest of this paper) status of a plant can be either favourable or unfavourable to the infection process, depending on the pathosystem (Huber & Thompson, 2007). This variable effect can be explained by the fact that plant N nutrition may have an influence on many factors involved in the epidemiological cycle. Such factors include the molecules involved either in host defence or in the virulence and aggressiveness of the pathogen, the quantity and nature of host N-based substrates acquired by the pathogen and the microclimate around the plant (through an effect on plant vigour and architecture). The N level of plant tissues has often been correlated to host susceptibility, one explanation being that at high plant N content more substrate is available for the development of the pathogen (Jensen & Munk, 1997, Neumann *et al.*, 2004, Walters & Bingham, 2007). In contrast, it has been shown in *Arabidopsis thaliana* that the constitutive and induced levels of some proteins involved in plant resistance to infection are higher at high N nutrition (Dietrich *et al.*, 2004). But other defence compounds and molecules acting as structural barriers against pathogens can be lowered at low C/N ratio, which are the consequence of a high N nutrition (Talukder *et al.*, 2005). It has also been suggested that plant soluble carbohydrate content – which is negatively correlated with N nutrition – has a positive influence on plant susceptibility (Hoffland *et al.*, 1999).

Although these contradictory effects highlight the need for pathosystem-specific studies, a general rule has been suggested (Solomon *et al.*, 2003): the development of biotrophic fungi would be enhanced by nitrate and inhibited by ammonium, while the contrary would be true for necrotrophic fungi. Regarding the influence of N nutrition on the necrotroph *Botrytis cinerea*, the situation is not as clear (Dik & Wubben, 2004). Higher plant susceptibility to *B. cinerea* was reported at high N fertilization rates in legumes (Davidson *et al.*, 2004), grape (R'Houma *et al.*, 1998), sweet basil (Yermiyahu *et al.*, 2006) and begonia (Pitchay, 2007). In three of these studies (R'Houma *et al.*, 1998, Yermiyahu *et al.*, 2006, Pitchay, 2007), the N source was nitrate or a mixture of nitrate and ammonium. In contrast, a high N nutrition seems to lower the level of disease in tomato (Verhoeff, 1968, Hoffland *et al.*, 1999). It has been reported that the susceptibility of bean to *B. cinerea* was 2.5 fold higher with an ammonium nutrition compared to a nitrate-based source of N (Huber & Watson, 1974). The explanation for this was that ammonium enhanced cell permeability and increased leaf exudates, both factors being favourable to infection (Huber & Watson, 1974). For grape, pruning of leaves and fruits lead to a reduction in infection, even at high N, suggesting that the effect of N was mainly to increase leaf surface, thus rendering the microclimate around infection sites more conducive to infection (R'Houma *et al.*, 1998). For sweet basil, the percentage of stems carrying sporulating lesions was higher at higher nitrate concentration in the fertigation solution, but the percentage of infected plants, the lesion size and the rate of disease progression were not affected (Yermiyahu *et al.*, 2006). For begonia, disease incidence was higher at 42 mmol.L⁻¹ N (brought as ammonium nitrate), a concentration hardly found in agricultural situations. However, between 1.7 mmol.L⁻¹ and 28 mmol.L⁻¹ N, the relationship between N nutrition and susceptibility was quadratic, with a maximum at 7 mmol.L⁻¹ N (Pitchay, 2007). Thus the effect of plant internal N content on *B. cinerea* infection and lesion growth appears to be highly dependent on the host species. This suggests that there could be a trade-off between a “trophic component” and a “defence component” of the host-pathogen interaction. For example, a high plant N status could raise the level of nutrients accessible to the pathogen, while at the same time enhance host defences. An unexplored hypothesis to explain the reported variability could be that the host-pathogen interaction could be differentially affected by N fertilisation depending on the strain of *B. cinerea*. The

response to N availability, *in vitro* and *in planta*, of *B. cinerea* strains of contrasting aggressiveness, as well as the effect of inoculum density, should help to better understand the nature of this interaction. In the present study, our objective was to confirm that the N content of nitrate-fed tomato plants has an influence on their susceptibility to *B. cinerea*, and to test the influence of initial inoculum density and fungal strain on the growth response of the fungus, both *in vitro* and *in planta*.

Materials and methods

Three experiments were conducted with tomato plants produced under differing N nutrition regimes between May and July 2008 (experiment E1), between June and August 2008 (experiment E2) and between March and May 2009 (experiment E3). Although comparable in its design, experiment E3 differed from E1 and E2 by the number of N treatments and strains of *B. cinerea* tested. Additionally, two *in vitro* tests with *B. cinerea* on contrasting nutrient medium were conducted in summer 2009.

Production of plant material and fertigation treatments

Tomato (*Solanum lycopersicum* var. *esculentum*) seeds (cv Swanson, De Ruiter Seeds, Saint Andiol, France) were sown in 1 cm³ rock wool cubes in a greenhouse. Ten days after sowing, the cubes, each containing one plantlet, were transferred onto rock wool blocks 7.5 x 7.5 x 6cm (Grodan®, Roermonds, the Netherlands). During the first month, the plants were fertigated twice a day with a standard commercial nutrient solution (Duclos international, Lunel, France). After that period, the plants (bearing 3-4 leaves) were placed on the top of 2-liter pots filled with a mixture (1:1 V/V) of vermiculite and pozzolana (inert crushed volcanic rock) to start the nutrition treatments. Thirty plants were used in each treatment. In experiments E1 and E2, three levels of NO₃⁻ concentrations were tested: 0.5 mmol.L⁻¹ NO₃⁻, 5 mmol.L⁻¹ NO₃⁻ and 15 mmol.L⁻¹ NO₃⁻; in E3, five levels were tested: 0.5 mmol.L⁻¹ NO₃⁻, 2 mmol.L⁻¹ NO₃⁻, 5 mmol.L⁻¹ NO₃⁻, 10 mmol.L⁻¹ NO₃⁻ and 20 mmol.L⁻¹ NO₃⁻. The equilibrium in electric charges was maintained by replacing nitrates by sulphates in the solutions with less nitrate. The concentration of other major nutrient elements was kept constant, at the following levels: 11 mmol.L⁻¹ K, 3.5 mmol.L⁻¹ Mg, 3.5 mmol.L⁻¹ Ca and 1 mmol.L⁻¹ P. Oligo-elements were also added at the following concentrations (in µmol. L⁻¹): 20.6 B, 0.5 Cu, 10.7 Fe, 11.6 Mn, 0.28 Mo, and 3.2 Zn. The plants were fertigated with a drip irrigation system (one dripper per pot) up to 6 times a day depending on the climatic demand, with one minute pulses. Three pots chosen at random were weighted continuously to evaluate their loss of water, and thus the climatic demand in the greenhouse. The pH was adjusted to 6 in each treatment by addition of H₂SO₄. Plants were grown with those solutions for four (in E1) or three (in E2 and E3) weeks and were then inoculated.

Evaluation of plant susceptibility to Botrytis cinerea

Two monoconidial strains of *B. cinerea* (BC1 and BC21), previously collected in commercial greenhouses and used routinely in the laboratory, were used in E1 and E2. Four additional strains (BC43, BC44, BC84 and NHPm4) were added in experiment E3. From previous work of the laboratory, strains BC1, BC43 and BC44 were known to have a high level of aggressiveness on tomato, while strains BC21, BC84 and NHPm4 had a medium to low level of aggressiveness (Ajouz, 2009, Ajouz et al., 2010). For each strain, inoculum was produced on potato dextrose agar medium (39 g L⁻¹, Difco, Detroit, USA) in a growth chamber (18°C night, 22°C day, and 14h daylight). Spores were collected in sterile distilled water from the surface of 14-day old cultures. Each suspension was filtered through a 30 µm mesh sterile filter to remove mycelium fragments and adjusted to the desired concentration with the help of a hemacytometer. Three infection concentrations were tested in E1 and E2: 10⁵ spores per mL (e5), 10⁶ spores per mL (e6) and 10⁷

spores per mL (e7). Only concentrations e6 and e7 were retained in experiment E3. The third, fourth, fifth and sixth leaves of 5 randomly selected plants were excised, leaving 5-10 mm petiole stubs on the stems. The wounds were inoculated with 10 μ L aliquots of a spore suspension. In E1 and E2, strain BC1 was inoculated on petioles 3 and 5 and strain BC21 on petioles 4 and 6, with different groups of plants for the different inoculum concentrations. A total of 15 plants were inoculated per N treatment. In E3, each plant was inoculated with one strain only, at concentration e6 on petioles 4 and 6 and at concentration e7 on petioles 3 and 5. A total of 30 plants were inoculated per N treatment. After inoculation, the plants were placed in a growth chamber for 7 days. The chamber was set at 21°C, 90% RH and 14h daylight. During this period, the plants were irrigated manually, twice a day, using the same fertilization solutions as those used before inoculation. Symptoms were assessed between the 3rd and 7th day after inoculation. The incidence of stem lesions and the length of developing lesions (in mm) were recorded daily. Area under the disease progress curves (AUDPC) were computed as described by Aissat et al. (2008) and Decognet

et al. (2009), as $AUDPC = [Y_1/2 + \sum_2^{n-1} Y_j + Y_n/2][I]$, where Y_j was the observed lesion length (in mm)

at the j^{th} observation time, n was the total number of observations, and I the interval between each observation (in days). These values were computed for individual pruning lesions for $n=5$ observations dates at daily intervals during the period from the 3rd to the 7th day after inoculation.

Plant nutrition assessment

At the end of the growing period in the greenhouse, just prior to the assay of susceptibility to *B. cinerea*, five additional plants were randomly sampled in each treatment for nutrient analysis. The wet and dry weights (after 72h at 70°C) of stem and leaves were measured. Subsamples were ground, calcined at 400°C for 12h and then mineralized in boiling H₂SO₄. In experiments E1 and E2, the K, Ca and Mg concentrations in the filtrates were measured with an atomic absorption spectrometer (Varian AA100). In all experiments, plant P, N and C content was assessed, with a spectrophotometer (Perkin-Elmer Lambda) for P and a gas analyzer (Thermo Finnigan 1112) for C and N.

Fungal growth in vitro

The 6 strains used in the inoculation treatments were grown on an agar medium amended with varying concentrations of NH₄NO₃. We used a minimal medium adapted from Weeds *et al.* (1998). It contained per litre: 2.5 g Na₃ Citrate, 5 g KH₂PO₄, 0.2 g MgSO₄, 15 g sucrose and 15 g Bacto™ Agar (Difco). Four doses of NH₄NO₃ were compared: 0, 0.1 [as in the minimal medium of Weeds *et al.* (1998)], 2 and 10 g.L⁻¹. For each medium, five 90mm diameter Petri plates (containing 15 mL of medium) were inoculated with a 2 μ L drop of spore suspension deposited in their centre. For all strains, the suspension was adjusted to 10⁷ spores.mL⁻¹. The plates were incubated at 21°C for 5 days and the colony diameter was recorded daily. Two independent repetitions of the test were conducted. There was no difference between these two experiments and the pooled data were used for analysis. The colony radial growth between day 3 and 4 was used for statistical analysis.

Data analysis

Significant differences between the experiments were assessed by analysis of variance. When significantly different, results from different experiments were treated separately. In the experiments with tomato plants, the latency period was calculated as the number of days between inoculation and lesion appearance on the stem. If a lesion was visible on the first day of symptoms assessment (3rd day after inoculation), the latency period was given a value of 2 days; if no symptoms were apparent on the last day of measurement, it was given a value of 7 days. For each inoculation point, the rate of lesion expansion was calculated as the average daily increase in lesion

length between the day of symptom appearance on the stem and the last day of measurement. As a result, the estimated rate of expansion of an individual lesion was based on 1 to 4 measurements depending on the date of appearance of the lesion. There was no systematic effect of the day of measurement on the rate of lesion expansion, thus the mean rate of lesion expansion was used in the statistical analyses as a variable following a normal law. For each strain, the effect of the inoculum concentration (e5, e6 and e7) and the nitrate nutrition were assessed by analysis of variance. Lesions on the same stem did not overlap and were assumed to appear and to grow independently. In each experiment, the number of inoculated wounds, for a given inoculum concentration and plant nutrition treatment, was 10. The SAS software package was used for data analysis.

Results

Fungal growth in vitro

All the strains were significantly affected ($p < 0.0001$) by the concentration of NH_4NO_3 in the agar medium (Fig. 1). On the medium containing no added N, their radial growth was strongly reduced and the mycelial mats were composed of sparse and very thin filaments which contrasted with the dense growth observed on the media supplied with N. On media amended with NH_4NO_3 , all strains were similarly affected by N concentration. For a given strain, radial growth was similar or slightly faster at $2 \text{ g.L}^{-1} \text{ NH}_4\text{NO}_3$ than at $0.1 \text{ g.L}^{-1} \text{ NH}_4\text{NO}_3$, and reduced at $10 \text{ g.L}^{-1} \text{ NH}_4\text{NO}_3$ (Fig. 1).

Plant growth and mineral content

As expected, the N fertigation treatments in the three experiments with tomato plants consistently resulted in contrasted dry matter contents of the plants at the time when they were used for the assays of susceptibility to *B. cinerea* (Table 1). It appeared from experiment E3 that the optimal concentration for growth was close to 10 mmol.L^{-1} . At higher N nutrition, plant N content still increased, but not plant weight. In experiments E1 and E2, the K, Mg and Ca contents were measured. They were positively correlated with the nitrate concentration in the fertigation solution (data not shown). Conversely, the phosphorous content was negatively correlated with nitrate nutrition. Plant C content did not vary significantly, except in E1 where a small decrease was noticed at high N (Table 1).

Overall disease severity

The AUDPC for the two groups of strains appeared rather different (Fig. 2). For fast growing strains there was a steep decrease of AUDPC between 0.5 mmol.L^{-1} and 10 mmol.L^{-1} and no significant difference between 10 and 20 mmol.L^{-1} . A quadratic regression gave a better fit to the data compared to a linear regression (r^2 of 0.33 and 0.21 respectively). For slow growing strains AUDPC was not significantly altered at low N nutrition rates, but decreased steeply between 5 mmol.L^{-1} and 20 mmol.L^{-1} at higher nitrate nutrition. A quadratic model did not improve the regression compared to a linear model (r^2 of 0.19 in both cases).

Kinetics of stem infection

There was an overall highly significant effect ($p < 0.0001$) of inoculum concentration and plant nutrition on the latency period before symptom appearance. Details for the different strains are given in Table 2. For BC1 and BC21, results were not different in experiments E1 and E2 ($p = 0.22$), and were pooled. For each strain, at each inoculum concentration, there was an effect of N nutrition on the delay before symptom appearance: high nitrate nutrition delayed symptoms by 0.5 to 2.5 days. This increase in latency period was more pronounced for less aggressive strains (table 2). An increase in inoculum concentration resulted in a shorter latency period, but the effect was more

pronounced at low N levels. The time necessary to obtain symptoms on 100% of the inoculated wounds also increased with N concentration (data not shown). After 7 days, all wounds showed stem lesions, with the exception of plants inoculated with strain BC21, for which lesions were observed on only 60 to 100% of the wounds, depending on the experiment and the N treatment.

Lesion expansion

The comparison of the rates of lesion expansion on tomato stems revealed contrasted patterns among strains of *B. cinerea* in response to plant nutrient status. For the more aggressive strains (BC1, BC43 and BC44), the rate of lesion expansion decreased with increasing nitrate concentrations in the fertigation solution up to 10-15 mmol.L⁻¹ (Fig. 3a). The response was similar for these three strains (p=0.12). In contrast, the strains with lower aggressiveness (BC21, BC84 and NHPm4) were stimulated by increasing N concentrations, up to 10-15 mmol.L⁻¹ (Fig. 3b). The response was not significantly different among these three strains (p=0.13). The effect of inoculum concentration on lesion expansion was only significant for BC21, BC43, BC44 and NHPm4. However, in each experiment, when data of all strains and N nutrition were put together, there was a highly significant effect of inoculum concentration on lesion growth rates (Fig. 4).

Discussion

Our results show that the overall severity of the disease, as assessed by the AUDPC, was consistently lower at high nitrate nutrition (between 10 and 20 mmol.L⁻¹) for six strains of *B. cinerea* differing widely in their aggressiveness to tomato. We thus confirm earlier results showing that a high N nutrition lowers the susceptibility of tomato to *B. cinerea* (Verhoeff, 1968, Hoffland *et al.*, 1999). However the correlation between N content and AUDPC was not linear, at least for aggressive strains. The C/N ratio of our plants decreased steeply from low to high N treatments, as a result of roughly constant C concentration and increasing N concentration in the plant (Table 1). Hence, we did not observe in our experiment a linear relationship between C/N and susceptibility, as proposed by Hoffland *et al.* (1999). Moreover, examining separately two key steps of pathogenesis (infection and lesion expansion) revealed a higher than anticipated level of complexity. The lesions of the three strains with lower aggressiveness expanded faster with increasing plant N content, while those of the three more aggressive strains were inhibited by high N content. In contrast, the increase with plant N concentration in the latency period before symptom appearance was more pronounced for less aggressive strains. This may explain why, *in fine*, the relationship between N nutrition and AUDPC was overall similar for both types of strains.

Contrasted intra-specific pathogen responses to plant nutritional status has already been reported in other pathosystems. However, the correlation between nutrient concentration and plant susceptibility is, for the various strains tested, always either positive or negative, but not opposite as we have observed here. For example, the infection efficiency of *Blumeria* (formerly *Erysiphe*) *graminis* on barley seedlings was, depending on the isolate, 2.5 to 7.8 times higher at 240 mg N per plant compared to 30 mg N per plant (Jensen & Munk, 1997). The inhibitory effect of calcium on *B. cinerea* has also been shown to depend on the isolate, but a decrease in fungal growth at high calcium concentration was always noted (Chardonnet *et al.*, 2000). Here, an opposite effect of plant N on the two types of strains used in our study was consistently observed in three independent experiments.

It has been suggested that high nitrate nutrition increases host susceptibility to biotrophic fungi and decreases that of necrotrophic fungi. One explanation could be that biotrophic pathogens rely on the nutrient content of the apoplast, or on nutrient transfer from the host cells by a haustorium, while necrotrophic fungi that kill cells have a better access to the host nutrients (Solomon *et al.*, 2003,

Divon & Fluhr, 2007). We have shown here that *in vitro* the strains responded positively, and similarly, to the N content of the substrate, at least up to a concentration of 2g.L^{-1} NH_4NO_3 (Figure 1). As the plant N content increased with increasing N in the nutrient solution, it could be hypothesized that any repression of fungal growth at the higher host N content was the result of a better ability of the plant to defend itself against the pathogen. This is consistent with reports that a high N nutrition can lead to production by the host of higher levels of some constitutive and induced defence molecules (Stout *et al.*, 1998, Dietrich *et al.*, 2004). The different responses of our strains to the host N status could be explained by the fact that infection strategies of *B. cinerea* (types and levels of secreted fungal toxins, cell wall degrading enzymes or oxalic acid) are strain-dependant (Siewers *et al.*, 2005, Choquer *et al.*, 2007). Thus the ability of the host to counteract, at high N content, the effect of pathogen metabolites could depend on the fungal phenotype. However, we have shown that, regarding primary infection and latency, the effect of plant N was comparable for all strains (Table 2): the delay before symptom appearance was higher at high plant N and lower at high inoculum concentration. Regarding early infection then, there appears to be no strain-specific effect of host N status.

A nitrogen deficit comparable to that imposed in the treatment with 0.5 mmol.L^{-1} in this study leads to a steep decrease of the nitrate and amino-acid content of the plant (Scheible *et al.*, 1997, Le Bot *et al.*, 2001), notably glutamine and glutamate which are the principal source of fungal N nutrition. Although secondary N sources can be used by the pathogen, N scarcity induces specific responses in fungi, ultimately sporulation (Divon & Fluhr, 2007). Thus, even for necrotrophic fungi such as *B. cinerea*, a decrease in host N resources could impair growth. Our hypothesis is that the increasing rate of lesion development of less aggressive strains with increasing N may be related to a reduction in competition for N substrates. As the total plant volume colonized by the mycelium of less aggressive strains is smaller than that colonized by more aggressive ones, N scarcity is likely to be more detrimental to the first group, as local resources are exhausted faster. The fact that low N reduced the latency period for both groups of strains can be explained if one assumes that, at that time, fungal growth relied mostly on the nutrient content of the spore (Divon & Fluhr, 2007). Thus the effect of high N availability could predominantly affect host defences – and not the pathogen nutrition – during early infection. Moreover, we have shown an unexpected decrease in lesion growth at higher inoculum concentration (Fig. 4). Usually, disease severity is higher when the conidial concentration in the infected wound is higher (see e.g. O'Neill *et al.* (1997)). This could indicate that the competition for N was higher when N resources were low, leading to less disease development and that conversely, it was attenuated when N resources were higher.

Conclusion

We have shown here that the effect of host N on *Botrytis* development depends on the strain and the concentration of inoculum leading to primary infection. One explanation for this could be that the fungal development depends both on trophic and defence processes, and that the availability of nutrients for the pathogen ultimately depends of its ability to explore new sources of unexplored plant tissues. Upon N scarcity, low substrate availability would impair growth of moderately aggressive isolates, those against which plant defences are more efficient, while resources available to aggressive strains would not be limiting, whatever the nutrition status of the host.

Acknowledgements

The authors wish to thank François de Bruyne, Magali Duffaud, Gisèle Riqueau, Michel Pascal for excellent technical assistance. This work is part of the French “programme national de recherches

PICléq” and the SYSBIOTEL program. It received financial support from INRA and the Agence Nationale de la Recherche (ANR). A grant for studies was also provided by the Higher Education Commission (HEC) of Pakistan.

References

- Aissat K, Nicot PC, Guechi A, Bardin M, Chibane M, 2008. Grey mould development in greenhouse tomatoes under drip and furrow irrigation. *Agronomy and Sustainable Development* **28**, 403–409
- Ajouz S, 2009. *Estimation of the potential of resistance of Botrytis cinerea to biofungicides*. Avignon, France: University of Avignon. Ph.D. thesis.
- Ajouz S, , Nicot PC, Bardin M, 2010. Adaptation to pyrrolnitrin in *Botrytis cinerea* and cost of resistance. *Plant Pathology*: in press.
- Chardonnet CO, Sams CE, Trigiano RN, Conway WS, 2000. Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. *Phytopathology* **90**, 769-74.
- Choquer M, Fournier E, Kunz C, Levis C, Pradier JM, Simon A, Viaud M, 2007. *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. *FEMS Microbiology Letters* **277**, 1-10.
- Davidson JA, Suresh P, Bretag TW, Lindbeck KD, Gali K-K, 2004. Biology and management of *Botrytis* spp. in legume crops. In: Elad Y, Williamson B, Tudzynski P, Delen N, eds. *Botrytis: Biology, Pathology and Control*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 295-318.
- Decognet V, Bardin M, Trottin-Caudal Y, Nicot PC, 2009. Rapid change in the genetic diversity of *Botrytis cinerea* populations after the introduction of strains in a tomato glasshouse. *Phytopathology*, **99**, 185-193.
- Dietrich R, Ploss K, Heil M, 2004. Constitutive and induced resistance to pathogens in *Arabidopsis thaliana* depends on nitrogen supply. *Plant Cell and Environment* **27**, 896-906.
- Dik AJ, Wubben JP, 2004. Epidemiology of *Botrytis cinerea* diseases in greenhouses. In: Elad Y, Williamson B, Tudzynski P, Delen N, eds. *Botrytis: Biology, Pathology and Control*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 319-33.
- Divon HH, Fluhr R, 2007. Nutrition acquisition strategies during fungal infection of plants. *FEMS Microbiology Letters* **266**, 65-74.
- Hoffland E, Beusichem MLv, Jeger MJ, 1999. Nitrogen availability and susceptibility of tomato leaves to *Botrytis cinerea*. *Plant and Soil* **210**, 263-72.
- Huber DM, Thompson IA, 2007. Nitrogen and plant disease. In: Datnoff LE et al., eds. *Mineral nutrition and plant disease*. Saint Paul, MN, USA: APS Press, 31-44.
- Huber DM, Watson RD, 1974. Nitrogen form and plant disease. *Annual Review of Phytopathology* **12**, 139-65.
- Jensen B, Munk L, 1997. Nitrogen-induced changes in colony density and spore production of *Erysiphe graminis* f sp *hordei* on seedlings of six spring barley cultivars. *Plant Pathology* **46**, 191-202.
- Le Bot J, Jeannequin B, Fabre R, 2001. Impacts of N-deprivation on the yield and nitrogen budget of rockwool grown tomatoes. *Agronomie* **21**, 341-50.
- Neumann S, Paveley ND, Beed FD, Sylvester-Bradley R, 2004. Nitrogen per unit leaf area affects the upper asymptote of *Puccinia striiformis* f.sp *tritici* epidemics in winter wheat. *Plant Pathology* **53**, 725-32.
- O'Neill TM, Shtienberg D, Elad Y, 1997. Effect of some host and microclimate factors on infection of tomato stems by *Botrytis cinerea*. *Plant Disease* **81**, 36-40.

- Pitchay DS, 2007. Impact of applied nitrogen concentration on growth of elatior Begonia and New Guinea Impatiens and susceptibility of Begonia to *Botrytis cinerea*. *Journal of the American Society for Horticultural Science* **132**, 193-201.
- R'Houma A, Chérif M, Boubaker A, 1998. Effect of nitrogen fertilizing, green pruning and fungicide treatments on *Botrytis* bunch rot of grapes. *Journal of Plant Pathology* **80**, 115-24.
- Scheible WR, GonzalezFontes A, Lauerer M, MullerRober B, Caboche M, Stitt M, 1997. Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell* **9**, 783-98.
- Shtienberg D, Elad Y, Niv A, Nitzani Y, Kirshner B, 1998. Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses. *European Journal of Plant Pathology* **104**, 753-63.
- Siewers V, Viaud M, Jimenez-Teja D, Collado IG, Gronover CS, Pradier JM, Tudzynski B, Tudzynski P, 2005. Functional analysis of the cytochrome P450 monooxygenase gene *bcbot1* of *Botrytis cinerea* indicates that botrydial is a strain-specific virulence factor. *Molecular Plant-Microbe Interactions* **18**, 602-12.
- Solomon PS, Tan KC, Oliver RP, 2003. The nutrient supply of pathogenic fungi; a fertile field for study. *Molecular Plant Pathology* **4**, 203-10.
- Stout MJ, Brovont RA, Duffey SS, 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. *Journal of Chemical Ecology* **24**, 945-63.
- Talukder ZI, McDonald AJS, Price AH, 2005. Loci controlling partial resistance to rice blast do not show marked QTL X environment interaction when plant nitrogen status alters disease severity. *New Phytologist* **168**, 455-64.
- Verhoeff K, 1968. Studies on *Botrytis cinerea*. Effect of soil nitrogen level and of methods of deleafing upon occurrence of *B. cinerea* under commercial conditions. *Netherlands Journal of Plant Pathology* **74**, 184-92.
- Walters DR, Bingham IJ, 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. *Annals of Applied Biology* **151**, 307-24.
- Weeds PL, Beaver RE, Long PG, 1998. New genetic markers for *Botrytis cinerea* (*Botryotinia fuckeliana*). *Mycological Research* **102**, 791-800.
- Yermiyahu U, Shamai I, Peleg R, Dudai N, Shtienberg D, 2006. Reduction of *Botrytis cinerea* sporulation in sweet basil by altering the concentrations of nitrogen and calcium in the irrigation solution. *Plant Pathology* **55**, 544-52.

Table 1 Dry matter (g), N and C content of 7-week old (experiment E1) or 8-week old (experiments E2 and E3) tomato plants, fertigated with solutions containing different NO₃⁻ concentrations. Data are means and standard errors of 5 replicate per treatment.

| N (mmol.L ⁻¹) | | 0.5 | 2 | 5 | 10 | 15 | 20 |
|---------------------------|----|------------|------------|------------|------------|-------------|------------|
| Dry matter (g) | E1 | 10.3±0.6 a | . | 35.8±1.0 b | . | 45.9±2.0 c | . |
| | E2 | 5.1±0.5 a | . | 20.4±0.7 b | . | 24.3±1.3 c | . |
| | E3 | 6.4±0.4 a | 11.4±0.2 b | 20.0±0.7 c | 29.1±1.7 d | . | 26.9±2.4 d |
| N (% dm) | E1 | 1.0±0.1 a | . | 2.0±0.1 b | . | 3.7±0.2 c | . |
| | E2 | 1.1±0.1 a | . | 1.9±0.1 b | . | 3.8±0.2 c | . |
| | E3 | 1.4±0.1 a | 1.8±0.1 ab | 2.2±0.1 b | 3.4±0.2 c | . | 4.1±0.2 d |
| C (% dm) | E1 | 39.7±0.1 a | . | 40.5±0.6 a | . | 38.4±0.34 b | . |
| | E2 | 39.7±0.1 a | . | 40.1±0.3 a | . | 39.1±0.2 a | . |
| | E3 | 37.8±0.2 a | 38.0±0.1 a | 37.8±0.2 a | 37.7±0.2 a | . | 36.8±0.6 a |

Different letters in a line indicate significant difference between treatments (SNK test, $\alpha=0.05$).

Table 2 Latency period (days) between inoculation of *Botrytis cinerea* and symptom appearance on the stem of tomato plants grown at various nitrate concentrations, for 6 different strains, and different inoculum concentrations (e5: 10⁵ spores.mL⁻¹, e6: 10⁶ spores.mL⁻¹ and e7: 10⁷ spores.mL⁻¹). Data are mean and standard errors of 20 replicates (Experiments 1 and 2) or 10 replicates (Experiment 3).

| Latency period (d) | | 0.5 mmol.L ⁻¹ | 2 mmol.L-1 | 5 mmol.L-1 | 10 mmol.L-1 | 15 mmol.L-1 | 20 mmol.L-1 |
|--------------------|----|--------------------------|--------------|--------------|---------------|-------------|--------------|
| Experiment 1 and 2 | | | | | | | |
| BC1 | e5 | 3.1±0.1 a,A | . | 3.3±0.1 a,A | . | 4.3±0.3 b,A | . |
| | e6 | 2.2±0.1 a,B | . | 2.3±0.1 a,B | . | 2.8±0.1 b,B | . |
| | e7 | 2.0±0.0 a,B | . | 2.0±0.0 a,C | . | 2.4±0.2 b,B | . |
| BC21 [Ⓢ] | e5 | 3.9±0.3 a,A | . | 4.0±0.1 a,A | . | 5.7±0.2 b,A | . |
| | e6 | 3.3±0.2 a,AB | . | 3.0±0.1 a,B | . | 4.7±0.3 b,B | . |
| | e7 | 2.9±0.3 a,B | . | 2.4±0.2 a,C | . | 4.7±0.3 b,B | . |
| Experiment 3 | | | | | | | |
| BC1 | e6 | 3.0±0.0 a,A | 3.0±0.0 a,A | 3.0±0.0 a,A | 3.0±0.0 a,A | . | 3.2±0.1 b,A |
| | e7 | 2.4±0.2 a,B | 2.6±0.2 ab,B | 2.9±0.1 ab,A | 2.8±0.1 ab,A | . | 3.1±0.1 b,A |
| BC43 | e6 | 2.8±0.1 a,A | 3.0±0.0 ab,A | 3.0±0.0 ab,A | 3.5±0.3 b,A | . | 3.3±0.2 ab,A |
| | e7 | 2.3±0.2 a,B | 2.9±0.1 ab,A | 3.0±0.0 ab,A | 3.0±0.0 ab,A | . | 3.6±0.4 b,A |
| BC44 | e6 | 2.7±0.1 a,A | 2.9±0.1 ab,A | 3.0±0.0 b,A | 3.0±0.0 b,A | . | 3.0±0.0 b,A |
| | e7 | 2.2±0.1 a,B | 2.4±0.2 a,B | 2.9±0.1 b,A | 2.9±0.1 b,A | . | 3.0±0.0 b,A |
| BC21 | e6 | 3.2±0.1 a,A | 3.6±0.4 a,A | 4.3±0.4 ab,A | 4.9±0.3 bc,A | . | 5.8±0.4 c,A |
| | e7 | 2.6±0.2 a,B | 3.0±0.0 a,A | 3.6±0.3 a,A | 4.7±0.4 b,A | . | 5.1±0.5 b,A |
| BC84 | e6 | 3.3±0.2 a,A | 3.2±0.2 a,A | 4.3±0.3 b,A | 5.1±0.2 c,A | . | 5.1±0.1 c,A |
| | e7 | 2.9±0.2 a,A | 3.1±0.1 a,A | 3.9±0.3 b,A | 4.8±0.2 c,A | . | 5.1±0.1 c,A |
| NHPm4 | e6 | 3.0±0.0 a,A | 3.3±0.3 ab,A | 3.9±0.2 bc,A | 3.6±0.3 abc,A | . | 4.2±0.3 c,A |
| | e7 | 2.7±0.2 a,A | 3.0±0.0 a,A | 3.1±0.1 a,B | 3.1±0.1 a,A | . | 3.8±0.3 b,A |

Lower case letters indicate significant differences between N treatments in a line, uppercase letters indicate significant differences between inoculum concentrations for a given strain, in a column (SNK test, $\alpha=0.05$)

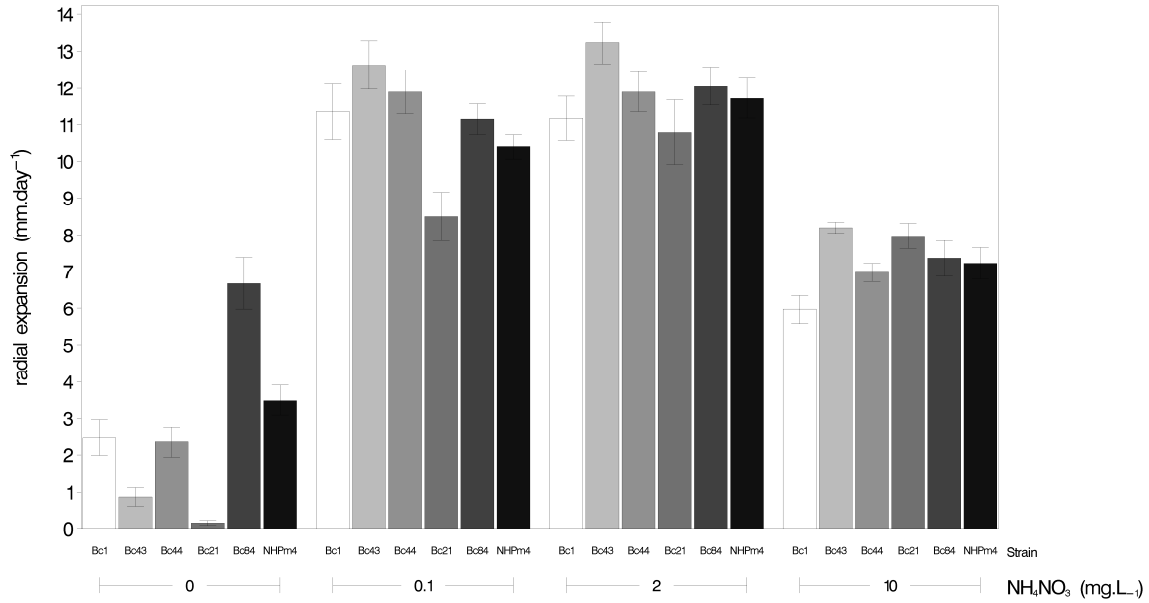


Figure 1 Radial growth of *Botrytis cinerea* strains on agar medium at different concentrations of NH₄NO₃. Bars represent the standard errors.

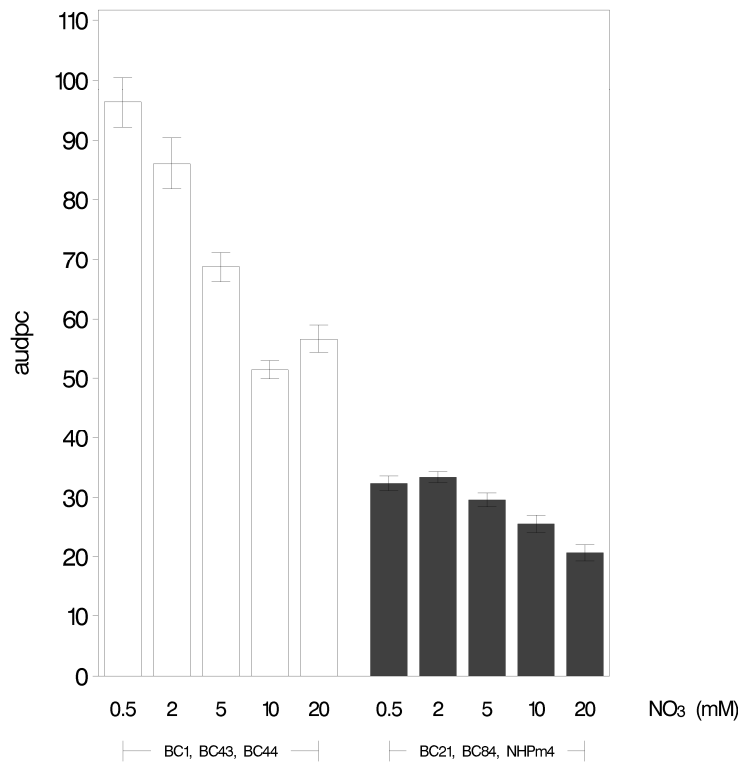


Figure 2 Area under disease progress curve (AUDPC) for stem lesions of *Botrytis cinerea* measured on tomato plants grown at various nitrate nutrition in the growing medium. Data were pooled for fast-growing strains (BC1, BC43 and BC44) and slow-growing strains (BC21, BC84 and NHPm4). Data are from experiment 3. Bars indicate standard errors.

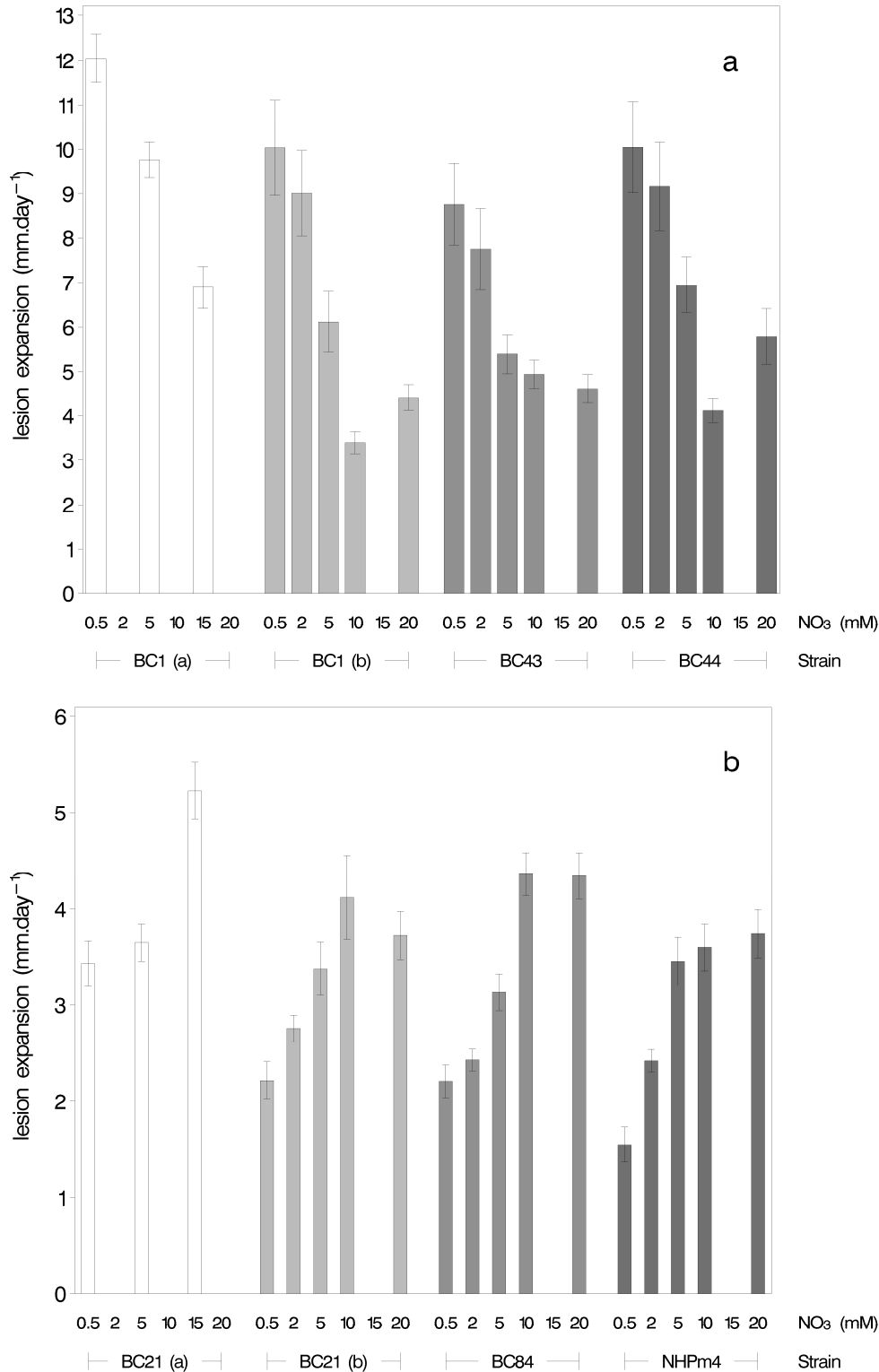


Figure 3 Daily rate of lesion expansion of 6 strains of *Botrytis cinerea* on stems of tomato plants grown at various nitrate concentration in the growing medium. A separation was made between fast-growing strains (Panel a: BC1 in experiment 1 and 2 (BC1a) and experiment 3 (BC1b) and BC43 and BC44 in experiment 3) and slow-growing strains (Panel b: BC21 in experiment 1 and 2 (BC21a) and experiment 3 (BC21b) and BC84 and NHPm4). Bars represent the standard errors.

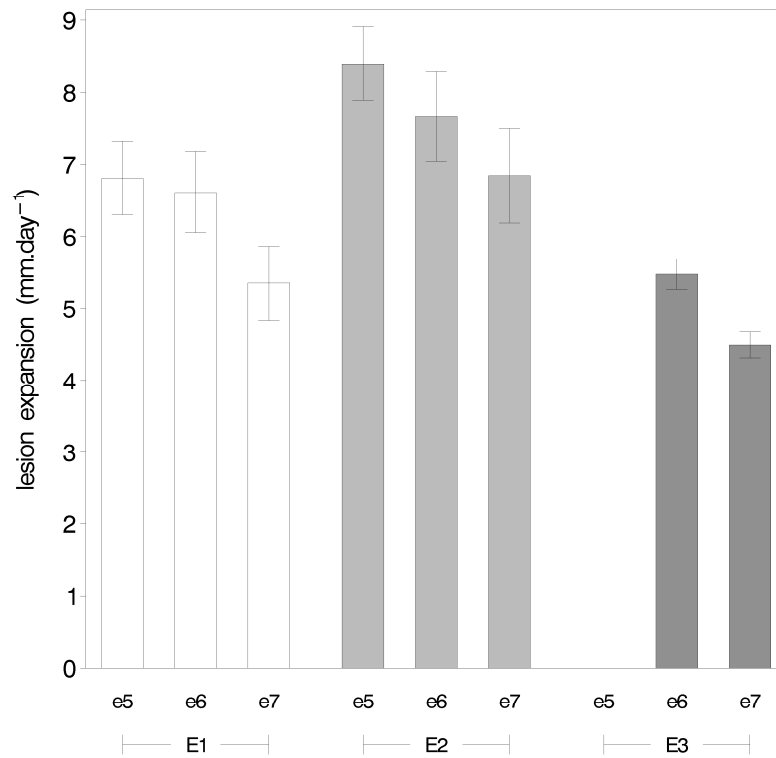


Figure 4 Daily rate of stem lesion expansion following the inoculation of leaf pruning wounds with spore suspensions of *Botrytis cinerea* containing 10⁵ spores.mL⁻¹ (e5), 10⁶ spores.mL⁻¹ (e6) and 10⁷ spores.mL⁻¹ (e7). Data from all the strains were pooled in each experiment, E1 to E3. Bars indicate standard errors.