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# Can plant sugars mediate the effect of nitrogen fertilization on lettuce susceptibility to two necrotrophic pathogens: *Botrytis cinerea* and *Sclerotinia sclerotiorum*?

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1 Can plant sugars mediate the effect of nitrogen fertilization on lettuce susceptibility to two  
2 necrotrophic pathogens: *Botrytis cinerea* and *Sclerotinia sclerotiorum*?

3

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14

1 **Abstract**

2 *Aims.* Nitrogen (N) fertilization is known to modify a plant's susceptibility to necrotrophic diseases.  
3 However, the effect of N nutrition on defence is not well known. It was hypothesized that not only  
4 molecules related to the N metabolism but also main sugars could mediate the effect of plant fertilization  
5 on its susceptibility to pathogens.

6 *Methods.* Two necrotrophic fungi, *Botrytis cinerea* and *Sclerotinia sclerotiorum* were inoculated on  
7 leaves of lettuce plants grown at 5 nitrate (NO<sub>3</sub><sup>-</sup>) fertilization levels, in three independent experiments.  
8 Variations in plant composition at the time of inoculation were linked to the size of lesions observed  
9 after 5-6 days.

10 *Results.* Both diseases were favoured by high NO<sub>3</sub><sup>-</sup> fertilization. However, the highest disease levels  
11 were not found in the same experiment for *B. cinerea* and *S. sclerotiorum*. Among the components  
12 measured, NO<sub>3</sub><sup>-</sup> and sucrose (SUC) were positively and negatively correlated to the two diseases in the  
13 three experiments, but the relationship between SUC and lesion size was more significant for *S.*  
14 *sclerotiorum*. Water content, N and total carbon (C) were also significantly correlated to the diseases,  
15 but the relationships were less straightforward. The ratios of SUC over total sugars and fructose (FRU)  
16 over total sugars fitted, very closely for *S. sclerotiorum*, a negative and positive exponential relationship  
17 respectively with lesion size. Absolute or relative glucose (GLU) levels were not linked to the diseases.  
18 *Conclusions.* Plant metabolic modifications induced by variations of N availability conferred the plant  
19 variable defence ability, which seemed, at least for *S. sclerotiorum*, mainly mediated by variations in  
20 host SUC and FRU levels. The generalization of these findings to other species would be of interest.

21

22 **Keywords:** *Lactuca sativa* L; plant disease; pathogenic fungi; sucrose; fructose.

23

## 1 **Introduction**

2 *Botrytis cinerea* Pers. Fr. and *Sclerotinia sclerotiorum* (Lib.) de Bary are two phylogenetically close  
3 necrotrophic ascomycetes (Amselem et al. 2011), sharing many common characteristics in their  
4 development and pathogenicity. Both feed and grow on previously killed plant host cells (Williamson  
5 2007; Bolton et al. 2006). Both fungi can infect a very wide range of plant species and, to kill their hosts,  
6 secrete a variety of compounds including oxalic acid, cell-wall degrading enzymes, peptidases, and a  
7 pool of toxic metabolites. These compounds allow the pathogen to modify the host redox status, perturb  
8 its defence, alter the cell integrity and macerate the plant tissues (Alghisi and Favaron 1995; Riou et al.  
9 1991; Godoy et al. 1990). Common patterns of host defence responses upon infection by the two  
10 necrotrophs have also been demonstrated (El Oirdi et al. 2011; Glazebrook 2005; Oliver and Solomon  
11 2010; Robert-Seilaniantz et al. 2011). They lead, on the one hand, to the local production of reactive  
12 oxygen species which trigger the hypersensitive response and ultimately programmed cell death. On the  
13 other hand, the plant activates the secretion of fungal enzyme antagonists, including inhibitors of cell  
14 wall degrading enzymes (Juge 2006), anti-fungal secondary metabolites, and cell wall strengthening  
15 molecules (Stotz et al. 2011; Kliebenstein 2004; van Baarlen et al. 2004). Whatever the similarities in  
16 infection strategies and stimulation of plant defences, the secretome diversity of both fungi allows them  
17 to adapt to their broad host range (Amselem et al. 2011; Choquer et al. 2007). Strain-specific  
18 aggressiveness, possibly linked to toxin synthesis and/or detoxification of plant defence metabolites, has  
19 been frequently reported (Choquer et al. 2007; Siewers et al. 2005). Although they are frequently  
20 associated in the field, especially in lettuce cropping systems, only a few comparative studies of  
21 symptom development of these two fungi have been conducted.

22 Upon infection, a massive change in the host's genome expression is triggered (Katagiri 2004), which  
23 coincides with an enhanced use of plant primary metabolites towards defence-oriented pathways and  
24 the use of energy and C and N skeletons for defence (Bolton 2009; Berger et al. 2007). Negative or  
25 positive correlations between plant sugar status and susceptibility to fungi have long been noticed (Levy  
26 and Cohen 1984; Horsfall and Dimond 1957), leading to the concept of low or high-sugar pathogens  
27 (Horsfall and Dimond 1957). Several studies have reported a decrease in photosynthetic activity and an  
28 increase in leaf cell-wall invertase activity after infections by either biotrophic or necrotrophic fungi

1 (Berger et al. 2004; Scharte et al. 2005; Kocal et al. 2008; Fotopoulos et al. 2003). A rise in the  
2 concentration of soluble sugars can induce the synthesis of defence related molecules (Johnson and Ryan  
3 1990; Ehness et al. 1997; Morkunas et al. 2011) and the onset of the hypersensitive reaction (Essmann  
4 et al. 2008). However, hexoses are also thought to be the principal source of C for the pathogen, and  
5 both *B. cinerea* and *S. sclerotiorum* secrete their own invertases to exploit the host sugar content (Jobic  
6 et al. 2007; Dulermo et al. 2009). Along with the host carbohydrate metabolism, the plant N status  
7 largely determines the outcome of an infection.. However the relationship between N availability and  
8 plant constitutive or induced immunity is not straightforward (Walters and Bingham 2007). Generally,  
9 higher concentrations of secondary metabolites active in plant defence, notably polyphenols, are found  
10 in plants grown at low N availability (Stout et al. 1998; Le Bot et al. 2009; Lou and Baldwin 2004).  
11 Nevertheless, the accumulation of secondary metabolites in N-deficient plants is not readily linked with  
12 decreased susceptibility to necrotrophs such as *B. cinerea* (van Baarlen et al. 2004) or *Alternaria solani*  
13 (Mittelstrass et al. 2006). Indeed, low N availability is known to limit the plant contents of amino acids  
14 and proteins, which could include constitutive or inducible pathogenesis-related proteins (Dietrich et al.  
15 2004). Also, the influence of the host N status on its ability to regulate nitric oxide synthesis, which has  
16 recently been reported as an important component of plant defence against *B. cinerea* and *S.*  
17 *sclerotiorum* (Asai and Yoshioka 2009; Perchepped et al. 2010), is not known. Furthermore, as for  
18 sugars, fungal pathogens retrieve N from their hosts, presumably with amino-acids as preferential source  
19 (Solomon et al. 2003). Whether the N content of the host can be limiting for pathogen growth is still a  
20 matter of debate (Bolton and Thomma 2008; Solomon et al. 2003). The availability of plant-based N  
21 compounds might however depend on the plant N status and the speed of fungal expansion, as suggested  
22 from disease progress observations (Lecompte et al. 2010; Newton and Guy 1998). As a result of these  
23 multiple processes, the effects of host N content on its susceptibility to necrotrophic pathogens are  
24 variable, either positive, negative, or neutral (Hoffland et al. 2000; Lecompte et al. 2010; Long et al.  
25 2000; Huber and Thompson 2007). Based on the above evidence that the mobilization of plant primary  
26 metabolism can markedly affect the outcome of the host-pathogen relationship, and given the  
27 interdependence of carbon (C) and N metabolism (Nunes-Nesi et al. 2010), it is surprising that very few  
28 studies have examined the combined effect of C and N availability on fungal disease development. An

1 experiment with two levels of N at ambient and elevated CO<sub>2</sub> concluded that high N and CO<sub>2</sub> reduced  
2 epidemics caused by *Cercospora* sp. on *Solidago rigida* (Strengbom and Reich 2006). However, in this  
3 work, the various C and N-based metabolites were not analysed with regard to disease severity. We  
4 hypothesized that the effect of N nutrition on epidemic severity could be partially due to modifications  
5 of the plant C status, especially its sugar content, and to its impact on triggering or fueling host  
6 immunity. The questions we addressed were: (i) which mathematical function(s) gave a consistent  
7 relationship between lettuce NO<sub>3</sub><sup>-</sup> fertilization and the symptoms caused by *B. cinerea* and *S.*  
8 *sclerotiorum* ; (ii) was the fertilization effect on each disease related to the plant N status or to the main  
9 plant sugars? We report an experimental study on lettuce grown at five N-supply levels, during three  
10 different seasons in a greenhouse, leading to marked variations in C and N contents. We assessed and  
11 compared the severity of disease caused by *B. cinerea* and *S. sclerotiorum* and related the development  
12 of lesions with total C, total N, NO<sub>3</sub><sup>-</sup>, sugars and other nutrients in plant tissues. The relative proportion  
13 of sucrose (SUC) and fructose (FRU) were closely correlated, negatively and positively respectively  
14 with lettuce susceptibility to *S. sclerotiorum*, while infections with *B. cinerea* were correlated most  
15 closely with plant NO<sub>3</sub><sup>-</sup> and total N.

## 16 **Materials and methods**

17

### 18 **Experimental design**

19

20 Three batches of 200 lettuce plants were grown from March 31<sup>st</sup> to May 30<sup>th</sup> 2009 (experiment E1),  
21 September 7<sup>th</sup> 2009 to November 11<sup>th</sup> 2009 (experiment E2) and November 16<sup>th</sup> 2009 to January 14<sup>th</sup>  
22 2010 (experiment E3). . For all experiments, seeds of cultivar Faustina (Rijk Zwaan) were sown in 1  
23 cm<sup>3</sup> rockwool cubes in a nursery greenhouse. Ten days after sowing, the cubes, each containing one  
24 seedling were transferred to 10 x 10 x 6 cm rockwool blocks (Grodan, Roermonds, The Netherlands)  
25 and placed in a second greenhouse dedicated to the experiments. Plants were then grown for  
26 approximately one month and irrigated twice a day with a standard commercial nutrient solution  
27 (Plantain, Duclos international, Lunel, France). After that period, the rockwool blocks (bearing plants  
28 with 3-4 developed leaves) were transferred on to the top of 2 L pots filled with a mixture (1:1 V/V) of

1 vermiculite and pozzalana (inert crushed volcanic rock) to start the nutrition treatments. The  
2 experimental design was monofactorial, with four randomized blocks of 10 plants per treatment. Five  
3  $\text{NO}_3^-$  concentrations were tested in the fertilization solution: 0.5, 2, 5, 10 and 20  $\text{mmol.L}^{-1}$   $\text{NO}_3^-$   
4 (abbreviated as mM in the rest of this paper). The composition of the five solutions, made up from  
5 simple salts, is given in Table1. At  $\text{NO}_3^-$  concentrations below 10 mM,  $\text{NO}_3^-$  ions were replaced by  
6 sulphates, by the use of potassium sulphate instead of potassium nitrate. At the highest nitrate level (20  
7 mM), the concentration of potassium nitrate was doubled. We considered that potassium nitrate was  
8 better than other  $\text{NO}_3^-$  salts to achieve the doubling of  $\text{NO}_3^-$  concentration in solution. The concentrations  
9 in solution of other macronutrients (calcium, magnesium and phosphorus) and trace elements (B, Fe,  
10 Cu, Mn) were kept constant. The ionic charge was neutral in all solutions when accounting for  $\text{Cl}^-$  and  
11  $\text{HCO}_3^-$  ions already present in the irrigation water, but the electrical conductivity was 40% higher at the  
12 highest  $\text{NO}_3^-$  level (20 mM). The pH of each solution was adjusted to 6.5 by adding  $\text{H}_2\text{SO}_4$ . Nutrient  
13 solutions were supplied via a fertigation network with an individual dripper into each pot. Three pots in  
14 an additional block with a 20 mM fertigation were continuously weighted to estimate daily water losses,  
15 which were replaced by fertigation pulses, up to six times a day, depending on the external radiation and  
16 the crop growth stage. All plants, whatever the nutrient solution, received the same amount of water.  
17 Excess water was lost in drainage. Plants were grown for three additional weeks with these different  
18  $\text{NO}_3^-$  concentrations in the fertigation solution. At the end of the period, the 70 day old plants were either  
19 used for inoculation with *B. cinerea* or *S. sclerotiorum* or for nutrient content assessment. Depending  
20 on the season, the greenhouse was either cooled or heated. The incoming radiation gradually increased  
21 from 1000 to 2300  $\text{J.cm}^{-2}$  in E1, decreased from 1800 to 750  $\text{J.cm}^{-2}$  in E2 and remained around 500  $\text{J.cm}^{-2}$   
22 in E3. Daily average air temperatures increased from 17 to 27°C in E1, fluctuated between 15 and  
23 25°C in E2 and decreased from 17 to 10°C in E3.

24

25 Analysis of plant components

26

27 Five plants were randomly selected from each N nutrition regime in the greenhouse for determination  
28 of plant fresh and dry weight and for analysis of different minerals and primary metabolites just before



1 inoculation. The plants were harvested at similar times of day in the three experiments, all around 8 am.  
2 The fresh and dry mass of the aerial parts were measured, while roots were discarded. A subsample of  
3 3 or 4 leaves from the intermediate crown were kept for plant analysis. Immediately after sampling, the  
4 leaves were placed in liquid N. Ten plant components (primary metabolites or elementary elements)  
5 were measured, namely: total N (N), total C (C), nitrate ( $\text{NO}_3^-$ ), glucose (GLU), fructose (FRU), sucrose  
6 (SUC), phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca). Sub samples of dry  
7 material were ground, calcined at 400 °C for 12 h and then mineralized in boiling  $\text{HNO}_3$ . The K, Ca and  
8 Mg contents were measured with an atomic spectrometer (Varian A220), and the P content with a  
9 spectrophotometer (Perkin-Elmer Lambda 2). Total N and C were measured with a gas analyzer  
10 (Thermo Finnigan EA1112), and  $\text{NO}_3^-$  with a  $\text{NO}_3/\text{NO}_2$  analyzer (5000 FIAstar). Soluble sugars were  
11 determined with an enzymatic method in a microplate reader, as proposed by Gomez *et al.*(2007).  
12 Hexoses (FRU + GLU) and total sugars (FRU + GLU + SUC) were computed from these analyses, as  
13 well as the C/N ratio.

14

15 Inoculation and disease assessment

16

17 Six strains of *B. cinerea* and one strain of *S. sclerotiorum* were used in this study. The strains of  
18 *B. cinerea* (BC1, BC43, BC44, BC21, BC84, NHPm4) were chosen for their contrasted aggressiveness  
19 on tomato (Lecompte et al. 2010), but without preliminary information on their aggressiveness on  
20 lettuce. For each strain, the inoculum was produced on potato dextrose agar medium ( $39 \text{ g L}^{-1}$  Difco,  
21 Detroit, USA) in a growth chamber (Heliofroid, Le Beausset, France) at 21 °C with a 14h photoperiod.  
22 For each strain of *B. cinerea* and *S. sclerotiorum*, one set of five plants per  $\text{NO}_3^-$  treatment (35 plants in  
23 total) was inoculated. On each of these 70 day old plants, three leaves of the middle crown were  
24 inoculated. A 5 mm diameter mycelial disk, excised from a 3-day old colony, was placed in the centre  
25 of each leaf. The leaf inoculations were made on intact plants. Following inoculation, the plants were  
26 placed in a growth chamber and incubated for seven days in conditions conducive to disease  
27 development (21 °C, RH above 85 %, with a 14h photoperiod). During this period, the plants were  
28 irrigated manually twice a day, using the same fertilization solutions as those used before inoculation.

1 Very few of the 1575 observed leaves failed to develop lesions, and 0 values were discarded. The size  
2 of the lesions was assessed 5 and 6 days after inoculation for *S. sclerotiorum* and *B. cinerea*,  
3 respectively. At these dates, the lesions had not yet covered the whole leaf surface. Each inoculated leaf  
4 was detached and photographed over a blue background. The image analysis software Assess 2.0 (APS  
5 Press, St Paul, MN, USA) was used to quantify the leaf area (mm<sup>2</sup>) and the lesion size (mm<sup>2</sup>).

6

7 Data analysis

8

9 For the 5 NO<sub>3</sub><sup>-</sup> nutrition levels and 3 experimental replicates, 1350 and 225 observations were analysed  
10 for *B. cinerea* and *S. sclerotiorum* respectively. The SAS statistical package was used for the data  
11 analysis. The first step was to analyse by general linear models (GLM) the effect of the three (or two)  
12 experimental factors related to *Botrytis cinerea* (or *Sclerotinia sclerotiorum*) lesion development: “NO<sub>3</sub><sup>-</sup>  
13 concentration in solution”, “experiment” , “*B. cinerea* strain” (for *B. cinerea* only), and their  
14 interactions. Significant differences among treatments were determined by the Neuman-Keuls test.  
15 Secondly, the type of mathematical function relating NO<sub>3</sub><sup>-</sup> concentration with disease severity was  
16 assessed. Six types of functions were tested, whose equations are as follows, where L is the lesion size  
17 (mm<sup>2</sup>), R the regressor, and a, b and c are parameters:

18 Linear:  $L = a + b * R$

19 Exponential:  $L = a * \exp(b * R)$

20 Power:  $L = a + b * R^c$

21 Logistics:  $L = \frac{a}{\left(1 + \left(\frac{a}{b} - 1\right) * \exp(-c * R)\right)}$

22 Michaelis-Menten:  $L = a * \frac{R}{b + R}$

23 Hill:  $L = a * \left(\frac{R^b}{c^b + R^b}\right)$

24

25 The Gauss-Newton method was used to estimate parameter values, using the NLIN procedure of the  
26 SAS package (with 449 degrees of freedom (df) for each regression model for *B. cinerea* and 74 for *S.*

1 *sclerotiorum*). For each disease, the model minimizing the mean square error of prediction (MSEP), was  
2 chosen.

3 Thirdly, the plant components linked to disease variability were investigated, using the fresh weight  
4 data. Variables whose distributions were highly skewed and significantly different from a normal  
5 distribution were log-transformed. As plant analyses and leaf susceptibility assays were not done on the  
6 same plants, the regressions had to be performed on mean values, providing 14 df for each model with  
7 the pooled data (for global multiple regression), and 4 df for regressions on individual plant components  
8 in each experiment. This means that for each N level in each experiment the mean lesion area observed  
9 on leaves was related to the mean nutrient content of 5 other plants that received the same nutrient  
10 solution. However as the trend of the relationship between plant nutrient content and susceptibility was  
11 similar for all strains, the *B. cinerea* inter-strain variability was not considered in this part of the analysis  
12 and the overall mean from 90 measurements was used: 3 leaves x 6 strains x 5 plants. Fifteen  
13 measurements (3 leaves x 1 strain x 5 plants) were used for *S. sclerotiorum*. In the linear regressions  
14 between lesion areas and individual plant component for each experiment, only those components which  
15 were, in the three experiments, highly correlated ( $r^2 > 0.7$ ) to disease severity were retained. Among  
16 these components, several showed high auto-correlation levels. Repeated GLM analysis – with the  
17 pooled data from the three experiments - between disease severity and plant components were thus  
18 performed, with all possible combinations of variable orders, and the type I sums of squares (SS) were  
19 analysed. When a significant F-value for a given variable was obtained whatever the order of its entry  
20 in the model, it was assumed to be independent of other variables.

21 On a fourth step, the same six functions described above were tested to relate the influence of the selected  
22 plant components on both diseases.

23

24 was tested.

## 25 **Results**

26

27 Lettuce growth, primary metabolites and ions contents

28

1 Leaf area increased asymptotically with  $\text{NO}_3^-$  concentration in the fertigation solution ( $p < 0.0001$ , Fig.  
2 1). Significant differences were found between experiments ( $p < 0.0001$ ), but in all cases no further  
3 increase was observed for concentrations beyond 10 mM.  $\text{NO}_3^-$  concentration also had a significant  
4 effect on fresh weight (g), dry weight (g) and plant water content ( $\text{g water g}^{-1} \text{ dm}$ ), following the same  
5 pattern, with no increase beyond 10 mM  $\text{NO}_3^-$ . Water content almost doubled with the treatments, from  
6  $8.5 \text{ g g}^{-1}$  at 0.5 mM  $\text{NO}_3^-$  to around  $17 \text{ g g}^{-1}$  at 10 and 20 mM  $\text{NO}_3^-$ , and was closely correlated with total  
7 N content (data not shown).  $\text{NO}_3^-$  nutrition had a very significant effect on the ten plant components  
8 measured, either on a fresh or on a dry weight basis (Table 2, data shown only on a fresh weight basis).  
9 All elements except Ca showed significant variability between experiments, and the “ $\text{NO}_3^-$ ” x  
10 “experiment” interactions were also always significant. Almost no  $\text{NO}_3^-$  was found in plants grown at  
11 very low  $\text{NO}_3^-$  concentrations, but the plant  $\text{NO}_3^-$  content increased exponentially with increasing  $\text{NO}_3^-$   
12 fertilization regimes. At the 10 and 20 mM  $\text{NO}_3^-$  regimes, plant  $\text{NO}_3^-$  content was much higher in E1,  
13 with nitrate N accounting for 15% and 30% of total N respectively as compared to 5-6% (at 10 mM)  
14 and 7-8% (at 20 mM) in the two other experiments. Similarly to plant  $\text{NO}_3^-$ , total N increased up to 20  
15 mM  $\text{NO}_3^-$ . In contrast with plant  $\text{NO}_3^-$ , N accumulation was lower in E1. The highest SUC levels were  
16 found at 2 mM  $\text{NO}_3^-$  and SUC decreased at higher  $\text{NO}_3^-$  regimes, while GLU and FRU contents increased  
17 up to 5 mM  $\text{NO}_3^-$  (Table 2). The variation in the plant total sugar content (SUC+GLU+FRU) with the  
18 fertilization regime was comparable in the three experiments, with an increase from 0.5 to 5 mM  $\text{NO}_3^-$   
19 and lower values at higher  $\text{NO}_3^-$  regimes (Fig. 2a). However the proportion of SUC in the sugar pool  
20 decreased sharply with  $\text{NO}_3^-$  nutrition up to 10mM, and appeared fairly different from one experiment  
21 to another, with a much higher proportion of SUC in E3 (Fig. 2b). Total C, P, K, Ca and Mg contents  
22 decreased, on a fresh weight basis, with increasing  $\text{NO}_3^-$  concentration in the nutrient solution (Table 2).  
23 The plant C content decreased exponentially with the  $\text{NO}_3^-$  regime and was highest in E3. In that  
24 experiment, on a dry weight basis, the C content did not change with  $\text{NO}_3^-$  regime, while it decreased  
25 strongly with  $\text{NO}_3^-$  nutrition in E1. On a dry weight basis, P and K plant content increased with increasing  
26  $\text{NO}_3^-$  concentrations (data not shown). The increased  $\text{K}^+$  concentration in nutrient solutions at 20 mM  
27  $\text{NO}_3^-$  did not have a systematic effect on plant K accumulation in this regime, as plant K contents were  
28 not higher at 20 mM  $\text{NO}_3^-$  compared to those at 10 mM  $\text{NO}_3^-$ , in the E2 and E3 experiments. With pooled

1 data from the three experiments, many of the plant components appeared significantly correlated. The  
2 most highly correlated components (on a fresh weight basis), were GLU and FRU ( $r=0.95; 0.75; 0.76$   
3 in resp. experiments E1, E2 and E3 respectively), Ca and C ( $r=0.92; 0.93; 0.80$ ), SUC and C ( $r=0.93;$   
4  $0.79; 0.81$ ) and Mg and K ( $r=0.5; 0.79; 0.89$ ). A strong negative correlation was also found between  
5 SUC and  $\text{NO}_3^-$  plant content ( $r=-0.75; -0.91; -0.87$ ). On pooled data, the plant water content was highly  
6 correlated with the C content ( $r=-0.96$ ) and SUC content ( $r=-0.81$ ). SUC was highly negatively  
7 correlated with FRU ( $r=-0.64$ ) but only slightly with GLU ( $r=-0.39$ ).

8

9 Disease development at different N nutrition levels

10

11 Overall lesion sizes were different for the two fungal pathogens, and for a given pathogen, they were  
12 significantly different from one experiment to another (Fig. 3a). Lesion size was greater in E1 for *B.*  
13 *cinerea*. Lesions caused by *S. sclerotiorum* appeared more severe in E2, whereas in that experiment  
14 lesions caused by *B. cinerea* were minimal. Necrotic lesions developed usually as an ovoid shape, being  
15 slightly wider towards the distal end of the leaf (Fig. 3b,c).

16 A GLM analysis relating the size of lesions caused by *B. cinerea* to the  $\text{NO}_3^-$  fertilization and experiment  
17 effects, along with their interaction, yielded a highly significant model ( $r^2=0.41$ , 1349 df,  $f=175$ ,  
18  $p<0.0001$ , Fig. 4a). Response curves were best fitted by a logistic function in E1 and a power function  
19 in E2 and E3. Whatever the model, the lesion size increased significantly in each experiment between  
20 10 mM and 20 mM  $\text{NO}_3^-$  in the nutrient solution. Experimental factors generated a 2.3 fold variation in  
21 disease variability. A small but significant ( $p<0.0001$ ) part of the observed lesion variability was due  
22 to a “strain” effect. When added to the GLM model, this third factor improved the model  $R^2$  very slightly  
23 (0.44 vs 0.41). The lesions caused by strain BC1 were 34% greater on average than those caused by the  
24 other five strains tested (1875  $\text{mm}^2$  vs 1394  $\text{mm}^2$ , data not shown). No significant differences were found  
25 between the five other strains (data not shown). The “strain x experiment” effect was not statistically  
26 significant ( $p=0.07$ ) and individual tests for each strain all indicated significantly greater lesion sizes in  
27 experiment E1. Strain variability from *B. cinerea* was not considered further.

1 The same GLM model fitting performed on lesions caused by *S. sclerotiorum* yielded comparable  
2 results: there was a highly significant effect of N fertilization level on lesion size ( $r^2=0.58$ , 224 df,  $f=58$ ,  
3  $p<0.0001$ ), with also highly significant effects of the individual experiments and the  $\text{NO}_3^-$  x experiment  
4 interaction. Lesion size increased with  $\text{NO}_3^-$  concentration in the fertigation solution, by a factor of 6.8  
5 from 950  $\text{mm}^2$  at 0.5 mM  $\text{NO}_3^-$  to 6500  $\text{mm}^2$  at 20 mM  $\text{NO}_3^-$  (Fig. 4b). Response curves were best fitted  
6 by a logistic function in experiment E1 and a Hill function in the two others. In contrast with what was  
7 observed for *B. cinerea* however, disease reached an asymptote at 10 mM and above: the disease was  
8 significantly greater at 20 mM  $\text{NO}_3^-$  in E3, but not statistically different between the two regimes in the  
9 first experiments..

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#### 11 Relationship between plant nutrient content and lesion development

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13 Leaf C and  $\text{NO}_3^-$  contents ( $\text{mg g}^{-1}$  fresh weight) were not normally distributed and so were log-  
14 transformed. Amongst the plant elements measured, only the N, logC, log $\text{NO}_3^-$ , SUC contents and the  
15 N/C ratio were consistently correlated with lesion areas ( $r^2>0.7$ ) in all three experiments, for the two  
16 diseases independently (Table 3). The total sugars, FRU, GLU or hexoses (FRU + GLU) contents, as  
17 well as the P, and Mg contents were not significantly correlated with any of the two diseases, while K  
18 and Ca were correlated, but not in all experiments, with lesions caused by *B. cinerea* and *S. sclerotiorum*  
19 respectively (Table 3). Additionally, the N/C ratio and the FRU/total sugars ratio also appeared highly  
20 significant in regressions for lesion areas for the two pathogens, in all three experiments (data not  
21 shown).

22 As the four identified individual factors (N, logC, log $\text{NO}_3^-$ , SUC) were correlated, we further analysed  
23 the data with type I SS assessment in repeated GLM models (24 possible combinations with 4 variables,  
24 see Materials and Methods section). For *B. cinerea*, the model with these four factors was significant  
25 ( $r^2=0.70$ , 14 df,  $F=5.8$ ,  $p=0.01$ ). The most significant factors were log $\text{NO}_3^-$  ( $p=0.04$  with type III SS)  
26 and N ( $p=0.08$  with type III SS). Analyzing the type I SS analysis, the logC and SUC effects appeared  
27 not to be independent of the log $\text{NO}_3^-$  effect. The N effect was independent from the log $\text{NO}_3^-$  effect, and  
28 in regression models where both  $\text{NO}_3^-$  and N were introduced, the parameter related to N was negative.

1 For *S. Sclerotiorum*, the model was highly significant ( $r^2=0.93$ , 14 df,  $F=31$ ,  $p<0.0001$ ). The most  
2 significant factors were SUC ( $p=0.004$  with type III SS) and N ( $p=0.06$  with type III SS). The  $\text{NO}_3^-$   
3 effect was not independent of the SUC effect, and the N effect was independent of the three other effects.  
4 Here again, the parameter associated to N in models was negative when the factor  $\text{NO}_3^-$  was introduced.  
5 The effect of logC was, for the two diseases, in most regressions not independent of the other effects.  
6 Individual regressions are presented for  $\log\text{NO}_3^-$ , N and SUC in Fig. 5. In all the experiments, the best  
7 model for the regression of *B. cinerea* lesions on  $\log\text{NO}_3^-$  was an exponential function, with increasing  
8 disease severity at higher plant  $\text{NO}_3^-$  content (Fig. 5a). However the fitted parameters were different from  
9 one experiment to another, and little was gained by analysing the symptoms on a plant  $\text{NO}_3^-$  basis rather  
10 than on a  $\text{NO}_3^-$  concentration in solution basis. Although a negative relationship between plant SUC  
11 content and lesion area was found in each experiment (Fig. 5b), the best fits were achieved with different  
12 functions for the various experiments.. Plant N content, isolated from the other plant components, was  
13 positively correlated with *B. cinerea* symptoms (Fig. 5c).

14 For *S. sclerotiorum*, although the relationships between lesion size and plant  $\text{NO}_3^-$  were very strong in  
15 each experiment, the overall regression showed poor predictive capacity at high plant  $\text{NO}_3^-$  content (Fig.  
16 5d). As stated earlier, there was a strong negative correlation between  $\text{NO}_3^-$  and SUC, which was closely  
17 correlated with disease intensity, either when considering individual experiments or the pooled data  
18 (Fig. 5e). The fitted functions were an exponential function for E1 and E2 experiments, and a linear  
19 function for E3. However, the MSE of the linear and exponential models in this later experiment were  
20 very close. The relationship between disease intensity and SUC explains the relatively weak symptoms  
21 in 2010, where SUC plant contents were high, regardless of the  $\text{NO}_3^-$  fertigation regime. As for *B.*  
22 *cinerea*, the plant N content had poor predictive capacity on the pooled data, but total N was positively  
23 correlated with disease, when including the nitrate fraction of total N (Fig. 5f). Relating the disease to  
24 the proportion of SUC within the total sugars pool did not greatly affect the observed effect of SUC  
25 alone on *B. cinerea* or *S. Sclerotiorum* (Fig. 6a,b). However, although FRU alone was very poorly  
26 correlated with disease intensity, the ratio of FRU on total sugars fitted very well the data, following an  
27 exponential function for *S. sclerotiorum* (Fig. 6c,d). Conversely, the ratio of GLU / total sugars did not  
28 give any fit to the data (data not shown).

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## Discussion

Examining the effect of fertilization treatments on the plants, we observed that  $\text{NO}_3^-$  concentration in the nutrient solution limited growth up to 10 mM  $\text{NO}_3^-$ , following a typical plateau curve. Doubling the concentration from 10 to 20 mM  $\text{NO}_3^-$  did not increase growth, but led to an accumulation of  $\text{NO}_3^-$  and of reduced N. These results are in line with previous reports showing  $\text{NO}_3^-$  accumulation in lettuce leaves when its uptake rate is higher than its assimilation rate (Maynard et al. 1976). The observed consequences of N shortage or excess on plant C and dry matter accumulation relative to the plant water content have also been reported before (Seginer 2003; Burns 1994; Cardenas-Navarro et al. 1999). Less is known about the effect of N nutrition on lettuce sugar content, but our observations seem consistent with general considerations on the coordination of C and N metabolism (Stitt et al. 2002). In the lowest  $\text{NO}_3^-$  regime (0.5 mM  $\text{NO}_3^-$ ), growth – and probably photosynthesis – were severely restricted, and the available C was stored as SUC as starch accumulation in lettuce is limited (Seginer 2003). For wheat, higher SUC content at low  $\text{NO}_3^-$  availability has already been reported (Lawlor et al. 1987). At higher regimes (2-5 mM  $\text{NO}_3^-$ ), growth was still N-limited, and the availability of sugars might have reflected the availability of photosynthetic products that were not involved in proteins and organic acids metabolisms. Sugars are also known to maintain osmolarity in lettuce in the absence of  $\text{NO}_3^-$  (Blomzandstra and Lampe 1985). Significant differences were found between our experiments. Leaf  $\text{NO}_3^-$  and potassium content at 10 and 20 mM  $\text{NO}_3^-$  were higher in experiment E1, while the SUC content relative to the total sugar pool was much higher in E3 (table 2). The high evaporative demand observed during E1 called for increased frequency and duration of irrigation events and, as a consequence of the constant ion concentrations in solutions, the plants were fed with relatively more  $\text{NO}_3^-$  and potassium in that experiment than in the others. Additionally, we consistently observed in the experiments a strong negative correlation between nitrate and SUC contents. These contrasting environments, in association with the nitrate nutrition treatments, allowed us to obtain a large range of C and N plant contents, which was helpful to assess the influence of primary metabolites on disease intensities.



1 Several differences were observed between *B. cinerea* and *S. sclerotiorum* infections. First, tissue  
2 colonization was much faster for *S. sclerotiorum*, which caused larger lesions in five days than *B.*  
3 *cinerea* in six days (Fig. 3).. As lesions of both fungi appeared at approximately the same time, 24-36h  
4 after inoculation, this was due to a higher mycelial expansion rate of *S. sclerotiorum*. The ability of *S.*  
5 *sclerotiorum* to achieve a quicker colonization of its host than *B. cinerea* had already been noticed on  
6 tomato stems (Gerlagh et al. 1996) and sunflower leaves (Dulermo et al. 2009), and mirrors the generally  
7 higher growth rate of *S. sclerotiorum in vitro*. However, although the experimental methods and  
8 environmental conditions in the growth chambers before and after inoculation were similar, the  
9 experimental effect on lesion size was not the same for the two pathogens.. Maximum lesion  
10 development for the two fungi was not found in the same experiments: lesions caused by *S. sclerotiorum*  
11 were larger in E2, while those caused by *B. cinerea* were larger – for all the tests on six strains - in “E1”.  
12 The incubation conditions which conferred the plant more efficient defence against *B. cinerea* were thus  
13 not necessarily optimal against *S. sclerotiorum*, and vice-versa. Furthermore, response curves of disease  
14 severity to host NO<sub>3</sub><sup>-</sup> nutrition were not identical: although both diseases were favored by high N  
15 fertilization, there was no increase of symptoms between 10 mM NO<sub>3</sub><sup>-</sup> and 20 mM NO<sub>3</sub><sup>-</sup> for *S.*  
16 *sclerotiorum*, while a significant increase in disease severity was found between 10 mM NO<sub>3</sub><sup>-</sup> and 20  
17 mM NO<sub>3</sub><sup>-</sup> for *B. cinerea*.. It can therefore be concluded that symptoms of *B. cinerea* and *S. sclerotiorum*  
18 on lettuce grown at various N levels differ significantly, although higher N fertilization generally  
19 increased the severity of both disease.

20 Another objective of our study was to assess the effect of plant composition on disease development,  
21 regardless of fertilization treatments. Regressions of disease severity on several plant components gave  
22 a much better fit to the data than did the NO<sub>3</sub><sup>-</sup> treatments. The severity of both diseases was related to  
23 the plant’s content of NO<sub>3</sub><sup>-</sup>, total N, SUC, and total C. The regressions on these four components  
24 explained 70% and 93% of overall disease variability in all three experiments for *B. cinerea* and *S.*  
25 *sclerotiorum* respectively. For *B. cinerea* the most significant factor was plant NO<sub>3</sub><sup>-</sup> content, while SUC  
26 and C effects on lesion size could not be separated statistically from the NO<sub>3</sub><sup>-</sup> effect. There was an  
27 influence of total N independent of that of NO<sub>3</sub><sup>-</sup>, illustrated by more severe *B. cinerea* infections in  
28 experiments where total N was lower and the nitric fraction of total N higher. Lesion size was negatively

1 correlated with the C/N ratio, which disagree with observations showing that disease incidence after  
2 inoculation of *B. cinerea* spores on tomato leaves was positively correlated to C/N (Hoffland et al. 1999).  
3 These results as a whole show that total N, total C or their ratio, when considered alone, are not reliable  
4 predictors of plant susceptibility to *B. cinerea*. We show here that, in lettuce,  $\text{NO}_3^-$  accumulation in  
5 leaves was associated with higher disease.

6 In contrast with what was observed for *B. cinerea*, susceptibility of lettuce leaves to *S. sclerotiorum* was  
7 more significantly linked to sugars than  $\text{NO}_3^-$ . We observed a negative exponential relationship between  
8 leaf SUC content and lesion size, which was quite similar in the three independent experiments (Fig.  
9 5e). Although FRU and GLU contents were negatively correlated with SUC, we did not observe any  
10 positive correlation between hexoses and susceptibility. Thus, either SUC or other metabolites whose  
11 variations mirror those of SUC are possibly linked with a decrease of lettuce susceptibility to *S.*  
12 *Sclerotiorum*, or a decreased aggressiveness of the pathogen. Also, ratios representing the proportion of  
13 SUC and FRU on the total sugar pool were closely linked to lesion size. It has been shown on sunflower  
14 cotyledons that *S. sclerotiorum* depletes the host from its SUC and FRU, but not its GLU (Jobic et al.  
15 2007), during the course of infection. The involvement of sugars in plant defense has been increasingly  
16 recognized in recent years. Sugars may act either by providing C skeletons for the synthesis of defensive  
17 secondary metabolites, and/or as signals for the induction of defence-related pathways (Berger et al.  
18 2007; Bolton 2009). In several plant-fungus interactions, SUC is known to be mobilized upon infection  
19 via an increase in acid invertase activities of both plant and fungal origins (Berger et al. 2004; Jobic et  
20 al. 2007). The use of SUC as a C source for the synthesis by the plant of defensive metabolites first  
21 requires its cleavage into FRU and GLU. If a significant involvement of SUC, via its initial  
22 transformation into hexoses, was necessary for fuelling the defence, then a lower susceptibility should  
23 have been found also at high hexose content; however neither GLU nor FRU were directly correlated to  
24 any of the two diseases. SUC is known to be a specific inductor of flavonoid biosynthesis in Arabidopsis  
25 (Solfanelli et al. 2006), and SUC-supplemented tissues activate the synthesis and accumulation of  
26 flavonoids in lupins challenged with *Fusarium oxysporum* (Morkunas et al. 2011). An enhanced  
27 production of anti-fungal metabolites in plants containing high SUC concentrations is compatible with  
28 our observations, SUC playing the role of a signaling molecule rather than that of a C source for

1 polyphenol synthesis. It also appeared that the respective proportions of FRU and SUC in the total sugar  
2 pool were even more closely related to the diseases than individual sugars. SUC and FRU were  
3 negatively correlated. This suggests that an induction of defense mediated by plant sugars might be  
4 controlled by the relative content in SUC and FRU, rather than their absolute concentration in the  
5 infected tissues. . Although a negative correlation between SUC content and lesion size was also found  
6 for *B. cinerea*, the relationship with SUC gave a poorer fit to the data than  $\text{NO}_3^-$  did.  
7 The main conclusion of our work is that, at least for one of the two necrotrophs in this study, the effect  
8 of N fertilization on susceptibility appeared statistically mediated by the metabolism of sugars. As sugar  
9 accumulation is specific in each plant species, and for a given species, in its different organs, it might  
10 explain why the variation of plant susceptibility to necrotrophs in response to N fertilization is complex.  
11 Genotypes with different sugar accumulation should be useful to test a general relationship between  
12 specific sugars and resistance to necrotrophs in lettuce and other species.

13

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## 20 **References**

21

22 Alghisi P, Favaron F (1995) Pectin-degrading enzymes and plant-parasite interactions. Eur J Plant  
23 Pathol 101:365-375 Amselem J, Cuomo CA, van Kan JAL, Viaud M, Benito EP, Couloux A,  
24 Coutinho PM, de Vries RP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L,  
25 Lapalu N, Plummer KM, Pradier JM, Quevillon E, Sharon A, Simon A, ten Have A,  
26 Tudzynski B, Tudzynski P, Wincker P, Andrew M, Anthouard V, Beever RE, Beffa R, Benoit  
27 I, Bouzid O, Brault B, Chen ZH, Choquer M, Collemare J, Cotton P, Danchin EG, Da Silva C,

1 Gautier A, Giraud C, Giraud T, Gonzalez C, Grossetete S, Guldener U, Henrissat B, Howlett  
2 BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Neuveglise C,  
3 Oeser B, Pearson M, Poulain J, Poussereau N, Quesneville H, Rasclé C, Schumacher J,  
4 Segurens B, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yandava C,  
5 Yarden O, Zeng QD, Rollins JA, Lebrun MH, Dickman M (2011) Genomic Analysis of the  
6 Necrotrophic Fungal Pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genetics  
7 7

8 Asai S, Yoshioka H (2009) Nitric Oxide as a Partner of Reactive Oxygen Species Participates in  
9 Disease Resistance to Necrotrophic Pathogen *Botrytis cinerea* in *Nicotiana benthamiana*. Mol  
10 Plant-Microbe Interact 22:619-629

11 Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene  
12 expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol Plant  
13 122:419-428

14 Berger S, Sinha AK, Roitsch T (2007) Plant physiology meets phytopathology: plant primary  
15 metabolism and plant-pathogen interactions. J Exp Bot 58:4019-4026

16 Blomzandstra M, Lampe JEM (1985) The role of nitrate in the osmoregulation of lettuce (*Lactuca*  
17 *sativa* L) grown at different light intensities. J Exp Bot 36:1043-1052

18 Bolton MD (2009) Primary Metabolism and Plant Defense-Fuel for the Fire. Mol Plant-Microbe  
19 Interact 22:487-497

20 Bolton MD, Thomma BPHJ (2008) The complexity of nitrogen metabolism and nitrogen-regulated  
21 gene expression in plant pathogenic fungi. Physiol Mol Plant Pathol 72:104-110

22 Bolton MD, Thomma BPHJ, Nelson BD (2006) *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and  
23 molecular traits of a cosmopolitan pathogen. Mol Plant Pathol 7:1-16

24 Burns IG (1994) Studies of the relationship between the growth rate of young plants and their total-N  
25 concentration using nutrient interruption techniques: theory and experiments. Ann Bot -  
26 London 74:143-157

27 Cardenas-Navarro R, Adamowicz S, Robin P (1999) Nitrate accumulation in plants: a role for water. J  
28 Exp Bot 50:613-624

1 Choquer M, Fournier E, Kunz C, Levis C, Pradier JM, Simon A, Viaud M (2007) *Botrytis cinerea*  
2 virulence factors: new insights into a necrotrophic and polyphageous pathogen. FEMS  
3 Microbiol Lett 277:1-10

4 Dietrich R, Ploss K, Heil M (2004) Constitutive and induced resistance to pathogens in *Arabidopsis*  
5 *thaliana* depends on nitrogen supply. Plant Cell Environ 27:896-906

6 Dulermo T, Rasclé C, Chinnici G, Gout E, Bligny R, Cotton P (2009) Dynamic carbon transfer during  
7 pathogenesis of sunflower by the necrotrophic fungus *Botrytis cinerea*: from plant hexoses to  
8 mannitol. New Phytol 183:1149-1162

9 Ehness R, Ecker M, Godt DE, Roitsch T (1997) Glucose and stress independently regulate source and  
10 sink metabolism and defense mechanisms via signal transduction pathways involving protein  
11 phosphorylation. Plant Cell 9:1825-1841

12 El Oirdi M, Abd El Rahman T, Rigano L, El Hadrami A, Cecilia Rodriguez M, Daayf F, Vojnov A,  
13 Bouarab K (2011) *Botrytis cinerea* manipulates the antagonistic effects between immune  
14 pathways to promote disease development in Tomato. Plant Cell 23:2405-2421

15 Essmann J, Schmitz-Thom I, Schoen H, Sonnewald S, Weis E, Scharte J (2008) RNA interference-  
16 mediated repression of cell wall invertase impairs defense in source leaves of tobacco. Plant  
17 Physiol 147:1288-1299

18 Fotopoulos V, Gilbert MJ, Pittman JK, Marvier AC, Buchanan AJ, Sauer N, Hall JL, Williams LE  
19 (2003) The monosaccharide transporter gene, AtSTP4, and the cell-wall invertase, At beta  
20 fruct1, are induced in Arabidopsis during infection with the fungal biotroph *Erysiphe*  
21 *cichoracearum*. Plant Physiol 132:821-829

22 Gerlagh M, Whipps JM, Budge SP, GoossenvandeGeijn HM (1996) Efficiency of isolates of  
23 *Coniothyrium minitans* as mycoparasites of *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*  
24 and *Botrytis cinerea* on tomato stem pieces. Eur J Plant Pathol 102:787-793

25 Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic  
26 pathogens. Annu Rev Phytopathol 43:205-227

1 Godoy G, Steadman JR, Dickman MB, Dam R (1990) Use of mutants to demonstrate the role of oxalic  
2 acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. *Physiol Mol Plant*  
3 *Pathol* 37:179-191

4 Gomez L, Bancel D, Rubio E, Vercambre G (2007) The microplate reader: an efficient tool for the  
5 separate enzymatic analysis of sugars in plant tissues - validation of a micro-method. *J Sci*  
6 *Food Agric* 87:1893-1905

7 Hoffland E, Jeger MJ, van Beusichem ML (2000) Effect of nitrogen supply rate on disease resistance  
8 in tomato depends on the pathogen. *PlantSoil* 218:239-247

9 Hoffland E, van Beusichem ML, Jeger MJ (1999) Nitrogen availability and susceptibility of tomato  
10 leaves to *Botrytis cinerea*. *PlantSoil* 210:263-272

11 Horsfall G, Dimond AE (1957) Interactions of tissue sugar, growth substances and disease  
12 susceptibility. *Z Pflanzenk Pflanzen* 64:415-421

13 Huber DM, Thompson IA (2007) Nitrogen and plant disease. In: Datnoff LE, Elmer WH, Huber DM  
14 (eds) *Mineral nutrition and plant disease*. APS Press, Saint Paul, MN, USA, pp 31-44

15 Jobic C, Boisson A-M, Gout E, Rasclé C, Fevre M, Cotton P, Bligny R (2007) Metabolic processes  
16 and carbon nutrient exchanges between host and pathogen sustain the disease development  
17 during sunflower infection by *Sclerotinia sclerotiorum*. *Planta* 226:251-265

18 Johnson R, Ryan CA (1990) Wound inducible potato inhibitor II genes enhancement of expression by  
19 sucrose. *Plant Molec Biol* 14:527-536 Juge N (2006) Plant protein inhibitors of cell wall  
20 degrading enzymes. *Trends Plant Sci* 11:359-367

21 Katagiri F (2004) A global view of defense gene expression regulation - a highly interconnected  
22 signaling network. *CurrOp Plant Biol* 7:506-511

23 Kliebenstein DJ (2004) Secondary metabolites and plant/environment interactions: a view through  
24 *Arabidopsis thaliana* tinged glasses. *Plant Cell Environ* 27:675-684

25 Kocal N, Sonnewald U, Sonnewald S (2008) Cell wall-bound invertase limits sucrose export and is  
26 involved in symptom development and inhibition of photosynthesis during compatible  
27 interaction between tomato and *Xanthomonas campestris* pv. *vesicatoria*. *Plant Physiol*  
28 148:1523-1536

1 Lawlor DW, Boyle FA, Young AT, Kendall AC, Keys AJ (1987) Nitrate nutrition and temperature  
2 effects on wheat - soluble components of leaves and carbon fluxes to amino-acids and sucrose.  
3 J Exp Bot 38:1091-1103

4 Le Bot J, Benard C, Robin C, Bourgaud F, Adamowicz S (2009) The 'trade-off' between synthesis of  
5 primary and secondary compounds in young tomato leaves is altered by nitrate nutrition:  
6 experimental evidence and model consistency. J Exp Bot 60:4301-4314

7 Lecompte F, Abro MA, Nicot PC (2010) Contrasted responses of *Botrytis cinerea* strains developing  
8 on tomato plants grown under different nitrogen nutrition regimes. Plant Pathol 59:891-899

9 Levy Y, Cohen Y (1984) A negative association between leaf sugar content and the development of  
10 northern leaf-blight lesions in sweet corn. Physiol Plant Pathol 24:247-252

11 Long DH, Lee FN, TeBeest DO (2000) Effect of nitrogen fertilization on disease progress of rice blast  
12 on susceptible and resistant cultivars. Plant Dis 84:403-409

13 Lou YG, Baldwin IT (2004) Nitrogen supply influences herbivore-induced direct and indirect defenses  
14 and transcriptional responses to *Nicotiana attenuata*. Plant Physiol 135:496-506

15 Maynard DN, Barker AV, Minotti PL, Peck NH (1976) Nitrate accumulation in vegetables. Adv  
16 Agron 28:71-118

17 Mittelstrass K, Treutter D, Plessl M, Heller W, Elstner EF, Heiser I (2006) Modification of primary  
18 and secondary metabolism of potato plants by nitrogen application differentially affects  
19 resistance to *Phytophthora infestans* and *Alternaria solani*. Plant Biol 8:653-661

20 Morkunas I, Narożna D, Nowak W, Samardakiewicz S, Remlein-Starosta D (2011) Cross-talk  
21 interactions of sucrose and *Fusarium oxysporum* in the phenylpropanoid pathway and the  
22 accumulation and localization of flavonoids in embryo axes of yellow lupine. J Plant Physiol  
23 168 (5):424-433

24 Newton AC, Guy DC (1998) Exploration and exploitation strategies of powdery mildew on barley  
25 cultivars with different levels of nutrients. Eur J Plant Pathol 104:829-833

26 Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation  
27 of plant carbon nitrogen interactions. Mol Plant 3 (6):973-996

1 Oliver RP, Solomon PS (2010) New developments in pathogenicity and virulence of necrotrophs. *Curr*  
2 *Op Plant Biol* 13:415-419

3 Perchepped L, Balague C, Riou C, Claudel-Renard C, Riviere N, Grezes-Besset B, Roby D (2010)  
4 Nitric oxide participates in the complex interplay of defense-related signaling pathways  
5 controlling disease resistance to *Sclerotinia sclerotiorum* in *Arabidopsis thaliana*. *Mol Plant-*  
6 *Microbe Interact* 23:846-860

7 Riou C, Freyssinet G, Fevre M (1991) Production of cell wall-degrading enzymes by the  
8 phytopathogenic fungus *Sclerotinia sclerotiorum*. *Appl Environ Microb* 57 (5):1478-1484

9 Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone crosstalk in plant disease and defense:  
10 more than just JASMONATE-SALICYLATE antagonism. *Annu Rev Phytopathol* 49:317-343

11 Scharte J, Schon H, Weis E (2005) Photosynthesis and carbohydrate metabolism in tobacco leaves  
12 during an incompatible interaction with *Phytophthora nicotianae*. *Plant Cell Environ* 28:1421-  
13 1435

14 Seginer I (2003) A dynamic model for nitrogen-stressed lettuce. *Ann Bot* 91:623-635

15 Siewers V, Viaud M, Jimenez-Teja D, Collado IG, Gronover CS, Pradier JM, Tudzynski B, Tudzynski  
16 P (2005) Functional analysis of the cytochrome P450 monooxygenase gene *bcbot1* of *Botrytis*  
17 *cinerea* indicates that botrydial is a strain-specific virulence factor. *Mol Plant-Microbe*  
18 *Interact* 18:602-612

19 Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P (2006) Sucrose-specific induction of the anthocyanin  
20 biosynthetic pathway in *Arabidopsis*. *Plant Physiol* 140:637-646

21 Solomon PS, Tan KC, Oliver RP (2003) The nutrient supply of pathogenic fungi; a fertile field for  
22 study. *Mol Plant Pathol* 4:203-210

23 Stitt M, Muller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A (2002) Steps  
24 towards an integrated view of nitrogen metabolism. *J Exp Bot* 53:959-970

25 Stotz HU, Sawada Y, Shimada Y, Hirai MY, Sasaki E, Krischke M, Brown PD, Saito K, Kamiya Y  
26 (2011) Role of camalexin, indole glucosinolates, and side chain modification of glucosinolate-  
27 derived isothiocyanates in defense of *Arabidopsis* against *Sclerotinia sclerotiorum*. *Plant J*  
28 67:81-93



- 1 Stout MJ, Brovont RA, Duffey SS (1998) Effect of nitrogen availability on expression of constitutive  
2 and inducible chemical defenses in tomato, *Lycopersicon esculentum*. J Chem Ecol 24:945-  
3 963
- 4 Strengbom J, Reich PB (2006) Elevated CO<sub>2</sub> and increased N supply reduce leaf disease and related  
5 photosynthetic impacts on *Solidago rigida*. Oecologia 149 (3):519-525
- 6 van Baarlen P, Legendre L, van Kan JAL (2004) Plant defence compounds against Botrytis infection.  
7 In Elad Y, Williamson B, Tudzynski P, Delen N (eds) Botrytis: Biology, Pathology and  
8 Control. Kluwer Academic Publishers, Dordrecht, The netherlands, pp143-161
- 9 Walters DR, Bingham IJ (2007) Influence of nutrition on disease development caused by fungal  
10 pathogens: implications for plant disease control. Ann Appl Biol 151:307-324
- 11 Williamson B (2007) *Botrytis cinerea*: the cause of grey mould disease. Mol Plant Pathol 8:561-580  
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1 **Table 1** Ionic concentrations (mmol L<sup>-1</sup>) of main nutrients in the fertilization solutions (Solutions S1  
2 to S5, with increasing NO<sub>3</sub><sup>-</sup> concentration from S1 to S5) used for lettuce production.

mmol.L <sup>-1</sup>	[NO3-]	[K+]	[Ca2+]	[Mg2+]	[SO42-]	[H2PO4-]
<b>S1</b>	0.5	11	3.25	3.5	7.25	1
<b>S2</b>	2	11	3.25	3.5	6.5	1
<b>S3</b>	5	11	3.25	3.5	5	1
<b>S4</b>	10	11	3.25	3.5	2.5	1
<b>S5</b>	20	21	3.25	3.5	2.5	1

3

1 **Table 2** Leaf content (mg g<sup>-1</sup> fw) of lettuce plants grown at five different NO<sub>3</sub><sup>-</sup> fertilization regimes, in  
 2 three independent experiments. Values are the mean of five observations. Symbols in the bottom lines  
 3 indicate significant differences between NO<sub>3</sub><sup>-</sup> treatments, experiments, and their interaction, in a  
 4 Student Newman-Keuls test: \*\*\* p<0.0001; n.s.: not significant

NO <sub>3</sub> <sup>-</sup> concentration in solution (mM)		N	NO <sub>3</sub>	C	SUC	GLU	FRU	P	K	Ca	Mg
		(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)
<b>0.5</b>	<i>E1</i>	1.13	0.02	38.4	4.99	1.67	1.24	0.43	4.51	0.51	0.31
	<i>E2</i>	1.94	0.04	45.6	4.43	2.12	2.25	0.69	3.50	0.92	0.22
	<i>E3</i>	1.37	0.03	45.4	5.71	0.53	0.46	0.71	3.55	1.04	0.37
<b>2</b>	<i>E1</i>	1.48	0.03	33.8	4.73	4.25	4.1	0.46	4.12	0.47	0.34
	<i>E2</i>	2.2	0.09	29.9	4.71	4.17	4.88	0.57	2.95	0.55	0.13
	<i>E3</i>	1.98	0.03	42.1	6.47	1.71	1.51	0.73	3.64	0.54	0.3
<b>5</b>	<i>E1</i>	1.82	0.34	25.8	1.46	5.62	6.71	0.45	3.57	0.38	0.23
	<i>E2</i>	2.29	0.41	24.2	3.09	4.12	5.94	0.51	2.23	0.27	0.11
	<i>E3</i>	2.45	0.15	30.9	5.43	3.25	3.71	0.62	2.89	0.59	0.17
<b>10</b>	<i>E1</i>	2.14	1.51	20.8	0.68	4.07	5.07	0.43	3.43	0.28	0.28
	<i>E2</i>	2.46	0.71	19.6	1.34	1.95	4.31	0.52	2.08	0.22	0.13
	<i>E3</i>	2.68	0.61	25.9	4.45	2.31	4.28	0.53	2.49	0.21	0.08
<b>20</b>	<i>E1</i>	2.62	3.37	20.4	0.61	3.66	4.64	0.45	4.28	0.26	0.18
	<i>E2</i>	2.76	1.07	19.7	1.32	1.34	3.58	0.40	1.76	0.17	0.12
	<i>E3</i>	2.89	0.95	25.1	3.65	1.71	3.67	0.53	2.56	0.33	0.11
<i>p for effects</i>											
NO <sub>3</sub> -		***	***	***	***	***	***	***	***	***	***
experiment		***	***	***	***	***	***	***	***	ns	***
NO <sub>3</sub> - x experiment		***	***	***	***	***	***	***	***	***	***

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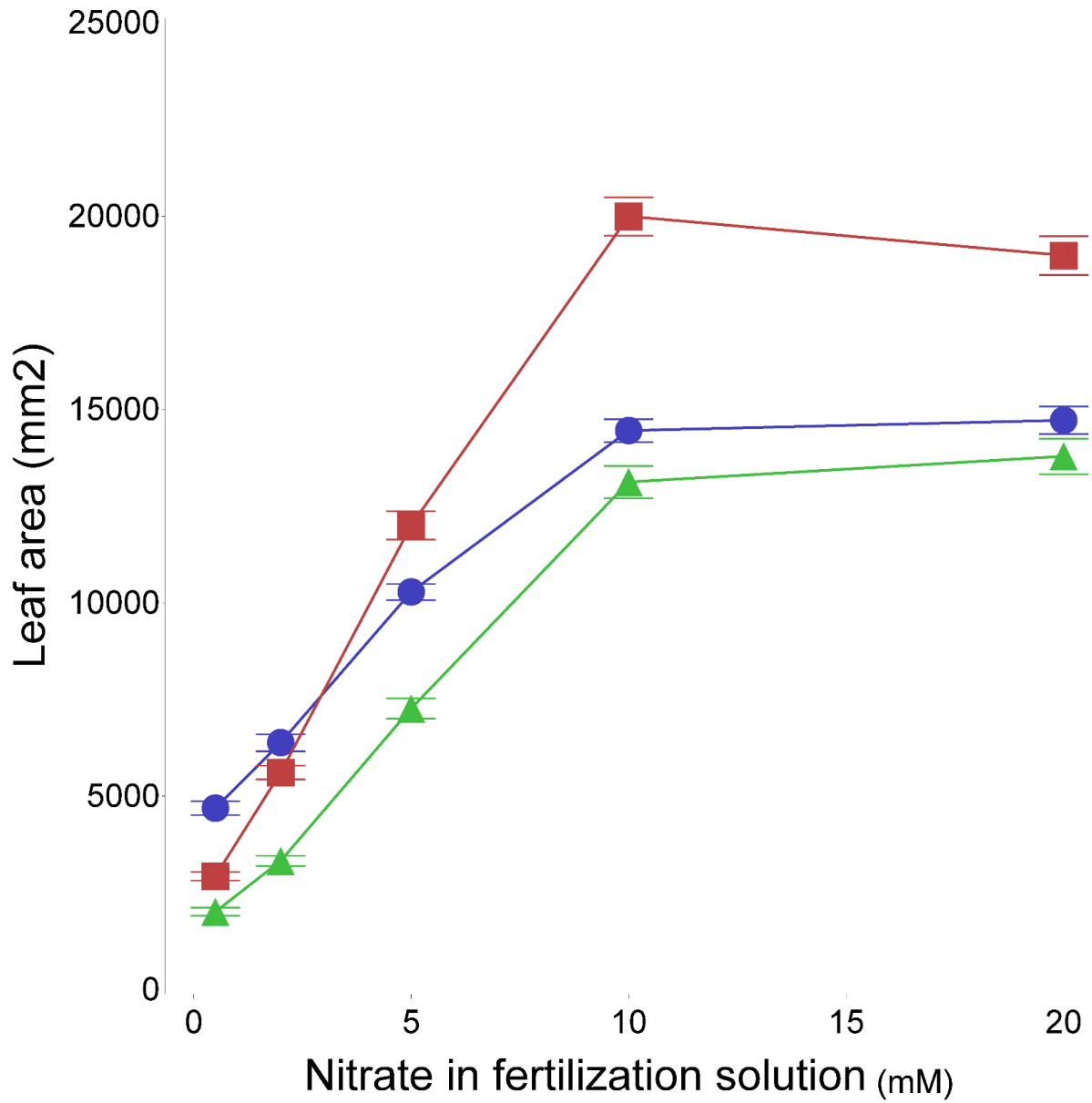
1 **Table 3** Coefficients of determination  $r^2$  and probabilities of significance of linear models relating lesion size to concentrations of elements in plants ( $\text{mg g}^{-1}$   
 2 fw). Bold  $r^2$  values indicate  $r^2$  beyond 0.7.

3

	N		NO3		C		SUC		GLU		FRU		P		K		Mg		Ca		Hexoses		Tot. Sugars		
	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$	
<i>B. cinerea</i>	E1	<b>0.96</b>	0.004	<b>0.90</b>	0.014	<b>0.85</b>	0.026	<b>0.74</b>	0.063	0.03	0.75	0.17	0.49	0.01	0.88	0.03	0.76	<b>0.78</b>	0.048	<b>0.91</b>	0.011	0.11	0.59	0.09	0.63
	E2	<b>0.95</b>	0.005	<b>0.96</b>	0.004	<b>0.86</b>	0.024	<b>0.83</b>	0.031	0.16	0.49	0.07	0.65	<b>0.93</b>	0.008	<b>0.96</b>	0.003	0.55	0.15	<b>0.86</b>	0.022	0.01	0.92	0.23	0.41
	E3	<b>0.76</b>	0.051	<b>0.83</b>	0.032	<b>0.77</b>	0.051	<b>0.83</b>	0.033	0.08	0.62	0.52	0.16	<b>0.72</b>	0.067	0.67	0.09	0.66	0.096	0.41	0.24	0.35	0.29	0.05	0.71
<i>S. sclerotiorum</i>	E1	<b>0.84</b>	0.028	<b>0.97</b>	0.002	<b>0.96</b>	0.004	<b>0.96</b>	0.004	0.12	0.57	0.33	0.31	0.01	0.88	0.25	0.38	<b>0.99</b>	3E-04	<b>0.96</b>	0.004	0.23	0.41	0.04	0.75
	E2	<b>0.73</b>	0.064	<b>0.80</b>	0.041	<b>0.78</b>	0.047	<b>0.92</b>	0.009	0.34	0.3	0.01	0.89	0.55	0.15	<b>0.73</b>	0.064	0.28	0.36	0.68	0.089	0.06	0.67	0.44	0.22
	E3	<b>0.83</b>	0.031	<b>0.97</b>	0.002	<b>0.93</b>	0.009	<b>0.92</b>	0.009	0.13	0.55	0.69	0.08	<b>0.92</b>	0.01	<b>0.84</b>	0.019	<b>0.82</b>	0.032	0.51	0.17	0.47	0.19	0.1	0.61

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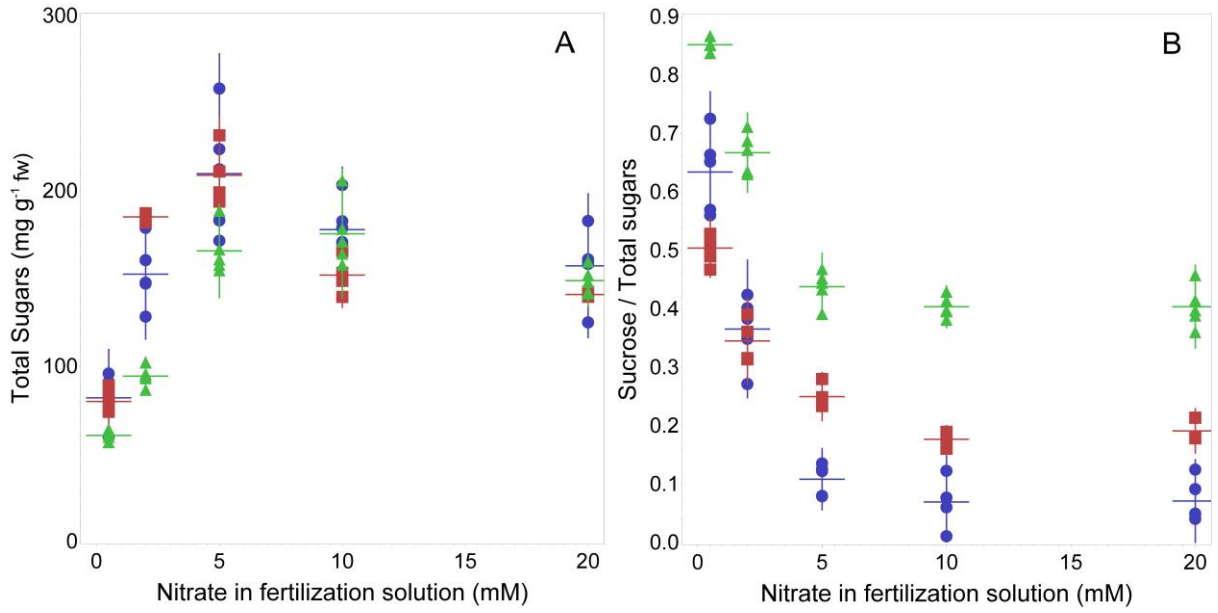
1 **Fig. 1** Leaf area of lettuce plants grown under different  $\text{NO}_3^-$  fertilization regimes. Each curve represents  
2 an independent experiment (blue dots: E1; red squares: E2; green triangles: E3). Vertical bars represent  
3 the standard error of the mean.



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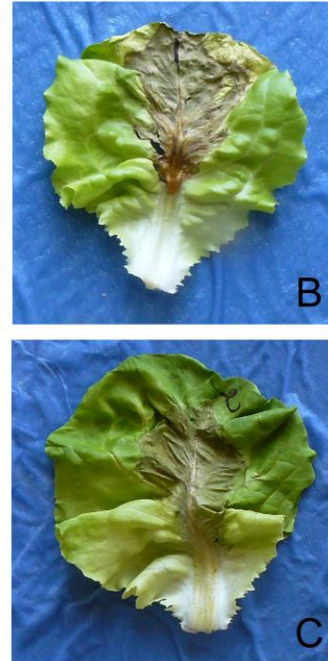
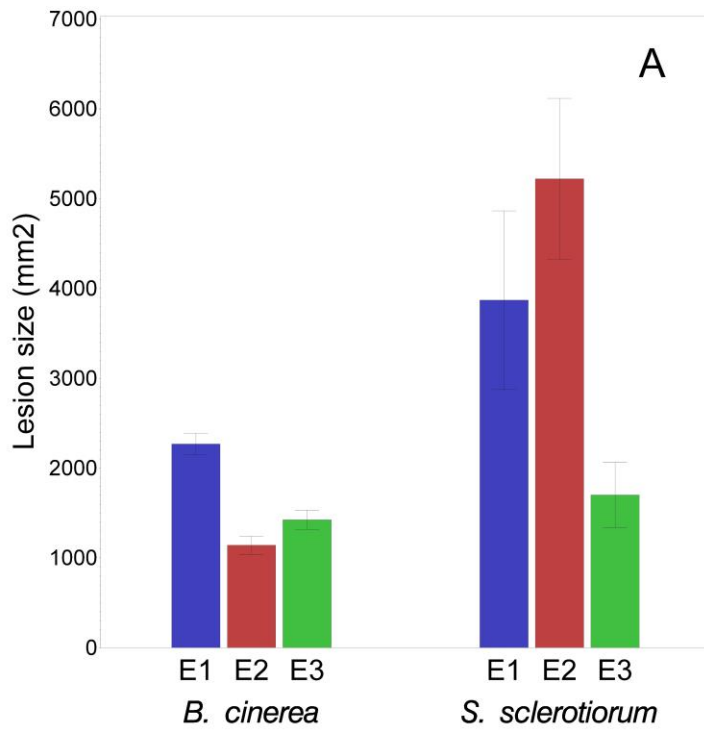
- 1 **Fig. 2** Total sugars (SUC+GLU+FRU, a) and ratio of SUC/total sugars (b) in leaves of lettuce plants
- 2 grown under different N regimes in three independent experiments (dots: E1; squares: E2; triangles:E3).
- 3 Horizontal bars represent the mean and vertical bars the standard error of the mean.



4

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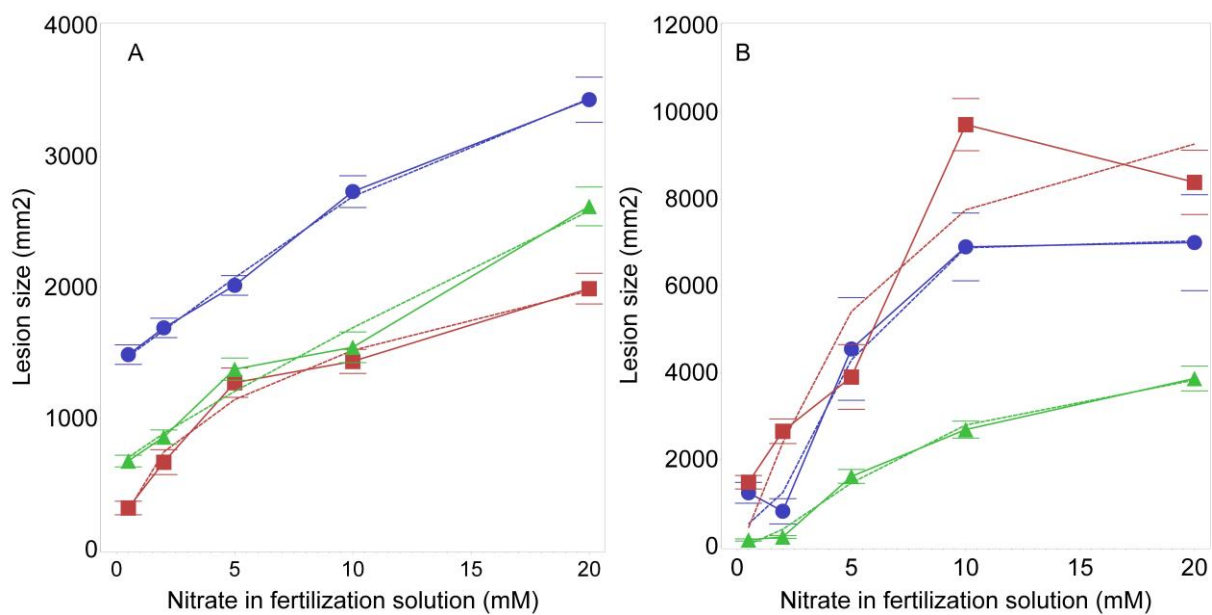
1 **Fig. 3** Overall disease assessment with pooled data, independently of  $\text{NO}_3^-$  regimes. a: Lesion size at 6  
2 days (*Botrytis cinerea*) or 5 days (*Sclerotinia sclerotiorum*) after infection by mycelia disks, in three  
3 independent experiments. Bars represent the mean  $\pm$  standard deviation. b: Lesions caused by *Botrytis*  
4 *cinerea* on lettuce leaves. C: Lesion caused by *Sclerotinia sclerotiorum* on lettuce leaves.



5

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1 **Fig. 4** Size of leaf lesions caused by *Botrytis cinerea* (a; 6 days after inoculation) and *Sclerotinia*  
 2 *sclerotiorum* (b; 5 days after inoculation) under different NO<sub>3</sub><sup>-</sup> fertilization regimes, in three independent  
 3 experiments (dots: E1; squares: E2; triangles:E3). Each symbol is the mean of 90 lesion measurements  
 4 for *B. cinerea* (pooled data for 6 strains) or 15 lesion measurements for *S. Sclerotiorum* (1 strain).  
 5 Horizontal bars represent the standard error of the mean. Continuous lines join the symbols, dotted lines  
 6 are statistical fits by non-linear functions. *Botrytis cinerea* response curves were fitted by a logistic  
 7 function (E1) and a power function (E2 and E3). *Sclerotinia sclerotiorum* response curves were fitted  
 8 by a logistic (E1) and a Hill function (E2 and E3).

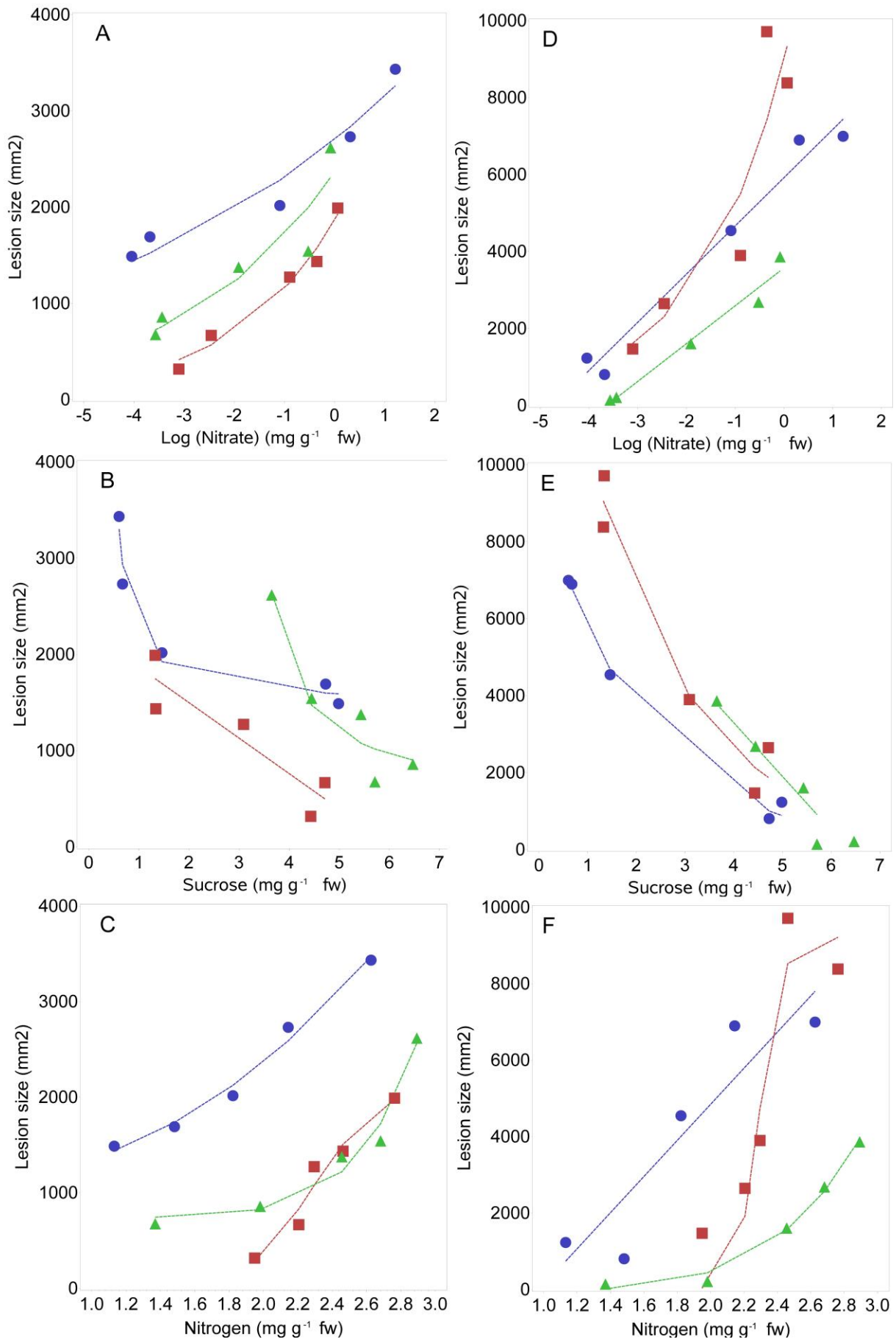


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1 **Fig. 5** Size of leaf lesions caused by *Botrytis cinerea* (a, b and c; 6 days after inoculation) and *Sclerotinia*  
2 *sclerotiorum* (d, e and f; 5 days after inoculation) on lettuce plants as a function of leaf contents (in  
3 mg.g<sup>-1</sup> fresh weight). a, d: log (NO<sub>3</sub><sup>-</sup>); b, e: SUC; c, f: N. Each symbol relates the mean of 5 plant  
4 analyses to the mean of 90 lesion measurements for *B. cinerea* (pooled data for 6 strains) or 15 lesion  
5 measurements for *S. Sclerotiorum* (1 strain). Different symbols and colours represent independent  
6 experiments (dots: E1; squares: E2; triangles:E3). Continuous lines join the symbols, dotted lines are  
7 statistical adjustments by non-linear functions. Details of fitted functions are given in the text.



1 **Fig. 6** Fungal lesions caused by *Botrytis cinerea* (a, b) *Sclerotinia sclerotiorum* (c,d) related to the ratio  
2 of SUC ( a, c) and FRU (b, d) on Total Sugars (SUC+FRU+GLU). Each symbol relates the mean of 5  
3 plant analyses to the mean of 90 lesion measurements for *B. cinerea* (pooled data for 6 strains) or 15  
4 lesion measurements for *S. Sclerotiorum* (1 strain). Different symbols and colours represent independent  
5 experiments (dots: E1; squares: E2; triangles:E3). Dotted black lines are fitted exponential functions on  
6 the pooled data for the three experiments ( $y=k_1*\exp(k_2*X)$ , with  $X=SUC/total\ sugars$  in a and c,  
7  $X=FRU/total\ sugars$  in b and d; a:  $k_1=2926, k_2=-1.88; F=55.8, p<0.0001, 15\ DF$ ; b:  $k_1=676, k_2=2.2,$   
8  $F=45.1, p<0.0001, 15\ DF$ ; c:  $k_1=9659, k_2=-3.2, F=44.1, p<0.0001, 15\ DF$ ; d:  $k_1=214, k_2=6.6; F=527,$   
9  $p<0.0001, 15\ DF$ ).

