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Research Article

# Nitrogen supply effect on lettuce response to *Botrytis cinerea* and *Sclerotinia minor*

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## Abstract

**Background information:** Cultural practices play an important role on the development of pathogens (Datnoff, et al. 2007). In this context, several authors have been interested in studying the effects of mineral nutrition on the resistance of vegetables and fruits to fungi during storage, especially nitrogen.

**The purpose of the study:** In this work we tested the effects of three contrasted regimes of nitrogen supply, with nitrate concentrations in the nutrient solutions of 2 mM (low), 10 mM (normal) and 20 mM (high) on the susceptibility of *Lactuca sativa* L towards *Botrytis cinerea* (*BC87*) and *Sclerotinia minor* (*SM)* during storage.

Once harvested, the outerleaves of the plants derived from the three nitrogen regimes were inoculated with either *Botrytis cinerea* (*BC87*) or *Sclerotinia minor* (*SM*). Data showed that the resistance to this two pathogens increase when plants were developed under low nitrogen concentration. This resistance observed is correlated with low values in oxidative stress indicators (MDA and $H_2O_2$) and high values in total phenols.

**More Information**

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**Keywords**: *Botrytis cinerea; Sclerotinia minor; Lactuca sativa* L; Nitrogen



## Introduction

In addition to oxygen, carbon dioxide and water, plants require at least 14 mineral elements for adequate nutrition [1]. A deficiency in any of these minerals reduces plant growth and crop yield. Plants usually acquire their minerals from the soil. Six mineral elements, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfide(S) are required in large quantities, while the chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo) are needed in small quantities.

Leafy vegetables occupy a very important place in human nutrition, however unfortunately they are a food group that contributes to a high consumption of nitrate by living beings. In the case of fertilizers excessive use, plants accumulate very large quantities of nitrate. Once humans consume these plants, this causes health problems. Therefore, efforts are guaranteed to minimize the accumulation of nitrates in leafy vegetables.

In general, nitrate accumulators vegetables belong to the families *Brassicaceae* (arugula, radishes and mustard), *Chenopodiaceae* (beets, chard and spinach), *Amaranthaceae* (Amaranthus), *Asteraceae* (lettuce) and *Apiaceae* (celery and parsley) [2].

The lettuce *lactuca sativa* L. is well known for its great ability to accumulate nitrate in the leaves, which can affect human health. Nitrate is considered as a possible precursor of carcinogenic nitrosamines [3,4]. However, it is still not consensus, it is not known precisely, if the intake of nitrate is beneficial or harmful to human health [5]. Plants grown in hydroponic systems showed higher levels compared to those grown in conventional systems [6].

In soil, nitrogen is found in organic form (humus) or inorganic ($NH_4^+$ ammonium, nitrate $NO_3^-$). Nitrogen in the form of nitrate ions is a soluble element, retained by the soil. Brought in large quantities, the excess is leached (dissolved and washed away by water flowing in the ground) and therefore lost for the plant. Nitrogen should be brought as much as possible, just before its absorption by the plant, in order to prevent leaching to groundwater.

Low needs of romaine lettuce on nitrogen are satisfied in summer and autumn by the mineralization of organic matter. Excessive nitrogen fertilization enhance *Botrytis*, delay pomaison and can cause nitrate accumulation in the leaves.

Indeed, cultural practices also influence the development of pathogens [7]. In this context, several authors have been interested in studying the effects of mineral nutrition on the resistance of vegetables and fruits to fungi.

Thus, an increase in contributions may according to the mineral element, to the plant and to the disease in question increase or decrease the incidence and severity of symptoms [8]. This diversity of effects of mineral fertilizers, especially nitrogen, is related to the fact that plant nutrition can influence the different levels of host-pathogen interaction. The nutrients supplied to the plant have an effect on the growth and development of crops, thus the architecture of plants. Excessive nitrogen fertilization may encourage a dense canopy and poorly ventilated, favors the development of pathogenic fungi. Similarly, nutrition has an effect on the metabolism of plants, including secondary metabolism, which includes the synthesis of molecules involved in defense against fungal diseases (such as total polyphenols).

Indeed, Navarette, et al. [9] have shown that excess nitrogen induces a sensitivity of lettuce *Botrytis cinerea* and *Sclerotinia sclerotirium*. On the other hand, Lecompte, et al. [10] observed an opposite effect in tomatoes. Indeed, the plants were more resistant to *Botrytis* under high nitrogen level.

Low nitrogen nutrition levels are therefore likely to reduce the severity of the damage caused by two fungal pathogens causing significant damage to lettuce.

The effect of the nitrogen source depends on the plant species and the conditions under which it is grown. For example, Verhoeff [11] found a decrease in the sensitivity of tomato to gray mold when grown in soil rich in nitrogen. Hoffland, et al. [12] found a positive correlation between the C/N ratio and the sensitivity of the leaves of tomato plants to *Botrytis cinerea* and attributed to varying levels of soluble sugars available in the plant. Hobbs and Waters [13], reported the opposite results with chrysanthemum flowers in these conditions. Sol [14] found that plants of *vicia faba* L. growing on medium containing ammonium as a nitrogen source are more susceptible to *Botrytis* than pushing nitric medium. In experiments studying the effect of nitrogen source on gray mold, it was found that the nitrogen source had no effect on the sensitivity of eggplant or pepper to gray mold. However, a greater proportion of nitrate in fertilizers is associated with a reduced incidence of the disease on cucumber plants [15].

So come the aim of this present work which is to find the appropriate dose of nitrogen, which guarantees romaine lettuce var *claudius* better resistance to the 2 fungi already used namely *Botrytis cinerea* and *Sclerotinia minor* during storage.

## Materials and methods

### Plant material and growing conditions

Lettuce plants of cultivar *Claudius* were grown from April to June 2012. Seeds were sown in 1 $cm^3$ rockwool cubes in greenhouse. Four weeks after sowing, the cubes, each containing one seedling, were transferred to the top of 2 L pots filled with a mixture (1:1 V/V) of vermiculite and pozzalana (inert crushed volcanic rock) to start the nutrition treatments [16].

The lettuce plants were grown at three N concentrations: 2 (low), 10 (normal) and 20 mM (high). The composition of the nutrient solution is as follows: 3.5 mM $MgSO_4$, 1 mM $KH_2PO_4$, 3.25 mM $CaCl_2$, 20.6 µM $H_3BO_3$, 0.5 µM $CuSO_4$, 11.6 µM $MnSO_4$, 0.28 µM $((NH_4)Mo_7O_{24})$, 3.2 µM ZnSO4 and 10.7 µM FeEDTA. At NO3 concentrations 2 and 10mM, the solution was added with 4mM $K_2SO_4$ and 2 mM $KNO_3$ for the first solution and with 10 mM $KNO_3$ for the second solution. At the highest nitrate level (20 mM), the concentration of potassium nitrate was doubled [16]. We considered that potassium nitrate was better than other $NO_3$ salts to achieve the doubling of $NO_3$ concentration in solution. Nutrient solutions were supplied via a fertigation network with an individual dripper into each pot. Plants were grown for four additional weeks under these different N regimes at a temperature of 22 °C/16 °C (day/night).

### Pathogen culture and inoculation tests on leaves

To assess the nitrogen supply effect on plant susceptibility to pathogens, we inoculated lettuce leaves with strain of *Botrytis cinerea* and strain of *Sclerotinia minor*. At harvest (52 days old plants), three leaves per plant from the three batches were inoculated. The strain was previously isolated from diseased lettuce plants in southern France and maintained in the Plant Pathology research unit of INRA Avignon. The inoculum was produced in three days on potato dextrose agar (39 g $L^{-1}$ Difco, Detroit, USA) in a growth chamber at 21 °C with 14 hours day/10 hours night photoperiod.

Once harvested, detached leaves, were placed in plastic Petri dishes, and inoculated in their centre with a mycelium plug, 5 mm in diameter. The Petri dishes were then placed in a growth chamber at 21 °C /16 °C and a photoperiod of 14h. The leaves were photographed every 24 hours up to 4 days after inoculation and lesion areas were assessed with "Image J" image analysis software (US National Institutes of Health, Bethesda, MD, USA).

### Oxidant status

The oxidant status of the plants growing under the three concentrations was studied using 2 indicators of oxidative stress: The malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$).

### Lipid peroxidation

Aliquots of 200 mg of frozen powdered leaves were

homogenized in 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 g for 15 min. 1 ml of supernatant was then vortexed with 1 ml of 20% (w/v) TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA), and the solution was heated for 30 min at 95 ºC. The samples were cooled on ice for 5 min and centrifuged again for 15 min at 10,000 g. Absorbance of the supernatant at 532 nm was corrected for non-specific absorbance by subtracting absorbance at 600 nm. Malondialdehyde (MDA) concentration was estimated using 155 $mM^{-1}$ $cm^{-1}$ as the extinction coefficient [17]. Assay was performed on 5 plants per treatment ($n$ = 5).

### Hydrogen peroxide ($H_2O_2$)

Aliquots of 200 mg of frozen powdered leaves were homogenized in 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 g for 15 min at 4 °C. Three aliquots of 50 µl of each tube was placed in a microplate ELISA (96 wells) with 50 µl of phosphate buffer (pH = 7) and 100 µl of KI. Each plate also contained increasing amounts of $H_2O_2$ to generate a calibration curve. The plate was shaken briefly, incubated at room temperature for 30 min and then the absorbance of each well was determined at 390 nm using a microplate reader (Power Wave HT from Bio Tek Microplate Spectrophotometer). The $H_2O_2$ content of the various samples were determined using the calibration curve [18]. Assay was performed on 5 plants per treatment ($n$ = 5).

### Determination of total polyphenols

The determination of the phenolic compounds in this study was done using the Folin-Ciocalteu method [19]. It implies that the oxidation of reactive tungsten oxide and molybdenum blue, the intensity of the blue color concentration polyphenol extracts. After extraction was charged, 125 µl extract mixed with 500 µl of distilled water and 125 µl of Folin-Ciocalteu reagent. After shaking, the samples were incubated for the first time for three minutes. Thereafter was added a solution of 1250 µl of $(Na)_2CO_3$ at 7%. The resulting mixture was adjusted to 3 ml with distilled water. A second incubation was performed for 90 min away from the lights. The absorbance reading at 760 nm was measured. Quantification of total phenols was made using a linear calibration curve obtained by a "Gallic acid" of extracted standard (AG) at increasing concentrations (from 25 $mg.l^{-1}$ to 500 $mg.l^{-1}$) [19]. The polyphenol content was expressed as mg of gallic acid per g of dry matter (mg AG $.g^{-1}$DM). Assay was performed on 5 plants per treatment ($n$ = 5). This assay was done on harvest day before inoculation and done after inoculation on day1, day 2 and day 4.

### Statistical analysis

Data were analyzed by analysis of variance followed by multiple means comparison which was performed using the Duncan test [20].

## Results

### Effect of nitrogen fertilization on leaf concentrations in total phenols before inoculation

Leaf total phenolics were negatively correlated with the concentration in N in the nutrient solution, being 89% higher in the 2 mM N treatment and 31 % lower in the 20 mM N treatment when compared to the 10 mM N treatment. (Table 1).

### Effect of nitrogen fertilization on susceptibility towards *Botrytis cinerea* and *Sclerotinia minor*

There was a strong increase in lesion surfaces from day 1 to day 4 in all treatments as the consequence of leaf inoculation by either *Botrytis cinerea* or *Sclerotinia minor* (Table 2). At the end of the trial (day 4), lesion surfaces appeared positively correlated to the concentration in N of the nutrient solution. It goes from 4.25 $cm^2$ in the 2mM N treatment to 8.79 $cm^2$ in the 20 mM N treatment for inoculation with *Botrytis cinerea* (Table 2), and from 9.04 $cm^2$ in the 2mM N treatment to 14.65 $cm^2$ in the 20 mM N treatment for *Sclerotinia minor* (Table 2).

Data shows that lettuce is more sensitive to *Sclerotinia minor.*

Table 2. Lesion surfaces on lettuce leaves caused by *Botrytis cinerea* (BC87) and *Sclerotinia minor (SM)* as a function of time and of N concentration in the nutrient solution (2, 10 and 20 mM). Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05 threshold.

**Table 1:** Concentrations in total phenolics of leaves of lettuce plants grown at three N concentrations (2, 10 and 20 mM) before inoculation by *Botrytis cinerea* and *Sclerotinia minor*.

| | Concentration in N of the fertilizing solution (mM) | | |
|---|---|---|---|
| | 2 | 10 | 20 |
| Total phenolics (mg EAG $g^{-1}$ DM) | 9.25 ± 0.15$^a$ | 5.42 ± 0.12$^b$ | 3.75 ± 0.24$^c$ |

Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05.

**Table 2:** Lesion surfaces on lettuce leaves caused by *Botrytis cinerea* (BC87) and *Sclerotinia minor (SM)* as a function of time and of N concentration in the nutrient solution (2, 10 and 20 mM).

| | Concentration in N of the fertilizing solution (mM) | | |
|---|---|---|---|
| | 2 | 10 | 20 |
| | *Botrytis cinerea(BC87)* | | |
| Lesion area, $cm^2$ | | | |
| Day 1 | 0.04 ± 0.003$^a$ | 0.03 ± 0.004$^a$ | 0.05±0.007$^b$ |
| Day 2 | 0.1 ± 0.03$^a$ | 0.11 ± 0.059$^a$ | 0.42±0.15$^b$ |
| Day 3 | 2.14 ± 0.21$^a$ | 3.42 ± 0.83$^b$ | 4.67±0.21$^c$ |
| Day 4 | 4.25 ± 0.41$^a$ | 4.9 ± 0.54$^b$ | 8.79± 0.93$^c$ |
| | *Sclerotinia minor(SM)* | | |
| Day 1 | 0.08 ± 0.009$^a$ | 0.08 ± 0.011$^a$ | 0.06±0.01$^b$ |
| Day 2 | 1.04 ± 0.15$^a$ | 1.5 ± 0.18$^b$ | 1.18±0.24$^a$ |
| Day 3 | 3.89 ± 0.37$^a$ | 4.43 ± 0.34$^b$ | 4.29±0.47$^{ab}$ |
| Day 4 | 9.04 ± 0.82$^a$ | 12.59 ± 0.98$^b$ | 14.65± 1.71$^c$ |

Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05 threshold.

### Effect of nitrogen fertilization on leaf concentrations in malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$)

At the end of trial day 4), the MDA contents increased significantly compared to day1. This increase is more accentuated following inoculation with *Botrytis cinerea*, especially in the presence of a concentration of 20mM N (8.58 µmol.g$^{-1}$ FM against 4.26 µmol.g$^{-1}$ FM in the presence of 2mM) (Table 3). This indicator correlates negatively with lesion area.

$H_2O_2$ content in leaves clearly appeared positively correlated with N concentration (Table 4).

At the end of the trial, $H_2O_2$ seems insensitive to the nitrogen treatment following inoculation with *Botrytis cinerea*. On the other hand, inoculated with *Sclerotinia minor*, the lettuce leaves seem more resistant in the presence of a low concentration of nitrogen 2mM ( 13.65 nmol.g$^{-1}$ FM against 29.59 nmol.g$^{-1}$ FM in presence of nitrogen 20 mM). This indicator correlates positively with data show by lesion area.

### Effect of nitrogen fertilization on leaf concentrations in total phenolics after inoculation by *Botrytis cinerea* and *Sclerotinia minor*

**Table 3**: Concentrations in malondialdehyde (MDA) of leaves of lettuce plants as a function of time and of N concentration in the nutrient solution (2, 10 and 20 mM) after inoculation by *Botrytis cinerea* and *Sclerotinia minor*.

| | Concentration in N of the fertilizing solution (mM) | | |
|---|---|---|---|
| | 2 | 10 | 20 |
| | *Botrytis cinerea(BC87)* | | |
| MDA, µmol.g$^{-1}$ FM | | | |
| Day 1 | 1.3 ± 0.06$^{a}$ | 4.54 ± 0.08$^{b}$ | 3.04±0.05$^{c}$ |
| Day2 | 3.07 ± 0.66$^{a}$ | 5.8 ± 0.84$^{b}$ | 8.77±1.4$^{c}$ |
| Day 4 | 4.26 ± 0.26$^{a}$ | 5.71 ± 1.49$^{a}$ | 8.58± 0.52$^{c}$ |
| | *Sclerotinia minor(SM)* | | |
| Day 1 | 1.18 ± 0.42$^{a}$ | 1.54± 0.29$^{a}$ | 0.66±0.12$^{b}$ |
| Day2 | 2.65 ± 0.56$^{a}$ | 2.91 ± 0.98$^{a}$ | 3.18±0.49$^{c}$ |
| Day 4 | 2.8 ± 0.43$^{a}$ | 7.65 ± 1.44$^{b}$ | 3.12± 0.32$^{c}$ |

Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05.

**Table 4**: Concentrations in hydrogen peroxide ($H_2O_2$) of leaves of lettuce plants as a function of time and of N concentration in the nutrient solution (2, 10 and 20 mM) after inoculation by *Botrytis cinerea* and *Sclerotinia minor*.

| | Concentration in N of the fertilizing solution (mM) | | |
|---|---|---|---|
| | 2 | 10 | 20 |
| | *Botrytis cinerea(BC87)* | | |
| $H_2O_2$, nmol.g$^{-1}$ FM | | | |
| Day 1 | 4.73 ± 1.21$^{a}$ | 5.07 ± 1.38$^{a}$ | 8.44±1.12$^{b}$ |
| Day 2 | 4.87 ± 1.43$^{a}$ | 8.3 ± 1.25$^{b}$ | 13.31±1.8$^{c}$ |
| Day 4 | 19.94 ± 4.79$^{a}$ | 20.12 ± 2.53$^{a}$ | 20.58± 4.18$^{a}$ |
| | *Sclerotinia minor(SM)* | | |
| Day 1 | 3.15 ± 0.9$^{a}$ | 3.92± 0.98$^{a}$ | 4.64±0.76$^{a}$ |
| Day 2 | 8.39 ± 2.88$^{a}$ | 9.76 ± 1.05$^{a}$ | 10.23±2.48$^{a}$ |
| Day 4 | 13.65 ± 2.45$^{a}$ | 19.67 ± 5.32$^{b}$ | 29.59± 4.04$^{c}$ |

Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05 threshold.

There was a strong increase in phenolics from day 1 to day 4 in all treatments as the consequence of leaf inoculation by either *Botrytis cinerea* or *S. minor* (Table 5). But the most remarkable increase was noted in the presence of a nitrogen deficit in the culture solution (2 Mm°. At the end of the trial (day 4), phenolics appeared negatively correlated to the concentration in N of the nutrient solution. Indeed it's ranging from 12.5 mg EAG g$^{-1}$ DM in the 2 mM N treatment to 6.3 in the 20 mM N treatment for inoculation with *Botrytis cinerea* (Table 5), and from 10.1 mg EAG g$^{-1}$ DM in the 2mM N treatment to 6.6 in the 20 mM N treatment for *Sclerotinia minor* (Table 5). This indicator correlates positively with data show by area lesion. It proves that resistance to pathogens is acquired following the enrichment with polyphenols.

This enrichment in polyphenols is a result of a low nitrogen fertilization (2mM) as already observed in leaves not inoculated with either *Botrytis cinerea* or *Sclerotinia minor.* (Table 1).

## Discussion

Lettuce is a vegetable widely consumed by human beings. However, this vegetable is sold as a 4$^{th}$ range product. During storage, lettuce leaves can be the target of several pathogens such as *Botrytis cinerea* and *Sclerotinia minor.* To combat those fungi and others, a range of strategies for prevention and control are currently employed. Cultural practices play a vital role [21]. In addition, lettuce is well known for its great ability to accumulate nitrate in the leaves, which could affect human health because this nutrient is considered a possible precursor of carcinogenic nitrosamines [4,5]. Thus, the nitrogen status of these bodies, marked by a decline in total nitrogen and nitrate levels in response to depleted nitrogen system (2 mM), justifies the choice of a low dose of nitrogen in the environment culture of lettuce. Indeed, fungal development

**Table 5**: Concentrations in total phenolics of leaves of lettuce plants grown at three N concentrations (2, 10 and 20 mM) after inoculation by *Botrytis cinerea* and *Sclerotinia minor*.

| | Concentration in N of the fertilizing solution (mM) | | |
|---|---|---|---|
| | 2 | 10 | 20 |
| | *Botrytis cinerea(BC87)* | | |
| Total phenolics (mg EAG g$^{-1}$ DM) | | | |
| Day 1 | 9.7 ± 1.2$^{a}$ | 5.7 ± 0.04$^{b}$ | 4.4±0.7$^{c}$ |
| Day 2 | 10.5 ± 0.3$^{d}$ | 5.84 ± 0.09$^{b}$ | 5.7±0.1$^{b}$ |
| Day 3 | 10.9 ± 0.21$^{e}$ | 6.8 ± 0.3$^{b}$ | 5.9±0.1$^{f}$ |
| Day 4 | 12.5 ± 0.41$^{g}$ | 6.9± 0.7$^{b}$ | 6.3± 0.3$^{b}$ |
| | *Sclerotinia minor(SM)* | | |
| Day 1 | 8.19 ± 0.1$^{a}$ | 4.4 ± 0.11$^{b}$ | 3.6±0.1$^{c}$ |
| Day 2 | 9.0 ± 0.3$^{d}$ | 5± 0.8$^{e}$ | 3.9±0.7$^{c}$ |
| Day 3 | 9.7 ± 0.7$^{f}$ | 5.3 ± 0.4$^{g}$ | 5.4±0.6$^{g}$ |
| Day 4 | 10.1 ± 0.2$^{h}$ | 6.59 ± 0.7$^{i}$ | 6.6± 0.71$^{i}$ |

Data represent means ± standard errors. $n$ = 5. Different letters indicate significant differences at $p$ = 0.05

depends on farming practices and weather conditions [21]. In this work we have proved that plants growing on environment nutrient additioned of 2mM nitrogen have a better resistance to *Botrytis cinrea* (BC87) and *Sclerotinia minor* (SM). This resistance is due to the enhancement observed in phenolics in presence of N 2mM (Table 1). In nature, polyphenols have a well-defined function: to defend the plant against attacks (UV, insects, fungi, diseases, etc. [22]). This resistance is confirmed by the MDA and $H_2O_2$ values. (Tables 3,4).

Moreover, it is well established that low nitrogen status is associated with an enrichment of secondary metabolites [16]. In agreement with this statement, our results also showed that nitrogen (2 mM) led to a leaves enrichment on total polyphenol. Indeed, the content in polyphenols, whose antioxidant properties can be searched in a process of improving the nutritional quality significantly falls as we increase nitrogen fertilization [22]. Indeed High N-fertilizer additions have been indicated to be the cause of decrease in fungal biomass [23,24].

Our results have clearly shown a decrease in the sensitivity of romaine lettuce to both fungi studied in response to the decrease in the concentration of nitrogen in the environment. These results are consistent with those of Navarette, et al. [9], which showed that the excess nitrogen causes a sensitivity to *Botrytis cinerea* and *Sclerotinia sclerotirium*. Low nitrogen nutrition levels are therefore likely to reduce the severity of damage caused by two fungal pathogens on salads.

However, other authors have well observed on tomato, opposite reactions where the agressivness of *Botrytis cinerea* strains were disadvantaged by high levels of nitrogen in the plant and little aggressive strains favored by these levels [10]. Hobbs and Waters [13], also reported the opposite results on chrysanthemum flowers under these conditions. Similarly, other researchers find a decrease in the sensitivity of tomato plants to *Botrytis* when they were grown on soil with high concentrations of nitrogen [11]. For its part, Sol (1967) found that plants growing *Vicia faba* on ammonia medium are more susceptible to *Botrytis* as fed with nitrate nitrogen. In other experiments, the nature of the nitrogen source has proved no effect on the sensitivity of eggplant or pepper to gray mold. However, a greater proportion of nitrate in fertilizers is associated with a reduced incidence of the disease in cucumber plants [14]. A recent study on tomato, has demonstrated that tomato sensitivity to *Botrytis* was greater for plants grown under low doses of nitrogen [25,26].

This work showed clearly that the dose of nitrogen 2 mM allowed leaves to gain a better resistance against both fungi, tested in this study, during storage. It showed also that the positive effects of low nitrogen are associated with low $H_2O_2$ and MDA and high total phenolics concentrations.

### Limitation of the study

During this work we were interested in studying the effect of nitrogen fertilization, applied during growth, on the response of romaine lettuce to pathogens during storage. To evaluate this effect, a few biochemical indicators were assayed. A molecular study will be essential to understand the mechanisms of the antioxidant response of romaine lettuce to fungi.

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