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Manipulating nitrogen fertilization for the management of diseases in the tomato greenhouse: what perspectives for IPM?

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Abstract: Although controlled conditions studies have shown that nitrogen nutrition can affect the susceptibility of tomato to certain pathogens, fertilization schemes for disease management at crop level remain to be designed. With this aim, a study has been conducted in an experimental greenhouse with cultural practices similar to those of commercial soilless production. The heated greenhouse was equipped with drip irrigation networks allowing the comparison of up to three different fertigation solutions (containing 4, 8 or 16mmol of NO_3^- per litre). In the first two years of the study (2010 and 2011), plants were inoculated with known spore concentrations of either *Botrytis cinerea* or *Oidium neolycopersici* and disease incidence and severity were recorded. Although variability was higher than in controlled conditions, these crop-level studies generally confirmed the influence of nitrogen fertilization on both diseases. Low nitrogen levels resulted in higher severity of *Botrytis* stem lesions while they decreased that of powdery mildew. In contrast, fruit yield from un-inoculated control plants did not differ significantly among the three nitrogen levels. More results should become available from similar studies conducted by other partners of a national collaborative project. For the design of health-enhancing fertilization schemes, further information will be needed on possible effects of nitrogen nutrition on the susceptibility of tomato to other pests and diseases and on the efficacy of various control methods used in IPM.

Key words: *Botrytis cinerea*, *Oidium neolycopersici*, *Solanum lycopersicum*, nitrate, soilless culture

Introduction

Nitrogen is routinely used as fertilizer to foster yield and crop quality. As nitrogen fertilization is also known to affect the susceptibility of host plants to pathogens (Huber & Thomson, 2007), it could possibly be modulated to reduce the impact of diseases, thus providing a tool to enhance IPM practices. However, attempts to design mineral fertilization schemes for disease management at crop level remain uncommon (Huber & Haneklaus, 2007).

Previous work in controlled conditions has shown that symptom development by two of the major pathogens of greenhouse tomato, *Botrytis cinerea* and *Oidium neolycopersici*, can be influenced by the level of nitrogen supplied to the plant (Lecompte *et al.*, 2010; Hoffland *et al.*, 2000). However, it remains to be established if similar effects can be observed at crop level in the more complex environment of a production greenhouse.

This paper presents our first results on studies evaluating the effect of nitrogen fertilization on plant health in conditions similar to those of commercial production.

Material and methods

Experimental set up and plant fertilization

Trials were conducted in 2010 and 2011 in a 300m² heated greenhouse compartment at the Experimental Domain of INRA Alénya in southern France. Tomatoes cv Climberley were grown in a soilless system in conditions similar to those of local commercial growers. The greenhouse was equipped with separate drip irrigation networks which allowed the delivery of up to three different fertigation solutions, repeated over 4 blocs in a random design.

Two levels of nitrogen fertilization were compared in 2010 (8 and 16mmol NO_3^- per litre of fertigation solution) and three levels in 2011 (4, 8 and 16mmol/l). The highest nitrogen level in the trials was similar to that used by many growers in local soilless production systems at the beginning of the growing season (before the start of fruit harvest). The concentrations of other major nutrients and oligo-elements were similar for the three fertigations solutions and their salinity was maintained by adjusting the concentration of $S0_4^{2-}$ and Cl^- ions. Fertigation was provided in excess of the plant's water needs and approximately 30% of the solution was drained-to-waste. During the experiments, samples were taken to control the mineral composition of the solution in the vicinity of the roots and that of the sap in axillary shoots.

In both years, the plants were trellised and pruned regularly according to local practice. Bumble bees were used for pollination and pest management was achieved through biological control. To avoid interference with the inoculation experiments, the use of fungicides against diseases was kept to a minimum.

Measurement of plant growth and yield

Samples of leaves were collected several times during the experiments to assess their fresh and dry weight, their surface, and for analyses of their mineral content. In addition in the 2011 trial, all above-ground parts were collected for two batches of three plants (at one month interval) in each of the fertilization treatments and similar measurements were carried out.

As tomato fruits were regularly harvested in the greenhouse according to local commercial practices, yield data were recorded for the duration of the 2011 experiment from each of 36 plants (12 per fertilization level) which were not used for inoculation experiments.

***Inoculation experiments with* Botrytis cinerea**

Two inoculation experiments were carried out each year after the beginning of fruit harvest (when the plants were 6-7 months old). For each inoculation date, batches of 28 (in 2010) and 12 (in 2011) plants per fertilization level were randomly selected and tagged. Leaves were removed from each plant and the wounds were inoculated with 10µl aliquots of a suspension containing 10^6 spores/ml. In the 2010 trial, we used two strains of *B. cinerea* based on their contrasted levels of aggressiveness to tomato in previous work in controlled conditions (Lecompte *et al.*, 2010). In 2011, only highly aggressive strain BC1 was used. The development of disease was recorded during the two weeks after inoculation.

***Experiments with* Oidium neolycopersici**

In the 2010 trial, no specific inoculation was carried out, but the incidence of naturally occurring powdery mildew was recorded on 320 plants (160 for each fertilization level). The frequency data were analyzed using a Chi² test to assess a possible effect of nitrogen fertilization on disease incidence.

In the 2011 trial, two inoculation experiments were carried out. In each test, two leaves on each of 12 plants per fertilization level were sprayed to run off with a suspension

containing 5000 spores of *O. neolycopersici* per litre. After 14 days (16 for the 2nd experiment), symptoms of powdery mildew were quantified. Each leaf was rated for global disease severity, using a 0-to-9 scale adapted from Bardin (1996). The number of mildew lesions was recorded and the surface of the leaves was measured (using image analysis software Assess 2, APS Press, St Paul, MN, USA) to express disease severity as a density of spots per cm² of leaf tissue. The diameter of 10 mildew spots was measured on each of 12 leaves (24 for Test 2) and the sporulation of the fungus was assessed by bulking 25 mildew spots into 2ml aliquots for each of 12 leaves (24 for Test 2). The spores were counted with the help of a haemocytometer and results were expressed as average numbers of spores per individual lesion. Data were analyzed with the help of the ANOVA module of Statistica software (Statsoft, Tulsa, OK, USA).

Results and discussion

Effect of nitrogen fertilization on the plant and on fruit yield

The fresh weight of both leaves and stems and the average leaf size tended to increase with increasing nitrogen level, although differences were not always statistically significant (data ± standard error presented for 2011 in Table 1).

In the 2011 experiment, cumulated marketable yields of tomatoes over a 10-week period (from May 2nd until July 11th) were respectively 21.07kg/m^2 (± 0.21 standard error), 21.08 (± 0.31) and 18.75 (± 0.96) for the plants receiving 16, 8 and 4mmol nitrate per litre of fertigation solution. The differences were not statistically significant (P = 0.11).

Table 1: Effect of nitrogen fertilization on the development of tomato plants in the 2011 trial.

Test #	**NO_3^- fertilization level** (mmol/l)	**Leaves per plant**	**Fresh weight of leaves** (g) (± standard error)		**Leaf size** (cm²)		**Fresh weight of stems** (g)
Test 1	4	18.3	665.5 ± 38.5	a	846.4 ± 91.5	a	558.6 ± 29.5
	8	18.3	714.8 ± 44.2	a	791.3 ± 78.4	a	592.9 ± 37.1
	16	20.0	874.4 ± 27.3	b	1174.1 ± 126.0	b	686.9 ± 27.0
			P < 0.05		P < 0.05		P > 0.05
Test 2	4	15.7	595.5 ± 114.8		736.2 ± 56.0		719.3 ± 73.1
	8	17.7	816.4 ± 58.3		819.0 ± 58.4		888.9 ± 25.5
	16	18.0	765.1 ± 98.9		911.0 ± 76.3		979.7 ± 119.5
			P > 0.05		P > 0.05		P > 0.05

For a given column and test, numbers followed by different letters are significantly different (Newman-Keuls tests; P = 0.05).

Effect of nitrogen fertilization on the development of grey mould

In all tests, stem lesions developed within days after the inoculation of pruning wounds with spores of *B. cinerea* (Figure 1). Symptoms caused by strain BC1 were more severe than those caused by strain BC21, confirming at crop level their difference in aggressiveness to tomato observed in earlier work in controlled conditions (Lecompte *et al.*, 2010).

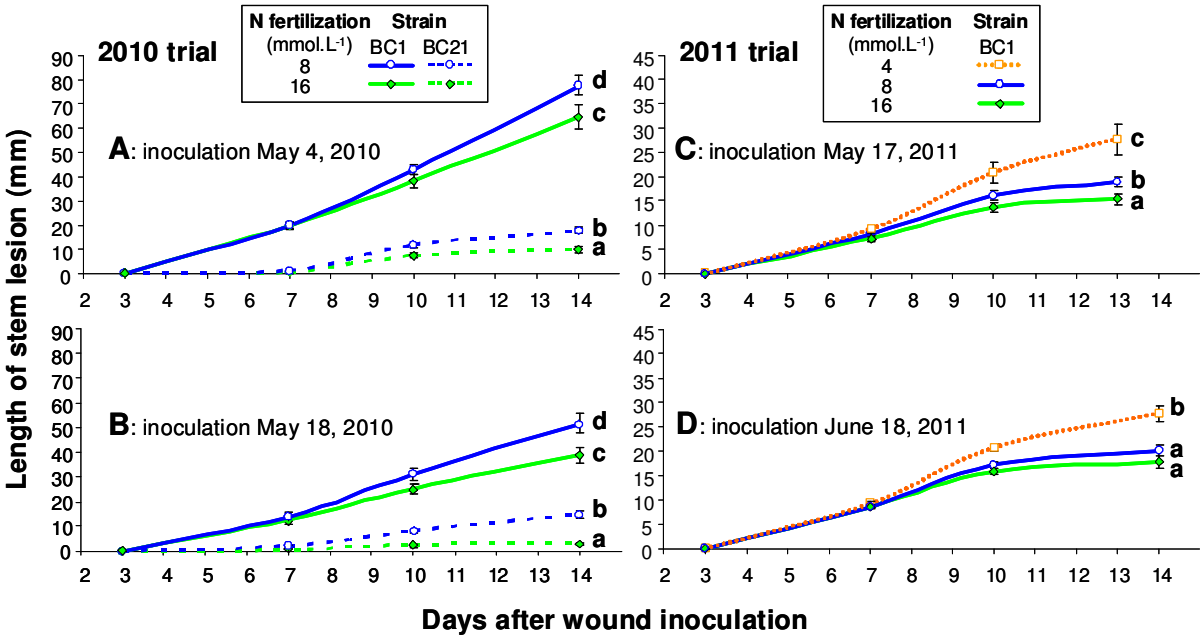

Figure 1: Effect of the nitrogen fertilization level (4, 8 or 16mmol NO_3^- per litre of fertigation solution) on the development of *Botrytis* stem lesions from pruning wounds inoculated with strain BC1 or BC21 of *B. cinerea*. Bars indicate the standard error of the mean (84 values per point in 2010 and 36 in 2011). For a given inoculation date, values on the last observation day followed by a different letter were significantly different (P = 0.05).

Regardless of the strain, disease severity in 2010 was significantly higher ($P < 0.05$) on plants receiving 8 than on those receiving 16mmol of nitrate per litre of fertigation solution (Figures 1-A and 1-B). In the 2011 trial, stem lesions were significantly longer on plants receiving the lowest nitrogen fertilization level (4mmol/l). However, the overall severity of disease was lower than in 2010 (possibly due to warmer and drier conditions, less conducive to the development of grey mould) and the differences between 8 and 16mmol/l were statistically significant in only one of the tests (Figures 1-C and 1-D).

Effect of nitrogen fertilization on powdery mildew

In the 2010 trial, the incidence of mildewed plants was significantly higher ($P = 0.03$; Chi^2 test) in the plots receiving 16mmol nitrate per litre of fertigation solution (85.6%) than in those receiving 8mmol/l (76.3%).

In the 2011 trial, disease severity on inoculated leaves decreased with decreasing nitrogen fertilization level, but the differences were statistically significant in the second test only (Table 2). Lesion density, lesion diameter and spore production were also significantly ($P < 0.05$) affected by the level of fertilization in the second test but not in the first one, possibly due to differences in sample size between the two tests. While spore production increased with increasing nitrogen fertilization, lesion density and lesion diameter were not significantly different for nitrate fertilization levels of 8 or 16mmol/l. These results are compatible with those obtained in controlled conditions by Hoffland *et al.* (2000).

Table 2: Effect of nitrogen fertilization on the development of powdery mildew on tomato leaves inoculated with *Oidium neolycopersici*.

Test # (date of inoculation)	**NO_3^- fertilization level** ($mmol.L^{-1}$)	**Disease index** [x] (scale from 0 to 9)	**Lesion density** [y] (nb / cm² of leaf tissue)	**Lesion diameter** [y] (mm)	**Spores per lesion** [y]
Test 1					
(5/4/2011)	4	2.58 ± 0.16	0.49 ± 0.07	3.53 ± 0.18	443 ± 60
	8	2.68 ± 0.20	0.60 ± 0.13	3.25 ± 0.28	307 ± 32
	16	3.13 ± 0.23	0.53 ± 0.08	3.48 ± 0.15	480 ± 59
		P>0.05	P>0.05	P>0.05	P>0.05
Test 2					
(10/5/2011)	4	1.25 ± 0.14 a	0.15 ± 0.03 a	2.63 ± 0.05 a	853 ± 99 a
	8	2.17 ± 0.24 b	0.39 ± 0.07 b	2.95 ± 0.07 b	1223 ± 164 a
	16	2.21 ± 0.19 b	0.32 ± 0.05 b	2.92 ± 0.06 b	1752 ± 166 b
		P<0.001	P<0.01	P<0.001	P<0.001

[x] Data are means for 24 leaves per fertilization level (± standard error).
[y] Data are means for 12 (Test 1) or 24 (Test 2) leaves per fertilization level (± standard error).
For a given column and type of plant tissue, numbers followed by different letters are significantly different (Newman-Keuls tests; P = 0.05).

Conclusions and perspectives

The results of this study confirmed at crop level that nitrogen fertilization may have an effect on the susceptibility of tomato plants both to *B. cinerea* and to *O. neolycopersici*. However, more variability was encountered than in controlled condition studies, attesting to the fact that complex interactions may be at play with environmental conditions. More information should become available from similar trials currently conducted in different regions of France by partners of collaborative project "FertiPro" coordinated by C. Raynal (Centre Technique Interprofessionnel des Fruits et Légumes).

If confirmed by other trials, the decrease in severity of powdery mildew with decreasing nitrogen and the small difference in yield between the highest and lowest fertilization levels suggest that nitrogen use by farmers could be advantageously reduced. However, the opposite effect observed for grey mould complicates the design of an ideal fertilization scheme. Strategies of precision farming could be envisioned to fine-tune the amount of nitrogen fertilization over the growing season not only on the basis of the physiological needs of the plant for fruit production, but also according to periods of relative risks (and available control methods) of grey mould and powdery mildew.

More information is also needed on possible effects of nitrogen fertilization on other pathogens and key pests of tomato, as well as on the efficacy of biocontrol agents and possibly on that of other methods used in IPM schemes. Finally, as other mineral nutrients may also affect tomato diseases (Elad & Volpin, 1993), a more comprehensive approach of fertilization may be needed for a maximum beneficial effect on IPM.

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