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Full Length Research Paper

Nitrogen and Calcium Dosage in Nutrient Solutions: Effect on *Fusarium Wilt*, Nutrition and Yield of Tomato Plants

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In the agricultural zone of Culiacán, Sinaloa, the tomato crop is affected by *Fusariumoxysporum* f. sp. *lycopersici* race 3 (Fol3), which decreases yield and leads to high economic costs associated with controlling this plant pathogenic microorganism; in addition to the ecological impact of soil and water contamination. Thus, the objective of the present study was to evaluate four nutrient solutions to assess their effects on the incidence and severity of fusarium wilt induced by Fol3, on the nutritional status of the crop, and on the commercial yield. Hybrid tomato plants of the round Imperial® and Roma Reserva® types were planted in substrate infested with *Fol*3. The nutrient solutions, based on the Steiner universal solution, were spiked with two concentrations of N-NO_3^- (12 and 9 moles m^{-3}) and two concentrations of Ca^{2+} (9 and 11 moles m^{-3}), which were combined to yield four nutrient treatment conditions. Statistical analysis did not show significantly different disease incidences among the four treatments; however, the severity of the disease was significantly reduced ($p<0.05$) in treatments containing 12 moles m^{-3} of N-NO_3^- and 11 moles m^{-3} of Ca^{2+} for Imperial hybrid plants, and no significant difference was observed ($p>0.05$) for the Reserva hybrid plants at different Ca^{2+} and NO_3^- concentrations. The nutritional status of the plants was adequate in the four nutrient solutions, and the fruit yield increased 38% in nutrient solution 2 for the Imperial tomato hybrids, while solution 1 (Steiner, 1984) inoculated with *Fol3* presented the best results for the Reserva tomato hybrid.

Keywords: *Solanumlycopersicum*, nutrient solution, *fusarium wilt*, commercial-quality produce

SCOPE AND LIMITATION

SCOPE. The study covers two mineral nutrients which affect the Fusarium wilt, according with other authors, in terms of object oriented yield particularly the tomato crop, semi-hydroponic and *Fol* incidence. Tomato crop, the most common hybrid used in the zone, specialized for the export trade, which attributes and quality were accepted for the customer, and can used the result for other zones whit semi-hydroponic system. *Fol* incidence show the scope of the disease on the crop and was measure the real problem in the zone of Culiacan, Mexico.

Limitations. The study will not cover the other important nutrients as K, Mg, P and S, neither physiology of the plant features such as flowering and quality of root. Fertility of

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soil is an important item in the local agriculture in general we have good soil system, the response of the plant was only measured by mineral plant analysis and parametric scales, and lack of the PCR identification of the pathogen and the response on specific ion channel of calcium and nitrogen behavior.

INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici*race 3 (*Fol*3) is considered the most significant pathogenic fungus in tomato crops due to the economic losses it causes when it infects plant roots (Agrios, 2008). To manage this fungus, resistant hybrids are recommended for planting, crop rotation, chemical control and biological control (Sánchez-Castro, 2006). In conditions of high pathogen prevalence, the use of grafting is recommended (Hall, 2014). Lemmens et al. (2004) reported that adequate use of fertilizers is most likely the best method of chemical control; the adequate use of nitrogen and calcium, aside from increasing yield, enriches the soil in a way that increases plant resistance to vascular diseases (Bletsos, 2006). However, ammonia-based nitrogen fertilizers increase the severity of fusarium wilt in tomato, whereas nitrate-based nitrogen fertilizers have the opposite effect (Borrero et al., 2012). On the other hand, the use of large doses of calcium-based nutrients in tomato crops has been shown to increase fruit production and decrease the occurrence of blossom end rot (Lazcano, 2000). The majority of experiments involving the application of fertilizers on the incidence and/or severity of fusarium wilt have been conducted in open fields; however, in protected-cropping systems, other factors also play a role, such as late crop practices and the design of small greenhouses without adequate ventilation, which causes crop damage (Rembialkowska *et al*., 2010). This type of damage is difficult to control because it is mostly influenced by weather and is less influenced by human knowledge or the training of human resources. Finally, little is known regarding the effects of managing nutrient solutions and their relation with the incidence and severity of pathogens on tomato. Therefore, the main objective of this paper is to describe the effect of different doses of nitrogen, in the form of nitrates, and ionic calcium on the incidence and severity of *Fol*3 in crops of the hybrid tomatoes round Imperial® and Roma Reserva® under greenhouse conditions, to identify possible nutritional imbalances or deficiencies in these crops and to identify any influences on the yield and commercial quality of the tomato fruit.

MATERIALS AND METHODS

Geographic location and climate. The experiment was conducted from 2014-2015 in the municipality of Culiacán, Sinaloa, México, latitude 24°73´35”, longitude 107°45´46” and 65 m above sea level, at the facilities of the Research Center for Food and Development, Culiacan station (Centro de Investigación en Alimentación y Desarrollo A. C., Unidad Culiacán). The dominant climate is a hot and dry steppe climate with rainy summers and an annual mean precipitation between 600 and 680 mm. The annual average temperature oscillates between 24 and 25°C, with maximums of 44 to 47°C and minimums of 1 to 2 °C.

Greenhouse characteristics. A multi-span greenhouse with medium technology was used: a completely automated greenhouse with window structure, interior ventilation, shade control and hydroponic fertigation with an area of 675 m^2 divided into four irrigation sectors, with an independent fertigation system for each sector. A temperature sensor (HOBO Pro series®) was installed inside the greenhouse to record the temperature every hour during the crop cycle.

Seeding and trasplanting. The tomato has been extensively used as a plant model species to evaluate nutrient solutions. Therefore, two types of tomato (*Solanumlycopersicum* Mill)plants were used: hybrid “round” Imperial® and “Roma” Reserva® type, which are both of indeterminate growth and susceptible to *Fol*3. Hybrid seeds were planted in polyurethane trays with 128 cavities and then grown for 45 days. The seedlings were transplanted into 20-L polyethylene bags with 10 kg of coconut fiber substrate in each bag with hydroponic irrigation. The plants were arranged with inter-plant distances of 0.4 m and double rows, with a distance of 1.80 m between rows, for a density of 2.8 plants per m^2.with 5 randomly selected plants in each treatment repetition. Crop management was conducted according to standard methods (Berenguer, 2003).

Nutrient solutions. The nutrient solutions were designed based on Steiner (1984) who designed an universal solution, which consist of balancing anions and cations and maintaining the hydrogen potential between 5.5 and 6.0 and the electric conductivity at 2.0 dS m^{-1}. The solutions were applied as individual treatments starting at the second week after the seedlings were transplanted. Normal balanced nutrition was calculated for all solutions, which included four variations (treatments). Nutrient solution number 1 was the Steiner (1984) universal solution (Table 1), which provides nitrogen in the form of nitrates (12 moles m^{-3}), phosphorus in the form of phosphates (1 mole m^{-3}), sulfur in the form of sulfates (7 moles m^{-3}), potassium (7 moles m^{-3}), calcium (9 moles m^{-3}), and magnesium (4 moles m^{-3}). A micronutrient solution was also applied weekly to all treatments via foliar application throughout the experiment [iron (1.12 ppm), boron (0.27 ppm), manganese (0.11 ppm), zinc (0,131 ppm), copper (0.037 ppm) and molybdenum (0.05 ppm)] (Moreno and Valdés, 2005). For nutrient solution number 2, the calcium concentration was increased, providing nitrogen in the form of nitrates (12 moles m^{-3}), phosphorus

Table 1: Nutrient solutions used in tomato hybrids in the experiment.

Solution	Anions (moles m^{-3})			Cations (moles m^{-3})			pH	CE
	NO_3^-	$H_2PO_3^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}		
1*	12	1	7	7	9	4	5.5-6.0	2
2	12	1	7	5.7	11	3.3	5.5-6.0	2
3	8	1.5	10.5	7	9	4	5.5-6.0	2
4	8	1.5	10.5	5.7	11	3.3	5.5-6.0	2

* Solution 1 is the universal Steiner solution, which was used as a reference; other solutions are modifications of the same, maintaining Steiner's (1964) balance criterion.

in the form of phosphates (1 mole m^{-3}), sulfur in the form of sulfates (7 moles m^{-3}), potassium (5.7 moles m^{-3}), calcium (11 moles m^{-3}), and magnesium (3.3 moles m^{-3}). For nutrient solution 3, the nitrogen was reduced to 8 moles m^{-3}, providing nitrogen in the form of nitrates (8 moles m^{-3}), phosphorus in the form of phosphates (1.5 moles m^{-3}), sulfur in the form of sulfates (10.5 moles m^{-3}), potassium (7 moles m^{-3}), calcium (9 moles m^{-3}), and magnesium (4 moles m^{-3}). Finally, solution 4 contained nitrogen in the form of nitrates (8 moles m^{-3}), phosphorus in the form of phosphates (1.5 moles m^{-3}), sulfur in the form of sulfates (10.5 moles m^{-3}), potassium (5.7 moles m^{-3}), calcium (11 moles m^{-3}), and magnesium (3.3 moles m^{-3}).

Inoculation of *Fusarium oxysporum f. sp. lycopersici* strain 3 (*Fol*3). The fungus*Fol*3 strain Folmx-19 was obtained from the collection of the Plant Pathology laboratory at CIAD-Culiacán and used in the present experiment. The fungus *Fol3* was activated and later increased as follows: initially, the strain was grown in Potato Dextrose Agar medium amended with chloramphenicol (0.25 g L^{-1}); later, a section of the actively growing fungus was cut and placed in a 1-L flask containing potato dextrose broth. The flask was shaken for 8 days at 110 rpm and 25 °C. Finally, the spores were counted using a haemocytometer to obtain a suspension of $1x10^6$ spores per milliliter. Prior to the inoculation, plant roots were injured 15 days after planting by inserting a spatula in the root zone and immediately applying 50 mL of the *Fol3* spore suspension (Carrillo-Fasio et al., 2003).

Assessment of the incidence and severity of *Fol*3. The incidence and severity of fusarium wilt was evaluated in five randomly selected plants from each treatment group. Disease incidence was assessed weekly after pathogen inoculation, using the equation Incidence = (∑Incidence of symptoms/Total number of assessed plants) x 100. The severity of *Fol*3 was assessed at the same time as the incidence using the hedonic scale proposed by Khaled et al. (2007). This scale consists of values that range between 0 and 3, where 0=healthy plant, 1=chlorotic plant, 2=wilted plant and 3=dead plant. The results were converted to severity values according to the following equation (Song et al., 2004):

$$\text{Severity}=\frac{\Sigma \text{ Severity of symptoms (0,1, 2 or 3) x Total number of assessed plants}}{\text{3 x Total number of plants}}$$

Chemical analysis of plant tissue. Twenty compound leaves were collected from the tomato plants for nutritional analysis in the laboratory. The selected leaf was the third completely developed leaf from the first plant canopy and was absent of disease. The leaves were washed with distilled water, left to air dry and then transferred to a drying oven (Baxter Scientific Products mod. 8620-SA, Melrose Park, I11) at 70°C for 24 h. Next, the sample was homogenized in a mill with a 40-micron sieve. Protein nitrogen content was determined using the standard method by AOAC (1998 a).

Determination of total nitrogen. The total nitrogen was determined according to method 988.05 by AOAC (2005): 0.2 g of dry sample was added to a Micro Kjeldahl flask, to which 1.5 g of catalytic mix (90% K_2SO_4 + 10% $CuSO_4$ $5H_2O$) and 5 mL of concentrated sulfuric acid were added. The mix was placed in a digester (Labconco mod. 60300 Kansas City, MO, USA) until a greenish-blue solution was obtained. The solution was cooled down and then diluted in 10 mL of distilled water and transferred to a distiller (Labconco mod. 60500 Kansas City, MO, USA). The flask was heated. When the solution took on a darkened color, it was neutralized with 50% sodium hydroxide (NaOH). At the same time, 5 mL of 4% boric acid and 3-4 drops of methyl red were added to a small flask. Both flasks were added to a Micro Kjeldahl flask until the color of the solution in the small flask changed from pink to yellow. Next, the solution was titrated with 0.1 N hydrochloric acid. The percentage of nitrogen was estimated with the following equation:

N (%)=(HCl spent x acid normality of HCl x 0.01401/sample weight)*100.

Determination of mineral elements. The assessed minerals were calcium, manganese, iron, potassium, copper and zinc, which were determined according to AOAC (1998 b). A 1g amount of each sample was weighed on an analytical scale (Denver Instrument Company AA-160, Denver, USA) using pre-weighed porcelain crucibles. The crucibles were placed in a muffle furnace (30400 Furnace Dubuque Iowa, USA) at 550°C for 12 h until complete conversion to white ash. For sample digestion, 5 mL of HCl was added to the crucible containing the samples and brought to volume with distilled water in a 100-mL flask. Readings of each element were made using an atomic absorption spectrophotometer (Varian mod. SpectrAA-220 Zeeman software with Ultra AA lamps. Victoria, Australia). Ca, Mg, Fe, Cu and Zn were determined using hollow cathode lamps at different wavelengths; however, K was determined by flame emission spectroscopy. The content of elements in the samples was quantified using the following formula:

$$\text{Percentage of element} = \frac{\text{Equipment reading (ppm)} \times \text{dilution factor}}{\text{Sample weight (g)}} \times 10^{-4}$$

$$\text{where Dilution factor} = \frac{\text{(mL of original dilution} \times \text{mL of final sol.)}}{\text{mL of final dilution aliquot}}$$

Phosphorus was determined using a colorimeter, as indicated by AOAC (1995). A sample of 1 mL was taken from the solution obtained from the digestion, to which 1 mL of ammonium molybdate, 1 mL of hydroquinone solution, 1 mL of 20% sodium sulfite and 4 mL of distilled water were added. The solution was allowed to sit for 30 min, and the absorbance of the sample was measured at 650 nm on a spectrophotometer (UV-visible mod. 6405 Jenway, Seoul, Korea) in 1-cm silica cells. A calibration curve was estimated from 0 to 100 ppm of phosphorus. The percentage of phosphorus was then estimated according to the above equation.

Fruit production. Fruits were harvested when they reached physiological maturity for each hybrid assessed. For the Imperial hybrid tomato, this process range in 4 weeks, with one harvest per week, whereas the Reserva hybrid was ranged for 11 weeks. Fruit production was quantified according to the weight in kg m^{-2} and size in inches (USDA, 1999) as extra-large (2”28/32 - 3”15/32), large (2”17/32 - 2”28/32), medium (2”9/32 - 2”17/32) and small (2”4/32 - 2”9/32). The discarded fruit comprised the nonmarketable portion of fruit with defects such as floral scar, deformed, sun damage, cracked, fruit smaller than the “small” size and fruit with blossom-end rot.

Statistical analysis. A repeated-measures treatment design was used with two nested factors (nitrogen and calcium) and one crossed factor (time) with weekly readings. The treatments consisted of 4 different nutrient solutions, with 4 repetitions each, for a total of 16 experimental parcels. The experimental unit consisted of 5 randomly selected plants in each experimental parcel. A comparison of treatment measurements was conducted using a Tukey test at 5% significance. The experimental design was a completely random arrangement. Data were submitted to a normal analysis of variance using the program Mini Tab version 15 (Minitab Inc., Philadelphia, USA). The effect of nitrogen and calcium was determined, as well as their interaction with the incidence and severity of the pathogen *Fol*3. Finally, a simple correlation analysis was performed between the variables (incidence and severity) and the applied levels of nitrogen and calcium.

RESULTS AND DISCUSSION

Greenhouse climate conditions. An average minimum temperature of 12.5°C and an average maximum temperature of 29.8°C were recorded. Crop management was performed according to standard methods (Rembialkowska *et al*., 2010; Nuño *et al*., 2007) to avoid the effect of factors other than nutrient solutions. Nuez *et al*. (2001), and Ho *et al*. (1993) mentioned that the optimum development of tomato crops occurs between 12°C and 30°C; however, for the development of *Fol*3, Cruz-Alcalá *et al*. (2000) mention that the optimal temperature varies between 27°C and 28°C.

Incidence and severity of fusarium wiltin Imperial hybrid tomato. Treatment with nutrient solution (Steiner, 1984) number 2, which contained 12 moles m^{-3} NO_3^- and 11 moles m^{-3} Ca^{2+}according to the scale proposed by Khaled *et al*. (2007), did not show lower incidence in the Imperial hybrid tomato compared with treatments 1 and 3. However, it did show a significant difference ($p<0.05$) when compared with treatment 4, which contained 8 moles m^{-3} NO_3^- and 11 moles m^{-3} Ca^{2+}. Regarding the results obtained for the incidence of *Fol*3, in a comparative study of two nutrition types in high technology greenhouses, the application of larger doses of N-NO_3^- was found to reduce the incidence of *Pythium* in the rhizosphere but without any significant differences among treatments ($p>0.05$). The first harvest month yielded an average of 8.58 kg m^{-2} under a system of open nutrition and high technology with an N-NO_3^- concentration of 201 ppm (14 moles m^{-3}) and a fruit yield of 9.97 kg m^{-2} with a nutrition system under the nutrient film technique (NFT) using a concentration of 229 ppm (16 moles m^{-3}) of N-NO_3^-. However, no pathogen was applied in the experiment; rather, only the nutrition was assessed, and no significant difference was found among the treatments ($p>0.05$) according to the Duncan test (Hao and Papadopoulos, 2002). Treatment with nutrient solution 2, which contained 12 moles m^{-3} N-NO_3^- and 11 moles m^{-3} Ca^{2+}, did show significant differences compared with

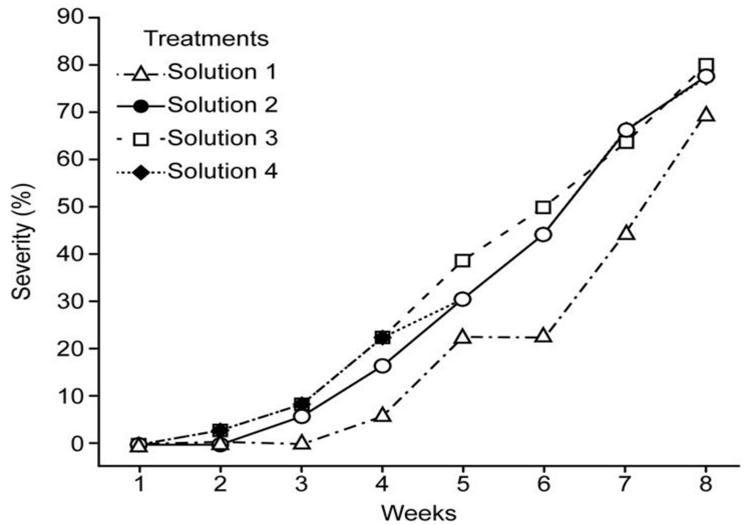

Figure 1. Severity of Fol3 over time in Imperial hybrid tomato.
* no significant difference was found among treatments.

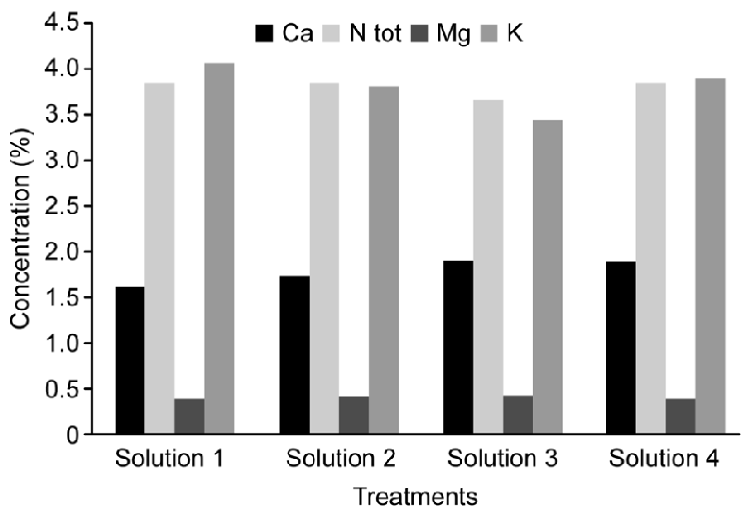

Figure 2. Nutrient element content in leaves of hybrid round Imperial type tomato.

solution 4, which contained 8 moles m^{-3} N-NO_3^- and 11 moles m^{-3} Ca^{2+}. This result is in accordance with those described by Altinok et al., (2013) who determined that fertilizing with $CaNO_3^-$ reduces the incidence of *Fol*3 and also increases the amount of dry matter compared to fertilizing with $(NH_4)^{-2}SO_4$, NH_4Cl and NH_4NO^{3-} as nitrogen sources. Martin *et al*. (1982) showed a reduction in severity of the bacterium *Pseudomonasin vitro* when a bactericide was added to a calcium carbonate mix, showing a synergistic effect against the pathogen. The severity was measured over time and showed a non significant difference ($p>0.05$) among treatments (Figure 1). Nutrient solution 2 performed less well than did the other treatments. Huber and Watson (1974), using a nutrient solution based on the one proposed by Hoagland, with 200 ppm (14 moles m^{-3}) of N-NO_3^- in 3 treatments, showed that the highest dose significantly reduced the severity of wilting by *Verticillium* in tomato ($p<0.05$).

Nutritional content of the Imperial hybrid tomato plants. The levels of mineral elements found in petioles were within the range recommended by Jones *et al*. (1991) (Figure 2) for the adequate development of tomato crops. The N/K ratio supplied by the nutrient solution is considered important due to the amount of nitrogen and potassium that it provides and the benefit to the crop yield when this ratio is high. In this study, it was determined that the N/K ratios in nutrient solutions 2 and 3 were 1.01 and 1.06, respectively, and that the plants treated with these

Table 2: Yield in kg m^{-2} of fruit according to size in hybrid round Imperial tomato type.

Fruit size	Solution 1	Solution 2	Solution 3	Solution 4
Discard	0.16^{a*}	0.14^{a}	0.26^{a}	0.02^{a}
Small (S)	0.53^{a}	0.47^{a}	0.50^{a}	0.17^{a}
Medium (M)	0.82^{a}	0.42^{a}	0.48^{a}	0.10^{a}
Large (L)	0.81^{a}	0.38^{a}	0.54^{a}	0.10^{a}
Extra large (XL)	1.01^{a}	0.48^{a}	0.60^{a}	0.19^{a}

Identical letters indicate no significant difference according to Tukey's test (p>0.05).

solutions presented the lowest incidence and severity of fusarium, whereas the solutions that had N/K ratios below 1 presented a higher incidence and severity of fusarium. This result is in agreement with findings by León Gallegos (2001), who recommends applying an N/K ratio of 1/1 during plant germination and development and subsequently increasing the ratio over the course of the crop cycle to meet nutritional demands and thereby ensure optimal tomato plant development.

Yield of Imperial hybrid tomato. The fruit production results did not present significant differences (p>0.05) between nutrient treatments. However, nutrient solution 2 produced the highest yield at 2.8 kg m^{-2} (Table 2), and treatment 1 (inoculated with *Fol*3) produced the lowest yield, 2.3 kg m^{-2},during the entire assessment. These results agree with those obtained by Hoffland *et al*. (2000), in which the susceptibility of tomato seedlings to *Fusarium oxysporum f. sp. lycopersici* strain 2 was assessed as a function of the concentration of nitrogen in plant tissues using three treatments of N-NO_3^-, with the lowest nitrogen amount being 14 moles m^{-3}. They did not observe significant differences in the amount of dry matter produced by the seedlings.

The commercial quality fruit was dominated by two fruit sizes, “large” and “extra-large” (Table 2). The amount of discarded fruit was higher following treatment with nutrient solution 4, which contained a low dose of calcium. This observation agrees with findings by Ho and White (2005), who have stated that the mineral intake of calcium by the fruit is somewhat dependent on uptake from the soil and that it is necessary to apply calcium to the fruit to prevent reductions in fruit yield. Bletsos (2006) mentions that applications of calcium combined with grafting produced an increase in the greenhouse production of eggplant. Thus, from a market point of view, the result is relevant, independent of the statistical significance. Hall (2014) noted that there is some variability in yield among varieties according to the degree of tolerance to vascular diseases.

Incidence and severity of fusarium wilt in Reserva hybrid tomato. Treatment with nutrient solution 1 (Table 1) presented a significant difference (p<0.05) with regard to the pathogen (Figure 3). In the other treatments, the incidence of the pathogen was constant: once the symptoms manifested, the development of the disease progressed very slowly, which allowed for assessment over 11 weeks after inoculation. (Figure 3) (Khaled *et al*., 2007). These results agree with those of Ehret *et al*. (2002), in which different fertilizer salts were used to inhibit powdery mildew disease (*Oidium neolycopersici*) in tomato plant leaves and no significant differences in disease control were observed after the application of calcium nitrate and calcium chloride. Likewise, Jones and Wolts (1997), who did not find significant differences between different doses of calcium carbonate with regard to protection against *Fusarium oxysporum f. sp. Lycopersici* race 1.

The reduced incidence and severity of *Fol*3 in treatment 1 (Figure 3 and 4) could be attributed to the potassium-calcium cation ratio, which was 0.78, 0.52, 0.78 and 0.52 for solutions 1, 2, 3 and 4, respectively. This outcome implies that with the lower K^+/Ca^{2+} ratio supplied to the plants by solutions 1 and 3, a lower dose of calcium and a low supply of N-NO_3^- were also given. This agrees with the report by Bar-Tal and Pressman (1996), who determined that an increase in blossom end rot in tomato fruit is associated with an increase in the K^+/Ca^{+2} ratio, such that when this ratio was 0.83, blossom rot was reduced by 0.38 kg $plant^{-1}$, and in the case of a K^+/Ca^{2+} ratio of 1.67, blossom rot was reduced by 0.73 kg $plant^{-1}$. When Ca^{2+} is increased without lowering K^+, blossom end rot is reduced by up to 0.12 kg $plant^{-1}$, indicating that the large dose of calcium in balance with potassium reduced this physiological disorder in tomatoes.

The severity and incidence of *Fol*3 showed a similar pattern (Figure 4). Better crop development was observed with nutrient solution 1, which contained 168 ppm (12 moles m^{-3}) of N-NO_3^-. This observation agrees with the results obtained by Arya and Kuwatsuka (1991), who applied three levels of N-NO_3^- (5, 10 and 20 mg of nitrogen per 100 g soil) in three types of soil infested with *Fusarium oxysporum* f. sp. *raphani.* In that study, it was determined that increasing concentrations of NO_3^- caused a reduction of $1x10^{-6}$ to $1x10^{-5}$ colony-forming units per gram of soil (cfu/g) in comparison to the treatment with a lower concentration of N. These researchers also mention that

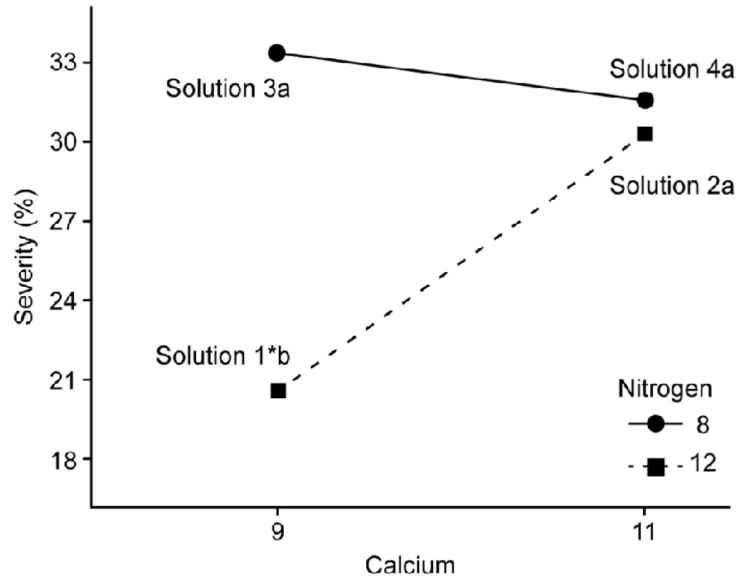

Figure 3. Interaction between calcium and nitrogen as nitrates in percent severity of Fusarium on Reserva hybrid tomato.
* Identical letters indicate there is no significant difference according to Tukey's test ($p<0.05$)

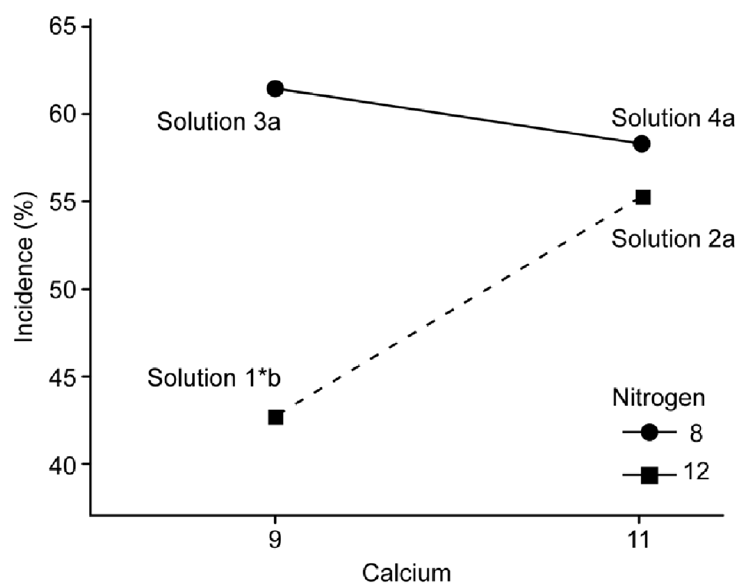

Figure 4. Interaction between calcium and nitrogen as nitrates in percent incidence of Fusarium on Reserva hybrid tomato. Identical letters indicate there is no significant difference according to Tukey's test ($p<0.05$).

applying nitrogen in the form of nitrates improved disease reduction in soil that contained high levels of calcium. Similarly, Zielińska and Michniewicz (2001) assessed the severity of *Fusarium culmorum* in wheat seedlings, applying a Hoagland nutrient solution with 200 ppm (14 moles m^{-3}) of calcium. The experiment was conducted in Petri dishes with 4 mM of complete solution (without calcium), to which 0, 2, 4 and 8 mM calcium were added. The best treatment was the one with the double dose of calcium.

The effect of the interaction between the NO_3^- and Ca^{2+} levels significantly ($p<0.05$) affected the severity and incidence of *Fol*3 in plants, as an incidence of 43% was observed for nutrient solution 1 (Table 3), and increasing the amount of calcium and lowering the nitrates relative to nutrient solution 1 led to a 62% incidence and increased

Table 3: Effect of levels of NO_3^- and Ca^{+2} in nutrient solution on incidence, severity and yield of hybrid tomato type "roma".

Nitrogen concentration	Ca^{+2} (moles m^{-3})	Incidence (%)	Severity (%)	Yield (kg m^{-2})
12 NO_3^- (moles m^{-3})	9	72.08^{b*}	49.31^{b}	11.49^{b}
	11	67.60^{a}	42.78^{a}	11.32^{b}
8 NO_3^- (moles m^{-3})	9	69.17^{b}	45.69^{b}	11.97^{b}
	11	72.59^{b}	53.06^{b}	24.25^{ab}
NO_3^-*Ca^{+2} (moles m^{-3})	*		*	ns

Means with identical letter in each column indicate there is no significant difference according to Tukey's test ($P \leq 0.05$).The symbol * in each column indicates statistically different values, ns = not significant.

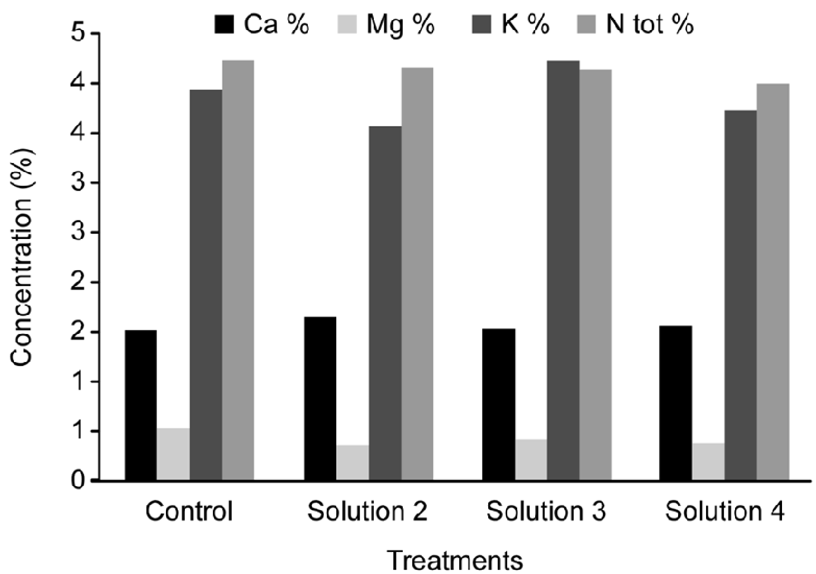

Figure 5. Content of nutrient elements in leaves of hybrid roma Reserva tomato type.
No comparison of means tests were done since all levels are within recommendations (Jones et al., 1991).

severity of the pathogen (Figures 3 and 4). Nutrient solution 1, which contained 12 moles m^{-3} of nitrogen in the form of nitrates, significantly ($p<0.05$) decreased the severity and incidence of the pathogen, although there was no significant difference in fruit yield (Table 3). One possible cause of the observed effect on the behavior of the disease (incidence and severity) is the absence of ammonium in the nutrient solution (Borrero *et al.*, 2012).

Nutritional content of the Reserva hybrid tomato plants. Plants treated with nutrient solution 1 (12 moles m^{-3} N-NO_3^- and 9 moles m^{-3} Ca^{2+}) also called "control" inoculated with *Fol*3 showed the lowest incidence and severity ($p<0.05$) of fusariumwilt attack (Figure 5), which may be due to the high doses of nitrogen in the form of nitrates. The effect of treatment with nutrient solution 2, which also contained 12 moles m^{-3} of N-NO_3^-, was distinct from the use of the fertilizer KNO^{3-}as a source of K^+. To supplement the N content, it was necessary to increase the amounts of potassium, which caused a decrease in Ca^{2+}. Bar-Tal and Pressman (1996) and Lazcano (2000) recommend a balance between cations and anions. Our findings suggest that there was a synergistic effect on the reduction of the pathogen with the application of higher doses of NO_3^- and Ca^{2+} via $CaNO^{3-}$ addition to balance nutrient solution 1 and meet Steiner's criteria.

Yield of the Reserva tomato hybrids. Treatment with nutrient solution 1 (12 moles m^{-3} N-NO_3^- and 9 moles m^{-3} Ca^{+2}) also called "control" inoculated with *Fol*3 showed the largest fruit size, with 48% classified as "large" and "extra-large" fruits compared with 25%, 30% and 30% for nutrient solutions 2, 3 and 4, respectively, (Figure 6) during 11 weeks of harvesting, although there were no significant

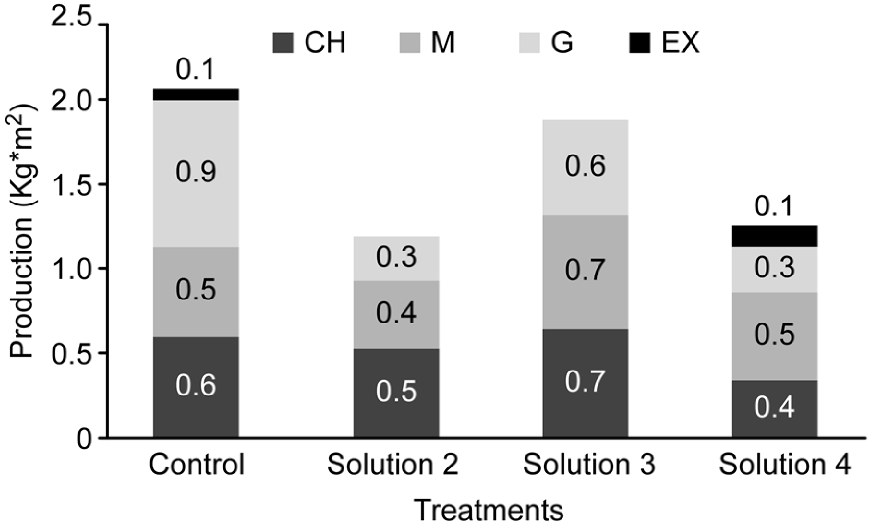

Figure 6. Fruit yield by size in hybrid roma Reserva tomato type, with 48% large and extra large fruit for solution 1 or control, 25% solution 2, 30% solution 3 and 30% solution 4.
* No significant difference was observed among treatments. Tukey's test (p>0.05)

differences in the amount of fruit in kg m^{-2}. This result is probably due to the effect described by Sarhan *et al*. (1982), who mention that fertilization with N-NO_3^- reduces the susceptibility of tomatoes to fusaric acid, the phytotoxin produced by *Fol*3. Altinok noted that treatments with increasing doses of N-NO_3^- significantly increased the amount of dry matter in tomato seedlings, compared with treatments with ammonium sulfate. Reserva hybrid tomato fruit production was reduced due to blossom end rot. In this hybrid, yields of 0.2 kg m^{-2} due to such damage with nutrient solution 4 and of 0.1 kg m^{-2} with all the other treatments have been observed. These observations are in agreement with results described by Terraza *et al.* (2008), who assessed two types of tomato (“round” and “Roma”) and found significant differences, as well as those of Romero *et al*. (2002), who attributed physiological production changes to the sudden influx of water and nitrogen. According to Sarhan *et al*. (1982), tomato crops show higher tolerance to fusariumwilt when $CaNO_3^-$ and $(NH_4)^{-2}SO_4$ are applied in high quantities. Sarhan *et al.* (1982) conducted an experiment to identify the effect of the amount of NO_3^- on attack by the pathogen by applying doses of 70, 280, 420, 630 and 1050 ppm and observing that the highest dose produced the best yield. Treatments below the optimal level (280 ppm or 20 moles m^{-3}) presented the same symptoms of incidence and severity, and disease severity decreased as the NO_3^- levels increased. The resistance response to pathogen attack on the Imperial and Reserva hybrid tomato plants can be attributed to the fact that each hydroponically grown species requires a specific nutrient solution (Steiner, 1984), and more studies are necessary to evaluate concentrations and genetic material to understand the different responses to the same nutrient solution.

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