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Performance of tomato rootstocks in False Root-knot Nematode (*Nacobbus aberrans*) infested soil

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Abstract

Nacobbus aberrans, known as the "false root-knot nematode", has drawn special attention since it affects large areas of tomato production in Latin America. The aim of this study was to evaluate two rootstocks, *Solanum lycopersicon* L. var. cerasiforme 'Carolina' and *S. lycopersicon* L. 'Maxifort', onto which *S. lycopersicon* L. 'Santa Clara' was grafted. In addition to these, non-grafted 'Santa Clara' plants were used as control. Grafting was carried out 30 days after sowing, both for scion and for rootstocks. Both the grafted plants and those used as control were grown in a greenhouse on two kind of substrate: without the presence of *N. aberrans* and inoculated with 5000 eggs. After 60 days of being inoculated, growth parameters, physiological stress indicators, and pathogen reproductive factor were evaluated. The parasitism caused changes in the metabolism of the plants. On grafted plants, flowering was delayed, and on plants exposed to nematodes such delay was even greater. The reproductive factors of the nematode were 3.68, 5.47 and 2.76 on non-grafted rootstocks, 'Carolina' and 'Maxifort', respectively, and they were classified as susceptible. The Maxifort rootstock has an invigorating effect on Santa Clara scion. It stimulates the apical growth and shows a great tolerance to the attack of nematodes as indicated by parameters like the accumulation of proline and the damage in the cell membranes. However, *N. aberrans* reproduces at its roots and increases its population, so its use as rootstocks must be carried out together with other practices that reduce the reproduction factor of the pathogen.

Keywords: Genetic control; Stress tolerance; Grafting; Rootstocks; *Solanum lycopersicon* L. Abbreviations: Fr_reproduction factor; FW_ fresh weight; MDA_malonildialdehido; RI%_reproduction index;

Introduction

Over the last few decades, world production of tomatos has increased significantly, a fact that is not entirely unrelated to the significant increase in research over the same period. This research includes the creation and introduction of new production techniques focused primarily on increasing productivity, increasing the size of the fruit (Guimarães et al., 2008), achieving a variety of fruit shapes and colours (Mattedi et al., 2014), and increasing the period of postharvest conservation, among other factors.

Although there have been important advances in tomato production, the crop still faces serious problems today, due to infestation by pests and diseases which cause high economic losses and make the tomato one of the vegetables that receive the highest amount of agricultural pesticides. Those relating to nematodes are of special importance.

Nacobbus aberrans (Thorne 1935), popularly known as the false root-knot nematode or rosary nematode, has been responsible for significant losses in tomato production in the Americas; this nematode has been reported in Peru, Bolivia, Chile, Argentina, Ecuador, Mexico and United States (Manzanilla-López et al., 2002). Once the pathogen is installed in the root, it induces the accumulation of starch, increases the levels of calcium oxalate, and causes

hypertrophy and hyperplasia which causes the rupture of the xylem and the phloem. In most cases, plants show symptoms such as wilting (during high temperatures), decreased growth and falling productivity (Inserra et al., 1985). In general, the resistance and persistence of the inoculum in the soil hinder pathogen control. Despite the above, there are techniques with a low environmental impact that have certain degree of efficiency in controlling the disease. A good example is grafting which gives rapid results without drastic changes to the production system, and can be learned by anyone.

Based on the above, the aim of this study is to evaluate characteristics of growth, development and physiology in the 'Santa Clara' tomato of the Santa Cruz group, grafted onto different rootstocks and cultivated both in uninfested soil, and in soil infested with the false root-knot nematode, *Nacobbus aberrans.*

Results and discussion

Sixty days after transplanting, the plants were removed in order to evaluate growth, response to stress and nematode reproduction in six different treatments:

• Ungrafted Santa Clara without nematode inoculation

• Ungrafted Santa Clara with nematode inoculation

• Santa Clara grafted onto 'Maxifort' without nematode inoculation

• Santa Clara grafted onto 'Maxifort' with nematode inoculation

• Santa Clara grafted onto 'Carolina' without nematode inoculation

• Santa Clara grafted onto 'Carolina' with nematode inoculation

Growth parameters

When it comes to the presence or absence of the false rootknot nematode, no difference was seen in accumulated shoot dry matter for the infested or uninfested root system sustaining the grafted or ungrafted plant. 'Maxifort' gave the greatest accumulated shoot dry matter on grafts of 'Santa Clara', on average 20.94 g, 41% more than the ungrafted 'Santa Clara'. In the case of grafts grown on 'Carolina' rootstock, the accumulation of shoot dry matter was lower, at 6.82 g on average (Fig 1 A).

For the rootstock 'Maxifort', there was no difference between plants grown in the presence or absence of *N. aberrans* in the root fresh weight, plants of 'Santa Clara' and 'Carolina' varieties, grown in the presence of the false rootknot nematode, displayed a lower value for root fresh weight (Fig 1 B).

The geatest values of stem diameter were found in the 'Maxifort' variety, followed by the ungrafted 'Santa Clara' and by 'Carolina'. For 'Maxifort', the plants grown in the presence of the false root-knot nematode had a larger diameter than those grown without the nematode (Fig 2).

When shoot dry matter, root fresh weight and stem diameter are compared, the grafted 'Santa Clara' grown on the 'Carolina' tomato were smaller, even when compared to the ungrafted 'Santa Clara' variety considered the control for pathogen susceptibility. For plant combinations where the Maxifort variety was used as rootstock, greater vigour was seen in the graft. The latter variety is an interspecific hybrid (Solanum lycopersicon x L. hirsutum) whose invigorating effect on the graft has been documented elsewhere (Albacete et al., 2015). In the treatments where N. aberrans was present, a reduction in growth was seen in the grafts and in the shoots of the ungrafted 'Santa Clara' variety, these differences being significant in some cases. The decrease in growth of the parasitised plants may have been caused by rupture of the xylem and phloem, which is a result of the N. aberrans infestation, and which impairs the flow of substances through the vascular bundles (Franco, 1994).

Cristobal et al. (2001) described and quantified in detail the nutritional changes that *N. aberrans* causes in tomato plants. According to those researchers, the parasite causes significant decreases in the levels of nitrogen, phosphorus, potassium, calcium and magnesium in both shoot and root tissue. Such decreases may represent a reduction in the rate of plant growth, since the low availability of these elements, considered essential to the organisms, reduces the rate of cell expansion and multiplication, and influences other mechanisms for development and metabolism in the plant (Taiz et al., 2006). According to some authors, the consequences of nematode infestation on growth may increase over time as the pathogen completes successive

cycles in the same plant and the parasitism intensifies (Doucet et al., 2005).

For the number of shoots, the grafts of 'Santa Clara' on 'Maxifort' had twice as many shoots than the ungrafted 'Santa Clara', and six times more than grafts of the same variety on 'Carolina' (Fig 3 A).

Figure 3 B compares the dry matter of shoots that were cut and placed in the greenhouse. The presence of N. aberrans caused no changes in the number of lateral shoots in any of the combinations under evaluation. However, in the case of 'Maxifort', the shoots obtained from plants grown in soil infested with the parasite generally presented shoots that were 12% lighter. According to Navarrete et al. (2000), the greater the vigour of the plant, the more the shoots should be thinned. This statement agrees with the results obtained in the present work, as the most vigorous rootstock used, 'Maxifort', provided a considerable increase in the number of shoots on the graft compared to the other plant combinations. According to Hartmann et al. (2001), there is a negative correlation between the weight of the axillary shoots and production, indicating that the growth of lateral shoots involves competition with fruit development.

For root tissue, the nematode parasitism caused a clear tendency for reduced soluble protein levels (Table 2), which in the case of the ungrafted plants reached significant values. Some authors state that the stress and damage to the root tissue cause a rapid reduction in the concentration of root protein (Zhu et al., 2002). This reduction is a result of a mechanism of proteolysis, which occurs to increase solute concentration in the cytosol, mainly to reduce osmotic potential and ensure the absorption of water and nutrients by the plants (Javot, 2002).

As for flowering precocity, it can be seen that treatments containing the ungrafted variety 'Santa Clara', were the first to issue floral racemes whether infested or not with N. aberrans. The remaining treatments, all grafted, whether onto the 'Maxifort' or 'Carolina' rootstock, flowered from 7 to 12 days later than the ungrafted plants, with treatments infested with the nematodes taking from 3 to 5 days longer. These results agree with those presented by other researchers. Loos et al. (2009) state that one of the main consequences of the practice of grafting is a delay to flowering, possibly caused by the need of the new plant combination to re-establish the connection between the vessels of the xylem and phloem. In any event, not only grafting, but the presence of N. aberrans in the roots also seems to contribute to delaying the plants from entering the reproductive stage, as all the cultivars being evaluated as rootstock, as well as the ungrafted plants, exhibited delayed flowering when grown in soil infested with the parasite.

Stress parameters

For the MDA content of the leaf tissue in the absence of *N. aberrans*, there is a difference between the ungrafted and grafted treatments, with the ungrafted. 'Santa Clara' cultivar having the highest concentrations of MDA (Fig 4 B). However, there was no difference in MDA content of the grafted or ungrafted plants for the absence or presence of the pathogen. In the root tissue, the ungrafted plants displayed higher values for MDA compared to those of the grafted treatments, with the presence of the nematode increasing lipid degradation in the roots of the ungrafted plants, having

Table 1. Classification of resistance response to *Naccobus aberrans* based on the reproduction index (RI%) in the nematode, calculated as the number of eggs per gram of root in one plant compared to the number of eggs per gram of root in a susceptible plant used as the reference standard (Hadisoeganda et al., 1982).

Condition	Reproduction index	
Highly resistant	RI<1%	
Very resistant	1%≤ RI< 10%	
Moderately resistant	10% ≤ RI< 25%	
Slightly resistant	25% ≤ RI< 50%	
Susceptible	RI ≥ 50%	

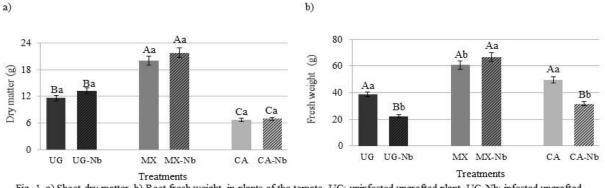


Fig. 1. a) Shoot dry matter, b) Root fresh weight, in plants of the tomato. UG: uninfested ungrafted plant, UG-Nb: infested ungrafted plant; MX: uninfested Maxifort, MX-Nb: infested Maxifort; CA: uninfested Carolina, CA-Nb: infested Carolina. Uppercase letters compare varieties for the same infestation condition. Lowercase letters compare the same variety for both infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test.

Table 2. Soluble protein content in root tissue in plants of the tomato cv. 'Santa Clara', ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, in soil infested with the nematode *N. aberrans* and in uninfested soil.

'Santa Clara' 'Carolina' 'Maxifort'					
Nematodes	ungrafted (mg.ml ⁻¹)	Rootstock (mg.ml ⁻¹)	rootstock (mg.ml ⁻¹)		
Presence	0.2836 Bb	0.3248 Ba	0.3765 Aa		
Absence	0.3443 Ba	0.3550 Ba	0.4236 Aa		
	CV = 14.38 %				

Uppercase letters on a line compare varieties for the same infestation condition. Lowercase letters in a column compare the same variety for two infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test.

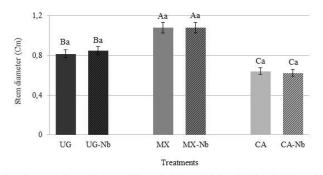


Fig. 2. Stem diameter in plants of the tomato. UG: uninfested ungrafted plant, UG-Nb: infested ungrafted plant; MX: uninfested Maxifort, MX-Nb: infested Maxifort; CA: uninfested Carolina, CA-Nb: infested Carolina. Uppercase letters compare varieties for the same infestation condition. Lowercase letters compare the same variety for both infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test. **Table 3.** Number of eggs and reproductive factor for nematodes in the root system of plants of the 'Santa Clara' tomato, ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, in soil infested with the nematode *N. aberrans*.

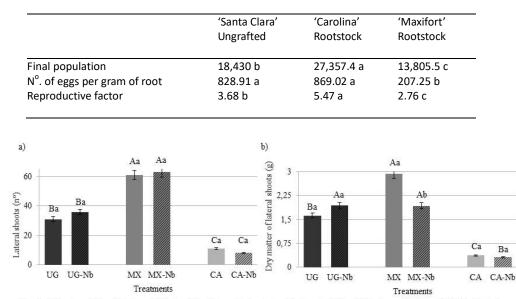


Fig. 3. a) Number of lateral shoots and b) Lateral shoot dry matter in plants of the tomato. UG: uninfested ungrafted plant, UG-Nb: infested ungrafted plant; MX: uninfested Maxifort, MX-Nb: infested Maxifort; CA: uninfested Carolina, CA-Nb: infested Carolina. Uppercase letters compare varieties for the same infestation condition. Lowercase letters compare the same variety for both infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test.

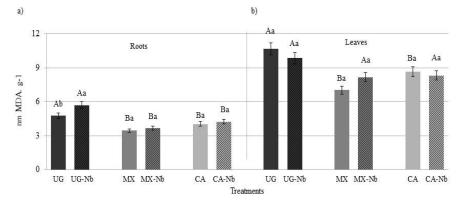


Fig. 4. Malondialdehyde (MDA) concentrations in the root (a) and leaf (b) tissue in plants of the tomato, UG: uninfested ungrafted plant, UG-Nb: infested ungrafted plant; MX: uninfested Maxifort, MX-Nb: infested Maxifort; CA: uninfested Carolina, CA-Nb: infested Carolina. Uppercase letters compare varieties for the same infestation condition. Lowercase letters compare the same variety for both infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test.

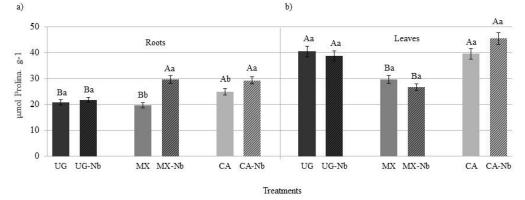


Fig. 5. Proline concentrations in the root (a) and leaf (b) tissue in plants of the tomato. UG: uninfested ungrafted plant, UG-Nb: infested ungrafted plant; MX: uninfested Maxifort; CA: uninfested Carolina, CA-Nb: infested Carolina. Uppercase letters compare varieties for the same infestation condition. Lowercase letters compare the same variety for both infestation conditions. Mean values followed by the same letter do not differ at 5% probability by Scott-Knott test.

no effect in the other treatments (Fig 4 A). The damage caused by the nematodes begins with rupture of the cells by the style, continues with the dissolution of the cell walls, and induces physiological changes as a consequence of the introduction of enzymes into the cell medium (Sijmons,

1993). The increase in lipid peroxidation in the treatment with ungrafted plants grown in the presence of the nematode, confirms the occurrence of damage that, besides having a negative effect by affecting normal tissue function, predisposes the plant to attack by other pathogenic organisms, all this contributing to a loss of productivity in the crop (Powell, 1972).

The plants grafted onto 'Maxifort' presented the lowest concentrations of proline in the leaves (Fig 5 B), the presence or absence of *N. aberrans* having little influence on the proline content of the leaf tissue within each of the grafted or ungrafted treatments being evaluated.

In the case of the roots, the grafted treatments grown in the presence of *N. aberrans* displayed greater concentrations of proline than the uninoculated treatments (Fig 5A). The higher proline levels in the inoculated treatments are probably a response to the damage caused to the tissue by the pathogen. Proline is an important defence constituent in the plant, and its action in the protection of membrane phospholipids to prevent their degradation has already been demonstrated (Samaras et al., 1995), as well as its participation in oxidative phosphorylation to generate ATP, where it helps to compensate for the negative effects of stress (Liang et al., 2013). Some authors propose that increases in this amino acid may be a result of the need of the plant to eliminate the excess of ammonia produced and released into the cell medium (Amini et al., 2005).

Nematode reproduction

When processing the roots to count the eggs for the final nematode population, no galls or eggs were found in the plants of species that had not been inoculated, demonstrating accuracy in preparing the substrate and care in conducting the treatments. However, in the inoculated treatments, large amounts of eggs were found per plant: 18,430 ('Santa Clara' ungrafted); 10,857 ('Maxifort') and 27,357 ('Carolina') (Table 3). By relating the final populations of the pathogen with the initial population, it could be seen that the reproductive factor in all the grafted and ungrafted treatments was greater than one. All were therefore classified as susceptible to Nacobbus aberrans, as established by Oostenbrink (1966). When relating the number of eggs to root size, it can be seen that the treatment which included the 'Maxifort' rootstock presented plants with the lowest number of eggs per gram of root. This result is important, since the use of this rootstock in areas infested with N. aberrans tends to compensate for the decreased efficiency caused by the pathogen in the root system. In the comparative research of cultivars in soils infested by nematodes, it is common for some researchers to use a different classification to that used by Oostenbrink (1966). Hadisoeganda et al. (1982), for example, have proposed a reproduction index (RI%) which relates the

number of eggs per gram of root of the different varieties studied to the number of eggs per gram of root of the standard or principal variety, in this case, the 'Santa Clara' tomato (828.91 eggs per gram of root). According to this classification (Table 1), the 'Maxifort' variety (25.01%) can be classified as *slightly resistant*, and 'Carolina' (104.86%) as *susceptible*.

Based on the above, the results should be analysed in an integrated way, and not simply as a function of one or other classification; a productive variety could be disregarded if it were classified as susceptible by Oostenbrink (1966), or similarly, a variety where the pathogen multiplies exponentially could be classified as resistant by Hadisoeganda et al. (1982) if the standard variety were highly susceptible.

It should be noted that despite the high vigour provided by the 'Maxifort' variety to graft growth, which could compensate for production losses caused by plant parasitic nematodes, the progressive increment in the population of N. aberrans after every crop might be a problem in the short and medium-term. This is because the increase in pathogen population in the soil can limit rotation with other susceptible crops, possibly even becoming a limitation to 'Maxifort'. It should be remembered that a high nematode population generates more lesions and a higher incidence of other pathogens that take advantage of these injuries to enter the roots (Perry et al., 2013). Rootstocks that display good characteristics of growth, productivity and tolerance to stress should therefore be tested together with other techniques that may contribute to a reduction in the growth of the pathogen in the field. In this way, the selection of rootstocks will be the result of the analysis of many factors, and not of a classification that considers one factor only.

Materials and methods

Location of the experiment

The trial was carried out in one of the greenhouses at INFIVE Instituto de Fisiología Vegetal (Institute of Plant Physiology), an institution that belongs to both the National University of La Plata and CONICET (National Council of Science and Technology), in La Plata, Argentina.

Plant material

The tomato cultivar used for grafting was 'Santa Clara 8000' of the Santa Cruz group. As for rootstocks, two were used: the 'Carolina' cultivar from the Cherry group, and the 'Maxifort', a commercial rootstock, carrier of the Mi gene. Besides, non-grafted plants of this cultivar were used as control and subjected to the same treatments. Both grafted and non-grafted cultivars were exposed to infested and noninfested soils.

Setting up the crop

Seedlings were produced in trays of 72 cells, previously filled with sterilised substrate comprising a mixture of perlite and vermiculite (1:1). For the supply of nutrients, the trays were

irrigated weekly with Hoagland nutrient solution (Hoagland et al., 1950).

From sowing until the time of grafting, the plants were kept in a growth chamber under controlled conditions: temperature: 25°C, illuminance: 12.000 Lux, photoperiod: 12 h.

At 30 days after sowing, the plants were grafted using a technique of bevelled cuts. A cut for producing the graft was made one cm above the first definitive leaf. Silicone clips were used to maintain close contact between parts of the grafted plants.

From grafting to transplanting, the plants were kept in the same growth chambers under the same conditions. The exception was humidity which was incressed to 99% in order to avoid transpiration and favour the production of new tissue. From the sixth day onwards, the humidity in the growth chamber started to be gradually reduced for acclimatisation of the grafted plants. After three more days the seedlings hardened off completely (Filgueira, 2008).

At 10 days after grafting, the most uniform seedlings were selected and transplanted to 10L pots and taken to a gable greenhouse with a polycarbonate roof under forced air ventilation, at a temperature of between 20 and 28°C. To fill the pots, a tindalized mixture of 75% soil and 25% sand was employed. The soil used was a vertic Argiudoll, with a pH of 5.5, 10 mg.kg⁻¹ total P, 3.5% organic matter, 2.0% total C, and 0.24% total N.

At one day after transplanting the seedlings, inoculation was carried out using 5000 eggs of *N. aberrans* per pot. The plants remained 60 days for later evaluation.

Obtaining the inoculum and inoculation with the nematode

The inoculum was extracted from tomato plants that were infested only with *N. aberrans*, according to Coolen (1979); the protocol for the extraction of nematodes from the roots was used in both the inoculation and quantification. The solution obtained was first homogenized and observed under an optic microscope and then diluted to reach a concentration of 1000 juvenile eggs per millilitre. For inoculation, three holes approximately two centimetres deep were opened beside the seedlings. After opening the holes, five millilitres of the solution were deposited in each pot; the holes were then closed using substrate from the pot.

Evaluated parameters

From the time of transplantation, the phenological status of the plants was observed weekly, and the lateral shoots quantified. To observe the phenological state, the presence of closed and open flower buds, and the emergence of small fruits were recorded for each plant; moreover 3 to 5 cm long lateral shoots were removed (Filgueira, 2008). The shoots were counted and placed in a forced air circulation oven (65°C) for 48 hours until reaching constant dry weight.

Sixty days after inoculation, the plants were removed from the pots and the following parameters quantified: a) Shoot dry matter; b) Root fresh weight; c) Stem diameter, 10 cm below the first cluster; d) Soluble protein content of the leaves and roots, as per the Bradford method (1976); e) Proline content of the leaves and roots following the method of Bates et al. (1973). The absorbance reading was taken at a wavelength of 520 nm. The proline content per unit fresh weight was calculated from the following equation: µmols proline.g⁻¹ FW = [(proline g.ml⁻¹ x mL toluene) / 115.5 μ g.pmol⁻¹] / [(g FW) / 5]. f) Oxidative degradation of the cell membrane by malondialdehyde (MDA) determination, according to the method described by Heath & Packer (1968). The absorbance reading was made by spectrophotometer (Shimadzu UV-160 A), at 532 and 600 nm. The MDA concentration was calculated using an extinction coefficient of 155 mM⁻¹.cm⁻¹, applying the following formula: MDA equivalents (n.mol.ml⁻¹) = [(A532 - A600) / 155000] 10⁶.; g) Final nematode population; h) Nematode reproduction factor, following the Oostenbrink method (1966); and i) Reproduction index, as per Hadisoeganda et al. (1982).

The reproduction factor (Fr) was calculated from the expression $Fr = Pf.Pi^{-1}$, where: Pf = final population (number of eggs extracted from the roots) and Pi (number of inoculated eggs). Plants where Fr = 0 were considered immune; where Fr < 1 were considered resistant, and where Fr > 1 were considered susceptible (Oostenbrink, 1966).

To calculate the reproduction index (RI), the number of eggs per gram of root for each treatment was divided by the number of eggs per gram of root of the 'Santa Clara 8000'. The value obtained for the RI indicated a condition of resistance or susceptibility, as shown in Table 1 (Hadisoeganda et al., 1982).

Experimental design

The study was carried out in a completely randomised design in a 3 x 2 factorial scheme with 10 replications per treatment. Three kinds of plants were used:

1) ungrafted 'Santa Clara 8000' tomato;

2) 'Santa Clara 8000' tomato grafted onto 'Maxifort' rootstock;

3) 'Santa Clara 8000' tomato grafted onto 'Carolina' rootstock.

All of them were exposed to two conditions of substrate: with and without the presence of the false root-knot nematode, *Nacobbus aberrans*.

All the results were submitted to analysis of variance (ANOVA), and where a difference was identified between treatments, they were compared by Scott-Knott multiple comparison test at a significance level of 5%.

Conclusion

Both under nematode and control, the rootstock 'Maxifort' has an invigorating effect on lateral shoot development and shoot dry matter. Conversly, and also under both conditions, the rootstock 'Carolina' has a reducing effect on those two variables.

Grafting the 'Santa Clara' tomato onto 'Maxifort' and 'Carolina' delayed flowering in the graft, with the parasitic *Nacobbus aberrans* further enhancing this delay in the grafted plants.

The rootstock 'Maxifort' displays moderate resistance to *Nacobbus aberrans*, and the rootstock 'Carolina' and ungrafted 'Santa Clara' are susceptible to the pathgogen. The reproduction factor of a pathogen is an important but insufficient piece of information when it comes to the selection of a rootstock. Although the pathogen can reproduce in 'Maxifort', the observed growth parameters and stress indicators suggest that this cultivar is a promising rootstock to be used in soils infested with *N. aberrans*. However, other complementary tools that focus on reducing the reproductive factor of *N. aberrans* should be tested against plant compositions containing this rootstock.

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