Performance of Green Power and Shincheonggang tomato rootstocks in *Ralstonia solanacearum* contaminated area

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Monteiro FP, Wamser AF, Ogoshi C, Valmorbida J, Cardoso DA, Perazzoli V 2020 – Performance of Green Power and Shincheonggang tomato rootstocks in *Ralstonia solanacearum* contaminated area. Plant Pathology & Quarantine 10(1), 28–43, Doi 10.5943/ppq/10/1/4

Abstract

The bacterial vascular wilt caused by *Ralstonia solanacearum* is an important limiting factor to tomato production in contaminated areas. To overcome this problem the performance of Green Power and Shincheonggang tomato rootstocks in an *R. solanacearum* highly infested area was determined by measuring their productivities and the disease incidences. Both rootstocks had higher production compared to the tomato plants without rootstocks. The first symptom of the tomato bacterial wilt was observed 20 days after the seedlings transplant. More than 90% of the tomato plants without rootstocks have died at the 92^o day after the transplant. At the end of the growing season was accounted for that 54% and 36% of Green Power and Shincheonggang rootstock plants died due to *R. solanacearum* infection, respectively. The area under the disease incidence progress curve for Green Power, Pay Pay, Shincheonggang and Compack were 3,643.50 ± 1,354.15; 11,959.50 ± 1,127.47; 1,638.00 ± 1,221.91 and 8,631.00 ± 728.98, respectively. The AUDIPC in both rootstocks were statistically different from the AUDIPC of plants without rootstocks. Despite the good performance of the rootstocks compared with plants without rootstocks, the *R. solanacearum* were found infecting them. Asymptomatic rootstocks plants of Green Power (74.91%) and Shincheonggang (98.44%) were infected by *R. solanacearum* confirmed through the vascular flow test. On average, the Green Power rootstock produced 115,925 fruits per hectare classified as extra AA and 160,333 fruits per hectare classified as extra A while Pay Pay plants without rootstocks produced 2,661 (extra AA) and 8,981 (extra A) fruits per hectare. The Shincheonggang rootstock produced 29,522 fruits per hectare classified as extra A fruits while Compack plants without rootstocks produced 416 fruits per hectare. The Compack plants without rootstock did not produce any extra AA fruits while the Shincheonggang rootstock produced 68,524 fruits per hectare. On the basis of the results, the rootstocks used herein are recommended as one component of a management program to control *R. solanacearum*.

Keywords – bacterial wilt – vascular disease – *Solanum lycopersicum*

Introduction

Ralstonia solanacearum, a widely distributed and economically important plant pathogen is considered a species complex with considerable variation in hosts, geographic origin, pathogenicity and physiological properties (Denny 2007). A review with historical facts and the current status of *R. solanacearum* of selected hosts in Brazil was published by Lopes & Rossato (2018). The bacterium is able to infect more than 53 botanical families which represent more than 200 host plant species, which includes commercial crops such as banana (*Musa* sp.), bean (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), chicory (*Cichorium endivia*), eggplant (*Solanum melongena*), eucalyptus (*Eucalyptus* sp.), geranium (*Pelargonium* sp.), ginger (*Zingiber officinale*), groundnut (*Arachis hypogaea*), melon (*Cucumis melo*), mulberry (*Morus* sp.), olive (*Olea europaea*), passion fruit (*Passiflora edulis*), pepper (*Capsicum annuum*), potato (*S. tuberosum*), scarlet eggplant (*S. aethiopicum* var. gilo), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), tomato (*S. lycopersicum*), watermelon (*Citrullus lanatus*) and zucchini (*Cucurbita* spp.) (Akiba et al. 1980, Hayward 1991, Jangir et al. 2018, Santiago et al. 2017). Formerly, the species was subdivided into six biovars based on carbohydrate metabolism (Hayward 1964) and five pathogenic races on the basis of host range (Buddenhagen 1962). Later, phylotypes were proposed together with sequevars (Fegan & Prior 2005). Four phylotypes (I, II, III and IV) which were associated with the geographic origin and eight clades with distinct evolutionary patterns were recognized in *R. solanacearum* (Wicker et al. 2012). The term sequevar (sequence variant) is used to designate infra-subspecific groups on the basis of endoglucanase gene sequencing (Fegan & Prior 2005). More than 50 sequevars has been reported (Peeters et al. 2013). In addition, Safni et al. (2014) using polyphasic analyses suggested the division of *R. solanacearum* species complex into three species: *R. pseudosolanacearum* sp. nov., *R. solanacearum* and *R. syzygii*, which were supported by Prior et al. (2016) by using genomic comparisons, proteomic analyses and metabolic characterization.

A review reports the prevalence of *R. solanacearum* race 1 and 3, biovar 1, 2 and 3 in the South of Brazil (Morais et al. 2015). Another study with 301 strains isolated from different hosts in Brazil reveals that the biovar 1 and 2, and the phylotype IIA and IIB are dominant in the South, which comprehends the states of Parana, Santa Catarina and Rio Grande do Sul (Santiago et al. 2016).

The most typical symptom of the bacterial wilt of tomato is the rapid wilt of the plant. In tomato plants, the disease appears in any developmental stage, but mainly during the formation of the first bunch. Once bacteria reach the wounds on the roots, they are housed in the conducting vessels of xylem, the vessel where the water flows from roots to the aerial part, and start to multiply producing a high concentration of viscous extracellular polysaccharides that block the xylem (Buddenhagen & Kelman 1964). The longitudinal cut of the stem at the bottom of affected plants can be performed to verify the browning of the vessels caused by the enzymes produced by the bacterium. When high inoculum conditions are present a white liquid may ooze from vessels after the transversal cut (Buddenhagen & Kelman 1964). In addition, the vascular flow test can confirm the bacterial tomato wilt caused by *R. solanacearum* (Lopes & Ávila 2005).

The persistence of *R. solanacearum* is high, once the bacteria established in soil they may survive for long period, for example, Stander et al. (2003) cultivating potato in an artificially infested field after five years of monoculture of potato, corn, bare-fallow and weed-fallow soils observed that 96%, 42%, 40% and 27% of plants wilted. Attempts to control the tomato bacterial wilt were performed to find resistant sources, but the resistance found has not been stable due to the existence of strains of the bacterium with different levels of virulence (Lopes et al. 1994, Wicker et al. 2007). Resistant genotypes identified so far do not present commercially desirable characteristics, the reason why it has been exploited mainly for use as rootstocks. Rivard et al. (2007) reported successful control of bacterial wilt by the use of CRA66 and HI7996 tomato rootstocks.

The objective of this work was to study the performance of Green Power and Shincheonggang tomato rootstocks in a highly *Ralstonia solanacearum* infested area by measuring their productivities and the disease incidences.

Materials & Methods

Experimental design and location

The experiment was performed in randomized blocks design with five replicates which one with 20 plants in a highly *R. solanacearum* contaminated area located at 26°49'06.6"S and 50°59'35.1"W during the season 2018-2019 in Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) at Caçador, Brazil. The commercial tomato rootstocks Green Power and Shincheonggang which are classified as resistant against *R. solanacearum* and the tomato without rootstocks Pay Pay and Compack were cultivated to determine their survival. Pay Pay and Compack were grafted on Green Power and Shincheonggang, respectively. The transplant of Green Power and Shincheonggang occurred in 22th October 2018 and 6th December 2018, respectively. After transplants, the remaining plants were kept in the greenhouse in high humidity conditions.

Isolate characterization and natural infection of other hosts

The characterization of biovar was performed as suggested by Huang et al. (2012) and host range was determined via natural infection by plating *Capsicum annum* and *Solanum tuberosum* in the same experimental area (26°49'06.6"S and 50°59'35.1"W), and through the analysis of spontaneous weed of *Solanum americanum*.

Ralstonia solanacearum diagnosis and disease incidence

Tomato plants were observed weekly after transplant to determine the appearance of the first wilted plant. Then, the evaluation was performed every three weeks. The area under the disease incidence progress curve (AUDIPC) was calculated as suggested by Campbell & Madden (1990). The vascular flow test and the longitudinal cut of the stem were performed to confirm the presence of *R. solanacearum* (Lopes & Ávila 2005) and the tetrazolium medium was used to determine the pathogenicity (Kelman 1954). The virulent wild type was irregularly-round, fluidal, white colonies which develop light pink centres (Kelman 1954).

Tomato fruit harvesting

During the production season, tomato fruits were harvested and classified as extra AA and extra A. For the cultivar Compack extra A fruits had a weight which varies between 100-150 g and extra AA were fruits that weight more than 150 g. For the cultivar Pay Pay extra A fruits had a weight which varies between 100-130 g and extra AA were fruits that weight more than 130 g.

Statistical analyses

The results were submitted to analysis of variance, when significant by the F test, the means were compared by the Tukey statistical test.

Results

The bacteria isolated were identified as *R. solanacearum* race 3, biovar 2 and caused the bacterial wilt disease in *C. annum*, *S. americanum*, *S. lycopersicum* and *S. tuberosum*.

The first symptom of the tomato bacterial wilt was observed 20 days after the seedlings transplant. Both tomato plants with rootstocks performed better than the tomato plants without rootstocks (Figs 1, 2). Some tomato plants with Green Power and Shincheonggang rootstocks considered resistant were susceptible showing symptoms of wilting disease and signs of the bacteria (Figs 3, 4). The AUDIPC for Green Power, Pay Pay, Shincheonggang and Compack were 3,643.50 ± 1,354.15; 11,959.50 ± 1,127.47; 1,638.00 ± 1,221.91 and 8,631.00 ± 728.98,

respectively. The AUDIPIC in both rootstocks were statistically different from the AUDIPIC of plants without rootstocks. On average, the difference of AUDIPIC between the rootstock Green Power and Pay Pay plant without rootstock was 328.24% and between Shincheonggang and Compack plant without rootstock was 526.92%. At the end of the growing season, 54% and 36% of Green Power and Shincheonggang rootstock plants died due to *R. solanacearum* infection, respectively. The destructive analysis of Green Power and Shincheonggang rootstock plants reveals that 74.91% and 98.44% of asymptomatic plants had *R. solanacearum* confirmed by the vascular flow test, respectively.

The formation of adventitious roots at the point of union between the rootstocks and the grafted plant was observed in the greenhouse and infield in high humid conditions (Fig. 5).

Plants with rootstocks were able to produce tomatoes fruits within commercial standards (Fig. 6). In a number of tomato fruits per hectare the Green Power rootstock produced on average 5,356.45% more fruits classified as extra AA and 1,785.25% more fruits classified as extra A than Pay Pay plants without rootstocks. In tons of fruits per hectare, the Green Power produced in average 3,157.69% and 1,757.00% more extra AA and extra A fruits compared to the Pay Pay without rootstocks. The Shincheonggang rootstock produced on average 7,096.64% more extra A fruits than Compack plants without rootstocks. The Compack plants without rootstock did not produce any extra AA fruits while the Shincheonggang rootstock produced on average 68,524.00 fruits per hectare. In tons of fruits per hectare, the Shincheonggang produced on average 8,160.00% more extra A than Compack plants without rootstocks. The Compack plants without rootstock did not produce any extra AA fruits while the Shincheonggang rootstock produced 13.73 tons of fruits per hectare.

Discussion

The rootstocks must not be indicated as the unique tactic aiming at the decrease of the bacteria population in the area, since it may allow *R. solanacearum* to increase the inoculum, even if the symptoms of wilt are not visible. Prior et al. (1996) previously reported that resistant plants may be heavily invaded by *R. solanacearum* without displaying wilt symptoms. The rootstock is indeed a technology which allows rentable tomato production in an *R. solanacearum* contaminated area, but they should be used before the disease pressure reaches high levels. Probably, the symptoms of the wilting disease will not be observed when the rootstocks are cultivated at low-pressure of *R. solanacearum*. Grafting tomato to manage bacterial wilt caused by *R. solanacearum* have been performed (Rivard et al. 2012), but the use of rootstocks cannot be an exclusive tactic to control the wilt disease, since the resistance can be overcome when the inoculum pressure in the soil is high or the strain find a way to get the resistance down (Lopes & Mendonça 2014, Lopes et al. 2015). Promising rootstock made of *Solanum torvum* may be another source for resistance (Singh et al. 2014). However, the level of control achieved is variable since the rootstocks react differently to the bacterial isolates (Lin et al. 2008, Wicker et al. 2007, Wang et al. 2000), but certainly, they delay the onset of the bacterial disease. Root wounding during transplanting (Kelman 1953), contaminated pruning knives, and the penetration of the cortex by root-knot nematodes (Lucas et al. 1955) are factors which affects the performance of the rootstocks. In addition, any incompatible agricultural practice that places the soil in contact with the region of union between the rootstock and the graft is incompatible with the planting of rootstock for the management of the bacterial wilt disease.

Successful rotation crops have been reported, for example, two seasons of non-host crops reduced wilt from 81% to 22-49% (Lemaga et al. 2001). The incorporation of the aerial part of pigeon pea (*Cajanus cajan*) and crotalaria (*Crotalaria juncea*) to the soil as a control method was successfully evaluated in greenhouse conditions by Cardoso et al. (2006). *Crotalaria falcata* as a component of crop rotation practices reduced the bacterial wilt incidence by more than 85% (Kakuhenzire et al. 2013). *Crotalaria spectabilis* and *Raphanus sativus* cv. melody grown as previous crops decreases the incidence of the disease (Deberdt et al. 2015). *Mucuna pruriens* monocropped reduce *R. solanacearum* population, but not the disease incidence (Adebayo et al.

2009). *Tagetes patula* was reported as a suppressive plant to *R. solanacearum* due to the production of thiophenes (Terblanche & Villiers 1998), which differ from *Tagetes minuta* considered a host of *R. solanacearum*. The rotation with corn could soil bacterial community composition and structure changed with *Acidobacteria* and *Actinobacteria* more abundant in the corn group rotation (Niu et al. 2017) which could lead to the control of the bacterial wilt disease. In addition, corn was considered immune to wilt (Smith 1939) and could be a suitable candidate for a rotation program. Successful eradication of potato wilt disease was already reported by Lloyd (1976), who suggests that a pasture rotation for two and half years minimum, control of tuber seed health and effective quarantine policy are efficient practices to control the bacterial wilt. Although there are numerous reports of the reduction in disease incidence in susceptible hosts after crop rotation practices (Navarro 1975, Smith 1944, Graham & Lloyd 1979), there are exceptions to this general rule, for instance, Jackson & Gonzalez (1981) reported that rotations with corn, cowpea, sweet potato, or wilt-resistant tomatoes, failed to reduce the incidence of disease on potatoes planted one year later, as compared with those planted on fallowed soil. The reason for that inconsistency may be due to the life cycle of *R. solanacearum* which include a saprophytic phase as in many other 'environmental pathogens' (Morris et al. 2009) and the bacterial ability to colonize plants that remain asymptomatic and weeds (Hayward 1991). Despite onions (*Allium cepa*) and garlic (*Allium sativum*) are not considered hosts for *R. solanacearum* (race 3, biovar II) they could support the bacteria survival under winter conditions that are unfavourable to this pathogen (Terblanche 2007), and could not be used in a crop rotation program.

Without discrimination of race or biovar numerous infected plants and weeds such as *Ageratum conyzoides*, *Amaranthus* spp., *Bidens pilosa*, *Brassica napus* cv. Oro, *Brassica oleracea* var. capitata, *Capsella bursa-pastoris*, *Celastrus orbiculatus*, *Cenchrus ciliaris*, *Cereus peruvianus* "monstruosus", *Chenopodium album*, *Cicer arietinum*, *Cichorium pamilium*, *Convolvulus arvensis*, *Cyphomandra betacea*, *Cyperus rotundus*, *Datura stramonium*, *Dopatrium* sp., *Erigeron floribundus*, *Eruca sativa*, *Eupatorium cannabinum*, *Euphorbia pelus*, *Galinsoga parviflora*, *Heliconia* sp., *Kalanchoe* sp., *Leucas martinicensis*, *Limonium* sp., *Ipomoea* sp., *Malva parviflora*, *Monochoria vaginalis*, *Oxalis latifolia*, *Pelargonium* sp., *Piper hispidinervum*, *Physalis angulata*, *Polygonum nepalensis*, *Portulaca oleracea*, *Raphanus raphanistrum*, *Rumex abyssinicum*, *Salpiglossis sinuata*, *Senecio vulgaris*, *Sida alba*, *Solanum americanum*, *S. cinereum*, *S. dulcamara*, *S. karsense*, *S. macrocarpon*, *S. melongena*, *S. nigrum*, *Sonchus oleraceus*, *Spergula arvensis*, *Stellaria sennii*, *Strelitzia reginae*, *Tagetes minuta*, *Talinum triangulare*, *Tropaeolum majus*, *Urtica dioica* and *Vigna sinensis* were already reported as hosts worldwide (Albuquerque et al. 2016, Álvarez et al. 2008, Graham & Lloyd 1978, Hayward 1975, Janse 1996, Martin & Nydegger 1982, Melo & Takatsu 1998, Lopes et al. 1997, 1999, 2002, Horita & Tsuchiya 2001, Olsson 1976, Pereira et al. 2001, Pradhanang & Momol 2001, Salcedo et al. 2017, Sikirou et al. 2015, Swanepoel 1992, Tomlinson et al. 2005, Tusiime et al. 1998, Wenneker et al. 1999, Wicker et al. 2007, Paz Zambrano 1990).

The bacteria could survive in the rhizosphere of some plants using the exudates as a nutrient source (Yao & Allen 2006), but it may not survive for long periods in vegetation-free soil (Granada & Sequeira 1983). In fact, Ayana & Fininsa (2016) observed that the population of bacterial pathogens form a declining trend but detectable in the rhizosphere soils and roots of presumable non-host crops at 30, 45, 60, 90 and 120 days after inoculation. However, evidence pointed that the *R. solanacearum* is capable to survive in prolonged nutrient scarcity for more than four months and retain the ability to cause disease when storage in the ultrapure water (Van der Wolf et al. 2005). Those bacteria are capable to survive for at least four years in river water (Alvarez et al. 2008) or irrigation ponds (Hong et al. 2008). The survival of *R. solanacearum* is strongly affected by soil type more than management, and could survive longer in clay than in sandy soils, but the disease progression may be higher in sandy soils (Stander et al. 2003). All those characteristics diminished the effect of crop rotation with non-host plants.

Combinations of different practices for the integrated management of bacterial wilt of tomato have been reported (Anith et al. 2004) and are recommended to preserve the rootstock technology.

Preponing or postponing transplantation times to the cooler period significantly decreased bacterial wilt disease incidence and increase the efficiency of biological control via bio-organic fertilizers fortified with pathogen-suppressive bacteria (Wei et al. 2015), because the low temperature can directly affect the virulence of *R. solanacearum* (Bocsanczy et al. 2014) and its population (Hong et al. 2008). Abiotic factors such as soil organic matter or clay content, pH, water holding capacity and temperature exert strong controls over microbial activities in soil (Van Veen et al. 1997) and could be a determinant to the success of a biological control. Competitive use of root exudates between *Bacillus amyloliquefaciens* and *R. solanacearum* decreases the pathogenic population density and effectively control tomato bacterial wilt (Wu et al. 2017). Rhizocompetence and antagonistic activity towards genetically diverse of *R. solanacearum* strains were detected on tomato plants by Xue et al. (2013). The use of *Pseudomonas fluorescens* to control the bacterial wilt of tomato (Khalequzzaman et al. 2002) and the utilization of bacteriophages to control *R. solanacearum* have been performed (Addy et al. 2012), such as the use of soilborne lytic *Podoviridae* phage (Elhalag et al. 2018) or the developing a bacteriophage cocktail for biocontrol of bacterial wilt (Wei et al. 2017). *B. amyloliquefaciens* and *Bacillus subtilis* strains and their derived bio-organic fertilizers have potential to control *R. solanacearum* (Ding et al. 2013). Endophytic bacteria from tomato was previously reported as antagonistic potential bacteria against *R. solanacearum* (Amaresan et al. 2012, Barretti et al. 2012).

Cow manure amendments may decrease the survival of *R. solanacearum* and clear shift the microbial community, which could improve a natural biological control (Messiha et al. 2007). Poultry and farmyard manure reduce incidence by fomenting the increase of microbial activity (Islam & Toyota 2004). Cow dung manure and Tef straw were the compost ingredients for FYM at 10% which were successfully used to suppress bacterial wilt and survival of *R. solanacearum* in the soil (Yadessa et al. 2010). A suppressive soil to *R. solanacearum* was previously reported by Shiomi et al. (1999). The application of bioorganic fertilizer enhances soil suppressive capacity against bacterial wilt of tomato (Liu et al. 2015) and some composts may enhance plant resistance against the bacterial wilt pathogen (Youssef & Tartoura 2013). A combination of poultry manure and solarization reduced the tomato bacterial wilt incidence (Baptista et al. 2006). The challenge is to maintain the soil healthy for crop production as suggested by Gamliel & van Bruggen (2016), and to increase the durability of the rootstock technology.

Fig. 1 – Average incidence of wilted plants of the tomato rootstocks Green Power and Shincheonggang and the tomato plants without rootstocks Pay Pay and Compack.

Fig. 2 – Tomato plants cultivated in the *R. solanacearum* contaminated area at 104° day from transplant. A Plants of Green Power tomato rootstocks. B Susceptibility of tomato plants Pay Pay without rootstock. C Plants of Shincheonggang tomato rootstocks. D Susceptibility of tomato plants Compack without rootstock.

Fig. 3 – Susceptibility of the rootstocks in the *R. solanacearum* contaminated area. A Green Power rootstock after 65 days from transplant. B Shincheonggang rootstock after 104 days from transplant.

Fig. 4 – Bacterial vascular flow test for rapid diagnosis of *R. solanacearum* from rootstocks.

Fig. 5 – Putative reason for the rootstock susceptibility. A Adventitious root formation at the point of the graft between the rootstocks and the grafted plant in a high humidity condition at the greenhouse. B Adventitious root at the point of the graft between the rootstocks and the grafted plant in the field.

Fig. 6 – Productivity of the rootstocks Green Power and Shincheonggang compared to plants without rootstocks cultivated in an *R. solanacearum* contaminated area. A-B Performance of Green Power rootstocks compared with Pay Pay plants without rootstocks. C-D Performance of Shincheonggang compared with Compack plants without rootstocks.

Application of chemicals may be part of the management program. For irrigation ponds, Hong (2005) suggested the use of chlorine at 2 mg/L for a bacterial concentration of 10^4 cfu/mL. Application of dazomet at 80 g/m² covered with a 40 µm polyethylene film reduces the population of *R. solanacearum* (Mao et al. 2017). Salicylic acid (SA) has been identified as a key element of wtl-mediated resistance to *R. solanacearum* (Denancé et al. 2013). Salicylic acid (SA) was able to induce sweet potato resistance against *R. solanacearum* through antioxidant enzymes (Yu et al. 2008). Use of both acibenzolar-S-methyl and *Pseudomonas fluorescens* by foliar sprays and soil drench (Abo-Elyousr et al. 2012), or chitosan and *Paenibacillus polymyxa* applied as a soil drench or seed treatment significantly reduced wilt incidence (Algarn et al. 2010). Successful use of thymol applied as a soil fumigant and acibenzolar-S-methyl as foliar spray applied together reduced the bacterial wilt in tomato (Hong et al. 2011). Application of acibenzolar-S-methyl enhances host resistance in tomato against *R. solanacearum* (Pradhanang et al. 2005). Liu et al. (2016) reported the efficiency of calcium cyanamide and other two inorganic nitrogenous amendments to suppress the survival of *R. solanacearum* in soil. Calcium nutrition also may suppress the disease progress in resistant tomato cultivars when the nutrient solution is supplemented with 4.4 mM of calcium (Yamazaki & Hoshina 1995). Amendment with urea and calcium oxide using 50 g urea mixing with 500 g calcium oxide per 16.2 kg of infested soil control *R. solanacearum* in ginger (Vudhivanich 2002). Copper sulfate and bleaching powder at 15 g/m² reduced wilt incidence by ≥ 50% (Verma & Shekhawat 1991). Soils treated with CaCO₃ (particles 0~1 mm) reduced the incidence of the bacterial wilt of *Tobacco* (He et al. 2014). Control of *R. solanacearum* through soil drenching with BABA (DL-3-aminobutyric acid) was also achieved (Hassan & Abo-Elyousr 2013). The application of silicon could mediate resistance in tomato against *R. solanacearum* through changes of soil microorganism amount and soil enzyme activity (Wang et al. 2013). A correlation between bacterial wilt resistance and translocation of sulfur, boron, calcium and nitrogen seems to exist in tomato (Hacisalihoglu et al. 2008). Soil pH and calcium content were key factors in determining soil bacterial communities (Niu et al. 2017). Attempting to control *R. solanacearum* have been reported such as the use of *Eichhorina crassipes* aqueous extract *in vitro* (Alemu et al.

2013). Phytochemicals have been exploited to control *R. solanacearum* in the greenhouse (Din et al. 2016). A method called biological soil disinfestation which induces an anaerobic condition to the soil by increasing microbial respiration through the incorporation of fresh organic amendments and by reducing re-supply of oxygen by covering with airtight plastic sheets may be promising (Messiha et al. 2007).

In this work was not possible to determine whether rootstocks are resistant or tolerant, because, the rootstock was not cultivated without grafting, a technique which may induce adventitious roots at certain conditions that can be a point of bacteria entrance. But as both rootstocks harbored *R. solanacearum* in asymptomatic plants and others have died, they seem to have tolerance against the bacteria. In addition, when the resistant rootstock is used as the scion to graft onto the susceptible rootstock, the “resistant” plant succumbed to the disease (Obrero et al. 1971). The rootstocks physically limit the movement of bacteria from the soil to the scion (Grimault & Prior 1994), acting as a filter, and the roots of the rootstocks seem to play a crucial role to induce tolerance against *R. solanacearum*. The bacterial *sequevar* molecular characterization was not performed. As the objective of the work was to explore the practical issues for the production of tomatoes in an area with a high inoculum of *R. solanacearum*, this aspect will be explored later. Therefore, the use of the rootstocks combined with the management practices discussed herein is indispensable for tomato cultivation in long term.

Acknowledgements

Authors are thankful to Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) and Foundation for Research and Innovation Support of the State of Santa Catarina (FAPESC) for financing and encouraging the execution of the project that gave rise to this publication. The authors also would like to thank the technician Jonas Borba Pereira for its assistance on this study.

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