



Pith Necrosis of Tomato Caused by *Pseudomonas viridiflava* May Not Decrease Production

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Authors' contributions

This work was performed in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2019/v4i430030

Editor(s):

(1) Dr. Magdaléna Valšíková, Professor, Department of Vegetables Production, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture (SUA), Nitra, Slovakia.

Reviewers:

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(2) Stefan Martyniuk, IUNG-PIB, Poland.

(3) Ningappa M. Rolli, BLDEA's Degree College, India.

Complete Peer review History: <https://sdiarticle4.com/review-history/52324>

Short Research Article

Received 01 September 2019

Accepted 02 November 2019

Published 13 November 2019

ABSTRACT

Aims: This study aimed to measure the losses in the production of tomato plants caused by the bacteria *P. viridiflava*.

Study Design: Experiments were performed in a completely randomized design with six replicates.

Place and Duration of Study: The study was conducted on the Caçador experimental station of the Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) from October to April during 2017/2018 and 2018/2019 crop season.

Methodology: Bacteria were isolated from tomato plants with pith necrosis symptoms, using nutrient agar. The isolated strain was identified by a scheme of tests for bacteria that emit fluorescence, known as LOPAT, and by sequencing the 16S rDNA region. Tomato plants were cultivated for two seasons during 2017/2018 and 2018/2019. In the first year the cultivar Paronset was cultivated and in the next season the experiment was performed with the cultivars Compack,

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Nagai, Paronset and Pizzadoro. During the production season, tomato fruits were harvested and the weight was accounted for. At the beginning of the first bunch formation, the stems of the plant were inoculated with wood sticks containing bacterial colonies removed from a 48h-Petri dish culture medium. Tomato plants cultivated as control treatments were not inoculated. At the end of the cultivation seasons, the stems were cut to analyze the pith necrosis progress.

Results: In both cultivation seasons, there was no decrease in the production associated with the pith necrosis caused by *P. viridiflava* EPAGRI BacPvT1 because the total weight of fruits harvested from inoculated plants was not statistically different compared to the non-inoculated plants. The disease progressed in all inoculated plants and adventitious root formation as external symptoms was observed.

Conclusion: The bacteria *Pseudomonas viridiflava* EPAGRI BacPvT1, one of the etiological agents of pith necrosis of tomato, may not decrease the production. Even causing some injuries, it may be not cause any damage.

Keywords: *Fluorescent bacteria; Solanum lycopersicum; tomato disease.*

1. INTRODUCTION

The Pith necrosis disease affects tomato plants causing necrosis and destruction of the pith. The symptoms commonly observed, as a reaction of the affected plants, are brown spots and cracks in the stem, and adventitious roots in unusual places, even inside the stem. Cut stems revealed brown coloured pith [1]. Attacked plants also present yellowing of the leaves and less development that may be related to lower productivity. However, damage associated with the disease is not clearly known since some authors report the disease with low aggressiveness and few expressive damages [2] while others suggest that pith necrosis may cause huge losses [3].

Besides *P. viridiflava* [1], the bacteria *Pseudomonas agglomerans* [4], *Pseudomonas corrugata* [5], *Pseudomonas mediterranea* [6], *Pseudomonas marginalis* [7], *Pseudomonas cichorii* [8], *Pseudomonas fluorescens* and *Xanthomonas perforans* [9] may cause the pith necrosis of tomato. Up to now, only *Pseudomonas corrugata*, *P. viridiflava* and *Pseudomonas mediterranea* were reported as the etiological agent of tomato pith necrosis in Brazil. The former was reported in the States of São Paulo [10], Rio Grande do Sul [11] and Goiás [5], *P. viridiflava* was reported in the State of Santa Catarina [1], and the latter was reported in the State of São Paulo [6].

In March 2017, Paronset tomato plants with pith necrosis symptoms were observed in commercial and experimental fields in Caçador, Santa Catarina state (Southern Brazil). The incidence of the disease was above 90% in affected fields [12]. After several trials, the bacteria *P. viridiflava*

was isolated and identified [1]. Therefore, this study aimed to measure the losses in the production of tomato plants caused by the bacteria *P. viridiflava*.

2. MATERIALS AND METHODS

2.1 Isolation and Characterization of *Pseudomonas viridiflava*

Bacteria were isolated from tomato plants with pith necrosis symptoms, using nutrient agar [13]. The isolated strain was identified by a scheme of tests for bacteria that emit fluorescence, known as LOPAT, which is a series of determinative tests – L, levan production; O, oxidase production; P, pectinolytic activity; A, arginine dihydrolase production; and T, tobacco hypersensitivity [14], and by sequencing the 16S rDNA region. The molecular identification was performed by amplifying the V3-V4 region of the 16S rDNA, using the primers 341F – CCTACGGGRSGCAGCAG [15] and 806R – GGACTACHVGGGTWTCTAAT [16].

Koch's postulates were performed with Paronset tomato plants grown in a greenhouse and in the field. In the greenhouse, tomato seeds were sown directly in the soil. At age of 30 days, using a needle, the plants were inoculated with 1 ml per plant of a suspension of 10^6 CFU/ml. Plants were grown for 7 days after inoculation. In the field trial, the bacteria were inoculated with a wood sticks at 15 cm height from the soil and kept growing for 60 days. The stems were then cut and sliced to see any possible colour modification of the pith compared to the healthy plants, and the bacteria were re-isolated to complete Koch's postulates.

The sequence was deposited in Genbank with the accession number MG396956. Using two storage methods, the isolate was kept in the culture media collections of the Phytopathology Laboratory at Caçador Experimental Station, Santa Catarina State, Brazil, identified by the accession number EPAGRI BacPvT1. The isolate was also stored in the Phytobacteria Culture Collection of the Instituto Biológico, São Paulo State, Brazil (WDCM 110) as IBSBF 3287. Further information about the isolate used herein can be found in [1].

2.2 Tomato Cultivation, Bacterial Inoculation and Fruit Harvesting

Tomato plants were cultivated for two seasons during 2017/2018 and 2018/2019. In the first year the cultivar Paronset was cultivated and in the next season the experiment was performed with the cultivars Compack, Nagai, Paronset and Pizzadoro installed in a completely randomized design with six replicates per treatment. At the beginning of the first bunch formation, the stems of the plant were inoculated with wood sticks containing bacterial colonies removed from a 48h-Petri dish with nutrient agar. Tomato plants cultivated as control treatments were not inoculated. During the production season, tomato fruits were harvested and the weight was accounted for. At the end of the cultivation seasons, the stems were cut to analyse the pith necrosis progress.

2.3 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.

3. RESULTS AND DISCUSSION

In both cultivation seasons, there was no decrease in the production associated with the pith necrosis caused by *P. viridiflava* EPAGRI BacPvT1 because the total weight of fruits harvested from inoculated plants compared to the non-inoculated plants was not statistically different ($p>0.05$) (Figs. 1 and 3). The disease progressed in all inoculated plants (Figs. 2 and 4), and adventitious root formation as external symptoms was observed.

High rates of natural infections occur along the tomato plant cycle [12], mainly after sprouts removal, a constant cultural practice in tomato crop. The possibility of pith necrosis cause losses in the tomato productivity still exist, because despite the bacteria *P. viridiflava* EPAGRI BacPvT1 may not cause any damage in the conditions described herein, other bacteria associated with pith necrosis might debilitate the tomato plants. In addition, *P. viridiflava* have a significant genetic variation [17], which can make arise a versatile pathogen leading to losses in the productivity of tomato.

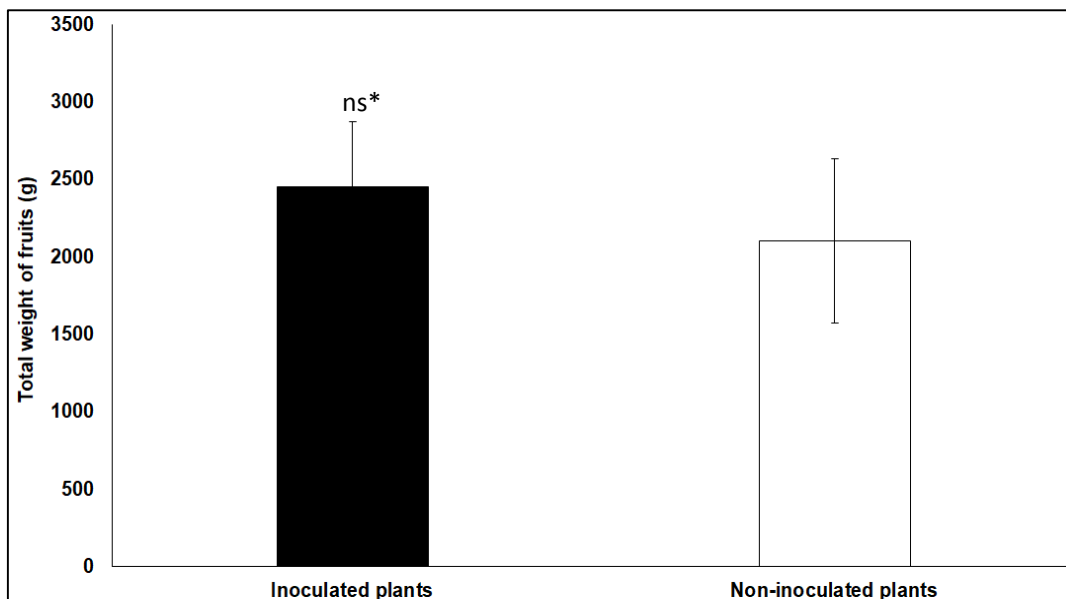


Fig. 1. The total weight of fruits from inoculated plants compared to the non-inoculated plants

* There was no statistical difference between treatments ($p>0.05$)



Fig. 2. Pith necrosis in tomato stems, cultivar paronset, inoculated with *P. viridiflava* compared with the non-inoculated plant

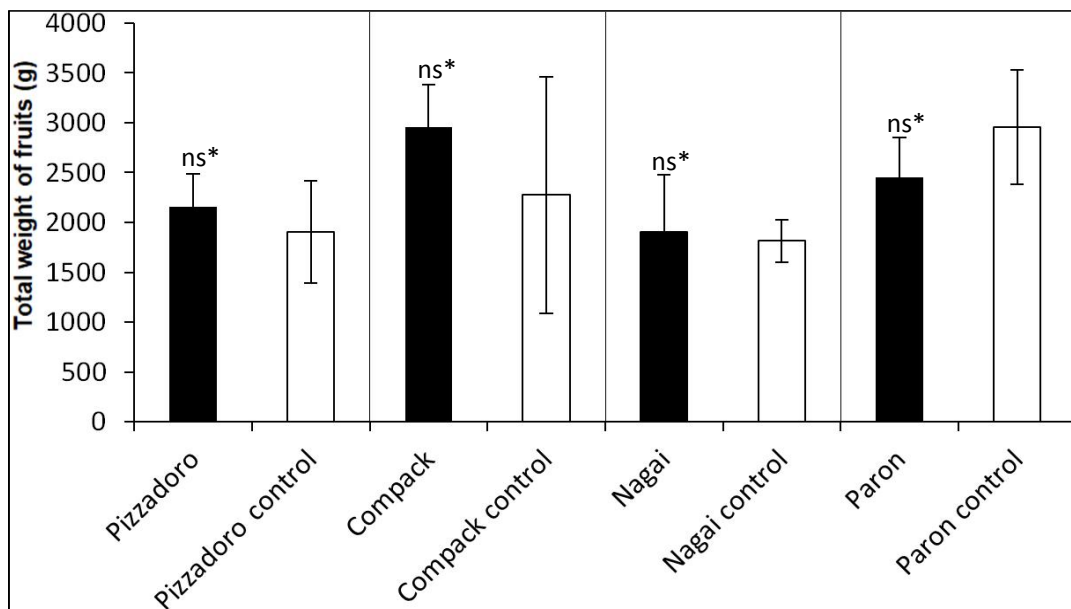


Fig. 3. The total weight of fruits from inoculated plants compared to the non-inoculated plants of the tomato cultivars pizzadoro, compack, nagai and paronset

* There was no statistical difference between treatments ($p > 0.05$)

Any visible symptom caused by a harmful organism is called injury, and any reduction in quality or quantity of production is called damage [18]. Then, although there are injuries, as such changes in the color of the pith, the affected tissues remain firm, which may not compromise the flow of nutrients.

The first sprouting removal takes place 45 days after tomato transplantation, and it represents the first gateway to the bacterium. After the first sprouting removal, the operation is done weekly, so every week the wounds are made on the plants, and consequently, new entrance doors are opened.



Fig. 4. Pith necrosis in tomato plants inoculated with *P. viridiflava* compared with the non-inoculated plants

A - Pith necrosis in tomato stems of cultivar paronset; B - Pith necrosis in tomato stems of cultivar compact; C - Pith necrosis in tomato stems of cultivar pizzadoro; D - Pith necrosis in tomato stems of cultivar nagai

Accounting a total of 12 sprouting removal until the apical tomato stem is removed. Thus, there is a temporal difference in the opening of wounds throughout the crop cycle that goes through different weather conditions that could favour different bacteria, and thus, with each new wound, a bacterium of a different species could infect the same plant.

Sometimes the pith necrosis may be coexisting with stem rot disease [19], caused by species of *Pectobacterium* that cause huge losses during tomato cultivation, which may lead the confusion about losses caused by pith necrosis.

4. CONCLUSION

The bacteria *Pseudomonas viridiflava* EPAGRI BacPvT1, one of the etiological agent of pith necrosis of tomato, may not decrease the production. Even causing some injuries, it may be not cause any damage.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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