engineering proceedings

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Proceeding Paper

Susceptibility of Twenty-Three Kiwifruit Cultivars to *Pseudomonas syringae* pv. *actinidiae* †

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† Presented at the 13th EFITA International Conference, online, 25–26 May 2021.

Abstract: One of the best methods to control plant disease is the use of resistant cultivars. The purpose of this study is to evaluate 23 kiwifruit genotypes and cultivars for susceptibility to four strains of Psa (biovar 3) in alaboratory setting. The results showed that all the bacterial strains were pathogenic. There was no statistical difference among the bacterial strains tested. None of the kiwifruit cultivars tested were immune to Psa. There was a statistical difference in the level of susceptibility among cultivars. The cultivars Sorelli and D495/312 were the most susceptible, while the cultivar A501/44 was the most resistant. However, the above results must be verified in field conditions.

Keywords: kiwifruit; *Pseudomonas syringae* pv. *actinidiae*; resistance

Citation: Thomidis, T.; Goumas, D.E.; Zotos, A.; Triantafyllidis, V.; Kokotos, E. Susceptibility of Twenty-Three Kiwifruit Cultivars to *Pseudomonas syringae* pv. *actinidiae*. *Eng. Proc.* **2021**, *9*, 33. https://doi.org/10.3390/engproc2021009033

Academic Editors: Dimitrios Aidonis and Aristotelis Christos Tagarakis

Published: 9 December 2021

1. Introduction

Greece is one of the major kiwifruit producers in the world. Bacterial canker is a dangerous kiwifruit disease. In 1984, *Pseudomonas syringae* pv. *actinidiae* (Psa) was isolated and described in Japan, followed by Italy and South Korea on cv. Hayward (*A. deliciosa*). In 2015, the disease was identified for the first time in a Greek kiwifruit orchard, Palios Mylotopos Pella, Northern Greece [1].

Because growers do not spend money on unnecessary control methods, such as fungicides, the utilization of resistant cultivars is critical in disease control. Even when fungicides are required, cultivar resistance can be a valuable supplemental management tool. Fruit trees have been screened for resistance to bacterial diseases using a variety of approaches. Screening cultivars for bacterial canker resistance with stem inoculation is a frequent practice. In the lab, the excised twig assay is also used to determine cultivar resistance to bacterial infections. Both approaches are easy to use, convenient, and allow for a large number of replications.

A research program was carried out at the Zeus Actinidia SA company (Karitsa, Katerini Pieria, Greece) on the selection of kiwifruit genotypes suitable for Greek climate conditions. Based on their agronomic characteristics, 19 of these were identified as promising genotypes (productivity, fruit quality, etc.). However, there was no information on these genotypes' susceptibility to bacterial canker disease caused by Psa. The goal of this study was to investigate the level of susceptibility of the above genotypes to Psa in laboratory. The screening test included four commercially used kiwifruit cultivars.

2. Materials and Methods

Psa (biovar 3) strains obtained from kiwifruit with bacterial canker symptoms from Pella, Northern Greece, were used.

2.1. Excised twig Assay

Jeffers et al. [2], Krzesinska and Azarenko [3], and Thomidis et al. [4] explained the procedures used in these investigations. To obtain a layer of 10 mm, nutrient agar with 1.5 g of boric acid, 8 mg of cephalexin, and 20 mg of cycloheximide per liter was poured in sterilized Pyrex jars (9 cm diameter and 12 cm height). They were then inoculated with one of the above Psa strains and incubated for 7 days at 25 °C in the dark. From each kiwifruit genotype and cultivar tested, one-year-old dormant shoots measuring 50 cm in length and 10–15 mm in diameter were collected. Twigs measuring 10 cm in length were cut, surface disinfected for 5 min with 10% home chlorine bleach (sodium hypochlorite; 4.8%), and then rinsed three times with sterile water. Twigs were clipped to a slant at the base and put upright into the growing culture using a flamed sharp knife. Jars were resealed and placed in incubators at 20 degrees Celsius for 7 days. The length of necrosis was calculated by subtracting the depth of agar from the overall length of necrosis that formed. This experiment was carried out two times. Each jar contained ten twigs. Each genotype/cultivar had nine jars, three for each Psa strain. As a control, two non-inoculated jars were used.

2.2. Excised Shoot Assay

An excised shoot assay described by Matheron and Matejka [5] and Thomidis et al. [4] was used in a second laboratory experiment. Woody shoot segments measuring 15 cm in length and 1.5–2 cm in diameter were gathered from each kiwifruit genotype, and cultivar was evaluated. They were disinfested by soaking them in 10% household chloride bleach (sodium hypochlorite; 4.8%) for 5 min and then rinsing them three times with sterile water. The segments were inoculated with bacterial cells after removing 6 mm of epidermis from the core. To avoid desiccation, the wound was coated with petroleum jelly and sealed with adhesive tape. The length of necrosis was measured after inoculated segments were cultured for 7 days at 20 °C in moist chambers. Each cultivar had nine duplicates of five segments, and each Psa strain had three. As a control, non-inoculated portions were employed. This experiment was carried out two times.

The experimental design employed in both tests was completely randomized throughout. One-way analysis of variance was used to analyze the data. Duncan's Multiple Range Test (P0.05) was used to compare treatment means.

3. Results and Discussion

Given the importance of bacterial canker for kiwifruit agriculture, it is critical for producers to understand the level of susceptibility of cultivars to that disease. The ability of the host to thwart the pathogen's progress is referred to as resistance. Host resistance is probably the most valuable agricultural control measure, and it works best when sanitation measures are also used. In the resistant cultivar, resistance is effective throughout the colonization stage [6,7].

The major goals of this study were to conduct laboratory studies to determine the sensitivity of 23 kiwifruit genotypes and cultivars to Psa (excised twig assay and excised shoot method). These approaches can be effective for determining Psa susceptibility [4,8] since they are reliable and rapid, allow for a lot of replication, and can be used to practically any type of woody plant host. However, if relative rather than absolute levels of disease are investigated, the inoculation of stems, excised twigs, and shoots might be a valuable procedure for measuring plant sensitivity to wood diseases. The explanation for this is because changes in the physiology of excised phloem tissue caused by physical detachment from the growing plant may modify resistance to colonization by wood diseases. Furthermore, direct inoculation of the cambium and inner phloem tissues only assesses resistance

mechanisms that are active once the pathogen has penetrated host tissue, obviating the need to test defense mechanisms present in the outer phloem tissue [9].

All of the bacterial strains were found to be harmful, according to the findings. There were no statistical differences between the bacterial strains that were studied (so the data were combined). All the kiwifruit genotypes and cultivars examined were infected by the bacterial strains tested (Table 1). There was a significant difference in susceptibility levels between genotypes and cultivars. Sorelli and D495/312 were the most susceptible, whereas A501/44 was the most resistant.

Table 1. The level of the susceptibility of 23 kiwifruit genotypes and cultivars on *Pseudomonas syringae* pv. *actinidiae* in laboratory.

Genotypes and Cultivars	"Excised Twig Assay"		"Excised Shoot Assay"	
Hayward	18.7 [x]	bcd [y]	14.0	abcd
Tsehelidis	17.0	abcd	14.7	abcd
Summerkiwi	19.3	bcd	15.0	abcde
Sorelli	20.7	cd	19.0	e
C496/447	17.3	abcd	15.0	abcde
C497/325	17.3	abcd	13.7	abc
A501/3	17.0	abcd	13.0	abc
B497/001	18.0	abcd	15.3	bcde
D495/312	21.3	d	18.0	de
A501/18	17.0	abcd	14.7	abcd
B501/22	18.3	bcd	15.7	cde
D4398/337	18.0	abcd	14.3	abcd
A501/44	15.0	ab	11.0	a
C497/352	16.0	abc	11.3	ab
D497/355	16.3	abc	12.0	abc
A501/103	16.7	abcd	13.3	abc
C497/76	19.0	bcd	13.7	abc
B484/56	17.3	abcd	15.7	cde
A501/28	18.0	abcd	12.7	abc
A497/25	18.0	abcd	16.0	cde
D498/325	19.7	bcd	14.0	abcd
B497/76	17.3	abcd	12.7	abc
C501/39	18.0	abcd	13.7	abc

[x] Values are the means of the two experiments; results were similar so the data were combined. The Psa strains showed statistically similar aggressiveness, so the data were combined. [y] Treatment means were separated by using Duncan's multiple range test ($p = 0.05$).

4. Conclusions

Based on the above results, the cultivars Sorelli and D495/312 should not be used when a kiwifruit orchard is established in areas where the bacterium Psa is present. In such areas, a good choice could be the cultivar A501/44. However, the above results must be verified in field conditions.

Author Contributions: Conceptualization, T.T. and D.E.G.; methodology, T.T. and D.E.G.; formal analysis, A.Z.; investigation, T.T. and D.E.G.; resources, E.K.; data curation, V.T.; writing—original draft preparation, V.T.; writing—review and editing, T.T. and D.E.G.; visualization, T.T.; supervision, T.T.; project administration, T.T.; funding acquisition, T.T. and D.E.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014–2020 (EPAnEK) under the call "Research-Create-Innovate", project number: T1EΔK-03107, entitled "Distribution of the bacterium *Peudomonas syringae* pv *actinidiae* in Greek Kiwifruit orchards and evaluation of cultural and chemical methods to control this disease".

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the agreement with the A.S. EPISKOPIS.

Acknowledgments: We would like to express our thanks to John Cullum for his technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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