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REACTION OF PLUM CULTIVARS AND ROOTSTOCKS TO BACTERIAL BLIGHT (*PSEUDOMONAS* SP.)

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Abstract

The bacterial blight caused by the Gram-negative bacteria Pseudomonas sp. is a significant problem in stone fruit orchards. The resistance or tolerance of the cultivars is one important strategy for disease control. In the frame of a study conducted with the support of the BNSF, administrative contract КП-06 М 46/2, was compared the reaction of plum cultivars and rootstocks, after inoculation with the bacterial pathogen Pseudomonas sp. Flowers and shoots of the cultivars 'Topgigant Plus' and 'Jojo' grafted on the two rootstocks - 'Docera 6' (Prunus domestica L. x Prunus cerasifera Ehrh.) and the seedling myrobalan plum rootstock (Prunus cerasifera Ehrh) were artificially inoculated. In addition, two years old rootstocks 'Docera 6', seedling P. cerasifera and 'Myrobalan 29C' (Prunus cerasifera) also were inoculated with bacterial suspension. The cultivar 'Topgigant Plus' had lower susceptibility to flower infection when grafted on the seedling rootstock than 'Docera 6'. The reaction of rootstocks fourteen days after inoculation showed a lesion diameter of 400 mm on 'Docera 6', 433 mm measured on 'Myrobalan 29C', and 41 mm on the seedling rootstock P. cerasifera.

Key words*: Artificial inoculation, 'Docera 6', 'Jojo', 'Myrobalan 29C', Pseudomonas sp., Topgigant Plus.*

INTRODUCTION

The genus *Prunus* contains over 400 species, including the European plum. *Prunus domestica* L. is a traditional fruit crop in Bulgaria (Bozhkova & Savov, 2016). The South Central Region represents 22.2% of the total area occupied by plum trees and this culture occupies third place after walnuts and sweet cherries concerning planted areas (Ministry of Agriculture and Food, 2021). Bacterial canker, caused by members of the *Pseudomonas syringae* species complex, can be a major limiting factor in the cultivation of *Prunus* spp. (Omrani et al., 2019; Vicente et al., 2004). The diseases of fruit trees caused by the strains of *P. syringae* are resulting in severe economic losses (Gomila et al., 2017; Lee et al., 2015; Young, 2010). Due to a lack of effective control measures, plant diseases caused by bacteria are a significant problem for the global horticultural industry (Sundin et al., 2016; Lee et al., 2023). Independently of the genotype used as rootstock, the most important cultural practice for avoiding tree losses caused by *Pseudomonas* and other wound parasites is to avoid damage to the stem (Hinrichs-Berger, 2004). The disease caused by bacteria of *Pseudomonas* sp. is primarily characterized by necrosis, gummosis and dieback of woody plant tissues. In addition, the pathogens colonize other plant tissues where they exist epiphytically or invade to cause leaf and fruit spots and blossom blight. These tissues can be reservoirs for later woody tissue infection (Crosse, 1966). The phytopathogenic bacteria *P. syringae* is associated with several plant species (Gašić et al., 2018; Kennelly et al., 2007; Lee et al., 2015; Ruinelli et al., 2019), including fruits and ornamental plants (Scortichini et al., 2003; Vicente and Roberts, 2007). It is a prevalent bacterial pathogen that can incite stem and leaf diseases in various crop plants, particularly in temperate regions (Scholz-Schroeder et al., 2001). *P. syringae* complex infects woody tissue, exhibits the symptoms of cankers, and eventually spreads to the entire wood and kills branches (Gomila et al., 2017; Perminow et al., 2018).

The rapid laboratory-based tests allowing screening for tree resistance is a major challenge underpinning the rapid development of new cultivars that resist pests and diseases (Hulin et al., 2018). Bacterial canker

(*Pseudomonas syringae* van Hall) is an important disease in most plum-producing countries. According to Ramming and Cociu (1991), plums grafted on peach rootstock are less susceptible than those on plum rootstock. 'Myrobalan' rootstock is less susceptible than 'Marianna' rootstocks. Nothing is known about the inheritance of resistance to bacterial canker in plums. It remains essential for future breeding work to find sources of resistance against bacterial canker. A resistance test was developed for sweet cherries, which could be adapted to plums (Santi et al., 2004).

The rootstock Marianna (a hybrid of *P. cerasifera* and a native plum), is reported as prone to infection (Wilson et al., 1953). Seedling myrobalan plum (*Prunus cerasifera*) is much more resistant as are most mazzard seedlings. The Myrobalan B, Purple Pershore and Mahaleb were supposed resistant. Grubb (1937) does not feel that there is any direct evidence that rootstocks affect the resistance of the scion. If such an effect does exist, it is thought to be due to the influence on vigor.

Differences in susceptibility to different parts of the disease cycle of *Ps. syringae* have been reported on many occasions. Growers have noted that certain cultivars in mixed plantings were more tolerant of infection than others. Lists have been published by many research centers giving the degree of susceptibility of the cultivars in their experimental plantings. These lists have varied widely depending on the area, the phase of the disease being considered, and the horticultural practices. The degree of resistance, or tolerance, of a particular cultivar, frequently depends on its stage of development concerning the time that inoculum is available. Because of differences in the time of infection and climatic conditions, cultivars listed as resistant in one part of the world are classified as very susceptible in another region. Within the *Prunus* species, apricot is listed as the most susceptible, sweet cherry and some plum cultivars as second, followed by nectarine (Barss, 1915; Cameron, 1962).

Our study aimed to compare the reaction of different tree parts of plum cultivars and rootstocks after artificial inoculation with the bacterial pathogen of *Pseudomonas* sp.

MATERIALS AND METHODS

Disease symptoms of bacterial blight were observed and the phytopathogen was collected from an experimental plum orchard at the Fruit Growing Institute-Plovdiv, Bulgaria. In the growing seasons of 2021 and 2022, a survey has been conducted to investigate the symptoms of plum canker, lesions on branches, twigs and shoots as well as angular spots on the leaves and blight on flowers. The symptomatic tissues from flowers, shoots and branches cut were into squares using a scalpel and the surface was disinfected with 75% ethyl alcohol. The samples were washed 3 times with distilled and sterile water, and tissues were placed on King's B media. A single colony of potential strain was transferred on King's B media after 48 h conducted for fluorescent pigment. The obtained isolates were infiltrated into a tobacco leaf's mesophyll to detect a tobacco hypersensitivity (HR) reaction. The bacterial suspensions was 10^8 cfu/ml, positive response to white necrosis at the infiltrated area 24 h after inoculation indicates the ability to induce an HR. To assess the pathogenicity of pathogens green plum fruits (cultivar 'Topgigant Plus'), which were surface disinfected were artificially inoculated with bacterial suspensions (10^6 cfu/ml) by injecting. For negative control were used green plum fruits injected with sterile water. All plum fruits were covered with plastic bags.

As a plant material in the experiment were used blossoms and shoots of the cultivars 'Topgigant Plus' and 'Jojo' grafted on the two rootstocks - 'Docera 6' (*Prunus domestica* L. *x Prunus cerasifera* Ehrh.) and the seedling myrobalan plum rootstock (*Prunus cerasifera* Ehrh) which were artificially inoculated.

The study used also two years old plants of the rootstocks 'Docera 6', the seedling *P. cerasifera* and the clonal 'Myrobalan 29C' (*Prunus cerasifera*).

For artificial infection of the blossoms were used 1-year-old shoots, from trees show no symptoms of diseases. A minimum of 200 flowers were used in each repetition for each cultivar/rootstock combination. They were cut in BBCH 57 phenophase and placed in water containers. The inoculation was done by

spraying the opened flowers (BBCH 65) with bacterial suspension (10^6 cfu/ml). Sprayed shoots were covered with moist plastic bags and maintained in favorable conditions (t = 23°C and 80-90% relative humidity) for 6 days. Symptoms development was monitored daily. After 5 days, the degree of attack was determined for each flower using a 5-grade scale:

0 - no symptoms;

1 - symptoms of necrosis on petals;

2 - symptoms of necrosis on pistil and receptacle;

3 - symptoms of necrosis observed on sepals;

4 - symptoms of necrosis observed on all flower parts.

An average degree of attack was calculated. The level of susceptibility of the flowers was formed in basic degree attack using a 6-grade scale:

0- immune (no infection);
1- resistant (single infected flowers);
2- low susceptible (1-10% infected flowers);
3- moderately susceptible (11-25% infected flowers);
4- susceptible (26-50% infected flowers);
5- highly susceptible (over 50%).

Plum shoots from the cultivars 'Topgigant Plus' and 'Jojo' grafted on 'Docera 6' and *P. cerasifera* were cut in the dormant period and placed in water containers. The shoots' surface was disinfected and inoculated with bacterial suspension (10^8 cfu/ml). Ten shoots in repetition were inoculated by sterile needle and injection of 25µl bacterial suspension.

In the dormant period two-year-old plants of the rootstocks 'Docera 6', 'Myrobalan 29 C' and seedlings *P. cerasifera* were planted in 3 liters plastic containers. The rootstock surface was disinfected and inoculated using the same methodology as the shoots infection. The shoots and rootstocks were incubated at 25°C for 2 weeks. To compare the reaction of the plum cultivars and rootstocks, lesion blight (mm) were measured in 7 and 14 days after inoculation.

Each artificial inoculation was done with 24h bacterial cultures plates on KB media and incubated at 23°C.

The pathogen was reisolated from all symptomatic tissues, to complement Koch's postulates and was compared to the original isolates.

RESULTS AND DISCUSSIONS

Bacterial blight in the experimental plum orchard in Plovdiv, Bulgaria, was identified by visual observations and confirmed by laboratory tests. A total number of nine isolates were collected from different trees in the orchard. Of these, only four isolates exhibit fluorescence under UV light. All the studied potential *Pseudomonas* spp. were Gram-negative and positive to the tobacco HR test. The tobacco leaves became hypersensitive 24 h after the introduction of the pathogen into the leaves. The positive reaction was observed in 3 days after inoculating green fruit plums with all tested strains.

Crosse and Benmett (1955) observed the damage caused by *Pseudomonas* spp. on plum orchards. The cankers on plums start as small, brown to reddish-brown spots that enlarge as water-soaked streaks. In the spring, the area between the streaks turns brown and the area becomes uniformly brown and moist (Wilson & Hewitt.,1939; Wormald., 1932). Wilson et al. (1939) also describe gum formation as follows: "As a rule, little if any, gum is exuded from the affected tissues but, a watery material may flow from cracks in the bark and cover the limbs. The absence of gum is particularly noticeable in the case of plums." Cracks appear around the margin of the canker as the dead area dries out.

From all isolates, one was selected for artificial infections. The strain number TG/D007 was gram-negative, had positive HR on tobacco, produced fluorescent pigment after 24 h cultivated on KB media and was positive for pathogenicity test on green fruits.

As a control variant was used the cultivar 'Stanley' grafted on *P. cerasifera* rootstock.

Disease severity in cultivar 'Jojo' grafted on *P. cerasifera* rootstock was 3.49% that was a high value in the experiment (Table 1). This cultivar/rootstock combination was with statistically significant difference compared to the other studied cultivars. For 'Jojo' grafted on 'Docera 6' was calculated 2.40% severity of the disease. The difference was statistically significant compared to the 'Jojo'/ *P. cerasifera*. The control cultivar showed 0.67% severity of the disease.

Table 1. Percentage of infected plum flowers

Cultivar/rootstock	Diseases severity (%)	Degree of attack (%)	Level of susceptibility
Jojo/Docera 6	2.40 b	49.17	susceptible
Jojo/*P. cerasifera*	3.49 a	88.0	high
Topgigant Plus/Docera 6	0.56 c	12.70	moderate
Topgigant Plus/*P.cerasifera*	0.32 d	5.41	low
Stanley/*P. cerasifera*	0.67 c	11.52	moderate

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

The disease severity for 'Topgigant Plus' cv. was a lower value compared to cultivar 'Jojo', grafted on 'Docera 6'. The difference was statistically significant.

The susceptibility level in the cultivars varies depending on the percentage of infected flowers of each cultivar. The cultivar 'Jojo' grafted on *P. cerasifera* reacted as highly susceptible with 88% infected flowers caused by *Pseudomonas* sp., while the grafted on 'Docera 6' was evaluated as susceptible to the disease - 49.17% infected plum flowers.

The cultivar 'Topgigant Plus' grafted on 'Docera 6' and 'Stanley' were evaluated as moderately susceptible to the bacterial pathogen. For both cultivars, the percentage of the infected blossoms was of low value - 12.70% for 'Topgigant Plus' and 11.52% for 'Stanley'. The percent of infected blossoms was the lowest for 'Topgigant Plus' when grafted on *P. cerasifera* and according to its reaction the cultivar/rootstock combination was evaluated as low susceptible to the *Pseudomonas* sp. phytopathogen. In this experimental stage the influence of the rootstocks on the reaction to flower infection is negligible.

The attack on flowers reported as blossom blighting occurs on plums (Anderson, 1956) but is not usually as severe as on sweet cherries (Wilson et al.,1939). In England, shoot wilt of plum is quite common and more frequently incited by *Ps. syringae* than by *Ps. mors-prunorum* (Crosse, 1954; Wormald, 1928). However, both can cause the appearance of similar symptoms (Wormald., 1931). The death of dormant buds is not usually important in domestic or Japanese plums.

The pathogenic *Pseudomonas* sp. strain was used for artificial inoculation of cut plum one-year-old shoots and their reaction was evaluated by measuring the infected part after 10 and 20 days. Non-significant difference was observed for the reaction observed for 'Topgigant Plus' shoots from the cultivar grafted on 'Docera 6' and grafted on *P. cerasifera*. Twenty days after infection, the measured lesion was longer - 500 mm for 'Topgigant Plus' cv. grafted on both rootstocks. A similar situation was observed for the cultivar 'Jojo', grafted on 'Docera 6'. Ten days after inoculation the infected part of the shoots was 42 mm, compared to the same cultivar grafted on *P. cerasifera* where the lesion was 37 mm.

Table 2. Lesion diameter (mm) of infected plum shoots

Rootstock	Cultivar	Lesion diameter (mm)	
		10 day	20 days
Docera 6	Togigant Plus	42 a	500 a
	Jojo	42 a	450 a
P. cerasifera	Topgigant Plus	45 a	500 a
	Jojo	37 a	475 a
	Stanley	40 a	400 a

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

The infected part of shoots 20 days after inoculation showed a 450 mm lesion for the cultivar grafted on 'Docera 6' compared to 475 mm for the cultivar grafted on the seedling rootstock. The control variant 'Stanley' showed the lowest result compared to other cultivar/rootstock combinations in the experiment. On the 10^{th} day after inoculation, the lesion part was 40 mm, and on the 20^{th} day, it was 400 mm. The screening of plum shoots showed non-significant differences between the studied cultivar/rootstock combinations and the control variant, 10 and 20 days after infection.

The plants of the plum rootstocks were inoculated with a pathogenic strain of *Pseudomonas* sp. and their reaction was evaluated by measurement of the lesion 10 and 20 days after inoculation. On the 10^{th} day was calculated an average value from 5 repetitions of each rootstock. The seedling rootstock reacted with a 33 mm lesion. Non-significant difference was observed between the seedling rootstock and 'Docera 6'. The lesion measured for 'Myrobalan 29 C' rootstock was 283 mm which was the highest value in this experiment.

Table 3. Lesions diameter (mm) of infected rootstock

Rootstock	Lesion diameter (mm)	
	10 day	20 day
Docera 6	83 b	400 a
P. cerasifera	33 b	41 b
Myrobalan 29 C	283 a	433 a

*Different letters in the same row/column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

Twenty days after inoculation a significant increase was observed for 'Docera 6' and the measured lesion was 400 mm. The result was similar to 433 mm measured for the rootstock 'Myrobalan 29 C'. The difference observed between *P. cerasifera* and both other rootstocks was statistically significant.

CONCLUSIONS

In this study, we report plum canker, lesions on shoots and blossom blight caused by members of the *Pseudomonas* sp. on plum orchards as responsible for relevant yield losses in Bulgaria. Isolated nine potential bacterial pathogens and one was selected for inoculation. The selected strain identified as *Pseudomonas sp.* was gram-negative and positive to the tobacco HR test. The pathogen was positive reaction 3 days after inoculating green fruit plums.

In our study, the flowers of cultivar 'Jojo' were evaluated as susceptible to *Pseudomonas* sp, while the flowers of 'Topgigant plus' reacted with low susceptibility to the bacterial pathogen. The value reported in shoot infection showed similar results.

The rootstock does not influence flower infection and lesions on shoots. Minimal differences between the cultivars grafted different rootstocks were observed. The reaction after shoots infection also showed similar results. The rootstocks 'Myrobalan 29 C' and 'Docera 6' were evaluated as more susceptible to *Pseudomonas* sp. than the seedling *P. cerasifera* rootstock.

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