DOI: 10.22620/agrisci.2022.35.009

SUSCEPTIBILITY OF THE PLUM CULTIVARS 'JOJO' AND 'TOGIGANT PLUS' TO SOME POSTHARVEST DISEASES

Diyana Aleksandrova*, Marieta Nesheva, Nataliya Dimitrova

Agricultural Academy, Fruit Growing Institute-Plovdiv
***E-mail: aleksandrova_diyana@abv.bg**

Abstract

Postharvest diseases cause significant losses in orchards before and after fruit harvest. The study aimes to compare the susceptibility of the plum cultivars 'Topgigant Plus' and 'Jojo' each grafted on the plum seedling rootstock *Prunus cerasifera* Ehrh. and the clonal rootstock 'Docera 6' (*Prunus domestica* L. x *Prunus cerasifera* Ehrh.) to infection with fungal pathogens. Three fungal pathogens *Botrytis cinerea* (Pers), *Penicillium* sp (Link) and *Monilinia* sp. (Aderh and Ruhl.) Honey were used for artificial inoculation. From each scion/rootstock combination, 30 fruits were infected by injecting 25 µl of conidial suspension. From the spread in the experimental orchard pathogens at the most significant frequency were isolated and used. All scion/rootstock combinations had the highest percentage of infected fruits after the inoculation with *Monilinia* sp. However, the results for the other two pathogens vary - the 'Topgiant Plus' cv. is more susceptible to the pathogen *Penicillium* sp, compared to 'Jojo' cv. On the other hand, the fruits of 'Jojo' cv. are more susceptible to the pathogen *Botrytis cinerea*.

Keywords: Docera 6, scion/rootstock combination, *Prunus domestica* L., *Prunus cerasifera* Ehrh., *Botrytis cinerea* (Pers), *Penicillium* spp. (Link), *Monilinia* sp.

INTRODUCTION

Fruits are fresh perishable products due to their high water content and water activity, which makes them susceptible to mechanical damage and various pathogens (Kumar et al ., 2018 г.). The rotting of fruits and vegetables usually damages about 20-25% of the harvested production (El-Ghaouth et al., 2004; Droby, 2006; Zhu, 2006; Singh & Sharma, 2007). A variety of pathogens can cause stone fruits to rot. *Monilinia*, *Rhizopus*, *Penicillium*, *Alternaria*, *Botrytis*, *Cladosporium*, *Colletotrichum* and *Stigmina* include some of the most common species causing fruit rot. Disease control could be achieved through breeding resistant cultivars, natural control or chemical control (Bailey & Hough 1975; Layne et al. 1996; Audergon et al. 1995; Dosba 2003; Bassi 2006; Myrta et al. 2006). The rootstock is almost as important as the correctly chosen cultivar. The interaction between the scion and the rootstock directly impacts yield, fruit quality and possibly the profitability of the cultivars and it is also responsible for premature tree death. Therefore, selecting the most suitable rootstock that works optimally in combination with horticultural practices and adaptability to the country's unique soil and climate is very important (Vachun 1995; Ercisli & Guleryliz 1995). One of the best methods for investigating varietal susceptibility is through artificial infestations. Snowdon et. al. (2008) and Walter et al. (2004) described resistance screening methods in apricots that can also be used for selecting resistance in plums. The same authors indicated that all methods detected cultivar differences in disease susceptibility. For most scenarios, there were annual differences, with cultivar × year interaction influencing. The most effective method studied was measuring the lesion area on artificially inoculated fruits after 72 hours.

The current study aimes to compare the

susceptibility of the two plum cultivars 'Jojo' and 'Topgigant Plus', grafted on two plum rootstocks – the seedling *Prunus cerasifera* Ehrh. and the clonal rootstock 'Docera 6' (*Prunus domestica* L. x *Prunus cerasifera* Ehrh.) through artificial infection with fungal pathogens.

MATERIALS AND METHODS

The typical symptoms for collected samples of post-harvest pathogens were soft rots, accompanied by collapse and water-soaking of parenchyma tissues and fruits flesh, followed by the appearance of conidia.

Three phytopathogens were isolated from infected plum fruits (*Prunus domestica* L.) of the cultivars 'Jojo' and 'Topgigant Plus' that were collected from the experimental field and during storage at the Fruit Growing Institute - Plovdiv. The fruits were surface sterilized with ethanol and washed three times with sterilized water. Then they were refrigerated at 90% humidity and monitored for sporulation. Pieces of the fruit flesh were cut from the surface of the infected tissue and placed on potato-dextrose agar in Petri dishes (90 mm). The pathogenicity of the isolated fungal cultures was confirmed using pathogenicity tests.

The pathogenicity of the selected potential isolates was demonstrated by inoculating surface-sterilized (96% ethanol) mature apples. Using a sterile scalpel, shallow cuts were made into the apple peels, and 10-day-old fungal cultures were inserted into the wounds. Sterile PDA plugs was used as a negative control. In the test, the inoculated sites were covered with transparent adhesive tape. The fruits were incubated at room temperature in a sterilized box.

For the artificial infection, from each rootstock combination, fruits were taken before consumer maturity. The fruits were surface sterilized with alcohol (ethanol 90%) and washed with sterilized water. Three fungal pathogens isolated from the plum orchard were used. In Petri dishes (90 mm) with potato-dextrose agar (PDA), the fungal cultures were grown for ten days in a thermostat at 25°C. Pathogens were traced for conidia formation. After the colonies development and conidia formation, the fungal cultures were flooded with 10 ml of sterile water, and with a scalpel, the surface of the medium was slightly scraped. The resulting suspension was filtered 2 times and brought to 10^6 conidia per milliliter (ml) with a hemacytometer. Each fruit was inoculated with 25 µl of the fungal suspension by puncturing and injecting it into the fruit flesh. For each variant of the experiment 30 fruits were inoculated in repetition. The fruits with injected sterile water were used as a negative control. The fruits were placed at 90% air humidity and 23°C constant temperature. On the second and fifth day after inoculation the fungal development was traced. The percentage of the infected part of the fruit was calculated on the basis of the infected/healthy part (diameter measured) relative share. The susceptibility of the fruit to the fungal pathogens was determined as an average value on the following scale:

0- Resistant - 0 to 10% infection
1- Moderately susceptible – 11 to 30% infection
2- Susceptible - 31 to 50% infection
3- Highly susceptible – infection over 50%

The method of inoculation and the scale of susceptibility were used and described in apricot (Nicotra, A., et.al. 2001)

The data were statistically processed using IBM SPSS Statistics 26 software and Analysis Tool Pak in MS Excel. One-way analysis of variance was performed using Duncan's Multiple Range Test (MRT).

RESULTS AND DISCUSSION

The symptoms of the positive pathogenicity test (necrotic lesions) were observed after 2 to 3 days of incubation on the fruits, with no observed necrosis in the control variant. The pathogens were reisolated from all inoculated samples, but not the negative controls.

The isolated fungal pathogens were identified as *Botrytis cinerea*, *Penicillium* sp. and *Monilinia* sp. Therefore, the pathogens were traced for sporulation on the agar medium. *Botrytis cinerea* forms a fast-growing mycelium on PDA of grey to black color, loose. Conidia vary in shape, - elliptical or ovoid, smooth. The pathogen causing the blue-green mould on fruits, *Penicillium* spp., was found to grow more slowly on the agar medium forming compact, entire colonies with a white periphery. The conidiophores have a broom-like structure, simple with several branches. The formed conidia are spherical to elliptical, unicellular, smooth without septa. The brown rot is caused by one of the species of the genus *Monilinia*. This pathogen forms slowly to moderately, developing entire terminal colonies on PDA. The spore formation is in concentric circles. The conidia are lemon-shaped. In all three fungi, the presence of conidia was observed around the tenth day of cultivation. The microscope BB.4260 BioBlue was used to obtain the morphological characteristic of the isolated fungal pathogens.

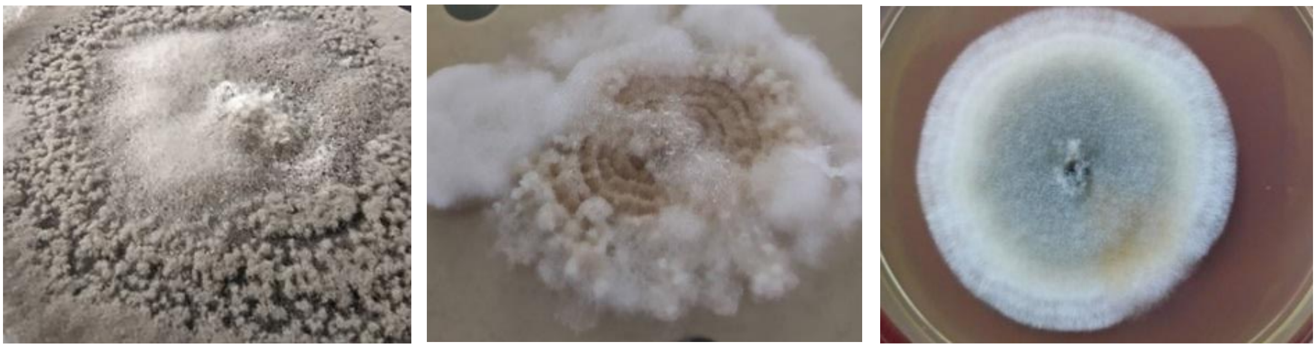

Figure 1. Isolated fungal phytopathogens of *Botritis cinerea* to the left, *Monilinia* sp. in the middle, and *Penicillium* sp to the right.

The diseases that occur during fruit storage can lead to significant fruit production losses and the cultivar susceptibility to each pathogen may differ. Since using fungicides after harvesting is not allowed in Bulgaria, all treatments are carried out in the orchards before the fruits harvest. Therefore, the cultivar susceptibility is an essential prerequisite to reduce production losses. As a result, on the third day after the artificial inoculation with *Penicillium* sp., the cultivar 'Topgigant Plus' grafted on 'Docera 6' had 7.97% infected fruits. The infected fruits of the same cultivar grafted on the seedling rootstock *Prunus cerasiefera* were 12.33%. On the fifth day of the artificial inoculation, the difference between the percent of infected fruits of the cultivar 'Topgigant Plus' grafted on 'Docera 6' and *Prunus cerasifera* was minimal - resp. 43.03% and 44.71%.

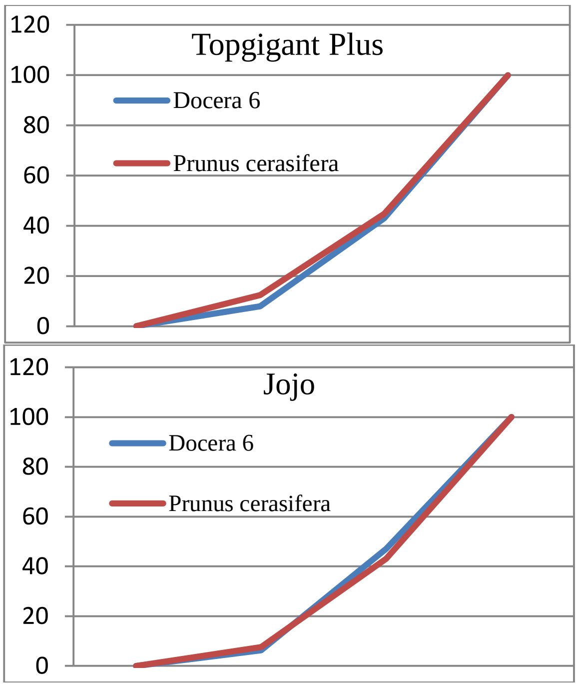

Figure 2. Percentage of infected surface on fruits inoculated with *Penicillium* spp.

The development of the blue-green mould on the fruits of 'Jojo' cv. was at a slower rate. The Jojo cv. had 6.24% infected part of the fruits when grafted on 'Docera 6', while the fruits obtained from the grafted on the other rootstock trees had 7.54% infected part. The results obtained on the fifth day indicate that when the cultivar is grafted on 'Docera 6', the infected part of the fruit (47%) is more significant than the same cultivar grafted on *Prunus cerasifera* (43.03%). Statistically non-significant differences were found according to the different rootstocks.

The reaction of the plum cultivars' fruits infected with *Monilinia* sp. indicates that the infected part of the fruits of 'Topgigant Plus' cv. grafted on 'Docera 6' was 51.46%, and the same cultivar grafted on the seedling *Prunus cerasifera* - 38.71%. This trend was preserved and at the next report on the fifth day, 60.76% of the fruits were infected for the 'Topgigant Plus' / 'Docera 6' and 48.05% for 'Topgigant Plus'/*Prunus cerasifera*.

The phytopathogen development dynamics on the fruits of 'Jojo' cv. is similar to the one of the other studied cultivar. When 'Jojo' is grafted on the 'Docera 6' rootstock, the infected part of the fruit was 51.99%. For the same cultivar grafted on *Prunus cerasifera*, the infected part of the fruits was 35.82%. The second report on the fifth day indicated that 'Jojo's' fruits were 71.13% infected, and when the cultivar is grafted on the other rootstock, it had a lower result - 48.05%. The two tested cultivars each had a low percentage of infection when grafted onto the seedling rootstock *Prunus cerasifera* but the differences were statistically non-significant.

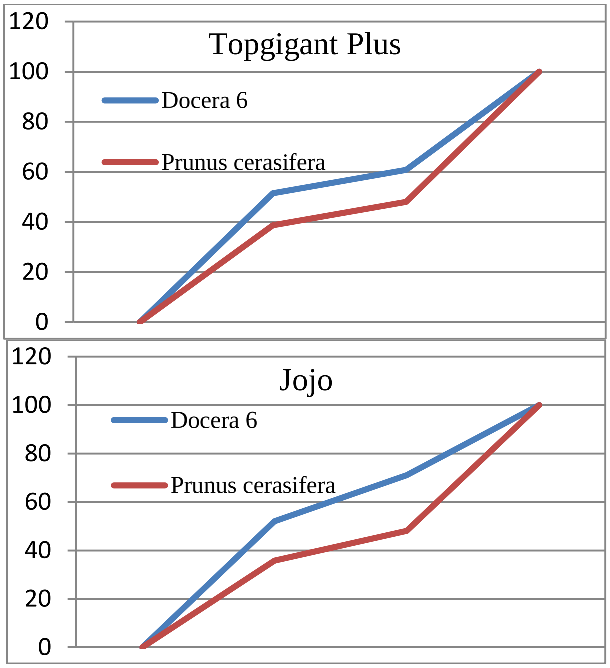

Figure 3. Percentage of infected surface on fruits inoculated with *Monilinia* sp.

After the inoculation with *Botrytis cinerea*, 'Topgigant Plus' cv. had minimum differences when grafted on both rootstocks - when grafted on 'Docera 6', 11.62% of the fruit surface was affected, and when grafted on *Prunus cerasifera* - 11.34%. During the second recording, the fungal pathogen developed more slowly in the plants grafted on 'Docera 6' (33.86%), while the fruits from the plants grafted on *Prunus cerasifera* seedling were reported with a higher percent affected surface (40.86%).

The 'Jojo' cv. showed lower values for the three types of infection when it was grafted on the seedling rootstock *Prunus cerasifera*. The other studied cultivar, 'Topgigant plus', responded with lower infection rates when grafted onto 'Docera 6' after inoculation with *Botrytis cinerea* and *Penicillium* sp.

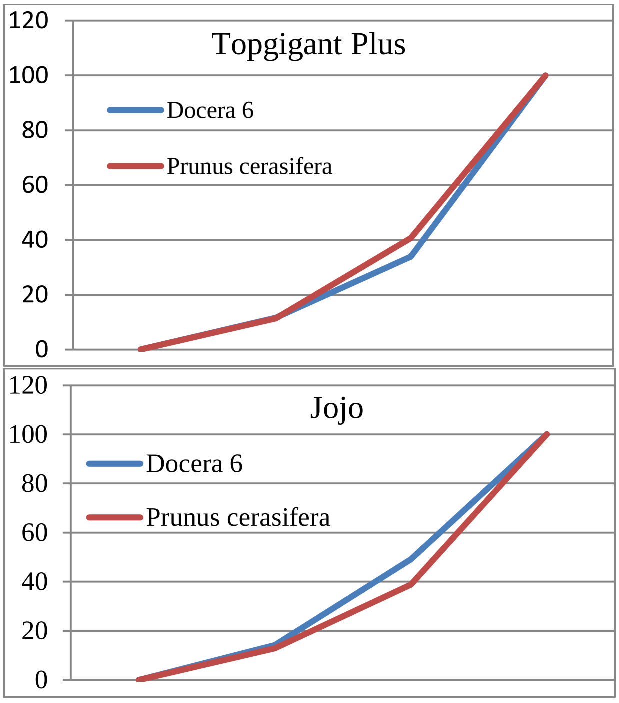

Figure 4. Percentage of infected surface on fruits inoculated with *Botrytis cinerea*

In our study, environmental factors such as temperature and humidity had no influence, and the inoculum was equal for all pathogens used. The fruits with high sugar content show a greater tendency to crack and provide better conditions for the growth of fungal pathogens. The high sugar content is cited as a possible reason when the fruits are more susceptible to infection. 'Jojo' cv. was reported to have higher sugar content, compared to 'Topgigant Plus' (Bozhkova, V., & Savov, P., 2018).

The obtained results after the artificial inoculation of the plum fruits indicated that the cultivar 'Topgigant Plus' grafted on 'Docera 6' was moderately susceptible to *Penicillium* sp. (25.49%) and *Botrytis cinerea* (22.74%), and to brown rot it reacted as highly susceptible (56.11%). The difference in the obtained values was statistically significant. The same cultivar, but grafted on *Prunus cerasifera* rootstock, reacted as susceptible after the inoculation with all three postharvest pathogens.

The difference was statistically significant for the highest percentage of infected fruit surface reported after infection with *Monilinia* sp. (43.38%). With 26.02% infected part of the fruit, the response to brown rot is the lowest result when comparing the three diseases. The fruits of 'Topgigant Plus' cv are the most susceptible after inoculation with *Monilinia* sp. followed by the pathogen *Penicillium* sp, with the lowest values after infection with *Botrytis cinerea* regardless of the substrate. Comparing the obtained values, according to the different rootstock, the cultivar grafted on 'Docera 6' had lower values than those grafted on *Prunus cerasifera*.

The fruits of 'Jojo' cv. grafted on 'Docera 6' reacted as moderately susceptible to the pathogens *Penicillium* sp. (26.62%) and susceptible to *Botrytis cinerea* (31.61%). For this scion/rootstock combination, the highest infection rate with *Monilinia sp (Aderh and Ruhl.) Honey* (60.94%) was recorded. The 'Jojo' cv. was determined to be highly susceptible to the disease, and the difference with the other two pathogens was statistically significant. The 'Jojo' cv., follows the trend described for the different rootstocks. The fruits with brown rot infection were calculated with 50.34% infected part, and the result was statistically significant. The cultivar was defined as moderately susceptible to the other two pathogens *Penicillium* sp. (22.44%) and *Botrytis cinerea* (25.34%).

Comparing 'Jojo' cv. grafted on the two different rootstocks, we can conclude that the variety grafted on *Prunus cerasifera* had lower infection values. On this basis, the degree of sensitivity to postharvest diseases of the plum also varies.

Table 1. Average percent infected fruit surface of ‘Topgigant Plus’ and ‘Jojo’ grafted on both rootstocks

Phytopathogen	Topgigant Plus/Docera 6		Jojo/Docera 6	
	% infected surface	Susceptability	% infected surface	Susceptability
Penicillium sp	25,49 b	moderate	26,62 b	moderate susceptible
Monilinia sp	56,11 a	high	60,94 a	high
Botrytis cinerea	22,74 b	moderate	31,61 b	susceptible
Phytopathogen	Topgigant Plus/*Prunus cerasifera*		Jojo/*Prunus cerasifera*	
	% infected surface	Susceptability	% infected surface	Susceptability
Penicillium sp	28,52 b	moderate susceptible	22,44 b	moderate
Monilinia sp	43,38 a	susceptible	50,34 a	high
Botrytis cinerea	26,02 b	moderate susceptible	25,83 b	moderate

*Values followed by different letters in the same column show significant differences according to Duncan’s Multiple Range test ($p < 0.05$)

CONCLUSION

After inoculation with the pathogen *Monilinia sp (Aderh and Ruhl.) Honey* causing brown fruit rot, the highest infection values were recorded in each of the rootstock combinations. For ‘Topgigant Plus’ cv., the next most aggressive disease is the pathogen causing green mould on the fruits *Penicillium* spp. The pathogen causing gray rot, *Botrytis cinerea*, appeared to be more harmful in the case of the ‘Jojo’ cv. grafted on both rootstocks.

ACKNOWLEDGEMENTS

This research work was carried out with the support of The Bulgarian National Science Fund (BNSF), project № КП-06-М 46/2 “Study of the new ‘Docera 6’ clonal rootstock impact on the agronomic characteristics and fruit quality” from 27.11.2020.

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