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Susceptibility of different grapevine cultivars to *Eutypa lata* isolate, causing agent of Eutypa dieback, originating from Serbia

Original Article

Sanja Živković
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
gajicsanja43@gmail.com (corresponding author)

Tanja Vasić
Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia
tanjavasic82@gmail.com

Jordan Marković
Institute for Forage Crops, 37251 Kruševac, Serbia
jordan.markovic@ikbks.com

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Abstract:

The experiment was established in the greenhouse during the two years. The grapevine cultivars used in experiment were Riesling blanc, Chardonnay, Banatski muskat and Gamay noir. The susceptibility of grapevine cultivars to the isolate of the fungi *Eutypa lata*, causing agent of eutyposis, was analysed. One isolate EL17 was used in the experiment. The highest susceptibility was found in Riesling blanc, Banatski muskat and Gamay noir cultivars, while Chardonnay cultivar was less susceptible to the isolates of fungus *Eutypa lata*.

Key words:

grapevine, Eutypa lata, cultivar susceptibility

Apstract:

Osetljivost različitih sorti vinove loze na izolat *Eutypa lata*, prouzrokovača eutipoze, poreklom iz Srbije

Eksperiment je izveden tokom dve godine u stakleniku. U eksperimentu su korišćene sorte vinove loze Rajnski rizling, Šardone, Banatski muskat i Game crni. Ispitivana je osetljivost sorti na izolat gljive *Eutypa lata* prouzrokovača odumiranja čokota vinove loze. U ogledu je korišćen jedan izolat EL17. Najosetljivijom se pokazala sorta Rajnski rizling, Banatski muskat i Game crni, a sorta Šardone pokazala se manje osetljivom na izolate gljive *Eutypa lata*.

Ključne reči:

vinova loza, Eutypa lata, osetljivost sorti

Introduction

The Eutypa dieback is one of the most harmful mycoses on the grapevine (*Vitis vinifera* L.) worldwide caused by fungal pathogen named *Eutypa lata*. This fungus can significantly reduce the yield and quality of the grapevines, but above all, it significantly reduces the longevity of the vine, as it causes partial or complete dying of the trunk (Bolay and Moller, 1977; Carter et al., 1985, 1994; Živković et al., 2012a, b).

In Serbia, during the period from 2010 to 2012, the occurrence the Eutypa dieback symptoms on the grapevines were observed. The symptoms first appear in the form of small, chlorotic and necrotic spots along the periphery of the leaves, deformation of the leaves, the appearance of shortened shoots, often with the so-called zig-zag internodes. In time, there is a partial or complete dying of the trunk of the vine (Carter at al., 1985; Živković et al., 2012a, b).

Fungus *E. lata* is a vascular pathogen that infects the grapevine through fresh pruning wounds. The infection occurs when askospores arrive at fresh cross cut sections and in the presence of water droplets penetrate the vascular tissue. The wounds are particularly sensitive immediately after pruning, although infections may occur even after seven weeks following the cut (Munkvold, 2001; Sosnowski et al., 2007a, b; Pitt et al., 2013).

Phytosanitary and chemical measures are used in the vineyards to suppress the degradation of grapevine. The use of preventive measures is reflected in the selection of the cultivars, the selection of the breeding form of the trunk, the time of the cutting and the removal and burning of diseased parts of the trunk. In a region where there are more hosts of *E. lata*, control of the disease cannot be done only by sanitary measures. Regular cutting and destruction of diseased trunk parts provide control of the inoculum within limits suitable for grapevine. Given that

there are no resistant grape cultivars to *E. lata*, planting of vineyards, it is recommended to plant tolerant cultivars, while avoiding sensitive cultivars. As a preventive measure of the fight, it is recommended to select one of the breeding forms, which involves the formation of a lower trunk and mixed cutting (Rolshausen et al., 2015; Sosnowski, 2016).

The main aim of this study was to determine the susceptibility of the grapevine cultivars to isolate of *E. lata* and finding potential sources of resistance for different grapevine cultivars. For the investigation, the four grapevine cultivars, the one domestic (Banatski muskat) and three foreign (Riesling blanc, Chardonnay, and Gamay noir) were selected. A test of the sensitivity of investigated grapevine cultivars to *E. lata* isolate was carried out by inoculation of unrooted cuttings.

Materials and methods

Samples and fungal isolation

During the period from 2010 to 2012, symptoms on the grapevine from 14 locations in Serbia, it was noticed a plant with dieback. Sampling was done in the second half of May and early June in vineyards aged 11 to 22 years. During the collection of samples of diseased plants in the field, fragments of the trunk and cordon of the grapevine, including diseased and healthy tissue, in size from 10 to 20 cm, were taken. Symptoms initially appeared as small, chlorotic and necrotic spots along the rim of the leaves, deformation of leaves and the appearance of shortened shoots, often with the so-called zig-zag internodes. Over time, partial or complete dying of the vines developed. To isolate the pathogen, diseased parts of grapevine plants were surface-sterilised with 5% sodium hypochlorite for 2 min, followed by three washes with sterile distilled water. Surface sterilised tissue was transferred to sterile filter paper and placed on potato dextrose agar (PDA) containing streptomycin and incubated at 24 °C in the 24 h UV light for 30 days. Single conidia were selected and transferred directly to the PDA plate according to the procedures described by Choi et al. (1999) and stored on PDA in tubes at 4 °C.

Of the total number of isolates obtained for this study, one isolate EL 17 (from cv. Cabernet Sauvignon) was selected.

Cultivar test

Cultivar test was performed according to the methods of Peros and Berger (1994). Unrooted cuttings of four cultivars Riesling blanc, Chardonnay, Banatski muskat and Gamay noir wereinoculated with mycelium of one isolate (EL17) in a randomised block design with four replication on eight cuttings. The day before the inoculation, 2-node cuttings, with the basal bud eliminated, were prepared and soaked in water overnight. A hole (5 mm in diameter, 5 mm deep) was drilled 4 cm below the upper bud, and a plug (5 mm diameter) of agar and mycelium, cut from a fresh culture on PDA medium was inserted into the hole, with the mycelium towards the wood. Plugs of PDA medium were used for the control. Just after inoculation, cuttings were inserted into pieces of rock wool, dimensions 50 x 70 x 30 cm. Five and ten weeks after inoculation, the plants were observed using the following scale: 0 (plants without symptoms), 1 (plants with few small and dried leaves), 2 (plants with higher number of small and dried leaves), 3 (plants with barely present leaves) and 4 (plants with dried shoots). After observing, the pieces were put into drained plastic containers, dimensions 12 x 12 x 30 cm, filled with substrate (Class 1) (without feeding) and finally placed on benches in a greenhouse. The plants were trimmed in February of the next vegetation cycle. Experiments were arranged in a complete randomised design. There were nine replicates of eight cuttings per treatment.

Five and ten weeks after bud break, plants were observed in the second vegetative growth cycle for each treatment of the cultivar experiment, evaluating the appearance of deformations and necrosis on leaves of inoculated plants. Calculation of the intensity of the disease on the shoots was done by direct proportion of the number of diseased leaves on inoculated cuttings and the number of leaves on control cuttings (Péros and Berger, 1994).

Statistical analysis

Statistical analysis was performed to determine the relationship between *E. lata* isolate and grapevine cultivar. Data were analysed by Analyse of variance (ANOVA) using the computerised software (PROC GLM, SAS, System, version 8.1; SAS Institute, Cary, NC). To satisfy the assumptions of the ANOVA, the arcsine transformation of the proportion was used (Y=2x arcsine $\sqrt{p}$). Homogeneity of groups was assessed using Duncan's test with p=0.05.

Results

By examining the vineyards in 14 locations where grapevine is cultivated in our country in the period from 2010 to 2012, symptoms of Eutypa dieback of grapevine have been observed. Samples were collected from 14 locations in Serbia.

During the vegetation, the diseased parts of the vines in vineyards become covered with a new leaf mass, so that the symptoms are more difficult to notice. Symptoms on the leaves of diseased plants are ex-

Table 1. Effect of grapevine cultivar on percentage of plants showing foliar symptoms and abnormalities after inoculation with *E. lata* isolate EL17

	Isolate EL17			
	Year n		Year n+1	
	5th week	10th week	5th week	10th week
Cultivar	Foliar symptoms (%)			
Riesling blanc	82.03 [a]	76.57 [ab]	80.21 [a]	65.63 [ab]
Chardonnay	44.53 [b]	48.44 [b]	56.25 [b]	61.46 [b]
Banatski muskat	86.72 [a]	79.69 [a]	72.92 [ab]	78.48 [a]
Gamay noir	74.22 [ab]	82.03 [a]	71.79 [ab]	77.08 [a]
Control	0.00 [c]	0.00 [c]	0.00 [c]	0.00 [c]

a-c: Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test at the p=0.05.

pressed in the form of tiny, chlorotic spots, arranged on the periphery of the leaves. The edges of the leaf are often spiked and bent toward the back, and with stronger infections, the leaf surface is mostly covered with necrotic spots. The central part of the leaf has a wobbly look. Over time, the leaves were dried and fall off early.

The susceptibility/resistance of different grapevine cultivars, to the selected isolate of *E. lata*, originating from Serbia, under greenhouse conditions was determined. Characterisation of morphological and molecular levels had previously been done on the fungi isolate. Assessment of susceptibility/resistance was carried out using methods of inoculation of unrooted cuttings of different cultivars of grapevine.

This research aimed to determine the susceptibility of different cultivars of grapevines to the isolate based on the appearance of symptoms on leaves and shoots, from the movement of growers on grapevine cuttings. Based on the results of this study, the method of inoculation of grape cuttings according to Peros and Berger (1994) will be used in the future.

Four grapevine cultivars were selected for testing: Riesling blanc, Chardonnay, Banatski muskat, and Gamay noir. Tested isolate caused eutyposis symptoms on grapevine cuttings. The reaction of the tested grapevine cuttings, as well as the estimated level of development of the disease after inoculation to selected *E. lata* isolate, are shown in **Tab. 1**.

The foliar expression of Eutypa dieback was compared between four cultivars of grapevine following inoculation by *E. lata* isolate EL17 (Tables 1). The numbers of cuttings showing foliar symptoms and other abnormalities were recorded five and ten weeks after inoculation in the first vegetative growth cycle and five and ten weeks after bud break in the second cycle vegetative growth cycle in cultivar experiments. All the cultivars inoculated exhibited foliar symptoms of the disease and the other abnormalities. In experiment, the effect of cultivar was highly significant. Some of the shoots showing necrotic leaves five weeks after inoculation showed normal growth ten weeks after inoculation and this phenomenon explained the decrease in some percentage. The differences between cultivars were less acute ten weeks after inoculation in first and ten weeks after bud break in second vegetative growth cycle (**Tab. 1**) indicating that the effects of the fungus appeared faster for some cultivars than for others.

The foliar symptoms occurred during the vegetative growth cycle on the inoculated unrooted cuttings. The smaller and necrotic leaves were similar to that observed in shoots of infected vines in the vineyard.

The cultivars had different responses in the test, indicating that cultivars differ in their susceptibility to fungal toxins and fungal invasion. The high susceptibility of cultivars Riesling blanc, Banatski muskat and Gamay noir was confirmed. Cultivars Riesling blanc, Banatski muskat and Gamay was considerably more affected than the Chardonnay cultivar, and the inoculation of cuttings caused a faster appearance of abnormal plants for Riesling blanc, Banatski muskat and Gamay noir than for the cultivar Chardonnay (**Tab. 1**).

The infected plants exhibited the typical symptoms of eutyposis on the shoots and leaves. Thus, on the inoculated cuttings of all grapevine cultivars studied, the intensity of the disease was similarly caused by isolate EL17 (**Tab. 1**).

Discussion

Eutypa lata is one of the most important phytopathogenic fungi that threaten vineyards, causing signifi-

cant economic damage (Pitt et al., 2013; Travadon et al., 2013). According to Munkvold et al. (1994) the Eutypa dieback is a very dangerous disease of the grapevine because there is no effective chemical protection, and after the advancement of this disease there is a decrease in the yield and dying of parts or whole infected trunks, leading to significant losses in production (Rolshausen et al., 2015).

In the world, there is a limited number of works on testing the susceptibility of different grapevine cultivars to this disease. Earlier studies of the susceptibility of different grapevine cultivars were based on apricot research that was very susceptible to *E. lata*, and very little was known about the colonisation of grapevine tissue by this fungus (Carter et al., 1985; Munkvold, 2001). For these reasons, Péros and Berger (1994) examined the aggressiveness of twelve *E. lata* isolates and the susceptibility of fifteen cultivars of grapevines in their experiments.

Sosnowski et al. (2007a) studied the virulence of twenty-eight *E. lata* isolates isolated from the grapevine, originating from Australia, and the susceptibility of three cultivars of grapevines Cabernet Sauvignon, Grenache and Merlot. Travadon et al. (2013) observed the susceptibility of seven commercial grapevine varieties (Cabernet Franc, Cabernet Sauvignon, Chardonnay, Merlot, Riesling, Shiraz and Thompson seedless) as well as the susceptibility of the interspecific hybrid of *Vitis labrusca* variety Concord by inoculation of one-year rooted cuttings during the dormant season.

Comparison of the obtained symptoms in this study with the symptoms of natural infections in Serbia, as well as with the descriptions of this phenomenon in literature, points to their exceptional similarity in the appearance and dynamics of development. The pathogenic isolate of *E. lata* was used in the sensitivity tests on grapevine cultivars, and it has been demonstrated that this species is capable of contaminating the unrooted cuttings of the grapevine and causing the appearance of symptoms characteristic of the dieback of the grapevine.

After the inoculation of unrooted cuttings under greenhouse conditions during the dormant season, after five and ten weeks, in the first vegetation cycle, the symptoms occurred. Symptoms were observed on inoculated plants as stunted, chlorotic leaves with curved edges, and chlorotic and necrotic patches along the periphery, which later spread over the entire surface, and subsequently caused drying and falling leaves. The shoots were significantly shortened due to slow growth and have a bright green color, often with the so-called zig-zag internodes. Symptoms on shoots which appeared on leaves under greenhouse conditions were similar to symptoms found in grapevines in nature. According to the literature data, leaf necrosis appearing on inoculated cuttings is identical to the necrosis of the leaf-induced fungi culture or to the eutypine toxin obtained by extracting leaves from infected grapevine plants from the vineyard (Tey-Rulh et al., 1991, loc. Cit. Péros and Berger, 1994; Mahoney et al., 2005; Jimenez-Teja et al., 2006; Trouillas and Gubler, 2010). According to Petzold et al. (1981) and Mur (1988), the appearance of leaf symptoms is a consequence of toxic products that move from the inoculation point to the point of growth (Travadon et al., 2013).

The studied *E. lata* isolate proved to be the most virulent on grapevine cultivars Riesling blanc cultivar, Banatski muskat, and Gamay noir compared to Chardonnay. On the other hand, the Chardonnay cultivar exhibits statistically significant resistance to the tested isolate. Péros and Berger (1994) observed the onset of symptoms five, seven and ten weeks after inoculation in the susceptibility of the cultivars. They concluded that the percentage of symptoms occurring during the fifth to seventh week increased by 70% and remained unchanged in the period from the seventh to the tenth week. Cultivars are variously susceptible to the toxin that fungus produce and invasion of fungi (Rumbos, 1985; Travadon et al., 2013). Many inoculated cuttings that exhibited symptoms in the first year did not exhibit symptoms in the second year. This is explained by the fact that the number of toxic products can vary and dilute depending on the development or growth of the shoots (Péros and Berger, 1994, 1999, 2003). Also, this phenomenon can be illustrated by the variation in the intensity of the observed symptoms in some cultivars, the ability to begin to develop regular shoots that overlap deformed shoots after the onset of symptoms. Foliar symptoms are initially confined to one span of infected vines, however, as the disease progresses symptoms my spread throughout the entire vine (Péros and Berger, 1994, 1999, 2003; Sosnowsli et al., 2007b; Živković et al, 2012a, b; Pitt et al., 2013; Travadon et al., 2013).

The artificial inoculation of the grapevine with isolate of *E. lata* gave preliminary information on the different susceptibility of four grapevine cultivars. Namely, by comparing the reactions of four grapevine cultivars to artificial *E. lata* infections, it was found that there were statistically very significant differences in the response of inoculated cuttings, which indicates the existence of a difference in the level of susceptibility. Péros and Berger (1994) in their studies suggest that the tested grapevine cultivars exhibited a different susceptibility to the *E. lata* toxin fungi and its invasion, which reflects on the intensity of the symptoms on their span. Also, by analysing the sensitivity of fifteen grapevine cultivars to *E. lata*, Péros and Berger (1994, 1999, 2003)

found that the cultivars Cabernet Sauvignon, Ugni Blanc, and Chasla were the most susceptible. A bit lower degree of susceptibility was revealed by the Merlot cultivar.

Travadon et al. (2013) have found in their experiments that the Thompson seedless cultivar has exhibited a high degree of susceptibility to the *E. lata* isolates examined. Likewise, there was no significant difference in the expressed susceptibility between the cultivars of Cabernet Sauvignon and Merlot.

Unfortunately, currently, there are no developed programs for selection of vines for disease resistance in our country. One of the contributions of this is the examination of the reaction of domestic commercial cultivars, as well as cultivars from the environment, to susceptibility to *E. lata* under conditions of artificial inoculation for the purpose of preventing the production of vineyard. Under experimental conditions, investigated isolate was able to cause typical symptoms on the tested grapevine cultivars.

Conclusion

Based on the results during the two-year investigation on susceptibility of four cultivars of the grapevines to the inoculation of *E. lata*, it can be concluded that the cultivars Riesling blanc, Banatski muskat and Gamay noir manifest high susceptibility compared to cultivar Chardonnay. All the cultivars inoculated exhibited foliar symptoms of the disease and the other abnormalities. The differences between cultivars were less acute ten weeks after inoculation in first and ten weeks after bud break in second vegetative growth cycle.

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