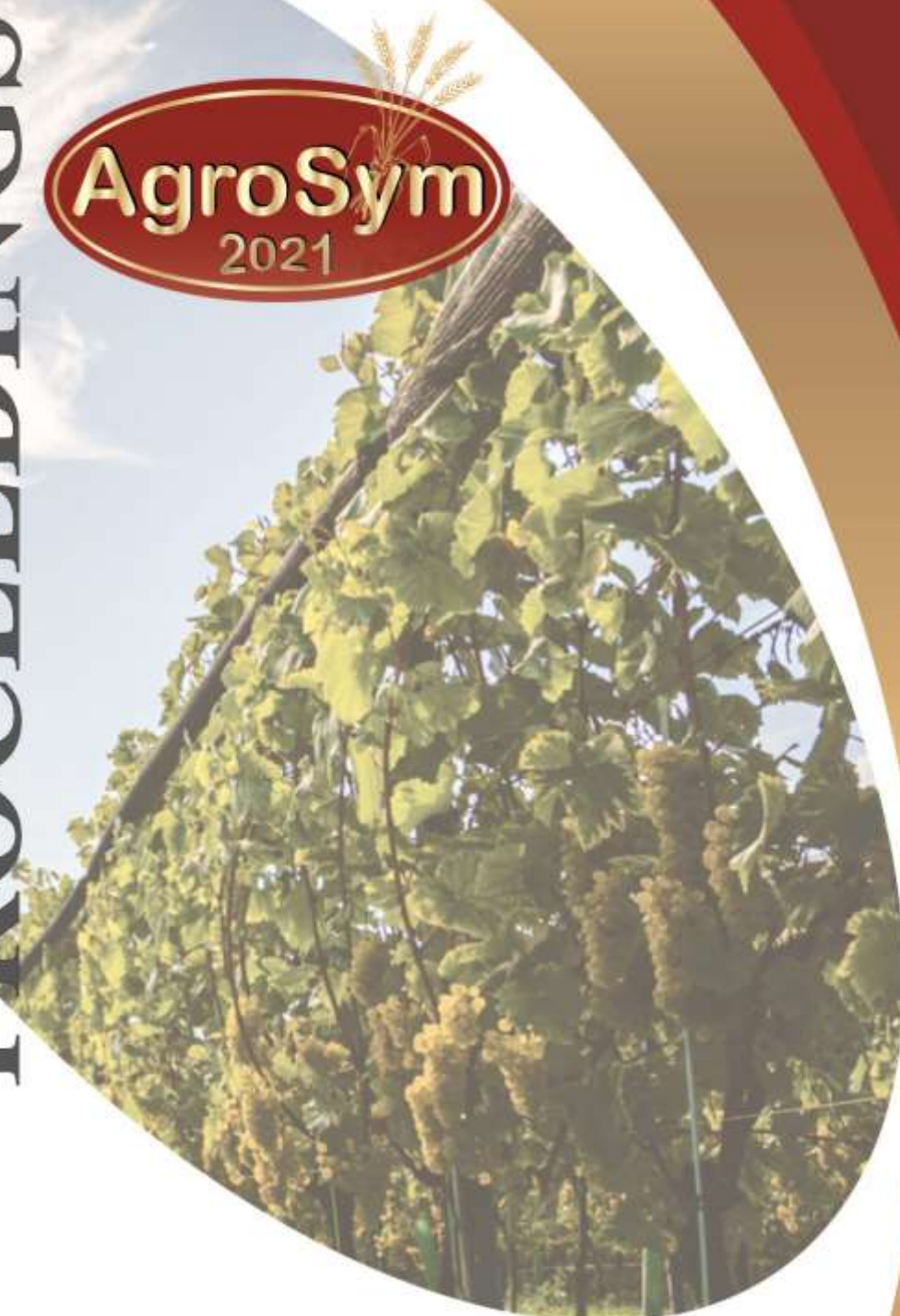


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MORPHOLOGICAL CHARACTERISTICS OF *EUTYPA LATA* ISOLATES FROM GRAPEVINE IN SERBIA

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Abstract

In an *in vitro* experiment, the morphological characteristics of three *Eutypa lata* isolates (EL129, EL153 and EL199), previously determined at the molecular level, and two reference isolates BX1.10 and 8F obtained from the Institute National de la Recherche Agronomique, INRA, France were observed. The following morphological features of the studied isolates were studied on the nutrient medium of potato dextrose agar (PDA): macroscopic (appearance, color and zoning of the front and back of the culture) and microscopic (vegetative organs of mycelium, reproductive organs of anamorphs - conidia, pycnidia, conidiophores and reproductive organs of teleomorphs - perithecium, ascus with ascospore). On potato dextrose medium (PDA) 24 h after placing, the studied *E. lata* isolates formed at the beginning a white mycelium. After 10 days, the mycelium had a cottony appearance, white color with a weak air growth. The entire colony developed evenly and had a fine diffuse edge. After 30 days, the face of the colony was white with a thick cotton aerial mycelium and a pale yellow conidial mass, which was excreted from the pycnidia. The studied *E. lata* isolates formed pycnidia, which were formed in culture. The pycnidia of the studied *E. lata* isolates were spherical or irregular in shape, from dark brown to black in color. The dimensions of the pycnidia were from 0.5 to 1 mm. Conidial mass was a cream to pale orange color on a PDA base. All studied isolates formed conidia in culture. None of the studied *E. lata* isolates, originating from the stem and branches of the grapevine from Serbia, as well as the control isolates, formed perithecia in culture.

Key words: *Isolates, breeding traits, substrates, Eutypa lata.*

Introduction

The grapevine dieback occurs in almost all countries of the world where vines are grown commercially. This is understandable given the wide range of host plants that *E. lata* fungus attacks. In addition to the wide distribution, grapevine dieback as an economically important grapevine disease is more significant in areas where annual rainfall is above 600 mm. On the other hand, it rarely occurs in areas where the average annual rainfall is less than 250 mm (Carter, 1994). In Serbia, this disease was first detected by Živković *et al.* (2012a, b).

E. lata is a pathogen of a large number of plants, from different genera of stone fruits, apples, berries and other fruits and causes great damage. The fungus is also isolated from a large number of ornamental, wild or woody plants. According to Carter *et al.* (1985) the grapevine (*Vitis* sp.) is the universal host for the distribution of *E. lata* to other species growing nearby.

Trouillas and Gubler (2010) studied the host circle of *E. lata* in the vineyard regions of California. The authors monitored the presence of *E. lata* perithecia on vine stems, in orchards

on apricot, almond, cherry, sweet cherry, apple and pear trees, as well as on natural hosts of large-leaved maple, willow and oleander. By phylogenetic analysis of the DNA sequences of the ITS region and the β -tubulin gene, the DNA-dependent RNA polymer gene (RPB2) was confirmed and identified by *E. lata* isolated from different plant hosts. The interspecific phylogenetic diversity according to Trouillas and Gubler (2010) does not match the geographical origin of the isolates, and the interspecific groups after different DNA phylogenies do not match. Significant phenotypic variations of *E. lata* isolates were detected on the vine, in terms of the length of ascospores and conidia as well as the levels of aggressiveness of isolates. The aim of this study was to describe the morphological (macroscopic and microscopic) properties of three isolates of *Eutypa lata* (EL129, EL153 and EL199) on a nutrient medium PDA.

Material and methods

The tested isolates were obtained from grapevine plants with grapevine dieback symptoms collected in the period 2015-2019. Sampling was performed in the main vine production areas on the territory of the Republic of Serbia from 14 sites: Dobričevo, Drenovac (Pomoravlje district), Praskovče, Lipovac (Nišavski district), Kobilje, Bela Voda, Krvavica, Suvaja, Trnavci, Tulež (Rasina district), Gudurica (South Banat district), Karbulovo (Bor district), Strezovac (Pčinja district), Sremski Karlovci (South Banat district). A total of 50 samples were taken and analyzed. After being brought to the laboratory, the samples were first washed with running water, and then fungal isolation was performed using standard phytopathological methods. Pathogen isolation was performed from grapevine stems and cordons. In order to remove surface impurities, parts of the stem and cordon were washed with running water for 2 h, and then cut into fragments 1 cm long. Fragments of the stem and cordon were cut at the junction of necrotic and healthy tissue, surface disinfected for 5 minutes in 5% sodium hypochlorite solution (NaOCl) (14% NaOCl, Superlab, Belgrade) and washed 3 times for 5 minutes in sterile distilled water. The fragments were transferred to sterile filter paper to remove excess fluid and then placed on a nutrient medium. Potato dextrose agar (PDA) with the addition of 300 μ l / l gentamicin sulfate was used to isolate the pathogen. This medium was prepared from 200 g of potatoes, 20 g of dextrose (Torlak, Institute of Immunology and Virology, Belgrade), 20 g of agar (Torlak, Institute of Immunology and Virology, Belgrade) and 1 l of distilled water (Dhingra and Sinclair, 1995). Petri plate with fragments were incubated in a thermostat at a temperature of $24 \pm 2^\circ\text{C}$, in the dark until the development of fungal colonies around the fragments, and incubated at 24°C in the 24 h UV light for 30 days. Individual conidia were selected and transferred directly to the PDA plate according to the procedures described by (Dhingra and Sinclair, 1995), and stored on PDA in tubes at 4°C . In this study, the macroscopic and microscopic morphological characteristics of 3 isolates (EL129, EL153, EL199) isolated from Serbia and 2 reference isolates BX1.10 and 8F obtained by the Institute National de la Recherche Agronomique, INRA, France, were studied. Of the macroscopic characteristics, the traits of colonies are described, such as the appearance, color and zoning of the face and back of the culture on the tenth day after transferring (Muntanola-Cvetković, 1987). Identification was done by pathogenicity test (Peros and Berger, 1994), morphology (Glave and Rogers, 1982; Glave *et al.*, 1982) and PCR methods (Lecomte *et al.*, 2000). Considering the microscopic morphological characteristics, the appearance of mycelium, the presence of asexual (conidia, pycnidia, conidiophores) and sexual reproduction (perithecia, asci, ascospores) were studied.

Macroscopic and microscopic morphological characteristics of 3 *Eutypa lata* isolates and 2 *E. lata* reference isolates were studied on a PDA medium, according to the method of Glawe *et al.* (1982). The appearance, color and zoning of the face and back of the culture were observed, followed by the appearance, structure, color and growth of the mycelium. Inoculation of the studied isolates on medium was performed by aseptic application of round fragments of colonies of pure cultures of the studied isolates with a diameter of 10 mm, in the center of Petri plate, which were then grown at 25 ° C, and under 24 h UV light for 30 days. In all 3 isolates of *E. lata* and 2 reference isolates of *E. lata*, the shape and dimensions of conidia were studied. The study of conidia was performed by observing 100 randomly selected conidia of the studied isolate, using a microscope at a magnification of 400 times (Olympus BX51 / BX52, Japan) and a digital camera (Olympus DP71, Japan). The average dimensions of the conidia were determined by measuring the length and width of 100 randomly selected conidia of the studied fungal isolates, grown on a PDA medium using a light microscope and a digital camera.

To monitor the formation of the teleomorphic stage, 3 studied *E. lata* isolates and 2 reference *E. lata* isolates were grown on PDA medium, cultures were grown in a thicker layer (40 ml of medium per Petri dish with a diameter of 100 mm), at a temperature of 25 ° C. Readings of the presence of teleomorphic formations were performed after 30 days, 6 and 12 months. Monitoring of the appearance of teleomorphs was also performed in nature. After removing the bark from the dead wood of naturally infected grapevine plants, fragments of dead wood were cut and the presence of a stroma with perithecia was observed under binocular microscope (Olympus SYX7, DFPLAPO 1 x 4).

Statistical analysis performed in order to determine the relationship between *E. lata* isolate and two reference isolates 8F and BX1.10. Data were analyzed by variance analysis (ANOVA) using the computerized 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 ($Y=2x \arcsin \sqrt{p}$). Homogeneity of groups was assessed using Duncan's test with $p = 0.05$.

Results and discussion

Examination of vineyards in several sites of grapevine cultivation in our country in the period from 2015 to 2019, revealed symptoms of grapevine dieback. Symptoms on the leaves of diseased vines are manifested in the form of small, chlorotic spots, distributed along the edge of the leaves, while the central part of the leaf blade had a wrinkled appearance. The edges of the leaves were worn and bent downwards, and in severe infections the surface of the leaves is mostly covered with necrotic spots. The shoots are light green in color, have a shortened appearance and the so-called zigzag rise of internodes.

On potato dextrose medium (PDA) 24 h after transferring, the studied *E. lata* isolates form the beginning of white mycelium. After 10 days, the mycelium was a cottony appearance, white color with a weak air growth. The entire colony develops evenly and were a fine diffuse edge. The back of the colony is cream in color, without fruiting structures (Figure 1). After 15 days, a dark pigment forms in the substrate. After 30 days, the face of the colony is white with a thick cotton aerial mycelium and a pale yellow conidial mass, which was excreted from the pycnidia. Macroscopic examination of colonies of *E. lata* isolates showed no differences in the morphological characteristics of the mycelium. Glawe *et al.* (1982) and McKemy *et al.* (1993) described cotton-white colonies of *E. lata* isolates on a PDA medium whose color changes to cream over time, which is in agreement with our results.

The studied *E. lata* isolates form pycnidia, which are formed in culture (Figure 1). The pycnidia of the studied *E. lata* isolates are spherical or irregular in shape, from dark brown to black in color. Conidial mass is a cream to pale orange color on a PDA base. The dimensions of the pycnidia were from 0.5 to 1 mm. Namely, under the influence of a change of 12 h of light - 12 h of darkness and at alternating temperatures of 19 ° C and 15 ° C, sporulation occurs after 30 days, which agrees with the statements of Carter (1994) and Munkvold (2001). The same authors state that in some cultures a gray pigment is formed after 15 days and the back of the colony turns black. Three to four weeks after placing, small black pycnidia appear in the culture under the influence of constant UV light. Conidia were excreted from the pycnidia in the form of a cream to an orange gelatinous mass (Rolshusen *et al.*, 2006; Trouillas and Gubler, 2010).

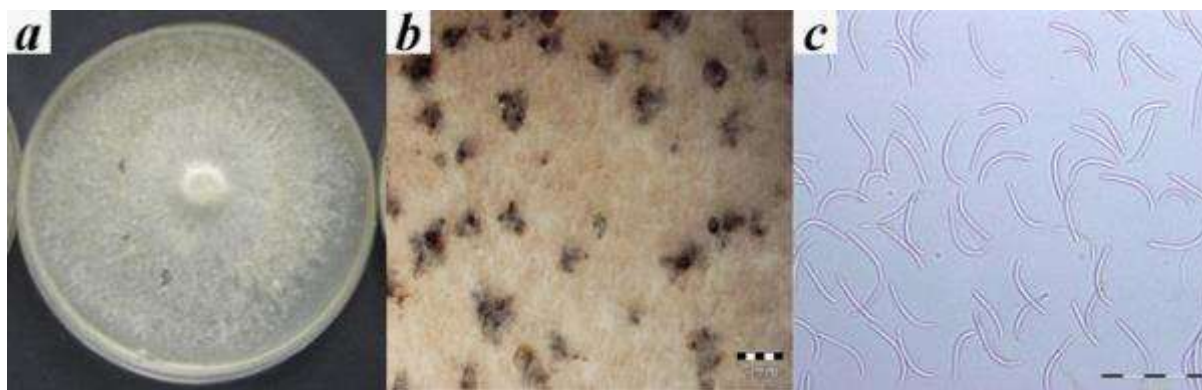


Figure 1. *Eutypa lata* EL129 isolate on PDA medium: a) Colony of isolates on PDA medium after 10 days of development; b) Stromatic formations of isolates in cultures 30 days old; c) Conidia EL153 in 30-day-old cultures.

In the studied *E. lata* isolates there are no morphological differences related to the appearance of conidia. All conidia were unicellular, unseptated, hyaline, elongated, and moderately curved with a flattened base (Figure 1). Conidia length and width were parameters that were individually statistically analyzed in order to determine suitability as a criterion for distinguishing between the tested *E. lata* isolates (Table 1). Based on data from the literature, the morphological characteristics of the anamorphic stage of the genus *Eutypa* were not sufficiently reliable traits for the identification of isolates up to the species level, although they were of great importance in the classification of the *Diatrypaceae* family (Trouillas *et al.*, 2010; Rolshausen *et al.*, 2014).

The genus *Eutypa* exhibits a very high similarity in the appearance of conidia, the ontogenesis of conidia, the characteristics of cultures, as well as the similarity of the teleomorphic stage with other genera within the fam. *Diatrypaceae*, such as the genus *Eutypella*, *Diatrype*, and *Diatrypella*. This makes it difficult to separate and identify the genus *Eutypa* (Glawe and Rogers, 1982; Rolshausen *et al.*, 2006; 2014). On the contrary, the morphological characteristics of the teleomorphic stage of these fungi, due to their high uniformity, are very important for the determination of pathogens up to the genus level. However, during the tour of the terrain in the vineyards, no teleomorph was found in any locality where the symptoms of the dieback of the grapevine were observed. Therefore, the characteristics of teleomorphs were not used in the determination of this pathogen. Investigations of the morphological characteristics of anamorphs of the fungus served to identify the cause of the grapevine dieback to the level of genus. The

morphological and biometric values of the anamorphs were in agreement and fully corresponded to the reference values from the literature considering the pathogen *E. lata*. Considering the morphological characteristics of anamorphs, unicellular, conical, hyaline and slightly bent conidia with a flattened base, 19.50- (24.69) -33.34 μm long and 1.20- (1.69) -2.74 μm wide, were observed on PDA medium (Table 1), which is characteristic of *E. lata* species. These results were in agreement with the results presented by other authors. Glawe *et al.* (1982) describe the conidia of *E. lata* as unicellular, conical, slightly bent and hyaline, measuring 25-74 $\mu\text{m} \times 1-2 \mu\text{m}$. Carter (1994) stated that conidia are 18-45 $\mu\text{m} \times 0.8-1.5 \mu\text{m}$ in size. Munkvold (2001) described the conidia of *E. lata* isolates as conical, straight or curved, very numerous, measuring 20-45 $\mu\text{m} \times 0.8-1.5 \mu\text{m}$. Rolshausen *et al.* (2006, 2014) state that the dimensions of conidia of *E. lata* isolates from different hosts were 23.6-35.00 $\mu\text{m} \times 1.8-4.7 \mu\text{m}$. In all tested isolates, conidia were formed in pycnidia, which is in agreement with the literature (Glawe and Rogers, 1982; Munkvold, 2001).

Table 1. Biometric values of conidia dimensions of the studied *Eutypa lata* isolates on PDA substrate

Isolate	Conidia length (μm)			Conidia width (μm)		
	Max	Min	Average*	Max	Min	Average*
EL 129	33.34	23.24	26.75	2.74	1.28	1.81
EL 153	23.10	19.50	20.94	1.81	1.20	1.54
EL 199	33.25	23.06	27.80	1.50	1.39	1.45
8F	26.25	22.54	24.33	2.19	1.58	1.94
BX1.10	24.99	20.04	23.62	2.16	1.28	1.71

*Average value of 100 repetitions

None of the studied *E. lata* isolates, originating from the stem and branches of the vine from Serbia, as well as the control isolates of *E. lata*, formed perithecia in culture, which agrees with the statements of Munkvold *et al.* (1993) and Munkvold (2001). Despite the very long storage of cultures until their complete exhaustion and observation on several occasions, after 30 days, 6 and 12 months, there was no formation of the teleomorphic stage. Also, the teleomorphic stage did not appear on grapevine plants inoculated with *E. lata* isolates, even after 27 months. Despite many years of monitoring this disease in vineyards, no teleomorphic stage has been found in nature. Glawe and Rogers (1982) and Carter (1994) stated that it is prevalent in vineyards older than 10 years in areas with frequent rainfall, and in vineyards where irrigation systems exist Munkvold *et al.*, (1993).

Conclusion

Macroscopic examination of cultures on a PDA medium showed that the studied *E. lata* isolates developed uniform colonies. In all studied isolates, conidia are formed in pycnidia from which a mucous mass (matrix) is secreted, which is a cream to a pale orange color. Isolates identified as *E. lata* had unicellular, smooth, hyaline, slightly bent, at the end narrowed, stringy conidia, 19.50- (24.69) -33.34 μm in length and width of 1.20- (1.69) -2.74 μm .

References

- Carter, M.V. (1994). Wood and root diseases caused by fungi. *Eutypa dieback*. In R. C. Pearson, and A.C. Goheen (ed.), Compendium of grape diseases, 3rd ed. APS Press. St. Paul, Minnesota: 32-34.
- Carter, M.V., Bolay, A., English, H. and Rumbos, I. (1985). Variation in the Pathogenicity of *Eutypa lata* (= *E. armeniaca*). Aust. J. Bot., 33: 361-366.
- DeScenzo, R. A., Engel, S.R., Gomez, G., Jackson, E.L., Munkvold, G.P., Weller, J. and Dhingra, O.D., J.B. Sinclair, J.B. (1995). Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida, (F).
- Dhingra, O.D., J.B. Sinclair, J.B. (1995). *Basic Plant Pathology Methods*. CRC Press, Boca Raton, Florida, (F).
- Glawe, D.A., Skotland, C.B., Moller, W.J. (1982). Isolation and identification of *Eutypa armeniaca* from diseased grapevines in Washington state. Mycotaxon 16: 123-132.
- Glawe, D.A., Rogers, J.D. (1982). Observations on the anamorphs of six species of *Eutypa* and *Eutypella*. Mycotaxon 14: 334-346.
- Lecomte, P., Peros, J.P., Blancard, D., Bastien, N., Delye, C. (2000). PCR Assays That Identify the Grapevine Dieback Fungus *Eutypa lata*. Applied and Environmental Microbiology 66 (10): 4475-4480.
- McKemy, J.M., Glawe, D.A., Munkvold, G.P. (1993). A hyphomycetous synanamorph of *Eutypa armeniaca* in artificial culture. Mycologia, 85(6): 941-944.
- Munkvold, G.P. (2001). *Eutypa dieback* of grapevine and apricot. Online. Plant Health Progress doi: 10.1094/PHP-2001-0219-01-DG. Accessed 20.12.2020.
- Munkvold, G.P., Duthie, J.A., and Marois, J.J. (1993). Spatial patterns of grapevines with *Eutypa dieback* in vineyards with or without perithecia. Phytopathology 83: 1440-1448.
- Muntañola-Cvetković, M. (1987). Opšta mikologija (General mycology). NIRO "Književne novine". Beograd.
- Peros, J.P., Berger, G. (1994). A rapid method to assess the aggressiveness of *Eutypa lata* isolates and the susceptibility of grapevines cultivares to *Eutypa dieback*. Agronomie 14: 515-523.
- Rolshausen, P.E., Mahoney, N.E., Molyneux, R.J., Gubler, W.D. (2006). A Reassessment of the Species Concept in *Eutypa lata*, the Causal Agent of *Eutypa Dieback* of Grapevine. Phytopathology 96: 369-377.
- Rolshausen, P.E., Baumgartner, K., Travadon, R., Pouzoulet, J. (2014). Identification of *Eutypa* spp. causing *Eutypa dieback* of grapevine in Eastern North America. Plant Dis. 98: 483-491.
- Trouillas, F.P., and Gubler, W.D. (2010). Host range, biological variation, and phylogenetic diversity of *Eutypa lata* in California. Phytopathology 100 (10): 1048-1056.
- Trouillas, F.P., Úrbez-Torres, J.R., and Gubler, W.D. (2010). Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102 (2): 319-336.
- Živković, S., Vasić, T., Anđelković, S., Jevremović, D., Trkulja, V. (2012a). Identification and Characterization of *Eutypa lata* on Grapevine in Serbia. Plant Disease 96 (6): 913.
- Živković, S., Vasić, T., Trkulja, V., Krnjaja, V., and Marković, J. (2012b). Pathogenicity on grapevine and sporulation of *Eutypa lata* isolates originating from Serbia. Romanian Biotechnological Letters. No 17(3): 7379-7388.