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UDK 630*181.45 : 582.47 Pseudotsuga menziesii (497.11 PK Kolubara)
Original scientific paper

THE CHANGES IN THE INTERNAL STRUCTURE OF *Pseudotsuga menziesii* (Mirb.) Franco NEEDLES UNDER THE INFLUENCE OF AIR-POLLUTION

Milorad Veselinović, Dragana Dražić, Mihailo Ratknić, Ljubinko Rakonjac, Vesna Golubović-Ćurguz, Nevena Čule, Suzana Mitrović[1]

Abstract: The deposits of Kolubara open-pit mining fields are under the direct influence of the largest power plants of this type in our country. This area is under the influence of air pollution due to the lignite mine exploitation, combustion and transport.

The scope of our research has been aimed at the *Pseudotsuga menziesii* (Mirb.) Franco resistance to air-pollution; taking into consideration the fact that the foliage represents the primary zone under the negative effect of air-pollutants on plant species, the tests have been conducted in order to establish how pollution affects the internal structure of Douglas fir needles.

The paper presents the results obtained by stereologic analyses of volume-density of mesophyllic cells, intercellular spaces, central cylinder, resin channels and vascular bundles through the cross-section of Douglas fir needles subjected to the direct influence of air- pollution in the area of Kolubara open-pit mine, as well as those from control localities on Jelova Gora and Juhor Mountain.

Based on the research results, the decrease in volume density of chlorenchyma and the increase of intercellular spaces are reported in Douglas fir needles in polluted area as opposed to those in control locality, and the differences are statistically justifiable.

Key words: air-pollution, biological recultivation by afforestation, needle anatomy

1 Milorad Veselinović, Ph.D, Dragana Dražić, Ph. D, Mihailo Ratknić, Ph.D, Ljubinko Rakonjac, Ph.D, Vesna Golubović-Ćurguz, Ph.D, Nevena Čule, B. Sc, Suzana Mitrović, B. Sc, Institute of Forestry, Belgrade, Serbia
Translation: Marija Stojanović

PROMENE U UNUTRAŠNJOJ STRUKTURI ČETINA *Pseudotsuga menziesii* (Mirb.) Franco POD UTICAJEM AEROZAGAĐENJA

Izvod: Odlagališta površinskih kopova na području PK Kolubarai se nalaze pod neposrednim uticajem najvećih energetskih postrojenja ovog tipa u našoj zemlji. Kao posledica eksploatacije, transporta i sagorevanja lignita ovo područje je pod svakodnevnim uticajem aerozagađenja

U okviru naših istraživanja koja su usmerena na istraživanja otpornosti vrste *Pseudotsuga menziesii* (Mirb.) Franco na aerozagađenja, a obzirom da je za delovanje vazdušnih polutanata lisna površina primarna zona negativnog uticaja na biljne vrste, vršena su i ispitivanja kako zagađenje utiče na unutrašnju strukturu četina.

U radu su prikazani rezultati stereoloških ispitivanja volumenskih gustina mezofila ćelija, intercelularnih prostora, centralnog cilindra, smonih kanala i provodnih snopića na preseku četina sa područja PK Kolubara koji su pod direktnim uticajem aerozagađenja i sa kontrolnih površina na Jelovoj gori i planini Juhor.

Na osnovu rezultata istraživanja utvrđeno je smanjenje volumenske gustine hlorenhima i povećanje intercelularnih prostora kod četina duglazije u zagađenoj sredini u odnosu na kontrolne i te razlike su statistički opravdane.

Ključne reči: aerozagađenje, biološka rekultivacija pošumljavanjem, anatomija četina.

1 INTRODUCTION

Douglas fir susceptibility to anthropogenic factors is the subject of the studies of numerous authors. Hermann and Lavander (1999) in their extensive paper on Douglas fir on authochtonous habitat, report that it is often the subject of very grave damages by various agents, stating only fungi and rodents, but not other anthropogenic factors, particularly air-pollutants. Nevertheless, there are some data that Dassler (1981b), speaking about susceptibility of individual important tree species to air-pollutants, classified Douglas fir as the species susceptible to SO_2. Studying the problems of species which are used in the urban environments and industrial zones Appelton et al. (2000) classified Douglas fir as the species tolerant to O_3, wheras Shreuder and Brewer (2001) came to the conclusion that the influence of the short exposure to a high chlorine concentration had an adverse physiological effect on the growth of *Pinus ponderosa* Dougl. Et Laws. needles and *Pseudotsuga menziensii* (Mirb.) Franco. The same gas increases the susceptibility of these species on other stressors, such as drought, reducing the photosynthetic foliage biomass.

Authors emphasize that *Pseudotsuga menziensii* is more susceptible to defoliation, and conclude that early defoliation in Douglas fir is a good indicator of the adverse effect of this gas after the first year.

Pseudotsuga menziesii (Mirb.) Franco has been used in REIK Kolubara for the first time for the recultivation by reforestation of the mechanically damaged soil (Šmit and Veselinović, 1996). The great adaptibility of this species has conditioned it to be among the conifer trees used for reforestation terrains antropogenically damaged in this way.

In mining-energy complex of Kolubara Basin, alongside with the soil degraded by surface lignite exploitation, energy and industrial facilities, such as thermal power plant in Crljeni, coal dryer, thermal power plants TENT A and TENT B in Obrenovac are lo-

cated, and one more thermal power plant in excavation site "Tamnava" Istočno polje is under construction. All these facts point out that this area is under the influence of air pollution as the consequence of combustion of great quantities of lignite which are used as the energy material for the work of the all aforesaid industrial facilites.

As a part of our investigations which are aimed at the studies of susceptibililty of *Pseudotsuga menziesii* (Mirb.) Franco species to air pollution, and regarding the fact that the foliage is the primary zone of the negative influence of the plant species for the activity of the air pollutants, the studies on the influence of pollution on the inner needle structure were done.

2 MATERIAL AND WORKING METHOD

For the analysis of influence of the air pollutants on the inner structure of needles the cultures established on the deposol of coal basin REIK Kolubara – strongly polluted Zs area with the greater number of pollutants in the air (SO_2, NO_3, CO); in Jelova gora – weakly polluted K1 area with the permitted SO_2 in the air; as well as Douglas fir culture established on the acid brown soil in Juhor – non-polluted K2 area.

For the studies in each experimental area with three dominant trees one-year-old and two-year-old needles in the autumn (October-November) and spring (April) were taken. The needle samples were taken from the middle of the crown from the side of the crown exposed to the source of pollution. The needle samples from the middle of the crown were also taken in the control areas. Only the green needles from the main axis of the branches were taken for the analysis. Upon taking in the field the needles were put into fixative. After fixation and dehydration in the laboratory conditions the tissue was impregnated with the araldite resin of low viscosity, and then the material was molded by putting the needles segments in the moulds in which the pure resin was previosly poured.

The material for histological studies was cut by LKB III ultramicrotom. For the experiments under the light microcope the sections 1 m thick, which were applied on the aforesaid tiles and dyed toluidin blue were used. The semi-thick sections were analysed in the laboratory of the Institute of Forestry in Belgrade under the microscope Laica Galen III by lens magnification 40×. The sections were recorded by mycroscopic CCCD camera (Topica TP-5001) in the appropriate formats (tif, bmp, cew, dwg), in the aim of the computer procession in Corel Draw, Photoshop CS (preparation) and AutoCad (measurements) environment.

The obtained and processed photos of sections were analysed by stereological methods. These methods enabled the quantitive appraisal of the dynamics of the alternation in the inner Douglas fir needles occured under the influence of the air pollution, long-term exposure to the effect or seasonal variations. By stereological study the following parameters were determined: volume density of the total needle mesophyll, healthy and injured mesophyll cells, intercellular space of the central cylinder, resin channels, and vascular bundles. Volume density of the studied needle parameter was determined by 30 photos obtained from the section of the one-year-old and two-year-old needles from the polluted and non-polluted zone by lens magnification 40 ×. The results of the measurements are synthesized in the appropriate formats of Excel software environment, and their statistics procession was done in the software package Statgraf. The average levels and average errors were determined, and the statistical significance of variant difference was determined by variance analysis (ANOVA – Duncan method).

3 RESULTS AND DISCUSSION

Volume density is the ratio between the area of the studied tissue and total area of the cross-section, i.e. percentage of this tissue in the total area of the cross-section of the needles. The volume density of chlorenchyma, intercellular space, vacular bundles, resin channels and epidermis was studied.

The volume density of chlorenchyma (Table 1) in autumn one-year-old needles is balanced between the experimental fields in polluted (Zs) and non-polluted areas (control K1 and K2 areas) and ranges between 58 and 59 percentages. The differences are minimal and insignificant per variances. In two-year-old needles the volume density of chlorenchyma is significantly less in the polluted area (Zs) – 55%, whereas in the control experimental areas (K1and K2) it is 61–63%, and the difference is statistically significant. In needles which lasted through the winter (the samples were taken in the spring), volume density of chlorenchyma of one-year-old needles is the smallest in the less polluted area (K1) – 50 %, wheras in the polluted area (Zs) it is 55%, but this difference is not significant. The differences between the one-year-old needles from the non-polluted area (K2), the volume density of which is 60%, is significant in the comparison with the needles from the less polluted area (K1). In two-year-old needles the difference in the volume density of chlorenchyma between the needles from the non-polluted area (K2) is also significant in the comparison with the needles from the polluted (Zs) and less polluted areas (K1).

By analysing the data one can see that in the winter time air pollution influenced the reduction of the volume density of chlorenchyma in the one-year-old and two-year-old needles from the polluted (Zs) and less polluted area (K1). It points to the fact that assimilative organs of the cultures in the polluted area (Zs) have sustained the same changes under the environmental influence, as well as the assimilative organs of the older cultures from the less polluted area (K1).

Table 1 - Volume density of chlorenchyma in one-year-old and two-year-old needles in the polluted area (Zs) and control areas (K1 and K2).

Experimental field	Needle age	Autumn	Spring
		Average value ± standard error	Average value ± standard error
Zs	one-year-old	0,5818 ± 0,0096[a]	0,5512 ± 0,0121[ab]
K 1	one-year-old	0,5921 ± 0,0025[a]	0,5073 ± 0,0471[a]
K 2	one-year-old	0,5850 ± 0,0031[a]	0,6077 ± 0,0071[b]
Zs	two-year-old	0,5532 ± 0,0277[a]	0,5258 ± 0,0203[a]
K1	two-year-old	0,6172 ± 0,0030[b]	0,5583 ± 0,0024[a]
K2	two-year-old	0,6333 ± 0,0079[b]	0,5948 ± 0,0061[b]

Multiple test of interval - values marked with the same letter in the column, do not show the difference at the level of importance $p<0,05$

Volume density of intercellular (Table 2) of the one-year-old needles collected in the autumn is the greatest in the polluted area (Zs) - 20 %, whereas in the non-polluted area (K2) it is 18%. In one-year-old needles which last through the winter (samples taken in the spring) volume density in the polluted area (Zs) is 24%, whereas in the less polluted area (K1) and in the non-polluted area (K2) it is significantly lower and the difference in the comparison with the polluted area (Zs) is significant. The results show that in the needles from the polluted area (Zs) volume density of

chlorenchyma was reduced and volume density of intercellular increased under the influence of air pollution.

Table 2 - Volume density of intercellular in needles the age of which ranges from one to two years from the polluted areas (Zs) and control areas (K1 and K2).

Experimental field	Needle age	Autumn	Spring
		Average value ± standard error	Average value ± standard error
Zs	one-year-old	0,2104 ± 0,0062b	0,2405 ± 0,0099c
K1	one-year-old	0,1923 ±0,0060a	0,1887 ± 0,0041b
K2	one-year-old	0,1964 ±0,0051ab	0,1666 ± 0,0079a
Zs	two-year-old	0,2052 ± 0,0115b	0,2403 ± 0,0089c
K1	two-year-old	0,1551 ± 0,0068a	0,2115 ± 0,0007b
K2	two-year-old	0,1848 ± 0,0028b	0,1728 ± 0,0066a

Multiple test of interval - values marked with the same letter in the column, do not show the difference at the level of importance p<0,05

Data in the Table 3 show that in the needles taken in the autumn volume density of the vascular bundles was greatest in the one-year-old needles of the older culture from the less polluted area (K1) and that the difference was statistically significant in comparison with the needles from polluted area (Zs) and non-polluted area (experimental field K2). In one-year-old needles in the polluted area (Zs) under the influence of air pollution the difference between the volume density of vascular bundles in comparison with both control areas (K1 and K2) was smaller, and the difference is statistically significant. However, in these needles collected after the period of reduced metabolycal activity the volume density of the vascular bundles in the needles from the polluted area (Zs) and both control ones (K1 and K2) were balanced. Therefore, the differences are not significant. In two-year-old needles (autumn samples) from the polluted area (Zs) the volume density of vascular bundles increased and it approached the volume density of the vascular bundles of the older culture. The difference between the volume density of the vascular bundles of the needles from the polluted area (Zs) and non-polluted area (K2) is statistically significant. It points to the fact that the independent influence of air pollution on the reduction of the volume density of the vascular bundles in these needles is significant. At the same time the obtained results point to the fact that two-year-old needles from the polluted area gain the features of old age due to air pollution.

Table 3- Volume density of vascular bundles in the needles the age of which ranges from one to two years from the polluted area (Zs) and control areas (K1 and K2)

Experimental field	Needle age	Autumn	Spring
		Average value ± standard error	Average value ± standard error
Zs	one-year-old	0,0648 ± 0,0020^{a}	0,0862 ± 0,0177^{a}
K1	one-year-old	0,0855 ± 0,0011^{c}	0,0797 ± 0,0008^{a}
K2	one-year-old	0,0776 ± 0,0009^{b}	0,0935 ± 0,0005^{a}
Zs	two-year-old	0,0747 ± 0,0016^{b}	0,0695 ± 0,0017^{a}
K1	two-year-old	0,0825 ± 0,0017^{b}	0,0911 ± 0,0001^{b}
K2	two-year-old	0,0738 ± 0,0014^{a}	0,1005 ± 0,0025^{c}

Multiple test of interval - values marked with the same letter in the column, do not show the difference at the level of importance p<0,05

The differences of the volume density of the resin channels (Table 4) in autumn samples of the one-year-old needles is not significant in the experimental samples, which means that the pollution did not affect the volume density of the resin channels in one-year-old needles. In two-year-old needles (autumn samples) the volume density of the resin channels is the smallest in the comparison with the other samples (Zs and K1). It points to the fact that air pollution influenced the increase of the volume density of the resin channels in the autumn samples.

In the needles sampled in the spring the volume density of the resin channels underwent significant changes. In one-year-old and two-year-old needles the volume density of the resin channels is smallest in the needles from the less polluted area (experimental field K1), whereas the difference in comparison with the ones from the polluted area (Zs) and non-polluted area (experimental field K2) is significant. In the one-year-old needles the difference in the volume density between the needles from the polluted area (Zs) and non-polluted area (experimental field K2) is also statistically significant. Air polluted also affected the reduction of the volume density of the resin channels.

Table 4 - Volume density of resin channels the age of which range from one to two years from the polluted area (Zs) and control areas (K1 and K2)

Experimental field	Needle age	Autumn	Spring
		Average value ± standard error	Average value ± standard error
Zs	one-year-old	0,0304 ± 0,0028[a]	0,0313 ± 0,0018[b]
K1	one-year-old	0,0273 ± 0,0039[a]	0,0244 ± 0,0002[a]
K2	one-year-old	0,0337 ± 0,0008[a]	0,0435 ± 0,0030[c]
Zs	two-year-old	0,0316 ± 0,0024[b]	0,0305 ± 0,0015[b]
K1	two-year-old	0,0261 ± 0,0027[b]	0,0186 ± 0,0001[a]
K2	two-year-old	0,0191 ± 0,0002[a]	0,0348 ± 0,0023[b]

Multiple test of interval - values marked with the same letter in the column, do not show the difference at the level of importance $p<0,05$

Volume density of the epidermis in the autumn samples (Table 5) in one-year-old and two-year-old needles from the polluted area (Zs) and less polluted area (K1) is equal and statistically significanly greater than the volume density of the epidermis of needles from the non-polluted area (K2). The volume density of the epidremis in needles which lasted through the winter (spring samples) did not undergo any changes in comparison with the autumn samples. Volume density of epidermis is the smallest in needles from the non-polluted area (K2). In the both cases air pollution affected the increase of the volume density of epidermis. Apple et al. (2002) showed that the Douglas fir needles of the older crowns are prone to lignification and the increase of the percentage of the epidermis. Therefore, it can be concluded that our results of the volume density of epidermis point to the fact that the needles from the polluted area (Zs) show the properties characteristic for the needles of the older trunks.

Table 5- Volume density of epidermis of needles the age of which ranges from one to two years from the polluted area (Zs) and control areas (K1 and K2)

Experimental field	Needle age	Autumn	Spring
		Average value ± standard error	Average value ± standard error
Zs	one-year-old	0,1195 ± 0,0041[b]	0,1101 ± 0,0033[b]
K1	one-year-old	0,1204± 0,0008[b]	0,1125 ± 0,0017[b]
K2	one-year-old	0,1080 ± 0,0016[a]	0,0887 ± 0,0005[a]
Zs	two-year-old	0,1200 ± 0,0048[b]	0,1161 ± 0,0024[b]
K1	two-year-old	0,1194 ± 0,0039[b]	0,1207 ± 0,0019[b]
K2	two-year-old	0,0891 ± 0,0003[a]	0,0971 ± 0,0012[a]

Multiple test of interval – values marked with the same letter in the column do not show the difference at the level of significance p<0,05

Air pollution influenced the decrease of the volume density of chholrenchyma and increase of the intercellular spaces in the needles in the polluted area (Zs), if they are compared with the volume density in the samples from the non-polluted area (K2) and the difference is statistically significant. This conclusion is in the accord with the results obtained by Ilijin (1995) for the species Picea omorica in the polluted area of Azotara in Pančevo. Volume density of some tissues, particularly of chlorenchyma, in the samples of less polluted area (K1) – older culture is similar with the volume density of tissues of the samples from the polluted area (Zs), which is in the accord with the results obtained by Apple et al. (2000, 2002) that the changes occur in the older cultures of Douglas fir in the structure of tissue of assimilating organs, particularly in chlorenchyma. In the same way, volume density of epidermis is significantly greater in the needles from the polluted area (Zs) and ones from the less polluted area (K1) than the volume density in the needles from the non-polluted area (K2). All these facts point to the conclusions that the changes of the percentage of some tissues in comparison with the total cross section of the needles in the trunks under the influence of air pollution give the picture of aging, which is reflected in the increase of the volume density of the vascular bundles and resin channels in two-year-old needles in the autumn samples both from the polluted area (Zs) and less polluted area (K1). Winner (1994) pointed to the fact that plants corresponded to the air pollution by leaf aging, which was reflected in the decrease of the mobility of stomata and photosythesis. According to the same author, it leads to the reduction of the distribution of nutritive matter from the crown, and thereby to the decrease of increment.

4 CONCLUSIONS

The results of the studies point to the following conclusions:

- For the effect of gas pollutants foliage is the primary zone of influence on the plant species.
- Air pollution influenced the decrease of the volume density of cholrenchyma and increase of the intercellular spaces, both in the needles in the more polluted area REIK Kolubara Zs and in the needles in the less polluted area of control areas K1 and Jelova gora.

- Under the influence of air pollutation volume density of epidermis is significanly greater in the needles from the area with the polluted environment (Zs) and control from the weakly polluted area (K1) in comparison with the control area (K2) from the non-polluted environment.
- The changes of volume density of some tissues under the influence of air pollutation give the picture of needle aging which is reflected in the increase of the volume density of vascular bundles, epidermis and resin channels in the two-year-old needles in the autumn samples from the both polluted (Zs) and less polluted area (K1).

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THE CHANGES IN THE INTERNAL STRUCTURE OF Pseudotsuga menziesii (Mirb.) Franco NEEDLES UNDER THE INFLUENCE OF AIR-POLLUTION

Milorad Veselinović, Dragana Dražić, Mihailo Ratknić, Ljubinko Rakonjac, Vesna Golubović-Ćurguz, Nevena Čule, Suzana Mitrović

Summary

In the mining-power complex of the Kolubara basin, alongside the soil degraded by the open-pit lignite exploitation, the power and industrial plants are situated. Therefore, this area is under the great influence of the air-pollution, which is the result of the combustion of the great quantities of lignite, used as the energy material for the industrial facilities.

Pseudotsuga menziesii (Mirb.) Franco has for the first time been used in the area of Kolubara to recultivate by afforestation mechanically damaged soil of this type (Šmit and Veselinović, 1996). The high adaptability of this species has conditioned its selection among the coniferous trees used for afforestation of anthropologically damaged terrain.

The species chosen for the influence of air -pollutants analysis have been planted on deposol of REIK Kolubara coal field - more polluted area Zs with a larger number of pollutants in the air (SO_2, NO_3, CO); on Jelova Gora - less polluted area K1 with permitted SO2 concentration in the air; and culture of Douglas-fir species on acid brown soil on Juhor mountain – the unpolluted area K2.

Volume density of the total pine needle mesophyll, of healthy and damaged mesophyll cells, central cylinder intercellular spaces, resin channels and vascular bundles have been determined by stereologic analysis. This method has enabled quantitative evaluation of change in the dynamics in internal Douglas-fir pine needle structure caused by air-pollution.

Based on the research results the conclusion has been reached that the changes in micromorphological parameters on the level of light microscope are consisted of the decrease in volume density of the total chlorenchyma and the increase in volume density of intercellular spaces and epidermis.

PROMENE U UNUTRAŠNJOJ STRUKTURI ČETINA *Pseudotsuga menziesii* (Mirb.) Franco POD UTICAJEM AEROZAGAĐENJA

Milorad Veselinović, Dragana Dražić, Mihailo Ratknić, Ljubinko Rakonjac, Vesna Golubović-Ćurguz, Nevena Čule, Suzana Mitrović

Rezime

Na području rudarsko energetskog kompleksa kolubarskog basena, pored površine zemljišta degradiranog površinskom eksploatacijom lignita, locirana su i energetska i industrijska postrojenja tako da je, kao posledica sagorevanja velikih količina lignita koje se koristi kao energetski materijal za rad industrijskih objekata, ovo područje pod velikim uticajem aerozagađenja.

Pseudotsuga menziesii (Mirb.) Franco je na području REIK Kolubara, prvi put na našim prostorima korišćena za rekultivaciju pošumljavanjem mehanički oštećenih ze-

mljišta ovog tipa (Šmit i Veselinović 1996). Velika adaptivnost ove vrste je uslovila, da ona uđe u izbor četinarskih vrsta koje su korišćene za pošumljavanje na ovaj način antropgeno oštećenih terena.

Za analizu uticaja aeropolutanata na unutrašnju strukturu četina odabrane su kulture podignute na: deposolu ugljenog basena REIK Kolubara – jače zagađena sredina Zs sa većim brojem polutanata u vazduhu (SO_2, NO_3, CO); na Jelovoj gori – slabije zagađena sredina K1 sa dozvoljenom koncentracijom SO2 u vazduhu; i kulturi duglazije podignutoj na kiselosmeđem zemljištu na Juhoru – nezagađena sredina K2.

Stereološkim ispitivanjem određivani su: volumenska gustina ukupnog mezofila četine, zdravih i ozleđenih ćelija mezofila, intercelularnih prostora centralnog cilindra, smonih kanala, provodnih snopića. Ova metoda je omogućila da se kvantitativno proceni dinamika izmena unutrašnje strukture četina duglazije koje su nastale pod uticajem aerozagađenja.

Na osnovu rezultata ispitivanja zaključeno je da su se promene mikromorfoloških parametara na nivou svetlosnog mikroskopa sastoje se u smanjenju volumenske gustine ukupnog hlorenhima i povećanju volumenske gustine intercelulara i epidermisa.

Reviewer: Prof. Dragica Vilotić, Ph.D, Faculty of Forestry, Belgrade, Serbia