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SHORT COMMUNICATION

Medial-vegetative proliferation of European larch *Larix decidua* Mill. cones

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ABSTRACT

Identification and analysis of proliferated cones in conifers has both theoretical and practical significance, particularly in systematic and taxonomic investigations, but also in the study of population and individual variability within a species. Comparative morphological-anatomical studies of reproductive organs can contribute to solving many issues in phylogeny and can identify abnormal phenomena in cone formation. In light of the value of such information, this paper presents the results of monitoring European larch *Larix decidua* Mill. cone proliferation in Serbia over a 24-year period, with the aim of studying cone apical meristem activity, which usually is limited. Cone proliferation was confirmed by comparative morphological and genetic-physiological analyses of cones from European larch growing in the Arboretum of the Faculty of Forestry in Belgrade, at ages of 41, 51, and 65 years old.

KEY WORDS

genotype, morphogenetic control, teratological phenomenon

Introduction

Cone proliferation in conifers is an exceptionally rare phenomenon in both natural and managed stands, because apical and diffuse meristems in cone structures usually have limited or determined growth. The limited growth of cones is achieved through the apical and diffuse meristem, and their growth is strictly controlled. The problem of this control is complex because it includes two types of morphogenetic controls. Interruption of limited growth – proliferation of cones includes both types of morphogenetic control, and consists of interconnected and not isolated processes. Intraspecific variability can either result from genetic or environmental factors (Borojević, 1981; Tucović and Stilinović, 1981; Mather and Jinks, 1982). Looney and Duffield (1958) attributed the mass formation of vegetatively proliferated Douglas-fir cones *Pseudotsuga menziensii* (Mirb.) Franco in North America to genetic factors. Tucović and Stilinović (1973) identified five types of proliferation of mature cones of *Cryptomeria* in Serbia, a unique and exceptional phenomenon occurring in plants of the *Pinophyta* division: medial-vegetative, medial-floral, medial-vegetative-floral, medial-vegetative-floral-vegetative-floral and medial-floral-vegetative*, which are induced by genetic factors (Isajev *et al*., 2000; Rudall *et al*., 2011).

*medial-vegetative: axial growth of fruits and formation of shoots with leaves; medial-floral: axial growth of fruits and formation of flowers; medial-vegetative-floral: axial growth of fruits and formation of shoots with leaves and flowers; medial-vegetative-floral-vegetative-floral: axial sprouting and formation of flowers and further sprouting and formation of shoots with leaves and flowers; and medial-floral-vegetative: axial sprouting and formation of flowers and shoots with leaves.

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The European larch *Larix decidua* Mill. trees in this study grow in a group of the same species in similar environmental conditions, so that it is unlikely differential environmental effects would cause cone proliferation in some but not other individuals. As a result, as in the previously noted studies, differences in cone proliferation most likely indicate genetic differences among these trees. Proliferated cones and other rare instances of inter-tree variation in trait expression are important in the study of genetic variability, as they help explain complex bioecological phenomena controlling tree characteristics (Rode, 1983). Medial-vegetative cone proliferation points to the homology of cone and vegetative shoot meristems, and that the existence of homology between reproductive and vegetative organs has a phylogenetic origin. Cone proliferation is a trait of several species of conifers and, just as for some other rare traits, it may be a genetic relic (such as is the case for vivipary, gigantism, nanism, *etc.*).

Material and methods

European larch cultivated in Serbia were monitored as part of the multiannual analyses of autochthonous and allochthonous woody plants in the University of Belgrade's Faculty of Forestry Arboretum. Only one tree with cone proliferation was identified, growing in a small group of trees of the same species in the arboretum, on a mild north-western slope of the Košutnjak forest, at an altitude of 108-124 m in the Banovo Brdo neighbourhood of Belgrade. The site's geographic position and coordinates are 44°48' E longitude and 20°28' N latitude. The site, as shown by the subspontaneous flora, belongs to the climatogenic community *Quercetum farnetto-cerris* Rud. The Faculty of Forestry Arboretum is on the outskirts of Belgrade, on the southern edge of the Pannonian Plain, and the site belongs to the subassociation *Quercetum-farnetto-cerris aculeatetosum* Jov. – an association of Hungarian oak and Turkish oak with butcher's broom, which also includes hornbeam, and is a natural part of the Košutnjak forest (Jovanović, 1956; Tomić, 2004; Ocokoljić, 2005). The climate of the site is similar to that of Belgrade, assessed as subhumid moist based on Thornthwaite's climate classification (Ocokoljić *et al.*, 2011, 2012). The soils consist of surface loess deposits overlaying Sarmat limestone and a layer of Sarmat clay. The bedrock consists of Urgonian limestones. An in-depth analysis of soil physical and chemical characteristics was based on a soil profile located on a plateau on the north-eastern slope of the Košutnjak forest indicates that the soil is a leached chernozem over loess and loess-like sediments. The soil morphology shows anthropogenic impacts affecting solum depth. The A horizon is 41 cm deep and has a notably granular structure. The soil has a loam texture with an optimal ratio of granulometric fractions (ISSS): sand-dust-clay 49.5%-38.2%-12.3%. Free carbonates were not detected in the A/C horizon (horizon transitional between A and C). The reaction of the soil is slightly alkaline 7.7-8.0 pH units in H2O. Humus content was 3.82% and total nitrogen was 0.2%, providing a satisfactory supply of nutrients. Significant nutrient reserves are also present in the A/C horizon, with a surplus of readily available phosphorus and potassium (Ocokoljić, 2000).

The tree selected for study and the adjacent control trees can provide information on the genetic characteristics that may be useful for species genetic improvement. Trees were measured to assess height, measured with a Blume Leiss altimeter; diameter at breast height measured using standard calipers; seed yield; ornamental quality; and tree vitality. These attributes were evaluated to assess variation across the species' range. Tree age was estimated based on reference data, *i.e.*, based on the date of tree planting. Tree vitality and ornamental quality were evaluated on 5-point ordinal scales. Vitality grades were assigned based on the following criteria: Grade 0: Dead or completely diseased; Grade 1: Severely diseased, damaged or decayed; Grade 2: Highly damaged and unhealthy individual with a high risk of dying, but still living (for example, trees with a dead top, trees with canker, with many decayed, mostly dead and damaged branches, with fungal disease or under heavy insect attack); Grade 3: Individuals of average vitality, with some dead, broken or damaged branches, but in which tree survival is not threatened and in which it is possible to improve vitality through remedial management; Grade 4: Healthy individuals, with normal development but some damage present (*e.g.*, some dead branches) or with minor stem defects due to growth in unfavourable conditions; Grade 5: Healthy, normally developed individuals without visible signs of damage. Ornamental quality was determined based on the following criteria: Grade 0: Individuals that have no aesthetic value, that are decayed or with severe defects (*e.g.*, due to disease or severe mechanical damage); Grade 1: Individuals whose location reduces their aesthetic value (*e.g.*, growing in a narrow space between other plants, located in a dense group, having very crooked stem form, *etc.*); Grade 2: Damaged individuals that have reduced aesthetic value, where they have poor stem form (*e.g.*, twisting stems), a dead crown, stem rot, or many damaged branches; Grade 3: Individuals of average ornamental value, with some defects (*e.g.*, dead or damaged branches), but whose appearance may be improved with management; Grade 4: Normally developed individuals with minor stem defects that slightly reduce aesthetic value; Grade 5: Trees with well-developed stem form, growing in a location, that are aesthetically suitable. Seed yield was evaluated by quantifying phenological observations on a five point scale, where 0 is a tree without cones (0% of branches bearing cones); 1 – a small number of cones (≤20%); 2 – low number of cones (>20-≤40%); 3 – moderate number of cones (>40-≤60%); 4 – abundant cones (>60-≤90%) and 5 – maximum number of cones (>90%). The comparative morphological analysis of the cones included three parameters: length, width, and the number of fertile cone scales. The cones were measured with a micrometer to the nearest 0.1 mm.

All biometric analyses were performed on samples large enough to meet the specified standard error of the mean. The study data were statistically processed using Excel and Statistica.

Results

Cone proliferation was identified and monitored at three times over a 24-year period for trees in the family *Pinaceae* Lindl., subfamily *Laricicoideae*, on a group of cultivated European larch trees in the Faculty of Forestry Arboretum. Proliferation was observed on only one European larch tree (tree No. 1 in Table 1), which in 1997 at 41-years-old had attained the height of 14.2 m and diameter of 24.6 cm, while the neighbouring trees at the same site (*Quercetum frainetto-cerris* s.l.) reached heights of 13.6 to 18.8 m, with diameters from 24.0 to 38.1 cm. In 2007, at the age of 51, height of tree No. 1 was 15.4 m and diameter 31.4 cm, while neighbouring trees reached heights of 14.1 to 18.9 m and diameters of 28.9 to 43.8 cm. In 2021 at the age of 56, tree No. 1 height was 15.9 m and diameter 32.0 cm, with neighbouring trees 14.5 to 19.2 m tall and 32.5 to 45.7 cm in diameter (Table 1).

The tree in which repeated cone proliferation was observed (tree No. 1) had excellent vitality and ornamental quality (5), and a high seed yield score of 4.67 (averaged over the three assessments). The average ratings of control trees were for: yield – 4.33; vitality – 4.41; and ornamental quality – 4.10. Trees No. 7 and No. 5 has the highest grade (5) for all analyzed variables. The lowest average grades were recorded for trees No. 6 (seed yield – 3.33) and No. 9 (ornamental quality and vitality for both variables – 3).

On tree No. 1, in which cone proliferation occurred, the incidence of proliferation ranged from 40% in 1997 to 60% in 2021 (Table 2). All proliferated cones in all study years were of the medial-vegetative type. Proliferated shoots varied from 4 to 27 cm long. Shoots formed by medial proliferation had a spiral distribution of buds and needles. None of the proliferated shoots produced short shoots with needles in bundles. Repeated cone proliferation over time on the same tree points to the hereditability of this characteristic in European larch.

Table 1.
Biometric characteristics of sampled European larch *Larix decidua* Mill. in the Faculty of Forestry Arboretum in Belgrade

Parameter Tree number	Age [years]	Tree height[m]	Diameter at breast height [cm]	Seed yield (0-5)	Ornamental quality (0-5)	Vitality (0-5)
1	41	14.2	24.6	5	5	5
	51	15.4	31.4	4	5	5
	65	15.9	32.0	5	5	5
2	41	16.4	27.0	4	5	4
	51	17.5	32.1	4	5	4
	65	18.0	34.3	3	5	4
3	41	16.2	36.2	5	5	4
	51	17.1	41.5	5	5	4
	56	17.5	44.4	5	4	3
4	41	17.3	38.1	4	4	4
	51	18.0	43.8	3	4	4
	65	18.3	45.7	4	4	4
5	41	18.3	21.6	5	5	5
	51	18.9	28.9	5	5	5
	65	19.2	32.5	5	5	5
6	41	17.3	24.0	4	4	4
	51	18.1	29.1	3	4	4
	56	18.6	33.4	3	4	4
7	41	13.6	30.2	5	5	5
	51	14.1	35.6	5	5	5
	65	14.5	38.7	5	5	5
8	41	18.0	30.0	5	5	4
	51	18.5	34.9	4	5	4
	65	19.1	38.8	5	5	4
9	41	15.3	26.8	4	3	3
	51	16.2	32.9	5	3	3
	65	16.4	38.4	3	3	3
10	41	16.5	33.0	5	4	4
	51	17.3	37.7	4	4	4
	65	18.2	42.1	5	4	4

Table 2.
Cone production and proliferation on a European larch *Larix decidua* Mill. tree in the Faculty of Forestry Arboretum in Belgrade

Year	Cone production		Typical cones produced		Cones with medial--vegetative proliferation	
	No.	[%]	No.	[%]	No.	[%]
1997	300	100	180	60	120	40
2007	250	100	137	55	113	45
2021	200	100	80	40	120	60

Typical cones and cones with proliferation differed in cone physical characteristics, as shown in the comparative morphological analysis presented in Table 3. Cones on the nine control trees where none were proliferated were shorter on average by 14%, 7% narrower and had 9% fewer fertile cone scales compared with proliferated cones (Table 3). Physical attributes of cones without proliferation (on tree No. 1) were within the range of values of cones from this tree that had proliferation. Statistical analysis of the absolute (standard deviation and error) and relative variability (coefficient of variation and its error) for all three physical attributes indicated greater variability in cones without proliferation (Tucović, 1995).

Analysis of variance for the three investigated variables of cones in three years (tree age 41, 51 and 65 years) confirmed the statistical significance determined by morphometric analyses. These results indicate that there are significant differences in cone attributes between the tree with cone proliferation and the control trees (Table 4).

The results of the analysis of variance for three cone attributes at three times (Table 4) showed significant differences between trees, but not between the repetitions and years, confirming the hypothesis that differences in cone attributes are genetic.

Table 3.

Comparative physical attributes of European larch *Larix decidua* Mill. cones with proliferation (tree No. 1) and without proliferation (trees No. 2-10) in the Faculty of Forestry Arboretum in Belgrade

Cone type	Min-max	$\bar{x} \pm S\bar{x}$	S ±Ss	V ±Sv
		Cone length [mm]		
Without proliferation	29-46	35.89 ±0.21	2.41 ±0.24	10.70 ±0.97
With proliferation	33-49	38.51 ±0.33	1.89 ±0.35	8.55 ±0.61
		Width of open cones [mm]		
Without proliferation	15-21	17.12 ±0.16	1.58 ±0.22	9.68 ±0.91
With proliferation	17-24	20.24 ±0.18	1.27 ±0.13	6.29 ±0.63
		Number of fertile scales per cone		
Without proliferation	41-52	46.11 ±0.13	1.78 ±0.19	7.89 ±0.69
With proliferation	44-55	48.44 ±0.21	1.14 ±0.15	6.22 ±0.43

$\bar{x}$ – medium values, S – standard deviations, V – coefficients of variation and $S\bar{x}$, SS, SV – errors

Table 4.

Analysis of variance of three physical attributes of European larch *Larix decidua* Mill. cones with proliferation and without proliferation measured in three different years in the Faculty of Forestry Arboretum in Belgrade

Variability factor	Sum of squares	F-ratio
	Cone length	
Factor A: tree	0.37122	14.68***
Factor B: year	0.14182	4.64*
Interaction: A×B	0.23334	5.48*
	Width of open cones	
Factor A: tree	0.17238	4.80***
Factor B: year	0.81435	2.37*
Interaction: A×B	0.51226	1.58*
	Number of fertile cone scales	
Factor A: tree	0.38122	12.89***
Factor B: year	0.14082	3.55*
Interaction: A×B	0.23349	3.17*

Discussion and Conclusions

This paper describes and analyses European larch medial-vegetative cone proliferation, which is essentially a medial (axial) proliferation. Cone proliferation takes place by activation of axial and diffuse meristems within the already formed cones, even though cones are structures with determinate growth, which ceases after cones reach maturity (Looney and Duffield, 1958; Zhuchenko, 1988; Yinyan and Shaoying, 1995). Thus, the axial and diffuse meristems of cones in some trees of this species can retain the ability to reactivate, *i.e.*, these meristems are potentially capable of forming vegetative shoots after cone formation. This potential for renewed development was apparent in this study by the activation of axial and sub-epidermal meristems that proceeded to produce vegetative shoots containing needles and attaining lengths of from several mm to several tens of centimetres.

The larch tree we observed producing proliferated cones grows in close proximity to a group of trees of the same species that failed to demonstrate cone proliferation, which indicates that cone proliferation was not due to external factors. The results of monitoring of cones' proliferation during 24 successive years on the same tree indicate a hereditary characteristic. Also, analysis of variance proves significant differences between trees, but not between repetitions and years, which confirms the assumption that this property is genetically determined. Cone proliferation by the same tree at three points in time over several decades points to the heritability of this trait in European larch. For this reason we conclude that cone proliferation is genetically based, which has both theoretical and practical significance in the study of population and individual variability of the species, as well as in systematic and taxonomic research.

Authors' contributions

M.O. – the research concept, laboratory and statistical analysis, manuscript preparation; Dj.P. – sample collection, laboratory analysis, manuscript corrections; N.G. – sample collection, manuscript corrections.

Conflicts of interest

There is no conflict of interest.

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STRESZCZENIE

Wierzchołkowo-wegetatywny wzrost szyszek modrzewia europejskiego *Larix decidua* Mill.

Wegetatywny wzrost (proliferacja) szyszek jest niezwykle rzadki, zarówno w drzewostanach odnawianych naturalnie, jak i sztucznie, ponieważ szyszki są tymi strukturami roślinnymi, których wzrost jest zazwyczaj ograniczony lub kontrolowany przez merystem wierzchołkowy i rozproszony.

Obserwacje i analizy dotyczące zjawiska proliferacji szyszek, w tym systematyczne badania taksonomiczne z tego zakresu, mające na celu poznanie zmienności populacyjnej i osobniczej pod względem tej cechy, mają duże znaczenie teoretyczne i praktyczne. Szczególnie badania z zakresu morfologii porównawczej i anatomii narządów rozrodczych pozwalają rozwiązać wiele istotnych zagadnień filogenetycznych oraz pomagają w wyjaśnianiu nieprawidłowości, które mogą występować w ich rozwoju.

W pracy przedstawiono wyniki 24-letniego monitoringu zjawiska proliferacji szyszek w grupie drzew modrzewia europejskiego (rodzina *Pinaceae* Lindl., podrodzina *Laricoideae*) rosnących w Arboretum Wydziału Leśnego w Belgradzie w Serbii (tab. 1). Badania miały na celu monitorowanie i badanie aktywności merystemu wierzchołkowego szyszki, którego wzrost zazwyczaj jest ograniczony. Stosując metody morfologii porównawczej i fizjologii genetycznej w odniesieniu do szyszek wytwarzanych przez grupę modrzewi europejskich w wieku 41 lat (w 1997 r.), 51 lat (w 2007 r.) i 65 lat (w 2021 r.), zjawisko wegetatywnej wielokrotnej proliferacji stwierdzono tylko u szyszek z jednego drzewa (tab. 3).

Analizowane drzewo rośnie w otoczeniu drzew tego samego gatunku na ograniczonym obszarze, co eliminuje możliwość wpływu czynników zewnętrznych na wegetatywny wzrost szyszek. Wytypowane i oznaczone drzewo modrzewia wytwarzało od 40% (1997 r.) do 60% (2021 r.) szyszek zdolnych do rozmnażania (tab. 2). Zaobserwowana proliferacja w każdym roku badań reprezen-

towała typ, który można określić jako wierzchołkowo-wegetatywny. Pędy, które powstawały w wyniku tej proliferacji, charakteryzowały się spiralnym układem pąków i igieł. Na żadnym z pędów nie zaobserwowano występowania skarłowaceń ze skupieniami szyszek.

Wyniki monitoringu proliferacji szyszek oraz analiza wariancji wykazały istotne różnice między badanymi drzewami dotyczące podstawowych parametrów szyszek, ale nie między powtórzeniami i latami (tab. 4), co wskazuje na dziedziczny charakter tej cechy u modrzewia europejskiego i potwierdza przypuszczenie, że cecha ta jest uwarunkowana genetycznie.