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Germination of Seeds and Cytogenetic Analysis of the Spruce in Differently Polluted Areas of Slovenia

By

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Summary

Bavcon J., Druškovič B. & Papeš D. 1994. Germination of seeds and cytogenetic analysis of the spruce in differently polluted areas of Slovenia. – Phyton (Horn, Austria) 33 (2): 267–277. – English with German summary.

An evaluation was performed of the damage to genetic material of the Norway spruce, *Picea abies* (L.) Karst., in twenty populations with different degrees of pollution. The factors as studied were seed germination and cytogenetic analysis.

The basic material consisted of spruce seeds gathered in twenty sites in the Republic of Slovenia. They were divided into three groups depending on the manner of pollution and our previous results.

The percentages of germination show significant differences between localities but the correlation with pollution is not explicit. Cytogenetic analysis of the root tips did not show a significant difference between individual sites.

A conclusion could be drawn that a constant natural selection probably exists through the elimination of sensitive genotypes of the Norway spruce and that the strongests only could remain in polluted areas.

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Zusammenfassung

BAVCON J., DRUŠKOVIČ B. & PAPEŠ D. 1994. Samenkeimung und cytogenetische Untersuchungen an der Fichte in unterschiedlich belasteten Gebieten Sloveniens. – Phyton (Horn, Austria) 33 (2): 267–277. – Englisch mit deutscher Zusammenfassung.

Die Schädigung von genetischem Material der Fichte, *Picea abies* (L.) KARST. wurde bei zwanzig Populationen mit unterschiedlicher Belastung von Luftverunreinigungen ausgewertet. Dabei wurde auf die Samenkeimung und cytogenetische Analysen eingegangen. Das Untersuchungsmateial waren Fichtensamen, welche an zwanzig Stellen in der Republik Slovenien gesammelt wurden. Sie wurden entsprechend der Art der Luftverunreinigungen und unseren früheren Untersuchungen in drei Gruppen geteilt. Die Keimungsraten zeigten signifikante Unterschiede zwischen den einzelnen Örtlichkeiten, wogegen die Korrelation mit den Luftverunreinigungen nicht deutlich war. Die cytogenetische Untersuchung der Wurzelspitzen zeigte keine signifikanten Unterschiede zwischen den Probestellen. Als Schlußfolgerung wird angenommen, daß wahrscheinlich eine ständige natürliche Auslese stattfindet, indem empfindliche Genotypen der Fichte eliminiert werden und nur die stärksten in den belasteten Gebieten ürig bleiben.

Introduction

Forest decay is currently an object of intensive studies as the forest with its numerous functions represents, ecologically speaking, the backbone of the land.

Forest decline due to gas was first reported in Slovenia in 1927, in the surroundings of the Žerjav lead smeltery (Ajnžik, 1968). The decline and decay of forests in the areas of known pollutants began to be systematically investigated towards the end of the 60's and the beginning of the 70's (ŠOLAR, 1978). In 1985, the first inventory was performed, i.e. a description of forests on the so-called forestry bioindicational grid, related also to the Middle European grid. The results showed that the Slovenian forests were considerably damaged (IGLG, 1989). Apart from the parameters studied by our foresters, a study of the cytogenetic bioindication was initiated in the same year. The question is of a method for an early detection of damage to the organisms due to the presence of genotoxic pollutants in the environment. It is aimed at determining the degree of threat at a given location (DRUŠKOVIČ, 1988a, b). Used as bioindicational tree was the Norway spruce whose chromosomes are large enough to allow a cytogenetic analysis (DRUŠKOVIČ, 1988a) and which is present almost everywhere in Slovenia (MARTINČIČ & SUŠNIK, 1969).

Our work was based on the results of the cytogenetic analysis performed on 5-years old spruces and some older fructiferous ones from our research area (DRUŠKOVIČ 1985, 1988a, b). The older trees were visually evaluated for damage (ŠOLAR et al. 1988, ŠOLAR, 1990). Measured was the sulphur content of their needles (KALAN, 1989). We proceeded by raising the question as to whether it would be possible to confirm the relationship between a polluted environment, the spruce seed germination, and degree of chromosomal damage in the seedlings grown from seeds from varyingly polluted areas.

Materials and Methods

The basic material seeds were those of the Norway spruce, *Picea abies* (L.) Karst. gathered in the fall of the seed year 1988.

According to the manner of pollution and the previous results (DRUŠKOVIČ 1988a, b; KALAN, 1989) from differently polluted areas in Slovenia, the seeds were divided into three hypotetical groups determined with respect to the visual damage, sulphur content and chromosomal aberrations:

I – extremely polluted, a local source of pollutants:

Žerjav, Jazbina at Mrdavs, and Jazbina at Halda (Mežica valley) N Slovenia, source of pollution: lead smeltery, visual damage: 4th (the highest) class, sulphur content: – 4 (ČAS & al. 1990), and chromosome damage: plus 3 to 4 (4th the highest class).

Retje (above Trbovlje by the Sava river), source of pollution: thermal power station only one last fructiferous spruce of the two still growing in the area, sulphur content 4th class.

II – polluted, a local source and remote influence of pollutants:

Koprivna, N Slovenia, possible sporadic effect of lead smeltery, visual damage plus 2 , sulphur content: 3 to 4 class.

Hanžej (Mežica valley), N Slovenia, sulphur content plus 3 class. Other data not available.

Brneško Sedlo (Pohorje), N Slovenia, a possible influence of the thermal power station in Šoštanj and a distant source, sulphur content: 3rd to 4th class, chromosome damage: plus 3rd class.

Repiško (Pohorje), N Slovenia, a possible influence of the thermal power station in Šoštanj, sulphur content: 2nd class, chromosome damage: up to 4th class.

Jezersko (Gorenjska), NW Slovenia, a remote influence of pollutants, sulphur content: 2nd class, chromosome damage: plus 3rd class.

Jelovica (Gorenjska), NW Slovenia, a remote influence of pollutants, chromosome damage: plus 3rd class.

III – less polluted, only a remote influence of pollutants:

Praznikarjevo (Drava valley), N Slovenia,chromosome damage: 2nd class.

Therefore, the present investigation was focused on total complex of pollutants acting in individual locations. The seeds were allowed to remain in the cones until these spontaneusly opened, then they were shaken out and their sails removed. They were kept in cool and dry conditions until the beginning of experiments.

In the first part of the experiment, germination of the spruce seeds was determined, in the second the seedling root tips were used to make squash preparations for cytogenetic analysis.

Dried seeds were germinated on damp filter paper in Petri dishes at a temperature of 20°C in daylight. The germinating seeds were first counted at the seventh day, then at the fourteenth, and finally at twenty first day (ISTA, 1985). For each locality four times one hundred seeds were placed in four Petri dishes. The entire germination was repeated twice (January 28, 1989, and March 23, 1989). Due to a lack of material, later series amounted to three times one hundred seeds only. Between the above dates, another attempt was performed on February 21, 1989 but was restricted to three sites only. (Table 1).

After the first week, the 1-2 cm long roots were cut off for cytogenetic analysis and placed in groups of ten in distilled water. The pretreatment with alpha-monobromonaphthalene for 24 hours at a temperature of 4° C resulted in clearly visible chromosomes so aberrations could easily be seen with a NU 2 light microscope at a magnification of x 790 and x 1250, respectively. After the pretreatment, the root tips were washed in tap water, fixed (in 3:1 alcohol and glacial acetic acid), and kept refrigerated until studied. They were stained according to the Feulgen technique, macerations were made in acetocarmine. The permanent preparations were frozen in liquid CO_2, rinsed first with alcohol and glacial acetic acid, 3:1, then in ethanol, and finally in butanol, and mounted in Euparal.

The metaphase chromosomal aberrations were divided into the so-called specific aberrations, representing the damage of individual segments of the chromosome, and the nonspecific ones in which marked changes occur in the morphology of a large part or even all of the chromosome (DRUŠKOVIČ, 1988a).

Specific aberrations included gaps, breaks, fragments, and rings; the nonspecific ones were chromosome connections and stickiness and amorphous chromatin masses. The so-called other aberrations: diplochromosomes, cleavage of the centromeres, fragmentation and chromatin disintegration were noticed too.

100 metaphases were analysed per slide. In our case, this was usually the maximum achievable number due to the small size of the root tips. Ten root tips were studied for each location, representing ten plants for each site.

The results were analysed statistically by using the computer program "Stat graphics" including the nonparametric method of the Kruskal-Wallis' test.

Results

Germination

According to our hypothesis, germination of seeds from highly polluted areas should be decreased because of possible damage to the reproductive region.

The external morphology of seeds from different locations showed no marked differences, except that those from Žerjav, a highly polluted area, were of better quality by sight, of quite uniform size than those from Praznikarjevo which belongs to the third, not heavyly polluted group. The sites of Praznikarjevo and Repiško, both slightly polluted, had more small seeds than the others. The seed from Brneško Sedlo, from the polluted group, had a conspicous, unusually light brown colour of the seed coating.

Seed germination percentage in individual localities showed that our hypothesis was not completely valid and that there were deviations (Table 1). The Agricultural Institute of Ljubljana considers an 80% rate of germination optimal in the Norway spruce.

In Retje, in the markedly polluted group, the percentage of germination at 28% was extremely low, indeed. Praznikarjevo, from the little polluted group, and Repiško, from the polluted one, had a rate of 48% and 64%, respectively, which lower than that of Žerjav with 65%, Jazbina at Mrdavs at 60% and Jazbina at Halde with 65%, both in the markedly polluted group. Brneško Sedlo at 91% and Koprivna at 89%, from the polluted group, both showed the highest percentage of germination. The two sites from Jelovica, 78% and 86%, though in the polluted areas, showed good germination even though they were lower than that from Brneško Sedlo and Koprivna.

All the sites were compared with each other by the Kruskal-Wallis test. It was found that there were differences among the localities (28. 1. 1989 sig. 0.00012, 21. 2. 1989 sig. 0.02 and 23. 3. 1989 sig. 0.0014) but the correlation with the degree of pollution was not prominent, with the exception of Retje where germination hardly appeared at all.

Table 1

Percentage of germination after 21 days (3 x 4 repetitions) for each locality.

Group/ Localities	28. 1. 1989 4 repetitions				21. 2. 1989 3 repetitions			23. 3. 1989 3 repetitions		
I Jazbina (Mrd.)	59	62	62	53	–	–	–	53	62	69
I Jazbina (hal.)	–	–	–	–	60	62	60	77	68	–
I Žerjav	66	74	50	45	–	–	–	61	73	85
I Retje	36	20	–	–	–	–	–	–	–	–
II Repiško	65	63	57	58	–	–	–	66	74	66
II Brneško	88	90	87	94	–	–	–	94	92	95
II Koprivna	85	85	86	84	–	–	–	98	94	92
II Hanžej	52	63	58	51	43	49	56	54	49	40
II Jezersko	53	62	59	56	–	–	–	59	56	65
III Jelovica 1	79	79	86	86	–	–	–	77	75	74
III Jelovica2	81	81	83	73	–	–	–	93	94	94
III Praznikar	50	53	24	–	50	45	52	58	48	–

Legend: I - markedly polluted group
II - polluted group
III - slightly polluted group

Cytogenetic analysis

To determine chromosomal aberrations, one must first get well acquainted with the karyotype (2n = 24, LOEVE & LOEVE 1972). The karyotype of the Norway spruce from different regions of Slovenia was determined in our laboratory (DRUŠKOVIČ, 1988a, 1989). The karyotype is comprised of three pairs of SATs, five pairs of metacentric and four pairs of submetacentric chromosomes. This number was obtained by several authors (Miyake 1903, SAX & SAX 1933, SANTAMOUR 1960). The description of the Norway spruce karyotype reported by HIZUME 1988 differed from ours in having five pairs with a secondary constriction (SAT), whereas in the sixth pair the secondary constriction appears in chromosome only.

The cytogenetic analysis of the root tips showed that the most frequent aberrations were those of the nonspecific type while the specific ones were less common. The so-called other aberrations appeared rarely. Among the nonspecific aberrations there often appeared chromosome stickiness, less frequently chromosome connections, while amorphous chromatin masses were very rare. In the specific group, fragments were seen most often, then breaks, gaps, while rings were very rare. The other aberrations such as fragmentations and chromatin degeneration, were rare.

The results obtained from different locations were subject to the Kruskal-Wallis test. It showed that there were no significant differences between different sites in the numbers of aberrant cells (sig. 0.71), the numbers of cells with specific aberrations (sig. 0.27), and the numbers of cells with nonspecific aberrations (sig. 0.13).

Discussion

Present-day conifers belong to ancient phylogenetic species which have succesfully competed with younger angiosperms and have thus managed to survive. Now, however, they are severly threatened by the excessive pollution of the environment (SCHÜTZ, 1985, JURAT & SCHAUB 1988, SCHULZE 1989, VAN DER STEGEN & MYTTENAERE 1991).

The commonest tree in Slovenia is the Norway spruce, which is intensively planted in large areas. Seed germination, meaning the ability of the tree to reproduce itself both on its home ground or in a larger territory, has a crucial role in the normal rejuvenation of the plantation. As we usually plant young seedlings in empty areas, natural rejuvenation is a fair indicator of the state of our forests. The extreme pollutional example of Žerjav in the Mežica valley proves that where there is no normal reproduction, seedlings brought in from the outside will likewise fail to prosper. There the environmental decline is acute because of the emissions from the lead smeltery. Natural rejuvenation has completely stopped and the once-present wood in the neighborhood has completely decayed. The many years of effort to reseed and reclaim the forest have proved in vain.

The present tests were designed to demonstrate whether such seed material could be obtained as would grow in this environment despite the damage and pollution. Our premise was that a weak plant was already a doomed one. KNIEBEL & LEBEN, 1981 (according to SCHOLZ, 1986) state the same: weaker individuals gradually disappear in a polluted environment. They could survive in an environment as long as pollution was a local phenomenon. SCHOLZ & GEBUREK, 1983, noticed that highly sensitive organisms seem to produce fewer seeds, which implies a partial loss of the gene pool. SCHÜTZ, 1985, reported that the resistance to pollution is no such thing as actual immunity; some species and individuals tolerate disturbance better than others, but this only postpones the moment of decline for a while.

Seed germination from different sites showed that our hypothesis was not completely valid and that deviations (Tab. 1) are involved that are not negligible.

The Kruskal-Wallis test did confirm the presence of significant differences between localities but these cannot be attributed to increased pollution only. They may occur due to various factors influencing pollination, fertilization, and later the development of seeds themselves. Among the markedly polluted group, Retje alone showed an extremely low percentage of germination, thus and so demonstrating the correlation with pollution. But all the other sites showed deviations explained more fully in the text.

Another object of our study was the morphology of the seedlings. Individual abnormalities were observed, yet their number was too low to allow any conclusions regarding the damage. The results show that there were three abnormal sprouts at the most in 400 samples from the markedly polluted group from Žerjav. This is in accordance with the limits set by the Agricultural Institute in Ljubljana when routinely testing seed germination, or it may even be better. Our data represent an interesting observation but cannot be ascribed much weight.

The second portion of the experiment consisted of observations of the chromosomes of meristem root tips. Cytogenetic analysis was applied to determine the degree of damage in the metaphase chromosomes. If, by way of comparison, aberrant cells are taken from individual sites (Tab. 2), the differences as stated between varyingly polluted areas are very small. Diversity is obvious already in individual localities. One could arrive at the same conclusion if comparing the specific aberrations and cells with nonspecific aberrations (Tab. 2). In all instances, the Kruskal-Wallis test confirms that there are no significant differences between sites.

In our analyses we took into account the total number of different aberrations (DRUŠKOVIČ, 1988a; MÜLLER et al. 1991). It is probably more legitimate to count only the number of aberrant cells, for natural selection it-

Table 2

Metaphase cells counts (N) with number of total aberrant cells, types of specific and nonspecific aberrations, and other types aberrations per chromosome.

N	Aber. cells	Spec. aber.	Nonsp. aber.	Other aber.	N	Aber. cells	Spec. aber.	Nonsp. aber.	Other aber.
Jazbina Mrdavs markedly polluted					Jazbina halda markedly polluted				
90	10	2	8		78	4	1	3	
100	8	1	7		100	3		3	
83	4	1	3		38	3	1 *	3	
100	7		7		100	11	3	8	
95	6 *	2 *	5		94	7		7	
100	6 *	2 *	5		64	6		6	
100	5	2	3		100	4	2	2	
100	8	2	6		70	2		2	
97	9 *	3 *	9		60	5		5	
80	6	2	4		62	5		5	
Žerjav markedly polluted					Retje markedly polluted				
74	11 *	4 *	8		77	11 *	4 *	5	3
100	8	2	6	1	100	3	1	2	
100	9	5	4	3	100	6	1	5	
92	4		4		97	3		3	
61	8		8		100	8 *	1 *	8	
85	5	1	4		93	11	1	10	
59	3		3		73	7	1	6	
40	9	3	6		63	10		10	
100	7 *	1 *	7		100	13	1	11	1
75	6 *	2 *	5		100	7		7	
Repiško polluted					Brneško sedlo polluted				
100	8	2	5	1	100	8	1	5	2
100	12 *	8 *	4	3	100	2 *		2	1 *
100	3		3		92	7 *	3 *	5	
62	7 *	2 *	6		27	2 *	3 *	2	
73	4		3	1	54	6		6	
79	1		1		32	3		3	
66	6 *	1 *	4	2	48	3		3	
93	8 *	2 *	7	1	37	5 *	3 *	4	
83	7	2	5		45	11 *	1 *	10	1
100	5	2	3		65	2 *		2	1 *

* two or more different types of aberration in one cell.

Table 2 (Continued)

N	Aber. cells	Spec. aber.	Nonsp. aber.	Other aber.	N	Aber. cells	Spec. aber.	Nonsp. aber.	Other aber.
Koprivna polluted					Hanžej polluted				
100	13*	2*	11	1	79	5		5	
100	8*	4*	6		100	9	2	7	
100	12	2	10		94	6		6	
81	4	1	3		75	6		6	
42	2		2		100	6	1	5	
51	12		11	1	92	9		8	1
90	6		5	1	89	7		7	
100	10*	3*	9		100	5	1	4	
54	6		4	2	100	9	1	8	
90	11*	2*	10		96	8		8	
Jezersko polluted					Jelovica polluted				
100	12		12		61	2		1	1
82	2	1	1		77	13		13	
100	3	3			77	3	1	2	
94	4	4			26	3		3	
48	4*	1*	4		100	5		5	
95	7	2	5		69	9	1	8	
100	8		8		88	6		6	
100	5		5		76	10	1	9	
100	10	1	6	3	43	9		9	
67	9		9		91	7*	1*	6	1
Jelovica polluted					Praznikarjevo slightly polluted				
72	3	2	1		77	15*	3*	13	1
100	4*	2*	3		100	11	1	10	
71	2	1	1		100	19*	5*	13	3
47	2	1	1		100	5	1	4	
100	6*	2*	5		62	4	1	3	
100	12		12		45	7		7	
70	8*	4*	7		68	6		6	
70	7		7		94	8*	1*	8	
71	6		6		100	10	1	9	1
66	10*	4*	7		82	5*	3*	3	

* two or more different types of aberrations in one cell.

self eliminates all those incapable of correcting the errors by reparative mechanisms.

Another question to be posed is how to evaluate the chromosomes changes seen in somatic cells. It cannot be supposed that they are hereditary. We can only conclude on the greater or lesser vitality of the spruce (DRUŠKOVIČ, 1988a; MÜLLER et al. 1991). The aim of the test was to determine whether the possibility of chromosomes damage to the seedlings could be anticipated at least to some extent, on the basis of cytogenetic damage to fully grown trees. In comparing the percentage of aberrations in 5-year spruces in 1988 (control 8.7%) with that in the seedlings of 6.5–10.9%, the number of aberrant cells remains at the level of the controls. This indicates that the damage is not hereditary but is merely the consequence of an individual spruce growing in a particular *milieu;* damage must, to some degree, be constantly repaired. In all likelihood, more vigorous specimens may develop more of healthy seed as the plant has the opportunity of developing active embryos.

Our results, however, show that such conclusions are highly questionable. On one hand, the genome of the Norway spruce, with its abundance of average genetic masses could be extremely sensitive to pollution (DRUŠKOVIČ, 1988a). On the other hand, ceaseless natural selection seems to eliminate the most sensitive organisms. Our results coincide with the decreasing in the variability of the species in highly polluted areas, as was also noted by KNIEBEL & LEBEN according to Scholz 1986.

The Norway spruce has as a protective mechanism, polyembryony, which is greatly controled by the mother's tree (ILLIES 1959), meaning that only the strongest, fittest embryos survive. This increases the chances of the seed to mature despite noxious influences which, if to many may lead to be empty seeds.

Proceeding from our results, it would be difficult to draw any conclusions on the influence of a polluted environment because of the numerous factors which may influence the plants in the course of an one year experiment. For more precise and better understanding it would be necessary to introduce an experiment covering several years so as to confirm or refute our results.

Literature

AJNŽIK J. 1968. Če kopre dov leti. – Koroški Fužinar 25–29.

ČAS M., GOLOB S. & AZAREV E. 1990. Prostorsko proučevanje in spremljanje pustošenja in propadanja gozdov ter spreminjanje namembnosti gozdnega prostora. – Interna publikacija IGLG, Ljubljana.

DRUŠKOVIČ B. 1985. Changes in plant populations around the lead smeltery. (In Slovenian). – Biol. Vestn. 33, 2: 23–38.

— 1988a. Cytogenetic bioindication I - Utilization of cytogenetic analysis for detection of the effects of genotoxic pollutants on forest trees. (In Slovenian). – Biol. vest. 36: 1–18.

— 1988b. Cytogenetic bioindication II - The kind of estimation of burdened genetic material and the assessment of menaced Norway spruce on a certain locality. (In Slovenian). – Biol. vest. 36: 31–44.

— 1989. Kariotipi slovenskih iglavcev. II. kongres biosistematikov Jugoslavije. – Izvlečki poročil 13–14. Gozd Martuljek.

Hizume M. 1988. Karyomorphological studies in the family *Pinaceae*. – Memoirs of the Faculty of Education Ehime University. 8:1 - 108., Nat. Sci. 8 : 1–108.

Iglg 1989. Gradivo za novinarsko konferenco. Interna publikacija. Ljubljana.

Illies Z. M.,1959. Weitere Mehrlingsuntersuchungen bei *Picea abies* (L.) Karst. Silvae genet. 8: 111–113.

International Seed Testing Association (Ista) 1985. – Seed Sci. Technol. 13.

Jurat R. & Schaub H. 1988. Effects of sulphur dioxide and ozone on ion uptake of spruce (*Picea abies* [L.] Karst.) Seedlings. – Z. Pflanzenernähr. Bodenk. 151: 379–384.

Kalan J. 1989. Obremenjenost slovenskih gozdov z žveplom. – Zbornik gozdarstva in lesarstva 34: 99–120.

Loeve A. & Loeve H. 1972. Cytotaxonomical atlas of the flora of Slovenia. – University of Colorado, Boulder, Colorado, USA.

Martinčič A., Sušnik F. 1969. Mala flora Slovenije. – Cankarjeva založba, Ljubljana.

Miyake K. 1903. On the development of the sexual organs and fertilization in *Picea excelsa*. – Ann. For. 17: 351–372.

Müller M., Guttenberger H., Grill D., Druškovič B. & Paradič J. 1991. A cytogenetic method for examining the vitality of spruces.-Phyton (Horn, Austria) 62: 507–520.

Santamour F. S. 1960. New chromosomes counts in *Pinus* and *Picea*. Silvae Genet. 9: 87–88.

Sax K. C. & Sax H. J. 1933. Chromosome number and morphology in the conifers. – J. Arnold Arbor. 14: 356–375.

Scholz F. 1986. Genetic effects of air pollutants. – 18th IUFRO World Congress. Proceedings 1: 286–293. Ljubljana.

— & Geburek T., 1983. Über die Wirkung saurer Niederschläge auf die genetische Struktur von Walclbaum Population. – VDI – Berichte.

Schulze E. D., 1989. Air pollution and forest decline in a spruce (*Picea abies*) – Forest. Sci. 244: 776–783.

Schütz J. Ph. 1985. Forest decay in a continental-wide polluted environment: control by silvicultural measures. Experientia 41: 320–325.

Šolar M. 1978. Vpliv onesnaženega zraka na gozdove v zgornji Mežiški dolini (Žerjav). Zdrav. vestn. 47: 269–272.

— 1990. The condition of the Slovene forest in 1989 and the damage degree-movement in the period between 1985–1989 – Basic Data. (In Slovenian). – G. V. 2: 85–90.

— & Pogačnik J., Hočevar M., Batič F., Jurc D., Anko B., Hrček D., Dručkovič B. 1988. Kako rešiti gozdove. Ljubljana.

Van der Stegen J. & Myttenaere C., 1991. Status of sulphur in foliage of Norway spruce (*Picea abies* [L.] Karst.) in relation to the mode of contamination. – Environ. Pollut. 69: 327–336.