depression. The results (*Table 2*) show that the selection is not very sensitive to penalty constant variation. Therefore it may be sufficient to use inbreeding depression as the penalty constant when GMS is applied for seed orchard selection. In a more complex case the identification of an adjusted penalty constant is similar to the simplified case; the corrections are mainly cancelled out. Thus, a penalty constant corresponding to inbreeding depression seems to be the most relevant value when GMS is applied for establishing a seed orchard.

Acknowledgement

This study was supported by Föreningen Skogsträdsförädling, SkogForsk, the Swedish Council for Agricultural Sciences, and the NICHOLSON's scholarship of the N. C. State University. We appreciate the support of the NCSU Tree Improvement Program and the use of their data. We would also like to thank TORE ERICSSON for his valuable comments.

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Clonal Differences and Relations Between Diameter Growth, Stem Cracks and Fungi in a 36-year-old Clonal Seed Orchard of Norway Spruce (*Picea abies* (L.) KARST.)

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(Received 28th March 2001)

Summary

A study was carried out during 1987 to 1999 in seed orchard of *Picea abies* (L.) KARST. located in central Lithuania to estimate differences in diameter growth, occurrence of stem cracks, bark beetle (*Dendroctonus micans* KUG.) attacks, tree dieback and fungal infections among 20 different clones. Those were represented by 1333 clonal grafts that, at the time of the study, were 36-year-old. The results had shown: 1) significant differences in diameter growth among the different clones; 2) significant differences in the occurrence of stem cracks among the different clones; 3) significant positive relationship between average diameter at breast height (d.b.h.) of the clone and amount of trees with the stem cracks within the clone ($r = 0.511$; $p < 0.05$); 4) that within the clone, trees of larger d.b.h. are more likely to have cracked stems. Analysis of the data by chi-squared tests revealed four significant ($p < 0.000001$) relationships: 1) in trees that possessed stem cracks occurrence of the dieback (66 dead out of 197, or 33.5%) was much higher than in trees without cracks (44 dead out of 1136, or 3.9%); 2) in trees that possessed stem cracks frequency of *D. micans* attack was much higher (52 attacked out of 197, or 26.4%) than in trees without cracks (10 attacked out of 1136, or 0.9%); 3) the survival rate of trees with the stem cracks that were attacked by *D. micans* was much lower (2 survived out of 52, or 3.8%) than survival rate of trees with the stem cracks that were not attacked by *D. micans* (129 survived out of 145, or 89.0%); 4) the trees that suffered both from stem cracks and *D. micans* attack were less likely to survive (2 survived out of 52, or 3.8%) than sound-looking ones which were attacked by the bark beetle (9 survived out of 10, or 90%). Among the fungal species, *Sarea resinae* KUNTZE and *Sarea dif-*

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formis FR. were the most common ones and were frequently found both in sound-looking stems and in stems with cracks.

Key words: *Picea abies*, clonal grafts, stem cracks, *Dendroctonus micans*, *Sarea resinae*, *Sarea difformis*.

Introduction

Forest seed orchard of Norway spruce (*Picea abies* (L.) KARST.) of Dubrava Forest Enterprise is located 5 km north of Kaunas, Lithuania (54.52N 23.55E). Establishing of the seed orchard was described in detail by RAMANAUSKAS and GRADECKAS (1967). According to their report, graft material for the seed orchard was collected during winter-summer 1963 from twenty *P. abies* trees of fast growth (age 50 to 150 years, diameter at breast height (d.b.h.) 29 cm to 70 cm, height 24 cm to 42 m), with exceptionally good stem and crown characteristics, in stands of highest quality class throughout the Lithuania. For the graft material only those trees were selected, which d.b.h. and height was exceeding an average d.b.h. and height of trees from the first quality class (bonität I) by at least 20% and 10%, respectively. The grafting was carried out during the spring-summer 1963 into 6-7-year-old *P. abies* trees. In spring 1964 to 1965, those were planted on former agricultural land characterized by rather rich clayish soil (RAMANAUSKAS and GRADECKAS, 1967). Consequently, the seed orchard comprises 20 clones, each represented initially by 73 ramets: in total 1460 trees were planted. Schematic distribution of clonal grafts in the forest seed orchard of Dubrava Forest Enterprise is presented in *figure 1.*

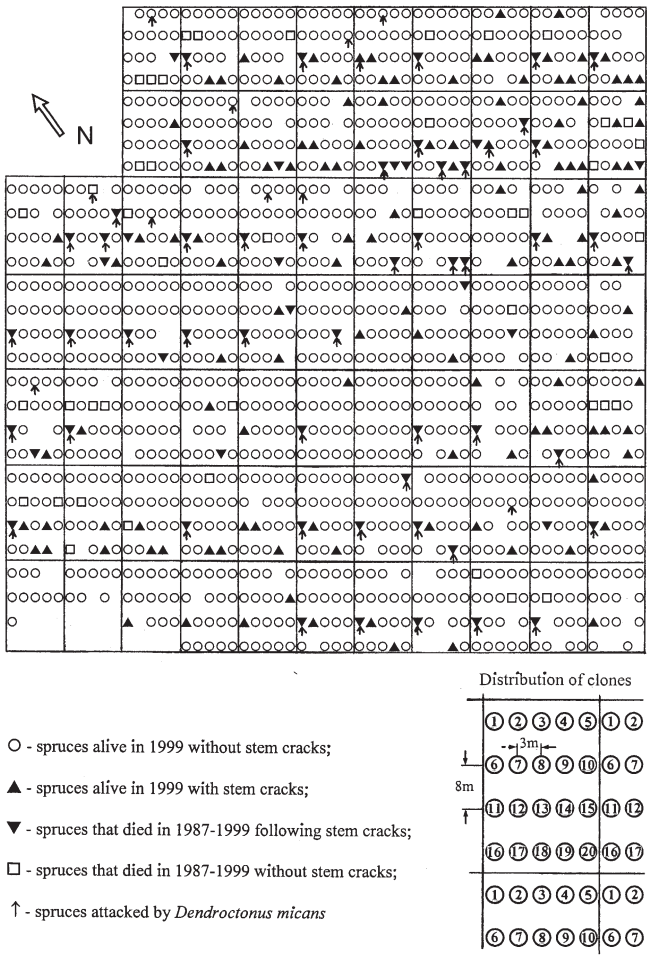

Fig. 1. – Spatial distribution of *Picea abies* trees in seed orchard of Dubrava Forest Enterprise in 1999. Symbols indicate trees planted in 1964 to 1965. Encircled numbers show clonal assignment. Based on RAMANAUSKAS and GRADECKAS (1967).

From the northwest, the seed orchard of *P. abies* borders the seed orchard of hybrid larch, and from the southeast, – the seed orchard of Canadian blue spruce. From the northeast and southwest, it is surrounded by the 10 m wide protective plantation composed of locust, maple, ash and lime trees.

In 1987, a number of spruces in the seed orchard showed dieback symptoms. Therefore the trees in the orchard were subject to close external inspection. By the end of 1987 it was found that: 86 trees (6.5%) possessed 2 m to 4 m long stem cracks (*Fig. 2*) and 45 of them died following the attack by the bark beetle *Dendroctonus micans* KUG.; 145 of the stems (10.9%) had 1 to 4 closed scars on the bark, each up to 20 cm in length (*Fig. 3*), but neither of them showed any dieback symptoms nor were attacked by the bark beetle; the occurrence of stem cracks and the incidence of the beetle attack differed sharply among the different clones VASILIAUSKAS (1989).

Fig. 2. – Over 3 m long crack on stem of *Picea abies* in seed orchard of Dubrava Forest Enterprise. Stem area affected by the crack is marked with paint (photo from 1987).

The present study has been undertaken in the seed orchard of Dubrava Forest Enterprise in spring 1999. Its main objectives were: 1) to monitor overall sanitary state of the seed orchard, especially in regard to the incidence of new stem cracks, attacks by *D. micans* and tree dieback; 2) to estimate differences in diameter growth among the clonal grafts of

Fig. 3. – Three 10 cm to 15 cm long closed scars on stem of *Picea abies* in seed orchard of Dubrava Forest Enterprise. Their margins are marked with paint (photo from 1987).

P. abies and to determine its possible influence on the occurrence of stem cracks; 3) to identify fungal species that inhabit sound-looking stems, stems with cracks and closed scars of different clonal grafts, and to provide implications on possible fungal role in the occurrence of the observed stem injuries.

Materials and Methods

At the time of initial investigation in 1987, every tree in the seed orchard was mapped onto field journal, and for each of them the presence/absence of cracks, scars and *D. micans* was recorded. Dying and dead trees were removed from the seed orchard. On living trees, margins of 20 stem cracks and those of 57 closed scars were marked with a paint (*Figs. 2, 3*). In January 1993, storm has broken 46 spruces in the forest seed orchard, eight among which (17.7%) had stem cracks.

Only the trees planted initially in years 1964 to 1965 were included into the present work, thus all the examined spruces were 42 to 43 years of age, and every of them were grafted 36 years previously. Trees that were replanted later, following death of initially planted ones, were excluded from the study. By spring 1999, out of 1460 spruces that were planted in the years 1964 to 1965, 1223 were alive. For each of them, the d.b.h. was measured and the stems were subject to close external examination. The appearance of 'old' cracks and closed scars (that were present in 1987) and the incidence of 'new' stem cracks (that appeared after 1987) was recorded. The symptoms of possible 'new' attacks by *D. micans*, as well as the symptoms of tree dieback were also looked for.

For fungal isolation and identification, samples of wood were taken from 26 stems. Those included eleven stems from the clone 18 (six with stem cracks and five sound-looking), five stems from the clone 19 (all with stem cracks), five stems from the clone 8 (all with closed scars) and five stems from the clone 9 (all sound-looking). From each stem three samples were taken, at approx. 0.5 m, 2.0 m and 3.5 m heights above the ground. While sampling stems with the cracks, an attempt was made to keep all three sampling points in crack's vicinity, at about 3 cm to 3.5 cm from its border. While sampling stems with the closed scars, an attempt was made to take one of the samples directly underneath the scar.

The sampling and fungal isolation were carried out as described in the previous studies (VASILIAUSKAS *et al.,* 1996; VASILIAUSKAS and STENLID, 1998). Each sample was taken by inserting an increment borer 6 cm to 9 cm deep into the stem. The bore cores were brought to the laboratory in sterilised glass tubes. All woody pieces were then surface sterilised by flaming and placed on Petri dishes containing Hagem agar (HA) medium: 5 g glucose, 0.5 g NH_4NO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 5 g malt extract, 20 g agar, 1000 ml H_2O, pH 5.5. Distance from the outer layer of wood at which the fungal growth occurred was measured. Fungal colonies were subcultured after 10 days to 15 days of growth and species in pure culture were identified by the staff of CBS in Baarn, the Netherlands.

A total of 25 isolates of *Sarea resinae* KUNTZE from 11 stems were included into vegetative compatibility tests. Seven of the stems possessed cracks and four did not. Vegetative compatibility tests were carried out as described previously (VASILIAUSKAS and STENLID, 1997), on vegetable juice agar (VA) medium: 5 g glucose, 200 mL Granini vegetable juice, 25 g agar, 800 mL H_20, pH 5.5. The tests were performed by cutting 4-mm discs of mycelium + VA from the margin of actively growing colonies and placing them pairwise 1 cm to 1.5 cm apart in the centre of 9 cm Petri dishes containing approx. 20 mL VA. These were incubated up to 120 days at room temperature and examined periodically. The isolates were paired in all combinations. Additionally, every isolate was self-paired as two pieces from the same mycelium.

For the d.b.h.'s of the trees measured, means and standard deviations were calculated, and their comparisons were performed using the *t*-statistics; occurrence of stem cracks among the clones was compared using the chi-squared tests (MEAD and CURNOW, 1986). In multiply comparisons carried out, confidence limits for p-values were reduced using the BONFERRONI correction (KREBS, 1999).

Results

Clonal assignment, status, number and d.b.h. of 36-year-old clonal grafts of *P. abies* in the seed orchard recorded by spring 1999 is shown in the *table 1*, and their spatial distribution in *figure 1*. Since the vigour and sanitary state of trees with closed scars did not differ in any respect from the sound-looking ones, these two categories in the following will be discussed as the trees without stem cracks.

Among the forty-four trees without stem cracks that died in 1987 to 1999, thirty-eight were broken by wind, one died following *D. micans* attack and the remaining five neither had the stem cracks, nor were attacked by the beetle, thus the reasons of their death are unknown. Among the sixty-six trees with the stem cracks that died in 1987 to 1999, forty-five died during the initial study in 1987 (see Introduction), five died during the subsequent years following *D. micans* attack in 1987, eight died during the subsequent years although were not attacked by *D. micans*, and another eight were broken by wind. Thus, in spring 1999 only 20 trees (or 23.3%) were alive out of 86 that possessed stem cracks in 1987.

Analysis of the above data using chi-squared test revealed four significant relationships. It was found that: 1) in trees that possessed stem cracks occurrence of the dieback (66 dead out of 197, or 33.5%) was much higher than in trees without cracks (44 dead out of 1136, or 3.9%); 2) in trees that possessed

Table 1. – Clonal assignment, number, status and stem diameters (d.b.h. ± s.d.) of 36-year-old *Picea abies* clones in seed orchard of Dubrava Forest Enterprise in 1999. Data reflects the trees that were planted in years 1964 to 1965.

Clone	Number (%) of ramets within each clone observed in 1987 – 1999					Mean d.b.h.± s.d.
	total	with scars	with cracks	attacked by *D. micans*[A]	dead[B]	in 1999, cm
1	66	2 (3.0)	2 (3.0)	1 (1.5)	1 (1.5)	31.9 ± 5.7
2	69	0	0	0	0	25.7 ± 5.3
3	73	5 (6.8)	6 (8.2)	5 (6.8)	2 (2.7)	29.2 ± 4.9
4	59	5 (8.5)	0	0	0	27.4 ± 5.9
5	68	35 (51.5)	9 (13.2)	2 (2.9)	2 (2.9)	31.5 ± 4.6
6	71	4 (5.6)	0	0	5 (7.0)	29.5 ± 3.9
7	70	0	0	0	12 (17.1)	29.2 ± 4.0
8	71	44 (62.0)	4 (5.6)	1 (1.4)	2 (2.8)	29.8 ± 3.3
9	67	7 (10.4)	3 (4.5)	1 (1.5)	5 (7.5)	31.6 ± 5.9
10	67	9 (13.4)	7 (10.4)	3 (4.5)	7 (10.4)	30.4 ± 4.3
11	70	7 (10.0)	56 (80.0)	37 (52.9)	39 (55.7)	30.8 ± 6.3
12	68	0	25 (36.8)	1 (1.5)	1 (1.5)	32.3 ± 5.5
13	66	2 (3.0)	0	0	1 (1.5)	28.3 ± 4.2
14	65	5 (7.7)	7 (10.8)	2 (3.1)	3 (4.6)	23.1 ± 4.8
15	65	1 (1.5)	6 (9.2)	0	3 (4.6)	30.4 ± 3.7
16	67	1 (1.5)	0	0	2 (3.0)	27.3 ± 2.3
17	58	6 (10.3)	0	0	3 (5.2)	21.8 ± 3.3
18	62	6 (9.7)	19 (30.6)	3 (4.8)	6 (9.7)	29.0 ± 7.6
19	69	4 (5.8)	44 (63.8)	4 (5.8)	12 (17.4)	34.3 ± 3.8
20	62	2 (3.2)	9 (14.5)	2 (3.2)	4 (6.5)	28.4 ± 4.7
Total	1333 (100)	145 (10.9)	197 (14.8)	62 (4.7)	110 (8.3)	29.1 ± 4.7

[A]) Among the 62 trees attacked by *Dendroctonus micans,* 52 (83.9%) possessed stem cracks; 50 (96.2%) of those have died until 1999. Among the remaining 10 trees that were free of cracks and were attacked by *D. micans,* only one (10%) has died until 1999.
[B]) Among the 110 trees that were dead, 66 (60%) possessed stem cracks.

stem cracks frequency of *D. micans* attack was much higher (52 attacked out of 197, or 26.4%) than in trees without cracks (10 attacked out of 1136, or 0.9%); 3) the survival rate of trees with the stem cracks that were attacked by *D. micans* was much lower (2 survived out of 52, or 3.8%) than survival rate of trees with the stem cracks that were not attacked by *D. micans* (129 survived out of 145, or 89.0%); 4) the trees that suffered both from stem cracks and *D. micans* attack were less likely to survive (2 survived out of 52, or 3.8%) than the sound-looking ones which were attacked by the bark beetle (9 survived out of 10, or 90%). The p-values of the chi-squared tests in all four cases were lower than 0.000001.

By the spring 1999, none of the cracks and closed scars, borders of which were marked with paint in 1987, showed any changes in their dimensions. No new attacks by *D. micans* were recorded in the forest seed orchard within that period of time. At the time of the present study, a total amount of trees with the stem cracks in the seed orchard was 131. This indicates that during 1987 to 1999 the cracks did appear on another 111 of stems that were sound-looking ten years previously. Analysis of diameter growth and occurrence of stem cracks in *P. abies* in the seed orchard has revealed a high number of significant differences in diameter growth among the different clones (*Table 2*). When all clones were compared in-between regarding the stem cracking frequency (19 comparisons were made for each clone), statistically significant differences in the occurrence of stem cracks most often were noted for the clone no. 11 (in 18 comparisons out of 19), the clone no. 19 (in 17 comparisons), the clone no. 12 (in 14 comparisons), and for the clone no. 18 (in 12 comparisons). The trees within these four clones suffered from stem cracks more often than the trees from the remaining sixteen clones (*Table 1*). Significant positive correlation has been detected between the average d.b.h. of the clone and amount of trees with the stem cracks within the clone ($r = 0.511$; $p < 0.05$). It was also found out, that within the clone, trees of larger d.b.h. are more likely to have cracked stems (*Table 3*).

Out of 78 samples that were taken for isolation of fungi, 67 (86%) gave fungal growth and 11 did not. Fourteen samples (18%) gave growth for two different fungi. Fungi were isolated from all 26 *P. abies* stems examined. Their species are presented in the *table 4*. *Sarea resinae* KUNTZE and *Sarea difformis* FR. were the most common ones and were commonly found in all three categories of stems examined: in sound-looking ones, in stems with cracks and in stems with closed scars (*Table 4*). In total, *S. resinae* was found in 10 trees with stem cracks out of 11 examined, and it was found in all 15 examined trees without stem cracks. *S. difformis* was found in 6 trees with stem cracks

Table 2. – Comparison by t-test of mean stem diameters among the 36-year-old clones of *Picea abies*. Diameters that were compared are presented in the *table 1* (Mean d.b.h. in 1999). In multiple (190) comparisons, consistent overall error rate was maintained by reducing confidence limits for p-values using the BONFERRONI correction (reducing the p value to p/190). Levels of significance are indicated as: * - difference significant at $p < 0.05$; ** - difference significant at $p < 0.01$; *** - difference significant at $p < 0.001$; n.s. - difference statistically not significant.

Clone	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
2	***																		
3	n.s.	*																	
4	**	n.s.	n.s.																
5	n.s.	***	n.s.	**															
6	n.s.	**	n.s.	n.s.	n.s.														
7	n.s.	*	n.s.	n.s.	n.s.	n.s.													
8	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.												
9	n.s.	***	n.s.	*	n.s.	n.s.	n.s.	n.s.											
10	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.										
11	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.									
12	n.s.	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
13	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	*	n.s.	n.s.	**							
14	***	n.s.	***	**	***	***	***	***	***	***	***	***	***						
15	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***					
16	***	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	***	***	*	***	n.s.	***	***				
17	***	**	***	***	***	***	***	***	***	***	***	***	***	n.s.	***	***			
18	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	***		
19	n.s.	***	***	***	n.s.	***	***	***	n.s.	***	n.s.	n.s.	***	***	***	***	***	***	
20	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	***	n.s.	n.s.	***	n.s.	***

Table 3. – Mean diameters (d.b.h. ± s.d.) and numbers of stems with and without cracks within the 36-year-old clones of *Picea abies* in seed orchard of Dubrava Forest Enterprise in 1999. Significant differences in d.b.h. among cracked and non-cracked stems within the clones are indicated as: * – significant at $p < 0.05$; ** – significant at $p < 0.01$; *** – significant at $p < 0.001$; all others – not significant. Confidence limits for p-values were reduced using the Bonferroni correction (reducing the p-value to p/13).

Clone	Mean d.b.h. ± s.d. of trees, cm (number of trees)	
	without stem cracks	with stem cracks
1	32.0 ± 5.8 (63)	30.0 ± 1.4 (2)
3	28.8 ± 4.8 (65)	33.5 ± 3.9 (6)
5	31.3 ± 4.3 (59)	32.9 ± 6.9 (7)
8	29.6 ± 3.2 (65)	33.0 ± 3.5 (4)
9	31.2 ± 5.6 (59)	40.0 ± 5.3 (3)
10	30.4 ± 4.3 (56)	31.0 ± 5.6 (4)
11**	26.1 ± 5.8 (13)	34.7 ± 3.6 (18)
12***	30.4 ± 5.9 (43)	35.6 ± 2.4 (24)
14*	22.4 ± 4.2 (58)	33.3 ± 2.3 (4)
15	30.2 ± 3.7 (57)	32.9 ± 2.9 (5)
18***	25.6 ± 5.5 (41)	38.4 ± 2.5 (15)
19	32.9 ± 4.8 (23)	35.2 ± 2.6 (34)
20	27.8 ± 4.3 (53)	35.2 ± 4.0 (5)

out of 11 examined, and it was found in 5 trees without stem cracks out of 15 examined. This shows that neither the incidence of stem cracks was influenced by the presence of these fungi, nor the occurrence of infections by these fungi were dependent on presence/absence of stem cracks.

S. resinae was isolated from 56 (72%) samples, eighteen of which were taken at 0.5 m height, twenty-one at 2.0 m height, and seventeen at 3.5 m height. *S. differmis* was isolated from 14 (18%) samples, five from both 0.5 m and 3.5 m heights, and four from 2.0 m height. This indicates rather uniform occurrence of both species over 0.5 m to 3.5 m height range within *P. abies* stems. Both *S. resinae* and *S. differmis* were found in stems of all four tree clones examined, thus clonal assignment of the host tree had no influence on the infection incidence by these fungi.

Among the 14 samples that gave growth for two different fungi, combination *S. resinae* + *S. differmis* was the most common, occurring in 7 cases (50 %). *Zalerion arboricola* BUCZACKI in all three cases was growing from the same sample as *S. resinae*, and *Arthrographis pinicola* SIGLER and YAMAOKA in both cases was growing from the same sample as *S. resinae*. The two remaining cases were: *S. resinae* + *Fomitopsis pinicola* (SCHW.: FR.) KARST., and *S. resinae* + *Beauveria bassiana* VUILLEMIN.

Table 4. – Isolation percentage of fungi from sound-looking stems of *Picea abies*, from stems with cracks and closed scars, and a number of trees examined in forest seed orchard of Dubrava Forest Enterprise.

Fungus	Stem condition, (no.)			
	sound	crack	scar	All
	(10)	(11)	(5)	(26)
Sarea resinae Kuntze	100	91	100	96
Sarea differmis Fr.	30	55	40	42
Zalerion arboricola Bucz.	–	18	20	12
Emericellopsis terricola v.B	10	–	–	4
Beauveria bassiana Vuill.	–	–	20	4
Fomitopsis pinicola Karst.	–	9	–	4
Arthrographis pinicola Sigl.	–	9	20	8

In 39 bore cores (70%) growth of *S. resinae* occurred from the outer layer of wood immediately close to the bark, and in another 17 samples fungus started to grow at 1 mm to 36 mm distance from the outer layer of wood. *S. difformis* showed growth from the outer layer of wood in 10 bore cores (67%), and in another 4 its growth occurred at 3 mm to 31 mm distance towards the centre of stem. Other fungi were also detected in the outer layers of the sapwood up to 11 mm deep.

Vegetative compatibility tests with the isolates of *S. resinae* had shown: 1) incompatible mycelial reactions (gap or reaction zone between two mycelia) among the isolates from different trees; 2) incompatible mycelial reactions among the isolates within 7 trees, five of which possessed stem cracks and two were without stem cracks; 3) compatible mycelial reactions (two mycelia growing into one entity as that in self-pairing controls) among the isolates within 4 trees, two of which possessed stem cracks and two were without stem cracks. Among the latter, two cases of compatibility were noted among the isolates sampled 2.0 m and 3.5 m high, one case among the isolates sampled 0.5 m and 2 m high, and one case among the isolates sampled 0.5 m and 3.5 m high. In the last case, sampled tree was without stem cracks.

Discussion

The study had shown that during the 12-years period (1987 to 1999) neither of the marked cracks had changed its dimensions noticeably, nor that any of the closed scars did develop into stem cracks or cankerous wounds. After 36 years of growth under rather similar environmental conditions, different clonal grafts of *P. abies* in most cases exhibited clear differences in diameter growth (*Tables 1, 2*). The results show that 111 'new' stem cracks appeared in the seed orchard during the 12 years, and that the fast radial growth might be an important factor predisposing *P. abies* trees to stem cracks (*Table 3*). There are reports from southern Scandinavia and Germany regarding the occurrence of very similar stem cracks in 15- to 35-year-old sparsely stocked *P. abies* plantations growing on rich soil; large width of annual growth-rings, low amount of latewood within the rings, high variation of growth-rings within the stem and droughts were suggested as possible causes of the injury (AIGNER, 1981; DIETRICHSON *et al.,* 1985; PERSSON, 1994). Our results correspond to this rather well, since the trees measured during the present work were of nearly similar age, therefore the ones with the larger diameter should contain wider annual growth-rings, and those indeed were more severely affected by the stem cracks.

This study has revealed three associations: 1) fast radial growth of *P. abies* and occurrence of the stem cracks; 2) occurrence of the stem cracks and incidence of *D. micans* attack; 3) incidence of *D. micans* attack and tree death. Although no 'new' attacks by the beetle were observed during the period 1987 to 1999, the presence of 131 trees with stem cracks makes the seed orchard prone to possible future outbreaks of the beetle. Also in forest stands, attacks by *D. micans* to *P. abies* trees with stem wounds might be frequent (VASILIAUSKAS *et al.*, 1996).

Apart of fast radial growth, tree provenance may be important in susceptibility of *P. abies* trees to stem cracks, while some provenances suffer form the damage more often than the others (DIETRICHSON *et al.*, 1985; PERSSON, 1994). According to PERSSON (1994), stem cracking in *P. abies* is partly under genetic control and may be different in different clones. Our work also revealed a number of significant differences regarding the frequency of stem cracks in different clonal grafts of *P. abies* (*Table 1*). This of course might be simply due to the fact that some tree genotypes grow faster than the others. However, our data shows that this relationship, although significantly positive, is not straightforward ($r = 0.511$; $p < 0.05$), so genetically determined factor for susceptibility of *P. abies* to stem cracks can not be excluded. It already has been suggested, that *P. abies* clones with a high predisposition to cracking should be removed from seed orchards (PERSSON, 1994).

Fungal impact on the occurrence of stem cracks remains unclear. *S. resinae* and its relative *S. difformis* were previously found both in sound-looking *P. abies* stems (HUSE, 1981), as well as in stems with mechanical damage (SOKOLOV, 1958; ŠČEDROVA, 1959; ROLL-HANSEN and ROLL-HANSEN, 1980). *S. difformis* has been also reported to inhabit sound-looking stems of *Pinus banksiana* LAMB. (BASHAM, 1966) and *Picea mariana* (MILL.) B.S.P. (BASHAM, 1973), and was isolated from stem cankers of *P. abies* and *Pinus sylvestris* L. (KUJALA, 1950; ŠČEDROVA, 1965; FYODOROV, 1985). *S. resinae* was commonly observed in stumps of *P. sylvestris* (MEREDITH, 1959, 1960). The characteristic for *S. resinae* and *S. difformis* is their growth in resin-soaked wood (MEREDITH, 1959, 1960; ROLL-HANSEN and ROLL-HANSEN, 1980).

Zalerion arboricola BUCZACKI, apart from the present work, was occasionally found in *P. abies* stems with mechanical injury (PAWSEY and STANKOVICOVA, 1974a, b; ROLL-HANSEN and ROLL-HANSEN, 1980) and probably is of minor importance. Among the rest of fungi that were identified during our study, *F. pinicola* is of certain interest. The fungus is an active decayer of wood, therefore its activity in damaged stems leads to losses of wood production even when tree survives the cracking of stem. The evidence is already available that *P. abies* stems with cracks frequently contain decay (AIGNER, 1981; DIETRICHSON *et al.,* 1985; PERSSON, 1994). It was surprising though, that during the present work only one tree yielded decay fungus. Moreover, it was *F. pinicola*, the species that seldom inhabits *P. abies* stems with mechanical wounds (VASILIAUSKAS, 1989; VASILIAUSKAS and STENLID, 1998). However, it may be of interest to note that in North America *F. pinicola* in many cases was found to be carried by the bark beetles from genus *Dendroctonus* (CASTELLO *et al.,* 1976; HARRINGTON *et al.,* 1981; PETTEY and SHAW, 1986). Nevertheless, the tree from which the fungus was isolated during the present study did not have any visible external symptoms of *D. micans* attack.

Vegetative compatibility tests with *S. resinae* provided limited evidence to suggest that the size of individual mycelium of this ascomycete within living trees of *P. abies* might be rather large, expanding over 2 m to 3 m. The evidence is already available that the individual mycelium of postfire ascomycete *Daldinia loculata* (LEV.) SACC. in stems of birch might also be large, reaching up to 2 m in size (JOHANNESSON *et al.*, 2001). However, the results of our vegetative compatibility tests should be regarded as preliminary. More detailed investigations are necessary and employing of molecular markers might prove useful in determining genetic relationships and population structure of *S. resinae* in living stems of *P. abies*.

Acknowledgments

We thank Dr. ULF OLSSON and Dr. ASKO LEHTIJÄRVI for valuable suggestions.

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Linkage Relationships as a Useful Tool to State Interspecific Gene Homology: Case Study with Isozyme Loci in *Austrocedrus chilensis (Cupressaceae)*

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(Received 3rd April 2001)

Summary

Linkage relationships among 12 polymorphic isozyme loci were analyzed in *Austrocedrus chilensis* (D. DON) FLORIN et BOUTELJE. Double or multiple heterozygous individuals were chosen from a general genetic inventory of 403 trees. Twenty-nine out of 66 possible pairwise combinations were found.

Between 21 to 187 macrogametophytes per tree were subjected to horizontal starch gel electrophoresis. Linkage was proved through individual and pooled data between four pairs of genes. Three of them showed a tight linkage: *Aat2~6Pgdh2* (frequency of recombination **R** = 0.122 ± 0.036), *Aat3~Sod* (**R** = 0.125 ± 0.021) and *Aat1~Pgi2* (**R** = 0.143 ± 0.030), while a moderate linkage was found with respect to the pair *Idh2~Skdh* (**R** = 0.333 ± 0.052).

The inheritance of two allozyme gene loci was additionally proved: *Aat2* and *Pgi2*, each locus with at least two alleles. Thus, two new isozyme markers are reported for this species.

Different considerations for the establishment of a correct homology of gene loci before doing interspecific comparisons are discussed. The relative migration distance of isozymes on zymograms is proven as non sufficient evidence of homology of the encoding genes between species. On the other hand, linkages are shown to be a good tool for this purpose due to the highly conservative arrangement of the genes among related species.

Key words: Gene homology, linkage, allozyme, inheritance, *Cupressaceae*, *Austrocedrus chilensis.*

Introduction

In the last two decades, the development of QTL marker methods led to the construction of genetic linkage maps for several agronomic crop plants and a few forest tree species with the aim to verify correlations between markers and QTLs of economic importance. The number of polymorphic allozyme markers is, however, usually not large enough in order to use this type of marker for the identification of QTLs.

On the other hand, it has been suggested that gene arrangements are highly conservative within a certain family or at least within a certain genus (GURIES et al., 1978; CONKLE, 1981; KING and DANCIK, 1983; STRAUSS and CONKLE, 1986; GONCHARENKO et al., 1998). Therefore, the study of linkages of allozyme markers could efficiently contribute to the understanding of the evolution and the phylogenetic relationships of different related species.

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