

Article

Impact of *Rhabdocline pseudotsugae* and *Phaeocryptopus gaeumannii* on the Selection of Suitable Provenances of Douglas Fir in Central Europe

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Abstract: Two diseases, Rhabdocline needle cast caused by *Rhabdocline pseudotsugae* Sydow, and Swiss needle cast caused by *Phaeocryptopus gaeumannii* (Rohde) Petr., recently became a severe threat to Central European Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands. Both pathogens infect assimilative organs causing needle chloritization and drop off. Pervasive infection by these pathogens has been recorded at the Hůrky provenance trial (Písek, South Bohemia, Czech Republic), established in 1971 as part of a series of experiments by International Union of Forest Research Organizations (IUFRO). The intensity and duration of needle cast sporulation were monitored using a Burkard spore trap, and the health status of 24 Douglas fir provenances from the original areal of distribution (British Columbia, Washington, Oregon) was evaluated under this trial. While comparing provenances, the following characteristics were surveyed: trunk volume, defoliation rate, and the difference in tree diameter between measurements in 2011 and 2016. A statistical evaluation was performed using the regression model and a decision tree. The highest sporulation rates on needles for both needle casts were observed from April to July. The Washington provenances 1069 North Bend, 1075 Enumclaw, and 1089 Cathlamet can be recommended for plantation, considering the provenances' satisfactory productivity and low extent of damage from needle casts, while the provenances such as 1104 Brookings, 1028 Merritt (due to high mortality) and 1010 Barrière, 1021 D'Arcy, and 1067 Skykomish (due to high defoliation) are not suitable for plantation under Central European conditions.

Keywords: *Pseudotsuga menziesii*; IUFRO provenance trial; climate change; Central Europe; invasive species; Rhabdocline needle cast; Swiss needle cast; health status

1. Introduction

During an examination of stands health in the neighborhood of the Hůrky research trial (Czech Republic) in 2012, infections of Douglas fir *Pseudotsuga menziesii* (Mirb.) Franco caused by pathogens *Rhabdocline pseudotsugae* Sydow and *Phaeocryptopus gaeumannii* (Rohde) Petr. were recorded for the first time. The occurrences of both needle casts were directly observed during the research trial itself. However, in 2011, when the trial had been evaluated by Kšír et al. [1], the needle cast problem was deemed insignificant. A repeated examination of the tree health status in 2015 confirmed the

regular occurrence of both needle casts in the surrounding stands, and an increased infection rate was also reasserted in the provenance trial, with apparent differences in the defoliation of individual provenances detected.

Douglas fir has been introduced to Central Europe by forest managers for its high production of commercially valuable products and also for its ability to stabilize forest stands, mainly against windthrow [2,3]. Various plantations of this species with undocumented origin were created in Europe during the 19th century, while first provenance-based research, oriented to survival rate, production, and quality, was performed in Germany in 1910 [4]. It is also considered as an alternative to the domestic Norway spruce (*Picea abies* (L.) Karst.) in areas where the quality of the litter has deteriorated [3]. In comparison to Norway spruce, Douglas fir has a smaller effect on soil nutrients and has a positive effect on humus production, soil pH, and can regenerate naturally under suitable silvicultural treatments [2,5,6].

Although Douglas fir has successfully regenerated naturally in a number of European locations, e.g., References [7–11], artificial renewal of partially adapted first-generation Douglas fir stands has had mixed success. The most significant limitation, however, is the low quality of seeds from European sources [12,13]. In contrast to seeds from North America, European seeds exhibit low germination rates [2,14] and low resistance to the Douglas fir seed chalcid *Megastigmus spermotrophus* Wachtl, 1893 [2,15]. Hence, stand renewal can often fail even after using the seed orchards, and European forest managers have no other practical alternatives than relying on imports from the US and Canada [12]. Ultimately, the use of seeds from local sources with pre-adaptation to the site conditions of Central Europe is most the desirable option [16].

The natural range of the Douglas fir is large, and that is reflected in its substantial intra-species variability. Two varieties are currently distinguished [17] as *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* (from here on *P. m.* var. *menziesii*) and *Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (from here on *P. m.* var. *glauca*). While *P. m.* var. *menziesii* grows closer to the Pacific coast at altitudes ranging from sea level to 1950 m a.s.l., *P. m.* var. *glauca* inhabits the more continental climate of mountainous and inland areas at altitudes of 250 m a.s.l. in the north to 3350 m a.s.l. in the south [18]. The main differences between the varieties are in the coloration of their needles, maximum age and size, growth rate and cycle, and resistance to climatic extremes and biological pests [19]. *P. m.* var. *menziesii* has the fastest growth and higher productivity and is usually more resistant to needle casts. However, it is more sensitive to winter transpiration and requires higher soil moisture to grow [20]. Alternatively, *P. m.* var. *glauca* grows more slowly and rarely reaches to 40 m height. It is frost-resistant and capable of enduring more massive shading and drought (arid conditions). However, due to its lower productivity, it is less used in Central European forestry [12,19].

In the domestic range of Douglas fir, fire is the decisive disturbance factor shaping the structure of local forests [21]. The selection pressures of abiotic factors such as frost and drought have contributed to the creation of a number of Douglas fir populations [22]. Among biotic pests, *Dendroctonus pseudotsugae* Hopkins, 1905 and *Orgyia pseudotsugata* have been shown to have a long-term impact on Douglas fir growth. Swiss needle cast (SNC) caused by *P. gaeumannii* emerged as a major disease during the 1980s and 1990s [23].

As for areas of non-natural occurrences in Europe, damage by late frost [24], winter transpiration [14] and late-spring frost [25] prevailed in the early phases of introduction (initial years). The resistance of certain provenances to frost can also be influenced by the early budding, which is a critical adaptive characteristic co-determining the length of the vegetation period and susceptibility to frost damage [25]. Campbell and Sorensen [26] have demonstrated that susceptibility to frost damage may increase due to the delay of budding by one week. The budding period is influenced primarily by elevation, geographic position, and distance from the ocean [22,26]. Usually, coastal provenances bud later (mid-May) than inland provenances (mid-April). Boyce [27] proposed such differences in the timing of budding as one of the reasons underlying the Douglas fir resistance to *P. gaeumannii* because a massive infection most often occurs before budding. Lavender and Hermann [19] considered the

influence of early budding (i.e., earlier entry into dormancy) as a possible explanation for greater drought resistance in populations from higher elevations.

Needle casts are an especially serious biotic constraint on Central European forests. For the last few decades, the most significant damage has been Rhabdocline needle cast (RNC) caused by *R. pseudotsugae*, while SNC has emerged more recently, e.g., References [28–36]. Both fungi cause changes in needle color and subsequent loss [29]. Although forests of all developmental phases are affected, 10- to 30-year-old stands are most susceptible [37].

R. pseudotsugae was first described by Weir [38] in North America. The highest spread occurred there in the mid-20th century [37]. The first damaging effect of RNC in Europe was recorded in Scotland back in 1922 [39], although there are reasonable grounds to presume that *R. pseudotsugae* existed there even before 1914 [40]. In subsequent years, it spread across England, Denmark, and the Netherlands to other states. Its first occurrence in the Czech Republic was recorded in the late 1930s [41]. Its presence has now been confirmed in 14 European countries [42].

R. pseudotsugae has a 1-year development cycle. First signs of attack are pale green flecks, which is changing to orange shade in late autumn and violet-brownish in winter. Fruiting bodies are formed on the underside of needles during April and ripen from May to July. Spores are spread by wind and infect leaf buds upon opening [43], often leading to the complete loss of new leaves in a given year. Infection can further spread to older needles [39,44].

SNC caused by *P. gaumannii* was first documented in 1925 on 20-year-old Douglas firs in Switzerland and Germany [45]. In the United States, this disease, found predominantly on Christmas tree plantations, was not considered a severe phytopathological problem until the early 1950s [46]. From 2003, it began spreading into forest stands in western Oregon and Washington [47,48]. *P. gaumannii* started to cause problems in New Zealand from the mid-1950s [31] becoming a limiting health factor for stands of various age classes [32].

The occurrence of the fungus has also been confirmed, for example, in Poland [33] and Bulgaria, where together with *R. pseudotsugae* it endangers the growing provenances [49]. It was not mentioned in the Czech Republic until 2002 [50]. Initial symptoms of SNC attack are very similar to RNC. Black fruit bodies began to break out through the stomata. In the second and third year, additional fruit bodies appeared on the green parts of the needles. After 3 years, the needles are shed [43]. Usually, fruiting bodies are more abundant on needles aged 3 years or older and are absent on younger foliage [27,51], but in the late 20th century, we have observed abundant fruiting bodies on current-year needles [37].

Previous provenance research identified areas in the natural region from where high-quality stands have originated with high production and growth rate in European conditions. But the increasingly visible climatic changes induce stress on the tree stands, and thus the requirement of a new wave of selection in European stands is unavoidable, and a mere production-oriented selection is no longer sufficient. Managers of Douglas fir in Europe require fast-growing and disease-resistant provenances. Although a higher resistance to needle cast has already been confirmed in *P. m. var. menziesii* [4,52], it exhibits poorer resistance to reduced soil moisture and humidity due to climate changes. Hence, it is crucial to identify factors associated with Douglas fir stands with both higher resistance to biotic and abiotic stresses with reasonable growth. The recorded infection of Douglas fir with pathogens *R. pseudotsugae* and *P. gaumannii* at the International Union of Forest Research Organizations (IUFRO) provenance trial at Hůrky in 2015 (Figure 1, Table 1) was thus used immediately to conduct a targeted study about the tree health status of all verified provenances and to determine the rates of their susceptibility to the two needle casts. Together with an evaluation of production criteria, it is now possible to perform a superior initial selection of provenances suitable for growing under the conditions of expected climate changes in Central Europe.

Table 1. Characteristics of evaluated provenances at the Hürky area.

State (USA), Province (Canada)	Provenance	Provenance Area	Elevation (m a.s.l.)	North Latitude	West Longitude	
British Columbia	1004	Stuie	Maritime	230	52°22'	126°00'
	1010	Barrière	Cariboo Trans	612	51°12'	120°10'
	1012	Klina Klini	Maritime	3	51°07'	125°36'
	1013	Revelstoke	Nelson	600	51°00'	118°12'
	1021	D'Arcy	Submaritime	275	50°33'	122°30'
	1025	Nimkish	Maritime	90	50°19'	126°53'
	1028 ×	Merritt	Zone Not Defined	870	50°04'	120°51'
	1030	Squamish	Maritime	15	49°47'	123°09'
	1033	Forbidden	Maritime	610	49°40'	125°09'
	1036	Alberni	Maritime	140	49°19'	124°51'
Washington	1043	San Juan	Maritime	215	48°35'	124°05'
	1049	Bacon Point	7–Skagit	500	48°36'	121°23'
	1050	Marblemount	7–Skagit	120	48°35'	121°24'
	1058	Lake Crescent	1–Hoh	305	48°04'	124°00'
	1061	Louella	1–Hoh	457	48°00'	123°05'
	1067	Skykomish	5–Kitsap	305	47°42'	121°20'
	1069	North Bend	8–Snoqualmie	150	47°28'	121°45'
	1075	Enumclaw	5–Kitsap	240	47°16'	121°56'
	1078 ×	Cle Elum	8–Snoqualmie	640	47°13'	121°07'
	1081 ×	Alder Lake	9–Toutle	430	46°48'	122°17'
Oregon	1089	Cathlamet	3–Twin Harbors	200	46°18'	123°16'
	1100	Grand Ronde	6	200	45°06'	123°36'
	1102 ×	Upper Soda	12	1000	44°23'	122°12'
	1103	Coquille	2	100	43°12'	124°10'
	1104 ×	Brookings	1	300	42°07'	124°12'

× standard International Union of Forest Research Organizations (IUFRO) provenance/IUFRO standards.



Figure 1. Map of provenances examined at the Hůrky trial in their natural range of occurrence in western North America; source: Google Earth Pro.

2. Materials and Methods

During 1966–1970, seeds from 182 autochthonous Douglas fir stands in the US and Canada were collected as part of an international experiment conducted by IUFRO. Samples were divided among 45 institutions from 30 countries [19]. Participating on behalf of the Czech Republic, the Forestry and Game Management Research Institute received seeds of 25 provenances, including five benchmark standards. In 1971, three experimental trials were found within the present-day Czech Republic. Evaluation of the susceptibility to the needle casts within provenances was possible at Hůrky due to active infection of Douglas firs by *R. pseudotsugae* and *P. gaumannii* in the provenance trial as well as nearby forests.

The Hůrky provenance trial is situated on a very gentle slope with a predominantly north-east exposure at 445 m a.s.l. (oak-beech forest altitudinal zone). Local mean annual temperatures reach 7.3–7.5 °C and mean annual precipitation is 550–575 mm. The soil type is oligotrophic brown earth. The absolute height yield class of Douglas fir at standing volume with bark per hectare 467 m³·ha⁻¹ is 36 [1].

Verified provenances (Table 1, Figure 1) originate from British Columbia (11), Washington (10), and Oregon (4). Provenances 1028, 1078, 1081, 1102, and 1104 constitute comparison benchmarks present in most trials of the international experiment [1]. The area of research trial is a regular rectangle of 1 ha divided into 100 parts (25 provenances in four repetitions), 10 × 10 m each. A total of 3700 nursery-raised 3-year-old saplings (2-year-old saplings for benchmarks) were planted in the spring

of 1971 at a spacing of 2×1.1 m in odd rows and 2×2 m in even rows. Since its planting in 1971, the number of individuals in the trial has gradually declined due to the fall in winter transpiration (1973), natural mortality, thinning (1987, 1996, 2006) aimed at releasing high-quality individuals and removing untrained and dry trees. The less number of trees at the planting age is due to the fast growth of Douglas fir, which overtakes all autochthonous trees.

In 2016, heights and breast-height diameters were measured to compare gains from the previous measurement in 2011 [1]. The height was measured at breast height and by a VERTEX III ultra-sound height-meter (accuracy of 0.1 m). Diameter breast height was measured by millimeter caliper in two perpendicular measurements (0.1 cm) respectively. Based on volume equations for Douglas fir [53], the volume of large wood with bark was calculated. Defoliation of all trees in the provenance trial was also assessed by consensus agreement of visual observations by three evaluators.

A Burkard automated spore trap (AMET, Velké Bílovice, Czech Republic) with datalogger Minikin THi for continual measuring temperature and air humidity were installed from 15 April to 31 of August 2015 in a location with strong infection pressure to determine the time and intensity of needle cast sporulation. An Olympus BX41 microscope with an Olympus WH10X/22 objective was used for sample processing. In addition, 10 Douglas fir tree samples were felled nearby the trial in monthly intervals, and branch samples from the upper and lower crown sections were collected from these to confirm the presence of needle cast. The presence of needle cast spores was determined in the laboratory under an Olympus 110AL 1.5X W061 binocular microscope ($4\times$ magnification). Determination of ascospores and conidia was carried out according to Butin [43].

Each tree's social standing within the stand was determined according to Konšel [54] using the grades 1–dominant, 2–co-dominant, 3–intermediary, 4–overtopped (viable), and 5–dying/dead. Defoliation rate was determined as an indicator of health status within five percentage point increments according to the grading system used for SANASILVA inventory [55]: undamaged tree (0%), slightly damaged (1%–25%), damaged (26%–50%), severely damaged (51%–75%), dying (76%–99%), and dead (100%). The occurrence of other fungal pathogens was also evaluated.

A statistical regression model was used to compare the provenances' health states [56]. In this case, the dependent variable was tree defoliation rate; the explanatory categorical variable was provenance, and the quantitative explanatory variables were trunk volume, diameter difference between measurements in 2011 and 2016, and the number of trees. As the data of the dependent variable did not follow a Gaussian normal distribution, a generalized linear model was applied as an alternative. Approximation using the Poisson distribution was not sufficiently reliable, even though defoliation had a specific and relatively small number of values. Given the positive values for defoliation and a histogram skewed to the right, a gamma distribution was used for approximation (gamma GLM).

Only trees belonging to classes 1, 2, and 3 [54] and forming the canopy level in both evaluations (2011, 2016) were included in the analysis. Cases with diameter difference exceeding $1.5\times$ inter-quartile span (46 individuals) were excluded because the marked increase in diameter was probably caused by gaps that formed in the surrounding canopy (usually due to death or harvesting of nearby trees). The entire 1104 Brookings provenance, as well as the 4th repetitions in provenances 1081 Alder Lake and 1103 Coquille, were removed due to high mortality.

All double interactions between factors were included in the initial model. Simplification was done according to Crawley [57] and Pekár, Brabec [56]. The significance of model factors was determined by analysis of variance using a conservative *F*-test at the usual levels of significance: $\alpha < 0.05$, $\alpha < 0.01$, and $\alpha < 0.001$. The resulting model (1) was selected by minimizing the Akaike information criterion according to the Occam's razor principle [57]. For the statistically significant categorical variable (provenance), factor levels were also compared using "treatment" contrasts [56,57], for which the provenance 1102 Upper Soda originating from the southern inland part of the Douglas fir range and with presumed higher resistance to a drier and warmer climate was selected for comparison. An advantage of this provenance also lies in the fact that it is one of IUFRO's benchmarks, thereby

allowing for potential future comparison with other trials of the international provenance experiment. Its average values for production and defoliation were also comparable. Provenances 1028 Merritt and 1104 Brookings were excluded from our analysis based on the previous evaluation, which was one of the worst due to the low number of individuals and high mortality ratio.

For evaluation of factors influence and sorting the provenances according to health status, the decision tree method was implemented. All significant factors from the previous regression analysis were adopted (without interactions), and the decision tree was created using the package called 'rpart' according to Burger [58]. The final decision tree was pruned and looked at the tree's complexity parameter. All analyses were performed in the R 3.3.2 environment [59].

3. Results

The results of evaluating *R. pseudotsugae* and *P. gaeumannii* sporulation by Burkard spore trap on the locality Hůrky in 2015 (Table 2) confirmed the most significant occurrence of *R. pseudotsugae* spores in June (40 pcs). Their presence on adhesive tapes of the spore trap and on collected sample branches was minimal by July (3 pcs). The occurrence of *P. gaeumannii* based on the numbers of spores captured on adhesive tapes and in samples was much more frequent (spores were registered on all sample collection dates with the exception of April). The highest number of spores (201 pcs) were collected in July. The first finding was recorded in late May, and the strongest infection pressure occurred at the end of June and in early July (i.e., sporulation occurred later than in *R. pseudotsugae*). Sporulation of *P. gaeumannii* occurred at lower temperatures in comparison with *R. pseudotsugae*.

Table 2. Timing and intensity (number of spores) of needle cast sporulation obtained from the Burkard automated spore trap on Hůrky trial in 2015.

	April	May	June	July	August	Total
<i>Rhabdocline pseudotsugae</i>	0	14	41	4	0	59
<i>Phaeocryptopus gaeumannii</i>	0	1	38	201	0	240

The presence of ascospores and conidia of *P. gaeumannii* and *R. pseudotsugae* was reaffirmed in 2016 on ten trees that were cut purposefully at the close neighborhood to the trial. The conidia of *R. pseudotsugae* were found only in a single May term, the ascospores were confirmed in April, May, and June. The *P. gaeumannii* conidia were not detected but ascospores were recorded on all samples, mainly in April, May, and July.

Average defoliation of trees on the provenance trial reached 37% (Figure 2). The greatest defoliation was observed in provenances 1028 Merritt (90%), 1010 Barrière (61%), and 1067 Skykomish (53%). In contrast, the best evaluations were seen in provenances 1069 North Bend (23%) and 1089 Cathlamet (24%). A total of 71 trees showed 100% defoliation indicating they all were dead.

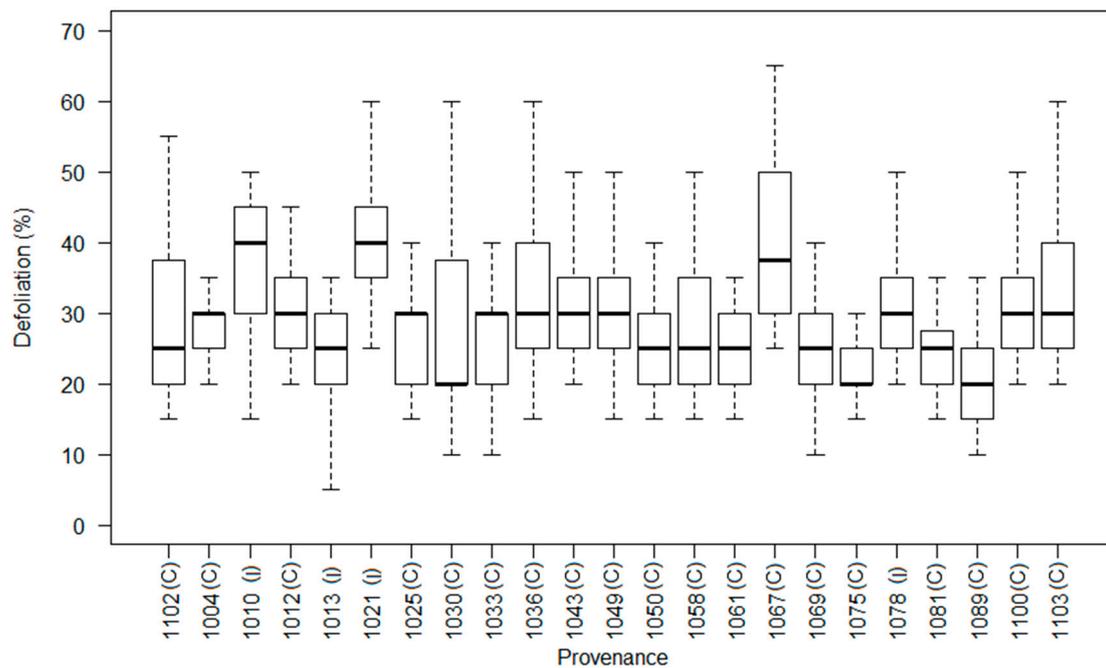


Figure 2. Box plot for defoliation rates of individual provenances. Whiskers represent 1.5 times the inter-quartile range. The letter above the provenance means coastal (C) or interior (I).

In the frame of comparison measurement in the provenance trial in 2016, 704 trees were measured in total. The lowest numbers of individuals were recorded (Table 3) in the provenances 1028 Merritt (15) and 1081 Alder Lake (18), whereas the most were observed at 1067 Skykomish provenance (45).

The average height of Douglas firs during the research trial was 30.1 m (Table 3). The most substantial heights were reached by the provenances 1075 Enumclaw (33.5 m), 1069 North Bend (32.7 m), and 1103 Coquille (32.4 m). The average heights of five other provenances also exceeded 32 m. The lowest average heights were documented in provenances including 1028 Merritt (18.3 m), 1067 Skykomish (25.2 m), and 1078 Cle Elum (25.9 m). The tallest tree (40.4 m) was observed at provenance 1075, while the shortest (16.2 m) one was present at provenance 1067.

The average diameter of all trees in the trial was 30.5 cm (Table 3). The absolutely lowest diameters were recorded at provenances 1078 Cle Elum (11.8 cm) and 1025 Nimkish (13.7 cm), whereas the thickest individuals were documented at provenances 1102 Upper Soda (55.9 cm), 1013 Revelstoke (53.9 cm), 1103 Coquille (53.3 cm), and 1075 Enumclaw (52.6 cm). The highest average values were found at provenances 1075 (37.7 cm) and 1069 North Bend (35.3 cm), while the least was at 1067 (22.4 cm) and 1028 (15.9 cm) provenance.

Standing volume with the bark of all Douglas firs evaluated within the trial was 765.84 m³. Average volume per tree was 1.09 m³. The most massive average volume was achieved by provenance 1075 Enumclaw (1.72 m³), while provenances 1028 Merritt (0.21 m³) and 1067 Skykomish (0.48 m³) had the smallest volumes.

Using a gamma GLM, the factors including tree volume, provenance, and diameter difference between 2011 and 2016, were determined as significant for defoliation rate, and the interaction of volume and provenance (gamma GLM $R^2 = 0.65$; Table 4) was also significant. Crown defoliation was significantly influenced by the volume of the evaluated trees. Defoliation showed a decrease with increasing volume (Figure 3; $r = -0.62$). The most defoliated trees had volumes around <1 m³. The relationship between defoliation and diameter difference was also inversely proportional (Figure 3; $r = -0.65$). Trees showing <30% defoliation have the most substantial diameter increase. The differences in defoliation rates among provenances are apparently clear in Figure 2. Undoubtedly, most defoliated provenances were 1010 Barrière, 1021 D'Arcy, and 1067 Skykomish.

Table 3. Mean quantitative characteristics determined.

State (USA), Province (Canada)	Provenance	Coastal (C)/Interior (I)	Number of Trees	Height (m)	Breast-Height Diameter (cm)	Standing Volume of One Tree with Bark (m ³)	Defoliation (%)	Number of Trees with 100% Defoliation	
British Columbia	1004	Stuie	C	27	32	31.9	1.16	34	2
	1010	Barrière	I	32	26	25.1	0.64	61	10
	1012	Klina Klini	C	27	31.3	31.4	1.12	31	1
	1013	Revelstoke	I	40	31.4	32.6	1.22	28	1
	1021	D'Arcy	I	24	28.7	29.8	0.95	48	3
	1025	Nimkish	C	30	30.6	29.2	0.99	32	2
	1028 ×	Merritt	I	15	18.3	15.9	0.21	90	14
	1030	Squamish	C	36	32.2	30.5	1.2	36	3
	1033	Forbidden	C	38	30.5	29.5	1.01	36	5
	1036	Alberni	C	27	32.3	34	1.36	34	1
1043	San Juan	C	32	29.9	29.6	1.02	38	3	
Washington	1049	Bacon Point	C	31	30.6	30.9	1.1	33	1
	1050	Marblemount	C	31	32.2	32.1	1.18	30	1
	1058	Lake Crescent	C	31	32.1	32.7	1.26	28	0
	1061	Louella	C	21	30.6	33.5	1.35	37	3
	1067	Skykomish	C	45	25.2	22.4	0.48	53	7
	1069	North Bend	C	29	32.7	35.3	1.42	23	0
	1075	Enumclaw	C	30	33.5	37.7	1.72	27	1
	1078 ×	Cle Elum	I	28	25.9	25.7	0.70	46	5
	1081 ×	Alder Lake	C	18	31.5	32.8	1.29	31	0
	1089	Cathlamet	C	31	31.9	33.5	1.27	24	0
Oregon	1100	Grand Ronde	C	26	30.8	33.0	1.21	34	3
	1102 ×	Upper Soda	C	31	28.8	30.4	1.07	39	4
	1103	Coquille	C	19	32.4	31.8	1.22	37	1
	1104 ×	Brookings	C	5	29.5	31.5	1.01	26	0
Σ/Average			704	30.1	30.5	1.09	37	71	

× standard IUFRO provenance/IUFRO standards.

Table 4. Resulting simplified gamma model of defoliation.

	Df	Deviance	Residual Df	Resid. Deviance	F	Pr (>F)	
NULL	558	86.412					
volume	1	42.627	557	43.785	728.487	2.20×10^{-16}	***
provenance	22	4.908	535	38.878	3.8122	2.23×10^{-8}	***
diameter difference	1	3.933	534	34.945	67.2096	1.98×10^{-15}	***
volume:provenance	22	2.903	512	32.042	2.2552	0.001	***
volume: diameter difference	1	1.337	511	30.705	22.8495	2.29×10^{-6}	***

Df—degrees of freedom; ***—statistically highly significant ($P \leq 0.001$).

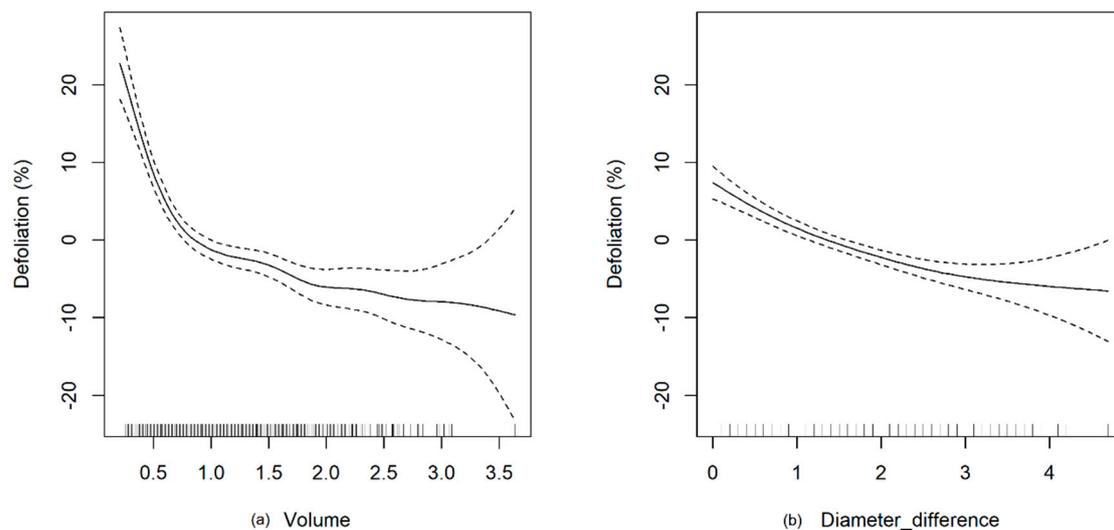


Figure 3. Relationship between defoliation and volume (a) resp. diameter difference (b), using non-parametric smoothers in generalized additive model. On the y-axis are the differences from the average defoliation = 37%.

The provenance was divided into several groups using the decision tree Figure 4, and the extent of impact on the health status caused by individual factors was assessed. Tree volume was substantially affected by the defoliation rate. Trees with a volume of over 1645 m^3 had the lowest defoliation. A second important factor for well-growing trees was thickness difference that implies little influence to the needle casts. So well-growing and thick trees were observed to have low defoliation. If the growth wasn't so good, the provenance might have a higher influence of needle cast. The provenance with the best growth increase and the lowest defoliation originates from British Columbia—1012 Klina Klini, 1025 Nimkish and Washington—1050 Marblemount, 1061 Louella, 1069 North Bend, and 1089 Cathlamet. On the other hand, provenance with a volume of less than 0.675 m^3 showed a higher level of defoliation and higher susceptibility to needle casts. The worst health status was documented for provenance 1010 Barrière and 1021 D'Arcy, which were strongly defoliated in spite of their excellent volume. So we can categorize them as sensitive to needle casts. If the volume of the trees is low, the defoliation is even higher. The more defoliated provenance was 1067 Skykomish, which also belonged to the worst growing provenances.

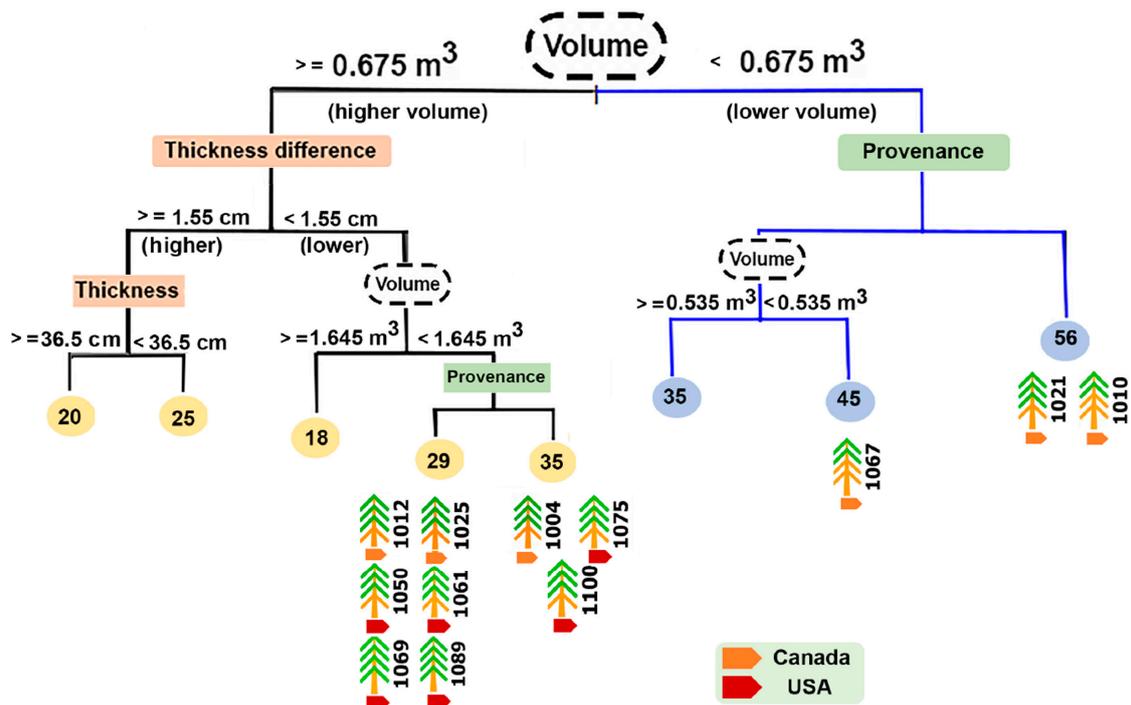


Figure 4. The decision tree based on the factors that influence the Central European Douglas fir stands health status. At the end of the nodes, the mean defoliation is mentioned. A higher number represents a more damaged tree. Healthier trees are on the left (black part of the decision tree) whereas more damaged trees are on the right side (blue part of the decision tree). Factor importance for splitting the arms decreases with the floor. Values dividing the nodes are stated for each arm. Selected typical provenances are stated with marking of country origin (Canada/USA).

4. Discussion

The Hůrky international provenance trial has previously been evaluated several times, e.g., [1,60]. However, a more in-depth observation of the health status of the provenances was never the subject of monitoring. In Czech territory, the most substantial losses at the IUFRO provenance trials occurred shortly after planting, during 1972/73 and 1975/76, due to winter transpiration. However, the losses were not so high among most provenances specifically at the Hůrky trial. The coastal provenances from Oregon and Washington were among the most damaged ones whereas those from the inland areas (e.g., from British Columbia) were damaged the least and demonstrated relatively good growth [20].

It is known that the formation and spreading of spores of *R. pseudotsugae* and *P. gaeumannii* are substantially influenced by temperature, wind, and air humidity [31–33,36,37,51]. Periods of spreading *R. pseudotsugae* spores at the Hůrky trial started at the end of April with the culmination in May to June, which is consistent with published data [43,61,62]. In the case of *P. gaeumannii*, the spores occur in May and June [43]. At the Hůrky trial, however, the highest occurrence of this needle cast was recorded only in late June and early July, which corresponds more closely to the findings in Poland [33].

In addition to the substantial influence of needle cast, there are other risks such as physiological drought and low moisture availability that is regarded as the main threat to Douglas fir stands in the coming decades. Growth can also be decreased due to drought damage, which was not apparent at first sight [19]. A partial solution may be to use inland provenances from drier areas of the North-Western US [19] that are less productive but more resistant to drought. However, the disadvantage for their planting in Europe is their greater susceptibility to needle cast, and especially to RNC.

In an evaluation of an international IUFRO trial in the Netherlands, Eilmann et al. [63] concluded that no provenance simultaneously fulfills the criteria of high production and high resistance to drought. They recommended giving preference to northern provenances at less dry locations and southern provenances at drier locations. At the Hůrky trial, the result is comparable, and at least

several production provenances with lower infection rate are available to choose from. In comparisons using contrasts, the provenances 1021 and 1030 were significantly different. Provenance 1021 had a volume close to the average for the trial. Such volume would correspond to the mean defoliation rate, but the defoliation rate determined actually varied significantly. A similar trend was demonstrated in provenance 1030, which produced high volume under average defoliation.

While comparing the results from measurements between 2011 [1] and 2016, there was a decrease in the number of trees in the research trial from 721 to 704. In 2016, 633 individuals were critical, and 71 were dying or dead. Kšír et al. [1] did not evaluate defoliation, and therefore it was not possible to compare the changes over the last five years. However, the current health status across the provenances can be considered slightly worse due to the co-influence of needle cast. Years with an extreme moisture deficit and drought stress only occurred recently and had an impact for the first time in the winter of 2015/16. Its impact then continued to the winter of 2016/17, too. The effect of drought on Douglas fir health status was therefore primarily eliminated for the analysis.

There is an apparent proportional relationship between volume and diameter increase between 2011 [1] and 2016 (Figure 4). In 2011, the smallest volume production had been documented in provenances 1028 Merritt, 1067 Skykomish, 1078 Cle Elum, and 1010 Barrière. The new results presented here are analogous. All mentioned provenances did not prove successful in the research trial. In contrast, provenances 1075 Enumclaw, 1069 North Bend, and 1089 Cathlamet, which had proven themselves healthy in the previous evaluation, can be recommended primarily due to their above-average production, favorable diameter growth, and minimal infection by pathogens. There are a number of other provenances in the trial with above-average production (e.g., 1036 Albern) while showing poorer health status. Moreover, further deterioration of their health status can be expected in the coming years due to their origin in the coastal areas of British Columbia and Washington (i.e., higher susceptibility to drought and autumn frosts).

Crown defoliation was negatively correlated with volume (-0.6) and thickness difference (-0.6). In addition, the vitality (growth potential) of the individual trees can also characterize the resistance of the tree, which is a useful phenomenon in relation to the resistance to against needle casts. Vitality was not directly measured in the current research, but it could be derived from growth rates. Thus, it is clear that the resistance of a tree to needle casts is more relying on the functional health status of it than a specific provenance that it belongs to. Nevertheless, some provenances showed better growth and more resistance to pathogens. These include provenances including 1069, 1075, and 1089, which were found to be appropriate in previous measurements [1]. Alternatively, the highest defoliation, the lowest volume, and the worst vitality were documented in provenances 1010, 1021 and 1067, which were previously not recommended by Kšír et al. [1] for further cultivation. However, in some rare cases, the extent of tolerance of some provenance against needle casts was demonstrated by variable and unexpected (higher and lower) defoliation rate relative to the volume found. For example, provenances like 1004, 1075 and 1100 showed the highest growth and a relatively higher defoliation rate than expected, and their higher susceptibility to needle casts can be assumed.

Many researchers examined the growth status of the different provenances in their published studies [20,31,32,49,60,63,64] but no one is considering the problems related to needle casts. Hence, we can only compare some aspects of our investigated trial with pre-existing studies. Four provenances (1025, 1050, 1069, and 1100) identical to those on the Húrky research trial are also represented in the range of 18 provenances evaluated in the Netherlands [63]. The results are, however, apparently contradictory due to different natural conditions. The 1025 provenance, somewhat below-average considering growth at the Húrky trial, was among the most productive one in the Netherlands in spite of its higher sensitivity to drought condition. In contrast, higher sensitivity to dry spells was also shown by provenance 1069 North Bend, which was among the best of the positively verified units at Húrky.

At research trials in Bulgaria, the needle casts caused by *R. pseudotsugae*, and *P. gaeumannii* appear to be the limiting factor for selecting the Douglas fir stands for planting. They were first found in

a Douglas fir trial at 17 years of age [64] when they occurred primarily in inland provenances. The results of evaluating the health status in the trial at 24 years of age [49,65,66] indicate that the coastal provenances from Oregon and Washington demonstrate faster growth than inland provenances (almost a third of the trees have died, and 10% had defoliation >60%) in the local conditions. Among the worst-rated provenances were, with no exceptions, from the interior such as Keremeos (Washington), Whitefish (Montana), Bates (Oregon), and Canyon City (East Oregon) that showed symptoms typical of needle cast together with decreased vitality [49]. The best-growing and more resistant to both needle casts [49,67], which is traditionally recommended for importing of Douglas fir reproductive material into Central Europe from above-mentioned source areas are the provenances namely Newhalem and Darrington from the Skagit seed zones in the western part of the Cascades. They are originated from an altitude between 500 and 1,167 m, which is similar to Central European conditions. Similar results were obtained from the coastal provenance Brookings from Oregon. Unfortunately, the use of them has been legally banned in Czech forestry at present.

We have additional data from Poland, where SNC was found on 3 out of 11 monitored trials [33]. In highly infested stands, defoliation exceeded above 45% in 90% of the trees. A higher infection pressure was determined in older (35 years) Douglas firs than in younger (17 years) trees. This does not correspond to the oft-stated most normal infection age of 10–30 years [37,43,68]. It is, however, partially confirmed by the results from the Hůrky trial, where high infection pressure in certain provenances was demonstrated in trees with almost 50 years of age. Still, the Polish research did not consider that the needle cast, caused by *P. gaeumannii*, is the most dangerous risk factor for young Douglas fir plantations [31,37,68]. Similar to the findings in the Hůrky research trial, Łakomy and Iwańczuk [33] have also recorded dying and dead trees. It is clear, however, that not all losses from recent years can be attributed solely to the influence of needle casts, as stress caused by drought and other pathogens contributed significantly to it.

The spread of pathogen *P. gaeumannii* has also been examined in New Zealand [31,32]. Seven provenances were evaluated at seven locations. Active infection (with fruiting bodies on more than 80% of needles) was determined at four locations. There were almost no observed differences in infection rates among provenances. However, the differences were prominent in infection intensity on the last year's needles. Kimberley et al. [32] demonstrated indirectly that, in their local conditions, *P. gaeumannii* is the most significant primary pathogen of Douglas fir. Simultaneously, they confirmed the importance of climatic factors for the spread of needle cast and a positive correlation between temperature and growth reduction. They determined the period of 8–15 years from infection start as the time showing the highest decrease in Douglas fir's growth gain (i.e., during the time of maximum infection pressure).

It is difficult to determine the underlying causes of the increasing magnitude of damage in recent years, primarily due to *P. gaeumannii* [32]. One explanation to consider is climate change that leads to weakening and subsequent increase in infection. Hansen et al. [37], who reported the infection in stands spanning of more than 120,000 ha in the coastal area of Oregon, confirmed this. The cause was apparently the origin of provenances from the higher altitudes and stands with lower natural resistance to the pathogen (probably partially from inland). A comparison of the above-mentioned study with the Hůrky trial is impossible because no data on tree defoliation was available. The difference in both pathogenic fungi attack among provenances was not evaluated, and so it is not possible to mention, which one is causing more damage and which provenances are more susceptible.

It is expected that the changing climatic conditions in Central Europe will probably result in an increased average temperature along with fluctuating and lower annual precipitation [69,70]. Hence, certain provenances from the inland areas may have higher potential applicability despite their lower productive capabilities and lower resistance to needle cast [4,36]. While there are no provenances known for the complete resistance to needle cast [4,52], provenance's with relatively high resistance and production capability are required. From the range at the Hůrky research trial, especially the provenances 1069 North Bend and 1089 Cathlamet can be considered can be considered viable, in this

respect. Conversely, provenances 1028 and 1078, which are among the poorest performing for growth and the most defoliated, appear unfavorable. Moreover, the positive evaluation of provenances 1061, 1069, 1075, and 1089 from the previous investigation supports the present claim [1].

Interestingly, earlier findings supported our claims as to the unsuitability of introducing *P. m.* var. *glauca* into Central European conditions due to its low production capability and high susceptibility to needle cast [10]. The inland provenances like 1010, 1021 are more susceptible than coastal ones. Whereas the coastal provenances were evaluated positively in most IUFRO research trials, the results from the Czech Republic are somewhat less optimistic. This is due to the adverse effects of the local spring frosts and physiological drought on young Douglas firs [25]. Findings from many parts of Europe [2,19] have aided in the selection of certain preferred geographic areas for importing seeds (mainly western Washington and northwestern Oregon). In addition [49] to selecting a suitable provenance, both needle casts can be partially reduced in stands by removing the most afflicted trees. This improves the microclimatic conditions to favor the remaining, healthy trees.

5. Conclusions

In a provenance trial with Douglas fir, we confirmed the occurrence of pathogens from the genera *Rhabdocline* and *Phaeocryptopus*. The highest sporulation intensity was recorded in June for *R. pseudotsugae* and June /July for *P. gaeumannii*. The determined defoliation indicates a somewhat poor health status of the Hürky provenance trial. Defoliation has a significant association with trunk volume in which the most defoliated individuals only rarely reached 1 m³. An inversely proportional relationship between defoliation and diameter gain was also observed. Among the compared provenances, it is essential to highlight the Washington provenances 1061 Louella, 1069 North Bend, 1075 Enumclaw, and 1089 Cathlamet. All of these have maintained satisfactory gains since the previous measurement in 2011 and were almost not affected at all by pathogens. Therefore, these appear most suitable for stands with similar climatic, soil, and topographic conditions. In contrast, the provenances 1010 Barrière, 1021 D'Arcy, and 1067 Skykomish, in addition to IUFRO standard 1078, can be considered unsuitable, demonstrating not only lower growth but also poorer health status. These results correspond well with the evaluation from 2011, with the exception of provenance 1036 Alberni, the current health status of which is considerably poorer and therefore should be placed among provenances that no longer should be planted.

As climate change becomes increasingly problematic in Europe, interest in replacing spruce with Douglas-fir is growing. However, as the area of Douglas-fir forest in Europe grows, the connectivity between stands also increases, along with the risk for pathogen attack. The risk posed by the RNC and SNC to Central European Douglas stands is likely to increase in upcoming years.

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