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Cumulative effect of needle cast on Scots pine saplings

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Abstract. Premature needle cast, affecting the needles on one-year old shoots, thus shrinks the size of green crown of Scots pines, and can cause reduction of increment or even death of trees, especially during first years of their growth. Aim of our study was to evaluate the lasting impact of pathological needle cast to young Scots pines and its implications for tree breeding. Assessment of needle cast damages in 5 grade scale and measurements of height were done repeatedly in Scots pine open pollinated progeny trial, consisting of 65 families, located in central part of Latvia. Proportion of saplings with different level of needle cast damage differed significantly between the years of impact: severely damaged were 51%, 30% and 17% of saplings in growing seasons 4, 5 and 6, respectively. Both the needle cast damage grade at current growing season, as well as height of the sapling before the needle cast infection (at the end of the third growing season) had a significant effect on its grade of needle cast damages in the next growing season. Cumulative level of needle cast damages at the age 4 to 6 years had significant negative effect on height of the saplings at the age of 12 years both at individual tree and family mean level, as well as on survival. Selection of best growing families in such trial would also lead to selection of genotypes least affected by the disease.

Key words: *Lophodermium* spp., fungal disease, repeated infection, survival.

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Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most important forest tree species in Latvia, it dominates in 34% of forests (Report of State Forest Service, 2015). Annual planting area reaches around 6,000 ha, that is 18–21% of the total forest regeneration area and about half (43–51%) from total planted area (Report of State Forest Service, 2015). Until the 90-s of the previous century planting of relative large 2-year-old bare root pine seedlings in high initial density (7,000-10,000 trees per ha) was the common forestry practice in Latvia (Bušs & Mangalis, 1971). Such density allowed improving the quality of stand by natural selection and high intensity of thinnings; thus trees with defects or unfavourable stem and branch traits (at least party genetically determined) were removed. Due to intensive tree breeding, leading to improvement of productivity and quality of trees (Jansons, 2005; Jansons *et al.*, 2006; Jansson *et al.*, 2016) as well as development of plant production technologies, leading to better survival and initial growth of planted trees, nowadays 3,000–3,500 seedlings per ha are planted. It means that resistance of tree to the impact of different negative environmental factors is becoming increasingly more important. Especially if considering, that mostly (74%) one-year

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old containerized seedlings with relatively small size (height 8–20 cm, root collar diameter 2–3 mm) are used for planting (Report of LVM, 2015). Therefore, notable investments had been made into protection of the plants against dendrophagous insects (mainly – large pine weevil *Hylobius abietis* L.) both in nursery and in forest, as well as in protection against browsing damages by cervids (Report of LVM, 2015). However, so far not much has been done to improve disease-resistance of trees against fungi. The impact of fungi might be increasing in future due to arrival of new invasive species in particular area (Drenkhan & Hanso, 2009) or due to increase of impact of already common fungi, caused by more favourable climatic conditions. *Lophodermium* needle cast had been a continuous threat to young Scots pine in nurseries and plantations across the northern Baltics and Scandinavia for a long time (Stenström & Arvidsson, 2001; Hanso & Drenkhan, 2007; Lilja *et al*., 2010; Bednářová *et al*., 2013). Besides, damage to forest stands could be the result of a complex of pathogens, for example, *Dothistroma* needle blight is often associated with *Lophodermium* needle cast at tree and stand level (Drenkhan *et al*., 2016). In Latvia *Dothistroma septosporum* (Dorog.) M. Morelet. was recorded in 2008 (Drenkhan & Hanso, 2009; Drenkhan *et al*., 2016). Also *Mycosphaerella dearnessii* M.E. Barr, *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter, *Diplodia pinea* ((Desm.) J.J. Kickx) can cause needle damages of pines in Europe (Drenkhan & Hanso, 2009; Bednářová *et al*., 2013; Adamson *et al*., 2015). Importance of foliar diseases is increasing due to gradual reduction of use of fungicides in forests (especially in relation to forest certification) as well as more favourable climatic conditions for this disease (Jansons *et al*., 2012).

Selection for resistance in tree breeding programs can reduce the susceptibility of planted trees (Eriksson *et al*., 2013). Differences of resistance of geographically distinct European provenances of Scots pine have been analysed, and the decreased resistance was detected for the most southern and western provenances; accordingly, the progenies of the northern and eastern origin suffered the least from needle cast infection (Björkmann, 1964; Squillace *et al*., 1975; Baumanis, 1983; Ostry & Nicholls, 1989; Kuzmina & Kuzmin, 2008; Vuorinen, 2008). Differences in susceptibility between the families within a provenance also had been detected (Baumanis, 1993; Jansons *et al*., 2008) indicating a potential for selection. However, as more traits are included into selection index, as smaller the improvement for each particular trait might become. Therefore it is important to assess the short and long-term impact of the disease before including it into selection program. It is known, that *Lophodermium* needle cast not only contributes to the mortality of young Scots pines, but also reduces growth of trees (Squillace *et al*., 1975; Martinsson, 1979; Jansons *et al*., 2008; Hanso & Drenkhan, 2012), like any other cause of defoliation. For instance, decline of the increments caused by defoliation by sawfly was reported for Scots pine in Latvia (Šmits *et al*., 2008) and in Finland (Lyytikäinen-Saarenmaa, 1999). Several authors pointed out that impact of defoliation on growth depends not only on the intensity of defoliation, but also other environmental factors (Kulman, 1971). Thus higher stress level to the young trees in future climatic conditions (e.g. due to altered precipitation regime) might increase the impact of the needle cast. Previous studies mostly had addressed short-term (1–3 year) impact of the disease; however, in forestry information on longer-term consequences of any influences is important. Therefore, aim of our study was to evaluate the lasting impact of pathological needle cast on young Scots pines and its implications for tree breeding. We hypothesized that impact of needle cast at young age (4–6 years) would have a significant negative effect on tree height and survival at selection age (12 years) and thus choice of best-growing families at this age would be sufficient to simultaneously select also for genotypes least affected by the disease.

Material and Methods

The study was carried out in open pollinated progeny trial of Scots pine. Trial consists of 65 families, it was established in central part of Latvia, near Daugmale (56°47´ N, 24°30´ E), in *Vacciniosa* forest type (Bušs, 1976) using one-year-old seedlings. Each family was planted in eight replicates of 10 trees (two rows of five trees), planting density 2 x 1.5 m. Tree height at age 3, 4, 5, 6, 7 and 12 years was measured. Crown closure of pines has been just started, and no thinning was done yet. Mean values (± 95% confidence intervals) were calculated for tree height at different age, and Pearson's correlation was used to asses relationship between these values. To estimate the role of tree height in its reaction to needle cast infection trees were divided in two approximately equal groups depending on their "initial" height (i.e. height at the end of the 3rd growing season, before the first needle cast damages): (1) trees which height exceeded average, further in text "high trees" and (2) trees which height was equal or less than average, further in text "low trees".

Premature needle cast was first observed in trial in the 4th growing season and needle cast damage assessment was done at five grade scale in the June of the 4th, 5th and 6th growing seasons (years 2008, 2009 and 2010, respectively), according to proportion of damaged needles on the previous season's terminal shoot: Grade 1 corresponded to 0–5%, Grade 2 to 6–35%, Grade 3 to 36–65%, Grade 4 to 66–95% and Grade 5 to 96–100% of the damaged needles. To assess the cumulative effect of disease in 3 successive years, the sum of 3 grades (obtaining values from 6 to 15) was used for each tree, denoting them as Total Grade. The proportion of severely damaged trees assessed by Total Grade > 9 was calculated per family. At the family mean level, linear regression was used to estimate the effect of the proportion of severely damaged trees to the height. The differences of tree height among Total Grades as well as among families were assessed using one way analysis of variance. The Chi squared test was used to evaluate (1) differences of trees' distribution in damage Grades among 3 years of assessments; (2) differences in trees' shifting process from one Grade in assessment of n-season to another Grade in assessment of n+1 season among high trees and low trees; (3) differences of survival among the Total Grades. At the individual tree level, relations between the Grades of current and previous season were assessed using Spearman rank correlation. At individual tree level, also the effect of Total Grade and genotype (family) to tree height at age 12 was estimated by generalized linear model (GLM) using tree height at age 3 as covariate.

Results and Discussion

The proportion of trees with different needle cast damage Grades differed significantly between the years of needle cast infection (Figure 1): severely damaged (Grade ≥ 4) were 51%, 46% and 17% of trees in growing seasons 4, 5 and 6, respectively, indicating that damage level has been decreased in period 2008–2010. These results are in agreement with studies in the south and central Estonia, where in 2008 epidemic of *Lophodermium seditiosum* Minter, Staley & Millar was noted (Drenkhan & Hanso, 2009). *Lophodermium* needle cast epidemic is often followed by several intermediate years, during which pines can (at least to some extent) renew their foliage, produce and relocate resources to the new growth or stores (Drenkhan & Hanso, 2009). On the contrary, Martinsson (1979), analysing resistance of Scots pine to *Lophodermium* needle cast for 3 successive years (1974–1976) at the same age of trees as in our study, observed the increase of damage level. He explained it as inability of weakened trees to grow out of the zone of strongest influence of the disease (closer to ground), as well as by the increase of the produced amount of pathogens' spores from a repeatedly infected

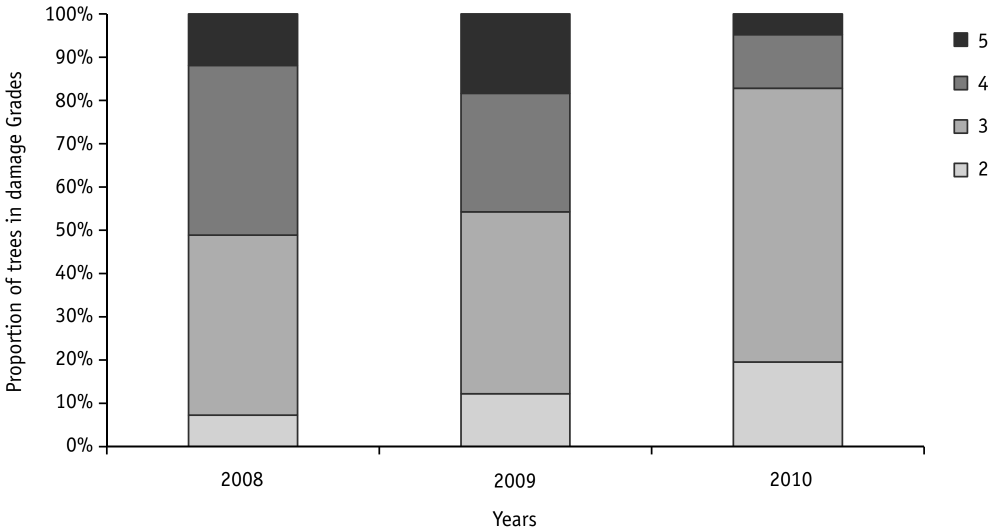

Figure 1. Distribution of trees in different needle cast damage Grades (2–5) in 2008–2010 (the 4th, 5th and 6th growing seasons, respectively).

needles. In our study the strong positive relationship (p < 0.001) between the tree damage Grade in the 4th and 5th, 5th and 6th, as well as 4th and 6th seasons has been detected, reaching r_s = 0.55, 0.53, 0.37, respectively. Similarly, strong positive correlations were found between damages in several successive years for Scots pine affected by *Lophodermium* needle cast (Martinsson, 1979), and for 2–6-years old Monterey pine (*Pinus radiata* D. Don) influenced by *Dothistroma* needle blight *Dothistroma pini* Hulbary (van der Pas, 1981).

Martinsson (1979) emphasized that in the third assessment season trees with little damages had been less affected also two years earlier, unlike the more severely damaged trees which assessment varied notably in the first observation season. Similarly in our trail inter-annual link between damage level (Grade) of a tree had been found. Moreover, this was influenced by tree height. The shifting of trees from damage Grade in the 4th season to damage Grade in the 5th season differed significantly (p < 0.01) between high trees and low trees: the proportion of trees which had been less damaged in the 5th season than in the 4th was twice greater for high trees than for low (34 and 17%, respectively), even so the proportion of trees which kept the same damage Grade in both seasons was similar in both tree height groups (50% and 45%, respectively). Similarly, significant (p < 0.001) differences between tree height groups in the proportion of trees changing their damage grade (higher, the same, lower) from one season to another were found also in analysis of tree in each of the 4th season's damage Grade separately (Figure 2). For instance, from high trees (H1) with damage Grade 3 in 4th season majority (85%) had the same or lower damage Grade in the next season, while from low trees (H2) with the same grade in 4th season the proportion of such tress was notably smaller (56%).

Similar tendencies were found for changes in damage Grade between 5th and 6th growing season, although differences between the groups of high and low trees were less pronounced, and were statistically significant (p < 0.001) only for trees

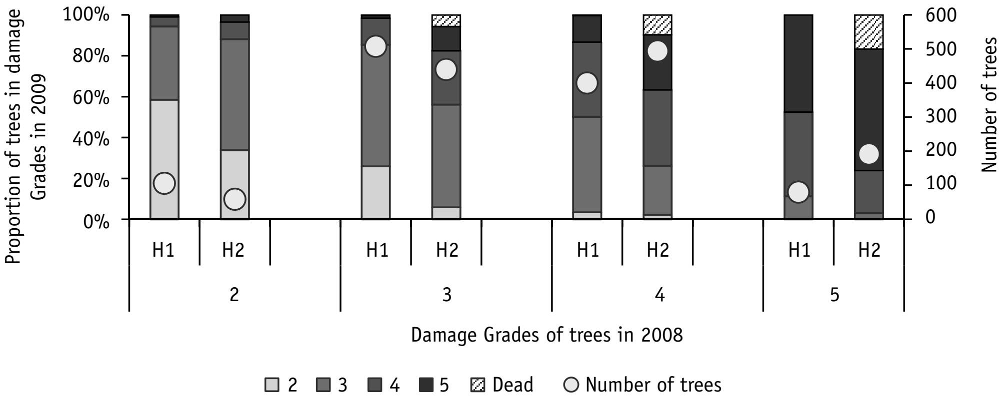

Figure 2. Distribution (shift) of high trees (H1) and low trees (H2) in different damage Grades (2–5) in the 5th growing season (2009) according to their damage Grade (2–5) in the 4th growing season (2008).

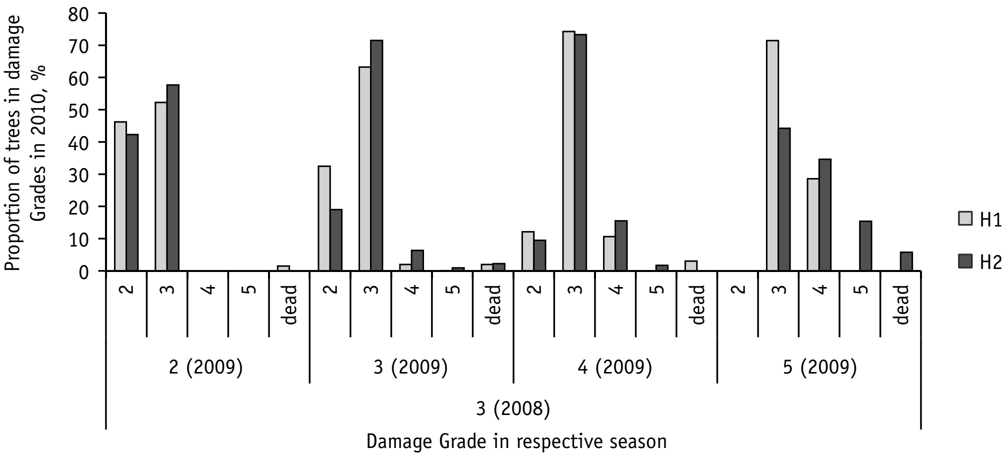

Figure 3. Distribution of trees in different damage Grades (2–5, and dead) in the 6th growing season (2010) according to their damage Grade (2–5) in the 5th growing season (2009). High trees (H1) and low trees (H2) of the damage Grade 3 in the 4th season's assessment are displayed.

in most frequently found damage Grades (3 and 4). Likewise, for majority of trees (damage Grades 3 in 4th and 5th growing season or damage Grade 4 in both growing seasons) damage Grade in the 6th season was dependent on damage Grades in each of two previous seasons as well as significantly ($p < 0.01$) affected by the initial height group (Figure 3).

Strong negative cumulative impact of pathological needle cast on survival was observed (Figure 4): mortality for trees which had the most severe damages in 3 consecutive years (Total Grade 15) reached 85% at age of 12 years. Contrary, none of the least damaged trees (Total Grade 6) had died during the study period; differences of survival among Total Grades were significant ($p < 0.001$). It

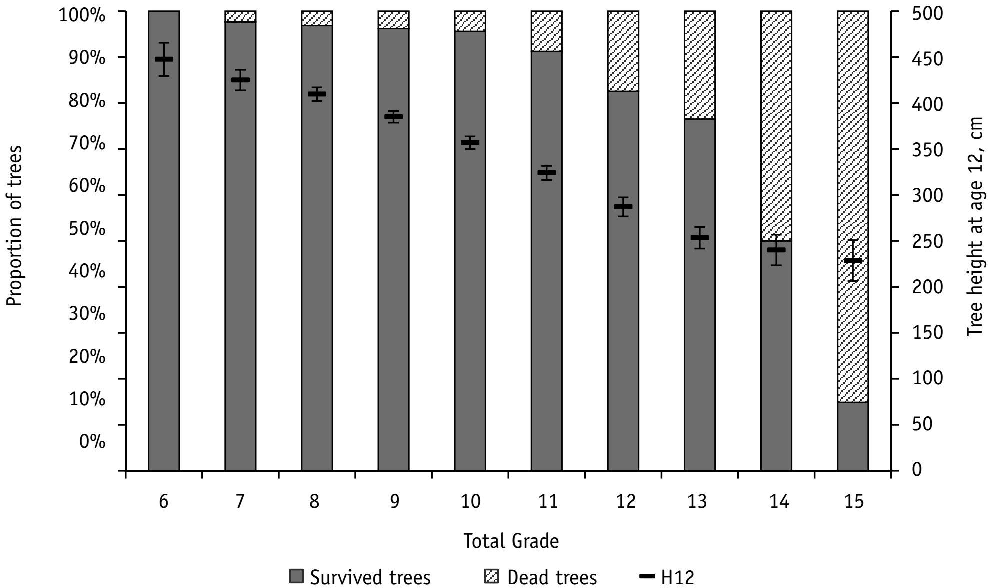

Figure 4. Distribution of survived and dead trees, and tree height (H12; ± confidence interval) according to Total Grades (6–15) at the age of 12 years.

indicates that trees weakened by needle cast had become more exposed to the negative impact to any abiotic or biotic factors such as competition for moisture, nutrients and light, impact of different fungus and insects *etc*. and consequently died.

Impact of needle cast could be altered by small initial height of trees in the trial (Table 1). Height of trees at age 3 was only 18.5 ± 0.31 cm and was a result of both small size of seedlings at the planting moment (length of above-ground part did not exceed 10 cm) and also by unfavourable environmental conditions – severe drought period from mid-April till the end of June in the first growing season. Furthermore, the soil preparation method (deep furrows) was not appropriate for the sandy soil in the area of the trial, and seedlings were at least partly covered with moving sand during first growing season.

Correlations between height of individual trees at different age were positive, strong and statistically significant ($p < 0.001$ for all values). Though Pearson's correlation coefficients between height at the age 4 and height at later measurements were slightly higher than between height at the age 3 and later measurements. Tree height measured before the needle cast infection (i.e. at age 3) and the current tree height (i.e. at age 12) was used in further analysis of relations between tree height and needle cast damages.

Mean height of trees differed significantly ($p < 0.001$) among the Total Grades – less severely damaged trees were higher than more severely damaged trees. Height of trees which had relatively less suffered from needle cast (Total Grade ≤ 9) was 402 ± 4.4 cm, while more damaged trees (Total Grade > 9) were notably and significantly ($p < 0.001$) lower (320 ± 4.7 cm). It is in agreement with previous studies, demonstrating significant impact of needle cast fungus *L. seditiosum* on both height and radial increment of Scots pine (Squillace *et al.*, 1975; Martinsson, 1979; Wühlisch & Stephan, 1986; Hanso & Drenkhan, 2012). This impact

Table 1. Correlation between tree height at different age.

Age of trees, years	Height ± confidence interval, cm	Pearson correlation coefficient	
		with height at age 3	with height at age 4
3	18.5 ± 0.31	-	-
4	36.9 ± 0.57	0.85	-
5	60.3 ± 0.97	0.74	0.95
6	94.1 ± 1.42	0.65	0.88
7	126.0 ± 1.77	0.62	0.85
12	358.2 ± 3.74	0.51	0.69

has been explained by the loss of nutrients stored in the shedding (affected) needles already relatively early in the season. Similar negative effect had been observed also in case of defoliation due to other causes e.g. sawfly feeding (Lyytikäinen-Saarenmaa, 1999). Current year needles, which mature after the shoot elongation, cannot support the current year height increment and can only partly support the radial increment (Clark, 1961; Ericsson *et al*., 1980), thus only the second and third year needle sets influence the current year growth significantly (Drenkhan *et al*., 2006; Kurkela *et al*., 2009). Therefore the assessment of proportion of the lost (damaged) needles of the previous season´s height increment, as used in our study, would provide information on the direct link between disease and growth of tree.

Cumulative impact of repeated defoliation on tree growth and, eventually, also survival had been explained not only by the mentioned direct effect, but also indirect (secondary) influences. Repeated defoliation leads to lower production of carbohydrates and thus: (1) gradual depletion of their reserves (Ericsson *et al*., 1980). Impact of this effect is more pronounced for smaller trees (Shaw & Toes, 1977); (2) decreasing amount of carbohydrates allocated to the roots of the tree, negatively affecting their development and absorption of nutrients and water (van der Pas, 1981). Impact of secondary effects are manifested with time-lag in comparison to the progress of the influence e.g. disease can be prolonged in comparison to the duration of the direct influence (Lyr & Hoffmann, 1967; Ericsson *et al*., 1980; van der Pas, 1981). This had been demonstrated by Hanso & Drenkhan (2012): in analysis of long-term historical data they found significant height growth decrease for 3–11-years old Scots pine during the *Lophodermium* needle cast epidemic year and two consecutive years. Also Kurkela *et al*. (2009) in the analysis of data from 12-year-old provenance trial concluded, that 50% needle loss resulted in height increment decrease by 35% in three successive years. Even five-year recovery period was needed for Sitka spruce after defoliation by European spruce sawfly, causing the reduction of height increment by 24–49% (Williams *et al*., 2003).

Prolonged influence of the disease leads to reduced total tree height also a number of years after the impact of the disease: differences among Total Grades in our trial were notable and significant ($p < 0.01$) both for group of trees with low and high initial height (Figure 5). Initially higher trees after being repeatedly severely damaged (great values of Total Grade, e.g. 15) were lower at the age of 12 years than less damaged (e.g. Total Grade 6) initially smaller trees (222 ± 31.3 cm and 437 ± 35.8 cm, respectively). More pronounced decrease in number of trees (survival) was observed for group with lower initial height and, simultaneously, with greater Total damage Grade. In

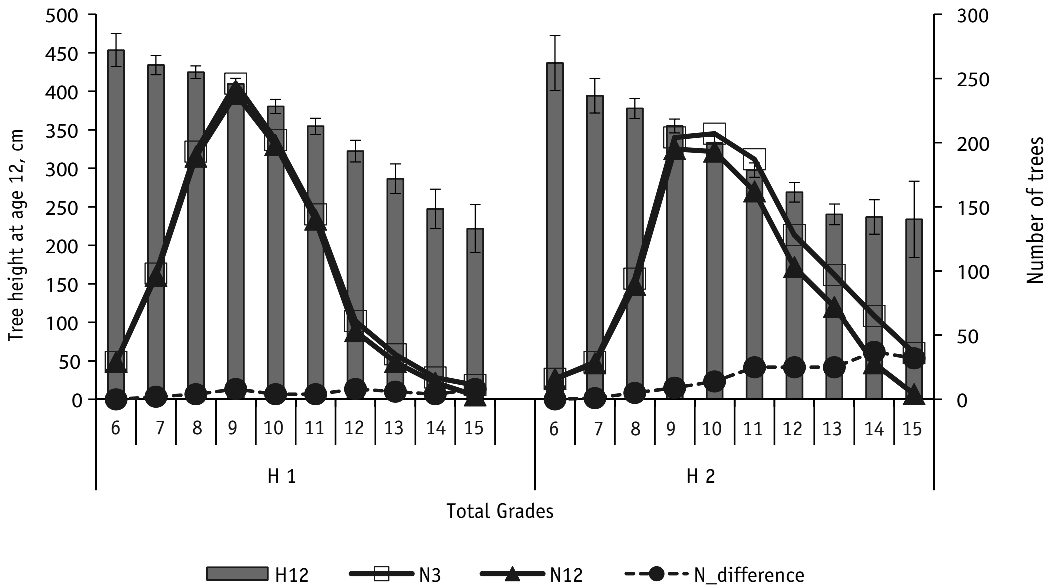

Figure 5. Number of trees at the age of 3 and 12 years (N3 and N12, respectively), difference between them (N_difference), and tree height at the age of 12 years (H12; ± confidence interval) according to Total Grades (6–15) for high trees (H1) and low trees (H2).

some cases, needle damage is intensified by attack of several pathogens, for example, in Estonia the epidemic of *L. seditiosum* converged with the serious spread of *D. septosporum* in young *P. sylvestris* plantations (Drenkhan & Hanso, 2009).

A similar approach to estimate the differences in impact of disease depending on dimensions of trees was used by Shaw & Toes (1977) for Monterey pine at age 8-10 years, damaged by *D. pini*. Cumulative diameter increment of uninfected, moderately and heavily infected trees was compared. Greater impact of the same infection level on trees belonging to group with small initial diameter than to trees in group with large initial diameter was found. In our study the height of high trees exceeded that of low trees in each of Total damage Grade, these differences were statistically significant ($p < 0.05$) for Total Grades 7–13.

Besides, when comparisons within size groups were carried out, Shaw & Toes (1977) denoted that small trees were damaged more severely: for large trees differences in radial increment after the impact between uninfected and moderately infected trees were 17%, and between moderately and heavily infected – 38%, while the differences for small trees were greater: 50% and 54%, respectively. In our study differences in Total Grade impact on tree height for high and low trees were less pronounced. These differences might be due to analysed traits, tree species or additional impact of meteorological conditions of particular years and damages caused by other pathogens.

Tree height at the age of 12 years in our trial was affected not only by the level of needle cast damage (Total Grade), but also significantly ($p < 0.001$) influenced by genetics (family) (Figure 6). It was confirmed by GLM analysis for individual trees using initial height (at the age 3) as covariate: final (12-year) height was significantly ($p < 0.001$) influenced both by the Total Grade and the family ($R^2 = 0.56$). Mean val-

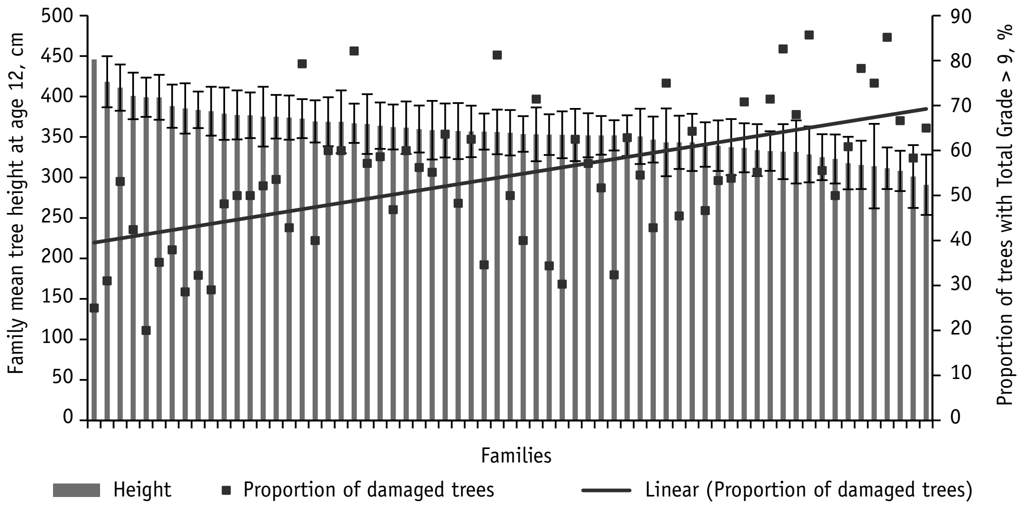

Figure 6. Relation between tree height (± confidence interval) at the age of 12 years and proportion of damaged trees with Total Grade > 9 at the family mean level.

ues for families ranged from 291 ± 37.3 cm to 446 ± 26.3 cm. The proportion of severely damaged trees (Total Grade > 9) per family ranged from 20% to 86%.

Similarly, strong genetic (family) effect was found for height and resistance to *Lophodermium* needle cast by Squillace *et al*. (1975) for 2–8-years old Scots pine in Netherlands, as well as by Stephan & Krusche (1986) for 9-year old Scots pine in Germany. Notable genetic variation in symptoms of Swiss needle cast (*Phaeocryptopus gaeumannii* (Rohde) Petrak) infection for Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) was found and author suggested, that families with higher tolerance (defined as continued tree growth in the presence of high disease pressure) could be selected (Johnson, 2002). Prospect to improve the tolerance *via* tree breeding was confirmed by relatively high heritability of this trait (Temel, 2002). However, economic feasibility of selection for resistance against, presumably, numerous diseases needs to be addressed before its inclusion into selection index. Family mean correlation between the proportion of trees estimated by Total Grade > 9 per family and tree height before the first infection (initial height) was moderate and lower than correlation between this proportion and final (12-year) height (r = –0.35 and r = –0.58, respectively, $p < 0.001$), demonstrating the lasting impact of the disease.

Conclusions

Size of Scots pine tree before the infection as well as genetics (family) determines the needle cast impact (proportion of the needles damaged) both at first and repeated occasions (years) with incidence of the disease. Impact of the diseases at the 4th–6th growing season significantly affects tree height at 12th growing season. Therefore, if the pathological needle cast has affected the trial, selection of faster growing families with higher survival at the age of 12 years would ensure also selection of genetic material less affected by the disease.

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