

Communication

The Effects of Frost Cracks and Large Poplar Borer Damage on Stem Rot in Hybrid Aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) Clones

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Abstract: Hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) plantations may produce valuable sawlogs for the growing timber market and contribute to carbon sequestration. However, environmental risks such as stem rot, the spread of which is facilitated by insect or frost damage, may reduce the proportion of valuable timber. It is important to understand the various factors affecting the spread of aspen rot to mitigate negative impacts with tree breeding. This study aimed to assess the impact of frost cracks and large poplar borer on stem rot in hybrid aspen clones in two clonal trials in Latvia. Genetic parameters for the traits were also estimated. The presence of insect passages substantially increased the probability of stem rot without distinct clonal differences. A negative and mainly insignificant correlation was observed between rot and stem cracking. The highest broad-sense heritability ($H^2 = 0.21$) and strong site-site genotypic correlation (0.86) showed that the probability of stem rot is genetically determined in the study material. Significant differences in diameter at breast height, the presence of stem rot, and its severity were found among the clones, albeit without undesirable positive correlation between growth and presence of decay. This indicated its potential to improve both productivity and rot resistance.

Keywords: wood decay; plantations; sunscald; heritability; genotypic correlation



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1. Introduction

The breeding of aspen hybrids between the European aspen and North American trembling aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) in the Baltic region and Nordic countries was initiated in the 1950s. A notable experimental base had been established since then and extensive knowledge accumulated over utilizing the high potential of this hybrid [1,2]. Hybrid aspen clones are grown for multiple purposes (for example, to produce sawlogs, pulpwood, or energy wood), and they provide raw materials for diversified forest product markets [1]. For first rotation plantations, a high yield of quality assortments with reduced harmful biotic and abiotic risks is desired [3,4]. Considerable damage is caused by stem rot, which is commonly associated with fungus species such as *Phellinus tremulae* (Bond) Bond et Boris. and *Phellinus ignarius* (L.:Fr) Quel. [5]. Furthermore, open growth conditions in plantations and the thin bark of hybrid aspen, particularly in the first few years, makes these trees susceptible to frost damage, including frost cracks caused by rapid temperature fluctuations during sunny winter days [6,7]. Regarding biotics risks, the large poplar borer (*Saperda carcharias*) commonly pierces tree stems [8].

Along with the direct negative impacts of cracks and insects on wood quality, they potentially serve as an entrance for fungal infections, thereby decreasing tree vitality and increasing mortality [9]. Rot is a common problem in forest stands in the region, affecting both hybrid and European aspen [10]. By understanding the factors influencing rot, we can learn how to limit it and select the most resistant clones [8]. Additionally, trees resistant to

rot are simultaneously more resistant to wind damage, which is becoming more frequent with the changing climate, contributing to higher mortality and potentially affecting carbon sequestration [11]. More rot-resistant trees in hybrid aspen plantations may ensure a larger volume of valuable timber. Such an outcome would, in turn, lead to higher carbon storage in wood products and substitution effect, and thus an overall higher climate change mitigation effect [12].

For better insights into rot resistance, hybrid aspen clonal plantations can provide the required data under controlled conditions. Although the clonal effect on resistance to stem rot, frost cracks, and insect damage separately has been indicated in several studies e.g., [13–15], our research focuses on the correlations between those damaging agents at the clonal level and the extent to which it is genetically determined. Therefore, this study aimed to assess the effect of frost cracks and large poplar borer damage on stem rot in hybrid aspen clones.

2. Materials and Methods

2.1. Study Site

The research was conducted in two adjacent hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) clonal trials in the central region of Latvia (56°27' N, 22°53' E), established with one-year-old containerized seedlings on abandoned agricultural land with fertile mineral soil and a normal moisture regime (Table 1). The experimental design was randomized blocks with 16-tree and 25-tree plots for each clone planted in six and four replications in two trials (Nos. 640 and 699), respectively, with 3 × 3 m spacing. A set of 5 clones (No. 36, 4, 41, 45, 44) was available for analysis in both trials, while trial 699 consisted of 13 clones in total (Figure 1). The studied clones were crosses between mother trees from botanical garden in central part of Latvia (*Populus tremuloides* Michx.; no information about origin) and local plus-trees across Latvia (*Populus tremula* L.).

Table 1. Characteristics of the studied hybrid aspen clonal trials 640 and 699 in Central Latvia.

Trial	640		699	
Age (years)	13		12	
Number of replications	6		4	
Plot design	25-tree block-plots		16-tree block-plots	
Number of analyzed clones	5		13	
Number of trees	457		677	
Number of harvested/remaining trees	202	255	265	412
Number of harvested trees with stem rot	149		207	
Number of harvested trees with insect passages	95		129	
Mean height ± standard deviation of harvested/remaining trees (m)	20.1 ± 2.3	20.2 ± 9.0	16.2 ± 8.1	15.5 ± 3.0
Mean diameter at breast height ± standard deviation of harvested/remaining trees (mm)	159.0 ± 30.7	159.0 ± 33.7	153.5 ± 29.1	155.5 ± 24.7
Proportion of harvested/remaining trees with stem cracks (%)	70.0	70.5	62.5	62.9

Stem frost cracks (hereafter: stem cracks) appeared in the winter of 2012/13, impacting the majority of trees—72% and 67% in trials 640 and 699, respectively. This was presumably caused by rapid temperature fluctuations during sunny winter days [13]. An inventory of the stem cracks was conducted in April 2013.

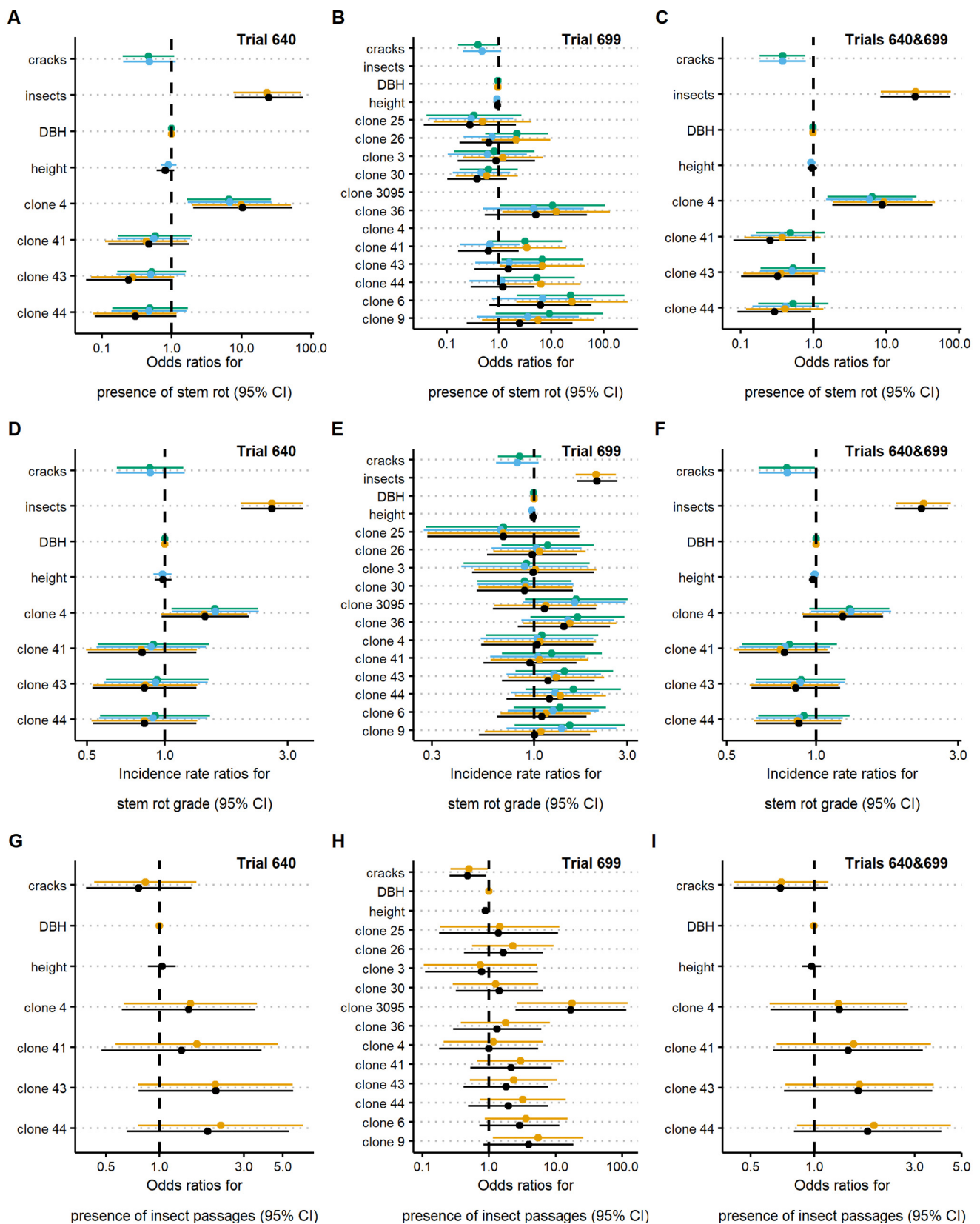


Figure 1. Forest plots of the multiple linear regressions (presented in different colors) with the presence of stem rot (A–C), stem rot grade (D–F), and presence of large polar borer passages (G–I) as dependent variables. Whiskers denote 95% confidence intervals (95% CI) of exponentiated coefficients. Non-overlap with the vertical intercept (dashed line) indicates statistically significant effect of an independent variable.

For all trees, height and diameter at breast height were measured at the age of 13 (trial 640) and 12 (trial 699) years in February 2020. In total, measurements from 457 and 677 trees were available in trial 640 and 699, respectively, after omitting dead and notably damaged trees (e.g., with lost top, damaged by agents other than the studied ones, etc.). Immediately following the inventory, systematic non-selective thinning was conducted in both trials whereby every second tree diagonally was removed, resulting in a negligible effect on the trial mean height (H) and diameter at breast height (DBH) (Table 1). The proportion of trees with stem cracks recorded in 2013 was similar between groups, ranging from 60–71% for harvested and 62–78% for remaining trees. For harvested trees, the presence of large poplar borer (*Saperda carcharias*) passages on stump surfaces (hereafter: insect passages) were identified. Stem rot stage on stump surface was visually assessed in a four-grade scale: (0) tree without signs of rot; (1) wood discoloration; (2) decolorized inner rings and rotting wood extending from the pith; and (3) a darkly pigmented response zone separating diseased from sound sapwood [16].

2.2. Data Analysis

For five clones present in both trials, joint-site analyses were conducted. The presence of rot (1 = present, 0 = absent) and rot grade as a function of the covariates were analyzed using a generalized linear mixed model (GLMM):

$$y_{ijklm} = \mu + M_{ijklm} + D_{ijklm} + C_i + r_{k(j)} + p_l + s_j c_i + \varepsilon_{ijklm}, \quad (1)$$

where y_{ijklm} is the response variable (presence of rot or rot grade) of the m th tree from the i th clone in the l th multiple-tree plot in the k th replication nested within the j th site, μ is the overall mean, M_{ijklm} is the fixed effect of the H or DBH measurement of the m th tree at the age of 13 years, D_{ijklm} is the fixed effect of the presence of damage (stem crack or insect passages) in the m th tree, C_i is the fixed clone effect, $r_{k(j)}$ is the random effect of the k th replication nested within the j th site, p_l is the multiple-tree plot effect, $s_j c_i$ is the random effect of clone $\times$ site interaction, and ε_{ijklm} is the residual error. To model the presence of rot, a binomial residual distribution with a “logit” link function was applied. For rot grade, a Poisson residual distribution with a “log” link function was used. Single-site analyses for trials 640 and 699 were conducted separately, applying a reduced form of Equation (1) with a random effect of replication r_k , without the clone $\times$ site interaction effect $t_j c_i$.

To estimate variance components for genetic parameter calculations, the following statistical model was used:

$$y_{ijklm} = \mu + S_i + R_{j(i)} + c_k + p_l + c_k s_i + \varepsilon_{ijklm}, \quad (2)$$

where y_{ijklm} is the observation on the m th tree from the k th clone in the j th replication nested in the i th site, μ is the overall mean, S_i is the fixed site effect, $R_{j(i)}$ is the random effect of the j th replication nested within the i th site, c_k is the random effect of the clone, p_l is the random effect of the multiple-tree plot, $c_k s_i$ is the random effect of the site and clone interaction, and ε_{ijk} is a residual error.

The broad-sense heritability was calculated as follows:

$$H^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_{GR}^2 + \hat{\sigma}_{GS}^2 + \hat{\sigma}_\varepsilon^2}, \quad (3)$$

where H^2 is broad-sense heritability and $\hat{\sigma}_G^2$, $\hat{\sigma}_{GR}^2$, $\hat{\sigma}_{GS}^2$, and $\hat{\sigma}_\varepsilon^2$ are the estimated variance components of the clone, clone $\times$ replication interaction, clone $\times$ site interaction, and residual, respectively [17].

Genotypic correlations between sites (type-B) were estimated using the following formula:

$$r_G = \frac{\widehat{Cov}_{(Gxy)}}{\sqrt{\hat{\sigma}_{(Gx)}^2 \times \hat{\sigma}_{(Gy)}^2}}, \quad (4)$$

where $\hat{\sigma}_{(Gx)}^2$ and $\hat{\sigma}_{(Gy)}^2$ are the genotypic (clone) variances for the same trait at two different sites and $\widehat{Cov}_{(Gxy)}$ is the estimated genotypic covariance between two sites [17]. The genotypic covariance was estimated by extending univariate statistical model in Equation (2) to bivariate model, where each trait in both trials formed a bivariate response variable.

Analyses were conducted in program R version 4.0.3. [18], using packages lme4 [19], emmeans [20], and MCMCglmm [21].

3. Results

3.1. Stem Rot Impacting Factors

The presence of stem rot in 13-year-old harvested trees was high in both trials—73.8% and 62.3% in trials 640 and 699, respectively, and similar to the proportions of harvested trees (62.9–70.5%) with previously recorded stem cracks (Table 1). Both stem damaging agents were poorly related in both trials separately—the presence of stem cracks was only statistically significantly correlated to rot in trial 699 ($p = 0.041$), where DBH was a covariate (Figure 1, Table S1); the effect on the severity of stem cracking (grade) was not significant ($p \geq 0.05$). However, when analyzing both trials together, the presence of stem cracks had a statistically significant effect on stem rot and its severity score ($p \leq 0.046$); trees with cracks had a lower probability of rot (Figure 2). Although statistically insignificant, this trend was present for all studied clones in both trials.

Contrary to their rather weak correlation with cracks, stem rot and its severity were strongly positively correlated with the presence of insect passages ($p < 0.001$). Trees with insect damage had a higher probability of rot (Figure 2), particularly in trial 699, where all trees with insect damage had stem rot. Conversely, insect passages did not have a remarkable correlation to stem cracking in trial 640. However, trees with cracks had a significantly lower ($p \leq 0.035$) probability of insect passages in trial 699 (Figure 2).

The mean diameter in both trials was similar for harvested and remaining trees (ca. 154–159 mm). Mean tree height was ca. 20 and 16 m in trials 640 and 699, respectively. Tree height was more homogenous than DBH among individual trees and clones in both trials. Accordingly, H was not found to be a statistically significant factor affecting the presence and severity of stem rot ($p \geq 0.105$). Its only significant effect was on the presence of insect passages in trial 699 ($p = 0.01$). DBH had a stronger and more significant effect on the presence of rot and insect passages and rot score in trial 699 ($p \leq 0.041$) yet a weak correlation to the corresponding traits in trial 640 and the joint-site analysis (Figure 1, Table S1).

A distinct clonal effect on the presence of rot and its score was observed in the trial 640 ($p \leq 0.0088$). In the trial 699, the presence of rot was significantly affected by a clone (genetics, $p \leq 0.046$) when DBH was a covariate (Figure 2). However, in the joint-site analysis of common clones, clones had a significant effect on both the presence and grade of rot ($p \leq 0.045$) (Figures 1 and 2; Table S1). No trend was observed between clonal mean tree growth and the presence of stem rot in either trial (Figure 3).

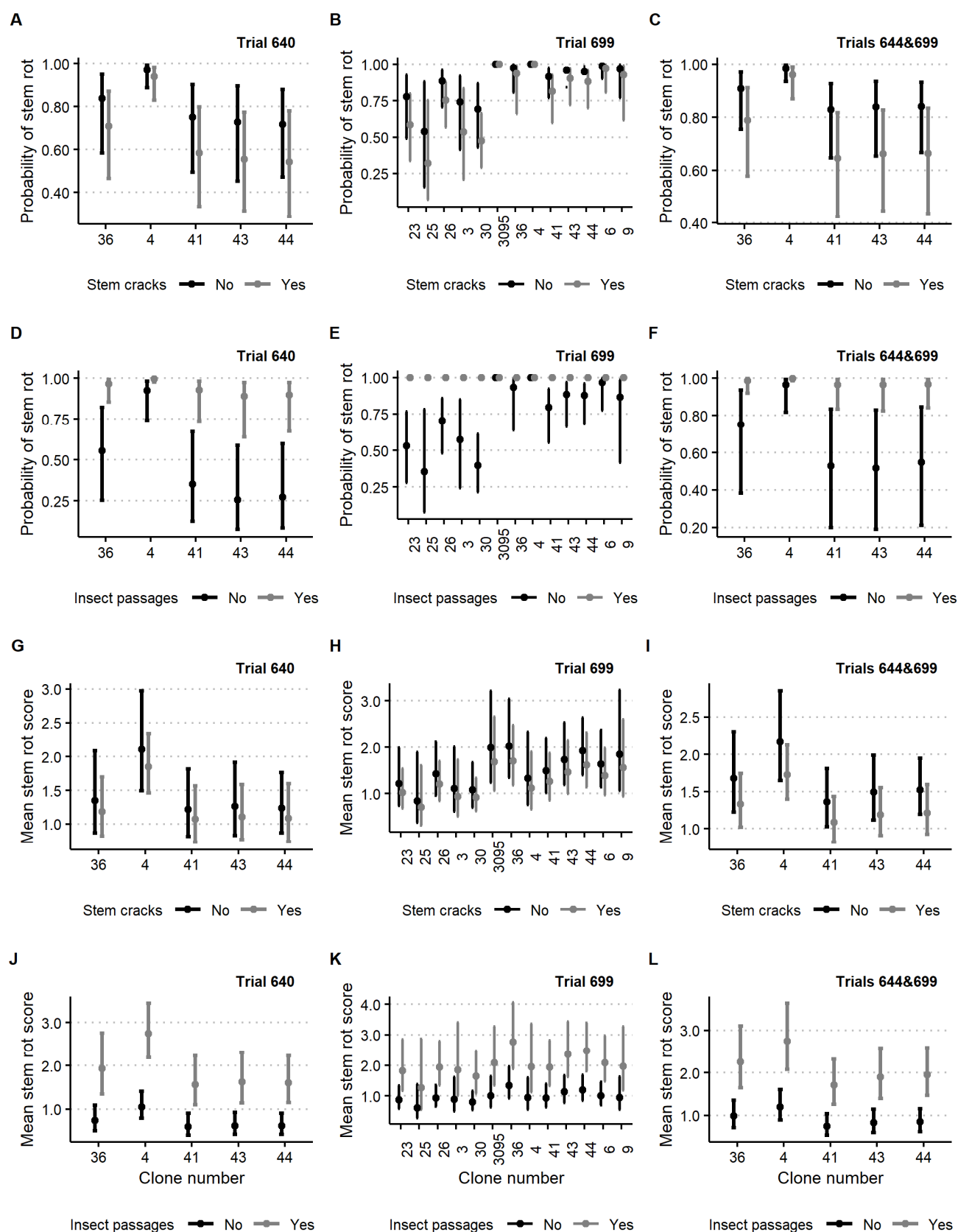


Figure 2. Clone mean probability of stem rot (A–F) and mean stem rot grade (score) (G–L) grouped by presence/absence of stem cracks and insect passages in the studied trials 640 and 699 and joint-site analyses.

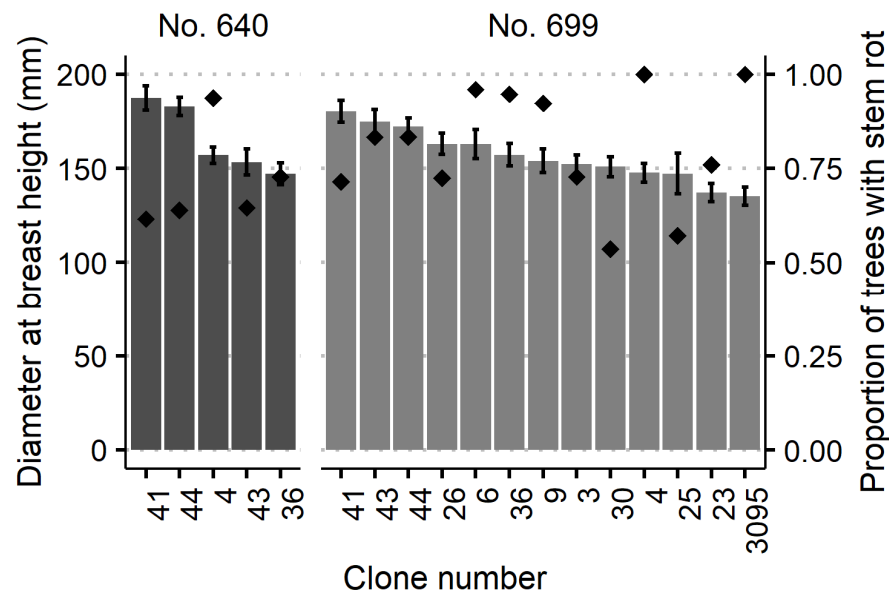


Figure 3. Clone mean diameter at breast height (bars; whiskers denote a 95% confidence interval) and the proportion of trees with stem rot (points) in the studied hybrid aspen trials 640 (age 13 years) and 699 (age 12 years).

3.2. Genetic Parameters

Joint-site calculations of broad-sense heritability H^2 showed a moderate genetic control concerning DBH and the presence of stem rot (0.17 ± 0.018 and 0.21 ± 0.178 , respectively), while estimates for stem cracks were weak (0.04 ± 0.056). For tree height and the presence of insect passages, H^2 was 0 (Table 2). Type-B genotypic correlations r_G between the trials followed the same patterns as those for H^2 : moderate to high estimates for DBH and stem rot (0.57 – 0.86 , respectively). However, there was almost non-existing genotypic covariance between trials for height, stem cracks, and insect passages ($r_G = -0.03$ – 0.02).

Table 2. Genetic parameters of the studied traits in the joint-site analyses of the hybrid aspen trials 640 and 699.

Trait	Broad-Sense Heritability $H^2 \pm \text{Standard Error}$	Type-B Genotypic Correlation $r_G \pm \text{Standard Error}$
Height	0.00 ± 0.11	-0.03 ± 0.55
Diameter at breast height	0.17 ± 0.02	0.57 ± 0.69
Stem rot	0.21 ± 0.18	0.86 ± 0.47
Stem cracks	0.04 ± 0.06	0.02 ± 0.86
Insect passages	0.00 ± 0.00	-0.02 ± 0.85

4. Discussion

The presence of stem rot in the harvested trees was rather high in both trials (63–74%). Since the trials were conducted on former agricultural land, the infection of the tree fungi could not be explained by transfer from the roots of the previous stand [22]. Potential causing agents may include fungi in the agricultural soil or anemochory from nearby stands [23]. Previous studies have found atypical fungi and early site colonizers, such as *Heterobasidion annosum*, to cause the decay of aspen [24,25]. Along with dead [26,27] or young, small branches with thin bark that are easy to penetrate [28], stem cracks may serve as an entrance for fungi [29]. However, our results showed a weak correlation between stem cracking and stem rot in both trials (Figure 1), suggesting other causes of stem rot and entrance via other types of bark imperfections [29] or even small damages not detected during the inventory. Some studies have reported genetic control of both stem rot [1] and cracks [1,13]. We found distinct clonal differences in the probability of stem rot

(Figures 2A–C and 3), supported by moderate heritability ($H^2 = 0.21$) and strong site-site genotypic correlations ($r_G = 0.86$). However, low heritability ($H^2 = 0.04$) and non-existing genotypic correlations between the trials ($r_G = 0.02$) suggested weak genetic control of stem cracking in the studied clones (Table 2). Still, genetic parameter estimates are only supportive to the results of multiple linear regressions and limited to the studied material, while larger set of clones would be necessary for general conclusions.

Conversely, the presence of insect passages was clearly an enhancing factor for the probability and severity of rot (Figure 1, Table S1). Moreover, all harvested trees in trial 699 with insect passages had stem rot, while 41% of trees without this damage had decayed. An earlier study of 48-year-old hybrid aspen also found a similar trend for higher numbers and greater areas of rot patches with increasing numbers of insect galleries [14]. This positive correlation between insect damage and rot was observed in all studied clones, particularly in trial 699, where all trees with insect passages had stem rot, regardless of the clone (Figure 2). Thus, the results stress the importance of biological control such as entomopathogens [30,31] along with selecting clones more resistant to stem rot to simultaneously reduce the potential damage of insects and decay.

The regression analysis (Figure 1) showed that stem cracking did not have a significant effect on the probability of insect passages (Figure 2), and trees with cracks tended to have a slightly lower incidence of insect damage, similar to the results concerning rot (Figure 1). Hence, it is unlikely that insects had entered through the cracks, suggesting ingress via other types of mechanical damage [29].

Overall, tree dimension had a weak correlation with stem rot (Figure 1), with no common trend over the trials. There was a tendency for a lower probability and severity of rot with increasing DBH in trial 699 (Table S1), similar to the observed negative correlation in an earlier study [14]. Although distinct differences in both DBH and the proportion of trees with stem rot were observed among the studied clones, there was no trend toward a higher risk of stem rot for larger trees (Figure 3). For instance, clone 3095 with the lowest mean DBH had 100% of trees with stem rot, while the clone with the highest mean DBH had an average rot presence level of ca. 70% (Figure 3). Similarly, a Finnish study found the superior clone concerning growth to be the most resistant to the pathogen *Neofabraea populi* [32]. Additionally, no positive correlation between stem cracking and DBH in young hybrid aspen has been reported previously [13].

Contrary to significant clonal effect on stem rot, estimated genotypic variance for tree height and the presence of insect passages was zero (Table 1). Nevertheless, heritability shows not only a genetically determined variance but is dependent on the site conditions [17]. Our results of site-site r_G for H show almost a non-existent genotypic-level correlation, hence the overwhelming environmental effects, particularly in trial 699, where clonal differences might not manifest themselves due to suppressed height growth (Table 1). However, high site-site r_G (0.86) for the presence of rot confirmed a rather stable genetic effect on this trait for the particular clones. Nevertheless, our estimates concerning genetic parameters were limited to the corresponding site conditions and should not be generalized, especially considering the limited number of clones (5), high estimated standard errors for r_G , and possibly masked genetic differences in tree height.

5. Conclusions

There were distinct clonal differences in probability and severity of stem rot in hybrid aspen, suggesting the potential to select genotypes with improved rot resistance. No positive correlation between tree growth and presence of stem rot indicates possibility to improve both traits simultaneously.

We did not observe a higher probability of stem rot in trees with frost cracks for the studied clones. Nevertheless, the presence of large poplar borer passages had the highest impact on the probability of rot without distinct clonal differences. Thus, biological control of insects along with selection of clones less susceptible to stem rot may significantly reduce decay and thus improve wood quality in hybrid aspen plantations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13040593/s1>, Table S1: Summary table of multiple linear regressions used to assess the impact of stem cracking and large poplar borer passages on stem rot.

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