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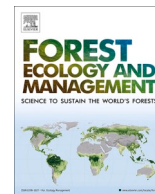Follow this and additional works at: https://digitalcommons.usu.edu/aspen_bib

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European aspen with high compared to low constitutive tannin defenses grow taller in response to anthropogenic nitrogen enrichment

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ABSTRACT

Boreal forests receive nitrogen-(N)-enrichment via atmospheric deposition and industrial fertilization. While it is known that N-enrichment can intensify interactions with natural antagonists, it remains poorly understood how genetic variability in plant defense chemistry can affect biotic interactions and height growth in N-enriched environments. We grew replicates of five low- and high-tannin *Populus tremula* genotypes, respectively, under three N-treatments (ambient, 15, and 150 kg N ha^{-1} yr^{-1}). We assessed shoot blight occurrence (i.e. symptoms caused by *Venturia* fungi) during four growing seasons, and tree height growth during the same period. Damage by *Venturia* spp. increased with N-addition during all years, likely due to enhanced foliar quality. Low–tannin plants showed higher incidences of *Venturia* infection than high-tannin plants, regardless of the N-input-level. Height responded to an N-by-tannin-group interaction, which occurred because high-tannin plants grew taller than low-tannin plants at the high N-treatment, but not under the other N-levels. This pattern indicates that innate resource investment into tannin production yields a positive effect on growth under N-enriched conditions. Given that N-deposition is increasing globally, our research suggests that further studies are needed to investigate how N-enrichment interacts with plant defense traits globally. Moreover, our research suggests that N-deposition may provide an advantage for well-defended, high-tannin plants; and further, that genetic diversity in plant defense may be a key mechanism by which plant populations respond to this change.

1. Introduction

During the past century, humans have greatly altered the supply of nitrogen (N) to terrestrial ecosystems (Galloway et al., 2008). Fossil fuel combustion and agriculture have increased global emissions of reactive N 3- to 5-fold during the past century, resulting in elevated levels of atmospheric N-deposition worldwide, including in N-poor boreal forests (Galloway et al., 2008). In addition to N-deposition, some boreal forests (e.g. in Scandinavia) are intentionally N-fertilized to increase their productivity (Lindkvist et al., 2011). Enrichment of boreal forests with anthropogenic N can have numerous consequences, such as increased net primary production, and plant susceptibility to herbivores and fungal pathogens, which may lead to shifts in plant community structures, and losses in plant diversity (Nordin et al., 1998; Bobbink et al., 2010). While community level changes in response to anthropogenic N-enrichment have received substantial attention, relatively little research has been directed to understanding intra-specific responses of tree populations.

The plant genus *Populus* has a widespread distribution in the northern hemisphere, including the boreal region. A number of tree species within this genus are valuable timber species due to their rapid regeneration and growth rates, and short rotation period, serving as a source for wood and paper products (Holeski et al., 2009). Because of their fast growth and ability to easily resprout after coppicing, *Populus* spp. and their hybrids are also increasingly managed for bioenergy (Lasch et al., 2010; Nikula et al., 2010; Derba-Maceluch et al., 2020). While *Populus* management has a longer history in North American, there is increased management focus on European aspen (*Populus tremula*) in Scandinavia due to its potential for rapid biomass production. Given the widespread use of *Populus* spp. in a variety of forestry contexts, there is great interest in how growth is impacted by a range of natural pests. One specific pest, *Venturia* spp. fungi, frequently infects *Populus*, which causes shoot blight disease (Martinez-Arias et al., 2019), and fungal infections may become more abundant in N-enriched boreal environments (Nordin et al., 1998; Bobbink et al., 2010). Diseased plants may show a reduced height growth and altered crown structure (Holeski et al., 2009), and thus

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infection can impair a tree's competitive ability for light, and reduce forest productivity. Anderson and Anderson (1980) described annual growth losses of approximately 8–10 cm in 3 year-old North American aspen (*Populus tremuloides*) shoots. Susceptibility to *Venturia* spp. has been shown to vary among *Populus* genotypes (Holeski et al., 2009; Albrectsen et al., 2010; Grady et al., 2015), and has been suggested to be influenced by a tree's constitutive foliar condensed tannin (CT) content (Holeski et al., 2009).

Foliar CT-levels can be highly variable among European and North American aspen genotypes (Lindroth and Hwang, 1996; Bandau et al., 2015), and may not only affect plant performance through providing defense against antagonists (Miranda et al., 2007; Holeski et al., 2009; Barbehenn and Constabel, 2011), but may also restrict plant growth due to their synthesis cost (Stamp, 2003). Numerous plant defense hypotheses predict trade-offs between growth and defense, whereby strategies that emphasize prioritization of growth are suggested to be associated with lower investment in defense chemicals (Stamp, 2003). Several hypotheses further expect that investment into growth or defense should be sensitive to nitrogen availability. For example, the Protein Competition Model predicts that the production of phenolic defense compounds is controlled by nitrogen availability, where high nitrogen availability leads carbon allocation into growth rather than phenolic defenses (Jones and Hartley, 1999). Such intra-specific trade-offs between growth and defense have been observed for multiple *Populus* sp. (Randriamanana et al., 2014; Bandau et al., 2015); and further, genetic variation in the susceptibility of *Populus* spp. to *Venturia* spp. has also been observed (Holeski et al., 2009; Grady et al., 2015). Research on how environmental change factors alter the occurrence of these pathogens across genetically diverse plant individuals (i.e. genotype × environment studies) remain relatively scarce (Grady et al., 2015), and it remains unclear whether intra-specific growth-defense relationships are responsive to anthropogenic N enrichment, and whether well defended genotypes have an inherent advantage or disadvantage under high nitrogen conditions (Bandau et al., 2015). Genotype by nitrogen enrichment experiments are worthy of attention because several studies have suggested that *Populus* spp. growth or population dynamics may be sensitive to environmental change factors (Lindroth et al., 2001), including anthropogenic nitrogen enrichment (Kochy and Wilson, 2001; Rogers et al., 2009).

We conducted an experiment to investigate how genotypic differences in defense (constitutive CTs) affect growth of European aspen in response to anthropogenic N-enrichment. We used a two phase common garden approach, whereby first a national scale common garden experiment was set up to identify and select five *Populus tremula* genotypes each with low and high foliar tannin concentrations. These ten genotypes were then propagated and used to create a new common garden experiment (i.e. phase 2), where replicates of these genotypes were subjected to three N-enrichment treatments. We measured the response of the genotypes by assessing leaf chemistry, height growth, and the occurrence of *Venturia* spp. fungi. We first hypothesized that low-tannin plants would be more susceptible to fungal infection than high-tannin plants; and further, that N-enrichment would increase plant damage due to changes in foliar chemistry (e.g. lower C:N ratio). Secondly, we hypothesized that N-enrichment and constitutive defenses would exhibit a positive interactive effect on plant growth, whereby plant growth responses to N-enrichment would be constrained when the plants are poorly defended and incur greater damage, and maximized when they are well defended and incur less damage. Testing these hypotheses provides a rare evaluation of the role of plant genetic diversity in determining how plant populations responds to a key anthropogenic change factor.

2. Material and methods

2.1. Plant material and experimental set-up

Our study utilized the Swedish Aspen collection, which consists of 116 *P. tremula* individuals collected from across Sweden, where replicated genotypes have been planted in two common gardens, one in Sävar in Northern Sweden (63.4°N), and one in Ekebo in Southern Sweden (55.9°N) (Luquez et al., 2008). Using this set-up, we screened the population for foliar CT-concentrations (method described below), and then selected 10 genotypes to be used in a new common garden experiment based on both their CT-levels (Fig. 1), and their availability in an existing tissue culture. This included five genotypes that consistently expressed lower and five genotypes that expressed higher levels of foliar CTs, regardless of the environment where they were grown (Bandau et al., 2015). We propagated the selected genotypes using invitro tissue culture, and initially grew the plants in a greenhouse. In early August 2010 we established a new common garden experiment on a clear-cut in Kulbäcksliden experimental forest (N 64° 9′ 8.02″, E 19° 35′ 12.09″). Thirty replicates of each genotype (i.e. 300 individuals) were randomly distributed across the site, and an additional row of aspen trees (N = 84) was planted around the experimental plants to prevent edge effects. The site was fenced to exclude hares and larger herbivores. A stand of mature aspens was present within 100 m of the site, providing a source of antagonists, e.g. fungal pathogens. During the winter of 2010/2011, an inter-annual peak in the vole population occurred, which resulted in the complete consumption of the aboveground biomass of every seedling that was planted (i.e. the height of the plants was unintentionally reset to 0 cm). Upon snowmelt of spring 2011, all plants began to grow new above-ground biomass.

In June 2011, plants were randomly assigned to one of three N-treatments: 0, 15, or 150 kg N ha^{-1} yr^{-1}, corresponding to ambient N, maximum N-deposition rates in the boreal region (Gundale et al., 2014), and high application doses used by the forest industry, respectively. Granulated NH_4NO_3 was applied to the soil within a 25-cm-radius around each plant. Annual fertilization began in 2011, and was divided into three applications each season (in May, June, and July), which continued for each year of the experiment, until 2015.

2.2. Leaf chemical analysis

On 16 July 2013, we harvested leaves from each plant for chemical analysis. We carefully sampled 5–10 of the most mature, fully expanded, undamaged leaves to obtain ca. 1.5 g leaf material. The harvested leaves were placed in a cooler, brought to the lab, freeze-dried to constant weight, and stored at −20 °C until further analysis. The leaves were ground to a fine powder on a ball mill (Retsch® MM 400, Retsch®, Haan, Germany), and the powder was then used for the quantification of CTs, total C and N (Kumordzi et al., 2014). Carbon and N concentrations were determined using dry combustion (LECO TruSpec CN Furnace, LECO Corporation, Lakeview, MI, USA; Gundale et al., 2012). Condensed tannins were extracted with 70% acetone + 10 mM ascorbic acid, and then quantified using the acid-butanol method (Porter et al., 1986) and procyanidin B2 ($C_{30}H_{26}O_{12}$; Sigma-Aldrich®, St. Louis, MO, USA) as a standard (Gundale et al., 2010). These leaf chemistry data were reported in a previous paper, which was focused on understanding litter decomposition rates (Bandau et al., 2017), and are therefore displayed in Supplementary Fig. 1 and Supplementary Table 1.

In addition to these leaf chemistry data, we assessed *Venturia* occurrence during the first week of August each year between 2012, and 2015. We screened all plants for the presence or absence of shoot blight symptoms caused by *Venturia* spp., i.e. blackened leaves and shoot
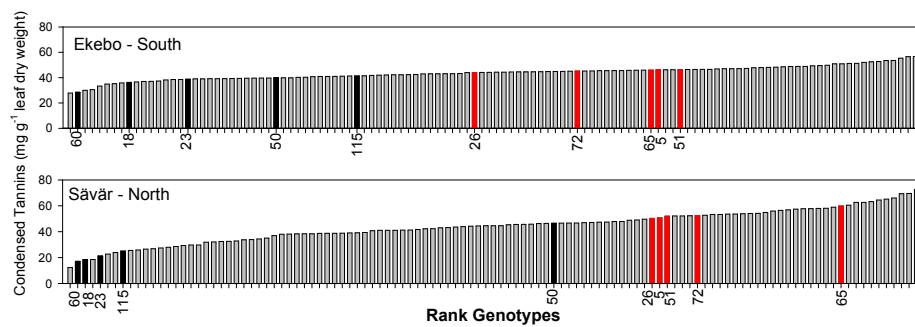
Fig. 1. A rank of foliar condensed tannin (CT) concentrations from genotypes of the Swedish Aspen (SwAsp) collection (Luquez et al., 2008). One hundred and sixteen (116) genotypes were grown in a Southern common garden in Ekebo, and 113 surviving genotypes in a Northern common garden in Sävar. Bars colored in black and red indicate low and high CT-genotypes that were selected for a new common garden experiment that tested the interaction between tannin group (Tgr) (high or low), and nitrogen addition treatments. Bars colored in grey are genotypes that were not selected for the current study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dieback characterized by a "shepherd's crook" appearance. We considered a plant as infected by *Venturia* spp., when a minimum of three branches showed the typical symptoms outlined above. During the final study year, 2015, we also recorded the height of each tree.

2.3. Statistical analysis

Differences in *Venutria* presence among N-treatments and tannin-groups were tested using 3-way log linear analyses with backward elimination. In these analyses, factors are removed from the best model when $p < 0.05$ for a given factor, and retained when $p > 0.05$. Effects that were retained were then followed up with separate χ^2-tests, where $p < 0.05$ confirmed a significant effect of that factor on *Venturia* occurrence.

To test the effect of N-addition, genotype, and tannin-group, and their interaction on tree height, we performed an ANOVA with a crossed and partially nested design, which include N–treatment, genotype nested within tannin group, and tannin-group as main factors, and N-treatment × genotype (within tannin group), as well as N-treatment × tannin group as interactive factors. Data were tested for assumptions of normality and homoscedasticity, and reasonably met these assumptions. Significant effects were followed with Tukey's *post hoc* tests across each N-treatment, and N treatment × tannin group combination. To test whether CT-concentrations and plant heights were correlated with each other, we used standard linear regression. All analyses were performed using IBM® SPSS® statistics, version 21 (Armonk, NY, USA).

3. Results

During the four-year observation period, we found that *Venturia* infection frequencies significantly increased with N-addition (Table 1,

Table 1
Final model terms retained in log linear analyses (i.e. a term is retained when $p > 0.05$) that evaluated the effect of nitrogen (N), constitutive tannin level (i.e. tannin-group; Tgr), and their interaction on the incidence of *Venturia* shoot blight in a *Populus tremula* common garden. The table shows the likelihood ratio, and degrees of freedom ($\chi^2_{(df)}$), as well as the final model terms retained on the left hand side of the table divide. Terms retained in the final model were individually followed up using χ^2-tests, for which the $\chi^2_{(df)}$, and significant P-values ($p < 0.05$) are displayed (on the right hand side of the table divide).

year	Log linear analyses			Follow-up χ^2-tests	
	$\chi^2_{(df)}$	P	retained terms	$\chi^2_{(df)}$	P
2012	2.87 (df: 4)	0.580	N × *Venturia*	19.22(df: 2)	0.000
			Tgr × *Venturia*	12.70 (df: 1)	0.000
2013	2.07 (df: 4)	0.723	N × *Venturia*	13.82(df: 2)	0.001
			Tgr × *Venturia*	6.93 (df: 1)	0.009
2014	5.43 (df: 6)	0.490	N × *Venturia*	8.03 (df: 2)	0.018
			–	–	–
2015	3.98 (df: 4)	0.408	N × *Venturia*	7.83 (df: 2)	0.020
			Tgr × *Venturia*	20.30 (df: 1)	0.000

Fig. 2). Infection was also more frequent in low- compared to high-tannin plants during all years, although not significant for 2014 (Fig. 2a-d, Table 1).

Tree height responded to N treatment, genotype, and tannin group, and there was an interaction between N and tannin group (Table 2, Fig. 3a). Specifically, trees subjected to the highest N addition rate grew taller on average than trees receiving low and no N-treatments. The interactive effect between N and tannin group on tree height occurred because high tannin plants grew taller than low tannin plants, but only under the highest N-treatment level. We also found a significant positive relationship between foliar condensed tannin concentrations measured near the beginning of the study (i.e. 2013), and height growth measured at the end of the study period (Fig. 3b).

4. Discussion

In support of our first hypothesis, we observed that low-tannin plants were more likely to show *Venturia* shoot blight symptoms than high-tannin plants. This supports findings by Holeski et al. (2009) who suggested that CTs could be responsible for genotypic differences in shoot blight susceptibility of *Populus tremuloides*. Consistent with the second part of our first hypothesis, we saw that *Venturia* infection rates increased with N-addition (Fig. 2). Higher infection frequencies in fertilized compared to unfertilized trees could have been attributed to an improvement of leaf quality for the pathogen, e.g. through an increase in leaf N content with N-addition (Supplementary Fig. 1c), as previously shown for fungal infection in other plant species (e.g. Nordin et al., 1998).

While high-tannin genotypes always showed higher levels of CTs compared to low-tannin genotypes, contrary to our expectation we did not observe a reduction in CT concentrations with N-addition, as one may have anticipated based on predictions of several plant defense hypotheses (Stamp, 2003), and which was evident when the same genotypes were grown under similar N-treatments but in a more protected environment that was free of antagonists (Bandau et al., 2015). Instead, we saw a significant increase in CTs with added N under the field setting of this study (Supplementary Table 1, Supplementary Fig. 1a). This may have been caused by stress induction of defenses caused by higher damage in fertilized plants. Although unstudied for *Venturia* spp., this explanation could be supported by observations of Miranda et al. (2007), who found an up-regulation of genes that encode enzymes for the synthesis of CTs in aspen leaves infected with rust fungi.

In agreement with our second hypothesis, we found that height was responsive to N-addition, and to an N-by-tannin-group interaction. All plants grew taller in response to added N, however, contrary to our expectation, low- and high-tannin plants did not differ in height under ambient and low N-conditions. Nevertheless, consistent with our hypothesis, high-tannin plants grew taller at the highest N-level compared to low-tannin plants (Fig. 3a). Further, foliar tannin concentration measured near the beginning of the study (2013) showed a significant relationship with our final height measurement (Fig. 3b). While it is
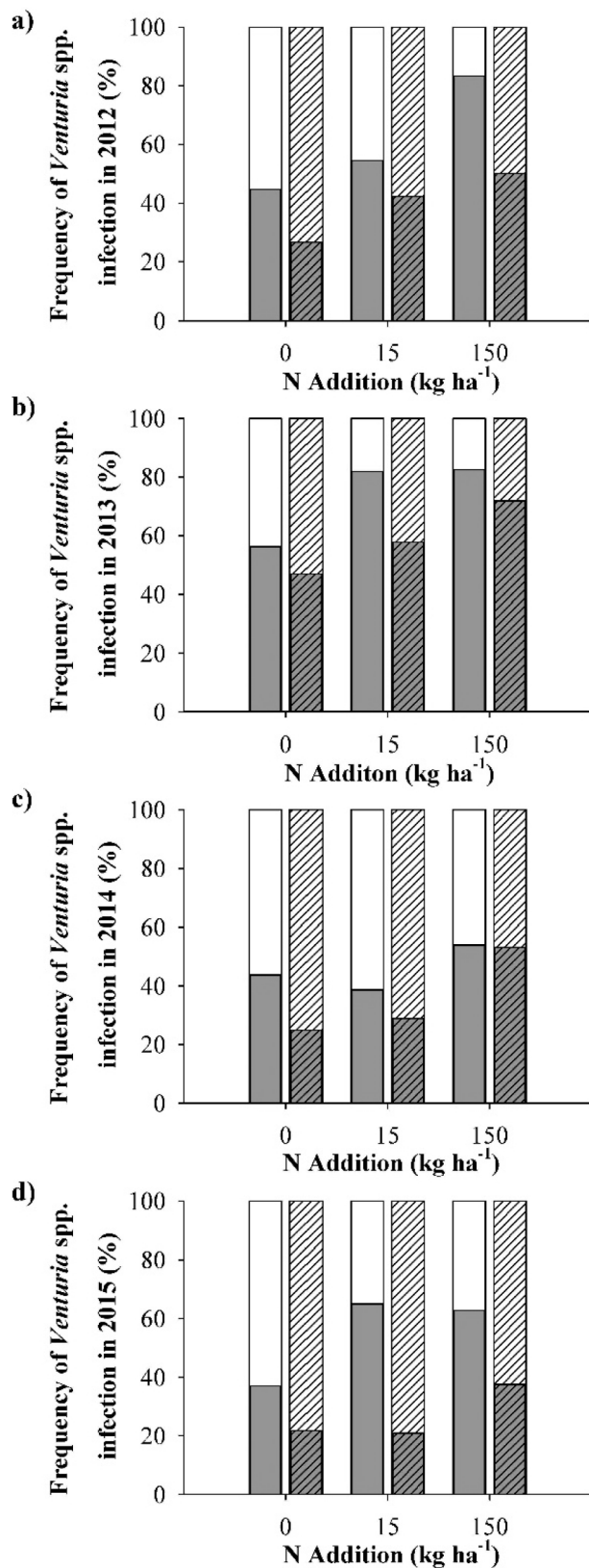
Fig. 2. Mean frequency of *Venturia* infection during August 2012 (a), 2013 (b), 2014 (c), and 2015 (d) for *Populus tremula* genotypes expressing either low (open bars) or high (hatched bars) intrinsic levels of foliar condensed tannins, subjected to three nitrogen addition levels. Gray and white indicates the presence and absence of infection, respectively.

Table 2

Results of a nested ANOVA testing the effects of nitrogen (N) addition, genotype (GT), and constitutive tannin level (i.e. tannin-group; Tgr), and their interactions on *Populus tremula* height growth measured in 2015, in a common garden set-up. Genotype was treated as a nested term within Tgr. F-values (F) are followed by significance levels: *** P < 0.001, ** P < 0.01, * P < 0.05. Significant results (P < 0.05) are displayed in bold.

	Numerator DF	F-value	P-value
Nitrogen	**2**	**48.72**	*******
GT (Tgr)	**8**	**6.66**	*******
Tgr	**1**	**9.37**	*******
GT(Tgr)*N	16	1.61	0.066
Tgr*N	**2**	**6.14**	******

*Denominator Degrees of freedom were 226 for each factor.

frequently emphasized in the literature that there is an inherent trade-off between defense and growth (Stamp, 2003), our data shows no such trade-off effects occurred, at least for the one class of defense compounds we considered (condensed tannins), and under the specific field setting where our study was performed, where the focal genotypes were exposed to abundant antagonists. Instead, our data suggest that in environments that promote high growth rates (e.g. environments subjected to high rates of anthropogenic N-enrichment), there is a substantial risk of being poorly defended due to greater susceptibility and risk of pathogen infection. Thus, while defenses may be biochemically costly to produce, these costs appear to only provide an advantage in certain contexts, where growing taller would provide a plant with a competitive advantage that likely compensates for the cost of defense (Koricheva et al., 2004).

Our study has several implications for understanding how plant populations with genetically diverse defense characteristics may respond to anthropogenic N-enrichment. First, we found that N-inputs increased *Venturia* infection frequencies, even at the relatively low addition rate that is representative of N-deposition rates in the boreal region (Fig. 2). This suggests that the N stoichiometry of *Populus* leaves is a likely control on pathogenicity. Secondly, we observed that high-tannin plants were frequently less infected than low-tannin plants, regardless of the level of N-addition. However, the benefit that defense provided to plant performance (i.e. as measured by height growth) differed depending on the degree of N-enrichment the plants experienced. These findings highlight the importance of genetic diversity in plant populations, which can help stabilize wild plant populations in response to environmental change (Rogers et al., 2020).

Populus tremula is not only a keystone species in the boreal region, but *Populus* sp. and their hybrids are also receiving increasing attention in bioenergy production (Lasch et al., 2010; Nikula et al., 2010; Derba-Maceluch et al., 2020), including a growing interest in Scandinavia. European aspen and other *Populus* spp., and their hybrids, regenerate very quickly after harvest from existing root systems, and achieve very rapid growth, allowing them to be managed under much shorter rotation periods than most conifers. For this reason *Populus* spp. are often managed intensively to maximize production. This can involve selection, breeding, or genetic modification of the fastest growing individuals (Andersson et al., 2003), and further involves managing those individuals in high fertility conditions to optimize their growth (Bettinger et al., 2009). However, *Populus* management systems frequently experience significant economic losses due to fungal pathogens and herbivores (Gruppe et al., 1999). Our data provides insights into how to further optimize biomass production for *Populus* spp. Specifically, breeding programs might wish to focus on individuals that are able to defend themselves well from local pathogens, particularly with high constituitive levels of foliar tannins. These types of tannin rich individuals are likely to provide a growth benefit, particularly when grown in high-fertility contexts, where higher foliar N contents correspond with higher rates of pathogen infection, which tannins can help defend against.
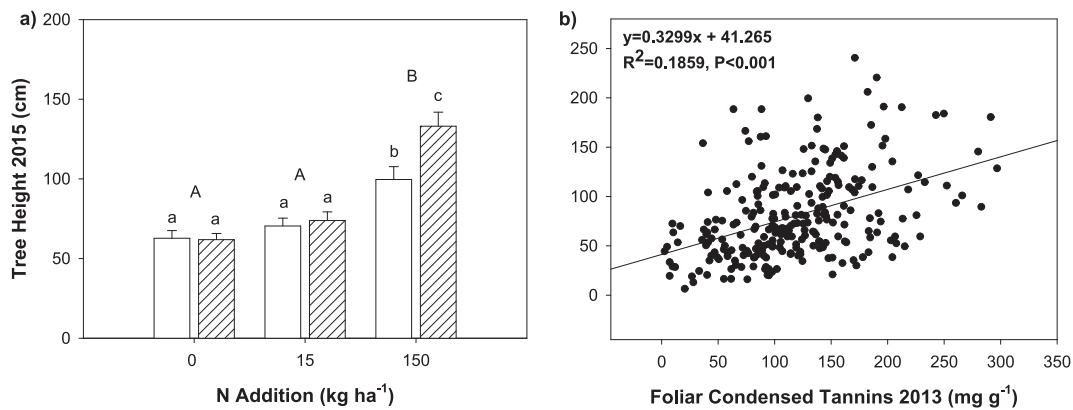
Fig. 3. Panel (a) shows the mean (±SE) tree height measured in 2015 of *Populus tremula* genotypes expressing either low (open bars) or high (hatched bars) intrinsic levels of foliar condensed tannins, subjected to three nitrogen addition levels. Lower or capital letters above individual bars or bar groups, respectively, indicate pairwise differences determined using Tukey's *post-hoc* tests. Panel (b) shows a regression of tree height measured in 2015 against foliar condensed tannins measured in 2013.

CRediT authorship contribution statement

MJG and BRA conceived of the study and set it up in the field. FB was responsible for data collection, analysis, and preparing a manuscript first draft. KMR was responsible for the condensed tannin analysis. All authors contributed to developing the final draft of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.118985.

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