Host genetics and environment shape fungal pathogen incidence on a foundation forest tree species, *Populus tremuloides*

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Host genetics and environment shape fungal pathogen incidence on a foundation forest tree species, *Populus tremuloides*

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Abstract

Diseases can markedly alter the ecological and economic value of poplars. To better understand poplar-pathogen interactions, we investigated the independent and interactive effects of tree genotype, soil nutrient limitation, and interspecific competition on incidence of powdery mildew (caused by the fungal obligate pathogen *Erysiphe adunca*) in a foundation tree species, trembling aspen (*Populus tremuloides*). We established a common garden of potted aspen saplings, incorporating five tree genotypes, two levels of soil nutrients (low and high), and two levels of competition (with and without grass). We then surveyed natural incidence of powdery mildew and aspen vigor (*i.e.*, growth). Incidence of powdery mildew varied among aspen genotypes, and variance in incidence shifted among environments in which the trees were grown. Added soil nutrients increased powdery mildew incidence on aspen, whereas grass competition had the opposite effect. Interestingly, grass competition either enhanced or dampened the variance in incidence of powdery mildew among tree genotypes, depending on soil nutrient levels. In addition, powdery mildew incidence was positively related to tree vigor. Our findings reveal strong genetic, environmental, and G x E effects of disease on a foundation tree species, and that particular environments can either enhance or diminish variation in responses among tree genotypes.

Key words: *Populus*, nutrients, competition, fungal pathogen, genetic variation
Introduction

Poplars (*Populus*) are widely distributed and genetically diverse tree species (Mitton and Grant 1996, Slavov and Zhelev 2010) with considerable ecological and economic importance. In natural and managed environments, poplars are attacked by numerous pathogens (Sinclair and Lyon 2005). Diseases can reduce poplar yield for fiber and biofuel production (Netzer et al. 2002), and restrict the geographic range of *Populus* species (Bell et al. 2015).

Both tree genetics and environmental context drive poplar-pathogen interactions. *Populus* genomics research has revealed several gene families that contribute to defense response (Duplessis et al. 2009). Soil nutrient limitation and competition with herbaceous plants are two factors that can significantly limit poplar establishment and growth in both natural and managed ecosystems (Dickmann and Stuart 1983), and environmental factors (*e.g.*, soil fertility, microclimate, and water supply) influence development of poplar diseases (Netzer et al. 2002, Bell et al. 2015). These genetic and environmental factors can interact, altering poplar-pathogen relationships (Netzer et al. 2002, Busby et al. 2014). Additional work is needed, however, to differentiate the effects of poplar genotype from those of environmental factors that influence plant growth and, consequently, poplar-pathogen interactions.

Trembling aspen (*Populus tremuloides*) is the most widely distributed forest tree species in North America and is highly genetically diverse (Mitton and Grant 1996). It is a foundation species with substantial impacts on dependent communities (Hillstrom et al. 2014). Many species and communities (*e.g.*, herbivores, pathogens, etc.) interact with and utilize aspen forests, and the mechanisms governing these interactions are tied to key aspen traits (Robinson et al. 2012).

Aspen vigor (*e.g.*, growth) is a key trait that influences ecological interactions. Vigor is governed by tree genetics, environment, and their interaction. For example, aspen genotypes vary
considerably in growth rates, and this variation is enhanced in nutrient rich soils (Osier and Lindroth 2006, Stevens et al. 2007a). In addition, harsh site characteristics such as competition and shade can decrease growth (Donaldson et al. 2006, Osier and Lindroth 2006). In accordance with the Plant Vigor Hypothesis (Price 1991), more vigorous aspen are preferentially attacked by herbivores (Robinson et al. 2012; Barker et al. unpublished data). While this framework has been tested mostly with plant-insect systems, it can also be applied to some plant-pathogen interactions. For example, fertilized willows, \textit{(Salix, sister genus to Populus)} were more susceptible to \textit{Melampsora} rust than less vigorous controls (Orians and Floyd 1997).

We investigated the independent and interactive effects of aspen genetics and environment on incidence of powdery mildew caused by the obligate pathogen \textit{Erysiphe adunca} (Sinclair and Lyon 2005). This fungus is prevalent in our study site and overwinters in chasmothecia on debris on the ground and leaves are initially infected following germination of spores in spring or early summer. Secondary cycles of spore production on leaf surfaces and infection occur, and by late summer and early fall a white coating of mycelium becomes visible on leaves. To date, powdery mildews and the factors that affect host susceptibility have been investigated primarily in herbaceous and woody foods \textit{(e.g., wheat, barley, and grape)}. Relatively little is known about how host genetics and environment interact to influence development of powdery mildew in forest tree species.

We were primarily interested in how the range of variation among genotypes in susceptibility to powdery mildew may vary across different environmental conditions and how tree vigor influences occurrence of disease. For our study, we assessed two environmental factors, soil fertility and interspecific competition. We then monitored incidence of powdery mildew on, and growth of, aspen genotypes grown in a common garden.
Materials and Methods

Aspen common garden

To determine the effects of host plant genetics, environment, and their interaction on host vigor and pathogen dynamics, we established an aspen common garden and monitored natural infection by powdery mildew. The garden was planted at the University of Wisconsin-Madison (Madison, Wisconsin, Dane County), in a completely randomized 5 x 2 x 2 factorial design with 24 replicate blocks (480 trees total) in July of 2013. We used five aspen genotypes, two levels of soil nutrients (low and high), and two levels of competition (presence/absence) with marsh reed grass (*Calamagrostis canadensis*).

We prepared experimental pots before planting the aspen replicates. The pots (Classic 2800) were filled with 18L of a soil and sand mixture (70% torpedo sand, 30% silt loam topsoil). An empty cell from a 64-cell planting tray was inserted into the middle of each pot as placeholder for the aspen replicate. In the high nutrient treatments (+N), we added 4.5 g/L of fertilizer (Osmocote Classic 8-9 month slow release, 18% N, 6% P, 12% K), mixing the fertilizer into the top 10 cm of soil in each pot. No fertilizer was added in the low nutrient treatments (-N).

In the competition treatments (+C), we added both marsh reed grass plants and seeds to the pots. On 24 April, we sowed grass seed (Prairie Restorations Inc.) into 32 planting trays (96 wells/tray with 10-15 seeds per well) in MetroMix (25-35% composted bark, coir, perlite, vermiculite, dolomite, lime, blue chip; Sun Gro Horticulture Canada Ltd.) in the greenhouse. The trays were watered regularly with a fertilizer (JR Peters INC, Peat-Lite fertilizer, 20% N, 10% P, 20% K) and water mixture (1.2 ml fertilizer/L). On 22-23 May, we also sowed 0.9 g of grass seed into each competition pot. Then, on 10 and 12 June, we transplanted 6 grass plugs into each pot. The grass was fully established by the time aspen were transplanted. The five aspen
genotypes (PG-1, PG-2, PI-3, Sau-3, and Wau-2) were originally collected from Sauk, Columbia, and Waushara counties in south-central Wisconsin. They vary widely in vigor (e.g., growth rate) and are genetically distinct (Stevens et al. 2007b). Plants were micropropagated from root tissue and grown in a greenhouse in MetroMix soil in 64-cell planting trays from 20 May to 19 June 2013. The plants were then moved outside and were transitioned to full sunlight with sequential shade cloth coverings (19 June 70% shade; 25 June 30% shade; 29 June full sun). On 2-3 July 2013 we transplanted the aspen to experimental pots.

Aspen vigor

We surveyed aspen vigor as measured by tree growth. Upon transplanting the trees, we measured the heights (from root crown to base of apical meristem) and basal diameters (2 cm above root crown) of each tree. We repeated these measurements in late fall of 2013. To quantify aspen growth, we calculated $\ln(diameter^2 \times height)_{final} - \ln(diameter^2 \times height)_{initial}$, a metric that correlates well with tree biomass measurements (Stevens et al. 2007b).

Powdery mildew survey and identification

Presence of white mycelium of a naturally occurring powdery mildew fungus became apparent on leaves by August of 2013 (Fig. 1). We then scored the incidence (0 = absence of mycelia on all leaves on the tree, 1 = visible mycelia on at least one leaf on the tree) of powdery mildew in the tree canopies on 16 October 2013. Thirty chasmothecia were collected from leaves of three trees in the garden, pooled, and DNA was extracted. The nuclear ribosomal internal transcribed spacer region was amplified using primers ITS5 and P3, and the resulting fragment of approximately 570 bp was then sequenced (GenBank KU361139). We then compared this sequence to other powdery mildew fungus sequences in GenBank.

Statistical analyses
To determine the independent and interactive influences of aspen genotype and environment on tree vigor and the incidence of powdery mildew (separately), we used both Gaussian (Y = tree growth), and binomial (Y = mildew presence/absence) regressions. For the binomial regression, we used an analysis of covariance (ANCOVA) including final tree size as a covariate to determine the effects of experimental factors (i.e., tree genotype, competition, and nutrients) on fungus incidence independent of tree size. To then explore the relationships between tree vigor (growth) and powdery mildew incidence, we used binomial regression with growth as the independent variable, powdery mildew presence/absence as the dependent variable, and block to account for spatial variation. All three regression models were performed in the stats package in R (R Core Team 2013).

Results

Aspen vigor

Tree growth was influenced by tree genotype, environment (competition and nutrients), and their interactions (Table 1, Fig. 2, $F_{(42,337)} = 125, P < 0.001, R^2 = 0.93$). Aspen genotypes varied moderately (1.14-fold difference) in growth, and tree growth increased by 2.4-fold with added nutrients, yet decreased by 0.6-fold with grass competition. The range of tree growth varied among aspen genotypes, depending on environmental conditions (i.e., genotypic range increased by 18% with added grass in nutrient-rich soils compared with aspen grown in nutrient-rich soils without grass).

Powdery mildew identification

Sequence comparison revealed ≥98% similarity with sequences of two strains: DDBJ LC028966 from *Populus nigra* in Hungary and GenBank AF011324 from *Populus* sp. in the United States. These two strains comprised the poplar subclade of *E. adunca* species complex as
reported by Takamatsu et al. (2015). The sequence we obtained was <87% similar to sequences of strains from the willow (*Salix*) subclade of the *E. adunca* complex (Takamatsu et al. 2015), and even less similar to sequences of strains of powdery mildew pathogens from other tree hosts.

**Powdery mildew incidence**

Powdery mildew incidence was influenced by tree genotype, environment, and their interactions, regardless of tree size (Table 1, Fig. 2, McFadden’s pseudo $R^2 = 0.44$). Aspen genotypes varied considerably (1.8-fold difference) in incidence of powdery mildew when averaged across environments. In addition, powdery mildew incidence increased by 1.3-fold with added soil nutrients ($P > 0.05$), yet decreased with grass competition in some environments.

Growth environment influenced the range of variation in powdery mildew incidence among aspen genotypes (GxE interactions; Table 1, Fig. 2). In low-nutrient soils, powdery mildew incidence varied by 4.9-fold among aspen genotypes grown without grass, but by only 1.2-fold for aspen grown with grass. Thus, in nutrient-poor soils, aspen genotypic variance was reduced with grass competition (Fig. 2 Bottom left). In contrast, the opposite trend occurred for trees grown in nutrient-rich soils: mildew incidence varied by only 1.3-fold among genotypes grown without grass, but by 2.2-fold for genotypes grown with grass (Table 1, Fig 2 Bottom right; significant GxNxC interaction). In addition, powdery mildew incidence was positively correlated with tree vigor ($X^2 = 188.25$, df = 1, $P < 0.001$, McFadden’s pseudo $R^2 = 0.42$).

**Discussion**

Poplar-pathogen interactions are driven by the independent and interactive effects of tree genetics and environment. We found that incidence of an aspen (*Populus tremuloides*) disease, powdery mildew (caused by the fungal obligate pathogen *Erysiphe adunca*), varies among tree genotypes, and variance in incidence depended on the environmental context in which the trees
were grown. Fertilization increased powdery mildew incidence on trees, whereas grass 
competition had the opposite effect but only in some environments (significant NxC interaction).
In addition, powdery mildew incidence was positively related to tree vigor. Our findings 
demonstrate that aspen genotype and environment interact to shape disease development. 

Aspen vigor

Aspen vigor as reflected by growth was influenced by tree genotype, soil fertility, 
interspecific competition, and interactions among these factors. As expected, tree growth 
increased with nutrient availability (*i.e.*, with added nutrients and without competition), which 
has been shown in previous studies on aspen (Osier and Lindroth 2006, Stevens et al. 2007a).
However, in contrast to earlier work (Osier and Lindroth 2006, Stevens et al. 2007a), variation in 
growth among tree genotypes was not significantly enhanced with nutrient availability.

Our results show that disease incidence was positively related to aspen vigor. Previous 
work on willow (Orians and Floyd 1997) and apple (Leser and Treutter 2005) has also shown 
that faster growing plants are more susceptible to some diseases, including rust (caused by 
*Melampsora* sp.) and scab (caused by *Venturia inaequali*), respectively. This link between 
disease and plant vigor may stem from increased susceptibility in actively expanding tissue 
(Gadoury, David and Seem 1995, Agrios 1997) and/or a tradeoff between plant growth and 
defense against pathogens. In addition, while we found a positive relationship between incidence 
of a biotrophic pathogen and host tree vigor, this relationship is complex and may not hold for 
necrotrophic pathogens. For instance, statements in the applied literature suggest that high tree 
vigor may reduce susceptibility to powdery mildews (ISO Plant Disease Clinic, 2011). In 
addition, Bagga and Smalley (1973) found that more vigorous (*e.g.*, fertilized) aspen were more 
resistant to a necrotrophic pathogen, Hypoxylon canker caused by *Entoleuca mammata*. 

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Powdery mildew incidence was variable in the garden (Fig. S1). Diseased trees had mycelia covering 1-30% of their leaf tissue at the time of the survey. While our method is efficient for surveying disease incidence in the field, it does not provide disease severity or development information.

Nitrogen fertilization likely enhances growth of foliar fungi by increasing nitrogen availability to the fungus (Jensen and Munk 1997, Chen et al. 2007) and/or by increasing microclimate humidity via changes in plant structure (e.g., denser foliage; Jenkyn 1976). In support of these mechanisms, nitrogen fertilization of host plants has been shown to increase incidence and severity of foliar biotrophic fungi, including powdery mildews (Huber and Watson 1974, Chen et al. 2007). Fertilization can also increase sporulation by powdery mildew fungi, and this effect varies with host plant genetics (Jensen and Munk 1997).

Along with changes in soil nutrient levels, biotic environmental factors, such as competition with other plants, can influence the availability of nitrogen to the host tree. Thus, our study had compounded effects between fertilization and grass competition, which were highlighted in that aspen grown in low nutrient soils with grass competition had the least incidence of powdery mildew, while aspen grown in high nutrient soils without grass competition had the highest incidence of disease.

While grass competition likely influenced powdery mildew incidence via availability of nutrients, additional mechanisms may have been involved. Competition with grass reduced tree size, making the aspen host a smaller target for powdery mildew inoculum (Morrison 1996). In support, the main effect of grass competition on powdery mildew incidence was insignificant when accounting for tree size. In addition, the smallest trees in the competition treatments had...
canopies partially surrounded by grass. Thus the grass, which is not a host for *E. adunca*, may have intercepted inoculum, limiting the spread of disease to these trees (Morrison 1996).

Variation in powdery mildew incidence may be related to genotypic and environmental variation in foliar chemistry. In particular, condensed tannins, which were strongly negatively correlated with *Venturia* shoot incidence on *P. tremuloides*, (Holeski et al. 2009), may play a role in pathogen defense (Barbehenn and Constabel 2011). Moreover, condensed tannins have a negative relationship with fertilization (Donaldson et al. 2006, Osier and Lindroth 2006, Stevens et al. 2007a). Thus, the fertilized trees in our experiment that were the most susceptible to powdery mildew likely had the lowest levels of condensed tannins. Further work is needed to address the separate and combined effects of vigor and defense on fungal incidence on poplar.

**Role of tree genetics**

We found that both tree growth and powdery mildew incidence varied strongly across aspen genotypes, suggesting that tree genetics plays an important role in tree vigor and disease resistance. These findings are supported in the literature; Robinson et al. (2012) found that tree size traits have a moderate heritability (0.39-0.56), and several gene families in *Populus* have been shown to be instrumental in pathogen response and plant defense (Duplessis et al. 2009).

**Genotype by environment interactions**

The dampening and enhancing of genotypic variation in disease incidence in a plant population due to variation in the environment has been shown in a related system. Busby et al. (2014) found that different *Populus angustifolia* genotypes harbor different communities of foliar fungal pathogens, and that tree genotypic effects were enhanced or dampened based on differences in the environment (*i.e.*, elevation). The effects of tree genetics and environment may vary in importance depending on the ecological relationship examined. Barbour et al. (2009)
found that macrofungal decay communities on tree bark were structured primarily through
changes in microhabitat, while host tree (Eucalyptus globulus) genetics and GxE interactions
played little to no role. Thus, the importance of GxE interactions are likely context-specific.

This work highlights the importance of the genetic make-up of populations and
environmental context in spreading disease. We found that in particular environmental
treatments (i.e., low soil fertility with grass competition), disease incidence was exceptionally
low, and variation in incidence among aspen genotypes was reduced. Under these and similar
conditions, disease impacts may be minimized. Yet, under high soil fertility conditions with
grass, the variation in disease incidence among aspen genotypes was enhanced. In this and
similar cases, selection could act against alleles that confer susceptibility to disease, potentially
altering the evolutionary trajectory of poplar populations.

In conclusion, we found that both host tree genetics and environmental factors related to
nutrient limitation influenced disease caused by a fungal pathogen of Populus. In particular, high
soil fertility increased tree vigor, which was positively related with disease incidence.
Environmental context played a key role in enhancing or dampening variation among genotypes
in pathogen susceptibility. In addition, the particular combination of environmental factors was
important (e.g., grass competition increases genotypic variation in high nutrient soils, yet
decreases variation in low nutrient soils). These findings emphasize the multiple, interacting
factors that can influence disease development in natural and managed poplar populations.

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References


Table 1. Effects of aspen genotype (G), soil nutrients (N), and grass competition (C) on tree growth (natural log transformed, ANOVA) and powdery mildew incidence (0 = absence, 1 = presence; ANCOVA). The ANCOVA includes final tree size (TS, natural log transformed) as a covariate. Statistically significant (P < 0.05) factors and interactions are shown in bold type.

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Figure 1. (A) White mycelial mats of powdery mildew pathogen *Erysiphe adunca* on upper surface of potted aspen leaves. (B) Magnified *Erysiphe adunca* chasmothecia on mycelial mat.

Figure 2. Norm of reaction plots of aspen genotype, soil nutrients, and grass competition (“-Comp” without and “+ Comp” with grass), on (top) tree growth (natural log transformed) and (bottom) powdery mildew incidence (0 = absence, 1 = presence). Points are the mean for 24 replicates with standard error bars.

Figure S1. The experimental garden, showing the variation in powdery mildew incidence among trees. Diseased trees had on average 1-30% infected leaf tissue.
Figure 1. (A) White mycelial mats of powdery mildew pathogen Erysiphe adunca on upper surface of potted aspen leaves. (B) Magnified Erysiphe adunca chasmothecia on mycelial mat. 395x285mm (300 x 300 DPI)
Figure 2. Norm of reaction plots of aspen genotype, soil nutrients, and grass competition ("- Comp" without and "+ Comp" with grass), on (top) tree growth (natural log transformed) and (bottom) powdery mildew incidence (0 = absence, 1 = presence). Points are the mean for 24 replicates with standard error bars.