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Incidence of *Venturia* shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype

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ABSTRACT

Quaking aspen (*Populus tremuloides*) is a foundation tree species in North American forests, as well as a valuable source of wood and paper products. Quaking aspen exhibits substantial genetic variation within and among natural populations in phytochemical compounds that influence both interactions with herbivores and ecosystem dynamics. The potential association of these phytochemicals with disease resistance, however, is unknown. Here we present the results of a “natural experiment” in a common garden of quaking aspen genotypes infected with shoot blight (*Venturia moreletii*). We found that the incidence of shoot blight varied by 10-fold among aspen genotypes, and was strongly and negatively correlated with constitutive foliar concentrations of condensed tannins. Selection factors that shape the genetic and phytochemical architecture of aspen populations may thus simultaneously influence aspen resistance to pathogen attack, with consequences for individual tree fitness as well as community organization, via “extended phenotype” effects.

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1. Introduction

Quaking aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America, with corresponding ecological and economic significance. This rapidly growing tree is a foundation species, playing prominent roles in the community and ecosystem dynamics of early successional forests throughout western and north-temperate North America (Alban and Perala, 1992; Pastor and Naiman, 1992; Romme et al., 1995; Hogg et al., 2005). Quaking aspen is dioecious and frequently reproduces asexually, creating clones that can persist for thousands of years (Perala, 1990; Mitton and Grant, 1996). In part because of its rapid growth, quaking aspen is commonly grown for commercial harvest of lumber and wood pulp, and is a promising biofuel feedstock (Chen and Wayman, 1989; Kauter et al., 2003).

Substantial variation exists among quaking aspen clones within and among natural populations for constitutive concentrations of two major classes of secondary compounds: phenolic glycosides (salicylates) and condensed tannins (Lindroth and Hwang, 1996; Donaldson et al., 2006). This variation can be attributed to developmental, genetic, and environmental (e.g., resource availability) factors, as well as to G × E interactions (Osier and Lindroth, 2001, 2004, 2006; Stevens and Lindroth, 2005; Donaldson et al., 2006). Moreover, phytochemical variation in an ecologically dominant species can have substantial effects on community members and ecosystem processes. In aspen, genetic variation in phenolic glycoside and/or condensed tannin production is known to affect insect preference and performance (Hemming and Lindroth, 1995; Hwang and Lindroth, 1997; Osier and Lindroth, 2001, 2006; Donaldson and Lindroth, 2007),

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influence mammalian browsing (Wooley et al., 2008), and alter nutrient cycling and litter decomposition rates (Madritch et al., 2006).

Despite the well-documented ability of phytochemicals such as phenolic glycosides and tannins to provide resistance against herbivores, much less is known about their potential to affect fungal pathogens. Quaking aspen is susceptible to a large number of pathogens, which can cause substantial damage, crown dieback, and/or widespread tree mortality (Hiratsuka, 1987; Webb, 1989; Brandt et al., 2003; Frey et al., 2004; Worrall et al., 2008). Pathogen infection has been the most frequent cause of mortality in some quaking aspen stands (Hill et al., 2005), and is thought to be partially responsible (via interactions with abiotic, climate-related variables) for widespread aspen dieback and reduced aspen forest growth in North America over the past two decades (Hogg et al., 2005).

Among the most common diseases affecting quaking aspen is shoot blight, caused by the fungus *Venturia moreletii* (syn. *Venturia macularis*, *Venturia tremulae* var. *grandidentatae*). This pathogen infects aspen leaves and stems on elongating terminal or lateral shoots in the spring. Symptoms include dark lesions that expand rapidly on shoots that wither and droop as they die and eventually become brittle. Within a few days after infection, a layer of conidiophores and conidia of the asexual anamorph of *V. moreletii*, known as *Fusicladium radiosum* var. *lethiferum* (syn. *Pollaccia radiosa*), develop on colonized tissues. Shoot blight is most damaging to young growth and the major effect of disease is reduction in height growth of the affected trees. In wet years *V. moreletii* may kill large numbers of the terminal shoots in aspen stands (Sinclair and Lyon, 2005; Blenis, 2007).

In July 2003, a young plantation of quaking aspen near Madison, WI, U.S.A., was subject to a major outbreak of *V. moreletii*. We took advantage of this “natural experiment” to address: 1) whether aspen exhibits genetic variation in resistance to *V. moreletii*, and 2) whether variation in resistance is associated with variation in constitutive phytochemistry.

2. Materials and methods

2.1. Study site and pathogen characterization

A common garden of quaking aspen was established at the Arlington Agricultural Research Station (University of Wisconsin–Madison), near Madison, Wisconsin (U.S.A.), in April of 2002. The garden contained replicates of multiple aspen genotypes, originating from several locations in south-central Wisconsin. Microsatellite analyses (C.T. Cole and R.L. Lindroth, unpubl. data) confirmed the unique genetic identity of each genotype. Trees were micropropagated in early 2001, then grown outside in pots (5 L) containing a 40–40–20 sand/field soil/perlite mix. In April 2002, aspens were transplanted to a plowed and disked common garden. The garden site was formerly an agricultural field, and the soil type is chiefly Plano Silt Loam. The common garden was established for long-term monitoring of aspen growth and foliar chemistry.

In mid-June, 2003, as part of our annual phytochemical survey, we collected healthy leaves from throughout the canopy of five individual trees from each of eleven aspen genotypes. Leaves were excised at the base of the lamina, and placed on ice until transferred to the laboratory. Leaves were then flash-frozen in liquid nitrogen, freeze-dried, and stored (–20 °C) until chemically analyzed.

In July 2003, numerous trees throughout the common garden exhibited symptoms of aspen shoot blight. In late July, we surveyed five trees within each of 11 genotypes (the same 55 individual trees that had leaves harvested for chemistry) for the incidence of shoots damaged by aspen shoot blight (expressed as a proportion and calculated for each individual tree as the number of symptomatic shoots/total number of shoots). These trees were randomly distributed within a portion of the common garden that had a relatively uniform distribution of blight symptoms. We also collected branch tips from 2 to 3 trees per genotype for culturing and identification of the fungal pathogen. The pathogen was identified on the basis of presence of mycelium and conidia of the anamorph, *F. radiosum* var. *lethiferum*, on leaves or shoot tips (Sivanesan, 1974). Of 25 trees assayed, 24 tested positive for *F. radiosum*.

2.2. Chemical analysis

The dried leaves were ground with a Wiley mill (40-mesh screen). We quantified aspen phenolic glycosides, including salicortin and tremulacin, using high-performance thin-layer chromatography methods described by Lindroth et al. (1993), with purified aspen phenolic glycosides as standards. Total phenolic glycoside concentrations were calculated as the sum of salicortin and tremulacin concentrations. We quantified condensed tannins using the acid butanol assay described by Porter et al. (1986), with purified aspen condensed tannins as a standard. Total nitrogen content was determined with a LECO elemental analyzer (St. Joseph, Michigan) using glycine *p*-toluenesulfonate as a standard.

2.3. Statistical analysis

The data for condensed tannins and nitrogen, as well as the proportion of blighted shoots per tree, were transformed (arcsine square root function) to meet normality assumptions prior to statistical analysis.

To ascertain whether genetic variation exists for concentrations of phenolic glycosides, condensed tannins, or nitrogen, we performed one-way analyses of variance (ANOVAs), each with genotype as a fixed factor and phytochemical concentration as the response variable. A Pearson product-moment test was performed to characterize the relationship between

total phenolic glycosides and condensed tannins across genotypes and genotype replicates. To assess the functional relationship between individual tree chemistry and the incidence of shoot blight, we regressed the proportion of blighted shoots per tree on each chemical parameter (salicortin, tremulacin, total phenolic glycosides, condensed tannin, and nitrogen, respectively).

To determine whether genotypes differed in their susceptibility to shoot blight, we performed a one-way ANOVA with genotype as a fixed factor and the proportion of blighted shoots per tree as a response variable. Ideally, an ANCOVA would also have been performed to determine the effects of genotype on blight susceptibility when variation in phytochemistry was taken into account. However, concentrations of phenolic glycosides, condensed tannins, and nitrogen were closely associated with genotype, thus precluding the opportunity to perform an ANCOVA with genotype and chemistry as factors and proportion of blighted shoots per tree as a response (Sokal and Rohlf, 1995). All data analyses were performed using Minitab 14.0 (State College, PA, USA).

3. Results

We found considerable genetic variation in total phenolic glycoside production, with genotype means that ranged from ~6 to 22% of leaf dry weight ($F_{10,42} = 9.50$; $p < 0.001$; Fig. 1). Similarly, substantial genetic variation existed in condensed tannin concentration, with genotype means that ranged from ~6 to 20% ($F_{10,42} = 28.49$; $p < 0.001$; Fig. 1). Genetic variation also existed for nitrogen concentrations ($F_{10,42} = 12.81$; $p < 0.001$; Fig. 1), although the range of variation (2.6–3.3%) was considerably less than for the secondary metabolites. We found significant variation associated with the natural source population from which the genets were derived for total phenolic glycoside concentrations ($F_{4,48} = 6.11$; $p < 0.001$), but not for condensed tannin or nitrogen concentrations ($F_{4,48} = 1.71$ and 1.54 ; $p = 0.163$ and 0.205 , respectively). Total phenolic glycoside and condensed tannin concentrations were negatively correlated ($r = -0.547$; $p < 0.001$). The concentrations of phenolic glycosides, tannins, and nitrogen in this study were consistent with patterns found in phytochemical surveys in later years (with no blight damage and minimal herbivory), supporting our contention that the data presented here represent constitutive levels of phytochemistry (Lindroth et al., unpublished data).

Substantial variation existed among genotypes in the incidence of shoot blight, with some genotypes experiencing up to 10 times higher rates of blight damage than others ($F_{10,42} = 3.08$; $p = 0.005$; Fig. 2). The incidence of blighted shoots per tree was also strongly functionally related to several chemical parameters. Fungal infection rates were positively related to total phenolic glycoside levels ($p = 0.009$; $r = 0.356$; Fig. 3A) but negatively related to condensed tannin concentrations ($p = 0.001$; $r = -0.443$; Fig. 3B). Blight incidence had no clear relationship with foliar nitrogen concentrations ($p = 0.778$; $r = 0.04$). We

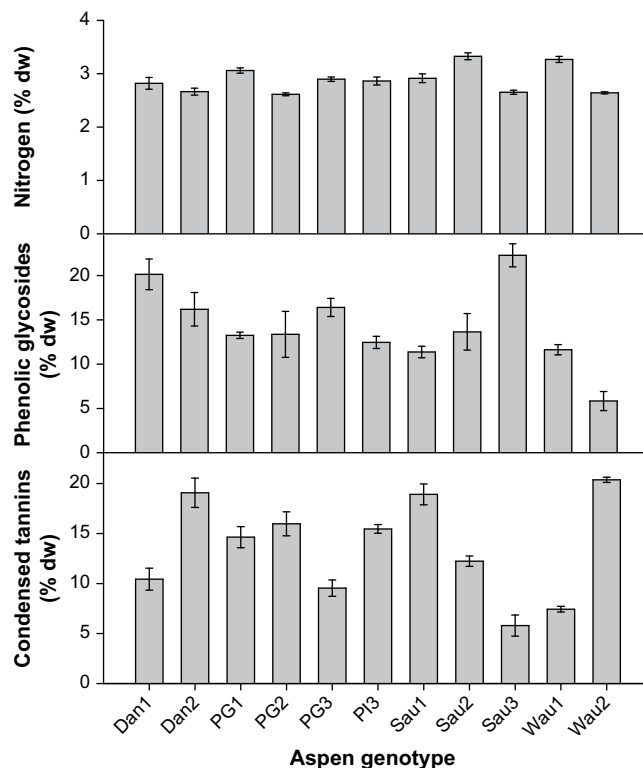


Fig. 1. Genetic variation in phytochemical composition of aspen genotypes (means \pm 1 standard error (SE); $N = 5$ trees per genotype).

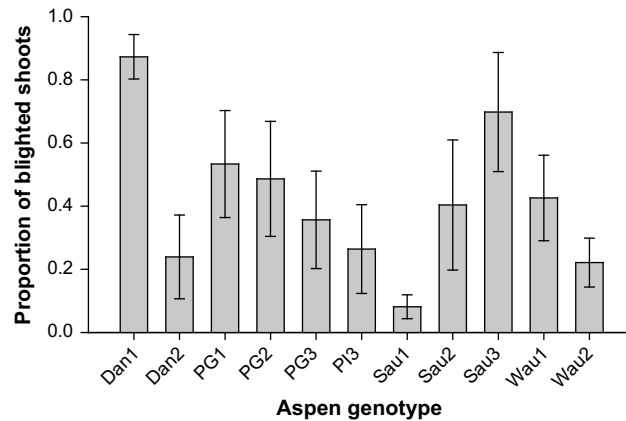


Fig. 2. Genotype means for the incidence of blighted shoots per tree (transformed). Bars represent 1 SE from the mean.

found no significant relationship between the natural source population of the common garden genotypes and the incidence of shoot blight ($F_{4,48} = 0.91$; $p = 0.466$).

4. Discussion

This study documents that quaking aspen genotypes are differentially resistant to *V. moreletii* (Fig. 2), and that resistance is positively related to constitutive condensed tannin concentration (Fig. 3). Genetic variation in resistance to *V. moreletii* has not previously been shown to exist in this host species (Sinclair and Lyon, 2005). In an analogous system (hybrid poplars), substantial genetic variation in resistance to other *Venturia* species was found, but no specific resistance traits were suggested (Newcombe and van Oosten, 1997).

4.1. Role of condensed tannins in resistance

No empirical evidence prior to this study has suggested that *V. moreletii* incidence is negatively associated with the constitutive concentration of condensed tannins in woody plants. In general, evidence in the literature that constitutive levels of phenolic compounds play a role in plant resistance to pathogens is rare (Witzell and Martin, 2008). However, previous studies of *Populus* species have shown that 1) condensed tannin production is induced following pathogen infection (Miranda et al., 2007), and 2) the concentration of condensed tannins in cells *post-infection* is negatively correlated with spread of a pathogen at the cellular level. Bloomberg and Farris (1963) assayed cells of *Populus trichocarpa* and *Populus canadensis* after the trees were inoculated at wound sites with *Cytospora chrysosperma* and found that growth of the canker was inversely related to the post-infection condensed tannin concentration. Przybyl (1984) assayed cells of *Populus nigra*, *P. trichocarpa*, and *P. maximowiczii* after they were inoculated with *Ceratocystis fimbriata* fungi, and found that cells resistant to the spread of fungal hyphae produced higher levels of condensed tannins more rapidly following inoculation than did the susceptible cells. Both of these studies suggest a mechanistic relationship between pathogen infection and levels of condensed tannins, but more research is needed to identify the specific processes involved.

Our previous work with aspen has shown that, with the possible exception of chrysomelid beetles (Donaldson and Lindroth, 2004), condensed tannins are ineffective as defenses against leaf-feeding insects (Lindroth, 2001). Results from this

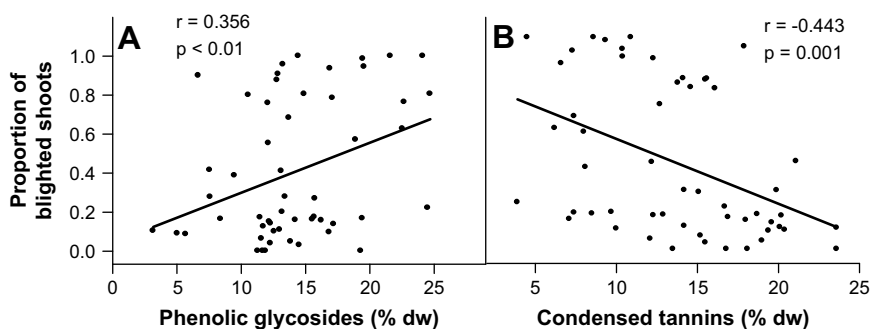


Fig. 3. Relationship of fungal pathogen incidence (transformed) to foliar chemistry. A. Total phenolic glycosides (salicortin + tremulacin), B) condensed tannins (transformed).

study support conclusions from studies of hybrid cottonwood, which suggest that in addition to their putative role as defenses against salicylate-adapted beetles, tannins may play important roles as resistance factors against fungal pathogens (Bailey et al., 2006; Schweitzer et al., 2008).

Our results also show that blight incidence is positively related to foliar phenolic glycoside concentrations. This result contrasts with those of several analogous studies (Hubbes, 1966; Kruger and Manion, 1994), which implicated phenolic glycosides as inhibitors of *Hypoxyton* infection in quaking aspen. We suggest that the positive association identified in this study is simply a spurious relationship, due to the negative correlation of condensed tannin and phenolic glycoside concentrations observed in this and other research with quaking aspen (Hwang et al., 1995; Lindroth and Hwang, 1996; Donaldson and Lindroth, 2007). While the two phytochemicals are both produced via the shikimic acid pathway, and are influenced by both genetic and the environmental factors, the extent to which the observed negative correlation between them is due to these influences and/or allocation of resources is unknown (Hemming and Lindroth, 1995; Osier and Lindroth, 2006).

4.2. Other potential mechanisms of resistance

Results of other investigations suggest involvement of specific mechanisms of disease resistance in: 1) poplars to pathogens other than *Venturia*, and 2) other tree hosts to *Venturia* pathogens. The draft genome of the black cottonwood (*P. trichocarpa* Torr. & Gray) includes numerous genes and gene domains associated with plant disease resistance (Tuskan et al., 2006). These include, for example, β -1,3-glucanase and chitinase genes and also putative orthologs of genes known to control disease resistance signaling (through a mechanism other than tannin production) in *Arabidopsis*. The potential relationship between the activity of specific genetic mechanisms of fungal disease resistance and the influence of particular secondary metabolites on infection and colonization is a promising area for future research.

4.3. Larger implications

4.3.1. Commercial production

In both aspens and hybrid poplars, genetic variation in resistance to *V. moreletii* may be an important consideration when planning the composition of plantations used for commercial harvesting. Commercial plantations are often grown as short-rotation, intensive cultures, where juvenile resprout tissue following harvest is particularly susceptible to damage from pathogens such as *Venturia*. Genetic variation in resistance allows breeders to select clones that are less susceptible to blight, and to artificially select and breed trees with increased resistance. Understanding of putative mechanisms of resistance (i.e., condensed tannins) could guide selection efforts. Because condensed tannins are negatively correlated with phenolic glycosides, which provide trees with resistance to some insect herbivores (e.g. Hwang and Lindroth, 1997), our findings also serve as an example regarding the risks of selection on a single trait. Selection for increased phenolic glycoside concentration, for example, could result in corresponding decreases in condensed tannin concentrations and impaired resistance to *V. moreletii*.

4.3.2. The extended phenotype

This research identified *V. moreletii* as a community member whose distribution is likely influenced by foliar chemistry. Thus, this work complements previous studies with aspen and related *Populus* species that have implicated phytochemistry as the key intermediary between tree genetics and herbivore resistance (Osier and Lindroth, 2001; Bailey et al., 2004; Donaldson and Lindroth, 2007; Wooley et al., 2008), multi-trophic interactions (Bailey et al., 2006; Müller et al., 2006), insect community organization (Bangert et al., 2006), and ecosystem processes (Madritch et al., 2006; Schweitzer et al., 2008).

Selection factors that shape the genetic and phytochemical architecture of aspen and related *Populus* populations can thus simultaneously influence resistance to pathogen attack, with potential consequences for individual tree fitness as well as community organization. For example, browsing by mammals can alter the genetic and chemical composition of aspen (Bailey et al., 2007) and cottonwood (Bailey et al., 2004) populations and may thereby modify resistance to pathogen attack. Moreover, because aspen shoot blight infects terminal meristems, trees that survive infection exhibit subsequent changes in branch architecture and canopy structure (Sinclair and Lyon, 2005; Blenis, 2007). Differential incidence of blight among aspen genotypes may thus have cascading effects on community members via alterations in tree architecture. Elk browsing on terminal growth in quaking aspen results in analogous shifts in tree structure, and has been demonstrated to affect insect herbivores and their avian predators (Bailey and Whitham, 2003). Studies with several other tree species have demonstrated that changes in tree architecture can markedly affect species richness and diversity of associated plant, insect, avian, and mammalian community members (Jennins, 1999; Liira et al., 2002; Hirose, 2005).

In conclusion, this natural experiment has demonstrated that quaking aspen exhibits constitutive genetic variation in resistance to the fungal pathogen *V. moreletii*, and that differential resistance is correlated with foliar chemistry. Genotypes with higher constitutive levels of condensed tannins are less susceptible to damage than genotypes with lower levels of the compounds. Genotypic variation in concentrations of condensed tannins could affect *Populus*-pathogen interactions and selection pressures in both natural populations and commercial plantations. More broadly, our results suggest that the selection factors that shape the genetic and phytochemical composition of an aspen population may have substantial, multifaceted consequences for *Populus* community dynamics, ecosystem processes, and evolutionary trajectories.

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References

- Alban, D.H., Perala, D.A., 1992. Carbon storage in lake states aspen ecosystems. *Can. J. For. Res.* 22, 1107–1110.
- Bailey, J.K., Whitham, T.G., 2003. Interactions among elk, aspen, galling sawflies, and insectivorous insects. *Oikos* 101, 127–134.
- Bailey, J.K., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Whitham, T.G., 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. *Ecology* 85, 603–608.
- Bailey, J.K., Wooley, S.C., Lindroth, R.L., Whitham, T.G., 2006. Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.* 9, 78–85.
- Bailey, J.K., Schweitzer, J.A., Rehill, B.J., Irschick, D.J., Whitham, T.G., Lindroth, R.L., 2007. Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. *Biol. Invas.* 9, 715–722.
- Bangert, R.K., Allan, G.J., Turek, R.J., Wimp, G.M., Meneses, N., Martinsen, G.D., Keim, P., Whitham, T.G., 2006. From genes to geography: a genetic similarity rule for arthropod community structure at multiple geographic scales. *Mol. Ecol.* 15, 4215–4228.
- Blenis, P.V., 2007. Impact of simulated aspen shoot blight on trembling aspen. *Can. J. For. Res.* 37, 719–735.
- Bloomberg, W.J., Farris, S.H., 1963. Cytospora canker of poplars: bark wounding in relation to canker development. *Can. J. Bot.* 41, 303–310.
- Brandt, J.P., Cerezke, H.F., Mallet, K.I., Volney, W.J.A., Weber, J.D., 2003. Factors affecting trembling aspen (*Populus tremuloides* Michx.) health in Alberta, Saskatchewan, and Manitoba, Canada. *For. Ecol. Manage.* 178, 287–300.
- Chen, S., Wayman, M., 1989. Continuous production of ethanol from aspen (*Populus tremuloides* Michx.) cellulose by co-immobilized yeast and enzymes. *Process Biochem.* 24, 204–207.
- Donaldson, J.R., Lindroth, R.L., 2004. Cottonwood leaf beetle (Coleoptera: Chrysomelidae) performance in relation to variable phytochemistry in juvenile aspen (*Populus tremuloides* Michx.). *Environ. Entomol.* 33, 1505–1511.
- Donaldson, J.R., Lindroth, R.L., 2007. Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. *Ecology* 88, 729–739.
- Donaldson, J.R., Stevens, M.T., Barnhill, H.R., Lindroth, R.L., 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). *J. Chem. Ecol.* 32, 1415–1429.
- Frey, B.R., Loeffers, V.J., Hogg, E.H., Landhäusser, S.M., 2004. Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. *Can. J. For. Res.* 34, 1379–1390.
- Hemming, J.D.C., Lindroth, R.L., 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103, 79–88.
- Hill, S.B., Mallik, A.U., Chen, H.Y.H., 2005. Canopy gap disturbance and succession in trembling aspen dominated boreal forests in northeastern Ontario. *Can. J. For. Res.* 35, 1942–1951.
- Hiratsuka, Y., 1987. Forest tree diseases of the prairie provinces. *Can. For. Serv. North. For. Res. Cent. Inf. Rep. NOR-X-286*.
- Hirose, T., 2005. Development of the Monsi-Saeki theory on canopy structure and function. *Ann. Bot.-London* 95, 483–494.
- Hogg, E.H., Brandt, J.P., Kochtubajda, B., 2005. Factors affecting interannual variation in growth of western Canadian aspen forests during 1951–2000. *Can. J. For. Res.* 35, 610–622.
- Hubbes, M., 1966. Inhibition of *Hypoxyylon prunatum* (Klotzsch) Cke. by aspen bark meal and the nature of active extractives. *Can. J. Bot.* 44, 365–386.
- Hwang, S.Y., Lindroth, R.L., Montgomery, M.E., Shields, K.S., 1995. Aspen leaf quality affects gypsy moth (Lepidoptera: Lymantriidae) susceptibility to *Bacillus thuringiensis*. *J. Econ. Entomol.* 88, 278–282.
- Hwang, S.Y., Lindroth, R.L., 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* 111, 99–108.
- Jennins, S.B., 1999. Assessing forest canopies and understory illumination: canopy closure, canopy cover and other measures. *Forestry* 72, 59–73.
- Kauter, D., Lewandowski, I., Claupein, W., 2003. Quantity and quality of harvestable biomass from *Populus* short rotation coppice for solid fuel use – a review of the physiological basis and management influences. *Biomass Bioenerg.* 24, 411–427.
- Kruger, B.M., Manion, P.D., 1994. Antifungal compounds in aspen: effect of water stress. *Can. J. Bot.* 72, 454–460.
- Liira, J., Zobel, K., Mägi, R., Molenberghs, G., 2002. Vertical structure of herbaceous canopies: the importance of plant growth-form and species-specific traits. *Plant Ecol.* 163, 123–134.
- Lindroth, R.L., 2001. Adaptations of quaking aspen for defense against damage by herbivores and related environmental agents. pp. 273–284. In: *Sustaining Aspen in Western Landscapes: Symposium Proceedings; 13–15 June 2000; Grand Junction, CO.U.S.D.A. Forest Service, Rocky Mountain Experiment Station. Proceedings RMRS-P-18. Fort Collins, CO.*
- Lindroth, R.L., Hwang, S.Y., 1996. Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). *Biochem. Syst. Ecol.* 24, 357–364.
- Lindroth, R.L., Kinney, K.K., Platz, C.L., 1993. Responses of deciduous trees to elevated atmospheric CO₂: productivity, phytochemistry and insect performance. *Ecology* 74, 763–777.
- Madritch, M.D., Donaldson, J.R., Lindroth, R.L., 2006. Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems* 9, 528–537.
- Miranda, M., Ralph, S.G., Mellway, R., White, R., Heath, M.C., Bohlmann, J., Constabel, C.P., 2007. The transcriptional response of hybrid poplar (*Populus trichocarpa* × *P. deltoides*) to infection by *Melampsora medusae* leaf rust involves induction of flavonoid pathway genes leading to the accumulation of proanthocyanidins. *Mol. Plant-Microbe Interact.* 20, 816–831.
- Mitton, J.B., Grant, M.C., 1996. Genetic variation and the natural history of quaking aspen. *Bioscience* 46, 25–31.
- Müller, M.S., McWilliams, S.R., Podlesak, D., Donaldson, J.R., Bothwell, H.M., Lindroth, R.L., 2006. Tri-trophic effects of plant defenses: chickadees consume caterpillars based on host leaf chemistry. *Oikos* 114, 507–517.
- Newcombe, G., van Oosten, C., 1997. Variation in resistance to *Venturia populina*, the cause of poplar leaf and shoot blight in the Pacific Northwest. *Can. J. For. Res.* 27, 883–889.
- Osier, T.L., Lindroth, R.L., 2001. Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *J. Chem. Ecol.* 27, 1289–1313.
- Osier, T.L., Lindroth, R.L., 2004. Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. *Oecologia* 139, 55–65.
- Osier, T.L., Lindroth, R.L., 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia* 148, 293–303.
- Pastor, J., Naiman, R.J., 1992. Selective foraging and ecosystem processes in boreal forests. *Am. Nat.* 139, 690–705.
- Perala, D.A., 1990. *Populus tremuloides* Michx. Quaking aspen. In: Burns, R.M., Honkala, B.H. (Eds.), *Hardwoods. Silvics of North America, vol. 2. Forest Service, United States Department of Agriculture, Washington, D.C.* pp. 555–569.
- Porter, L.J., Hrstich, L.N., L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25223–25230.
- Przybyl, K., 1984. Pathological changes and defense responses in poplar tissues caused by *Ceratocystis fimbriata*. *Forest Pathol.* 14, 183–191.

- Romme, W.H., Turner, M.G., Wallace, L.L., 1995. Aspen, elk, and fire in northern Yellowstone National Park. *Ecology* 76, 2097–2106.
- Schweitzer, J.A., Madritch, M.D., Bailey, J.K., LeRoy, C.J., Fischer, D.G., Rehill, B.J., Lindroth, R.L., Hagerman, A.E., Wooley, S.C., Hart, S.C., Whitham, T.G., 2008. From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. *Ecosystems* 11, 1005–1020.
- Sinclair, W.A., Lyon, H.H., 2005. *Diseases of Trees and Shrubs*, second ed. Cornell University Press, Ithaca, New York.
- Sivanesan, A., 1974. *Venturia macularis*. *Descriptions of Pathogenic Fungi and Bacteria*, vol. 403. Commonwealth Mycological Inst., Kew, Surrey, England, pp. 1–2.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry*, third ed. W.H. Freeman and Company, USA.
- Stevens, M.T., Lindroth, R.L., 2005. Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). *Oecologia* 145, 298–306.
- Tuskan, G.A., DiFazio, S., Jansson, S., et al., 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313, 1596–1604.
- Webb, S.L., 1989. Contrasting windstorm consequences in two forests, Itasca State Park, Minnesota. *Ecology* 70, 1167–1180.
- Witzell, J., Martin, J.A., 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens—past experiences and future prospects. *Can. J. For. Res.* 38, 2711–2727.
- Wooley, S.C., Walker, S., Vernon, J., Lindroth, R.L., 2008. Aspen decline, aspen chemistry, and elk herbivory: are they linked? *Rangelands* 30, 17–21.
- Worrall, J.J., Egeland, L., Eager, T., Mask, R.A., Johnson, E.W., Kemp, P.A., Shepperd, W.D., 2008. Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. *For. Ecol. Manag.* 255, 686–696.