Host plant genetics affect hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection

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Abstract: Recent studies have shown effects of host plant genetics on community and ecosystem processes, which makes understanding the impacts of genetically based traits on hidden or non-apparent organisms more important. Here we examined links among genetic variation in hybrid cottonwoods, plant phytochemistry, and twig fungal endophytes (i.e., a common hidden organism). We found three major patterns: (1) twig fungal endophyte infection was positively related to the introgression of Fremont cottonwood (*Populus fremontii* S. Wats.) RFLP genetic markers, (2) condensed tannin concentration in twig bark tissue was negatively correlated to the introgression of Fremont genetic markers, and (3) fungal endophyte infection was negatively related to condensed tannin concentration in twig bark. These data demonstrate that plant genotype can impact hidden ecological players (i.e., fungal endophytes) resulting in community and ecosystem consequences.

Key words: ecological genetics, fungal endophytes, hidden players, hybridization, *Populus*, tannins.

Résumé : Comme de récentes études ont démontré les effets de la génétique de la plante hôte sur les processus communautaires et écosystémiques, la compréhension des impacts de caractères basés génétiquement sur des organismes cachés ou non apparents devient plus importante. Les auteurs examinent, chez des hybrides de peupliers deltoïdes, les liens entre la variation génétique, la phytochimie, et les endophytes fongiques (c.à.d., organismes généralement cachés). Ils retrouvent trois patrons principaux: (1) l'infection par des champignons endophytes des rameaux est positivement reliée à l'introgression de marqueurs génétiques RFLP du peuplier de Frémont (*Populus fremontii* S. Wats.); (2) la teneur en tannins condensés dans les tissus de l'écorce des rameaux est négativement corrélée avec l'introgression de marqueurs génétiques Frémont, et (3) l'infection par les endophytes est négativement reliée à la teneur en tannins condensés dans l'écorce des rameaux. Ces données montrent que le génotype du plant peut avoir des impacts sur des acteurs écologiques cachés (c.à.d., des endophytes fongiques) susceptibles d'affecter les communautés et les écosystèmes.

Mots clés : génétique écologique, endophytes fongiques, acteurs cachés, hybridation, *Populus*, tannins.

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Introduction

The roles of hidden players (i.e., species or organisms, such as fungal endophytes, whose direct observation is problematic) in communities and ecosystems are only beginning to be understood (Clay and Holah 1999; Arnold et al. 2003). Understanding the factors that affect these hidden players and the roles they play in community assembly and ecosys-

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tem processes is a major frontier in ecology (Thompson et al. 2001). To address the potential genetic basis of a plant–endophyte interaction, in the present study, we utilized a well-studied cottonwood system in which the introgression of genes from Fremont cottonwood (*Populus fremontii* S. Wats.) into narrowleaf cottonwood (*Populus angustifolia* James) results in a very predictable change in the concentration of condensed tannins (Schweitzer et al. 2004; Bailey et al. 2004), which are known to inhibit many fungi (Gallardo and Merino 1992; Grime et al. 1996; Heal et al. 1997; Driebe and Whitham 2000; Schweitzer et al. 2004). Thus, we sought to understand how genetic-based variation in a major plant defense might affect the interactions between a dominant tree and a hidden player.

Fungal endophytes inhabit the aerial parts of plants without producing symptoms of disease and are present across the taxonomic range of vascular plants. The effects of symbiotic endophytic fungi represent a continuum from parasitic to neutral to mutalistic interactions (Saikkonen et al. 1998). They have been shown to deter herbivory (Clay 1996; Clay and Schardl 2002), increase drought tolerance (Elmi and

West 1995), improve nutrient uptake (Malinowski et al. 2000), and alter ecosystem processes (Rudgers et al. 2004). Of central importance in the regulation of endophyte activity is host phytochemistry. The manipulation of the endosymbiont's chemical environment by the plant is an important mechanism for controlling the degree of endophyte activity within the host (Espinosa-Garcia et al. 1996) and the quality of the symbiosis (Schulz et al. 1999).

In contrast to the paradigm that the effects of genes or specific genetically based plant traits become more diffuse across higher levels of organization (i.e., population, community, and ecosystems), recent studies have shown that specific plant traits can have pronounced direct and indirect effects that extend beyond the individual or population to affect the dependent community and ecosystem processes (Madritch and Hunter 2002), that is, have extended phenotypes (Whitham et al. 2003; Bailey et al. 2004; Schweitzer et al. 2004). For example, in a cottonwood hybridizing complex (*P. angustifolia* × *P. fremontii*), condensed tannins are under strong genetic control, affect the foraging of beavers (Bailey et al. 2004), and explain approximately 60% of the variation in net N-mineralization rates, an ecosystem-level process (Schweitzer et al. 2004). In other studies, genes of large effect influence traits such as plant architecture (Bradshaw and Stettler 1995), phenology (Frewen et al. 2000), and the number of queen ants tolerated by workers (Krieger and Ross 2000); they also determine whether a fungus is a pathogen or a mutualist (Freeman and Rodriguez 1993). These traits are especially important when they impact dominant or keystone species because, as community and ecosystem drivers, the extended phenotypes of genes of large effect are likely to be pronounced (Bailey et al. 2004).

Using observational and experimental approaches, we examined how symbiotic fungal endophyte infection was related to the genetic variation among pure and hybrid cottonwoods. Specifically we addressed three main questions: (1) does fungal endophyte infection differ among pure species and their hybrids? (2) Is fungal endophyte infection related to host plant phytochemistry? (3) Is fungal endophyte infection related to introgression of Fremont cottonwood alleles related to fungal endophyte infection?

Materials and methods

Observational and experimental surveys
Cottonwoods are the dominant species of western riparian forests (Rood and Mahoney 1990). Along the Weber River, Utah, the ranges of Fremont cottonwood (*P. fremontii*) and narrowleaf cottonwood (*P. angustifolia*) overlap in a 13-km zone where the two species readily hybridize and introgress unidirectionally (Keim et al. 1989; Martinsen et al. 2001). In the field, F₁ hybrids cross only with narrowleaf cottonwoods to form backcross hybrids. Because of this unidirectional genetic introgression, hybridization represents a genetic continuum between Fremont and narrowleaf cottonwoods.

Preliminary studies in this system indicated that endophytic fungi are more abundant and diverse in bark tissue than in leaf tissue. We quantified fungal endophyte infection of 35 cottonwood trees from the field at three sites in the hybrid zone in November 2002. We randomly selected 15 Fremont, 5 F₁, and 15 backcross/narrowleaf trees from

these sites. F₁ hybrids are rare in the hybrid zone and therefore we collected fewer of these tree types. From these randomly selected field trees, we collected twigs to assay for fungal endophyte infection. Because fungal infection level increases linearly with age (Bernstein and Carroll 1977; Stone 1987), we collected a single 7- to 10-cm twig sample from each tree from a terminal branch standardized by height of branch insertion on the main trunk.

To experimentally test the potential genetic components of our observational patterns, and remove the effects of environmental variation, we collected twig samples from a 12-year-old common garden with randomly distributed genotypes established in Ogden, Utah. We collected 2-year-old twig samples from 20 genotypes within the common garden (i.e., 5 from each species and tree type: Fremont, F1 hybrid, backcross, and narrowleaf) in which the genetic composition of the trees had been determined using RFLP molecular techniques with 35 species-specific alleles (Martinsen et al. 2001). The introgression of species-specific Fremont alleles (owing to unidirectional introgression) allows us to determine plant genotype and level of introgression. The twig samples were collected using the same methods as were used in the observational studies.

The twig samples from field and common garden trees were assayed for fungal endophyte infection. Collected samples were stored in cool conditions until they were returned to the lab where they were processed within 24 h. From the 7- to 10-cm twig sample, three 1-cm-long subsamples were taken from below the 3-year old terminal bud scar, that is, from 2-year-old growth. This ensured that all samples had equal temporal exposure to endophyte inoculum. To prevent contamination, isolations were performed aseptically in a laminar flow hood, where twigs were surface-sterilized by a 5-min immersion in 70% ethanol with vigorous agitation for 1 min., and a 5-min immersion in 50% commercial bleach, followed by several rinses in sterile distilled water. After sterilization, twigs were incubated in sterile Petri plates on potato dextrose agar (Difco). Plates were examined every second day under a dissecting microscope (Deckert et al. 2001). This made it possible to confirm endophytic hyphal growth as it emerged and distinguish it from the hyphae of contaminating fungi. Endophytic growth was characterized as present or absent after 1 month of incubation at room temperature. Because we were interested in the general response of fungal endophytes to host plant genetics and phytochemistry, fungal endophytes were not identified to species or morphotype. The fungi that emerged followed the normally observed pattern of endophyte assemblages: domination by a few abundant morphospecies with numerous isolations of many rare morphospecies. The dominant morphospecies were sterile and therefore difficult to identify by traditional methods. Identification of the isolates was not deemed crucial to fulfill the objectives of this study because (1) our interest was in the generalized effect of cottonwood hybridization on fungal endophytes, (2) fungal assemblages were potentially different at each site, and (3) common garden sampling controlled for assemblage differences.

Chemical analysis
To determine a potential mechanism for differences in fungal infection, we quantified concentrations of the princi-

pal secondary metabolites (phenolic glycosides and condensed tannin; Zucker 1982; Palo 1984; Lindroth and Hwang 1996) in the same samples that were assayed for fungal endophytes in the common garden. Twigs were collected on dry ice, lyophilized, and ground in a Wiley Mill until fine enough to pass through a 40-mesh screen. The phenolic glycosides salicortin and HCH-salicortin were quantified using high performance thin layer chromatography (Lindroth et al. 1993), using purified salicortin and HCH-salicortin as standards. Condensed tannin levels were measured on 70% acetone extracts of the twig samples using the butanol–HCl method of Porter et al. (1986). Narrowleaf cottonwood condensed tannins purified by the method of Hagerman and Butler (1980) were used as the reference standard.

Statistics

For the observational field data, we used a χ^2 test to compare the frequency of fungal endophyte infection among genotypes. Backcross hybrids and narrowleaf cottonwoods are indistinguishable in the field and therefore were pooled for analysis. Logistic regression was used for analysis of relationships among continuous and categorical variables, such as introgression or phytochemistry, against probability of infection. Simple linear regressions were used for analysis of relationships between introgression and phytochemistry data. All data used in the statistical analysis were transformed such that there was no significant lack of fit, no autocorrelation among residuals (Durbin–Watson test), and no inequality of variances based on the distribution of residuals. The figures show actual non-transformed data.

Results

Observations in the wild

Our observations within the Weber River hybrid zone showed that Fremont cottonwoods had significantly greater fungal infection than F_1 and backcross/narrowleaf cottonwoods based on an equal expectation among tree types. In the field, symbiotic fungal endophytes infected 43% more Fremont cottonwoods than expected. In contrast, F_1 hybrids and backcross/narrowleaf cottonwoods had 95% and 74% lower infection than expected (Fig. 1; $n = 35$ trees, df = 2, $\chi^2 = 5.7$, $p < 0.05$). Rather than any single species of fungal endophyte being affected, these observational data suggested that plant traits related to cottonwood hybridization might have a generalized effect on fungal endophytes. These observational data suggest that genetic factors related to plant hybridization are in part responsible for these patterns. To experimentally test our findings in the wild, we repeated our studies in a common garden where we could largely eliminate environmental effects to study the potential genetic impacts of plant hybridization on endophytes.

Common garden studies

In the common garden we confirmed our observations in the wild that Fremont cottonwoods had the greatest endophyte infection. With the RFLP data we were able to trace the introgression of Fremont cottonwood molecular markers where we found a significant positive correlation with fungal endophyte infection (Fig. 2A; $n = 20$ genotypes (5 from each crosstype), df = 1, $r^2 = 0.54$, $\chi^2 = 12.22$, $\beta = 6.30$, $\rho < 0.05$).

Fig. 1. Fungal endophytes infect pure and hybrid cottonwoods differently. In the wild, endophyte infection is greater than expected on Fremont cottonwoods and less than expected on narrowleaf cottonwoods and their naturally occurring hybrids. Two-year-old cottonwood twig samples were collected at three sites along a 13-km reach of the Weber River. Overall, Fremont cottonwoods had 43% greater infection than expected, while F1's and backcross narrowleaf cottonwoods had 95% and 74% less infection than expected, respectively. These observational data suggest a genetic basis to fungal endophyte infection.
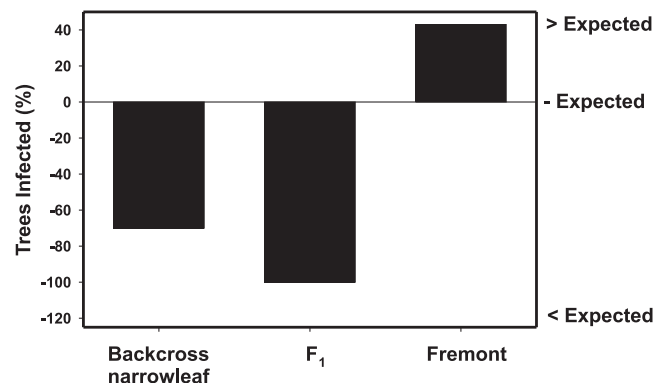
Very few narrowleaf cottonwoods were infected, whereas 80% of Fremont cottonwoods were infected.
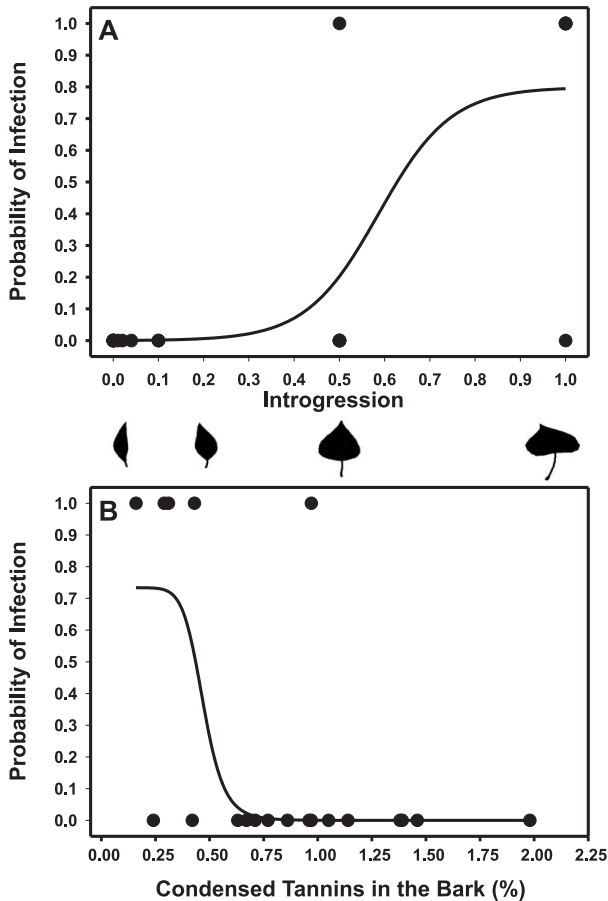
We found that fungal endophyte infection was negatively correlated with the concentration of condensed tannins (Fig. 2B; df = 1, $r^2 = 0.54$, $\chi^2 = 6.91$, $\beta = -4.4$, $\rho < 0.05$). Fungal endophyte infection showed no significant relationship to salicortin, HCH-salicortin, or total phenolic glycosides (salicortin + HCH-salicortin) ($\chi^2 = 0.23$, $p = 0.63$; $\chi^2 = 0.0$, $p = 1.0$, $\chi^2 = 0.23$; $p < 0.63$, respectively). Because this study was conducted in the common garden where all pure and hybrid cross types are present, and because the age of the tissue sampled was controlled, we were able to specifically address the links among plant genotype, secondary chemistry, and a generalized response of fungal endophytes. Overall, our results showed that fungal endophyte infection is related to condensed tannin concentration in the bark tissue of cottonwoods.

As fungal endophyte infection was negatively related to the concentration of condensed tannins in the bark and positively related to the proportion of Fremont markers, we predicted that tannin concentrations of individual genotypes would be negatively correlated with the proportion of Fremont markers contained in those trees. Using the same genotypes from the common garden, we regressed the concentration of condensed tannins against the proportion of Fremont markers and found a significant relationship (Fig. 3; df = 1,19, $r^2 = 0.78$, $F = 61.58$, $p < 0.05$), demonstrating that hybridization and introgression affect ecologically functional traits and interactions between species.
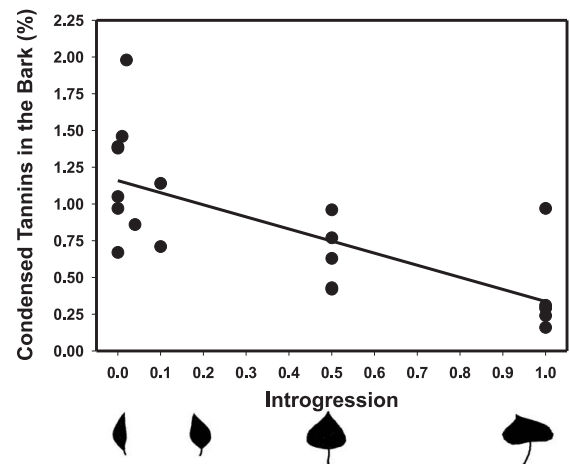
Discussion

Overall, these data linked host plant genetics, condensed tannins, and symbiotic fungal endophyte infection. While our studies did not focus on any single fungal endophyte species, our studies quantified the general relationship between host plant genetics, phytochemistry, and fungal

Fig. 2. Cottonwood genetics are related to fungal endophyte infection. (A) In a common garden with trees of known genotype, genetic introgression of Fremont markers was significantly correlated with fungal endophyte infection. There was a greater probability of fungal endophyte infection in tree genotypes high in Fremont markers. (B) Using the same genotypes from the common garden, the probability of fungal endophyte infection was negatively correlated to condensed tannin concentration in the bark.

Fig. 3. A strong relationship exists between introgression of Fremont cottonwood markers and condensed tannin concentration in the bark of cottonwood genotypes (Log(tannins) = 0.1654 – 1.5243 (introgression)). Overall, these data linked host plant genetics, condensed tannins, and fungal endophyte infection.

endophyte infection. Our findings showed that genetic variation in a dominant plant can have a general effect on fungal endophytes. For example, as introgression of Fremont alleles decreased through unidirectional backcrossing, the probability of infection declined. These data demonstrated that hybridization is an important mechanism for the transmission of genetically based and ecologically functional traits that influence the presence of other organisms, including nonapparent groups such as endophytes. Introgression of Fremont cottonwood markers was strongly and negatively related to the concentration of condensed tannins and positively related to endophytes, indicating that these compounds may be a chemical mechanism that inhibits fungal colonization in cottonwoods. Because linkage disequilibrium among genes from the two hybridizing species is likely to be strong, one could argue that the percentage of condensed tannins in the bark will be correlated with all the other traits by which the parent species differ, thus endophyte infection cannot be ascribed to any one of these traits. Interestingly, nitrogen, salicortin, HCH-salicortin, and condensed tannins

all differ among the parent species and their hybrids (Schweitzer et al. 2004) and only condensed tannins were related to the probability of fungal endophyte infection, suggesting that tannins were a mechanistic link.

In combination with other studies documenting the effects of condensed tannins on beaver herbivory (Bailey et al. 2004), fine root production (Fischer et al. unpublished data), and the availability of soil nutrients (Schweitzer et al. 2004), this study demonstrates the general importance of condensed tannins. Plant polyphenols, such as condensed tannins, are a class of secondary compounds that are involved in plant pigmentation, ultraviolet protection, and allelopathy, as well as a deterrent to herbivores and pathogens (Zucker 1982; Palo 1984; Hemingway and Karchesy 1989; Ossipov et al. 2001). They have been shown to retard leaf litter decomposition by reducing palatability to arthropods and microbes and by forming protein-binding complexes (Gallardo and Merino 1992; Grime et al. 1996; Heal et al. 1997; Driebe and Whitham 2000; Schweitzer et al. 2004). These processes and their reputed ability to inhibit net nitrification have led researchers to believe that they can regulate plant available nutrient pools (Rice and Pancholy 1973; Basaraba and Starkey 1966; Northup et al. 1998; Hättenschwiler and Vitousek 2000; Kraus et al. 2003). The multiple functions of condensed tannins indicate that their production may be adaptive under many circumstances (Kraus et al. 2003).

We are aware of only three studies that specifically examined the role of condensed tannins on microorganisms, none of which examined endophytes specifically. In reviews of the anti-microbial properties of condensed tannins, both Scalbert (1991) and Field and Lettinga (1992) found that multiple genera of microorganisms, including fungi, yeasts, and bacteria, were susceptible to the toxicity of condensed tannins, some of which were completely inhibited by their presence. More recently, Bending and Read (1996) found that while ericoid fungi are able to degrade polyphenols, ectomycorrhizal fungi are largely inhibited by tannins as a result of the inactivation of cell wall enzymes. The negative association between bark chemistry and fungal endophytes

that we observed reveals another group of microorganisms that are inhibited or influenced by the concentration of condensed tannins.

Although there is much that we do not understand about fungal endophytes and their interactions with plant genetics (Saikkonen et al. 1998; Arnold et al. 2003), we believe this study provides an example of the potential impacts of genetic introgression on clandestine organisms (i.e., hidden players; Thompson et al. 2002) such as fungal endophytes. Depending upon the relative importance of these hidden players, we expect these effects could be magnified throughout the community and ecosystem. For example, plant resistance traits to the gall aphid, *Pemphigus betae*, do not just affect this specific aphid, they affect a much larger community of organisms, ranging from microbes to vertebrates. Because galling aphids provide food and shelter for many other organisms, their presence or absence has a ripple effect on the community (Dickson and Whitham 1996). Genetically modified organisms could have similar unexpected effects. For example, Howard et al. (2004) showed that male medaka fish, genetically modified with salmon growth hormone, possess "overwhelming mating advantage" relative to male medaka fish without this "Trojan" gene, which could lead to the population extinction of unmodified medaka fish. Clearly, there can be large consequences for genetically modified species even if it is only a single trait, such as growth hormone or plant chemistry, that is being modified.

References

Arnold, A.E., Mehia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., and Herre, E.A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. U.S.A. **100**: 15649–15654.

Bailey, J.K., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Martinsen, G.D., and Whitham, T.G. 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. Ecology, **85**: 603–608.

Basaraba, J., and Starkey, R.L. 1966. Effect of plant tannins on decomposition of organic substances. Soil Sci. **101**: 17–23.

Bending, G.D., and Read, D.J. 1996. Effects of soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. Soil Biol. Biochem. **28**: 1595–1602.

Bernstein, M.E., and Carroll, G.C. 1977. Internal fungi in old-growth Douglas-fir foliage. Can. J. Bot. **55**: 644–653.

Bradshaw, H.D., Jr., and Stettler, R.F. 1995. Molecular genetics of growth and development in *Populus*. IV. Mapping QTL's with large effects on growth, form, and phenology traits in a forest tree. Genetics, **139**: 963–973.

Clay, K. 1996. Interactions among fungal endophytes, grasses, and herbivores. Res. Popul. Ecol. **38**: 191–201.

Clay, K., and Holah, J. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science (Washington, DC), **285**: 1742–1743.

Clay, K., and Schardl, C. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. **160**: S99–S127.

Deckert, R.J., Mellville, L.H., and Peterson, R.L. 2001. Structural features of a *Lophodermium* endophyte during the cryptic life-cycle phase in the foliage of *Pinus strobus*. Mycol. Res. **105**: 991–997.

Dickson, L.L., and Whitham, T.G. 1996. Genetically-based plant resistance traits affect arthropods, fungi, and birds. Oecologia, **106**: 400–406.

Driebe, E.M., and Whitham, T.G. 2000. Cottonwood hybridization affects tannin and nitrogen of leaf litter and alters decomposition. Oecologia, **123**: 99–107.

Elmi, A.A., and West, C. P. 1995. Endophyte infection effects on stomatal conductance, osmotic adjustment, and drought recovery of tall fescue. New Phytol. **131**: 61–67.

Espinosa-Garcia, F.J., Rollinger, J.L., and Langenheim, J.H. 1996. Coastal redwood leaf endophytes: their occurrence, interactions and response to host volatile terpenoids. *In* Endophytic fungi in grasses and woody plants: systematics, ecology, end evolution. *Edited by* S.C. Redlin and L.M. Carris. APS Press, St. Paul, Minn. pp. 101–120.

Field, J.A., and Lettinga, G. 1992. Toxicity of tannic compounds to microorganisms. *In* Plant polyphenols, synthesis, properties, significance. *Edited by* R.W. Hemingway and P.E. Laks. Plenum Press, New York. pp. 673–692.

Freeman, S., and Rodriguez, R.J. 1993. Genetic conversion of a fungal pathogen to a non-pathogenic endohytic mutualist. Science (Washington, DC), **260**: 75–78.

Frewen, B.E., Chen, T.H.H., Howe, G.T., Davis, J., Rhode, A., Boerjan, W., and Bradshaw, H.D., Jr. 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics, **154**: 837–845.

Gallardo, A., and Merino, J. 1992. Nitrogen immobilization in leaf litter at two mediterranean ecosystems of SW Spain. Biogeochemistry, **15**: 213–228.

Grime, J.P., Cornelissen, J.H.C., Thompson, K., and Hodgson, J.G. 1996. Evidence of a causal connection between anti-herbivore defense and the decomposition rate of leaves. Oikos, **77**: 489–494.

Hagerman, A.E., and Butler, L.G. 1980. Condensed tannin purification and characterization of tannin-associated protein. J. Agric. Food Chem. **28**: 947–952.

Hättenschwiler, S., and Vitousek, P.M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. **15**: 238–243.

Heal, O.W., Anderson, J.M., and Swift, M.J. 1997. Plant litter quality and decomposition: an historical overview. *In* Driven by nature. Plant litter quality and decomposition. *Edited by* G. Cadisch and K.E. Giller. Cab International, Wallingford, UK. pp. 3–32.

Hemingway, R.W., and Karchesy, J.J. 1989. Chemistry and significance of condensed tannins. Plenum Press. New York.

Howard, R.D., DeWoody, J.A., and Muir, W.M. 2004. Transgenic male mating advantage provides opportunity for Trojan gene effect in fish. Proc. Natl. Acad. Sci. U.S.A. **101**: 2934–2938.

Keim, P., Paige, K.N., Whitham T.G., and Lark, K.G. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*:

occurrence of unidirectional introgression. Genetics, **123**: 557–565.

Kraus, T.E.C., Dahlgren, R.A., and Zasoski, R.J. 2003. Tannins and nutrient dynamics of forest ecosystems – a review. Plant Soil, **256**: 41–66.

Krieger, M.J.B., and Ross, K.G. 2002. Identification of a major gene regulating complex social behavior. Science (Washington, DC), **295**: 328–332.

Lindroth, R.L., and Hwang, S-Y. 1996. Diversity, redundancy and multiplicity in chemical defense systems of aspen. Recent Adv. Phytochem. **33**: 25–56.

Lindroth, R.L., Kinney, K.K., and Platz, C.L. 1993. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry and insect performance. Ecology, **74**: 763–777.

Madritch, M.D., and Hunter, M.D. 2002. Phenotypic diversity influences ecosystem functioning in an oak sandhills community. Ecology, **83**: 2084–2090.

Malinowski, D.P., Alloush, G.A., and Belesky, D.P. 2000. Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. Plant Soil, **227**: 115–126.

Martinsen, G.D., Whitham, T.G., Turek, R.J., and Keim, P. 2001. Hybrid populations selectively filter gene introgression between species. Evolution, **55**: 1325–1335.

Northup, R.R., Dahlgren, R.A., and McColl, J.G. 1998. Polyphenols as regulators of plant-litter-soil interactions in Northern California's pygmy forest: a positive feedback? Biogeochemistry, **42**: 189–220.

Ossipov, V., Haukioja, E., Ossipova, S., Hanhimäki, S., and Pihlaja, K. 2001. Phenolic and phenolic-related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. Biochem. Syst. Ecol. **29**: 223–240.

Palo, R.T. 1984. Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. J. Chem. Ecol. **10**: 499–520.

Porter, L.J., Hrstich, L.N., and Chan, B.G. 1986. The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin. Phytochemistry, **25**: 223–230.

Rice, E.L., and Pancholy, S.K. 1973. Inhibition of nitrification by climax ecosystems. II. Additional evidence and possible role of tannins. Am. J. Bot. **60**: 691–702.

Rood, S.B., and J.H. Mahoney. 1990. Collapse of riparian poplar forests downstream from dams in western prairies: probable causes and prospects for mitigation. Environ. Manag. **14**: 451–464.

Rudgers, J.A., Koslow, J.M., and Clay, K. 2004. Endophytic fungi alter relationships between diversity and ecosystem properties. Ecol. Lett. **7**: 42–51.

Saikkonen, K., Faeth, S.H., Helander, M., and Sullivan, T.J. 1998. Fungal endophytes. A continuum of interactions with host plants. Ann. Rev. Ecol. Syst. **29**: 319–343.

Schulz, B., Rommert, A.K., Dammann, U., Aust, H.J., and Strack, D. 1999. The endophyte-host interaction: a balanced antagonism? Mycol. Res. **103**: 1275–1283.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Martinsen, G. D., Hart, S.C., Lindroth, R.L., Keim, P., and Whitham, T.G. 2004. Genetically based trait in a dominant tree affects ecosystem processes. Ecol. Lett. **7**: 127–134.

Scalbert, A. 1991. Anti-microbial properties of tannins. Phytochemistry, **30**: 3875–3883.

Stone, J.K. 1987. Initiation and development of latent infections by *Rhabdocline parkeri* on Douglas-fir. Can. J. Bot. **65**: 2614–2621.

Thompson, J.N., Reichman, O.J., Morin, P.J., Polis, G.A., Power, M.E., Sterner, R.W., Couch, C.A., Gough, L., Holt, R., Hooper, D.U., Keesing, F., Lovell, C.R., Milne, B.T., Molles, M.C., Roberts, D.W., and Strauss, S.Y. 2001. Trends Ecol. Evol. **51**: 15–24.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M., Wimp, G.M., Fischer, D.G., Bailey, J.K., Lindroth, R.L., Woolbright, S., and Kuske, C.R. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. Ecology, **84**: 559–573.

Zucker, W.V. 1982. How aphids choose leaves: the role of phenolics in host selection by a galling aphid. Ecology, **63**: 972–981.