Effects of soil type, fertilization and drought on carbon allocation to root growth and partitioning between secondary metabolism and ectomycorrhizae of *Betula papyrifera*

NATHAN M. KLECZEWSKI,1,3,4 DANIEL A. HERMS2 and PIERLUIGI BONELLO1

1 Department of Plant Pathology, The Ohio State University, 201 Coffey Road, Columbus, OH 43210, USA
2 Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691, USA
3 Corresponding author (nkleczew@indiana.edu)
4 Present address: Department of Biology, Indiana University, 1001 East 3rd Street, 147 Jordan Hall, Bloomington, IN 47405, USA

Summary Paper birch (*Betula papyrifera* Marsh) seedlings were grown in a greenhouse in either subsoil or topsoil in factorial combination with two fertilization and drought regimes to investigate how different soil environments and nutrient availability drive belowground partitioning between growth, secondary metabolism and ectomycorrhizal (EM) associations, and impact drought tolerance of paper birch. Root and total seedling dry biomass, starch, soluble sugars, soluble phenolics, lignin and EM abundance were quantified. In unfertilized topsoil, total plant biomass and root biomass were approximately nine times higher than in unfertilized subsoil, but the root weight ratios did not differ between soils. Root soluble phenolics and lignin were higher in unfertilized subsoil than in unfertilized topsoil, whereas EM abundance was significantly higher in unfertilized topsoil than in unfertilized subsoil. In topsoil, fertilization decreased root biomass and EM abundance and increased root phenolics and lignin. In contrast, fertilization of subsoil increased root biomass but decreased root phenolics and lignin, while EM abundance was unaffected. In both soil types, fertilization reduced root weight ratios. Across soil types, EM abundance was negatively correlated with root soluble sugars, root phenolics and lignin, but this was driven mainly by the responses in the topsoil treatment. Our results show that soil fertility mediates carbon tradeoffs among defense, growth and EM associations.

Keywords: growth–differentiation balance hypothesis, lignin, mycorrhizae, optimal allocation, phenolics, phenotypic plasticity.

Introduction

In plants, fixed carbon is translocated to organs and modules (allocation), and divided among components within those modules (partitioning) (Dickson and Isebrands 1993). The ability of plants to regulate patterns of C allocation and partitioning to maximize the acquisition of limiting resources is a fundamental mechanism by which plants acclimate to environmental variation (Chapin 1991). For example, when soil resources such as water and nutrients are low, plants may divert more C belowground to augment root growth (Ingestad and Ågren 1991, Glynn et al. 2007) and facilitate associations with mycorrhizal fungi (Slankis 1974, Mudge et al. 1987, Davies et al. 1996). In resource-limited environments, carbon partitioning to constitutive secondary metabolism often increases, which enhances resistance to attack by herbivores as well as stress tolerance (Chapin 1991; Herms and Mattson 1992). Tradeoffs at the organ level (i.e., between above- and belowground sinks) as well as within organs (i.e., between primary and secondary metabolism) occur due to plant limitations in carbon acquisition and demands within individual sinks. While these tradeoffs have important implications for a plant’s ability to establish and tolerate environmental stress, surprisingly little is known about their regulation.

Several models have been proposed to explain how environmental variation may affect C allocation and/or partitioning (Stamp 2003). The optimal allocation model (Ingestad 1988, 1991) predicts that plants increase C allocation towards organs that increase the uptake of limiting resources in a given environment. For example, when soil nutrients limit basic physiological processes (e.g., photosynthesis, growth), optimal allocation theory predicts that a plant will increase its root weight ratio. Conversely, optimal allocation theory predicts that plants will respond to nutrient addition by decreasing their root weight ratios, and that below- and aboveground growth rates will eventually stabilize at levels optimal for a given environment (Hirose 1987, Ingestad and Ågren 1991, Shipley and Meziane 2002).

Plasticity in patterns of C allocation and partitioning also translate into variation in secondary metabolism (Glynn et al. 2007). The growth–differentiation balance hypothesis
GDBH proposes that tradeoffs between primary (growth) and secondary (defense/stress tolerance) metabolism emerge from developmental constraints in growing plant cells, and direct competition for resources between primary and secondary metabolism in mature cells (Herms and Mattson 1992). A parabolic response emerges because (i) net assimilation rate, the balance between carbon gain (photosynthesis) and loss (respiration, exudation, etc.), is less sensitive to resource limitation than is relative growth rate (Luxmoore 1991, Glynn et al. 2007); (ii) growth processes impose high resource demands relative to secondary metabolism; and (iii) growth diverts resources from secondary metabolism and vice versa (Chapin 1989). The GDBH predicts that when moderate resource limitation constrains relative growth rate to a greater degree than net assimilation rate, as in moderately nutrient-poor soils (B in Figure 1a), photosynthate accumulates in tissues and becomes available to support secondary metabolic processes, resulting in higher levels of constitutive secondary metabolites in tissues. In nutrient-rich soil, the high resource demands of rapid growth are predicted to limit secondary metabolism (C in Figure 1a). In extremely nutrient-limited environments, both growth and secondary metabolism are predicted to be constrained by energy and resource limitations (A in Figure 1a).

Ectomycorrhizal (EM) associations are the dominant mycorrhizal form for many temperate forest trees (Richards 1987). EM fungi benefit their hosts by increasing access to nutrients (Tobar et al. 1994, Ruiz-Lozano 2003, Caravaca et al. 2004, Hobbie and Colpaert 2004) and water (Mudge et al. 1987, Davies et al. 1996, Ruiz-Lozano 2003) as well as by decreasing the likelihood of infection by some soil-
borne pathogens (Marx and Davey 1967). However, these benefits come at the expense of a significant portion of a tree’s C budget, which is partitioned within roots to support the physiological needs of EM fungi (Smith and Read 1997, Nehls and Hampp 2000, Egerton-Warburton and Allen 2001, Nehls et al. 2007). An estimated 20–25% of net photosynthesis is allocated to roots and partitioned to EM fungi (Nehls 2008).

Growth of mycorrhizal fungi and the process of host colonization are sensitive to variation in soil nutrient content (Bruns 1995, Treseder and Allen 2002, Baxter and Dighton 2005), perhaps because of their dependence on host C. Treseder and Allen (2002) proposed a conceptual model that describes the growth responses of mycorrhizal fungi in relation to soil nutrient content (Figure 1b). As more N or P is available to the fungi but still limiting to the plant, the host allocates more C to roots and increases partitioning to its mycorrhizal symbionts, thus increasing mycorrhizal colonization (A in Figure 1b). Once plant growth is no longer constrained by N or P availability, mycorrhizal growth is reduced as the host partitions less C to support the mutualism (C in Figure 1b). Thus, mycorrhizal growth is predicted to be maximized under conditions of intermediate N or P availability, where nutrients are still limiting to plant growth (B in Figure 1b). Thus, mycorrhizae are expected to increase plant survival and fitness in nutrient-poor soils as fungal hyphae are more effective at accessing, and competing for, nutrients. The benefits of enhanced nutrient uptake are reduced in nutrient-rich soils, where the C costs of maintaining mycorrhizae increase even to the point that the symbiosis can become parasitic (Jones and Last 1991). Reduced C partitioning to mycorrhizae in nutrient-rich soils may assist in initial seedling growth, but also may make seedlings more prone to future stresses such as drought or pathogen attack.

Metabolic tradeoffs between growth and secondary metabolism/defense may also have important implications for the regulation of EM associations. Secondary metabolites such as soluble phenolics may be toxic to EM fungi (Javaid 2007) or limit host root colonization and penetration (Malajczuk et al. 1982, Weiss et al. 1999, Voigt et al. 2000). Lignin, a phenolic polymer, can limit penetration and ingress of fungal pathogens (Vance et al. 1980) and may have similar effects on mycorrhizal colonization, although the ability of EM fungi to degrade lignin, as a group, is still debated (Cairney and Burke 1998, Seppanen et al. 2007, Koide et al. 2008, Baldrian 2009).

The optimal allocation, GDBH, and Treseder and Allen models provide a useful framework to interpret patterns of C allocation and partitioning in plants. We examined how different soils and fertilization interact to affect patterns of C allocation and partitioning in the roots of paper birch seedlings and utilized these models to assist in our data interpretation, but did not seek to explicitly test these models. Drought was imposed on some seedlings at the end of the study to determine whether initial patterns of C allocation influenced drought tolerance. Specifically, we assessed patterns of biomass allocation and partitioning to C storage (starch and soluble sugars), root secondary metabolites (total soluble phenolics and lignin) and EM abundance.

Paper birch was selected because it is a pioneer tree species common to the Midwestern USA (deJong 1992), is highly plastic in response to nutrient availability (Ingestad 1982, Ingestad and Ågren 1991) and has well-characterized associations with EM fungi (Barter 1957, deJong 1992, Smith and Read 1997). Although data obtained from seedling experiments may not translate to adult trees, the survival of pioneer tree species such as paper birch is strongly influenced by soil conditions and mycorrhizal associations. In addition, in managed settings fertilizer and/or mycorrhizal fungi are added to soils and potting media in an attempt to increase outplanting success. Thus, understanding the mechanisms that influence seedling growth and stress tolerance can be useful both in an ecological context and in developing management practices that enhance seedling stress tolerance and growth with minimal chemical inputs.

The specific predictions tested were: (i) fertilization will reduce seedling root weight ratios and increase total seedling biomass; (ii) growth (biomass) will be negatively correlated with the concentration of secondary metabolites; (iii) fertilization will decrease EM abundance; and (iv) drought tolerance will be higher in seedlings with greater root weight ratios or EM abundance.

Materials and methods

Plant material

Paper birch seed (F.W. Schumacher Inc., Sandwich, MA) was stored in the dark at 4 °C for 2 months prior to germination. The seed was surface sterilized in 10% (v/v) chlorine bleach solution for 60 s, followed by rinsing in distilled H2O. Seeds were sown in flats containing autoclaved, water-saturated AgSorb (Oil Dri Inc., Chicago, IL) and placed in a light bank. Light banks were equipped with eight 40-W cool fluorescent bulbs (16/8 photoperiod), which provided 500 μmol photons m−2 s−1 at the soil surface. Room temperature ranged from 20 to 23 °C, and flats were watered twice a week to maintain adequate soil moisture. Seedlings were transferred to 1.9-l pots once they had produced two true leaves, which occurred ~4 weeks after sowing.

Soil processing and assessment of initial nutrient and EM status

Soils were obtained from the Ohio Agricultural Research and Development Center (OARDC, Wooster, OH; 40°81’ N, 81° 94’ W). Subsoil was obtained from a pipeline construction site at a depth of 3 m and stored in a bulk pile outdoors for 3–4 months until its incorporation in this experiment. Topsoil was obtained from a nearby agricultural field and was also stored in bulk as described for subsoil. Soil samples were analyzed at the STAR lab at OARDC (http://oarcd.osu.edu/star-
lab/default.asp) to quantify soil pH (Thomas 1996), and 2 M KCl-extracted soil samples were used to determine inorganic nitrate nitrogen (Mulvaney 1996, Gelderman and Beegle 1998) and ammonia nitrogen (Mulvaney 1996). Inorganic, available phosphorus (weak Bray) was quantified following PI extraction (Kuo 1996, Frank et al. 1998). Exchangeable potassium, magnesium and calcium were quantified following ammonium acetate extraction (Helmke and Sparks 1996, Warncke and Brown 1998), while organic matter content was calculated as loss on ignition (Combs and Nathan 1998).

The OARDC is surrounded by fragments of mixed hardwood forest typical of the eastern USA. Consequently, immigration of EM propagules from the surrounding area into the soil storage sites was expected (Baar et al. 1999). However, since the topsoil contained more than twice the organic matter found in the subsoil (Table 1) and originated from the upper portions of a former agricultural field, we assumed that these soils supported different communities and abundances of EM fungi. We tested this assumption prior to starting the study by characterizing the EM propagule community in the two soils. Propagule density was measured by baiting the soils with newly germinated paper birch seedlings, followed by calculation of the most probable number (Porter 1979). Briefly, soils were collected and separated into eight subsamples of ~1 l of soil. Soils were diluted in a 10-fold series with sterile AgSorb [100, 10, 1, 0.1% (v/v) soil]. Four hundred milliliters of this soil was then placed into 500-ml pots. A single, aseptically germinated 1-month-old paper birch seedling was planted at the center of each pot. Five seedlings per dilution level were placed in the light bank described above and harvested after 3 months. Roots were analyzed for the presence and number of EM tips, followed by morphological and molecular characterization as described below.

**Experimental design**

Two-leaf-stage birch seedlings were transferred to 3.79-l pots containing either air-dried subsoil or topsoil on Day 1 and moved to the greenhouse, which was supplemented with high intensity discharge lighting set to deliver 450 µmol photons m$^{-2}$ s$^{-1}$ at canopy level on a 16/8 photoperiod. Greenhouse temperature was maintained at 22 °C. Pots were watered manually to capacity by drenching with 300 ml H$_2$O daily on Days 4–8 and again on Days 65–69. Moreover, 0.65 g of granular 30:10:7 NPK (Arbor Green; Davey Tree, Kent, OH) in 500 ml H$_2$O daily on Days 1–3, 35–39, 45–49, 54–57, 64–68, and 73–77. Drought events of relatively short duration are common in many environments and can drastically impact seedling survival or stress tolerance. We sought to replicate this situation by imposing a progressive drought that would limit water availability without causing seedling death. To do this, we reduced water availability for half of the seedlings in each soil/fertilization combination (total of 48 seedlings) in 6-day increments over the final 18 days of the study (from 300 ml to 250, 125 and 75 ml H$_2$O day$^{-1}$). Seedling pre-dawn water potentials were measured on 10-cm segments taken near the seedling apex on Day 138. After carefully removing stems and attached leaves with a razor blade, plant material was sealed in individual plastic bags and placed in a cooler containing ice. Water potential was measured with a pressure bomb (Model 600; PMS Instruments Co., OR) under minimal lighting.

**Harvesting and sample preparation**

Trees were destructively harvested on Days 140–141. At harvest, seedlings were separated into foliage, stem and root components. Stems were severed from roots at the soil line and oven dried for 14 days at 60 °C. Soil was removed from roots by carefully washing over a 2-mm sieve placed on top of a plastic tub under running tap water. Care was taken to ensure that all roots were collected from the sieve and tub following washing of the roots. Subsamples of ~25% of seedling canopy and root system were flash frozen in liquid nitrogen in the greenhouse, lyophilized, ground into powder in liquid nitrogen with a mortar and pestle, and stored at −20 °C until needed for chemical analyses. The remaining foliage and roots were oven dried for 14 days at 60 °C. The dry masses of oven-dried and lyophilized tissues were combined and used to calculate total plant biomass and carbon allocation patterns.

**Table 1. Topsoil and subsoil initially differed in pH, organic matter content (%), textural characteristics (%) and major inorganic nutrients (μg g$^{-1}$).**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Organic matter</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>NH$_3$ N</th>
<th>Nitrate N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsoil</td>
<td>7.6</td>
<td>2.3</td>
<td>11.3</td>
<td>59.2</td>
<td>26.1</td>
<td>2.6</td>
<td>54</td>
<td>16</td>
<td>88</td>
<td>2409</td>
<td>232</td>
</tr>
<tr>
<td>Topsoil</td>
<td>7.4</td>
<td>5.7</td>
<td>19.1</td>
<td>58.8</td>
<td>15.7</td>
<td>1.1</td>
<td>174</td>
<td>220</td>
<td>936</td>
<td>1996</td>
<td>437</td>
</tr>
</tbody>
</table>
Chemical analyses: sugars and starch

Total soluble sugars were extracted from 100 mg lyophilized root tissue using 6 ml methanol/chloroform/water (12:5:3 v/v/v) as the extraction solvent (Liu et al. 2005). Following extraction, an additional 4 ml of distilled H2O was added to the samples; the samples were vortexed for 30 s and allowed to phase-separate for 4 h at 4 °C. Two hundred microliters of water phase extract was further diluted to 1 ml with distilled H2O and sugars measured on a Spectronic Genesys II spectrophotometer (Thermo Fisher Scientific) at 620 nm against a standard curve of D-glucose (Sigma Aldrich) using the anthrone method (Mcready et al. 1950). Starch was calculated by digesting 250 mg of lyophilized root tissue with potassium iodide/perchloric acid against a standard of pure starch (J.T. Baker Chemical) (Hansen and Moller 1975, Rose et al. 1991).

Chemical analyses: secondary metabolites

Total root soluble phenolics and lignin were extracted and analyzed following the methods of Bonello et al. (1993). Briefly, 100 mg of ground, lyophilized root tissue was twice extracted with 500 μl of 100% HPLC-grade methanol by incubating each extraction overnight at ~20 °C. Samples were centrifuged for 5 min at 12,000×g and the methanol removed. Extracts were used to quantify total phenolics using the Folin–Ciocalteau method (Bonello and Pearce 1993). The remaining pellets were used to quantify lignin following the procedures of Bonello et al. (1993).

Assessment of EM colonization

Morphological characterization (morphotyping) of colonized roots was conducted at 40× magnification using a dissecting microscope (Goodman et al. 1996). Root tip cross-sections were obtained to confirm the mycorrhizal nature of the root tips by embedding samples in LR White resin (Bonello et al. 1991) and thin-sectioning (10 μm) with an American Optical Model 820 rotary microtome (Buffalo, NY). Staining of cross-sections with toluidine blue allowed for visualization of the Hartig net and mantle (Bonello et al. 1991).

Root systems were cut into 2- to 5-cm sections and individual samples were hand mixed. Subsamples approximating 25% of the total root system were haphazardly selected from mixed samples. A total of 200 live root tips were counted per sample and used for morphological and molecular characterization. EM abundance is calculated as the percentage of colonized root tips out of the 200 total tips. Mycorrhizal root tips were counted for each sample to determine abundance. EM species richness or diversity was not calculated because only two EM morphotypes were found.

In order to putatively identify EM fungal taxa colonizing birch roots, 10–15 EM tips were picked in triplicate for each EM morphotype. Fungal DNA was extracted from colonized tips using the Nucleospin DNA extraction kit (Macherey-Nagel, Duren, Germany). PCR of the fungal ribosomal internal transcribed spacer (ITS) unit was carried out using ITS1F and ITS4 primers (Invitrogen) (Gardes and Bruns 1993) and LightCycler FastStart DNA Master Plus SYBR Green I PCR reagents (Roche) in a Roche LightCycler (Basel, Switzerland). PCR parameters were as follows: pre-incubation 95 °C–10 min; amplification, 35 cycles of 95 °C–10 s, 55 °C–5 s and 72 °C–30 s. Purified PCR products were sequenced at the Plant-Microbe Genomics Facility (The Ohio State University, Columbus, OH; pmgf.osu.edu). Sequences were manually edited using Chromas Lite v2.01 (Technelysium Pty Ltd) and submitted to NCBI GenBank for comparison against deposited fungal ITS sequences using BLAST. Matches were considered positive when sequence identity was ≥97% and E-values were 0.0.

Statistical analyses

All statistical analyses were carried out using SPSS v.15 (SPSS Inc., Chicago, IL). Data were examined using the explore function of SPSS to assure that they met assumptions of normality. EM colonization was subjected to an arcsine transformation while total phenolics and lignin were log transformed prior to analyses. Multivariate analysis of variance (MANOVA) was carried out to determine overall significance of individual factors and factor combinations. We report Pillai’s trace statistic following Scheiner and Gurevitch (2001). This statistic has been shown to be more robust than Wilks’ lambda, which is another commonly reported multivariate statistic. After identification of significant treatments and interactions (soil, fertilization, drought and the soil×fertilization interaction), univariate analyses of variance (ANOVA; Proc GLM; Type III sum of squares) were carried out on individual variables. Following identification of significant treatment effects, means were separated using the protected least significant difference (LSD) test. Non-parametric (Spearman) pairwise correlations are presented to highlight associations between EM abundance and other variables within and between soil types.

Results

Assessment of initial EM propagule densities

The most probable number procedure indicated that the two soils were strikingly similar in number of viable EM fungal propagules (sterile Agsorb control: 0; subsoil: 41; topsoil: 47 propagules/100 ml soil), and corresponding EM fungal colonization of roots was also similar (control: 0; subsoil: 34±2%; topsoil 39±2%). ITS sequences of EM fungi baited from the soils indicated that dominant viable propagules in air-dried subsoil belonged to a Tomentella sp. (GenBank accession EU202697.1; 698 bp query sequence, identity=97%, E=0.0), while air-dried topsoil was characterized by the presence of a Tomentella sp. (GenBank accession DQ068971; 700 bp query sequence, identity=99%, E=0.0) and an unidentified Pezizalian fungus (GenBank accession DQ469743.1; 779 bp...
Table 2. Results of MANOVA of treatment effects and their interactions on measured variables of B. papyrifera. Soil type, fertilization, drought and the interaction of soil type and fertilization significantly affected measured variables. The larger the Pillai’s trace, the more the given effect contributes to the overall model.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pillai’s trace</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>0.79</td>
<td>15.3</td>
<td>*</td>
</tr>
<tr>
<td>F</td>
<td>0.73</td>
<td>10.7</td>
<td>*</td>
</tr>
<tr>
<td>D</td>
<td>0.41</td>
<td>3.92</td>
<td>*</td>
</tr>
<tr>
<td>S×F</td>
<td>0.64</td>
<td>6.96</td>
<td>*</td>
</tr>
<tr>
<td>S×D</td>
<td>0.21</td>
<td>1.08</td>
<td>0.40</td>
</tr>
<tr>
<td>F×D</td>
<td>0.26</td>
<td>1.39</td>
<td>0.20</td>
</tr>
<tr>
<td>S×F×D</td>
<td>0.20</td>
<td>0.99</td>
<td>0.48</td>
</tr>
</tbody>
</table>

S, soil type; F, fertilization; D, drought.

*P<0.05.

query sequence, identity=99%, E=0.0). Both of these fungal taxa are known to form EM.

Effects of soil type, fertilization and drought on plant growth patterns

As we do not present final soil nutrient content or tissue nutrient content, we use final plant biomass to gauge fertility of our soils. All results and figures are arranged accordingly.

Soil type, fertilization and drought treatment had significant effects on overall seedling growth and growth patterns (Tables 2 and 3). In subsoil, fertilization increased total plant biomass by 459% (Figure 2A). In addition, fertilization of subsoil resulted in a 328% increase in root biomass but a 16% reduction in root weight ratios (Figure 2B and C). Therefore, while overall plant growth increased with fertilization, a smaller proportion of the plant biomass was allocated to roots (Figure 2C).

Unfertilized topsoil seedlings were nearly 900% larger than unfertilized subsoil seedlings (Figure 2A), indicating large differences in initial resource levels between these soil types. In topsoil, fertilization did not affect total plant biomass (Figure 2A). In contrast to subsoil, fertilization in topsoil decreased root biomass by 28%. However, similarly to subsoil, fertilization of topsoil decreased root weight ratios by 16% (Figure 2C).

The drought treatment reduced seedling pre-dawn water potential (control: −1.23±0.06 MPa; drought: −1.63±0.070 MPa; F[1,53]=17.1, P<0.05), indicating that the treatment was implemented effectively. Drought also had significant effects on all measures of seedling growth (Table 3). Drought treatment resulted in a 27% reduction in total plant biomass (Figure 3A) and reduced root biomass by 32% (Figure 3B) and root weight ratios by 11% (Figure 3C).

Effects of soil type, fertilization and drought on root chemistry

Seedlings grown in subsoil had higher root sugar concentrations (subsoil: 95.3±5.9 mg g⁻¹ DM; topsoil: 71.1±5.2 mg g⁻¹ DM) (Table 3). Root starch was similarly affected by soil type (subsoil: 86.6±4.5 mg g⁻¹ DM; topsoil: 54.5±3.9 mg g⁻¹ DM) (Table 3). Fertilization decreased root starch (control: 84.9±3.9 mg g⁻¹ DM; fertilization: 56.2±4.4 mg g⁻¹ DM) (Table 3).

Fertilization and soil type interacted to affect the concentrations of root secondary metabolites and lignin (Table 3). In subsoil, fertilization decreased root soluble phenolics by 33% but increased their concentration by 33% in topsoil (Figure 2D). Lignin showed a very similar response to fertilization (Figure 2E).

Drought increased root soluble sugars by 23% (Figure 3D) but did not affect any other measured aspects of plant chemistry (Table 3).

Effect of soil type, fertilization and drought on EM abundance

EM abundance was higher in unfertilized topsoil than unfertilized subsoil (Figure 2F). Fertilization did not affect EM abundance in subsoil but decreased EM abundance by ~60% in topsoil (Figure 2F). These contrasting trends are reflected in a significant interaction between soil type and fertilization (Table 3). Drought had no effect on EM abundance (Table 3).

Identity of EM fungi present on root systems

At the end of the greenhouse experiment, the root systems supported two morphotypes that were not restricted to particular treatment combinations. BLAST analysis indicated that these morphotypes were highly similar to a single Tomentella sp., i.e., GenBank accession DQ068971, the same as one of the two detected in the initial assessment (morphotype 1: 708 bp query sequence, identity=99%, E=0.0; morphotype 2: 620 bp query sequence, identity=98%, E=0.0). Thus, it is likely that the two morphotypes represented different developmental stages of the same organism.

Correlations between EM abundance and other measured variables

Overall, EM abundance was negatively correlated with root soluble phenolics, lignin and sugars, but not with total plant
biomass (Table 4). However, the nature of relationships between variables changed when viewed within soil treatments. Within subsoils, there were no relationships between EM abundance and root soluble sugars, phenolics or lignin, but there was a positive relationship with total plant biomass (Table 4). Relationships between variables differed in topsoil, where EM abundance was unrelated to total plant biomass but negatively correlated with root soluble sugars, phenolics and lignin (Table 4).

**Discussion**

This study showed that (i) variations in soil environment can cause significant shifts in C allocation to roots and subsequent partitioning to root secondary metabolism (phenolics and lignin) and EM colonization; (ii) fertilization can change the direction of those responses; and (iii) soil type and fertilization had no effects on the seedlings’ response to drought. Lastly, the study provides correlative evidence suggesting a role for root secondary metabolites such as phenolics and lignin in the regulation of EM fungi.

Our first prediction, derived from the optimal allocation model (Hirose 1987, Shipley and Meziane 2002), was supported in nutrient-deficient subsoil, where seedling biomass and root biomass increased while root weight ratios decreased in response to fertilization, indicating reduced carbon allocation to the root system. At the same time, the concentrations of root soluble phenolics and lignin decreased. This response is consistent with our second prediction and the GDBH (Herms and Mattson 1992), which models a negative correlation between growth and secondary metabolism when growth is more nutrient limited than is net assimilation. In topsoil, fertilization did not affect total seedling biomass. However, seedling biomass in fertilized and unfertilized topsoil was more than twice that of plants in fertilized subsoil. We recognize that additional soil factors (bulk density, texture, organic matter) may have influenced seedling growth patterns between soils. However, as final seedling biomass is a good indicator of nutrient availability and uptake by
plants, we believe this result shows that nutrient requirements for growth were fully met by topsoil, and that fertilization simply saturated the environment. In topsoil, fertilization also affected patterns of C allocation, decreasing root weight ratios as we predicted, while decreasing root biomass. Furthermore, root secondary metabolism (phenolics and lignin) showed the opposite response in topsoil, increasing with fertilization. Although physiological responses to super-optimal nutrient levels fall outside the range modeled by the GDBH (Herms and Mattson 1992), we show that such environments may cause a decrease in overall root growth and increase in secondary metabolism that appear consistent with this model.

Our third prediction that fertilization would result in decreased EM colonization was validated for topsoil but not subsoil. In the Treseder and Allen (2002) model, fertilization is predicted to increase mycorrhizal growth as long as nutrient supplies remain limiting to plant growth. Nutrient limitations to seedling growth in subsoil were certainly relaxed by fertilization, as seedling biomass increased substantially. In addition, the observed reduction of root weight ratios with

Table 4. Spearman’s correlations between EM abundance and four variables showing significant associations in at least one case. Significant correlations are highlighted in bold. Relationships between growth and C partitioning to EM in roots differed between soil types.

<table>
<thead>
<tr>
<th>EM abundance</th>
<th>Total plant dry biomass (g)</th>
<th>Root soluble sugars (mg g⁻¹ DM)</th>
<th>Root phenolics (mg g⁻¹ DM)</th>
<th>Root lignin (mg g⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.029ᵃ</td>
<td>−0.316</td>
<td>−0.287</td>
<td>−0.226</td>
</tr>
<tr>
<td></td>
<td>0.796ᵇ</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>0.339</td>
<td>0.057</td>
<td>0.125</td>
<td>0.018</td>
</tr>
<tr>
<td>Subsoil</td>
<td>&lt;0.05</td>
<td>0.747</td>
<td>0.504</td>
<td>0.922</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Topsoil</td>
<td>0.199</td>
<td>−0.642</td>
<td>−0.489</td>
<td>−0.349</td>
</tr>
<tr>
<td></td>
<td>0.447</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>42</td>
</tr>
</tbody>
</table>

DM, dry biomass.

ᵃCorrelation coefficient.
ᵇP-value.
ᶜDegrees of freedom.

Figure 3. Drought impacted C allocation patterns and partitioning to soluble sugars in roots. (A) Total plant dry biomass, (B) root dry biomass, (C) root weight ratio and (D) root soluble sugar concentration. Error bars are standard error of the mean. Results of statistical analysis are reported in Table 3.
fertilization of subsoil is predicted by optimal allocation theory to occur when nutrient limitations on plant growth are met. We hypothesize that fertilization of subsoil increased nutrient availability to a point approaching full match to growth requirements, where C limitations to mycorrhizal fungi (reflected in EM abundance) are predicted to be minimal. In nutrient-rich soils, the model of Treseder and Allen (2002) predicts that mycorrhizal colonization will increase with nutrient addition, as C is thought to be diverted away from the root system and, consequently, the mycorrhizal association. This pattern was evident in topsoil, where fertilization resulted in reductions in root biomass and root weight ratios (Figure 2B and C) and EM colonization (Figure 2F).

There are two major points to note regarding our assessment of EM in this study and its relationship to root sugars. First, the model of Treseder and Allen predicts that mycorrhizal growth will be impacted by C availability in roots. We did not fully measure EM growth, which would include extramatrical hyphae. However, only a single EM fungal species was found on the roots; thus, EM colonization should be a reliable surrogate for EM growth. We were surprised that our most probable number assay of soils yielded such low EM fungal diversity and that only a single EM fungus was found on seedling roots at the end of the greenhouse study. Reductions in propagule numbers and diversity likely resulted from the somewhat prolonged outdoor storage of the soils prior to their use. Alternatively, the presence of only one EM fungal species on the host root systems at the end of the experiment may indicate that Tomentella is able to colonize host roots while displacing other EM fungi in our system, e.g., the Pezizalian fungus identified among the initial fungi.

Second, we did not analyze sugar uptake by Tomentella and therefore could not assess whether direct carbon limitation is one component in the regulation of this mycorrhizal fungus. Sugar content of roots is highly dynamic as carbon is rapidly partitioned between metabolic processes. In the case of nutrient-rich soils, reserve carbon (e.g., starch) is diverted from nutrient capture (i.e., root growth and mycorrhizal associations) to aboveground growth, but root soluble sugar could remain essentially unchanged because root growth rates are reduced or plants limit EM fungus access to the soluble sugars (e.g., by altering invertase activity) (Nehls et al. 2007).

Patterns of acclimation and stress tolerance of paper birch

In this study, drought reduced total seedling biomass, root biomass and root weight ratios. However, it did not interact with either soil type or fertilization to affect patterns of C allocation or partitioning (Table 3), which suggests that soil type and/or fertilization treatments did not affect the ability of seedlings to respond to drought, perhaps because of inherently low drought tolerance of paper birch.

Growth reduction is expected under drought because water limitation reduces photosynthetic rates (Ranney et al. 1991). While relatively small, the reduction in root weight ratios seen in this experiment is contrary to models of optimal allocation and known responses of woody plants to drought (Bloom et al. 1985, Ingestad 1988, 1991, Kozlowski 2002, Hale et al. 2005). However, other studies have observed that effects of drought on carbon allocation do not always conform to predictions of optimal allocation. For example, drought stress decreased C allocation to roots and increased root turnover of European beech (Meier and Leuschner 2008) and Holm oak (Chiatante et al. 2005). We speculate that the magnitude of drought imposed in this study was sufficient to restrict C acquisition and preferential allocation of C to seedling roots, resulting in decreased overall growth rates and increased death of fine roots.

Potential regulation of EM colonization by root phenolics

We found that proportionally less C was allocated to roots (expressed as lower root biomass and root weight ratio) in nutrient-rich, fertilized topsoil (Figure 2B and C), and this was associated with accumulation of root soluble phenolics (Figure 2D) and lignin (Figure 2E) and lower EM abundance (Figure 2F) (see also Table 4). Our results are consistent with those reported by Weiss et al. (1999), who documented a negative association between root phenolics and EM colonization of several species of conifer. Furthermore, root phenolics have been suggested to limit Hartig net formation in birch (Feughly et al. 1999). To our knowledge, no previous studies have positively demonstrated that lignin is directly responsible for reduced root colonization by EM fungi. However, lignin has been linked to decreased colonization by arbuscular mycorrhizal fungi (Schwob et al. 2000) and has been postulated to be a potential regulator of EM colonization (Malajczuk et al. 1982). Furthermore, the role of lignin in preventing ingress by pathogenic fungi is well known in numerous systems (Vance et al. 1980). Overall, EM fungi appear to have a limited capacity to degrade lignin (Cairney and Burke 1998, Baldrian 2009), which would lend credibility to the idea that this plant cell wall component plays a role in the regulation of EM colonization. We suggest that root soluble phenolics and/or lignin, induced by high fertility, have the potential to suppress EM fungal colonization under those conditions, at least with this Tomentella sp. However, other EM fungi may be less responsive to root phenolic or lignin content and therefore may not respond in the same manner.

Potential for integration of conceptual models

Historically, models of resource allocation between growth, defense and mutualisms like EM associations have been proposed in isolation. Glynn et al. (2007) proposed an integration of the optimal allocation model and GDBH. In an experimental test, they found that willows acclimated to nutrient limitation by increasing root weight ratios, but net assimilation rate also increased, thereby relaxing carbon constraints on secondary metabolism, which therefore increased. Once plants had fully acclimated to nutrient limitation, the equilibrium allocation state was characterized by high net assimilation rate, root weight ratio and secondary.
metabolism but a low growth rate, which is consistent with
the integrated whole-plant response to stress predicted by
Chapin (1991). Our results suggest that the optimal allocation
model and the GDBH may be further integrated with models
of mycorrhizal growth such as that proposed by Treseder and
Allen to provide better predictive power of whole-plant acclima-
tion to heterogeneous soil environments.

Acknowledgments

We thank Peter Avis, Keith Clay, Richard Phillips, Heather Reynolds
and two anonymous reviewers for insightful manuscript suggestions.
We thank Bryant Chambers, David Snodgrass, Duan Wang, Justin
Whitehill and Jim Vent for their assistance with many aspects of
this work. This research was supported by USDA Forest Service National
Urban and Community Forestry Advisory Grant No. 03-DG-
1124425-428 to D.A.H. and P.B., a Tree Research and Education
Endowment Fund John Z. Duling Grant to P.B. and D.A.H., an Ohio
Agricultural Research and Development Center SEEDS Graduate
Student Grant to N.M.K., and by State and Federal funds appropri-
ated to the Ohio Agricultural Research and Development Center,
The Ohio State University. All experiments comply with the laws of
the USA.

References

rhizal colonization of Pinus muricata from resistant propagules
Baldrain, P. 2009. Ectomycorrhizal fungi and their enzymes in soils:
is there enough evidence for their role as facultive soil sapro-
Barter, B.W. 1957. Studies of the Bronze Birch Borer, Agri
host plant response to ectomycorrhizal diversity. Mycorrhiza
16:363–392.
in primary roots of Scots pine challenged in vitro with Cylindrocar-
papilla response in primary roots of Scots pine challenged in vitro
39:213–228.
Bonello, P., W. Heller and H. Sandermann. 1993. Ozone effects on
root-disease susceptibility and defense responses in mycorrhizal
and nonmycorrhizal seedlings of Scots pine (Pinus sylvestris L.).
Bruns, T.D. 1995. Thoughts on the processes that maintain local spe-
Caimey, J.W.G. and R.M. Burke. 1998. Do ecto- and ericoid mycor-
Caravaca, F., D. Figueras, J.M. Barea, C. Azcon-Aguilar and A.
Roldan. 2004. Effect of mycorrhizal inoculation on nutrient aqui-
sition, gas exchange, and nitrate reductase activity of two Mediterrane-anoctothonous shrub species under drought stress.
Chapin, F.S. 1989. The cost of tundra plant structures: evaluation of
41:29–36.
Quercus ilex L. seedlings to drought and fire. Plant Biosyst
139:198–208.
Combs, S.M. and M.V. Nathan. 1998. Soil organic matter. Recom-
mented Chemical Soil Test Procedures for the North Central Region.
Missouri Agricultural Experiment Station, Columbia, MO, p 53–58.
Davies, F.T., S.E. Svenson, J.C. Cole, L. Phavaphutanon, S.A.
Duray, V. Olaide-Portugal, C.E. Meyer and S.H. Bo. 1996.
Non-nutritional stress acclimation of mycorrhizal woody plants
exposed to drought. Tree Physiol. 16:985–993.
deJong, P.C. Betula: its morphology, evolution, classification, and
distribution with a survey of recent work. Presented at Betula:
Dickson, R.E. and J.G. Isebrands. 1993. Carbon allocation termin-
74:175–177.
Egerton-Warburton, L. and M.F. Allen. 2001. Ecto- and ectomy-
rhizas in Quercus agrifolia Nec. (Fagaceae): patterns of root
colonization and effects on seedling growth. Mycorrhiza
11:283–290.
Frank, K., D. Beegle and J. Denning. 1998. Phosphorus. Recom-
ended Chemical Soil Test Procedures for the North Central Region.
Missouri Agricultural Experiment Station, Columbia, MO, p 21–23.
Feugly, L., D.G. Strullu, P. Poupart and P. Simonneau. 1999. In-
duced defence responses limit Hartig net formation in ectomycor-
Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced speci-
sificity for basidiomycetes—application to the identification of
Gelderman, R.H. and D. Beegle. 1998. Nitrate-nitrogen. Recom-
mented Chemical Soil Test Procedures for the North Central Region.
Missouri Agricultural Experiment Station, Columbia, p 17–20.
2007. Testing the growth–differentiation balance hypothesis: dy-
176:623–634.
1996. An manual of concise descriptions of North American ec-
tomycorrhizas: including microscopic and molecular characteriza-
tion. Mycologue Publications, Sidney, BC.
Hale, B.K., D.A. Herms, R.C. Hansen, T.P. Clausen and D.A.
Duray, Olaide-Portugal, C.E. Meyer and S.H. Bo. 1996.
Non-nutritional stress acclimation of mycorrhizal woody plants
exposed to drought. Tree Physiol. 16:985–993.
Hirose, T. 1987. A vegetative plant growth model: adaptive signi-
CARBON ALLOCATION AND PARTITIONING IN BETULA PAPYRIFERA


Downloaded from treephys.oxfordjournals.org at Ohio State University Libraries on October 4, 2010