IS NATURAL DEFENSE CAPACITY CORRELATED WITH ALLOCATION OF DRY MASS TO THE STEM IN LOBLOLLY PINE?

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Abstract—In addition to selecting loblolly pine (*Pinus taeda* L.) genotypes for superior growth, the concept of customized genetic selection may apply where tree vigor is threatened by insects and disease. A study conducted with seedlings from 15 loblolly pine genotypes found significant correlation between phenolic production and foliage mass when dry mass allocation to the stem was relatively low. Validation of this relationship in trees was attempted among four genetic sources of juvenile loblolly pine. Tree biomass allocation information was collected from six individuals per genotype at age 5 years in 2009. Five years later in January 2015, foliage and branch tip tissues were assessed for total phenolic concentrations. While tree biomass allocation differed significantly, total phenolic concentrations were similar among the four loblolly pine genotypes. Observations during this study suggest that our effort to validate this relationship in trees should be repeated during the growing season when carbon demands are at their highest and in stand conditions that exclude the possibility of light limitations to carbon fixation.

INTRODUCTION

Phenolic compounds are an important source of both naturally occurring and inducible plant defenses (Lattanzio and others 2006). Past research has reported that among many plant species, both forms of phenolic defense are heritable (Agrawal 1999, Datta and Lal 2012, Witzell and Martín 2008). For example, Danielsson and others (2011) compared levels of naturally occurring and induced terpene and phenol compounds in response to inoculation with *Heterobasidion annosum s.l.* among Norway spruce (*Picea abies* L.) genotypes that exhibited high or low susceptibility when challenged by this pathogen. Constitutive phenolic profiles, but not terpene profiles, differed between the two levels of *Heterobasidion* susceptibility, with a negative relationship between astringin compound content and susceptibility. Between 5 and 15 days after inoculation with *Heterobasidion,* there was an increase in catechin concentration in the less susceptible Norway spruce genotypes. Gene expression analyses indicated that genes involved in the phenylpropanoid, epicatechin, and catechin pathways were activated by inoculation with *H. annosum*.

Carbon allocation among plant components has been characterized by a hierarchy that meets the carbon demand of foliage and stem before the root system and secondary metabolites (Dickson 1991). More recently in a compilation of data collected from 63 forested ecosystems, Litton and others (2007) revised this model. They suggested that carbon allocation to foliage and maintenance respiration is relatively constant regardless of stand environment, but that the fraction of fixed carbon allocated to the stem, roots, and other entities varies by stand factors. Furthermore, with the carbon needs of foliage and respiration met, a larger fraction of fixed carbon is allocated to wood production in the stem and branches when soil resources are plentiful. Alternatively, poor soil fertility and repeated water deficit shift carbon allocation away from wood production toward root system growth. Therefore, in most forest settings, after the carbon support of foliage and respiration are met, the fate of fixed carbon varies by an array of factors that influence the availability of essential resources.

We propose that within the context of the carbon allocation model outlined by Litton and others (2007), genotype exerts some control on the carbon allocation pattern of loblolly pine (*Pinus taeda* L.) (Bongarten and Teskey 1987, Li and others 1991, Stovall and others 2013), and selection of loblolly pine genotypes with a high capacity to produce constitutive or induced defenses would benefit commercial forestry in the South on sites where insect attack or disease are anticipated. Greenhouse experimental results recently

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showed that stem total phenolic concentration and dry mass allocation patterns differed significantly among 15 families of commercially deployed loblolly pine seedlings (Singh 2012). Furthermore, stem total phenolic concentration was correlated with both stem dry mass fraction and total foliage dry mass, so that as dry mass allocation to the stem decreased, the relationship between stem total phenolic concentration and foliage mass became more robust. A large foliage dry mass could benefit phenolic defense capacity by producing ample photosynthate for phenolic biosynthesis (Aspinwall and others 2011). Our objective was to determine if this relationship between total phenolic concentration, dry mass allocation to the stem, and foliage dry mass in seedlings would also be present in trees. Using existing allometric data from four genotypes of loblolly pine at age 5 years and total phenolic concentrations of plant tissues collected at age 10 years in January 2015, we hypothesized that the genotypes characterized by significantly lower stem dry mass allocation would have significantly higher total phenolic concentrations and that they would be positively correlated with foliage dry mass.

MATERIALS AND METHODS

Four rapidly growing sources of loblolly pine were planted at a 1.8 m x 4.9 m spacing as container-grown seedlings at the Louisiana State University AgCenter Hill Farm Research Station near Homer, LA, in January 2005. Each source was replicated 12 times in 0.06-ha plots with four plots per source assigned to one of three blocks by soil textural attributes contributing to drainage. Two sources were open-pollinated, half-sibling families originating in the eastern United States (7-56 and 8-103). The other two sources were clones propagated by rooted cuttings, also originating in the eastern United States (CL9 and CL93). Two of the sources were characterized by a compact crown shape (7-56 and CL93), and two of the sources (8-103 and CL9) were characterized by a broad crown shape.

In August 2009, one tree in one randomly chosen plot per source in each block was destructively harvested so that the dry mass of all foliage age classes, the stem, and the branches could be determined after total tree height and diameter at breast height (dbh) measurements. From these data, total aboveground dry mass was calculated and allometric equations were developed to predict aboveground dry mass and its distribution for all trees per plot in 2009. Allometric equations and growth measurements of all trees per plot were applied and plot means were calculated to predict mean tree aboveground total and component dry masses and the percentage of dry mass allocated to foliage, stem, and branches by tree in 2009.

In late February to early March 2010, five fascicles from the first flush of 2009 were sampled from five trees in all experimental plots and pooled by plot. Foliage samples were oven dried to equilibrium at 70°C, ground to pass a 0.85 mm^2 screen, and evaluated for macronutrient and micronutrient concentrations (A&L Laboratories, Memphis, TN).

In January 2015, two sunlit shoots from two randomly selected trees per plot used for the development of allometric equations were sampled. Shoots were taken from the mid-crown by telescoping pruning shears. First-flush foliage grown in 2014 and the terminal 10 cm (excluding buds) of branches were excised from the shoots, pooled by tissue and plot, freeze dried, and ground to pass through a 0.50 mm^2 screen. Processed tissues were analyzed for total phenolic concentration by a modification of the Folin-Ciocalteu method originally described by Singleton and Rossi (1965) and adapted by Booker and Maier (2001) for loblolly pine. Total phenolic concentration was expressed as micrograms of catechin equivalents per milligram dry weight.

Mean dry mass allocation to foliage, stem, and branches at age 5 years; mean percentage of dry mass allocated to foliage, stem, and branches at age 5 years; and mean foliage and branch tip total phenolic concentrations at age 10 years were evaluated by analysis of variance using a randomized complete block design with three blocks. Means by source were evaluated by the Tukey pair-wise comparison test. Relationships between tree dbh and total phenolic concentrations were evaluated by Pearson correlation coefficients. The *F*-statistics and mean differences were considered significant at an α level of 0.05.

RESULTS AND DISCUSSION

At age 5 years, the fraction of dry mass allocated to the stem was significantly greater for 8-103 (65 percent) and CL9 (62 percent) compared to 7-56 (50 percent) and CL93 (45 percent). At the same time, the fraction of dry mass allocated to foliage was significantly lower for 8-103 (18 percent) and CL9 (21 percent) compared to 7-56 (33 percent) and CL93 (43 percent). A similar pattern was observed for foliage dry mass, with that of 7-56 (2.7 kg) and CL93 (3.1 kg) being significantly greater than that of 8-103 (0.9 kg) and CL9 (1.2 kg). These foliage dry mass observations follow a similar trend in measurements of tree leaf area conducted at ages 4 and 5 years by Osbon and others (2012). The aboveground total dry weight of 7-56 (8.8 kg) was significantly greater than that of 8-103 (5.2 kg) and CL9 (5.4 kg) but not CL93 (7.2 kg). One reason for the large aboveground dry mass of 7-56 was its significantly larger branch size compared to the other three loblolly pine sources. As anticipated, the strong positive relationship between leaf area and tree growth (Vose and Allen 1988) was beneficial to stemwood and branch production in 7-56 and stemwood production in CL93.

Mean total phenolic concentrations of foliage and branch tissues were 70.2 ± 2.2 (standard error) and 56.1 ± 0.7 µg catechin mg^{-1}, respectively. These values are within the range of total phenolic concentrations reported by other investigators for loblolly pine seedlings and saplings. For example, seedlings grown under ambient light in a greenhouse between May and October had 39.2 and 22.3 µg catechin mg^{-1} in the foliage and stem, respectively (Gebauer and others 1998). Booker and Maier (2001) assessed the total phenolic concentration of a single cohort of loblolly pine seedling foliage. In September of the year of fascicle initiation, total phenolic concentration averaged 35 µg catechin mg^{-1}. In February, July, and September of the next year, total phenolic concentrations averaged 65, 88, and 100 µg catechin mg^{-1}. Aspinwall and others (2011) found that in October, the foliar total phenolic concentration of nine sources of genetically improved 2-year-old loblolly pine ranged between 104 and 130 µg catechin mg^{-1}. Results of these studies indicate that the phenolic concentration of foliage is greater than that of woody tissue, and the total phenolic concentration of foliage can be expected to increase over time.

We hypothesized that phenolic defense production would be hindered under conditions of high dry mass allocation to the stem and limited foliage dry mass accumulation. The 8-103 and CL9 sources of loblolly pine produced a relatively large fraction of stem dry mass and a relatively small fraction of foliage dry mass. We expected, therefore, to see lower total phenolic concentrations for 8-103 and CL9 compared to 7-56 and CL93. Total phenolic concentration among the four sources of loblolly pine did not differ significantly in foliage ($Pr > F = 0.4571$) or branch tips ($Pr > F = 0.8129$) (fig. 1). Clearly, a new effort to validate the relationship that we observed among total phenolic concentration, fraction of dry mass allocated to the stem, and foliage dry mass in seedlings will be required. The present effort provides insight for this second validation effort.

For the foliage tissue, but not the branch tip tissue of CL9, we found a significant negative relationship ($r^2 = 0.9700$, $Pr > F = 0.0033$) between total phenolic concentration and tree size represented by dbh (figs. 2a and b). In contrast, correlations between total phenolic concentration and tree growth were not observed for 7-56, CL93, or 8-103. Aspinwall and others (2011) also found departure in this relationship between loblolly pine clones grown in two locations with similar site indices but different stocking levels. They suggested that where stocking was high, resource constraints caused by intraspecific competition reduced carbon fixation such that it met the carbon demands of growth but not phenolic biosynthesis. Where stocking was less competitive, ample resources benefited both tree growth and phenolic production.

The accessibility of fixed carbon is driven by light availability in the crown. Trees in our study will reach canopy closure within the next 3 years, and thus intraspecific competition for light and other essential resources is currently high (Personal communication. Michael Blazier. 2015. Associate Professor, LSU AgCenter, Hill Farm Research Station, 11959 Highway 9, Homer, LA. 71040). Application of Aspinwall and others' (2011) theory to the relationship between total phenolic concentration and tree growth in our study indicates that at the time of tissue sampling for total phenolics, CL9 may have been experiencing one or more resource deficits that limited carbon allocation to phenolic biosynthesis. A similar problem was not apparent for 7-56, CL93, and 8-103.

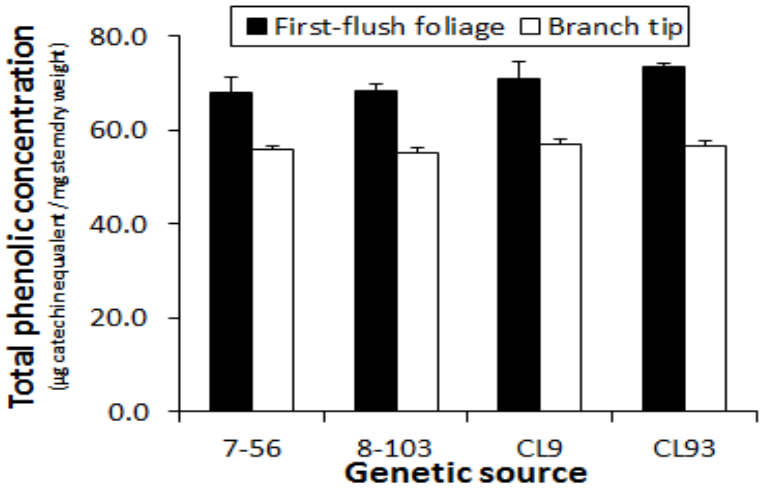

Figure 1—Total phenolic concentration means of foliage from the first flush and branch tips of four genetic sources of 10-year-old loblolly pine in January 2015. Bars represent one standard error of the mean.

In the dormant season of 2009, foliar nutrition was sufficient for all sources of loblolly pine (table 1). Also, while annual precipitation was 12 percent less than normal in 2014, this year was not characterized by drought (NDMC 2015). Thus, it is unlikely that nutrition or water limited the synthesis of phenolic compounds in the 2014 first-flush foliage or branch tips that were sampled in January 2015. Because the trees in our study were approaching canopy closure, it is possible that light availability, and thus whole-crown carbon fixation, was negatively affected by interaction between crown architecture and tree size.

The wide crown shape combined with the lower leaf area of CL9 compared to 7-56 and CL93 may have caused CL9 to reach a plateau of whole-crown carbon fixation with the approach of canopy closure. In contrast, it is possible that the compact crown shapes of 7-56 and CL93 aided light interception by the 2014 first-flush foliage. As a result, high leaf area and the way that leaf area was displayed in 2014 could have prolonged the period when fixed carbon satisfied the both tree growth and phenolic synthesis in 7-56 and CL93.

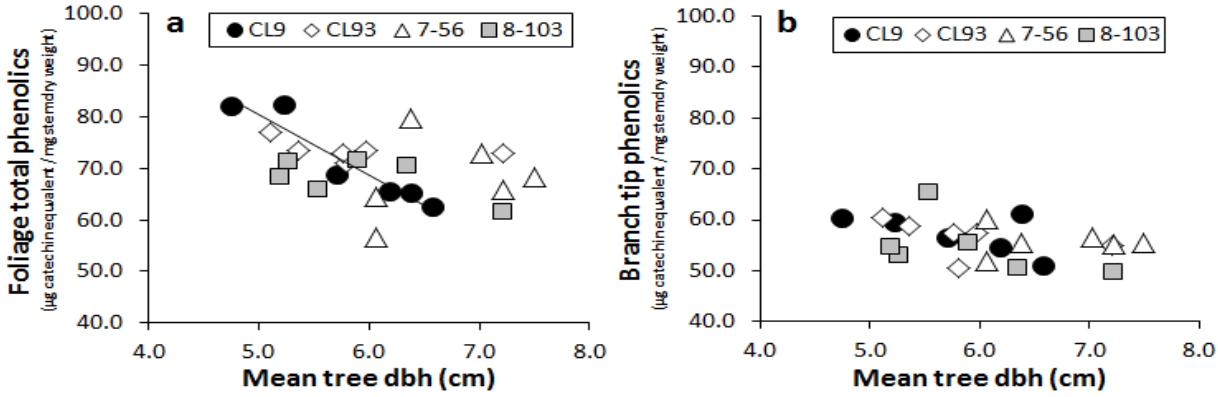

Figure 2— Relationship between mean tree diameter at breast height (dbh) at age 5 years and total phenolic concentration of (a) first-flush foliage and (b) branch tips at age 10 years for four genetic sources of loblolly pine. For the first-flush foliage of CL9, this relationship was statistically significant (r^2 = 0.9700, $Pr > F$ = 0.0033).

Table 1—Foliar concentrations of plant-essential mineral nutrients in four genetic sources of 5-year-old loblolly pine located at the Louisiana State University AgCenter Hill Farm Research Station near Homer, LA

Mineral nutrient[1]	Genetic source			
	CL9	CL93	7-56	8-103
Nitrogen (%)[2]	1.3 (0.1)	1.4 (0.1)	1.3 (0.1)	1.3 (0.1)
Phosphorus (%)	0.10 (0.03)	0.11 (0.01)	0.10 (0.02)	0.10 (0.01)
Potassium (%)	0.58 (0.08)	0.55 (0.06)	0.58 (0.07)	0.55 (0.08)
Calcium (%)	0.19 (0.03)	0.22 (0.07)	0.21 (0.04)	0.20 (0.03)
Magnesium (%)	0.09 (0.01)	0.10 (0.02)	0.08 (0.01)	0.09 (0.02)
Boron (ppm)	30 (9)	31 (10)	32 (8)	33 (6)
Zinc (ppm)	40 (5)	37 (6)	39 (5)	39 (5)
Manganese (ppm)	462 (163)	591 (120)	491 (172)	455 (77)
Iron (ppm)	66 (21)	74 (22)	73 (39)	67 (20)
Copper (ppm)	4 (1)	15 (42)	4 (1)	4 (2)
Aluminum (ppm)[3]	339 (59)	340 (42)	397 (82)	385 (67)

[1] Foliage is from the first flush of 2009 and was collected at age 5 years in late February to early March 2010. Values represent the mean and standard deviation of foliage sampled from the upper crown of five trees per plot.

[2] %: percentage; ppm: parts per million.

[3] While aluminum is not plant essential, it is included for informational purposes.

The dissimilar relationship between foliar phenolic concentration and dbh of CL9 and 8-103 cannot be explained by observations made in this study. Knowledge of root system architecture in addition to that of the crown, however, might reveal additional factors responsible for the potential carbon limitation to phenolic biosynthesis as CL9 tree size increased. For example, root system excavations at the time of aboveground biomass determinations showed that CL9 averaged fewer first-order lateral roots in a 0.5 m^3 block of soil around the stem that was 0.5 m deep compared to 8-103, 7-56, and CL93 (n=6; CL9: 8; 8-103: 17; 7-56: 20; CL93: 12). If the number of first-order lateral roots was correlated with root system expansion, then soil resource acquisition may have been lower for CL9 than 8-103. A subsequent negative effect on photosynthesis could have led to reduced carbon allocation to phenolic synthesis in CL9 compared to 8-103.

Foliage and branch tips were sampled for phenolic analyses outside the normal growing season, when the metabolic activity of woody tissues is low (Blanche and others 1992). During the dormant season, foliage physiology is closely tied to air temperature, with the potential for photosynthesis rates to be nearly as high as those during the growing season (Tang and others 2003). The difference in dormant-season metabolic rates between branch tip and foliage tissues could account for the observation of a significant negative relationship between total phenolic concentration and dbh in foliage, but not branch tips, of CL9.

CONCLUSION

Our present goal was to determine if a relationship between total phenolic concentration and dry mass allocation pattern among 15 genetic sources of loblolly pine seedlings would be observed among a different set of 4 genetic sources of loblolly pine trees. Validation of this relationship in trees would justify development of an index of dry mass allocation pattern that could be used to screen genetic sources of loblolly pine for natural phenolic defense capacity. Ultimately, this index would help land managers choose the loblolly pine genotype best suited for planting where the risks of insect attack and disease are high. Aboveground dry mass allocation pattern differed among our four genetic sources of loblolly pine planted in early 2005. Foliage and branch tip total phenolic concentrations, however, did not differ significantly among genotypes. Regression between total phenolic concentration and mean tree dbh indicated that the impact of crown shape on light interception, and therefore carbon fixation, might have been greater for one genotype than the other genotypes.

This effort also provides insight about how a second validation effort should be conducted. First, genotypes should be chosen that have genetically controlled dry mass allocation differences. Because aboveground dry mass allocation pattern and total phenolic production are under some degree of genetic control, a lag in time between measurements of these attributes is acceptable. It is critical, however, that the tested genotypes are in forest stand environments free of soil resource and light limitations. The resolution of differences in total phenolic concentration among genetic sources will likely be increased by sampling in the active growing season rather than the dormant season. Also, because its total phenolic concentration increases over time and is relatively high, mature foliage rather than developing foliage seems to be the best tissue for total phenolic surveys.

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