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RAPID COMMUNICATION

Evolutionary Potential of Root Chemical Defense: Genetic Correlations with Shoot Chemistry and Plant Growth

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Abstract Root herbivores can affect plant fitness, and roots often contain the same secondary metabolites that act as defenses in shoots, but the ecology and evolution of root chemical defense have been little investigated. Here, we investigated genetic variance, heritability, and correlations among defensive phenolic compounds in shoot vs. root tissues of common evening primrose, *Oenothera biennis*. Across 20 genotypes, there were roughly similar concentrations of total phenolics in shoots vs. roots, but the allocation of particular phenolics to shoots vs. roots varied along a continuum of genotype growth rate. Slow-growing genotypes allocated 2-fold more of the potential pro-oxidant oenothein B to shoots than roots, whereas fast-growing genotypes had roughly equivalent above and belowground concentrations. Phenolic concentrations in both roots and shoots were strongly heritable, with mostly positive patterns of genetic covariation. Nonetheless, there was genotype-specific variation in the presence/absence of two major ellagitannins (oenothein A and its precursor oenothein B), indicating two different chemotypes based on alterations in this chemical pathway. Overall, the presence of strong genetic variation in root defenses suggests ample scope for the evolution of these compounds as defenses against root herbivores.

Keywords Allocation · Below-ground · Chemical defense · Growth · Phenolics · Roots · Trade-off

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Introduction

Roots and shoots often both contain defense phytochemicals (Rasmann and Agrawal, 2008), suggesting that roots are chemically defended against herbivores. However, allocation patterns among shoots vs. roots can vary widely depending on the class and function of chemical compounds investigated, plant family and species, plant chemotype within a species, and damage history (Kaplan et al., 2008). Optimal defense theory predicts that allocation patterns are adaptive and can illuminate the forces driving natural selection for chemical defenses, but there are still relatively few investigations comparing chemical defense levels in above vs. belowground tissues (van Dam, 2009). Furthermore, although root defense chemicals may evolve if there is heritable variation that affects plant fitness, genotypic variation for root chemicals, and potential trade-offs with levels of shoot defenses, have been little explored.

Here, we quantified the concentrations of defensive phenolic compounds in the shoots and roots of 20 genotypes of common evening primrose *Oenothera biennis* L. (Onagraceae). We focused on ellagitannins, a group of phenolics that have a high potential for toxicity via their oxidative capacity (Salminen and Karonen, 2011). We examined the genetic relationships between defense investment and growth by asking three questions: 1) Are phenolic concentrations and plant growth rate in shoots and roots heritable? 2) What is the genetic covariation

within and among phenolic concentrations in shoots vs. roots? 3) Are there tissue-specific trade-offs between defense levels and plant growth rate?

Methods and Materials

Oenothera biennis has high genetic variability for functional traits, including life-history strategy, biomass allocation among roots vs. shoots, and secondary chemistry (reviewed in Johnson, 2011). Secondary chemicals are dominated by phenolics, in particular hydrolyzable tannins, ('ellagitannins') ranging in size from dimers to undecamers (Karonen et al., 2010). Variation in life-history strategy and the abundance of phenolics has been linked to herbivory by both aboveground insects (Johnson et al., 2009) and belowground mammals (Parker et al., 2010), but to date the chemical profiles of phenolics in above vs. belowground tissues of *O. biennis*, and their relationship to plant growth strategy, have not been reported.

We conducted a controlled growth experiment with 20 genotypes from separate *O. biennis* populations around Ithaca, New York (distinguished by microsatellites, Johnson et al., 2009), to investigate the interactions between plant growth rate and defensive chemistry. We germinated seeds from each genotype (N=3–6 per genotype) and grew them for 51–57 d on an open-air rooftop patio on the Cornell University campus, after which we collected, froze, lyophilized, and finely ground the leaf and root tissues from each plant. We analyzed phenolics in above and belowground plant tissues with high-performance liquid chromatography with a diode array detector following the methods of Johnson et al. (2009), where hydrolyzable tannins were quantified in pentagalloyl glucose equivalents (280 nm acquisition wavelength) and flavonoid glycosides in quercetin equivalents (349 nm). Plant growth rate was calculated as the dry biomass of roots plus shoot tissues divided by the number of days the plant had been alive.

We used restricted maximum likelihood (REML) to estimate the variance explained by plant genotype for each phenolic compound, total phenolics, and plant growth rate, with genotype included as a random effect, and significance tested using log-likelihood ratio tests. We calculated broad-sense heritability as $H^2=V_g/V_T$, where V_g is the total genetic variance (additive and nonadditive) and V_T is the total phenotypic variance (genetic and environmental). We assessed genetic covariation among traits using Pearson correlation coefficients on least squares mean values for all 300 pairwise combinations of phenolic correlations across genotypes (N=20 genotypes per correlation). The genetic covariance among traits was calculated according to the equation: $cov_g=r_g(G_{11}G_{22})^{0.5}$, where r_g is the genetic Pearson correlation coefficient between two traits, and G_{ii} is the genetic variance of each trait from REML. The statistical significance of genetic covariances was assessed as the P-value from the t-statistic of r_g. Binomial expansion tested whether the frequency of significant genotypic correlations differed from random expectations, and ANOVAs determined whether the strength of significant correlations differed among shoots and roots.

Results

Concentrations of total phenolics (i.e., sum of individual phenolics) and two ellagitannins comprising the bulk of the phenolics (≥68 %, oenothein B and A) were similar across shoots and roots (Fig. 1, Table S1). The composition of minor phenolics, however, differed. Shoot tissues contained chlorogenic acid and at least seven flavonoids that were not in root tissues, whereas root tissues contained at least eight ellagic acids that were not in shoot tissues (Table S1).

There was significant genetic variation for growth rate and phenolic concentrations. All but one of the phenolic groupings (a small group of unidentified flavonoids) were significantly heritable, with nearly 70 % of the variation in total above- and belowground phenolics attributable to plant genotype (Table S1). Qualitatively, four genotypes contained virtually no oenothein B in shoots, instead containing predominantly oenothein A (Fig. 1A, B). Plant growth rate varied 3-fold among genotypes (Fig. 1D–F), with half attributable to heritable variation (H^2=0.50, P<0.001).

There were mostly positive patterns of genetic covariation among phenolics. Out of 300 pairwise genetic associations among phenolics, we found 54 significant correlations (P≤0.05), 43 of which were positive (Table S2, overall P< 0.001, binomial expansion test). Genetic correlations among phenolic compounds generally were stronger within root tissues (mean r=0.29±0.04) than correlations within shoot tissues (mean r=0.04±0.04), or between shoot and root tissues (mean r=0.09±0.02; $F_{2,297}$, P<0.001, ANOVA). Phenolics also were positively correlated with plant growth rate, both within shoot tissues (mean r=0.26±0.05) and within root tissues (mean r=0.39±0.07), with no difference in the strength of these correlations across shoots vs. roots ($F_{1,23}$, P=0.154, ANOVA).

Slow-growing genotypes contained roughly twice as much oenothein B in their shoots vs. root tissues, whereas fast-growing genotypes had equivalent levels in shoots and roots (Fig. 1D).

Discussion

Our study demonstrates that although total levels of defense compounds may be similar across roots and shoots, this

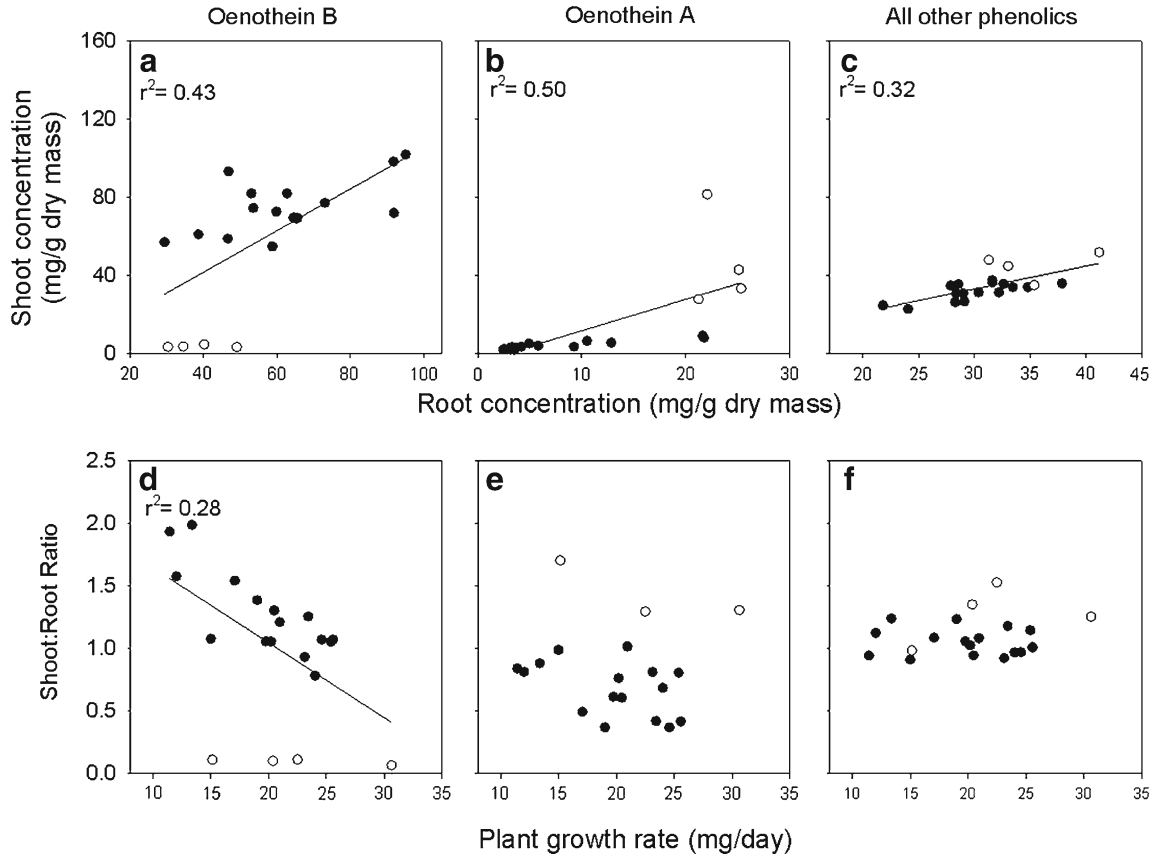

Fig. 1 Correlations between the concentrations of the major ellagitannins oenothein B and A and all other phenolics in shoots vs. root tissues of evening primrose, *Oenothera biennis* (Panels A–C). Each symbol is a mean value for an individual genotype (N=20). Four genotypes (open symbols) produced distinct shoot chemical profiles that were abundant in oenothein A but generally lacked its precursor oenothein B. Panels D–F show correlations between the ratio of phenolics in shoot over root tissues vs. the mean growth rate (mg/day) for each genotype. All regression lines are significant at P<0.05

pattern may underestimate differential patterns across genotypes. For example, one of the findings in our study was the observation of two *Oenothera biennis* chemotypes based on the two major compounds oenothein B and A. Twenty percent of genotypes produced essentially no oenothein B in their shoots, instead producing only oenothein A. Oenothein B is the dimeric precursor to the trimer oenothein A (Karonen et al., 2010). Thus, we posit the presence of a genetic polymorphism affecting either the structure or regulation of the enzyme that causes trimerization of oenothein B to oenothein A. Genotypes lacking oenothein B in their shoots retained it in their roots, and we found ellagic acids only in root tissues while flavonoids were only in leaf tissues. These differences in above vs. belowground phytochemical profiles could result from physiological constraints on allocation patterns or from differential selection pressures.

Our estimates of heritability and genetic variation for growth and phenolic compounds are similar to other published values in *O. biennis* (Johnson et al., 2009), indicating strong genetic differentiation for life history strategy and chemical defense. However, we saw few of the growth-defense trade-offs that define allocation costs to defense, and we did not find significant evidence of negative trade-offs among phenolic compounds. Instead, we found generally positive covariation among compounds, and a generally positive relationship between chemical defense and genotype growth rate. Similarly, other studies have failed to detect a negative relationship between growth and defense, or shown a positive relationship (Koricheva, 2002), consistent with analyses showing that the linkages between herbivory, chemical defense, and growth rate are complex and constrained by numerous genetic and physiological constraints (Carmona et al., 2011). Nevertheless, slow-growing genotypes invested more heavily in aboveground defenses, suggesting a tissue-specific trade-off that could be indicative of an adaptive response to aboveground vs. belowground herbivory that needs further exploration. Overall, the presence of strong genetic variation in root phytochemistry of *O. biennis* suggests ample raw material for natural selection to drive the evolution of root chemical defenses against root herbivores.

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