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Root Secondary Metabolites in *Populus tremuloides*: Effects of Simulated Climate Warming, Defoliation, and Genotype

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Abstract

Climate warming can influence interactions between plants and associated organisms by altering levels of plant secondary metabolites. In contrast to studies of elevated temperature on aboveground phytochemistry, the consequences of warming on root chemistry have received little attention. Herein, we investigated the effects of elevated temperature, defoliation, and genotype on root biomass and phenolic compounds in trembling aspen (*Populus tremuloides*). We grew saplings of three aspen genotypes under ambient or elevated temperatures (+4–6 °C), and defoliated (by 75%) half of the trees in each treatment. After 4 months, we harvested roots and determined their condensed tannin and salicinoid (phenolic glycoside) concentrations. Defoliation reduced root biomass, with a slightly larger impact under elevated, relative to ambient, temperature. Elevated temperature decreased condensed tannin concentrations by 21–43% across the various treatment combinations. Warming alone did not alter salicinoid concentrations but eliminated a small negative impact of defoliation on those compounds. Graphical vector analysis suggests that effects of warming and defoliation on condensed tannins and salicinoids were predominantly due to reduced biosynthesis of these metabolites in roots, rather than to changes in root biomass. In general, genotypes did not differ in their responses to temperature or temperature by defoliation interactions. Collectively, our results suggest that future climate warming will alter root phytochemistry, and that effects will vary among different classes of secondary metabolites and be influenced by concurrent ecological interactions such as herbivory. Temperature- and herbivory-mediated changes in root chemistry have the potential to influence belowground trophic interactions and soil nutrient dynamics.

Keywords Aspen · Biomass · Elevated temperature · Defoliation · Genetic variation · Roots · Salicinoids · Tannins

Introduction

Secondary metabolites in plant roots are important regulators of terrestrial community structure and function, as well as ecosystem processes. They can mediate interactions between roots and soil organisms (e.g., arthropods, nematodes, microbes), by serving as chemical defenses, and can influence plant productivity and fitness through plant-soil feedback interactions (Rasmann and Agrawal 2008; Tsunoda and van Dam 2017). Climate warming is predicted to affect ecological processes that are mediated by plant secondary chemicals (Jamieson et al. 2012; Holopainen et al. 2018; Rubert-Nason and Lindroth 2021). For example, in trembling aspen (*Populus tremuloides* Michx.), elevated temperature alters leaf chemical defenses, which in turn influence larval development times and food conversion efficiencies of a prominent defoliating insect, *Malacosoma disstria* Hübner (Jamieson et al. 2015). Although multiple experimental studies have explored how warming affects defense compounds in aboveground tissues, few such studies have focused on roots, and none has addressed roots of woody plants. Until now, research on the belowground effects of experimental warming in woody plants has been limited to primary and structural phytochemicals such as proteins,

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sugars, cellulose and lignin (e.g., Chen et al. 2008; Zhou et al. 2011).

Climate warming is likely to alter root secondary chemistry directly, through effects of temperature on plant metabolic processes (e.g., carbon fixation, Jamieson et al. 2012). Elevated temperature can decrease concentrations of carbon-based secondary metabolites in plant tissues by decreasing allocation of carbon to their production, through differential impacts on growth and nitrogen uptake (Jamieson et al. 2012; Salazar et al. 2020), or via passive dilution caused by faster growth under warmer conditions (Holopainen et al. 2018). Warming-mediated effects on secondary phytochemicals (e.g., phenolics, latex) have been reported in leaves of multiple species (e.g., Jamieson et al. 2015; Lemoine et al. 2017; Sobuj et al. 2018) and in roots of *Taraxacum officinale* F.H.Wigg. (Huang et al. 2020).

Climate warming may also alter root secondary chemistry indirectly, by changing the intensity of aboveground biotic interactions such as insect herbivory. Elevated temperature can increase herbivory by accelerating insect metabolic rates and voltinism, increasing insect population densities, and stimulating compensatory feeding in response to warming-mediated decreases in plant nutritional content (Bale et al. 2002; Jamieson et al. 2012, 2015; Deutsch et al. 2018). Herbivore damage to aboveground plant tissues can induce phytochemical changes in roots, such as terpenoids in *Solanum lycocarpum* A.St.-Hil. (Mundim et al. 2017). Chewing of foliage by caterpillars upregulates the jasmonate pathway, which can lead to increased concentrations of defense chemicals in roots that in turn influence the performance of belowground organisms interacting with the roots (Karssemeijer et al. 2020). Biotic stressors such as herbivory and disease can further interact with climate change factors to influence concentrations of plant secondary metabolites (Mikkelsen et al. 2015; Lama et al. 2016).

In this study, we investigated the effects of elevated temperature and defoliation on root biomass and concentrations of ecologically important phenolics in trembling aspen genotypes. Specifically, we focused on condensed tannins and salicinoid phenolic glycosides, compounds that mediate ecological interactions and provide defense against herbivores in aspen (Lindroth and St. Clair 2013; Rubert-Nason and Lindroth 2021). We addressed three primary questions: (1) What is the effect of elevated temperature on root biomass and concentrations of tannins and salicinoids? (2) Does warming influence the effects of aboveground defoliation on root biomass and phenolics? (3) Do aspen genotypes respond similarly or differently to temperature and defoliation treatments?

Methods and Materials

Experimental Design We applied two temperature (ambient, elevated) and two defoliation (none, 75%) treatments to 2-year old containerized trembling aspen trees. The trees were grown in eight greenhouse rooms (four per temperature treatment). Because aspen vary genotypically in their responses to environmental variables (Barker et al. 2019), we investigated three aspen genotypes (PG1, male; PI3, female; Wau2, male) from south-central Wisconsin, USA. Trees were clonally propagated from meristems by tissue culture following the method of Sellmer et al. (1989) and allowed to grow for two summers before use. Experimental treatments were arranged in a split-plot design, with temperature crossed at the whole-plot level and defoliation and genotype as sub-plots. Trees were planted into individual 8-L pots filled with a mixture containing equal volumes of Fafard germination mix (peat moss, perlite, vermiculite; MA, USA), Turface MVP (calcined clay; IL, USA), sand, and local farmland soil. Trees were supplied with filtered tap water as needed. For the first 2 weeks after transplanting, we also supplied 500 mL of ¼-strength Hoagland's complete nutrient solution to each plant. In the third and fourth weeks, we watered trees twice weekly, once with 500 mL of ¼-strength Hoagland's solution, and once with 500 mL of filtered water. Beyond week five, we watered three times per week, fertilizing once per week with 1/8th strength Hoagland's solution. Measurements were collected on three defoliated trees and four non-defoliated trees per genotype in each of 8 rooms (168 trees in total). Saplings used in this study were part of a larger project investigating the effects of warming, defoliation and genotype on aboveground tree physiology, growth and phytochemistry (M.A. Jamieson, K.F. Raffa and R.L. Lindroth, unpublished data).

Temperature Treatment Saplings were grown for 4 months, from mid-April through mid-August, with a 15:9 h day:night temperature cycle. Ambient temperature (control) rooms reflected spring/summer temperatures in Madison, Wisconsin (Table S1), with daytime temperatures starting at 18 °C and increasing to 24 °C over the four-month experiment, and nighttime temperatures ranging from 15 to 17 °C. Elevated temperature rooms were maintained at 4 °C above the ambient condition during the first 2 weeks of the experiment, and 6 °C above the ambient condition for the remaining time. These elevated temperatures were selected to approximate conditions projected to occur in southern Wisconsin forests within the next 75–100 years (Kling et al. 2003; Wuebbles and Hayhoe 2004), particularly during especially warm years in a 1.5–2 °C average global warming scenario (Hoegh-Guldberg et al. 2018). Each greenhouse room was climate-controlled independently using a forced-air heating and ventilating system regulated to the diurnal temperature conditions specified in the Supplementary Material (Table S2). Because our experiment utilized potted trees that were surrounded by

air, the air and soil experienced similar temperature dynamics.

Defoliation Treatment We imposed a standardized defoliation treatment using a combination of gypsy moth (*Lymantria dispar* L.) feeding and mechanical leaf cutting, as described by Cope and Lindroth (2018). Initially, five fourth-stadium gypsy moth larvae per tree were deployed onto half of the trees exposed to each temperature condition. All trees were enclosed in white no-see-um mesh bags in order to contain the insects; trees without insects were also enclosed in bags to control for potential bag effects. Larvae fed for 3 days, resulting in ~10% defoliation. Subsequently, we removed the larvae and clipped (using pinking shears) each leaf on each tree to a uniform 75% defoliation by area (simulating the magnitude of herbivore damage expected during a gypsy moth outbreak; Donaldson and Lindroth 2008). This combination of insect and mechanical defoliation exposes all trees to insect salivary elicitors that may induce chemical defenses, while standardizing the magnitude and pattern of leaf damage. To synchronize defoliation with leaf phenology, we applied the defoliation treatments in elevated temperature rooms on the seventh week of the experiment and in ambient temperature rooms on the eighth week of the experiment.

Root Harvest We harvested trees after 4 months of warming treatment. Whole root balls were washed free of potting media with lukewarm (30–40 °C) tap water in tubs, collecting fine root material over a sieve (~2 mm, to minimize loss of fine roots). Clean roots were drained of excess water on a rack, then freeze-dried and weighed. Whole dry root balls (including course and fine roots) were ground to USDA 20-mesh size with a Wiley mini-mill (Swedesboro, NJ, USA) and stored at −20 °C.

Chemical Analyses Condensed tannins were extracted into acetone/water (7:3) with ascorbic acid (10 mM) and quantified using the acid-butanol colorimetric assay (Porter et al. 1986), with modifications and referencing to condensed tannins that were purified from *P. tremuloides* as described by Rubert-Nason and Lindroth (2019). Salicinoids were extracted into methanol, and salicortin, salicin, tremulacin and tremuloidin concentrations were quantified using ultra-high performance liquid chromatography with mass spectrometry as described by Rubert-Nason et al. (2018).

Statistical Analyses Statistical analyses were performed using *R* 3.2.4 (http://www.r-project.org/) and JMP Pro 11 (SAS Institute Inc., Cary, NC, USA). We evaluated the collective effects of warming, defoliation, and genotype on root phenolic compounds using principal component analysis (PCA). Principal factors were computed from the matrix of total condensed tannin and salicinoid concentrations using the *princomp* function in the '*stats*' package (*R* Development Core Team). The effects of warming, defoliation, and genotype on the composite phenolics were determined by multivariate analysis of variance (MANOVA). We also evaluated treatment effects on phenolic compounds and root biomass using general linear mixed models (GLMMs), with temperature, defoliation, genotype, and their interactions as fixed effects and greenhouse room as a random effect. Differences among specific treatment combinations were evaluated using post hoc Tukey-HSD tests.

Graphical vector analysis (GVA; Koricheva 1999) was employed to evaluate the extent to which changes in phytochemical concentrations observed under the different treatment conditions could be attributed to phytochemical production versus biomass production. We conducted the GVA as described by Couture et al. (2017) and Li et al. (2020). Briefly, relative changes in phenolic concentrations and contents (concentration multiplied by root biomass) were calculated by dividing each measurement by its corresponding control (ambient temperature, non-defoliated) value. The relative changes in tannin and salicinoid concentrations (*Y*-axis) were plotted against the relative changes in tannin and salicinoid contents (*X*-axis), and vectors indicating the magnitude and direction of shifts were superimposed.

Results

Root biomass production was influenced by temperature, defoliation, genotype, and their interactions (Fig. 1, Table 1). Warming alone did not alter root biomass. Defoliation, however, decreased root biomass, and the magnitude of that decrease was slightly larger at elevated temperature (32–36%) than at ambient temperature (28–34%) (temperature × defoliation interaction, Table 1). Root biomass, and the negative effect of defoliation on root biomass, varied across the three aspen genotypes (defoliation × genotype interaction, Table 1).

Warming, defoliation, and genotype altered composite phenolic profiles (condensed tannins and salicinoids) in aspen roots, both independently and interactively (Fig. 2). Of the three treatments, warming produced the strongest clustering in the PCA plot. The effect of warming on phenolic profiles was driven primarily by decreases in condensed tannins and varied between defoliation treatments (temperature × defoliation interaction). Composite phenolic profiles were also influenced by defoliation and genotype, as well as the interaction between those factors (variable separation magnitudes between defoliation treatments within genotypes).

Warming and defoliation influenced condensed tannin concentrations in aspen roots (Fig. 3; Table 1). Warming decreased tannin concentrations by 21–43% across defoliation treatments and genotypes. Defoliation minimally affected tannin concentrations under ambient temperature, but increased

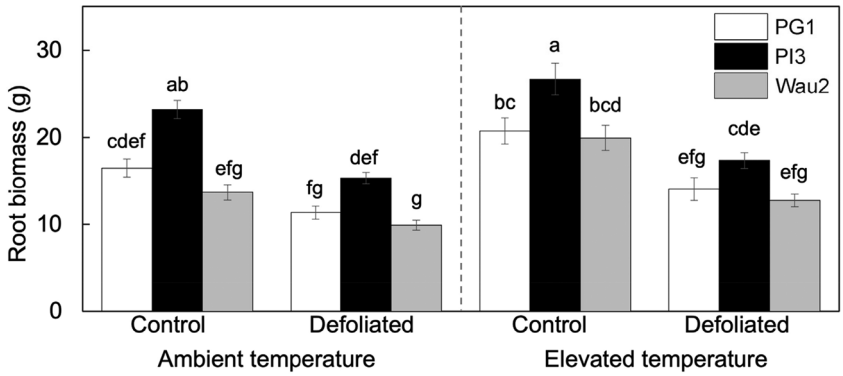

Fig. 1 Effects of temperature, defoliation, and genotype on aspen root biomass. Different lower-case letters indicate significant differences among temperature × defoliation × genotype combinations ($P < 0.05$)

concentrations by an average of 17% under elevated temperature (temperature × defoliation interaction). The defoliation response direction and magnitude varied from −13% to +29% among aspen genotypes (defoliation × genotype interaction).

Warming alone did not alter total salicinoid concentrations in roots, when averaged across defoliation and genotype treatments (Fig. 3; Table 1). As observed for tannins, warming influenced the effects of defoliation on total salicinoid concentrations. At ambient temperature, defoliation decreased total salicinoid concentrations by an average of 9%, but at elevated temperature, defoliation had no significant impact (temperature × defoliation interaction). Expression of total salicinoids in roots varied among aspen genotypes and with how they responded to defoliation (defoliation × genotype interaction).

Of the four major salicinoids (salicortin, salicin, tremulacin and tremuloidin) quantified in aspen roots, salicortin accounted for up to 90% of the total concentration (Fig. 4). Relative to salicortin, concentrations of salicin and tremulacin were approximately one order of magnitude lower, and concentrations of tremuloidin were three orders of magnitude lower. Concentrations of individual salicinoids, as observed for total salicinoids, did not respond to warming when averaged across defoliation and genotype treatments (Fig. 4; Table 1). However, warming altered salicortin concentrations via interaction with defoliation; foliar damage caused a small decrease in salicortin levels under ambient, but not elevated, temperatures. This effect contributed substantively toward the temperature × defoliation interaction observed for total salicinoids in Fig. 3. Defoliation decreased concentrations of salicin, tremulacin and tremuloidin (but not salicortin) in aspen roots, causing the overall decline in total salicinoid concentrations. Levels of individual salicinoids varied among the three genotypes, and responses of tremulacin to warming and defoliation varied among the genotypes.

Graphical vector analysis (Fig. 5) revealed possible mechanisms responsible for observed changes in phenolic concentrations. For condensed tannins, reduced concentrations under the ambient-defoliated, elevated-control, and elevated-defoliated treatments were a consequence of reduced tannin biosynthesis, relative to trees in the ambient-control treatment. For total salicinoids, reduced concentrations under the ambient-defoliated and elevated-defoliated treatments were similarly a result of reduced synthesis. In contrast, lower levels of salicinoids in the elevated-control treatment, relative to the ambient-control treatment, were due to a tissue dilution effect. Response patterns for condensed tannins and salicinoids were similar across all genotypes (Fig. S1).

Table 1 Analysis of variance evaluating the influence of elevated temperature (*T*), defoliation (*D*), genotype (*G*) and their interactions on aspen root biomass and phenolic concentrations

Factors		Root biomass		Condensed tannins		Total salicinoids		Salicortin		Salicin		Tremulacin		Tremuloidin	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Temperature	1,6	2.77	0.147	21.77	**0.003**	3.54	0.118	4.82	0.071	0.01	0.948	2.79	0.146	0.02	0.892
Defoliation	1,150	149.11	**<.001**	1.19	0.277	3.96	**0.021**	1.45	0.230	9.58	**0.002**	16.27	**<.001**	10.05	**<.001**
Genotype	2,150	57.69	**<.001**	2.61	0.077	21.49	**<.001**	32.92	**<.001**	32.30	**<.001**	7.54	**<.001**	47.11	**<.001**
T × D	1,150	3.91	**0.050**	7.02	**0.009**	4.54	**0.038**	4.64	**0.033**	0.03	0.856	1.31	0.254	0.16	0.694
T × G	2,150	1.10	0.334	1.00	0.371	1.44	0.225	1.58	0.209	2.12	0.123	8.06	**<.001**	2.81	0.064
D × G	2,150	3.14	**0.046**	4.31	**0.015**	3.28	**0.035**	2.85	0.061	0.55	0.577	5.67	**<.001**	2.43	0.092
T × D × G	2,150	0.34	0.710	0.10	0.903	0.04	0.866	0.06	0.938	4.26	**0.016**	1.87	0.158	4.26	**0.016**

Significant treatment effects ($P < 0.05$) are shown in bold

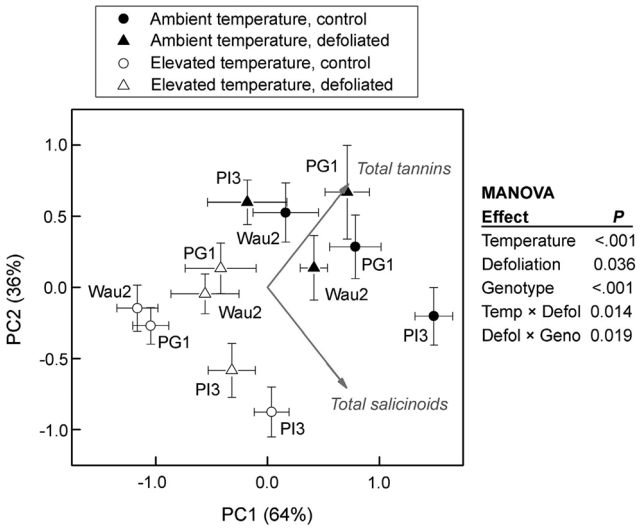

Fig. 2 Principal component analysis (PCA) depicting the effects of temperature, defoliation, and genotype on composite phenolic profiles in roots. Principal components (PC) 1 and 2 explained 64% and 36% of the variation, respectively. Individual points correspond to the average component score ± 1*SE* for each temperature × defoliation × genotype combination; the directions of gray arrows indicate increasing condensed tannin and salicinoid concentrations. Statistical differences among treatments were determined using MANOVA

Discussion

Under a 1.5–2 °C mean global warming scenario, climate models predict more frequent and intense "heat waves" in mid- and high-latitude regions, intermittently exposing forests to temperatures that are 4–6 °C greater than those documented at the present time (Hoegh-Guldberg et al. 2018). Our study explored how such 4–6 °C increases in temperature, independently and interactively with defoliation and plant genotype, influence biomass and phenolic secondary metabolites in aspen roots. To our knowledge, this is the first manipulative experiment to examine how warming affects expression of phenolic compounds in tree roots. We found that warming consistently altered phenolic concentrations in roots of three different aspen genotypes, either independently or via interaction with aboveground defoliation. Our study extends a small body of research demonstrating that warming and aboveground herbivory alter concentrations of ecologically relevant secondary metabolites in the roots of woody plants and highlights the need to consider aboveground factors when studying impacts of warming on belowground ecosystems.

Warming decreased the concentrations of phenolic compounds in aspen roots, primarily by lowering the concentrations of condensed tannins. In contrast to our findings on the impacts of experimental warming, total phenolics in roots of Scots pine (*Pinus sylvestris* L.) from diverse populations increased along a latitudinal gradient of increasing mean annual temperature (MAT; Zadworny et al. 2017). We speculate that the different responses of phenolics to warming may reflect differences in plant biochemistry and physiology, tissue heterogeneity, and duration and magnitude of warming. Diverse responses to warming have also been observed in other root compounds. For example, experimental warming did not affect lignin levels in roots of hardwoods (Zhou et al. 2011), but decreased levels in roots of the gymnosperm *Pseudotsuga*

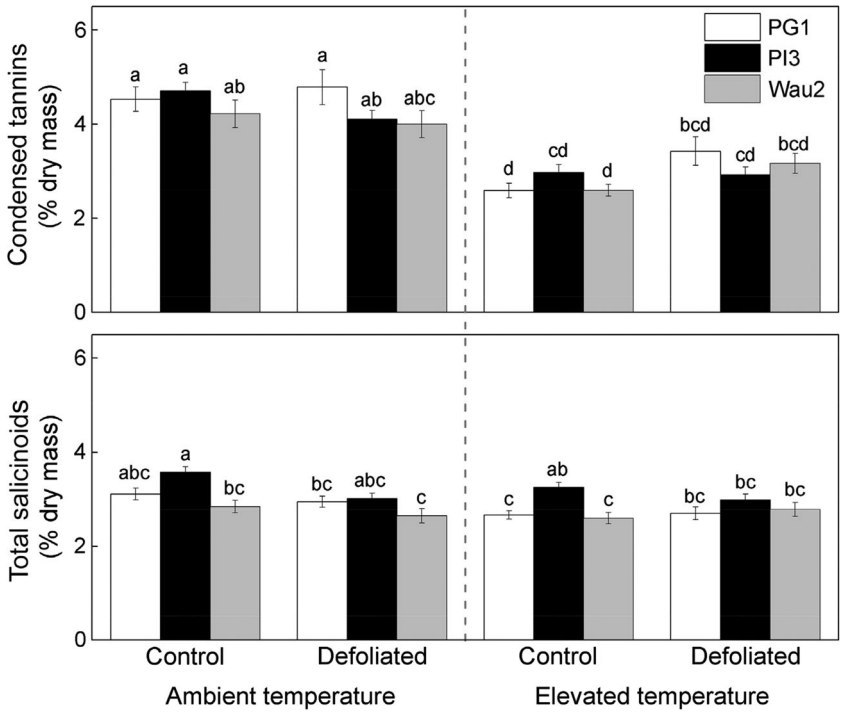

Fig. 3 Effects of temperature, defoliation, and genotype on concentrations of condensed tannins (± 1*SE*) and total salicinoids (Σsalicortin, salicin, tremulacin, tremuloidin; ± 1*SE*) in aspen roots. Within each chemical class, different lower-case letters indicate significant differences among temperature × defoliation × genotype combinations ($P < 0.05$)

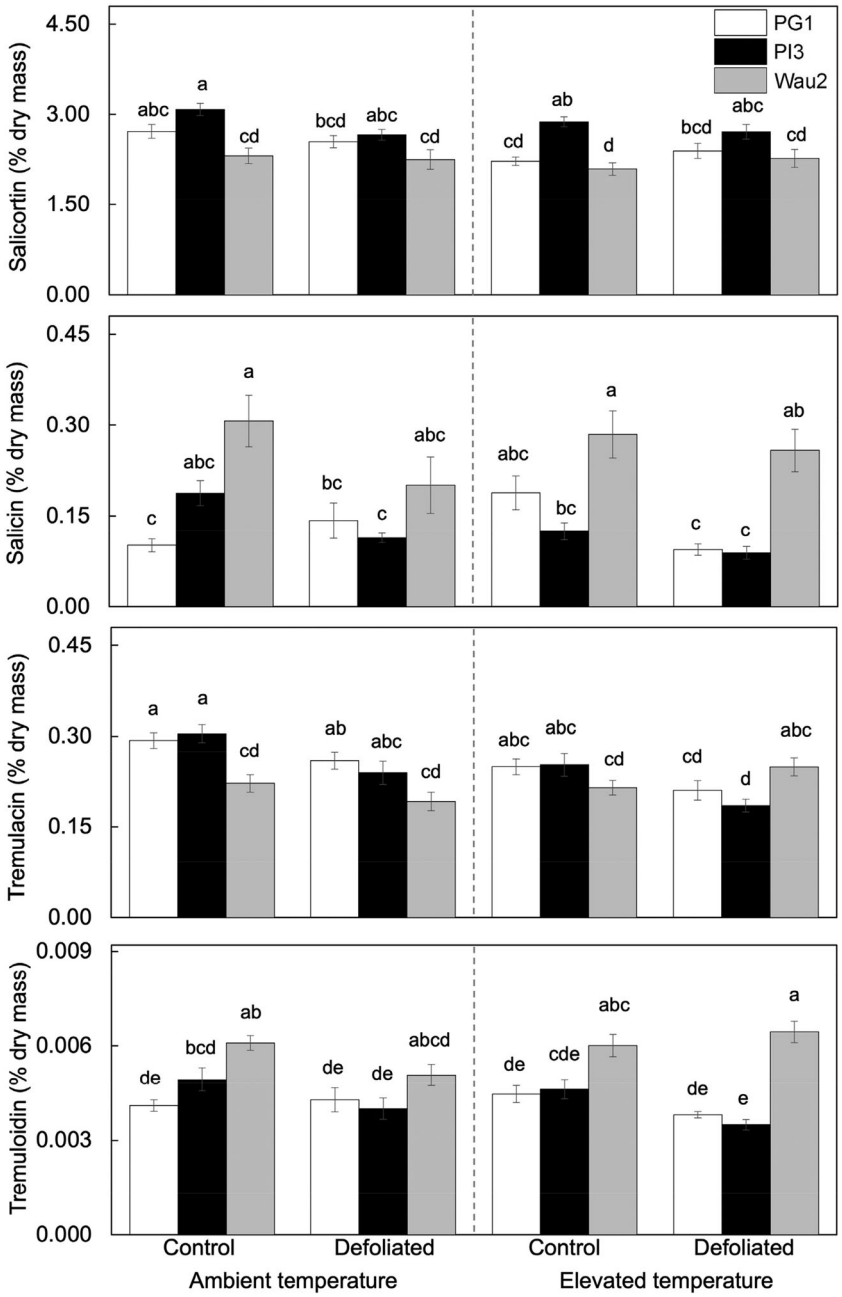

Fig. 4 Effects of temperature, defoliation, and genotype on individual salicinoids in aspen roots. Within each compound, different lower-case letters indicate significant differences among temperature × defoliation × genotype combinations ($P < 0.05$). Note that the y-axis scaling is different for each compound, reflecting different levels of each compound in the roots

menziesii (Mirb.) Franco (Chen et al. 2008) and increased levels in roots of C_3 and C_4 grasses (Suseela et al. 2017).

In our study, graphical vector analysis suggests that the negative effect of warming on aspen root phenolics was due to decreased phenolic (especially tannin) biosynthesis rather than passive dilution by accruing biomass. Dilution may be expected to occur because of increased root growth under warmer conditions. However, we observed only a modest increase in root growth due to warming and only in non-defoliated trees. Observations from *Picea asperata* Mast. also suggest that warming exerts minimal effects on root biomass in forest ecosystems (Li et al. 2015). A potential explanation for warming-mediated decreases in phenolic biosynthesis is a shift in metabolic processes underlying allocation of carbon to structural versus defense compounds (Jamieson et al. 2012). In addition, soil nitrogen is more bioavailable under warmer conditions (Salazar et al. 2020), and increased soil nitrogen decreases concentrations of condensed tannins in aspen roots (Stevens et al. 2014).

Warming altered the responses of aspen root phenolics to experimental defoliation (temperature × defoliation interactions). Root tannin concentrations did not respond to defoliation at ambient temperature but increased slightly following defoliation in the warmer environment. Although warming

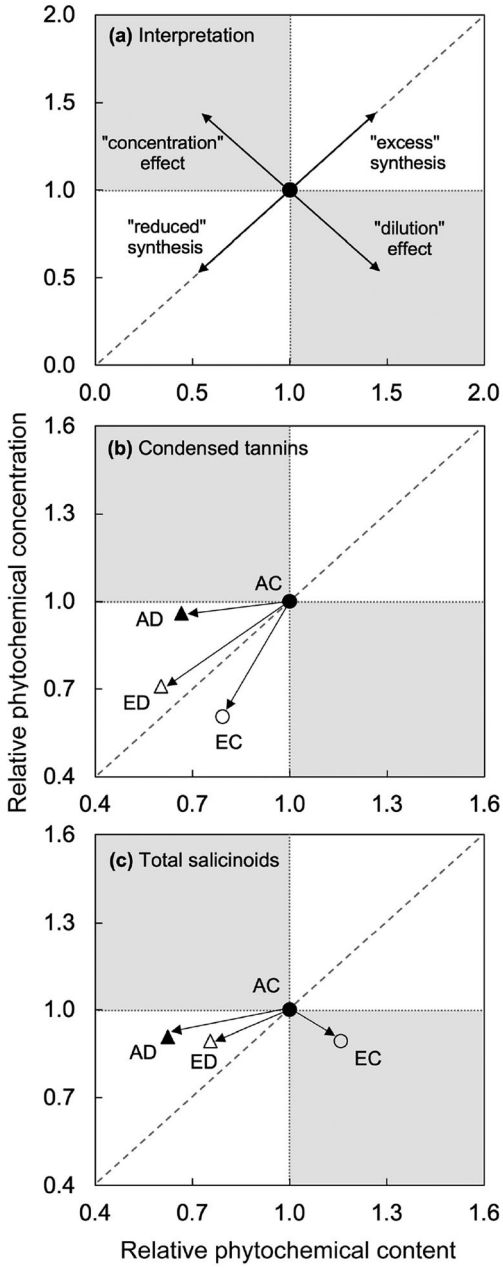

Fig. 5 Graphical representations illustrating independent and interactive effects of temperature and defoliation on the production of condensed tannins and total salicinoids in aspen roots, relative to the control conditions (AC). (**a**) As described by Koricheva (1999), vectors in the upper left quadrant indicate a concentration effect, vectors in the lower left quadrant indicate reduced biosynthesis, vectors in the lower right quadrant indicate a dilution effect, and vectors in the upper right quadrant indicate excess biosynthesis. For (**b**) condensed tannins and (**c**) total salicinoids, each point represents the mean value for three genotypes. *AC* ambient temperature, control (non-defoliated), *AD* ambient temperature, defoliated, *EC* elevated temperature, control, *ED* elevated temperature, defoliated

alone did not affect root salicinoid concentrations, it altered how salicinoids responded to defoliation. Findings from a prior study (Stevens et al. 2014) examining the effects of defoliation and soil nutrients on phenolics in aspen roots suggest that the varied responses of root phenolics to defoliation at different temperatures may be related to increases in soil nitrogen availability that occur at increased soil temperature (Salazar et al. 2020). The processes governing temperature × defoliation interaction effects are complex, however, and cannot be adequately explained from the limited information available in published literature. Furthermore, the relatively small magnitudes of the temperature × defoliation effects observed in an extreme warming and defoliation scenario suggests that the interplay of these variables may be of minimal ecological importance.

Condensed tannin and salicinoid concentrations in roots were substantially lower than levels in foliage of aspen (e.g., Jamieson et al. 2015; Cope et al. 2019), similar to patterns described elsewhere (Dettlaff et al. 2018; Rubert-Nason and Lindroth, 2021). The negative effect of warming on condensed tannin concentrations that we observed in roots mirrors reductions in foliar condensed tannins of aspen and birch observed in an earlier, field-based manipulative warming study (Jamieson et al. 2015). In contrast, warming had no effect on salicinoid concentrations in roots in our study, similar to effects on leaves in the work by Jamieson et al. (2015). These combined results suggest that future warming will concomitantly decrease condensed tannins in aboveground and belowground tissues but have minimal effect on salicinoids.

Phenolics protect plants against a variety of abiotic and biotic stressors. Condensed tannins likely protect *Populus* against oxidative stress and pathogen attack and may deter some herbivores (Rubert-Nason and Lindroth 2021). Salicinoids primarily function to defend *Populus* against herbivory (Lindroth and St. Clair 2013). In this study, a 4–6 °C temperature increase decreased root condensed tannin concentrations by up to 43%, while exerting minimal impacts on salicinoid concentrations. Similar response patterns have been reported in aboveground aspen tissues responding to a variety of stressors, with condensed tannins generally responding more strongly to environmental factors than salicinoids (Lindroth and St. Clair 2013; Barker et al. 2019). Changes in root condensed tannin concentrations can potentially influence trophic interactions and nutrient dynamics in the rhizosphere. Condensed tannin concentrations of ~4% dry mass can decrease litter decomposition rates by ~50% in temperate mixed forests (Madritch et al. 2006), and condensed tannin levels explained 55–65% of the variation in soil nitrogen mineralization among cottonwood stands (Schweitzer et al. 2004). Warming-mediated decreases in condensed tannin concentrations in tree roots therefore have the potential to accelerate decomposition and release of nitrogen from roots. Whether, and the extent to which, these effects occur in a warming scenario should be explored further.

In summary, our results indicate that climate warming will influence the biomass and secondary chemistry of plant roots directly, through biochemical and physiological processes, and indirectly, through interactions of warming with biotic

factors such as herbivory. Changes in root chemistry are likely to affect belowground ecosystem processes through their effects on interactions among plant roots, microbes, herbivores, and organic matter within the rhizosphere. Considering the interactive effects of warming, aboveground herbivory and plant genotype on root phenolics reported here, and the known roles of root phenolics in multiple rhizosphere processes, climate warming will likely influence the ecological associations and perhaps genetic structures of aspen populations via complex invertebrate-plant-soil feedbacks.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01259-w.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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